# Growth at the edge of the niche: An experimental study of the harmful alga *Prymnesium parvum*

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### Abstract

The haptophyte *Prymnesium parvum* forms harmful blooms toxic to fish in coastal and inland waters. Its growth in relation to niche factors is poorly characterized for the low salinities found in the inland waters in which *P. parvum* blooms have occurred. The specific growth rate of *P. parvum* as a function of temperature and salinity was determined in nutrient-sufficient cultures with low salinity. Additionally, phosphorus-limited growth was determined at low salinity and temperatures at or below 20°C. In nutrient-sufficient cultures, decreasing salinity from 4 g $L^{-1}$ to 0.5 g $L^{-1}$ reduced the growth rate of *P. parvum*. The estimated optimal temperature for growth decreased with decreasing salinity from about 27°C at salinities above 10 g $L^{-1}$, to about 24°C at 4 g $L^{-1}$, to about 22°C at 0.5 g $L^{-1}$. In phosphorus-limited experiments, the half-saturation concentration for growth was less than 0.02 $\mu$mol $L^{-1}$ under most conditions. The phosphorus-saturated growth rate was 0.84 $d^{-1}$ at 4 g $L^{-1}$ salinity and 20°C, and it was reduced at lower salinities and temperatures. The salinity–temperature interaction found here weakens the negative effect of low temperature on growth at low salinity and might partially explain why blooms of *P. parvum* occur in the winter months in inland waters of the southwestern United States. However, the relatively slow growth of *P. parvum* at low temperature and salinity suggests that additional factors should be examined, such as reduced effects of competitors, pathogens, and grazers during winter.

Harmful algal blooms are among the water-quality problems receiving growing attention from aquatic scientists. There is a perception that the frequency of such blooms has increased over recent decades (Hallegraeff 1993; Sunda et al. 2006), perhaps due to ongoing eutrophication, aquaculture, and other anthropogenic changes. In some cases, the species responsible are classified as invasive because they have become abundant in locations where they were previously unnoticed. Examples include the invasion of warm, shallow lakes in temperate climate zones by the cyanobacterium *Cylindrospermopsis raciborskii* (Briand et al. 2004; Wiedner et al. 2007), as well as the focus of this paper: the haptophyte *Prymnesium parvum*, which is apparently invading brackish inland waters of the southwestern United States (James and De La Cruz 1989; Edvardsen and Paasche 1998; Roelke et al. 2007).

Studies of niche factors and resources likely to limit population growth may improve our understanding and ability to predict the spread of invasive, harmful algal species. Such approaches have long been applied in algal ecology (Rhee 1982), and quantitative information on responses to niche factors and resources is available for many species (Tilman et al. 1982; Grover 1989). However, this information is sparse for many harmful algal species of contemporary interest, including *P. parvum*.

Previous studies of the growth of *P. parvum* in relation to physical and chemical niche factors covered salinities characteristic of estuarine and coastal waters, up to about 35 g $L^{-1}$, with very few observations below 4 g $L^{-1}$ (Larsen et al. 1993; Larsen and Bryant 1998; Baker et al. 2007). Lower salinities of 1–3 g $L^{-1}$ are common in most of the brackish inland waters of the southwestern United States where *P. parvum* blooms have occurred, and thus previous research provides only a limited characterization of its growth at salinities characteristic of such inland waters. Furthermore, there are no published studies that provide parameter estimates for commonly used kinetic models of nutrient-limited growth, such as the Monod model and its modifications (Istvánovics et al. 2000).

A lack of information about the specific growth rate of *P. parvum* at low salinities and nutrient concentrations hinders quantitative analysis of the potential for harmful blooms in many inland waters. Predictive, mechanistic models of bloom dynamics conventionally describe algal growth using a maximal rate that depends on physical factors multiplied by saturation terms that describe nutrient limitation (Chapra 1997). This study provides the necessary information for such models. Specific growth rates, abundances, and toxicity to fish under nutrient-sufficient conditions were measured in a factorial experiment at temperature and salinity levels characteristic of inland waters of the southwestern United States where harmful blooms have occurred. Additional experimental treatments compared specific growth rates at the ionic composition of seawater to specific growth rates at an ionic composition simulating inland waters. At selected combinations of temperature, salinity, and ion composition, specific growth rates were measured for a range of low-phosphorus (P) concentrations. Although the physical and

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chemical niche has been studied for many algae, growth is usually characterized under conditions near optimum or across a wide range. Here, we focus on growth of *P. parvum* at the edge of the niche, in terms of salinity, an uncommon approach but one that may be valuable for understanding invasive species (Briand et al. 2004).

## Methods

*General culture methods*—The strain of *P. parvum* culture used in this study (Culture Collection of Algae at the University of Texas at Austin, UTEX LL 2797) was isolated by J. Glass from a bloom in Texas and used in previous laboratory studies (Baker et al. 2007; Grover et al. 2007). For stock cultures, an artificial seawater (ASW) medium was prepared according to Kester et al. (1967), diluted to a working salinity of 5.8 g L$^{-1}$ in ultrapure water (Millipore Milli-Q, 18 MΩ cm$^{-1}$), and then enriched with f/2 levels of nitrogen (N), P, trace metals, and vitamins (MacLachlan 1973). In the trace-metals solution, an equimolar amount of ferric chloride was substituted for ferrous ammonium sulfate due to the toxicity of ammonium to *P. parvum* (Grover et al. 2007). An additional medium simulated inland waters of western Texas affected by *P. parvum* using an ion composition similar to Lake Whitney, Texas, which was ascertained during an active bloom. This artificial lake-water (ALW) medium was modified from ASW as follows: the same molar concentration of Mg was added as MgSO$_4$ instead of MgCl$_2$ to produce higher sulfate concentrations; eightfold higher additions of CaCl$_2$·2H$_2$O were used; and eightfold higher additions of NaHCO$_3$ were added aseptically after autoclaving. For both ASW and ALW, full-strength preparations of the basal salts were diluted as needed with ultrapure water to achieve experimental salinities.

