Limnol. Oceanogr., 50(3), 2005, 883–895

Does the red tide dinoflagellate *Karenia brevis* use allelopathy to outcompete other phytoplankton?

Julia Kubanek[1] *and Melissa K. Hicks*
School of Biology and School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332-0230

Jerome Naar
Center for Marine Science, University of North Carolina at Wilmington, Wilmington, North Carolina 28409

Tracy A. Villareal
Marine Science Institute, University of Texas at Austin, Port Aransas, Texas 78373

Abstract

Monospecific blooms of phytoplankton can disrupt pelagic communities and negatively affect human health and economies. Interspecific competition may play an important role in promoting blooms, and so we tested (1) whether the outcome of competition between the red tide dinoflagellate *Karenia brevis* (ex *Gymnodinium breve*) and 12 co-occurring phytoplankters could be explained by allelopathic effects of compounds released by *K. brevis* and (2) whether waterborne, lipophilic molecules, including brevetoxins, are involved. Nine of 12 phytoplankton species were suppressed when grown with live *K. brevis* at bloom concentrations. *K. brevis* extracellular filtrates or lipophilic extracts of filtrates inhibited six of these nine species, indicating allelopathy. However, these inhibitory effects were weaker than those experienced by competitors exposed to live *K. brevis.* Brevetoxins at ecologically reasonable waterborne concentrations accounted for the modest inhibition by *K. brevis* of only one competitor, *Skeletonema costatum.* The addition of brevetoxins also caused significant autoinhibition, reducing the maximum concentration of *K. brevis.* Allelopathy is one mechanism by which *K. brevis* appears to exhibit competitive advantage over some sympatric phytoplankters, although unidentified compounds other than brevetoxins must be involved, in most cases. *K. brevis* was also susceptible to competitive exclusion by several species, including *Odontella aurita* and *Prorocentrum minimum,* known to thrive during *K. brevis* blooms. Although field experiments are required to assess whether allelopathy plays a fundamental role in bloom dynamics, our results indicate that allelopathy occurs widely but with species-specific consequences.

Competition is one of the dominant forces structuring communities, including marine pelagic communities (Hutchinson 1961). The production and release of compounds that inhibit competitors, a process known as allelopathy, is a mechanism of interference competition that is hypothesized to be important among phytoplankton (Smayda 1997), affecting species succession (Keating 1977), especially under eutrophic conditions (Maestrini and Bonin 1981). Allelopathy may be a successful strategy for phytoplankton species that occur in dense blooms, maximizing the concentration of allelopathic compound(s) exposed to competitors and minimizing the opportunity for the evolution of resistance in competitors because of the infrequent occurrence of these blooms (Lewis 1986). However, few studies have unambiguously established that allelopathy occurs among co-occurring phytoplankton species, and this is the case for several reasons. First, using mixed culture competition experiments (e.g., Schmidt and Hansen 2001) or field observations of species composition (e.g., Vardi et al. 2002), it is impossible to distinguish allelopathy from other mechanisms, such as exploitation competition. Second, if cell lysates or extracts of one phytoplankton species are added to a second species in lab experiments, intracellular phytoplankton compounds that would not be expected to contact competitors are likely to confound allelopathic effects (e.g., Freeberg et al. 1979). Third, it is difficult to control nutrients in experiments in which extracellular filtrates are added to live cultures to test for growth inhibition (e.g., Rengefors and Legrand 2001), which could lead to possible misinterpretation of effects. Fourth, allelopathic compounds may be released at low concentrations, at different concentrations during different growth stages, and potentially in response to cues by competitors, making their isolation and identification challenging. Finally, high-nutrient, high–cell concentration, small-volume lab experiments that test competitive outcome between pairs of species are unlikely to represent what happens in the field (Maestrini and Bonin 1981; Legrand et al.

[1] Corresponding author (julia.kubanek@biology.gatech.edu).

Acknowledgments

This research was supported by NSF grant OCE-0134843 to J.K. M.K.H. was supported as a summer undergraduate research assistant by the Camille and Henry Dreyfus Foundation Environmental Chemistry Program. Nutrient analyses were funded by NOAA MERHAB grant MER 02-627A to T.A.V., and ELISA analyses were funded by COP-NOAA MERHAB, NIEHS PO1 ES10594-03, and the CDC-FDOH U50/CCU423360-01 to J.N. We thank P. Tester, R. Pierce, M. Hay, and E. Litchman for advice in planning experiments and two anonymous reviewers for suggestions that improved the manuscript. We also thank E. Prince, A. Prusak, E. John, D. Collins, and A. Lenzo for lab assistance and T. Riggens for advice about culturing phytoplankton.

2003). Nevertheless, because phytoplankton generally exist under flow regimes dominated by viscous rather than turbulent forces (Wolfe 2000), the release of noxious substances that kill or repel competitors to maximize one's access to essential nutrients and light remains a viable and undertested hypothesis for the dominance of certain species. Allelopathy may be especially effective for slow-growing dinoflagellates, which may be inferior exploitation competitors.

In the Gulf of Mexico, the unarmored dinoflagellate *Karenia brevis* (ex *Gymnodinium breve*) forms almost monospecific blooms in autumn months and, increasingly, at other times of the year, with cell concentrations of thousands to millions per liter (Tester and Steidinger 1997). Elsewhere, such as in the western North Atlantic, blooms of *K. brevis* are rare, but they have on occasion altered phytoplankton species composition (West et al. 1996). *K. brevis* produces brevetoxins, polyketide secondary metabolites that are neurotoxic to mammals (Baden 1989) and that are implicated in shellfish, finfish, and seabird mortalities (Steidinger et al. 1973; Tiffany and Heyl 1978; O'Shea et al. 1991). How *K. brevis* natural products, including brevetoxins, mediate interactions with small planktonic organisms is not known. Unfortunately, the descriptor ''toxic,'' applied to *K. brevis*, may imply that brevetoxins kill competitors. However, prior to this study, there has been no direct test of whether natural concentrations of waterborne brevetoxins are allelopathic, although *K. brevis* cell lysates and intracellular extracts were shown to have negative effects on some phytoplankters (Freeberg et al. 1979).

In this study, we asked whether *K. brevis* can outcompete Gulf of Mexico phytoplankton, and if so, whether allelopathy contributes to competitive outcome. We compared the growth of 12 phytoplankton species, alone and with live *K. brevis* at bloom concentrations, with extracellular filtrates of *K. brevis* or with lipophilic extracts of these filtrates, under nutrient-rich laboratory conditions. We also incubated phytoplankton species with purified brevetoxins to directly test whether brevetoxins at reasonable waterborne concentrations affect the growth of competitors. We sought to understand whether competitive exclusion and allelopathy as a mechanism of interference competition have the potential to explain how *K. brevis* populations can occasionally be maintained in such dense, nearly monospecific blooms.

Materials and methods

Phytoplankton—We chose sympatric species known to cooccur temporally with *K. brevis* (except *Rhizosolenia* cf. *setigera*, available as an isolate from the Arabian Sea) and that tolerate similar light, temperature, and nutrient conditions. All species were obtained as nonaxenic strains from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP) (Table 1). All species were maintained at 22°C with a 12 : 12 h light : dark cycle in a Percival incubator with Philips F32T8/TL741 Universal/Hi-Vision fluorescent bulbs mounted vertically, producing irradiance of 100–145 μmol m^{-2} s^{-1} throughout the incubator (measurement using Biospherical Instruments light meter model QSL2100). Species were grown in autoclaved F/2 + silicate media (Sigma-Aldrich) made with filtered Maine seawater (salinity 36 parts per thousand). Preliminary growth curves were prepared using both in vivo chlorophyll fluorescence measurements (hereafter ''fluorescence'') (Turner Designs TD-700 calibrated with chlorophyll *a* standard) and visual cell counts using an Olympus IX-50 inverted microscope with a Palmer–Maloney settling chamber. Stock cultures were consistently transferred to fresh media once stationary phase was reached.

Preparation of extracellular filtrates and lipophilic extracts—Filtrates to be used in allelopathy experiments were prepared no more than 2 h before their use in experiments in order to minimize decomposition of compounds. *K. brevis* strains CCMP 718 and 2228 (both originally isolated from the Gulf of Mexico) were grown in one- or two-liter conical flasks as described above. Once in log to stationary phase, aliquots were taken for cell counts, and cultures of *K. brevis* were slowly filtered with gentle stirring through a 5-μm Millipore Isopore TMTP membrane using an Amicon ultrafiltration unit, until approximately 10% of the starting volume remained. Microscopy was used to verify that *K. brevis* cells did not pass through the membrane and that cells remaining in the unit were intact. The *K. brevis* filtrate was then filtered through a Millipore 0.22-μm syringe filter to remove bacteria. An aliquot of filtrate was frozen for brevetoxin measurement by enzyme-linked immunosorbent assay (ELISA; *see* below). Lipophilic extracts of *K. brevis* were prepared from extracellular filtrates, but without the 0.22-μm filtration step, by extraction with ethyl acetate (3 × 500 ml per 1 liter filtrate). Extracts were combined and solvent removed by rotary evaporation. An aliquot of extract was frozen for brevetoxin measurement. Brevetoxins were purified from *K. brevis* cultures as previously described (Bourdelais et al. 2004).

