GLOUCESTER MARINE GENOMICS INSTITUTE

Quantitative PCR (qPCR) Summary Report

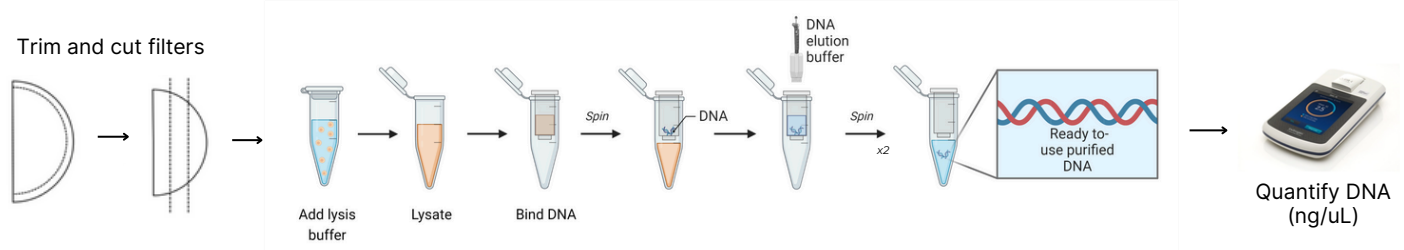
Project Summary

A quantitative PCR (qPCR) assay was optimized for the detection of *Morone saxatilis* (striped bass). A total of 206 filters (180 field and 26 filtration controls) were extracted and processed with the optimized assay. The 180 field filters originated from 140 unique environmental samples with 41.4% (n=58) returning a positive detection of striped bass DNA. Quality assurance and control was conducted with 46.2% (n=12) of filter controls yielding positive detection.

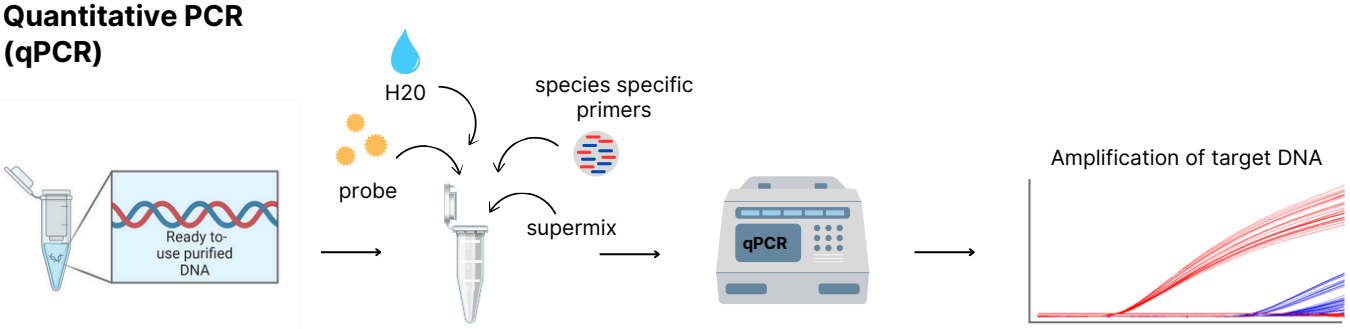
Methods

In quantitative PCR, species-specific primers and a fluorescent probe attach to a fragment of DNA unique to the target species, which is exponentially amplified over ~40 PCR cycles and measured by detecting the fluorescence emitted from the probe at each cycle. Fluorescence in a sample indicates the target species was present, while no fluorescence indicates target species absence. In positive samples, the thermocycler detects the cycle at which a fluorescent threshold is reached (Cycle threshold; Ct value). The Ct value is representative of the quantity of target DNA that was present in the environmental sample, with lower Ct values indicating a larger quantity of target DNA. Quantitative PCR offers semi-quantitative results—the estimated number of DNA copies in the environmental sample can be calculated with a standard curve. This calculation provides an estimate of how much DNA from the target species was present in the original sample but is not necessarily indicative of the number of individuals. If you have questions regarding laboratory procedures or quantitative PCR, please contact us for more information.

