Supplemental Materials

Table S1: Homogeneity of variance test (Levene) estimated for the data obtained from analyzing hemolytic activity in six different *Gambierdiscus* species found in the Caribbean. The analysis examined the differences among species, isolates, growth phase, and species vs. growth phase interaction. For these analyses the data from all three growth phases were considered as part of the same analysis. The differences in hemolytic activity among species in log, late log - stationary phase, and mid-stationary phase were also analyzed separately. The data indicated that only growth phase differences across all species met the homogeneity of variance requirements. The remaining data were therefore analyzed using a Kruskal-Wallis non-parametric ANOVA.

Source	Degrees of Freedom	F-value	P-value
species	5	22.45	6.44×10^{-17}
strains	53	2.365	8.31×10^{-05}
Growth phase	2	0.032	0.97
species*growth phase	17	6.09	1.82×10^{-10}
species (log phase)	5	7.14	4.59×10^{-05}
species (late log – early stationary phase)	5	7.14	4.5×10^{-05}
species (mid-stationary phase)	5	7.14	4.59×10^{-05}

Table S2: Non-parametric Kruskal-Wallis ANOVA test was performed to determine if hemolytic activity was significantly different among species when the log, late log - early stationary, and mid-stationary phase data were considered together or separately. The results indicated that each of these analyses were highly significant confirming that interspecific differences in hemolytic activity exist. This was true whether the EC_{50} data for all three growth phases were combined or considered separately.

Source	H-Statistic	df	P-value
Species (all three phases considered together)	69.95	5	1.05×10^{-13}
Species (log phase)	24.74	5	0.000156
Species (late log – early stationary phase)	20.32	5	0.001088
Species (mid-stationary phase)	27.88	5	3.84×10^{-05}

Table S3. A. Non-parametric multiple comparison test to determine which species had statistically different hemolytic activity. The data indicated that hemolytic activity in *G. carolinianus* was statistically lower than all the other species tested. *Gambierdiscus* ribotype 2 and *G. ruetzleri* were the two most toxic species and were significantly different from *G. caribaeus*, which had similar toxicities as *G. belizeanus* and *G. carpenteri*. Analyzing each growth phase separately weakened the analysis to the degree that only differences between *G. carolinianus* and certain other species were statistically significant (data not shown). B. The same non-parametric multiple comparisons test based on the EC_{50} data normalized to biovolume. These data again support at least three hemolytic activity groups consisting of (a) *G. carolinianus*; (b) *G. belizeanus*, *G. caribaeus*, and *G. carpenteri*; and (c) *G. ruetzleri* and *Gambierdiscus* ribotype 2.

A.

Comparison Groups	Obs dif	Critical dif	difference
G. caribaeus-G. *carolinianu*s	37.09	25.963	TRUE
G. belizeanus-*G. carolinianus*	62.33	50.279	TRUE
Gambierdiscus ribotype 2-*G. carolinianus*	108.11	50.276	TRUE
G. carpenteri-*G. carolinianus*	61.83	38.399	TRUE
G. ruetzleri-*G. carolinianus*	107.67	59.842	TRUE
G. belizeanus-*G. caribaeus*	25.24	48.572	FALSE
Gambierdiscus ribotype 2-*G. caribaeus*	71.02	48.572	TRUE
G. carpenteri-*G. caribaeus*	24.74	36.138	FALSE
G. ruetzleri-*G. caribaeus*	70.57	58.416	TRUE
Gambierdiscus ribotype 2-*G. belizeanus*	45.78	64.907	FALSE
G. carpenteri-*G. belizeanus*	0.5	56.211	FALSE
G. ruetzleri-*G. belizeanus*	45.33	72.568	FALSE
G. carpenteri-*Gambierdiscus* ribotype 2	46.28	56.211	FALSE
G. ruetzleri-*Gambierdiscus* ribotype 2	0.44	72.568	FALSE
G. ruetzleri-*G. carpenteri*	45.83	64.907	FALSE

B.

Comparison Group	Obs dif	Critical dif	difference
G. belizeanus-*G. caribaeus*	3.79	48.571	FALSE

G. belizeanus-G. carolinianus	41.06	50.271	FALSE
G. belizeanus-G. carpenteri	1.55	56.211	FALSE
G. belizeanus-Gambierdiscus ribotype 2	49.33	64.907	FALSE
G. belizeanus-G. ruetzleri	59.61	72.568	FALSE
G. caribaeus-G. carolinianus	37.27	25.962	TRUE
G. caribaeus-G. carpenteri	2.24	36.133	FALSE
G. caribaeus-Gambierdiscus ribotype 2	53.12	48.571	TRUE
G. caribaeus-G. ruetzleri	63.47	58.416	TRUE
G. carolinianus-G. carpenteri	39.51	38.399	TRUE
G. carolinianus-Gambierdiscus ribotype 2	90.45	50.276	TRUE
G. carolinianus-G. ruetzleri	100.67	59.841	TRUE
G. carpenteri-Gambierdiscus ribotype 2	50.88	56.211	FALSE
G. carpenteri-G. ruetzleri	61.16	64.907	FALSE
Gambierdiscus ribotype 2-G. ruetzleri	10.27	72.568	FALSE

