Supplementary materials for Park G, Dam HG. 2021. Cell growth gene expression reveals a direct fitness cost of grazer-induced toxin production in red tide dinoflagellate prey. Proc. R. Soc. B 20202480.
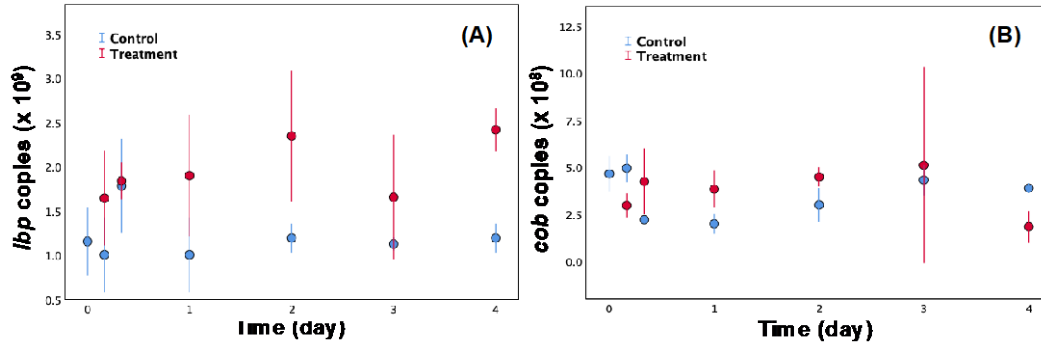**https://doi.org/10.1098/rspb.2020.2480**

Appendix S1A.

Details of qPCR primers used in this study. The primers sxt007F and sxt008R were used to generate PCR product of *sxtA* fragment.

Function	Gene	Name	Sequence 5' – 3'	Length	Reference
Toxin	*sxtA*	sxt007F	ATGCTCAACATGGGAGTCATCC	750 bp	Stüken 2011
	sxtA	sxt008R	GGGTCCAGTAGATGTTGACGATG		Stüken 2011
	sxtA	sxtA4F	CTGAGCAAGGCGTTCAATTC	125 bp	Murray 2011
	sxtA	sxtA4R	TACAGATMGGCCCTGTGARC		Murray 2011
	sxtG	559F	GACGGGAACGGCTACAA	65 bp	Orr 2013
	sxtG	605R	GCTCGAAGATCGGGTCCT		Orr 2013
Growth	*cyc*	AlexcyclinF1	CCAGGCTCAGCGGCTACGT	128 bp	Zhuang 2013
	cyc	AlexcyclinR1	AGCATCTCCGTGTGGCGATACT		Zhuang 2013
Reference	*lbp*	AlexLBPNF	GCGTGACATGAGCGGCTACAT	207 bp	Zhuang 2013
	lbp	AlexLBPNR1	TTGGAGCGGCGGCAGAACAT		Zhuang 2013
	cob	cobF	TCCCATTTTTCCCTTTCWTT	212 bp	Wiese 2014
	cob	cobR	ATTTTTGTTGGGCACAGCTT		Wiese 2014

Appendix S1B.

qPCR efficiency ($10^{(-1/slope)}$), R^2 (coefficient of determination of the regression), and melting temperature (Tm: °C) of *Alexandrium catenella* strain BF-5.

Strain	Function	Gene	qPCR efficiency	R^2	Tm
BF-5	Toxin	*sxtA4*	1.974	0.997	81.2±0.20
	Toxin	*sxtG*	1.976	0.991	77.7±0.19
	Growth	*cyc*	1.705	0.999	85.2±0.30
	Reference	*lbp*	1.850	0.999	82.3±0.17
	Reference	*cob*	1.874	0.999	82.5±0.16

Appendix S1C. Gene expression of references genes (A) *lbp* (luciferin-binding protein) and (B) *cob* (cytochrome b) in the control and treatment. Error bars represent ± 1 standard deviation of the mean. The expression level of *lbp* was more stable with the maximum fold-change of 1.77 in the control and 1.47 in the treatment, compared to that of cob (2.49-fold and 2.78-fold, respectively). Hence, the *lbp* gene was chosen to normalize the gene expression levels in the control (ANOVA, $p>0.07$) and treatment (ANOVA, $p>0.259$).