DNA Extraction



Quantitative PCR (qPCR)



Contact Us

tim.odonnell@gmgi.org
www.gmgi.org

Results Format

All data collected during the DNA extraction and qPCR of samples are included with this report and located in Results.xlsx. There are several columns of data within the spreadsheet, and brief explanations of each are detailed below.

Unique sample ID

Mean Ct of positive replicates when target species DNA was detected with qPCR

Average number of DNA copies of target species across qPCR replicates

Detection of target species in the sample

Sample ID	Sample Type	Mean Ct	Number of Replicates	Mean Copy Number	Mean Copy Number Normalized	Detection
4/7/2024_Sample_Name	Blank		0			Absent
3/22/2024_Sample_Name	Field	32.24	6	56.84	1.76	Present
9/23/2024_Sample_Name	Field	38.15	1	1.03	0.31	Present
5/20/2024_Sample_Name	Field	36.06	6	8.47	0.98	Present

Number of replicates (out of six) with positive detection of target species

Normalized Copy Number (log10)

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Quality Assurance and Control

Quality assurance and control is of the utmost importance at GMGI, and precautions are taken at several steps throughout laboratory processing (filtration controls processing, no template controls for qPCR, and inhibition controls) to test for potential contamination and produce high quality data.

No Template Controls

Six replicates of no template controls (NTC) are included with each qPCR plate where nuclease-free water is used in place of eDNA to test for potential contamination during qPCR. Of 20 qPCR plates and 120 NTC replicates, none exhibited positive target amplification.

Filtration Controls

A total of 26 blank samples were processed with qPCR (Figure 1). Of those, 12 (46.2%) returned a positive detection for striped bass DNA. 14 samples (53.8%) yielded no detection of striped bass DNA. If a blank sample returns a positive detection of target DNA, the sample is re-run to ensure the validity of the positive detection. Further information, including copy number and mean Ct of blank samples, are presented in Table 1.

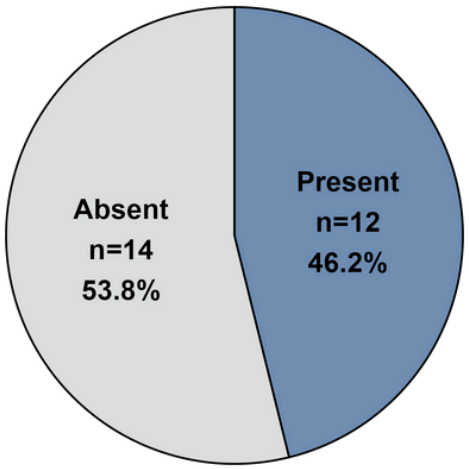

Figure 1: Percent detection of striped bass DNA in filter controls.

Inhibition Statement

Many environmental samples contain substances like humic, fulvic, or tannic acids that can potentially inhibit DNA polymerase activity. To account for this, each sample is screened for PCR inhibition by "spiking" target DNA at a concentration equal to the positive controls in two qPCR replicates to ensure that environmental contaminants are not inhibiting the reaction. An inhibited sample is defined as exhibiting a Ct value greater than 2 cycles relative to the positive control. Inhibition was not detected in any samples (Figure 2).