Quantification of brevetoxins—A competitive ELISA (Naar et al. 2002) was used to determine the concentration of type II brevetoxins (e.g., PbTx-2, PbTx-3, PbTx-9) in culture samples, extracellular filtrates, and filtrate extracts. Briefly, cultures and filtrates were sonicated for 15 min, whereas extracts were suspended in 1 ml of phosphate-buffered saline (0.1 mol L^{-1}, pH 7.4, containing 0.1% Tween 20 and 0.5% gelatin) and then sonicated. Each sample was serially diluted and applied to an ELISA plate previously treated with a brevetoxin–bovine serum album conjugate. Internal standards of PbTx-2 were also added to ELISA plates, and following reaction with anti-brevetoxin antibodies, amplification, and visualization, absorption at 492 nm was recorded using a Biotech fluo600 plate reader. Concentrations of brevetoxins were calculated by interpolation of a standard curve generated using PbTx-2.

Competition experiments—Three major experiments were conducted. In experiment 1, each tube containing 19.0 ml autoclaved fresh F/2 + silicate seawater media and either 5.0 ml of ultrafiltered and then sterile-filtered F/2 + silicate media (treatment A [control]), or 5.0 ml of *K. brevis* CCMP 2228 culture at early stationary phase with added F/2 + silicate nutrients (resulting in an average starting *K. brevis*

Table 1. Phytooplankton used, effects of *K. brevis* on growth of these species relative to controls, and effects of competition with these species on growth of *K. brevis* strain CCMP 2228 (NS, not significant; ND, experiment not done).

Species and taxon	Strain No. (CCMP)	Source of isolate	Effects of competition with live *K. brevis* (expt 1)	Negative effects of *K. brevis* filtrates (expts 1–2)	Negative effects of *K. brevis* extracts (expt 3)	Negative effects of brevetoxins (expt 3)	Effects of competition on *K. brevis* (expt 1)
Akashiwo sanquinea (dinoflagellate)	1740	Belize, Caribbean Sea	Maximum growth rate lower ($p=0.0017$) Lag before exponential growth longer ($p=0.054$)	NS	NS	Linear growth rate lower ($p=0.020$ for data to day 21)	*K. brevis* cell concentration lower ($p=0.017$ on day 7)
Amphora sp. (diatom)	129	Turks and Caicos Islands, Atlantic Ocean	Lag before exponential growth longer ($p<0.0001$)	Maximum concentration lower for two filtrates ($p=0.045$, 0.035)	NS	NS	*K. brevis* cell concentration lower ($p=0.026$ on day 14)
Asterionellopis glacialis (diatom)	137	Gulf of Mexico	Maximum concentration lower ($p=0.0075$)	Maximum concentration lower for two filtrates ($p=0.0026$, 0.042)	NS	NS	*K. brevis* cell concentration lower ($p=0.0051$ on day 7)
Chlorella capsulata (chlorophyte)	244	U.S. Virgin Islands, Caribbean Sea	NS	ND	ND	ND	*K. brevis* cell concentration lower ($p=0.0003$ on day 7)
Odontella aurita (diatom)	1796	Gulf of Mexico	NS	ND	ND	ND	*K. brevis* cell concentration lower ($p=0.0003$ on day 7)
Peridinium sp. (dinoflagellate)	626	Puerto Rico, Caribbean Sea	Linear growth rate lower ($p<0.0001$) Cell concentration lower ($p<0.001$ on days 10 and 20)	NS	NS	NS	NS
Prorocentrum mexicanum (dinoflagellate)	687	Florida Keys, Atlantic Ocean	Linear growth rate lower ($p<0.0001$) Cell concentration lower ($p<0.001$ on days 10 and 20)	Maximum concentration lower for one filtrate ($p=0.0011$)	NS	NS	NS
Prorocentrum minimum (dinoflagellate)	695	Florida Everglades	Maximum growth rate lower ($p=0.0008$)	NS	NS	NS	*K. brevis* cell concentration lower ($p=0.0003$ on day 7)
Rhizosolenia cf. *setigera* (diatom)	1694	Gulf of Oman Arabian Sea	Cell concentration lower ($p=0.0030$ on day 7)	Maximum growth rate lower for one filtrate ($p=0.054$)	NS	NS	*K. brevis* cell concentration lower ($p=0.0001$ on day 20)
Rhodomona lens (cryptophyte)	739	Bahamas, Atlantic Ocean	Linear growth rate higher ($p<0.0001$) Cell concentration higher ($p<0.016$ on day 20)	NS	NS	NS	*K. brevis* cell concentration lower ($p<0.0001$ on day 7)
Skeletonema costatum (diatom)	775	Gulf of Mexico	Maximum growth rate lower ($p=0.035$)	Maximum concentration lower ($p=0.0072$)	Maximum concentration lower for two extracts ($p=0.029$, 0.0047)	Maximum concentration lower ($p=0.039$)	*K. brevis* cell concentration lower ($p=0.0003$ on day 7)
Thalassiosira sp. (diatom)	1055	U.S. Virgin Islands, Caribbean Sea	Linear growth rate lower ($p<0.0001$ for data to day 10) Cell concentration lower ($p<0.001$ on days 10 and 20)	NS	Maximum concentration lower for one extract ($p<0.0001$)	NS	*K. brevis* cell concentration lower ($p=0.0002$ on day 20)

concentration of 2,400 cells ml^{-1} in experimental tubes; treatment B), or 5.0 ml of *K. brevis* CCMP 2228 extracellular filtrate (from same culture as ''B'' with added nutrients; treatment C) was inoculated with 1.0 ml of test phytoplankton species, except for slow-growing *Akashiwo sanguinea, Peridinium* sp., and *Prorocentrum mexicanum,* for which 2.5 ml inoculum and 17.5 ml fresh media were used. Five replicate tubes of each treatment were prepared for each species, and blocked replicates were haphazardly placed in the incubator. Positions in the incubator were changed and tubes were inverted every 1–2 d. One-milliliter samples from each tube were withdrawn by sterile pipette after inverting tubes twice, after approximately 10 s, 24 h, and 3, 5, 7, 10, 14, 20, and 28 d, and were preserved with Lugol's solution for cell counts by microscopy using a Palmer–Maloney settling chamber. Cells were not counted for treatment C if no significant differences were detected between treatments A and B. After 7 and 20 d, pH was measured using EM ColorpHast strips, and an additional 1.0-ml sample was collected and frozen for nutrient analysis.

In experiment 2, only those phytoplankton (10 species) for which an effect of treatment B (mixed culture) was observed in experiment 1 were subjected to culturing with and without various extracellular filtrates of *K. brevis.* Phytoplankton were grown with the addition of 5.0 ml of ultrafiltered and then sterile-filtered F/2 + silicate seawater media (treatment A [control]) or with one of three filtrates with added F/2 + silicate nutrients, from *K. brevis* CCMP 2228 (log phase; 3,961–7,117 cells ml^{-1}; treatment B), CCMP 2228 (stationary phase; 3,033–7,163 cells ml^{-1}; treatment C), and CCMP 718 (stationary phase; 544–2,352 cells ml^{-1}; treatment D). In other respects, experimental units were identical to experiment 1. In order to compensate for the likely decomposition or cellular uptake of allelopathic compounds throughout the experiment, 5.0 ml of freshly prepared extracellular filtrates of *K. brevis* or of media (control) were added at multiple intervals, after 0, 7, 14, and 21 d. Fluorescence measurements were made after approximately 30 s, 24 h, and 3, 5, 7, 10, 14, 21, and 28 d (and 35 and 42 d for *P. mexicanum*) using 5.0 ml of culture withdrawn with a sterile pipette; placed in a clean, sterile test tube for measurement; and then returned to the experimental tube. Preliminary experiments confirmed that for all of the phytoplankton species used in these experiments, linear relationships (r^2 = 0.75–0.99) existed between visual cell counts and fluorescence measurements (data not shown). After 30 s and 7 d, pH was measured as described above.

In experiment 3, the same 10 species as in experiment 2 were subjected to additions of lipophilic extracts of *K. brevis* extracellular filtrates or purified brevetoxins. Experimental units were prepared as above, except all components were reduced in volume for a total culture volume of 6.25 ml, enabling fluorescence to be measured directly with whole tubes without withdrawing samples by pipette. Treatments consisted of test species grown with extract of filtrate of F/2 + silicate seawater media (treatment A [control]); extract of extracellular filtrate of *K. brevis* CCMP 2228 taken during log phase (5,910 cells ml^{-1}; treatment B); extract of filtrate of *K. brevis* CCMP 2228 taken during stationary phase (1,400 cells ml^{-1}; treatment C); extract of filtrate of *K. brevis* CCMP 718 taken during stationary phase (1,810 cells ml^{-1}; treatment D); or pure brevetoxins (PbTx-2, PbTx-3, and PbTx-9 in the ratio of 100 : 10 : 1; total concentration of brevetoxins 8.8 ng ml^{-1} once added to cultures; treatment E). This concentration of brevetoxins was chosen to match the total extracellular concentration of type II brevetoxins from *K. brevis* cell and filtrate additions in experiment 1; the proportions of each brevetoxin approximated typical toxin profiles of *K. brevis* (Roszell et al. 1990). However, waterborne brevetoxins may not occur in these proportions during blooms. If interconversion or selective degradation of brevetoxins occurs in the field once brevetoxins are waterborne, then this is likely to have occurred in our experimental tubes as well. Extracts or brevetoxins were added after 0, 7, and 14 d. The amount of extract (dissolved in 10 μl dimethyl sulfoxide [DMSO]) added to each tube corresponded to that obtained from a volume of filtrate representing 20% of the volume of each culture (i.e., an amount equivalent to the filtrate added to each tube in experiments 1 and 2). Fluorescence was measured after approximately 10 s, 24 h, and 2, 4, 7, 10, 14, 17, 21, and 28 d (and 35 and 42 d for *P. mexicanum*). Preliminary experiments established that the use of DMSO did not significantly interfere with phytoplankton growth, whereas similar quantities of ethanol reduced the maximum concentration of *A. sanguinea, Asterionellopsis glacialis, P. mexicanum, Prorocentrum minimum, Skeletonema costatum,* and *Thalassiosira* sp. by 79–99% (data not shown).