Appendix S2. Summary of two-way ANOVA for dependent variables: cell concentration, toxin content, net growth rate, toxin composition, and relative gene expression during the grazing assay. Factors in the ANOVA are absence or presence of grazer *Acartia hudsonica*, time (seven levels; 0, 4, 8, 24, 48, 72, and 96 hr), and the interaction of treatment and time. SS(III), type III sum of squares; df, degrees of freedom; MS, mean sum of squares for ANOVA; F, statistic for ANOVA test; p, significance of the ANOVA test; η^2, partial eta squared.
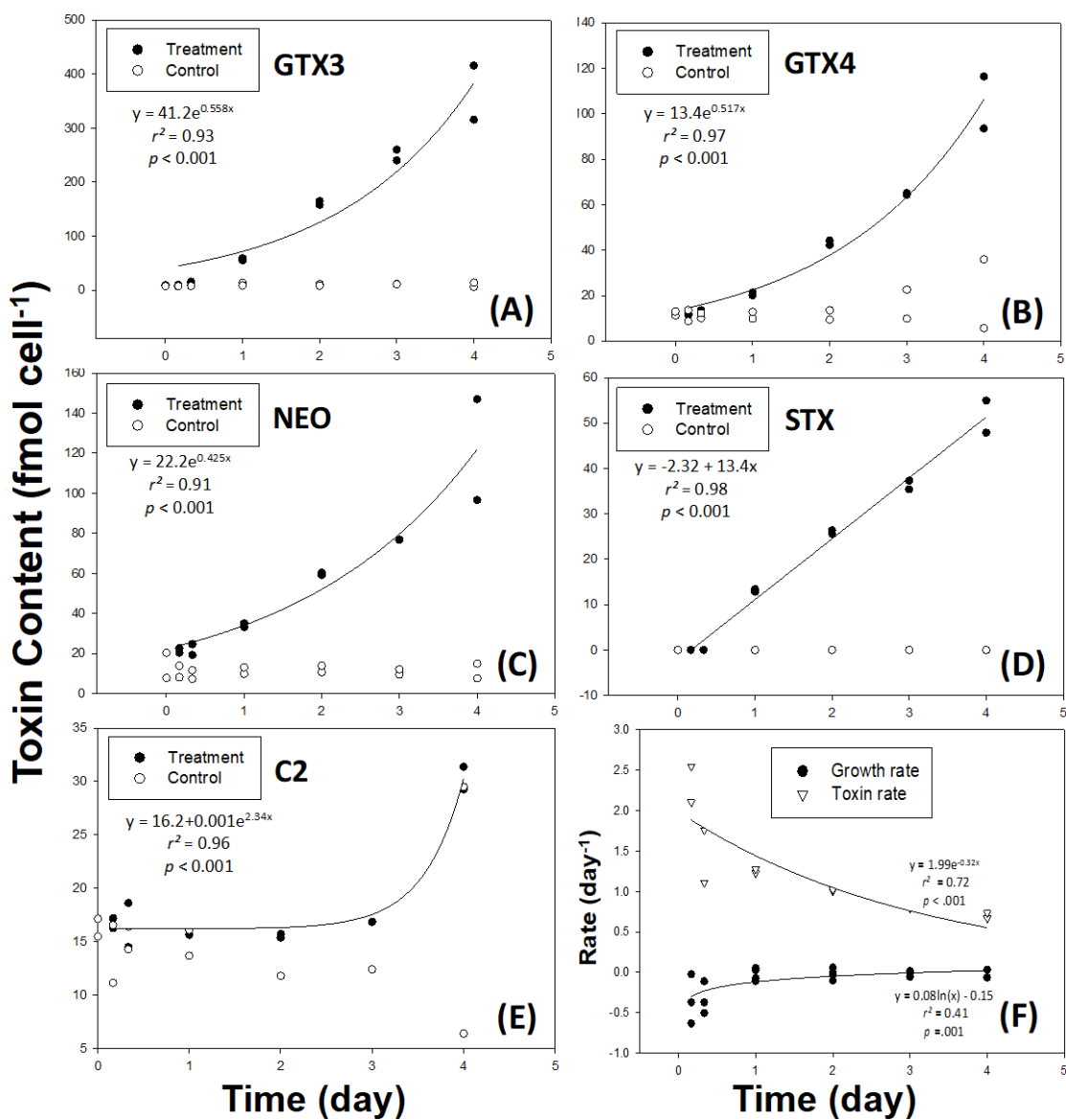
Cell Concentration (cells ml^{-1})						
Source	SS(III)	Df	Mean Square	F	p	η^2
Day	709	6	118	2.97	0.017	0.30
Treatment	8257	1	8257	207	0.001	0.83
Day × Treat.	3905	6	651	16.3	0.001	0.70
Residual	1674	42	40			
Total	60032	56				
Cell Toxin Content (fmol cell^{-1})						
Source	SS(III)	Df	Mean Square	F	p	η^2
Day	610	6	102	4.09	0.014	0.64
Treatment	700	1	700	28.2	0.001	0.67
Day × Treat.	169	6	28	1.13	0.395	0.33
Residual	348	14	25			
Total	7713	28				
Net Growth Rate (d^{-1})						
Source	SS(III)	Df	Mean Square	F	p	η^2
Day	0.373	5	0.075	5.61	0.001	0.44
Treatment	0.358	1	0.358	26.9	0.001	0.43
Day × Treat.	0.011	5	0.002	0.169	0.972	0.02
Residual	0.479	36	0.013			
Total	1.368	48				