Stock cultures of *P. parvum* were maintained by monthly transfers in an incubator at 20°C and on a 12:12 h light:dark photoperiod with an irradiance of about 150 μmol m$^{-2}$ s$^{-1}$. This photoperiod was used for all stock and experimental cultures because blooms typically begin in Texas at approximately this photoperiod in autumn. Irradiance of experimental cultures was also 150 μmol m$^{-2}$ s$^{-1}$, a value similar to average water-column irradiances in Texas lakes (Grover and Chrzanowski 2004). Illumination and culture-vessel positions were adjusted based on irradiance values measured with a photon flux meter (LiCor model LI-1400) in several areas inside of the incubators.

*Nutrient-sufficient experiment*—This experiment determined the growth rate and toxicity of *P. parvum* in response to temperature and low levels of salinity, along with additional treatments that examined the ion composition of the medium. All nutrients were provided at the high concentrations used in stock cultures to exclude limitation. To quantify curvilinear or unimodal responses to temperature and salinity, and their interactions, a duplicated 3 × 3 factorial experimental design was used with salinities of 0.5, 1.0, and 4 g L$^{-1}$, and temperatures of 11°C, 20°C, and 29°C (Table 1). For this design, ASW medium was used.

Table 1. Design for the nutrient-sufficient experiment. Ion composition was artificial seawater (ASW) or lake-water (ALW) medium. There were duplicate cultures for each combination of medium, temperature, and salinity.

| Ion composition | Salinity (g L$^{-1}$) | Temperature (°C) |
|---|---|---|
| ASW | 0.5 | 11, 20, 29 |
| | 1 | 11, 20, 29 |
| | 4 | 11, 20, 29 |
| | 17.8 | 20 |
| ALW | 0.5 | 20 |
| | 1 | 20 |
| | 4 | 20 |
| | 17.8 | 20 |

Additional treatments at 20°C were added to compose a duplicated 4 × 2 factorial design with four salinities (0.5, 1, 4, and 17.8 g L$^{-1}$) crossed with two ion compositions (ASW or ALW media). The high-salinity treatment of 17.8 g L$^{-1}$ was included to facilitate comparison to a similar experiment done over a higher salinity range (Baker et al. 2007).

After diluting basal salts to the experimental salinity, NaNO$_3$ was added. Other nutrients (NaH$_2$PO$_4$, NaHCO$_3$, vitamins, trace metals) were added aseptically after autoclaving (filter-sterilization through Nalgene 0.2-μm, nylon syringe filters). The same concentration of NaHCO$_3$ was added to all cultures of a given medium (ASW vs. ALW), so that dissolved inorganic carbon (DIC) availability would not be affected by the experimental adjustments of salinity. All ALW cultures received 2480 μmol L$^{-1}$ of bicarbonate, and all ASW cultures received 379 μmol L$^{-1}$ of bicarbonate. One-liter borosilicate glass flasks were filled to a working volume of 800 mL, and each flask was inoculated with 100 cells mL$^{-1}$ of *P. parvum* from stock cultures in late exponential phase grown in the corresponding ion composition at the stock salinity of 5.8 g L$^{-1}$. Inoculated flasks of appropriate salinity were then distributed to incubators at different temperatures to achieve the treatment combinations in the experimental design. Flasks were mixed daily by gently swirling, and positions within incubators were rotated daily.

Samples of experimental batch cultures were taken on days 2–4, 7, 9, 11, 14, 17, and 21. Populations reached stationary phase during this time. At each sampling, aliquots of 5 mL were preserved with 0.15 mL of Lugol's iodine (Throndsen 1978) for counts of *P. parvum*. Stock and experimental cultures were not axenic, so aliquots of 10 mL were preserved with 0.5 mL of formalin for bacterial counts. Cell concentration of *P. parvum* was obtained by direct microscopic counts, using sedimentation chambers and inverted microscopy (Margalef 1969). Cell concentrations of bacteria were obtained by direct counts of cells stained with acridine orange, collected on polycarbonate filters, and examined with epifluorescence microscopy (Hobbie et al. 1977). Maximum bacterial abundance in experimental cultures reached 2.65 × 10$^7$ cells mL$^{-1}$, although most cultures were below this, with an average abundance of 7.84 × 10$^6$ cells mL$^{-1}$. Bacterial data were not further analyzed. Specific growth rate (μ) of *P. parvum*

in each culture was estimated by regressing the natural logarithm of cell concentration against time for days 2 to 9. Stationary cell concentration was taken as the average for days 17 and 21. On day 21, pH was determined with a calibrated electrode.