Nutrient analyses—Nitrogen (nitrate + nitrite), phosphorus (orthophosphate), and silicate were measured for replicate 1 of each treatment of each species within experiment 1 (sampled after 7 and 20 d). A 20-fold dilution using 18-megaohm deionized water was necessary to bring the chemistries into an effective working range. Samples were analyzed on a LaChat QC 8000 ion analyzer with computer-controlled sample selection and peak processing.

Statistical analyses—Nonlinear regression with an *F* test (GraphPad Prism 4.0) was used to compare growth curves of treatments within each experiment, calculating means and standard errors for various growth parameters (*see* below) across replicates but without blocking treatments for each replicate. In most cases, a Gompertz (bacterial growth) sigmoidal curve successfully fitted the data, and the following parameters were compared between controls and each treatment: initial concentration (never significantly different between treatments), maximum growth rate, maximum concentration, and lag before the onset of exponential growth. In a few cases exponential growth was a better fit, and in these cases the parameters compared by *F* test were the initial concentration (never different) and exponential rate constant. For those species for which nonlinear regression failed to fit the data, linear regression was attempted, comparing slopes and Y intercepts of control and treatment data, and occasionally two-tailed *t*-tests were used to compare cell concentrations of control and treatment data at specific times during an experiment. Figures depict means ± 1 standard error.

Results

Competition with live Karenia brevis—In experiment 1, of the 12 phytoplankton species grown in the presence of live *K. brevis,* nine species were suppressed relative to each of these species grown alone (Fig. 1; Table 1). One species, *Rhodomonas lens,* was enhanced threefold by the presence of *K. brevis* ($p = 0.016$ on day 20). The growth of two species (*Chlorella capsulata* and *Odontella aurita*) was unaffected by *K. brevis.*

Of the nine negatively affected species, three (*Peridinium* sp., *P. mexicanum,* and *Thalassiosira* sp.) were >95% suppressed when grown with live *K. brevis* (Fig. 1; Table 1). For the six other negatively affected species, significant differences included (1) smaller maximum growth rates for *A. sanguinea, P. minimum,* and *S. costatum* when grown with live *K. brevis* than when grown alone; (2) lower maximum concentrations for *A. glacialis* and *Rhizosolenia* cf. *setigera*; and (3) longer lag before the onset of exponential growth for *A. sanguinea* and *Amphora* sp. (Fig. 1; Table 1). By light microscopy on preserved samples, qualitative differences in cell morphology were observed for *A. glacialis,* with cells grown with live *K. brevis* appearing more rounded and with irregular edges relative to cells grown alone. Similarly, *Rhizosolenia* cf. *setigera* cells grown with live *K. brevis* appeared shorter than when grown alone, although these effects were not quantified.

As measured by ELISA, the stock culture of *K. brevis* used to inoculate the *K. brevis* treatment in experiment 1 contained 263 ng ml^{-1} type-II brevetoxins, corresponding to 17 pg cell^{-1} intracellular brevetoxins. The extracellular filtrate generated from this culture contained 44 ng ml^{-1} brevetoxins, which were diluted to 8.8 ng ml^{-1} once added to experimental tubes.

Phytoplankton growth affected by K. brevis *extracellular filtrates*—Of the nine species that were negatively affected by the presence of live *K. brevis,* suppression by *K. brevis* extracellular filtrates (with enriched nutrients) was observed for five species in experiments 1 and 2 (Figs. 1, 2; Table 1). The maximum concentration of *Amphora* sp. was 25–27% lower when grown with two of four *K. brevis* filtrates, relative to controls. *A. glacialis* maximum concentration was 27–36% lower when grown with two *K. brevis* filtrates, relative to controls. *P. mexicanum* had a 28% lower maximum concentration when grown with filtrates of log-phase strain CCMP 2228, relative to controls. *Rhizosolenia* cf. *setigera* had a 43% lower maximum growth rate when grown with filtrates of early stationary-phase strain CCMP 2228, relative to controls. Finally, *S. costatum* grew to a 45% lower maximum concentration when filtrates of early stationary-phase strain CCMP 2228 were present versus absent (Figs. 1, 2; Table 1).

R. lens, which thrived when grown with live *K. brevis,* also seemed to benefit from extracellular filtrates of *K. brevis,* although to a lesser degree. Maximum concentration of *R. lens* was 41–119% greater when grown with filtrates of log- and stationary-phase strain CCMP 2228 and stationary-phase strain CCMP 718 than in controls ($p < 0.002$ for all; Fig. 2).

Enhancement of growth when in the presence of *K. brevis* filtrates was also observed for some phytoplankton species that were not enhanced in the live cell competition experiment. Specifically, *Amphora* sp., *Peridinium* sp., *P. minimum, S. costatum,* and *Thalasiossira* sp. all had greater maximum concentrations in the presence of some *K. brevis* filtrates than controls, with the magnitude of statistically significant effects ranging from 17% to 380% ($p < 0.0001$–0.039; Figs. 1, 2).

ELISA analysis of each of the filtrates used in experiment 2 indicated nondetectable (<1 ng ml^{-1}) quantities of type II brevetoxins. Unfortunately, *K. brevis* cells were not analyzed by ELISA for this experiment, so it is unclear whether these *K. brevis* cultures produced no brevetoxins or retained them in cells. Nevertheless, some allelopathic effects were observed (*see* previous).

Phytoplankton growth affected by lipophilic extracts of K. brevis *filtrates*—ELISA analysis of the stock cultures, filtrates, and filtrate extracts used in experiment 3 indicated that for *K. brevis* strain CCMP 2228 in log phase, used for treatment B of this experiment, concentrations of intracellular and extracellular brevetoxins were equal, each measuring 32 ng ml^{-1} (representing 5.4 pg cell^{-1} intracellular brevetoxins). The lipophilic extract of the extracellular filtrate also contained brevetoxins corresponding to 32 ng ml^{-1} of extracted material, indicating that extraction efficiency of brevetoxins from filtrates was 100%, as expected. When diluted in experimental tubes for experiment 3, this extract contributed 6.4 ng ml^{-1} brevetoxins, added once a week for 3 weeks. *K. brevis* stationary-phase strain CCMP 2228 used for treatment C had 24 ng ml^{-1} (17 pg cell^{-1}) intracellular brevetoxins, 8.0 ng ml^{-1} extracellular brevetoxins, and again, 100% of brevetoxins were extracted from the extracellular filtrate to the lipophilic extract (contributing 1.6 ng ml^{-1} brevetoxins with each addition). For treatment D, *K. brevis* stationary-phase strain CCMP 718 had equal amounts of intracellular and extracellular brevetoxins, each 2.0 ng ml^{-1} (1.1 pg cell^{-1}), and all of the extracellular brevetoxins were successfully transferred to the lipophilic extract (contributing 0.4 ng ml^{-1} brevetoxins with each addition). As expected, the F/2 seawater media, its filtrate, and the lipophilic extract of this filtrate used as a control in experiment 3 contained no brevetoxins by ELISA analysis.

Two competitors, *S. costatum* and *Thalassiosira* sp., were negatively affected by lipophilic extracts of extracellular filtrates of *K. brevis* (Fig. 3; Table 1). The maximum concentrations of *S. costatum* and *Thalassiosira* sp. were reduced by 12% and 33%, respectively, when grown with extracts of log-phase strain CCMP 2228 filtrate, relative to controls. Additionally, *S. costatum* maximum concentration was suppressed 12% by extracts of stationary-phase strain CCMP 2228 filtrate and 15% by a mixture of purified brevetoxins at 8.8 ng ml^{-1} added weekly (Fig. 3; Table 1).

