Establishment of quantitative PCR methods for the quantification of geosmin-producing potential and Anabaena sp. in freshwater systems

Supplementary Information

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Figures and tables below provide supplementary evidence for the main text.

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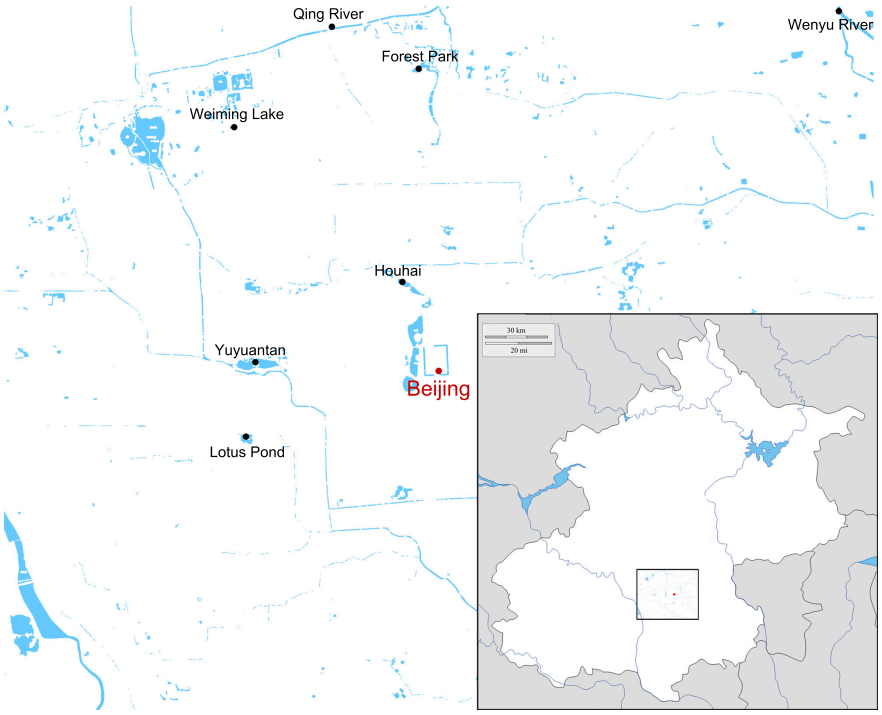

Fig. S1: The location of field sampling sites.

Field sites

Seven sites were chosen to evaluate the applicability of the two qPCR assays. Five freshwater aquaculture ponds included Weiming Lake (**WML**), Forest Park (**FP**), Yuyuantan (**YYT**), Houhai (**HH**) and Lotus Ponds (**LP**), and two rivers were included: Wenyu River (**WYR**) and Qing River (**QR**), spread over five districts in Beijing, China (Fig. S1).

Geosmin component

The geosmin concentration of all culture samples collected during the simulated bloom was determined by GC-MS. Intracellular geosmin increased along with the bloom stage and cell density, whereas extracellular geosmin increased during the first seven weeks and then decreased to a low level. Intracellular geosmin concentrations ranged from 1×10^2 to 1×10^5 ng L^{-1}. Most geosmin (83%-100%) was intracellular (Table S1).

Table S1: The geosmin component in culture samples. Extra- and intra- denote extracellular and intracellular geosmin concentration, respectively (ng L^{-1}); intra-portion is the intracellular fraction of total geosmin.

Date (day)	AE1 extra-	AE1 intra-	AE1 intra-portion	AE2 extra-	AE2 intra-	AE2 intra-portion
1	4.9	191	97.50%	15.3	906.4	98.34%
4	87.5	423	82.86%	73.1	2,150.7	96.71%
6	9.0	799.8	98.89%	25.5	4,200.9	99.40%
8	73.0	1,323.3	94.77%	232.5	8,955.7	97.47%
13	190.0	4,867.4	96.24%	294.6	31,874.4	99.08%
16	32.4	11,498.4	99.72%	237.3	30,616.3	99.23%
20	161.1	25,131.5	99.36%	1,312.4	42,078.8	96.98%
23	428.6	17,993.2	97.67%	2,043.3	18,556.4	90.08%
27	729.5	26,197.0	97.29%	2,783.0	18,643.0	87.01%
30	527.0	79,501.8	99.34%	2,416.2	73,727.4	96.83%
36	2,381.0	104,830.0	97.78%	1,365.1	88,458.5	98.48%
44	10,138.0	82,235.5	89.02%	5,493.8	69,928.2	92.72%
51	384.5	67,369.8	99.43%	6,235.7	77,520.6	92.55%
57	56.7	82,066.6	99.93%	248.2	37,136.9	99.34%
65	299.5	163,120.5	99.82%	582.6	111,916.5	99.48%