Acute toxicity was assessed for samples taken on day 21 by completing 48-h bioassays with <48-h-old fathead minnows (*Pimephales promelas*) according to U.S. Environmental Protection Agency (EPA) test method 2000.0 (USEPA 2002). The fish were fed newly hatched *Artemia nauplii* 2 h prior to test initiation. Their survival was assessed with whole-culture samples and at a series of six successive dilutions (0.5 at each step) from 50% to 1.56% (duplicate batches of five fish per dilution level). Reconstituted hard water (APHA 1998) was used as the dilution water and as a control, and additional controls were prepared by diluting samples of sterile culture media (ASW, ALW without algae). The percentage dilution of the whole sample causing 50% mortality of subjects ($LC_{50}$) was estimated using Probit (Finney 1971) or Trimmed Spearman–Karber (Hamilton et al. 1977) methods, as appropriate. To compare toxicity among cultures with different populations of *P. parvum*, the percentage $LC_{50}$ values were then multiplied by stationary cell concentration to estimate $LC_{50}$ as the concentration of cells causing 50% mortality of subjects. For example, a culture with a stationary cell concentration of $4.9 \times 10^5$ cells mL$^{-1}$ was estimated to kill 50% of subjects when diluted to 7.9% of its original concentration, giving a calculated $LC_{50}$ of $3.9 \times 10^4$ cells mL$^{-1}$.

Specific growth rate ($\mu$) was analyzed using multiple regression, where the independent variables of temperature and salinity were centered on their means to reduce collinearity. A full model was fitted to data from salinities of 0.5 to 4.0 g L$^{-1}$ with quadratic and interaction terms for temperature and salinity, a categorical variable for ion composition, and linear and quadratic interaction terms between ion composition and salinity. Interactions between ion composition and temperature could not be estimated given the experimental design.

To allow the unimodal response to temperature to be asymmetric, temperature data were transformed using the equation (Baker et al. 2007):

$$T_{transform} = \exp\left(\frac{\theta(T-20)}{20}\right) \quad (1)$$

where $T$ is temperature (°C), and $\theta$ is a transformation parameter, the value of which was chosen to maximize fit ($R^2$) of the full model. A best subset regression scheme was then used to find a simpler regression model with acceptable fit using Mallow's $C_p$ statistic (Kleinbaum et al. 1998), excluding models having higher-order terms without the corresponding linear terms. Residual plots were examined for failures of the regression assumptions. Two-way factorial analysis of variance (ANOVA) was used to analyze $LC_{50}$ in relation to salinity and ion composition for a subset of cultures in which acute toxicity to fish was detected.

*Nutrient-limited experiments*—These experiments used a short-term batch culture method (Tilman and Kilham

1976) to determine the responses of specific growth rate ($\mu$) to limiting concentrations of P under selected combinations of temperature, salinity, and ion composition. Prior to inoculation of experimental cultures to determine P-limited growth kinetics, populations of *P. parvum* were preconditioned to reduce cellular P storage. This preconditioning was done by growth to stationary phase in media with reduced P concentration (3.6 $\mu$mol L$^{-1}$, 1/10 of the f/2 concentration). Preconditioned cultures for experiments at 20°C were grown for 4 weeks; those for experiments at 10°C were grown for 8 weeks due to slower growth.

With one exception, preconditioning cultures were duplicated for all experiments. Periodically, samples of 5 mL were taken from preconditioning cultures and preserved with Lugol's iodine for counts of *P. parvum*. At the end of preconditioning, samples of 50 mL were filtered (0.2 $\mu$m) for soluble reactive phosphorus (SRP) analysis (Strickland and Parsons 1972). Samples of 50 mL were also filtered onto GF/F filters and stored frozen for later determination of particulate phosphorus (PP) by wet digestion with persulfate (Menzel and Corwin 1965) followed by SRP analysis. Samples of 100 mL were filtered onto precombusted GF/F filters for determinations of particulate C and N with a Perkin-Elmer CHN analyzer. Inoculation of preconditioning cultures sometimes carried non-negligible amounts of dissolved P to experimental cultures from inoculation, and such carryover was calculated from SRP measurements and added to the experimental P concentration.

Cell quota of P at the end of preconditioning was calculated as PP divided by cell concentration. Particulate composition data were not obtained for the experiment in ASW medium at 20°C and 4 g L$^{-1}$. A three-way factorial ANOVA was used to analyze cell (i.e., particulate) composition of preconditioned cultures in relation to temperature, salinity, and ion composition.

After dilution of basal salts to obtain the experimental salinity, vitamins, trace metals, and NaNO$_3$ were added at f/2 concentrations. After autoclaving, NaHCO$_3$ and NaH$_2$PO$_4$ were added aseptically (filter-sterilization through Nalgene 0.2-$\mu$m, nylon syringe filters); the same concentrations of NaHCO$_3$ were added to ASW and ALW media in the nutrient-sufficient experiment. For the experiment in ASW medium at 20°C and 4 g L$^{-1}$ salinity, 24 flasks were prepared and autoclaved with 600 mL of media each. Four replicates were then prepared for each target concentration of P: 0, 0.01, 0.1, 0.5, 1, and 10 $\mu$mol L$^{-1}$. Cultures for the remaining experiments were prepared similarly, except that 12 flasks were used, giving duplicate cultures at each target P concentration. Each experimental culture was inoculated with 100 cells mL$^{-1}$ of *P. parvum* from a preconditioning culture grown under the same temperature, salinity, and ion composition.