When grown with purified brevetoxins at 8.8 ng ml^{-1}, the growth rate of *A. sanguinea* was only 27% of that of controls (Fig. 3; Table 1). When grown with lipophilic extracts of *K. brevis* filtrates, the growth rate of *A. sanguinea* was either enhanced 2.4-fold (extract of log-phase strain CCMP 2228 filtrate, $p = 0.00061$) or was not affected (extracts of sta-

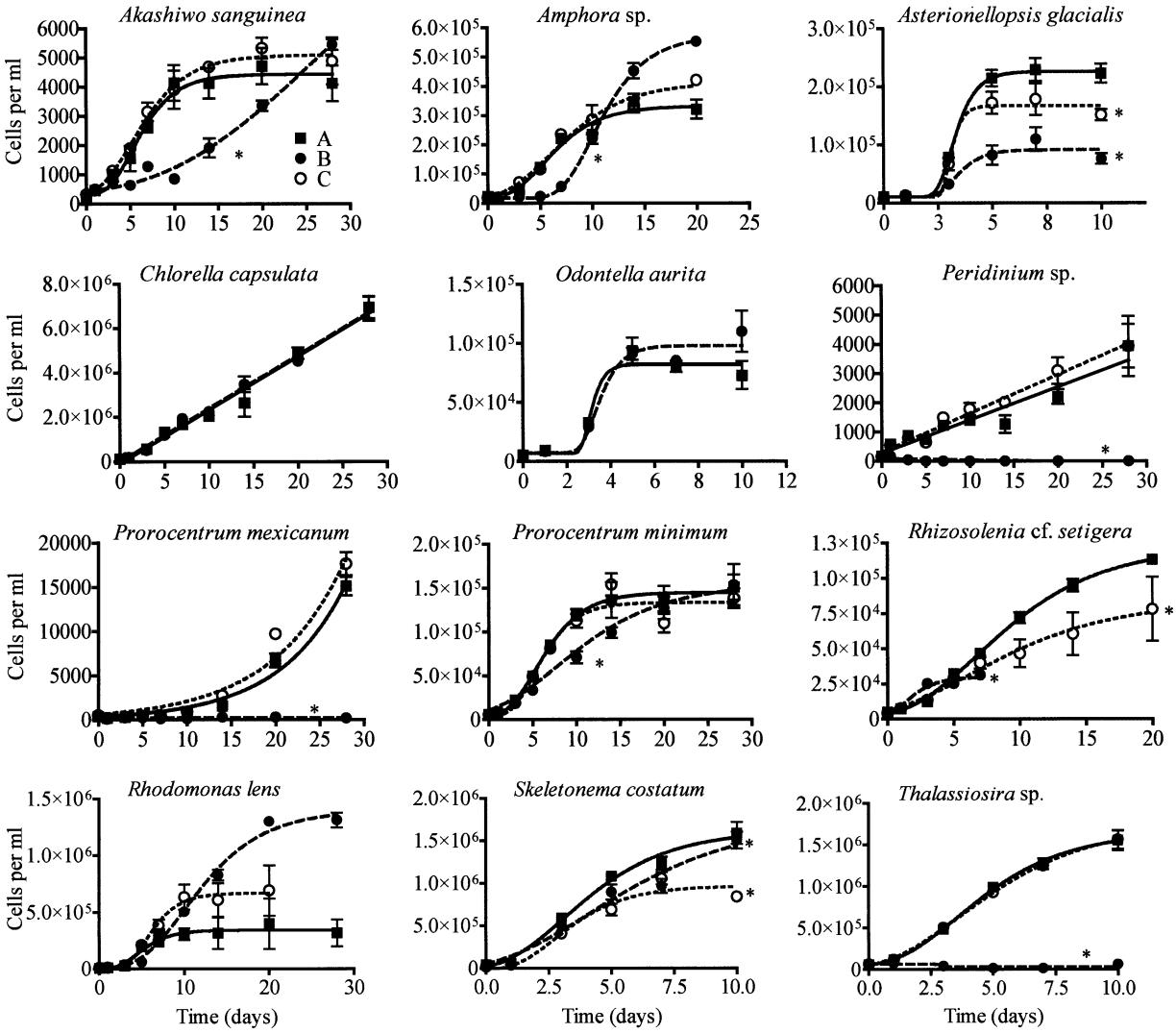

Fig. 1. Effects of *Karenia brevis* on the growth of competitors (experiment 1). Phytoplankton were grown with (A) filtrate of F/2 media in seawater (control), (B) live *K. brevis* strain CCMP 2228, or (C) one-time addition of extracellular filtrate of early stationary-phase strain CCMP 2228 ($n = 5$). Cells were counted under light microscope. Asterisks (*) indicate significant suppression of treatments relative to controls ($p \leq 0.05$).

tionary-phase strains CCMP 2228 and 718, $p \geq 0.52$ for both). Growth of two other species was also enhanced by extracts of *K. brevis* or by brevetoxins: *A. glacialis*, enhanced 52–94% by extracts of log- and stationary-phase strain CCMP 2228 filtrates (concentration at day 17, $p = 0.010$–0.014) and by brevetoxins (concentration at day 17, $p = 0.031$), although not by the extract of stationary-phase strain CCMP 718 (concentration at day 17, $p = 0.46$); and *P. mexicanum*, whose maximum concentration was enhanced 13% by the extract of stationary-phase strain CCMP 2228 filtrate ($p = 0.016$) but was not affected by other extracts nor by purified brevetoxins (Fig. 3).

K. brevis (strain CCMP 2228) was relatively unaffected by lipophilic extracts of extracellular filtrates of its own strain (that contributed 1.6–6.4 ng ml^{-1} brevetoxins), but its maximum concentration was suppressed by 24% by extracts of filtrates of stationary-phase CCMP 718 ($p = 0.014$) and suppressed 38% by purified brevetoxins at 8.8 ng ml^{-1} ($p = 0.017$; Fig. 3).

K. brevis *growth affected by other phytoplankters*—During experiment 1, in which the growth of various phytoplankters was assessed in the presence and absence of live *K. brevis*, we were able to measure the effects of this competition on *K. brevis* (Fig. 4; Table 1). Although we started the experiment with *K. brevis* concentrations at bloom levels (2,400 cells ml^{-1}), *K. brevis* grown without competitors went through an experimental growth phase, stabilizing at six times this concentration after 10 days (Fig. 4A). Five competitors (*C. capsulata, O. aurita, P. minimum, R. lens,* and *S. costatum*) drastically reduced *K. brevis* growth, with *K. brevis* cell concentrations peaking after 3 d and then declining to 0–1% of control cell numbers (Fig. 4A). When cell concentrations were compared by unpaired *t*-tests at various

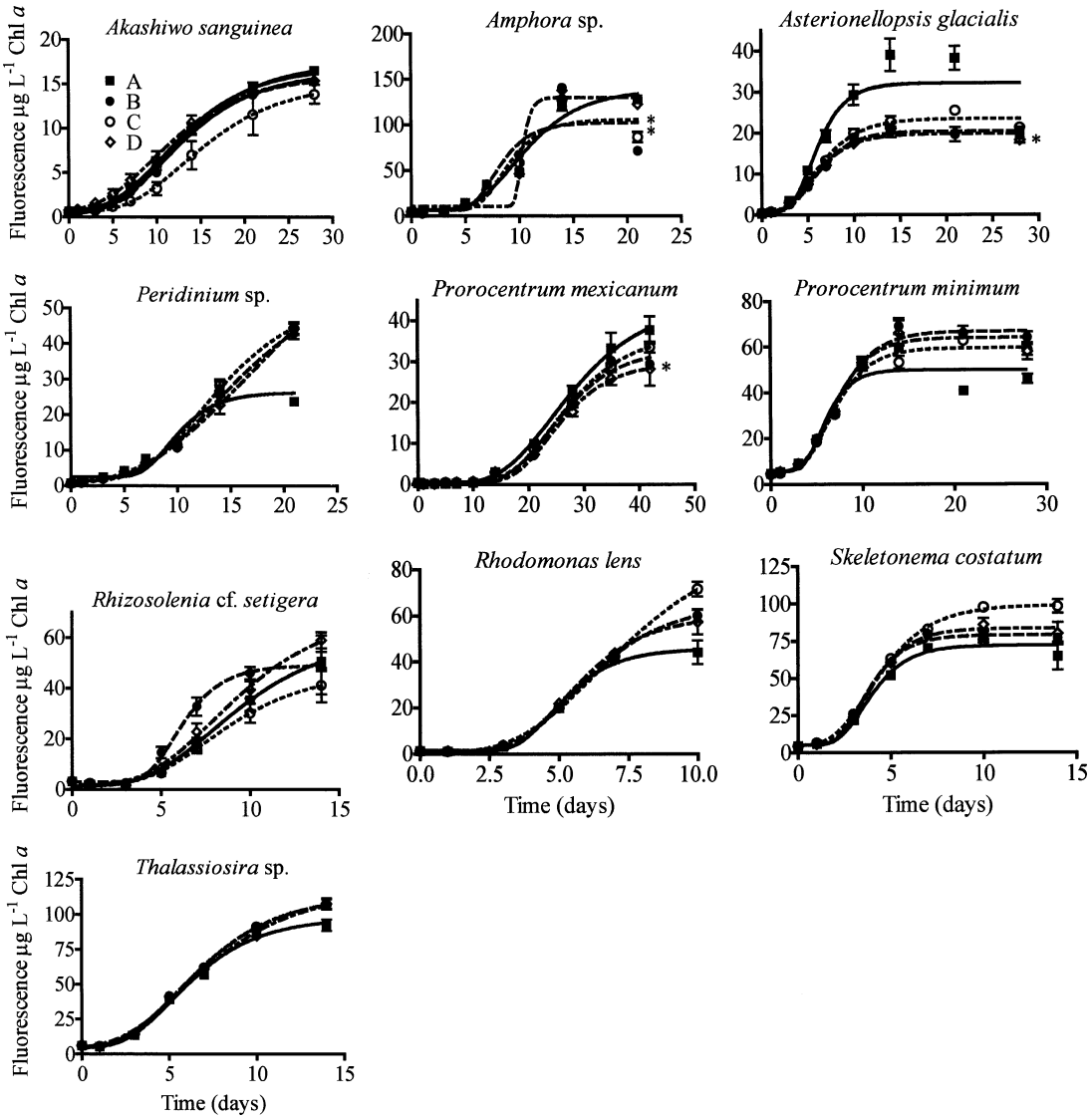

Fig. 2. Effects of *Karenia brevis* extracellular filtrates on the growth of other phytoplankton species (experiment 2). Phytoplankton were grown with weekly addition of (A) filtrate of F/2 media in seawater (control), (B) extracellular filtrate of log-phase strain CCMP 2228, (C) extracellular filtrate of stationary-phase strain CCMP 2228, or (D) extracellular filtrate of stationary-phase strain CCMP 718 ($n = 5$). Cell concentrations were assessed by fluorescence measurement of culture aliquot (μg L^{-1} chlorophyll *a*). Asterisks (*) indicate significant suppression of treatments relative to controls ($p \leq 0.05$).