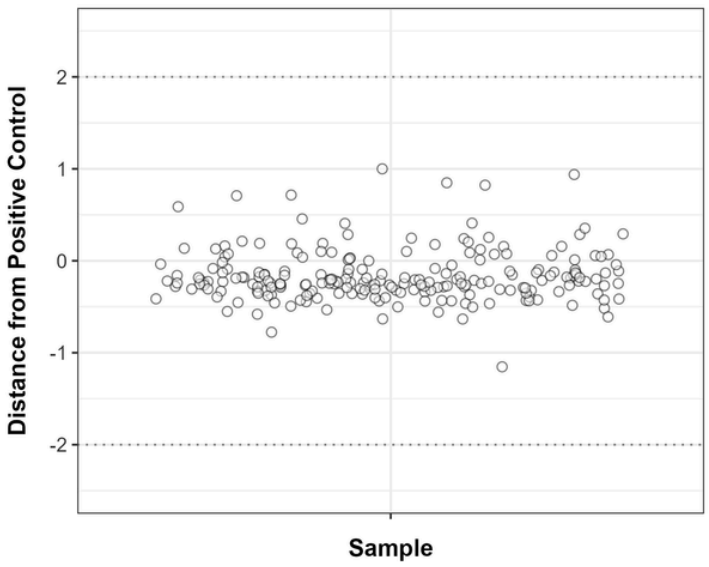

Figure 2: Deviation of samples from positive control

Contact Us
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www.gmgi.org

Filtration Controls

Table 1: Summary of detection data of positive filter control samples

Sample	Mean Ct	Number of Replicates	Mean Copy Number	Mean Copy Number Normalized	Detection
2024-01-17 DI Blank #2	32.52	6	47.09	0.54	Present
2024-01-18 DI Blank #1	38.08	2	1.07	0.05	Present
2024-01-18 Tap Blank #1	36.99	4	2.25	0.12	Present
2024-01-24 DI Blank #1	36.46	1	3.24	0.16	Present
2024-01-26 Tap Blank #2	37.82	1	1.28	0.07	Present
2024-02-06 Tap Blank #1	36.33	1	3.53	0.17	Present
2024-02-06 Tap Blank #2	34.14	6	15.62	0.37	Present
2024-02-21 Tap Blank #1	39.34	1	0.46	0	Present
2024-03-18 Tap Blank #1	37.42	5	1.68	0.09	Present
2024-03-22 Tap Blank #1	38.42	1	0.85	0.04	Present
2024-05-28 Field Blank #1	36.94	2	2.33	0.13	Present
2024-05-28 Lab Blank #1	38.65	1	0.73	0.03	Present

Note: Detection of target DNA in filter controls may indicate that environmental samples filtered in the same batch are subject to contamination. If you have questions in regards to blank samples and potential contamination of samples, please contact us.

Results

Presence/Absence

A total of 180 field filters were processed with qPCR originating from 140 environmental water samples. Of the 140 unique samples, striped bass DNA was detected in 58 (41.4%) and 82 samples (58.6%) yielded no detection.

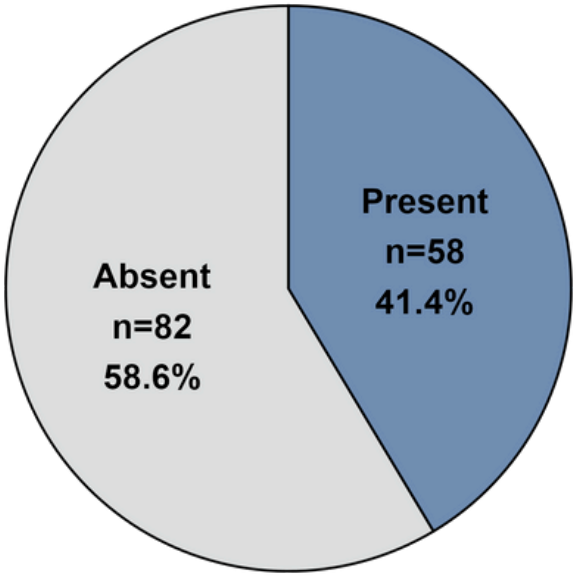

Figure 3: Percent detection of striped bass DNA in field samples

Copy Number & Outlier Detection

The log-normalized copy number depicts the prevalence of striped bass DNA by sample. If multiple filters were used on a single sample, the log-normalized copy number was calculated from taking the sum of copy number across all filters. Samples with normalized copy number values 1.5*IQR greater than the third quantile or 1.5*IQR less than the first quantile were flagged as outliers and depicted in red.