Date (day)	AE1 extra-	AE1 intra-	AE1 intra-portion	AE2 extra-	AE2 intra-	AE2 intra-portion
72	19.1	174,100.7	99.99%	69.2	116,592.6	99.94%

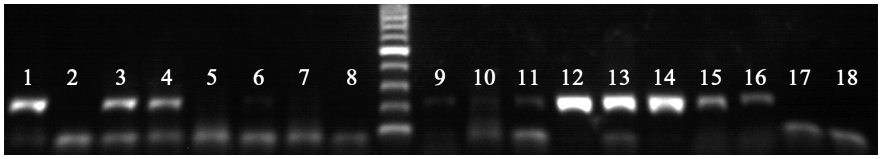

Fig. S2: Gel results of conventional PCR using ARG primers. Lanes: 1, FACHB-170; 2, FACHB-190; 3, FACHB-245; 4, FACHB-251; 5, FACHB-319; 6, FACHB-362; 7, FACHB-380; 8, FACHB-1096; 9, FACHB-1194; 10, FACHB-1199; 11, FACHB-1219; 12, FACHB-1239; 13, FACHB-1250; 14, FACHB-1255; 15, FACHB-1263; 16, FADC-0001; 17, FADC-0002; 18, nuclease-free water.

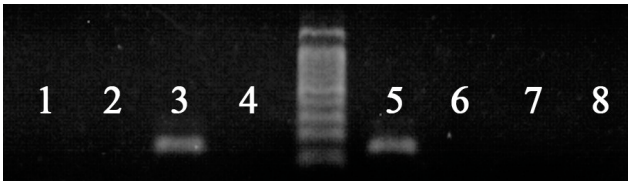

Fig. S3: Gel results of conventional PCR using GSG primers. Lanes: 1, FACHB-1199; 2, FACHB-1219; 3, FACHB-1239; 4, FACHB-1250; 5, FADC-0001; 6, FACHB-1255; 7, FACHB-1263; 8, FADC-0002.

Primer specificity

Genomic DNA from 30 *Anabaena* strains and 17 other strains was tested with the *ARG* primers AN03/06 by conventional PCR and gel electrophoresis. The available electrophoresis results are shown in Fig. S2.

The specificity of *GSG* primers 173AF/AR was tested using conventional PCR with seven *Anabaena* strains and one *Microcystis* strain, coupled with gel electrophoresis (Fig. S3). GC-MS comparisons indicated that the primers amplified *GSG* in geosmin-producing *Anabaena* strains.