times during the experiment, significant differences were observed for all five treatments versus controls, starting on the seventh day (Table 1). A sixth species, *Amphora* sp., also resulted in early termination of growth, reducing *K. brevis* to 1% of control cell concentration by day 14 (Fig. 4A; Table 1).

A second group of phytoplankton also suppressed *K. brevis* growth, but did so less dramatically (Fig. 4B). The presence of *A. sanguinea* and *A. glacialis* resulted in lower *K. brevis* concentrations early in the experiment (e.g., 37–40% lower on day 7), followed by recovery at day 20. *Rhizosolenia* cf. *setigera* and *Thalassiosira* sp. did not reduce *K. brevis* maximum concentration, but did cause *K. brevis* to peak about 1 week earlier than controls and then crash when *K. brevis* concentrations in controls were at their maximum (Fig. 4B; Table 1).

Two species, *Peridinium* sp. and *P. mexicanum*, did not affect *K. brevis* growth. No significant differences were observed for maximum growth rate, maximum concentration, or lag before exponential growth began for *K. brevis* grown with and without each of the *Peridinium* sp. and *P. mexicanum* ($p > 0.5$ for all comparisons; Fig. 4C).

Nutrients and pH—Mass spectrometric measurements on samples from experiment 1 indicated that, although variable across treatments, nutrients were not likely to be seriously limiting (Web Appendix 1: http://www.aslo.org/lo/toc/vol_50/issue_3/0883a1.pdf). Summarizing these data, nitrate/

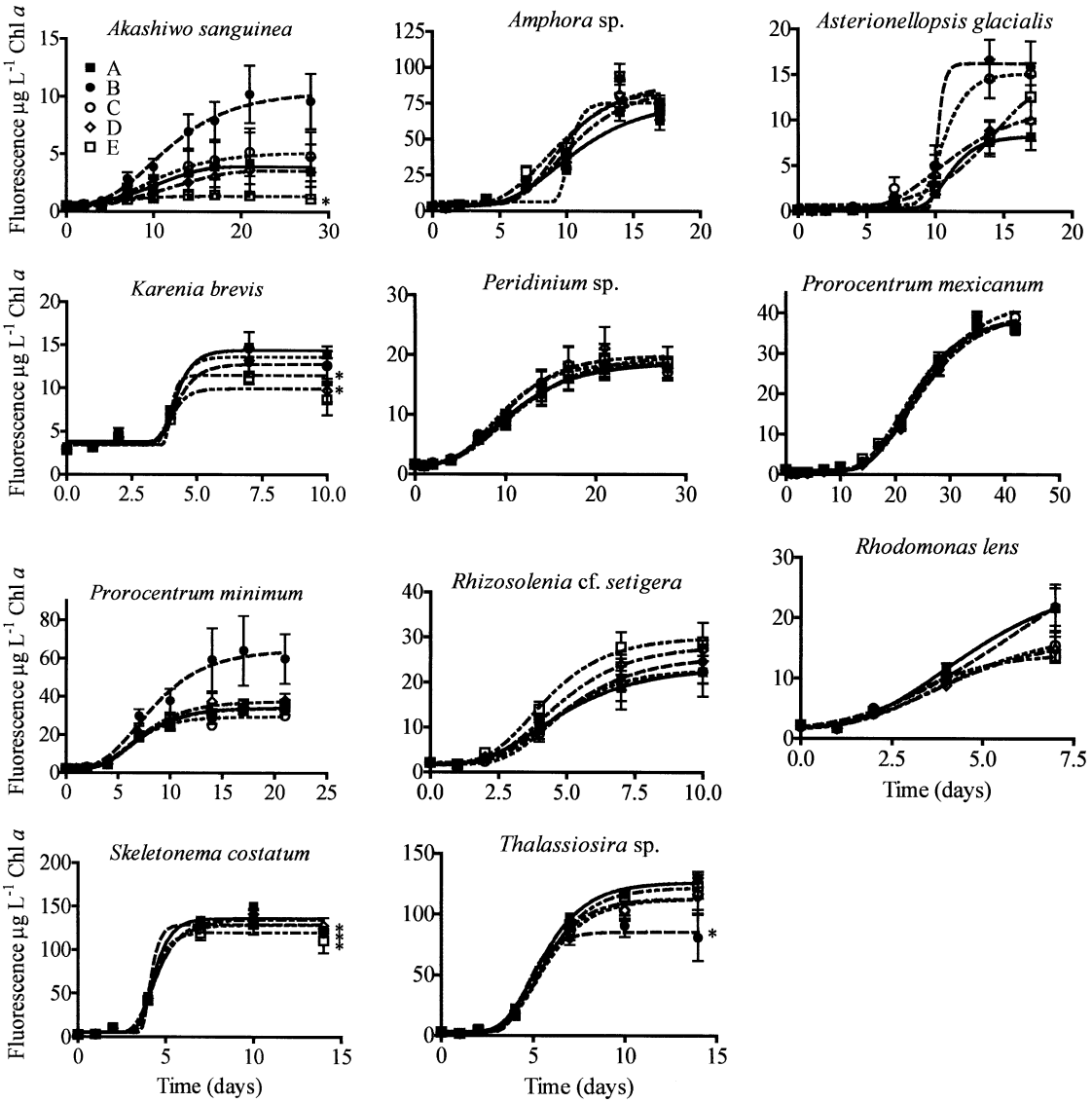

Fig. 3. Effects of lipophilic extracts of *Karenia brevis* extracellular filtrates and brevetoxins on the growth of other phytoplankton species (experiment 3). Phytoplankton were growth with weekly addition, dissolved in DMSO, of (A) extract of filtrate of F/2 media in seawater (control), (B) extract of extracellular filtrate of log-phase strain CCMP 2228, (C) extract of extracellular filtrate of stationary-phase strain CCMP 2228, (D) extract of extracellular filtrate of stationary-phase strain CCMP 718, or (E) PbTx-2, PbTx-3, PbTx-9 (100 : 10 : 1) totaling 8.8 ng ml^{-1} in cultures (n = 5). Cell concentrations were assessed by fluorescence measurement of culture (μg L^{-1} chlorophyll *a*). Asterisks (*) indicate significant suppression of treatments relative to controls ($p \leq 0.05$).

nitrite levels always exceeded 45 μmol L^{-1} and usually exceeded 150 μmol L^{-1} (fresh F/2 + silicate seawater media had 883 μmol L^{-1}), orthophosphate was 1.5–27.5 μmol L^{-1} (fresh media had 36.3 μmol L^{-1}), and silicate always exceeded 12 μmol L^{-1} (fresh media had 107 μmol L^{-1}). Throughout experiments 1–3, the pH of seawater and media was 6.5, and the pH of extracellular filtrates of *K. brevis* cultures (and control filtrates) was 7.0–8.5. During experiment 1, the pH of experimental cultures ranged from 6.5 to 9.0, with some species being under consistently slightly basic conditions (*O. aurita*, 8.5–9.0; *C. capsulate* and *P. minimum* 8.0–9.0) in all treatments. Only *Peridinium* sp. appeared to have a variable pH that was potentially dependent on treatment, with treatments A (control) and C (*K. brevis* filtrate) having pH values of 6.5–7.0 and with treatment B (addition of live *K. brevis* cells) having a pH of 8.5. Temporal pH patterns were not evident (data not shown).