Experimental cultures were incubated for 4 d, mixed daily by gently swirling, and positions of flasks within the incubators were rotated daily. Samples of 100 mL were taken daily from each experimental flask and preserved with Lugol's solution. Subsamples from preserved samples were settled in large-volume sedimentation chambers (50–100 mL) and counted with an inverted microscope to

Table 2. Acute (48-h) cellular toxicity ($LC_{50}$) of *P. parvum* to juvenile *Pimephales promelas* in relation to salinity and temperature. Each sample is a combination of salinity (g $L^{-1}$) and media type (ASW or ALW). All cultures were grown at 20°C.

| Salinity (g $L^{-1}$) | Medium | $LC_{50}$ (cells $mL^{-1}$) |
|---|---|---|
| 4 | ASW | 39,000 |
| 4 | ASW | 47,000 |
| 4 | ALW | 32,000 |
| 4 | ALW | 67,000 |
| 17.8 | ASW | 52,000 |
| 17.8 | ASW | 56,000 |
| 17.8 | ALW | 65,000 |
| 17.8 | ALW | 22,000 |

determine cell concentration. On day 4, samples of 50 mL were filtered (0.2 $\mu$m) for SRP analysis to evaluate nutrient depletion below the target concentration during the incubations.

Samples from the first day were very sparse, with unacceptable counting errors, so $\mu$ was estimated by regressing the natural logarithm of cell concentration against time for days 2–4. Specific growth rate was analyzed in relation to P concentration using two kinetic models: the conventional Monod model (Tilman et al. 1982; Grover 1989),

$$\mu = \frac{\mu_{max}S}{K_\mu + S} \qquad (2)$$

where $S$ is nutrient concentration, $\mu_{max}$ is the maximal specific growth rate, and $K_\mu$ is the half-saturation constant; and a modified Monod model with threshold concentration nutrient concentration ($S_T$) required for positive growth rate (Istvánovics et al. 2000):

$$\mu = \frac{\mu_{max}(S - S_T)}{K_\mu + S - S_T} \qquad (3)$$

These kinetic models were fitted with nonlinear least-squares regression, taking as independent variable the average of the target P concentration corrected for carryover from the preconditioning culture and the measured SRP concentration on day 4. The modified Monod model (Eq. 3) was fitted for all experiments, except for experiments in which negative estimates of the threshold $S_T$ were obtained, in which case, the conventional Monod model (Eq. 2) was fitted.

## Results

*Nutrient-sufficient experiment*—Specific growth rate ($\mu$) showed a unimodal function of temperature and an increasing function of salinity (Fig. 1). Asymmetry of the temperature response was represented by transforming temperature according to Eq. 1 with parameter $\theta = 0.7$. After conducting the best subsets regression analysis, a predictive model for $\mu$ was selected with five terms, including all linear and quadratic terms for temperature and salinity, and a temperature-salinity interaction:
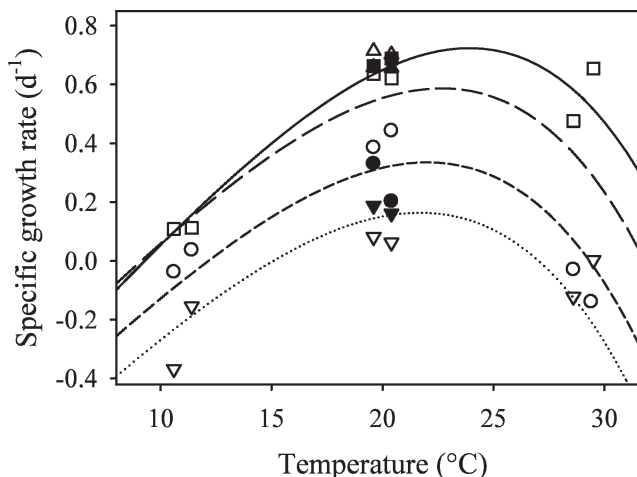


Fig. 1. Specific growth rate of *P. parvum* in the nutrient-sufficient experiment. Symbols show growth rate under different salinity and ion composition: Triangles = 0.5 g $L^{-1}$; circles = 1.0 g $L^{-1}$; squares = 4 g $L^{-1}$. Open symbols = ASW medium; closed symbols = ALW medium. Curves show the fitted regression model (Eq. 4) for different salinities: solid curve = 4 g $L^{-1}$; long-dashed curve = 2 g $L^{-1}$; short-dashed curve = 1 g $L^{-1}$; dotted curve = 0.5 g $L^{-1}$.

$$\mu = -3.531 + 0.02534(\sigma - 1.833) - 0.06311(\sigma - 1.833)^2$$
$$+ 7.468 \exp\left[0.7\left(\frac{T-20}{20}\right)\right]$$
$$- 3.414 \exp\left[1.4\left(\frac{T-20}{20}\right)\right]$$
$$+ 0.1697(\sigma - 1.833) \exp\left[0.7\left(\frac{T-20}{20}\right)\right] \qquad (4)$$

where $\sigma$ is salinity (g $L^{-1}$), and $T$ is temperature (°C). The predictive model fit well ($R^2 = 0.901$) and was significant ($F_{5,18} = 32.5$, $p < 0.001$). Terms not included in the predictive model involved ion composition and its interaction with salinity and were not statistically significant (partial *F*-tests, df = 1, 15, $p > 0.3$). Terms included in the predictive model were statistically significant (partial *F*-tests, df = 1, 18, $p < 0.01$) with two exceptions: the linear salinity term (partial *F*-test, df = 1, 18, $p = 0.71$) was included because the marginal quadratic salinity term (partial *F*-test, df = 1, 18, $p = 0.052$) was also included. Eliminating the quadratic salinity term produced residual plots that strongly suggested curvature in the response to salinity, so the term was retained. The highest predicted $\mu$ from Eq. 4 was 0.72 $d^{-1}$ at a temperature of 24°C and salinity of 4 g $L^{-1}$, the upper salinity limit for applicability of this model.