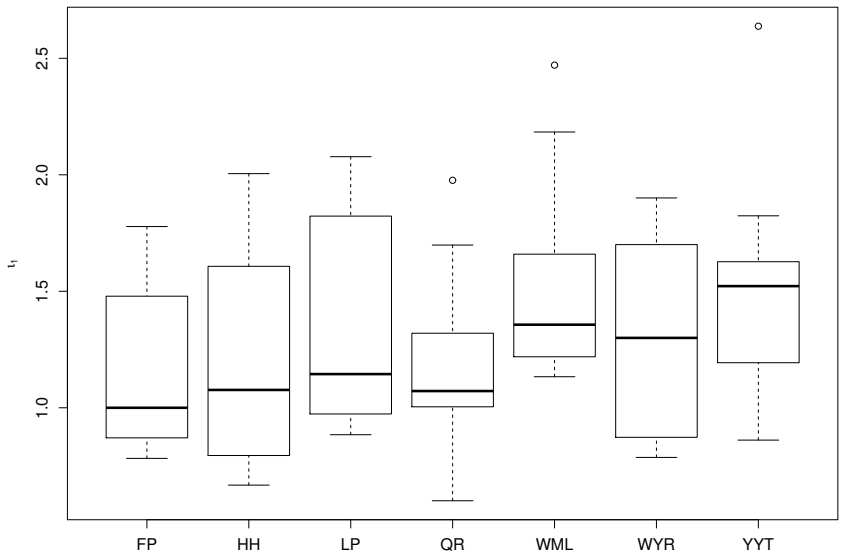

Fig. S4: ANOVA test for the ARG qPCR assay evaluating the effect of background biomass.

Validation on field samples

The 63 field samples used to validate the two qPCR assays were spiked with different concentrations of FADC-0001 (*Anabaena spiroides*) cells. Two ANOVA tests evaluated the effects of background biomass. The logarithmic *ARG* copy density normalized by cell density ranged from 1.17 (**FP**) to 1.54 (**QR**) (Fig. S4). The site effect was not significant (F=1.003, p=0.43). The logarithmic *GSG* copy density normalized by intracellular geosmin ranged from 4.96 (**QR**) to 5.7 (**WML**) (Fig. S5), with no significant site effect (F=2.27, p=0.053).

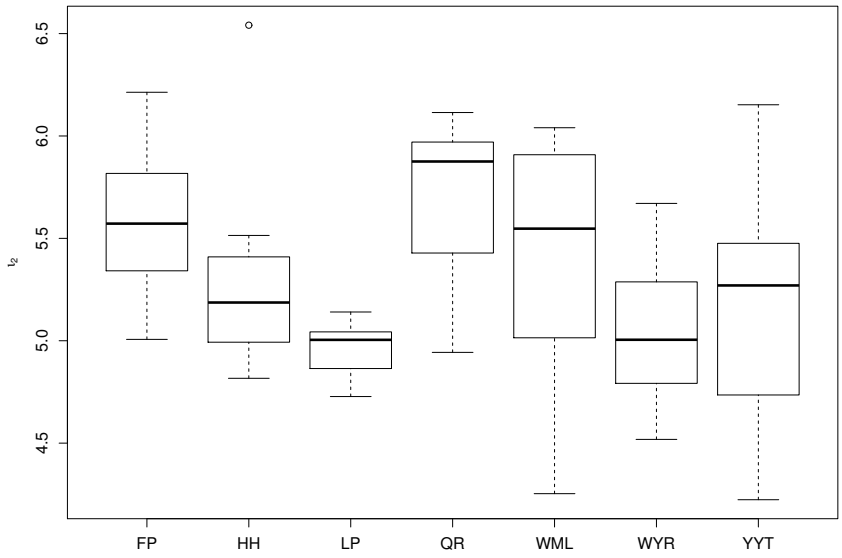

Fig. S5: ANOVA test for the GSG qPCR assay evaluating the effect of background biomass.