Discussion

Nine of 12 phytoplankton species experienced reduced growth (lower growth rate, lower cell concentration, or delayed growth) when exposed to high concentrations of live *K. brevis* in mixed cultures, relative to these species grown alone (Fig. 1; Table 1). Allelopathy appears to be one competitive mechanism used by *K. brevis*, since its extracellular filtrates and/or extracts of filtrates reduced the growth of six

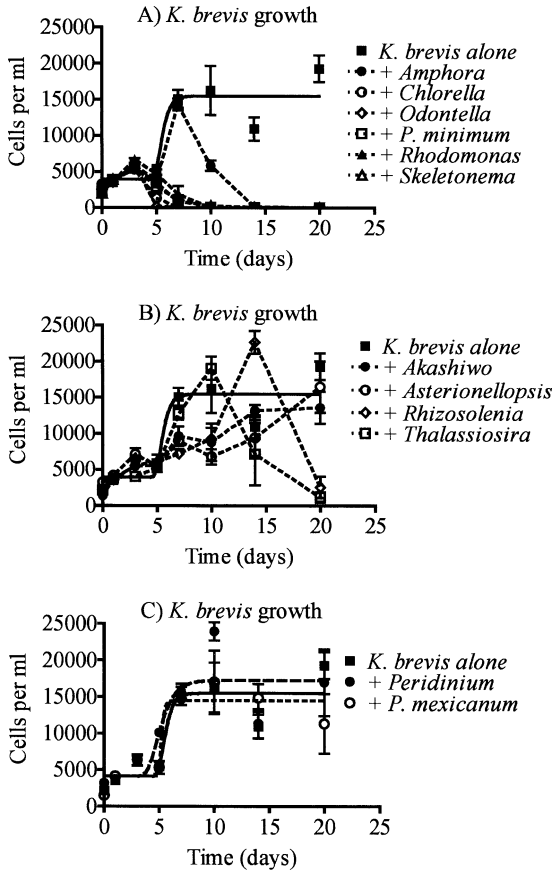

Fig. 4. Effects of other phytoplankton species on growth of *Karenia brevis* (experiment 1). (A) Strong suppression of *K. brevis* contrasted with *K. brevis* grown alone; (B) Mild suppression of *K. brevis*; and (C) No suppression of *K. brevis* ($n = 5$). Cells were counted under light microscope.

of nine phytoplankton species that were suppressed by live *K. brevis* (Figs. 1-3; Table 1).

K. brevis dramatically outcompeted two species in mixed culture: *Peridinium* sp. and *P. mexicanum*, neither of which ever grew beyond starting concentrations when paired with *K. brevis* (Fig. 1), and neither of which significantly suppressed *K. brevis* growth (Fig. 4C). In addition, *Thalassiosira* sp. was completely eliminated by competition with *K. brevis* in mixed cultures (Fig. 1), but *K. brevis* also suffered from the presence of *Thalassiosira* sp., growing to similar concentrations as controls but terminating about 1 week earlier than *K. brevis*–only controls (Fig. 4B). From our experiments, there is no evidence for an allelopathic mechanism to explain the dominance of *K. brevis* over *Peridinium* sp., since neither extracellular filtrates of *K. brevis*, nor extracts of *K. brevis* filtrates, nor purified brevetoxins at reasonable waterborne concentrations (8.8 ng ml^{-1} of PbTx-2, PbTx-3, and PbTx-9 [100 : 10 : 1]) affected *Peridinium* sp. (Figs. 1–3). *P. mexicanum* and *Thalassiosira* sp., on the other hand, were each significantly suppressed by one *K. brevis* filtrate (Fig. 2) and one filtrate extract (Fig. 3), respectively, although purified brevetoxins had no effect on either species (Fig. 3). The 28–33% reduction in maximum concentration caused by filtrates and extracts was modest compared to the 96–99% suppression by live *K. brevis*. It appears that allelopathy, mediated by *K. brevis* compounds other than brevetoxins, contributes at least partially to the competitive dominance of *K. brevis* over these two species when *K. brevis* is at bloom concentrations. In the Gulf of Mexico, *Peridinium* and *Thalassiosira* spp. occur year-round (Curl 1959; Turner and Hopkins 1974) and are therefore likely to interact with *K. brevis*. *P. mexicanum* is also found in the Gulf of Mexico, with the strain used in this study initially isolated in South Florida, but there are no reports of how any of these three species compete with *K. brevis* in the field.

Several phytoplankters were negatively affected but not completely eliminated by competition with live *K. brevis* (Fig. 1), as well as by suppressing *K. brevis* either partially (Fig. 4B) or completely over several days (Fig. 4A). Of these, the fall–winter seasonality of *Rhizosolenia* cf. *setigera* and *A. glacialis* (=*Asterionella japonica*) overlaps with the typical *K. brevis* bloom season (Curl 1959; Turner and Hopkins 1974; Tester and Steidinger 1997), and *R. setigera* has been found at high concentrations during *K. brevis* blooms (Gunter et al. 1948). Allelopathy appears to be used by *K. brevis* against these two competitors and against a third diatom, *Amphora* sp.; however, the negative effects of filtrates were not as strong as the suppression that was observed with live *K. brevis*. This is not surprising, since allelopathic compounds are likely to be continually produced and released by live *K. brevis*, and in fact may increase in concentration as *K. brevis* population density increases, whereas the filtrates, and the allelopathic compounds therein, were added either once or weekly. In addition to being weaker than the effects of live *K. brevis*, allelopathic effects on *Amphora* sp., *A. glacialis*, and *R.* cf. *setigera* also differed for various filtrates of *K. brevis*. For example, two *K. brevis* filtrates, both from strain CCMP 2228, one filtered during *K. brevis* log phase and the other during early stationary phase, significantly suppressed *A. glacialis* (Figs. 1–2), whereas full stationary-phase filtrates did not inhibit this species (Fig. 2). Brevetoxin concentration in these filtrates varied from nondetectable (<1 ng ml^{-1}) in the two inactive filtrates and in one inhibitory filtrate, to 8.8 ng ml^{-1} in another inhibitory filtrate. However, purified brevetoxins added to *A. glacialis* cultures weekly at 8.8 ng ml^{-1} enhanced rather than suppressed *A. glacialis* (Fig. 3). Therefore, brevetoxins cannot have been responsible for the allelopathic effects of *K. brevis* filtrates on *A. glacialis*. None of the lipophilic extracts of *K. brevis* filtrates or purified brevetoxins inhibited *Amphora* sp., *A. glacialis*, or *Rhizosolenia* cf. *setigera*, suggesting that allelopathic effects observed in the filtrate experiments were possibly due to highly water-soluble, volatile, or unstable compounds in filtrates that were not successfully extracted with a lipophilic solvent.

P. minimum is known to occur off the west coast of Florida (Millie et al. 1997), where impacts of *K. brevis* blooms are greatest. West et al. (1996) reported that during an unusual *K. brevis* bloom off North Carolina, *P. minimum* increased in concentration. In our mixed culture experiment, *P. minimum* reduced *K. brevis* concentration by 99.6% within 10 days (Fig. 4A). The maximum growth rate of *P. minimum* was in turn reduced by 45% by competition with *K. brevis*, although *P. minimum* eventually reached the same

concentration as controls, in which it was grown alone (Fig. 1). That *P. minimum* was a superior competitor to *K. brevis* in the laboratory appears to parallel its ability to thrive during *K. brevis* blooms (West et al. 1996). However, an allelopathic mechanism for the slowed growth of *P. minimum* was not evident, since *K. brevis* filtrates and filtrate extracts did not suppress *P. minimum* (Figs. 1–3). In fact, *P. minimum* was significantly enhanced by three of four *K. brevis* filtrates. Similar enhancements by filtrates were observed for *Peridinium* sp., *S. costatum,* and *Thalassiosira* sp. (Fig. 2) but not by lipophilic extracts of filtrates (Fig. 3). Nutrient analyses of samples from experiment 1 indicated that overall, nutrients were not seriously limiting in our treatments (Web Appendix 1); however, for phytoplankters that are relatively immobile in culture (e.g., diatoms) or for large cells that have a small surface-to-volume ratio, nutrient limitation in the immediate vicinity of individual cells could have occurred between times when experimental tubes were inverted, every 1–2 d. This may have resulted in enhanced growth in filtrate-addition treatments (to which nutrients were supplemented), whereas the lipophilic extract additions were unlikely to lead to this artifact.

For *R. lens,* which grew better not only with *K. brevis* filtrates (Fig. 2) but also in the presence of live *K. brevis* (Fig. 1), the observed effects are not likely to have been simply caused by nutrient enrichment of filtrates. It is possible that *K. brevis* produces substances that stimulate *R. lens* growth (which may be highly water-soluble, since *K. brevis* lipophilic extracts did not stimulate *R. lens*), or *R. lens* may be better able to use nutrients in the form released by *K. brevis* upon its decline, which coincided with the enhancement of *R. lens,* than it was able to use F/2 nutrients when grown alone.