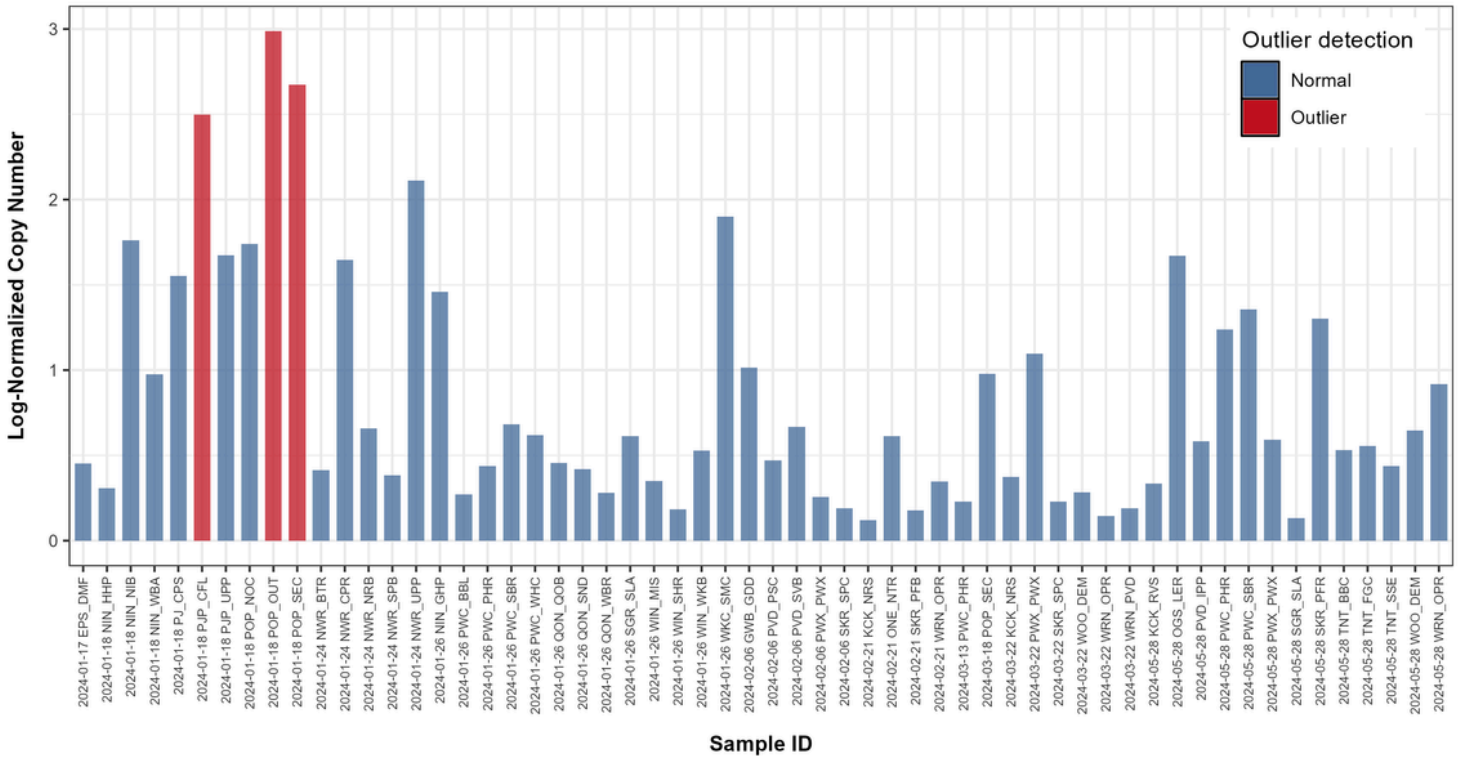

Figure 4: Log-Normalized copy number of striped bass DNA by sample.

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