Using 48-h static bioassays to assess acute toxicity to *P. promelas*, only samples from cultures with salinities of 4 or 17.8 g $L^{-1}$, grown at 20°C, were toxic. Other cultures were not detectably toxic (i.e., survival of fish in 100% sample water did not differ from survival in control water). Cultures that were toxic to fish had significantly higher (*t*-test, $t = 9.98$, df = 26, $p < 0.001$) average stationary cell

Table 3. Cell quotas and stoichiometry from P-limited preconditioning cultures of *P. parvum*. Cultures were grown under the indicated temperature, salinity, and ion composition (ASW = artificial seawater; ALW = artificial lake water). Ranges are given for duplicate cultures, except where the asterisk (*) indicates a single culture.

| Temperature (°C) | Salinity (g L$^{-1}$) | Ion composition | P quota (range, fmol cell$^{-1}$) | Cellular C:P (range) | Cellular N:P (range) |
|---|---|---|---|---|---|
| 10 | 1 | ASW | 335–341 | 69–81 | 1.3–1.4 |
| 10 | 1 | ALW | 82* | 1001* | 26* |
| 10 | 3 | ASW | 447–481 | 103–121 | 5.1–5.9 |
| 10 | 3 | ALW | 246–268 | 98–121 | 6.0–6.5 |
| 20 | 1 | ASW | 288–298 | 71–95 | 4.5–5.2 |
| 20 | 1 | ALW | 15–17 | 152–168 | 17–19 |
| 20 | 3 | ASW | 6.7–6.8 | 457–458 | 39–40 |
| 20 | 3 | ALW | 3.7–4.3 | 520–588 | 44–45 |

concentration of *P. parvum* than those that were not detectably toxic ($5.0 \times 10^5 \pm 1.5 \times 10^5$ cells mL$^{-1}$ for toxic cultures, $3.8 \times 10^4 \pm 9.1 \times 10^4$ cells mL$^{-1}$ for nontoxic cultures, mean $\pm$ SD). For many cultures not detectably toxic, stationary cell concentrations of *P. parvum* were below the LC$_{50}$ cell concentrations of toxic cultures, which ranged from 2.2 to $6.7 \times 10^4$ cells mL$^{-1}$ (Table 2). Toxic cultures also had significantly higher (*t*-test, $t = 5.43$, df = 26, $p < 0.001$) pH than those that were not detectably toxic ($9.5 \pm 0.41$ for toxic cultures, $7.5 \pm 0.97$ for nontoxic cultures). For toxic cultures, LC$_{50}$ was not significantly related to salinity, ion composition, or their interaction (ANOVA, *F*-tests, df = 1, 4, $p > 0.5$).

*Nutrient-limited experiments*—Preconditioning cultures were intended to reduce cellular P storage in *P. parvum* populations prior to inoculation into experimental cultures for determination of P-limited growth kinetics. After successful preconditioning, the cell quota of P can be interpreted as the minimal quota at which the population growth rate goes to zero (Grover 1989). Data on P quotas and cellular stoichiometry suggest that successful reduction of P storage occurred under some conditions but not others (Table 3). Cell quotas of P were less than 20 fmol cell$^{-1}$ for three sets of preconditioning cultures (Table 3), and all of these cultures had cellular C:P and N:P ratios exceeding the Redfield ratios of 106 and 16, respectively. Cell quota was somewhat higher at 82 fmol cell$^{-1}$ in the unreplicated preconditioning culture at 10°C, 1 g L$^{-1}$ salinity, and

ALW medium, but both cellular C:P and N:P ratios exceeded the Redfield ratios. These results suggest successful reduction of stored P by precondition in these four cases, and they suggest that the minimal P quota for *P. parvum* can approach about 4 fmol cell$^{-1}$. For all other experimental conditions, cell quotas of P in preconditioning cultures exceeded 200 fmol cell$^{-1}$, and cellular C:P and N:P ratios ranged from lower than the Redfield ratios to slightly higher. These results suggest that preconditioning did not successfully reduce stored P in these four cases.

For two experiments, parameter estimates for $K_\mu$ and $S_T$ were not biologically meaningful, i.e., estimates were negative. These cases occurred at 10°C and salinities of 1 and 3 g L$^{-1}$ in ASW medium. In the remaining experiments, biologically meaningful parameter estimates were obtained (Table 4; Fig. 2). In three cases, estimates of $S_T$ were positive. Otherwise, estimates of $S_T$ were negative, so Eq. 2 was fitted.

Estimates of $\mu_{\max}$ increased with temperature and salinity (Table 4), although they were not well predicted by regression Eq. 4 as developed from the nutrient-sufficient experiment. With one exception, estimates of $K_\mu$ and $S_T$ were low, indicating that P-dependent growth was saturated at concentrations lower than about 0.05 $\mu$mol L$^{-1}$. In general, estimates of $K_\mu$ were higher for growth in ASW medium than in ALW medium. For cultures grown at 20°C and 1 g L$^{-1}$ salinity in ASW medium, the estimated $K_\mu$ approached 0.5 $\mu$mol L$^{-1}$.

Table 4. Summary of nonlinear regression fit of all nutrient kinetics experiments. Cultures were grown under the indicated temperature, salinity, and ion composition (ASW = artificial seawater; ALW = artificial lake water). Maximal growth rate ($\mu_{\max}$), half-saturation constant ($K_\mu$), and threshold ($S_T$) estimates are from nonlinear regressions.