Appendix S2. Continued

GTX3						
Source	SS(III)	df	Mean Square	F	p	η^2
Day	919	6	153	9.66	0.001	0.81
Treatment	532	1	532	33.5	0.001	0.71
Day × Treat.	153	6	26	1.61	0.216	0.41
Residual	222	14	16			
Total	7713	28				

GTX4						
Source	SS(III)	df	Mean Square	F	p	η^2
Day	518	6	86	1.79	0.172	0.44
Treatment	386	1	386	8.04	0.013	0.37
Day × Treat.	249	6	42	0.86	0.544	0.27
Residual	673	14	48			
Total	7713	28				

NEO						
Source	SS(III)	df	Mean Square	F	p	η^2
Day	672	6	112	8.71	0.001	0.79
Treatment	782	1	782	60.8	0.001	0.81
Day × Treat.	192	6	32	2.49	0.075	0.52
Residual	180	14	13			
Total	7713	28				

STX						
Source	SS(III)	df	Mean Square	F	p	η^2
Day	356	6	59	415	0.001	0.99
Treatment	448	1	448	3136	0.001	1.00
Day × Treat.	356	6	59	415	0.001	0.99
Residual	2	14	0.14			
Total	7049	28				

C2						
Source	SS(III)	df	Mean Square	F	p	η^2
Day	340	6	57	0.644	0.695	0.22
Treatment	137	1	137	1.56	0.232	0.10
Day × Treat.	118	6	20	0.224	0.962	0.09
Residual	1231	14	88			
Total	7713	28				

Appendix S2. Continued

			sxtA4			
Source	SS(III)	df	Mean Square	F	p	η^2
Day	2045	5	409	4.91	0.002	0.41
Treatment	3040	1	3040	36.5	0.001	0.50
Day × Treat.	1127	5	225	2.70	0.036	0.27
Residual	3000	36	83			
Total	38024	48				
			sxtG			
Source	SS(III)	df	Mean Square	F	p	η^2
Day	2041	5	408	11.4	0.001	0.61
Treatment	4760	1	4760	133	0.001	0.79
Day × Treat.	1123	5	225	6.27	0.001	0.47
Residual	1289	36	36			
Total	38024	48				
			cyc			
Source	SS(III)	df	Mean Square	F	p	η^2
Day	4357	5	871	13.1	0.001	0.65
Treatment	2241	1	2241	33.7	0.001	0.48
Day × Treat.	221	5	44	0.665	0.652	0.09
Residual	2393	36	66			
Total	38024	48				

Appendix S3. Toxin profiles (A-E) of *Alexandrium catenella* in controls and treatments. Daily toxin production and growth rates in the treatments (F). Data in (F) are derived from the data shown in Figs. 1 C and 1D in the manuscript. Lines are regression fits.

Appendix S4. Calculation of defense fitness cost, grazing rate (g), mean cell concentration (Cm), and ingestion rate (I) calculated from Frost's equations and this study (g', Cm', and I') during the experiment. Units of all terms are given in the text.

	Time (d)	0.17	0.33	1	2	3	4
$Cell_{control}$	Mean	248	244	265	298	318	394
	SD	6	11	28	44	13	51
$Cell_{treatment}$	Mean	240	226	222	217	214	210
	SD	12	6	12	15	6	12
$\mu_{control}$	Mean	-0.062	-0.078	0.054	0.083	0.079	0.112
	SD	0.157	0.134	0.108	0.070	0.014	0.034
$\mu_{treatment}$	Mean	-0.264	-0.301	-0.118	-0.070	-0.052	-0.044
	SD	0.297	0.083	0.054	0.033	0.010	0.014
RGE $cyc_{control}$	Mean	0.362	0.824	0.954	0.948	0.673	0.691
	SD	0.204	0.380	0.187	0.194	0.231	0.206
RGE $cyc_{treatment}$	Mean	0.300	0.567	0.603	0.625	0.437	0.416
	SD	0.053	0.059	0.102	0.118	0.056	0.017
$\Delta\mu$	Eq. 4	0.202	0.222	0.172	0.154	0.132	0.156
Cost proportion		0.171	0.312	0.368	0.340	0.351	0.398
Fitness cost	Eq. 7	0.035	0.069	0.063	0.052	0.046	0.062
G	Frost (1972)	0.202	0.222	0.172	0.154	0.132	0.156
g'	Eq. 6	0.167	0.153	0.108	0.101	0.086	0.094
g' vs. g	**% difference**	**17**	**31**	**37**	**34**	**35**	**40**
Cm	Frost (1972)	245	238	236	233	231	229
Cm'	Eq. 9	245	241	243	246	248	259
I	Frost (1972)	1218	1325	1018	900	763	894
I'	Eq. 8	1009	923	666	627	530	609
I' vs. I	**% difference**	**17**	**30**	**35**	**30**	**30**	**32**