S. costatum, the most common Gulf of Mexico diatom year-round (Saunders and Glenn 1969), accounting for 29–99% of eukaryotic phytoplankton cells in the Tampa Bay region during the fall season (Turner and Hopkins 1974), was slightly suppressed by *K. brevis* (Fig. 1), but in turn caused catastrophic effects on *K. brevis,* reducing its cell concentration by 99.9% by day 20 (Fig. 4A). The continued dominance of *S. costatum* in coastal waters and its association with *K. brevis* blooms in the Gulf of Mexico and the western North Atlantic (Gunter et al. 1948; Turner and Tester 1989) may be partly due to its superior competitive ability. Yet the modest suppression of *S. costatum* by *K. brevis* had a clearly allelopathic origin: one extracellular filtrate of *K. brevis* (the only one with appreciable quantities of brevetoxins, contributing 8.8 ng ml^{-1} brevetoxins to experimental cultures) inhibited *S. costatum* at least as much as did live *K. brevis* (Fig. 1); *K. brevis* extracts (contributing 6.4 and 1.6 ng ml^{-1} brevetoxins), as well as purified brevetoxins added weekly to *S. costatum* cultures at 8.8 ng ml^{-1}, suppressed *S. costatum* concentration 12–15% relative to controls (Fig. 3). *S. costatum* appears to be one phytoplankter negatively affected by *K. brevis,* for which brevetoxins actually explain the observed suppression. Nevertheless, in mixed culture with *S. costatum, K. brevis* was itself much more inhibited than was *S. costatum* (Figs. 1, 4A), indicating that the allelopathic effect of brevetoxins from high cell concentrations of *K. brevis* fails to win out, at least under nutrient-rich laboratory conditions, against the competitive ability of *S. costatum.*

Brevetoxins added at 8.8 ng ml^{-1} significantly inhibited only two additional species, *A. sanguinea* and *K. brevis* itself (strain CCMP 2228) (Fig. 3). Interestingly, this strain of *K. brevis* was also inhibited by lipophilic extracts of the extracellular filtrate from another *K. brevis* strain (CCMP 718) contributing 0.4 ng ml^{-1} brevetoxins, but not by two lipophilic extracts of filtrates of its own strain contributing 6.4 and 1.6 ng ml^{-1} brevetoxins. Similarly, *A. sanguinea,* which was strongly inhibited by pure brevetoxins at 8.8 ng ml^{-1}, was not inhibited by the same concentration of brevetoxins added as part of a *K. brevis* filtrate (Fig. 1), and was in fact stimulated by extracts of filtrates that contained added 6.4 ng ml^{-1} brevetoxins to its cultures (Fig. 3). Apparently, when included within the complex mixture of organic compounds released by *K. brevis* into seawater, brevetoxins within filtrates and extracts caused no significant negative effects. It is possible that *K. brevis* releases compounds that modulate or negate the effects of brevetoxins toward *A. sanguinea* and itself. In fact, Bourdelais et al. (2004) recently discovered a new *K. brevis* metabolite that counteracts the ichthyotoxicity of PbTx-3 when administered directly in water.

Only two species were not affected at all by *K. brevis* in this study: *C. capsulata* and *O. aurita,* the latter having been observed to increase in abundance during *K. brevis* blooms (West et al. 1996) and commonly found in the Gulf of Mexico (as *Biddulphia aurita*; Curl 1959; Saunders and Glenn 1969). We found no reports regarding *C. capsulata* dynamics during *K. brevis* blooms, but Turner and Hopkins (1974) reported *Chlorella*-like organisms to be abundant year-round (although not in every year studied) in the Tampa Bay area, an area commonly affected by red tides. *C. capsulata* and *O. aurita* caused *K. brevis* concentration to be reduced to $<1\%$ of controls (Fig. 4A). Initial cell concentrations can affect competitive outcome (e.g., Uchida et al. 1999). In our mixed culture experiments, starting concentrations of *C. capsulata* and *O. aurita* exceeded the concentration of *K. brevis,* but because *K. brevis* cells are much larger than the other two species, the initial biovolume of *K. brevis* was actually twice that of *C. capsulata* and 1.2 times that of *O. aurita,* yet *K. brevis* was completely eliminated.

Freeberg et al. (1979) reported allelopathic effects of *K. brevis* toward 18 of 28 phytoplankters tested. However, that study used *K. brevis* filtrates and extracts from lysed cell cultures, such that intracellular compounds not normally found in seawater were included in treatments. Additionally, because filtrates were not enriched with media prior to use in experiments, full-strength media controls had higher nutrient concentrations than treatments, which could have lead to false-positive results, and variance was not reported nor were statistics used. Nevertheless, for the two species (*A. glacialis* and *S. costatum*) in our study that were also targeted by Freeberg et al. (1979), our findings of allelopathic effects of *K. brevis* against both these species were predicted by the earlier work. Species-specific allelopathic effects appear to be common (Arzul et al. 1999; Sugg and VanDolah 1999). Since producers of allelopathic compounds are likely to have evolved resistance to their own allelopathic agent(s), it seems reasonable that other species could also evolve re-

sistance, or allelopathic compounds may have evolved to target specific competitors in the first place.

Although our finding that many but not all competitors are inhibited by *K. brevis* appears to mirror some previous field observations, we cannot conclude that the effects reported herein account for competitive outcomes in the field. Obviously, nutrient conditions used in our experiments far exceeded what would be expected in the field, which could either have led to overproduction of allelopathic substances or masked intense exploitation competition that might dominate interactions in the field (Maestrini and Bonin 1981). Competitive outcome can also be strongly affected by starting cell concentrations (Pratt 1966; Uchida et al. 1999) and irradiance (Hegarty and Villareal 1998), and cultured strains can undergo metabolic changes over time, such that allelopathic potential may be lost (Gentien and Arzul 1990). In our competition experiment between live *K. brevis* and other phytoplankters, we inoculated both species on the same day at concentrations that enabled them to rapidly enter exponential phase, which may have biased the outcome toward discovering allelopathy that affects species mainly in the early part of their growth curve. This could have prevented us from gaining insights into some aspects of species succession. Possibly most restrictive, our practically motivated decision to test pairwise interactions certainly sacrificed our ability to detect indirect, additive, or synergistic interactions among multiple species. Understanding the community-wide effects of competition and allelopathy requires field experimentation, or at least the use of field assemblages of phytoplankton (e.g., Fistarol et al. 2003). With so little information previously available on the allelopathic potential of *K. brevis* or on the importance of brevetoxins in competition, the laboratory-based data reported herein at least provide testable hypotheses regarding *K. brevis* allelopathy in the field.

One major question in harmful algal bloom research has been whether phytoplankton toxicity plays a role in establishing or maintaining blooms. Smayda (1997) concluded that, so far, there is no evidence for the involvement of phytoplankton toxins in bloom formation, given that some bloom species produce toxins but many do not. Because we started our competition experiment with bloom concentrations of *K. brevis* cells, our findings may relate mostly to established *K. brevis* blooms and what forces maintain or disrupt these blooms. From our data, it is clear that brevetoxins at reasonable waterborne concentrations affected very few competing phytoplankters, but that allelopathy in general was fairly common. Because some filtrates were allelopathic to some phytoplankton species and other filtrates were allelopathic to others species, several yet-unidentified *K. brevis* compounds are probably involved. In laboratory experiments, Sugg and VanDolah (1999) and Tillmann and John (2002) reported that the well-known toxins produced by *Prorocentrum lima* and *Alexandrium* spp. (okadaic acid and saxitoxins, respectively) could not explain the outcome of competition with multiple species, and that other, unidentified allelopathic compounds appeared to be important. Identifying allelopathic agents is of great interest, in order to test their effects in manipulative experiments under realistic conditions.

Allelopathy may also act against *K. brevis*, if used as a strategy by other phytoplankters. Martin and co-workers (e.g., Perez et al. 1997) have published extensively on a suite of compounds produced by chlorophytes that inhibit *K. brevis* in culture. To date, allelopathic effects have been attributed to two fatty esters common to many taxa, although whether these compounds affect *K. brevis* at ecologically relevant concentrations is uncertain. In the current study, six phytoplankters strongly inhibited *K. brevis* and another four led to milder but significant suppression (Fig. 4), but none of the species tested were the ones reported by Martin and co-workers. We cannot yet predict whether the mechanism of this inhibition is based upon exploitation of resources or interference.

Bacteria are known to co-occur with phytoplankton, in some cases contributing by unknown mechanisms to increased phytoplankton toxin production (Doucette 1995). Bacteria could play a role in competitive interactions between phytoplankters, although to our knowledge, there is no report of this being tested by manipulative experiments. In the current study, we chose to use nonaxenic strains of phytoplankters to maintain realistic levels of brevetoxin production by *K. brevis* and to attempt to mimic field conditions, although the bacteria present in culture may not have been related to bacteria in the field. One disadvantage of including nonaxenic strains was the possibility that bacteria may have contributed to the decomposition of allelopathic agents, although this may occur in the field as well, diminishing the effectiveness of allelopathy in natural communities. If, on the other hand, *K. brevis*–associated bacteria released allelopathic agents affecting some competitors, we would have recorded this as allelopathy by *K. brevis*; if growth of a competitor was suppressed because its associated bacteria were disrupted by *K. brevis* allelopathic compounds, we would have recorded this as allelopathy against the competitor. Although this may have led us to a flawed understanding of the proximate mechanisms involved in allelopathy, the ultimate effects (suppression of a competitor by waterborne chemicals) would have been detected using our design. However, we considered it important to distinguish allelopathy from pathogenic infection by live bacteria, and so we removed actual bacterial cells before adding filtrates to test phytoplankters. Thus, if *K. brevis* normally interferes with competitors by inoculating them with pathogenic bacteria harbored by *K. brevis*, we would not have been able to distinguish this from exploitation competition. In addition, if the presence of a competitor normally induces the production or release of allelopathic compound(s) by *K. brevis*, we would have observed suppression in the mixed culture experiment, but missed the induced allelopathy in the filtrate- and extract-addition experiments. Finally, if some phytoplankters were mixotrophic, ingesting phytoplankton cells (Tillmann 2003), or interfered with each other via cell contact rather than by waterborne chemicals (Uchida et al. 1995), these mechanisms could not have been separated in our experiments from exploitation competition. Therefore, we are conducting experiments to explore possible mechanisms of interference competition other than constitutively expressed allelopathy.