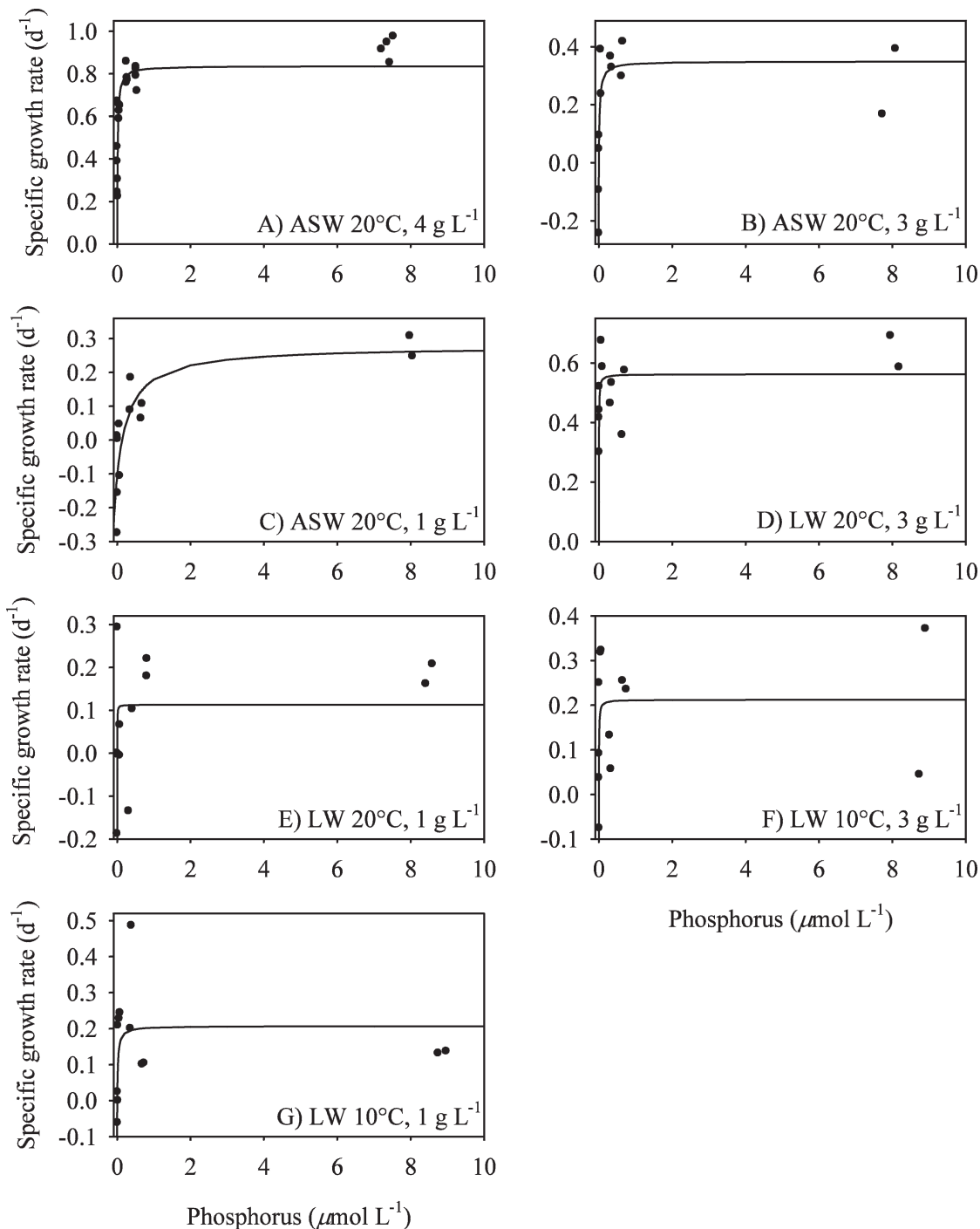| Growth conditions | | | Parameter (estimate$\pm$SE) | | |
|---|---|---|---|---|---|
| Temperature (°C) | Salinity (g L$^{-1}$) | Ion composition | $\mu_{\max}$ (d$^{-1}$) | $K_\mu$ ($\mu$mol L$^{-1}$) | $S_T$ ($\mu$mol L$^{-1}$) |
| 10 | 1 | ALW | 0.21$\pm$0.057 | 0.022$\pm$0.031 | N/A |
| 10 | 3 | ALW | 0.21$\pm$0.056 | 0.0055$\pm$0.0129 | 0.0011$\pm$0.0071 |
| 20 | 1 | ASW | 0.28$\pm$0.079 | 0.47$\pm$0.342 | 0.13$\pm$0.00276 |
| 20 | 1 | ALW | 0.11$\pm$0.047 | 0.0026$\pm$0.0051 | 0.0016$\pm$0.0031 |
| 20 | 3 | ASW | 0.35$\pm$0.058 | 0.024$\pm$0.025 | N/A |
| 20 | 3 | ALW | 0.56$\pm$0.040 | 0.0032$\pm$0.0019 | N/A |
| 20 | 4 | ASW | 0.84$\pm$0.048 | 0.013$\pm$0.0046 | N/A |

Fig. 2. Specific growth rates of *P. parvum* in nutrient-limited experiments. Curves show the fitted kinetic model (Eq. 2 or 3), the parameters of which are given in Table 4.