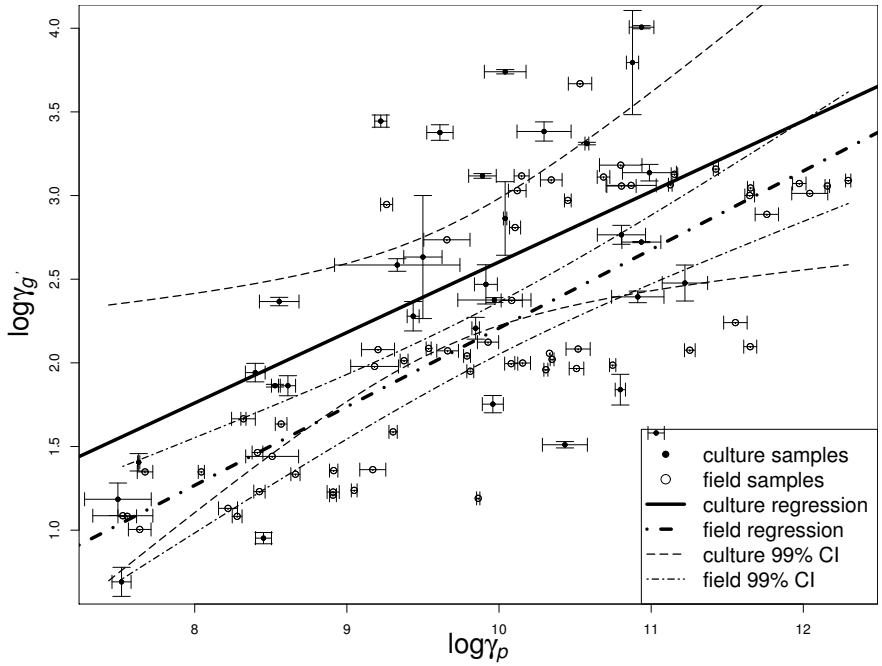

Fig. S6: Quantification of GSG copy numbers by qPCR and their relationship with extracellular geosmin concentration measured by GC-MS. Points represent 32 culture samples and 63 spiked field samples; error bars show standard deviations. Lines show log-log regressions and 99% confidence intervals.

Extracellular geosmin and *GSG* density

The *GSG* density of 32 culture samples and 63 field samples was compared with geosmin concentration measured by GC-MS. The intracellular geosmin concentration was more closely related to *GSG* density than extracellular geosmin. Fig. S6 shows the comparison with extracellular geosmin. The log-log regressions for culture and field samples were:

$$\begin{aligned} \gamma_p &= 0.4214\gamma_{g'} - 1.114 \quad (r^2 = 0.253,\ p < 0.01) \\ \gamma_p &= 0.4697\gamma_{g'} - 2.490 \quad (r^2 = 0.622,\ p < 0.01) \end{aligned} \tag{S1}$$

Here, γ_p is *GSG* copy number and $\gamma_{g'}$ is extracellular geosmin concentration. The two regression lines are shown in Fig. S6.

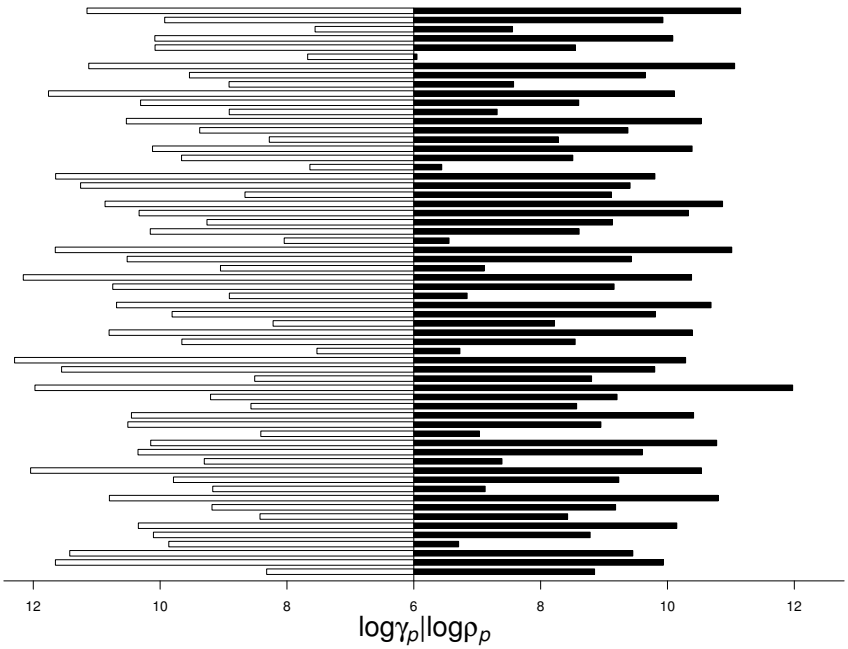

Fig. S7: qPCR variance analysis comparing ARG and GSG copy numbers in field samples.

ARG and GSG density in field samples

The *ARG* and *GSG* densities showed high consistency in both culture and field samples, although they were amplified separately (Fig. S7).