In conclusion, we found that the red tide dinoflagellate *K.*

brevis uses species-specific allelopathic strategies to interfere with the growth of six of 12 phytoplankters tested. *K. brevis* was itself inhibited by several competitors. Release of brevetoxins into seawater explained the modest inhibition by *K. brevis* of only one competitor, *S. costatum*, indicating that other allelopathic compounds have yet to be discovered. In almost all cases in which *K. brevis* suppressed competitors, allelopathic effects of *K. brevis* filtrates and extracts were weaker than the effects of live *K. brevis* cells, indicating that (1) allelopathic compounds may decompose over periods of days to weeks in seawater; (2) some allelopathic compounds may be highly water-soluble or volatile and thus may be lost during extraction; and (3) other mechanisms, including exploitation competition, microbial infection, cell–cell contact, and allelopathy induced only when *K. brevis* is in the presence of competitors, are also likely to be important.

References

ARZUL, G., M. SEGUEL, L. GUZMAN, AND E. ERARD-LE DENN. 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. J. Exp. Mar. Biol. Ecol. **232:** 285–295.

BADEN, D. G. 1989. Brevetoxins: Unique polyether dinoflagellate toxins. FASEB J. **3:** 1807–1817.

BOURDELAIS, A. J., S. CAMPBELL, H. JACOCKS, J. NAAR, J. L. C. WRIGHT, J. CARSI, AND D. G. BADEN. 2004. Brevenal is a natural inhibitor of brevetoxin action in sodium channel receptor binding assays. Cell. Molec. Neurobiol. **24:** 553–563.

CURL, H., JR. 1959. The phytoplankton of Apalachee Bay and the northeastern Gulf of Mexico. Publ. Inst. Mar. Sci. **6:** 278–320.

DOUCETTE, G. J. 1995. Interactions between bacteria and harmful algae: A review. Nat. Toxins **3:** 65–74.

FISTAROL, G. O., C. LEGRAND, AND E. GRANELI. 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar. Ecol. Prog. Ser. **255:** 115–125.

FREEBERG, L. R., A. MARSHALL, AND M. HEYL. 1979. Interrelationships of *Gymnodinium breve* (Florida red tide) within the phytoplankton community, p. 139–144. *In* D. L. Taylor and H. H. Seliger [eds.], Toxic dinoflagellate blooms. Elsevier.

GENTIEN, P., AND G. ARZUL. 1990. Exotoxin production by *Gyrodinium* cf. *aureolum* (Dinophyceae). J. Mar. Biol. Assoc. U.K. **70:** 571–581.

GUNTER, G., R. H. WILLIAMS, C. C. DAVIS, AND F. G. W. SMITH. 1948. Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August 1947. Ecol. Monogr. **18:** 309–324.

HEGARTY, S. G., AND T. A. VILLAREAL. 1998. Effects of light level and N : P supply ratio on the competition between *Phaeocystis* cf. *pouchetii* (Hariot) Lagerheim (Prymnesiophyceae) and five diatom species. J. Exp. Mar. Biol. Ecol. **226:** 241–258.

HUTCHINSON, G. E. 1961. The paradox of the plankton. Am. Nat. **95:** 137–145.

Keating, K. I. 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science **196:** 885–887.

LEGRAND, C., K. RENGEFORS, G. O. FISTAROL, AND E. GRANELI. 2003. Allelopathy in phytoplankton—biochemical, ecological and evolutionary aspects. Phycologia **42:** 406–419.

LEWIS, W. M., JR. 1986. Evolutionary interpretations of allelochemical interactions in phytoplankton algae. Am. Nat. **127:** 184–194.

MAESTRINI, S. Y., AND D. J. BONIN. 1981. Allelopathic relationships between phytoplankton species. Can. Bull. Fish Aquat. Sci. **210:** 323–338.

MILLIE, D. F., O. M. SCHOFIELD, G. J. KIRKPATRICK, G. JOHNSEN, P. A. TESTER, AND B. T. VINYARD. 1997. Detection of harmful algal blooms using photopigments and absorption signatures: A case study of the Florida red tide dinoflagellate, *Gymnodinium breve*. Limnol. Oceanogr. **42:** 1240–1251.

NAAR J., A., AND OTHERS. 2002. A competitive ELISA to detect brevetoxins from *Karenia brevis* (formerly *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid. Environ. Health Perspect. **110:** 179–184.

O'SHEA, T. J., G. B. RATHBUN, R. K. BONDE, C. D. BUERGELT, AND D. K. ODELL. 1991. An epizootic of Florida manatees associated with a dinoflagellate bloom. Mar. Mammal. Sci. **7:** 165–179.

PEREZ, E., W. G. SAWYERS, AND D. F. MARTIN. 1997. Identification of allelopathic substances produced by *Nannochloris oculata* that affect a red tide organism, *Gymnodinium breve*. Biomed. Lett. **56:** 7–14.

PRATT, D. M. 1966. Competition between *Skeletonema costatum* and *Olisthodiscus luteus* in Narragansett Bay and in culture. Limnol. Oceanogr. **11:** 447–455.

RENGEFORS, K., AND C. LEGRAND. 2001. Toxicity in *Peridinium aciculiferum*—an adaptive strategy to outcompete other winter phytoplankton? Limnol. Oceanogr. **46:** 1990–1997.

ROSZELL, L. E., L. S. SCHULMAN, AND D. G. BADEN. 1990. Toxin profiles are dependant on growth stages in cultured *Ptychodiscus brevis*, p. 403–406. *In* E. Graneli, B. Sundstrom, L. Elder, and D. M. Anderson [eds.], Toxic marine phytoplankton. Elsevier.

SAUNDERS, R. P., AND D. A. GLENN. 1969. Diatoms. Memoirs Hourglass Cruises **1:** 1–119.

SCHMIDT, L. E., AND P. J. HANSEN. 2001. Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: Effect of cell concentration, growth phase and pH. Mar. Ecol. Prog. Ser. **216:** 67–81.

SMAYDA, T. J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. **42:** 1137–1153.

STEIDINGER, K. A., M. A. BURKLEW, AND R. M. INGLE. 1973. The effects of *Gymnodinium breve* toxin on estuarine animals, p. 179–202. *In* D. F. Martin and G. M. Padilla [eds.], Marine pharmacognosy. Academic Press.

SUGG, L., AND F. M. VANDOLAH. 1999. No evidence for an allelopathic role of okadaic acid among ciguatera-associated dinoflagellates. J. Phycol. **35:** 93–103.

SYKES, P. F., AND M. E. HUNTLEY. 1987. Acute physiological reactions of *Calanus pacificus* to selected dinoflagellates: Direct observations. Mar. Biol. **94:** 19–24.

TESTER, P. A., AND K. A. STEIDINGER. 1997. *Gymnodinium breve* red tide blooms: Initiation, transport, and consequences of surface circulation. Limnol. Oceanogr. **42:** 1039–1051.

TIFFANY, W. J., AND M. G. HEYL. 1978. Invertebrate mass mortality induced by a *Gymnodinium breve* red tide in Gulf of Mexico waters at Sarasota, Florida. J. Environ. Sci. Health **A13:** 653–662.

TILLMANN, U. 2003. Kill and eat your predator: A winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat. Microb. Ecol. **32:** 73–84.

———, AND U. JOHN. 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: An allelochemical defence mechanism independent of PSP-toxin content. Mar. Ecol. Prog. Ser. **230:** 47–58.

TURNER, J. T., AND T. L. HOPKINS. 1974. Phytoplankton of the Tampa Bay system, Florida. Bull. Mar. Sci. **24:** 101–121.

———, AND P. A. TESTER. 1989. Zooplankton feeding ecology: Copepod grazing during an expatriate red tide, p. 359–374. *In* E. M. Cosper [ed.], Phytoplankton blooms. Springer.

UCHIDA, T., S. TODA, Y. MATSUYAMA, M. YAMAGUCHI, Y. KOTANI,

AND T. HONJO. 1999. Interactions between the red tide dinoflagellates *Heterocapsa circularisquama* and *Gymnodinium mikimotoi* in laboratory culture. J. Exp. Mar. Biol. Ecol. **241:** 285–299.

———, M. YAMAGUCHI, Y. MATSUYAMA, AND T. HONJO. 1995. The red-tide dinoflagellate *Heterocapsa* sp. kills *Gyrodinium instriatum* by cell contact. Mar. Ecol. Prog. Ser. **118:** 301–303.

VARDI, A., D. SCHATZ, K. BEERI, U. MOTRO, A. SUKENIK, A. LEVINE, AND A. KAPLAN. 2002. Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr. Biol. **12:** 1767–1772.

WEST, T. L., H. G. MARSHALL, AND P. A. TESTER. 1996. Natural phytoplankton community responses to a bloom of the toxic dinoflagellate *Gymnodinium breve* Davis off the North Carolina coast. Castanea **61:** 356–368.

WOLFE, G. V. 2000. The chemical defense ecology of marine unicellular plankton: Constraints, mechanisms, and impacts. Biol. Bull. **198:** 225–244.

Received: 12 July 2004
Accepted: 20 January 2005
Amended: 27 January 2005