## Discussion

Species that become abundant where previously unnoticed, including invasive species, likely encounter conditions at the edge of their niche. Traditionally, however, ecophysiological studies have measured growth at near optimal conditions, or over broad ranges that do not focus on the edge of the niche. This study focused on the growth performance of the harmful alga *Prymnesium parvum* at the low-salinity edge of its niche. For the strain studied here, a previous study estimated an optimal salinity for specific growth rate of 22 g $L^{-1}$ (Baker et al. 2007), and studies of other strains suggest optimal growth at salinities of 8–34 g $L^{-1}$ (Larsen et al. 1993; Larsen and Bryant 1998). Here, specific growth rate was found to decrease strongly as salinity decreased from 4 to 0.5 g $L^{-1}$. A lower limit of salinity for population increase appears to lie between 0.5 and 1 g $L^{-1}$. In the range of 1–2 g $L^{-1}$, characterizing
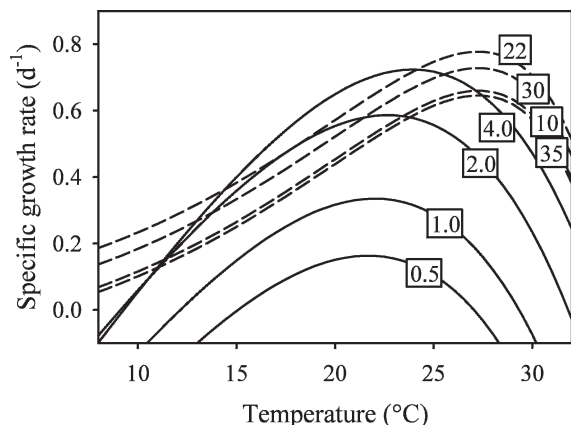
Fig. 3. Comparison of the regression model for specific growth rate fitted to the nutrient-sufficient experiment (Eq. 4, solid curves) with a previous model fitted to specific growth rate in a similar experiment at a higher salinity range (Baker et al. 2007, dashed curves). Numbers in the boxes are the salinity (g $L^{-1}$) for each curve.

many of the brackish inland waters in the southwestern United States where *P. parvum* has recently been problematic, rates of population growth reached a modest range of 0.1–0.3 $d^{-1}$.

Previous studies of *P. parvum* at higher salinities suggest a relatively high optimum temperature for population growth, estimated to be 27°C for the strain used in this study (Baker et al. 2007) and 15–30°C for other strains (Larsen et al. 1993; Larsen and Bryant 1998). For growth of this strain at higher salinities, Baker et al. (2007) derived a regression function similar to Eq. 4. A comparison of these two regressions (Fig. 3) suggests that low salinity reduces the growth of *P. parvum* to a greater extent at high temperatures than at low temperatures. Thus, the optimum temperature for growth apparently decreases as salinity decreases, reflecting a statistically significant interaction of temperature and salinity. Optimal temperatures for growth approaching 30°C found in other studies at salinities greater than 4 g $L^{-1}$ are hard to reconcile with the tendency for *P. parvum* to bloom during cooler weather in Texas and other parts of the southwestern United States. The salinity–temperature interaction found here shifts the optimum temperature for growth at salinities of 1–2 g $L^{-1}$, perhaps making blooms less likely during warm weather in this region and more likely in cooler weather.

The growth performance of *P. parvum* at low-P concentration might also contribute to bloom formation in inland waters. Though extensive studies of nutrient limitation in most Texas lakes affected by *P. parvum* have not been done, nearby lakes are often but not continually P-limited (Grover et al. 1999; Grover and Chrzanowski 2004). The nutrient-saturated, maximal growth rates of *P. parvum* are modest compared to many other algal species grown at similar temperatures (Tilman et al. 1982; Grover 1989; Grover et al. 1999), but growth near maximal is maintained at low-P concentrations under some conditions (Table 4; Fig. 2). In particular, P concentrations permitting half the maximum growth rate (i.e., the sum $K_\mu + S_T$) range

from 0.003 to 0.007 $\mu$mol $L^{-1}$ when *P. parvum* is grown under low-salinity conditions in ALW medium that mimics the ion composition of inland waters. Half-saturation concentrations found here for *P. parvum* are similar to those for diatoms and green algae identified as having high competitive ability for P (Tilman et al. 1982; Grover 1989), and they are lower than those of some diatoms, green algae, and cyanobacteria found in Texas lakes (Grover et al. 1999). High competitive ability under P limitation is consistent with high expression of putative phosphate transporter genes in *P. parvum* (La Claire 2006).

High competitive ability for P by *P. parvum* appears to be enhanced by the ion composition of brackish inland waters, which are rich in divalent cations and bicarbonate compared to seawater. When *P. parvum* is grown under low salinity with the ion composition of seawater, $K_\mu + S_T$ values exceed 0.01 $\mu$mol $L^{-1}$. Thus, *P. parvum* appears to be physiologically pre-adapted to invade P-limited brackish inland waters. Few other studies have examined the influence of salinity and ion composition on nutrient-limited growth kinetics of algae. For three diatom species studied, salinity and ion composition had species-specific influences on nitrate- and ammonium-limited growth kinetics (Saros and Fritz 2000). Given the widespread occurrence of brackish and saline inland waters in arid and semiarid climates, similar information would be helpful for other algae occurring in such regions.

The nutrient-limited experiments relied on a preconditioning phase to reduce cellular P storage. Based on P quotas and stoichiometry, preconditioning was successful in four cases and unsuccessful in another four. All of the unsuccessful cases involved either low temperature or low salinity, at which growth was so slow that the anticipated P depletion did not occur. Such failure of preconditioning is an inherent risk of examining growth under suboptimal conditions. For two cases of unsuccessful preconditioning, no estimates of growth kinetic parameters were obtained due to high variability of specific growth rates. For another case of unsuccessful preconditioning (growth at 20°C, 1 g $L^{-1}$ salinity in ASW medium), the estimated value of $K_\mu + S_T$ far exceeded all other such estimates (Table 4) and probably is unreliable. For the last case of unsuccessful preconditioning (growth at 10°C, 3 g $L^{-1}$ salinity in ALW medium), the estimated value of $K_\mu + S_T$ is within the range of estimates obtained when preconditioning was successful. For one final set of conditions (growth at 20°C, 4 g $L^{-1}$ salinity in ASW medium), data on cellular P composition were not obtained, so the success of preconditioning cannot be evaluated, but the value of $K_\mu + S_T$ is within the range of estimates obtained when preconditioning was successful.

