Methods for Additional Screening of Samples Yielding Only 18S rRNA Gene Amplicon in Initial Multiplex:

Samples which yielded only an 18S rRNA gene amplicon in the initial multiplex were further screened with additional PCRs using the sxtA4 primer pair 166F/680R. 166F targets a conserved region within sxtA4 of both *Pyrodinium* and *Alexandrium* spp (see sequence alignments under "sxtA4 Primer Variability" below). As the initial multiplex PCR was performed in the same tube that contained the single cell, all genomic DNA was still present in the tube for subsequent PCRs. These additional PCRs utilized as template the original multiplex PCR prepared as follows: 2 ul of a 1:5 dilution of the original multiplex; 20 ul undiluted; 2ul undiluted;14 ul cleaned PCR following the genomic DNA clean-up method of the GeneClean Turbo Clean-Up kit (MoBio). The PCRs that utilized 2 ul of a 1:5 dilution of the original multiplex PCR as template were the equivalent of the nested PCR used to screen the multiplex PCRs that yielded bands indicative of 18S rRNA gene and *sxtA4*. PCRs were conducted in 25 ul volumes using the conditions and thermocycling conditions described for the nested PCRs performed with the 166F/680R primers on samples that yielded an *sxtA4* amplicon in the initial multiplex. PCRs were also performed on the initial multiplex samples that yielded only the 18S rRNA gene amplicon using the F1/680R primers alone using 2 ul undiluted and 2 ul of a 1:5 dilution of the original PCR as template. Reagents and thermocycling conditions were the same as those used for the 166F/680R nested PCR.

Table S1. STX congeners detected in toxic *P. bahamense* lab isolate from the Indian River Lagoon.

ESF ID #	Culture	Sample Name	Total PST in µg STX eq./L culture by HPLC-FL)	Congeners Present
19-468	Pyrodinium bahamense	1 = No Copper, High Light, t= 1 day, Day Phase	41.28	GTX 5, STX
19-469	Pyrodinium bahamense	2 = 1 uM Cu, High Light, t= 1 day, Day Phase	38.44	GTX 5, STX
19-470	Pyrodinium bahamense	3 = 10 uM Cu, High Light, t = 1 day, Day Phase	69.34	GTX 5, STX
19-471	Pyrodinium bahamense	4 = No Copper, Low Light, t = 1 day, Day Phase	35.84	GTX 5, STX
19-472	Pyrodinium bahamense	5 = 1 uM Copper, Low Light, t = 1 day, Day Phase	32.47	GTX 5, STX
19-473	Pyrodinium bahamense	6 = 10 uM Copper, Low Light, t =1 day, Day Phase	38.74	GTX 5, STX
19-474	Pyrodinium bahamense	7 = No Copper, High Light, t = 5 day, Dark Phase	130.66	GTX 5, STX
19-475	Pyrodinium bahamense	8 = 10 uM Cu, High Light, t = 5 day, Dark Phase	93.39	GTX 5, STX
19-476	Pyrodinium bahamense	9 = 1uM Cu, High Light, t = 5 day, Dark Phase	207.87	GTX 5, STX
19-477	Pyrodinium bahamense	10 = No Copper, Low Light, t = 5 day, Dark Phase	104.30	GTX 5, STX
19-478	Pyrodinium bahamense	11 = 1 uM Cu, Low Light, t = 5 day, Dark Phase	106.19	GTX 5, STX
19-479	Pyrodinium bahamense	12 = 10 uM Cu, Low Light, t = 5 day, Dark Phase	174.68	GTX 5, STX

Methods for Toxin Analysis: Toxin analysis was performed at the State University of New York College of Environmental Science and Forestry. The cellular material was combined with 250 µL of 1% acetic acid in water and freeze-thawed three times to extract PSTs. Extracts were clarified by centrifugation at 16,000×g and the supernatant analyzed in triplicate using HPLC-Fluorescence with post-column chemical oxidation to determine PSTs. All toxins were quantified in STX equivalents, using STX standards acquired from Canada's National Research Council, as well as secondary in-house standards. The method detection limit was 0.48-0.58 µg STX eq/L, which is comparable or better than other established methods. Two PST congeners were evident in the samples. Identity was determined by matching retention times of standards, including GTX 1, 2, 3, 4, 5, dcGTX 2,3, C1/C2, STX, dcSTX, NEO, and LWTX-1,2,3,5,6.

Table S2. Percent Identity between two representative sequences from clone sequences of individual P. bahamense cells and published *P. bahamense* sequences.

	LW24_7 (-15bp)**	LW24_1	PR46_6 (-15)**	PR46_9
MN431957.1*	95% (358bp)	98% (358bp)	95% (358 bp)	99% (358bp)
GBXF01000001.1 TSA	96% (502bp)	98% (517bp)	96% (502bp)	99% (517bp)

*MN431957.1 is a 358 bp sequence in GenBank.

** indicates 15 bp deletion in sequence

sxtA4 Primer Variability: The following representative sequences illustrate the sequence differences in the sxtA4F1 and sxtA4166F primers between *Pyrodinium* and *Alexandrium* spp. *Pyrodinium* sequences include clone sequences from both genomic DNA and cDNA from the toxic lab isolate amplified with the 007F/sxtA4680R primers. Yellow highlight indicates sxtA4F1 primer binding region; base differences between *Pyrodinium* and *Alexandrium* are indicated in bold, with *Pyrodinium* sequence in brackets. Green highlight indicates sxtA4166F primer region.

>IRL_sxtADNA 1_007/680R

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Representative 18S rRNA gene sequences for alignment as referred to in the Discussion. "IRL" refers to
Indian River Lagoon sequences from this study.
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