Table S4. Growth rate and EC_{50} cell estimates ± 1 standard deviation for *G. caribaeus* and *G. carolinianus* isolates from the different regions grown at 27°C. Region 1 = western Gulf of Mexico and Caribbean. Region 2 = eastern Gulf of Mexico and Caribbean, and Region 3 = continental shelf off the coast of North Carolina, USA.

Growth rate ± SD in Log, Late Log – Early Stationary and Mid-Stationary Phase							
n	**Species**	Log	St deviation	Late log – early stationary	St deviation	Mid-stationary	St deviation
	G. caribaeus						
15	Region 1	0.16	0.05	0.07	0.04	0.01	0.01
11	Region 2	0.16	0.03	0.09	0.03	0.01	0.01
	G. carolinianus						
5	Region 1	0.18	0.04	0.09	0.02	0.04	0.02
5	Region 2	0.17	0.05	0.10	0.03	0.06	0.02
5	Region 3	0.23	0.05	0.10	0.03	0.05	0.01
	G. ruetzleri						
1	Region 1	0.25		0.07		0.03	
1	Region 3	0.19		0.14		0.05	
		EC_{50} cells ± SD in Log, Late Log and Stationary Phase					
n	*G. caribaeus*						
15	Region 1	133.48	42.29	91.78	34.83	183.33	60.63
11	Region 2	263.67	43.82	176.37	38.47	293.93	39.94
	G. carolinianus						
5	Region 1	171.27	33.08	109.23	28.41	222.03	32.46
5	Region 2	1673.20	856.89	1468.84	798.49	1773.97	888.30
5	Region 3	10432.87	950.32	9878.46	990.46	11153.48	873.38
	G. ruetzleri						
1	Region 1	12.20		6.78		20.34	

1	Region 3	69.16	42.71	80.68

Table S5: The data for *G. caribaeus* and *G. carolinianus* were further analyzed with respect to differences in hemolytic activity among regions where the isolates originated and with respect to log, late log-early stationary and mid-stationary growth phase. A Levene homogeneity of variance test indicated that only the *G. carolinianus* regional comparisons failed to meet the homogeneity of variance tests.

Source	df	F	P
Growth phase (*G. caribaeus*)	2	1.09	0.340
Growth phase (*G. carolinianus*)	2	0.04	0.957
Region (*G. caribaeus*)	1	0.47	0.493
Region (*G. carolinianus*)	2	12.16	6.78×10^{-5}

Table S6. Results of an ANOVA testing whether the regional differences in hemolytic activity within *G. caribaeus* were significantly different. The data indicated that regional differences in hemolytic activity were statically significant when the data for all three growth phases were combined.

Source	df	Sum Sq	Mean Sq	F	Pr(>F)
Region (*G. caribaeus*)	1	217391	217391	57.17	9.36×10^{-11}
Residuals	73	277565	3802.26	NA	NA

Table S7. A Kruskal-Wallis non-parametric ANOVA was conducted to estimate whether hemolytic activity varies significantly by region for *G. caribaeus* and *G. carolinianus* and for *G. carolinianus* alone. The analysis again supported a significant difference in hemolytic activity among regions for both species.

Source	H-Statistic	df	P
Region (both species)	71.95	2	2.37×10^{-16}

Region (*G. carolinianus*)	34.54	2	3.15×10^{-8}

Table S8. Comparison test for differences among regions in hemolytic activity examined the *G. carolinianus* data in more detail. The results indicated that region 3 was significantly different from regions 1 and 2, but that regions 1 and 2 were not statistically different. The failure to detect a significant difference among regions 1 and 2, despite an order of magnitude difference in average hemolytic activity was likely due to the low power of the non-parametric test.

Comparison Group (*G. carolinianus*)	Obs dif	Critical dif	difference
Region 2 - Region 1	10.93	11.48	FALSE
Region 3 – Region 1	27.96	11.48	TRUE
Region 3 – Region 2	17.03	11.48	TRUE

Table S9. An ANOVA testing whether hemolytic activity varied with growth phases in *G. caribaeus* only. The results indicated that hemolytic activity varied significantly with growth phase**.**

Source	df	Sum Sq	Mean Sq	F	Pr(>F)
Growth phase (*G. caribaeus*)	2	134349	67174.3	13.42	1.12×10^{-5}
Residuals	72	360607	5008.44	NA	NA