For the temperature and salinity ranges of 10–20°C and 1–4 g $L^{-1}$, the nutrient-sufficient experiment predicts that specific growth rate increases with both of these factors. Qualitatively, estimates of the maximal growth rate $\mu_{max}$ from nutrient-limited experiments agree with this prediction. Quantitatively, however, estimates of $\mu_{max}$ consistently exceed the predictions of the regression Eq. 4 developed from the nutrient-sufficient experiment. Preconditioning may be responsible for this difference. Inocula for the nutrient-sufficient experiment were taken directly from stock

cultures grown at 20°C and 5.8 g L$^{-1}$ in ASW or ALW media without preconditioning, and thus they experienced shifts in temperature and salinity at the start of the experimental incubations. Inocula for the nutrient-limited experiment were preconditioned by extended growth under the experimental temperature and salinity conditions. The fact that higher nutrient-saturated growth rates were apparently produced by preconditioning suggests that *P. parvum* might acclimate to suboptimal conditions over several generations. Such acclimation deserves further study, and it could enhance the capability of this species to form blooms under suboptimal growth conditions.

Toxicity of *P. parvum* is associated with nutrient limitation (Johansson and Granéli 1999; Granéli and Johansson 2003), and for the strain studied here, growth under suboptimal temperature and salinity previously led to enhanced toxicity (Baker et al. 2007). In the current study, toxicity to fish was detected only at salinities of 4 g L$^{-1}$ or higher. Populations cultured at lower salinities were not detectably toxic. The low abundance of most nontoxic cultures might explain such lack of toxicity. Most (75%) of the nontoxic cultures had cell concentrations below $2.2 \times 10^4$ cells mL$^{-1}$, the lowest LC$_{50}$ estimated from bioassays of acute toxicity to fish, and many (40%) of the nontoxic cultures had much lower cell concentrations of <1000 cells mL$^{-1}$. Even if cells of *P. parvum* were actively producing toxins, sparse populations might not accumulate enough toxins to cause mortality of fish during short-term bioassays. On the other hand, two nontoxic cultures had cell concentrations exceeding $10^5$ cells mL$^{-1}$. Dense populations of *P. parvum* with little or no toxicity have been observed in field experiments and lake monitoring (D. L. Roelke unpubl.; Schwierzke et al. in press). Together, these observations suggest that high abundance is a necessary but not sufficient condition for fish-kills to occur, and that regulation of toxin production also plays a role. These observations also suggest that toxin production and allelopathy are not necessary for high abundance, i.e., blooms, to occur.

The P-limited growth kinetics found here imply that only a severe depletion of P would reduce the potential for blooms to occur. Perhaps somewhat paradoxically, available evidence suggests that since specific growth of *P. parvum* becomes saturated at low-P concentrations, nutrient additions might often do little to increase its population growth, while reducing toxicity (Johansson and Granéli 1999; Granéli and Johansson 2003), and even reducing bloom initiation by stimulating competitors (Roelke et al. 2007; Errera et al. 2008).

As expected, growth performance is reduced at the low-salinity edge of the niche for *P. parvum*. Nevertheless, under many low-salinity conditions, its specific growth rate is sufficient to produce blooms that are toxic to fish. Blooms of *P. parvum* in the southwestern United States occur at water temperatures of 10–20°C and salinities of 1–2 g L$^{-1}$ (Roelke et al. 2007; L. Schwierzke unpubl.), where a growth rate of 0.1–0.3 d$^{-1}$ can be expected. A bloom population of $4 \times 10^4$ cells mL$^{-1}$ (L. Schwierzke unpubl.) can thus be reached from a background population of $10^2$ cells mL$^{-1}$ in as little as 20 d, if population losses are

negligible. Predictions of growth rates can be constructed by using Eq. 4 to describe the maximal growth rate ($\mu_{max}$) of *P. parvum* as a function of temperature and salinity, multiplied by a saturation term derived from Eq. 2 to describe phosphorus-limitation. This is a standard representation of algal growth in water-quality models (Chapra 1997). The resulting growth equations provide a reasonable model for short-term dynamics within a single bloom (D. L. Roelke et al. unpubl.). A longer-term model of annual dynamics incorporating several other processes was less successful when compared to observations in one lake (Grover et al. in press).

Ecologists often characterize growth performance under conditions close to optimal for the species they study. As a practical matter, growth rates are lower and more variable as the edge of the niche is approached, making their measurement difficult. However, there may also be an implicit view that species will most likely be abundant under conditions near the center of their niche; that is, that the fundamental and realized niches (Hutchinson 1958) do not differ much. For *P. parvum*, the realized niche where it occurs in brackish inland waters appears to lie along the edge of the fundamental niche, possibly as a result of its competitors (Roelke et al. in press) or natural enemies (Schwierzke et al. in press). Similarly, the tropical cyanobacterium *Cylindrospermopsis raciborskii* appears to be invading inland waters at midlatitudes along the low-temperature edge of its niche (Briand et al. 2004; Wiedner et al. 2007). Growth at the edge of the fundamental niche might be relevant more generally for other invasive and nuisance species, especially given global changes in climate, hydrology, and nutrient loading.

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