Table S10. Comparison test for differences in hemolytic activity among the various growth phases of *G. caribaeus*. The data indicated that the difference between log, late log - early stationary, and mid-stationary phases were significantly different from one another. The log and mid stationary phases, however, did not show a statistically significant difference in hemolytic activity**.**

Comparison Groups (*G. caribaeus*)	diff	lower	upper	p adj
Log vs. late log - early stationary phase	-61.76	-109.67	-13.86	0.009
Log vs. mid-stationary phase	41.22	-6.67	89.13	0.11
Late log - early stationary vs. mid-stationary phase	102.99	55.08	150.89	6.60×10^{-6}

Table S11. The results of an ANOVA testing whether hemolytic activity varied significantly with growth phase in *G. carolinianus*. The results again indicated no significant difference in hemolytic activity with growth phase.

Source	df	Sum Sq	Mean Sq	F	Pr(>F)
Growth phase (*G. carolinianus*)	2	2389103.2	1194552	0.052	0.949
Residuals	42	961011804	22881233	NA	NA

Table S12. Levene Homogeneity of variance test for the temperature experiment *G. caribaeus* and *G. carolinianus* were grown at 20, 24, 27, and 31^{o}C. The MTX EC_{50} values were estimated in log, late log -early stationary and mid-stationary phase for both species. The only data set which failed the homogeneity of variance test was the temperature data set for *G. caribaeus*.

Source	df	F	P
Temperature (both species combined)	3	0.032	0.992
Growth phase (both species combined)	2	0.037	0.962
Temperature*Growth phase (both species combined)	11	0.016	1
Temperature (*G. caribaeus*)	3	8.616	8.55×10^{-5}
Growth phase (*G. caribaeus*)	2	0.013	0.986
Temperature*Growth phase (*G. caribaeus*)	11	1.963	0.053
Temperature (*G. carolinianus*)	3	0.051	0.984
Growth phase (*G. carolinianus*)	3	0.051	0.984
Temperature*Growth phase (*G. carolinianus*)	11	0.024	1

Table S13. A. The temperature data for *G. caribaeus* failed the homogeneity of variance test, hence a Kruskal-Wallis non-parametric ANOVA was used to test whether hemolytic activity varied with temperature. All three growth phases were included in the analysis at each temperature. The data indicated that hemolytic activity significantly increased with increasing growth temperatures. B. A multiple comparison test was undertaken using the same data set to test which temperatures exhibited statistically significant different levels of hemolytic activity. The data indicated that the statistical significance in the analysis reported in 13A was due to the differences in hemolytic activity between the isolates grown as 20 and 27 ℃.

A.

Source	df	H-Statistic	P
Temperature (*G. caribaeus*)	3	9.060	0.028

B.

Comparison Groups (*G. caribaeus*)	Obs dif	Critical dif	difference
20°C-24°C	4.83	16.82	FALSE
20°C-27°C	17.36	16.82	TRUE
20°C-31°C	13	16.82	FALSE
24°C-27°C	12.53	16.82	FALSE
24°C-31°C	8.16	16.82	FALSE
27°C-31°C	4.36	16.82	FALSE

Table S14. A) Temperature experiment ANOVA which tested whether hemolytic activity in *G. caribaeus* varied significantly with growth phase. The analysis indicated that hemolytic activity did indeed increase significantly with an increase in growth temperature. B. A multiple comparisons test was done using the same data set to determine which growth phases contained statistically different levels of hemolytic activity. The test specifically examined the difference in hemolytic activity between log and late log - early stationary phase, log phase and mid-stationary phase, and between late log - early stationary and mid-stationary phase. The results indicated that the only significant difference in hemolytic activity was between in late log, early stationary phase and mid stationary phase.

A.

Source	Df	Sum Sq	Mean Sq	F	Pr(>F)
Growth phase (*G. caribaeus*)	2	44094.1	22047.05	4.346	0.017
Residuals	57	289115.9	5072.209	NA	NA

B.

Comparison Groups (*G. caribaeus*)	diff	Lower	Upper	p adj
Transition from log to late log - early stationary phase	-27.1	-81.29	27.09	0.456
Transition from log to mid-stationary phase	38.95	-15.24	93.14	0.203
Transition from late log -early stationary to mid-stationary phase	66.05	11.85	120.24	0.013

Table S15. ANOVA test on the individual effects and interactions of temperature and growth phase on hemolytic activity in *G. carolinianus*. The results indicated that there was no significant difference among the isolates of *G. carolinianus*.

Source	Df	Sum Sq	Mean Sq	F	Pr(>F)
Temperature (*G. carolinianus*)	3	4615937	1538646	0.047	0.986
Growth phase (*G. carolinianus*)	2	3684998	1842499	0.056	0.944
Temperature * Growth phase (*G. carolinianus*)	6	739404.8	123234.1	0.003	1
Residuals	60	1.95×10^9	32482229	NA	NA