Supplementary Information

Table S1. Gene regions across the nuclear (nu-) and mitochondrial (mt-) genomes used for developing diagnostic molecular assays targeting zebra mussel (*Dreissena polymorpha*), quagga mussel (*D. rostriformis*), or both. The number of copies of each gene per haploid nuclear genome is estimated from the assembled zebra mussel nuclear genome from NCBI (BioProject: PRJNA533175), the copies of each gene per mitochondrial genome is estimated from the assembled zebra mussel mitochondrial genome from NCBI (BioProject: PRJNA533175) and the assembled quagga mussel mitochondrial genome from NCBI (BioProject: PRJNA666063 – accession MW080914).

Target Gene	Number of Assays	Number of Copies per Genome	Source for assay development (References)
nu-18S	2	30	[14], [48]*
nu-28S	2	20	[49]
nu-H2B	2	19	[19], [34]
nu-H1	1	19	[19]
nu-MetRS	1	6	[19]
mt-16S	4	1	[16], [34], [50], [51]
mt-COI	16	1	[16], [17], [18], [22], [34], [48]* [50], [52]*, [53], [54]*, [55]*, [56]
mt-Cyt *b*	1	1	[16]
mt-repeat region	0	~50-100	This study

* Indicates studies implementing loop-mediated isothermal amplification (LAMP) or light transmission spectroscopy (LTS) methodologies

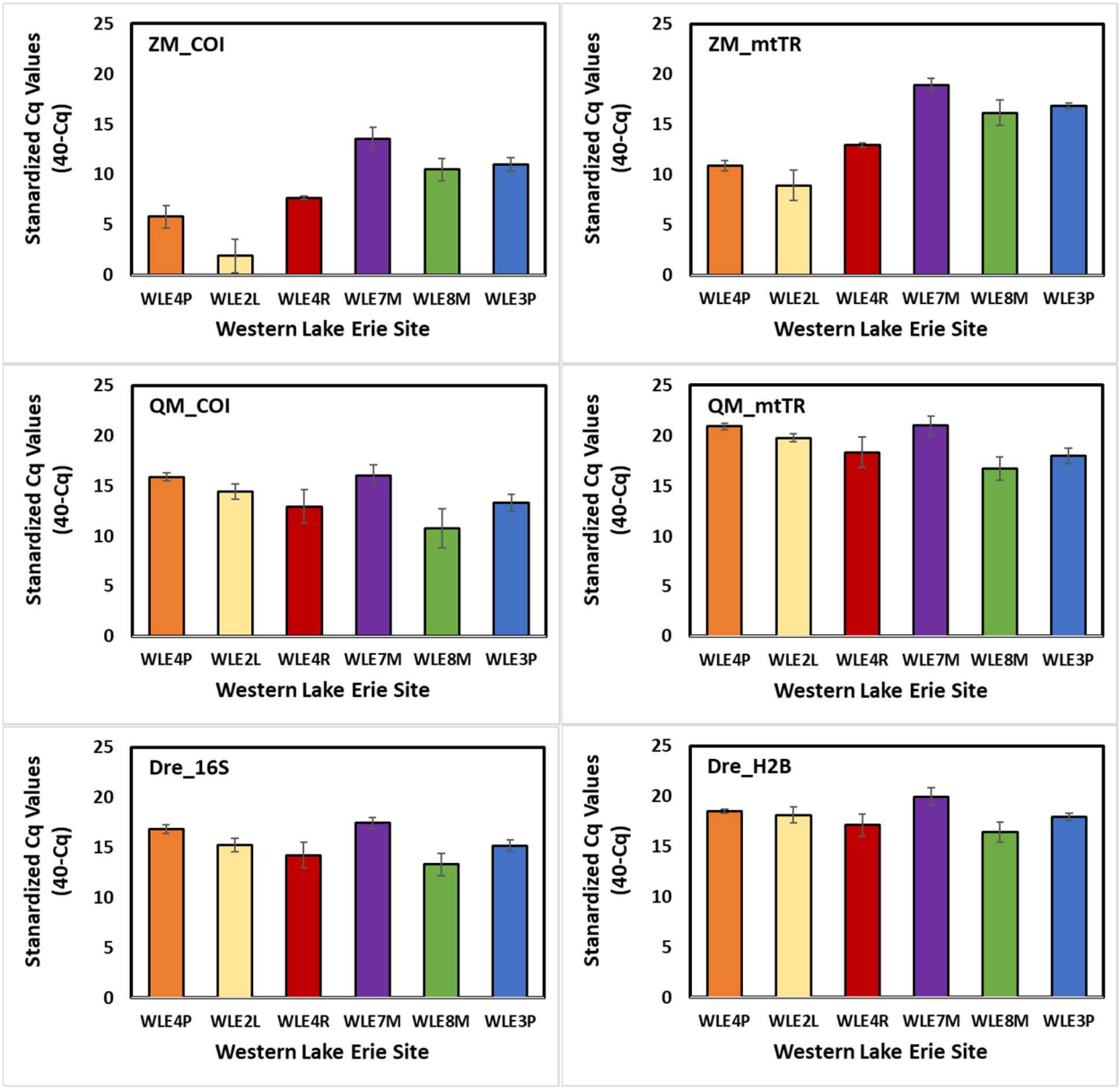

Figure S1. The Standardized Cq values (± standard deviation) across water samples collected from six sites in western Lake Erie for ZM_COI, ZM_mtTR, QM_COI, QM_mtTR, Dre_16S, and Dre_H2B.

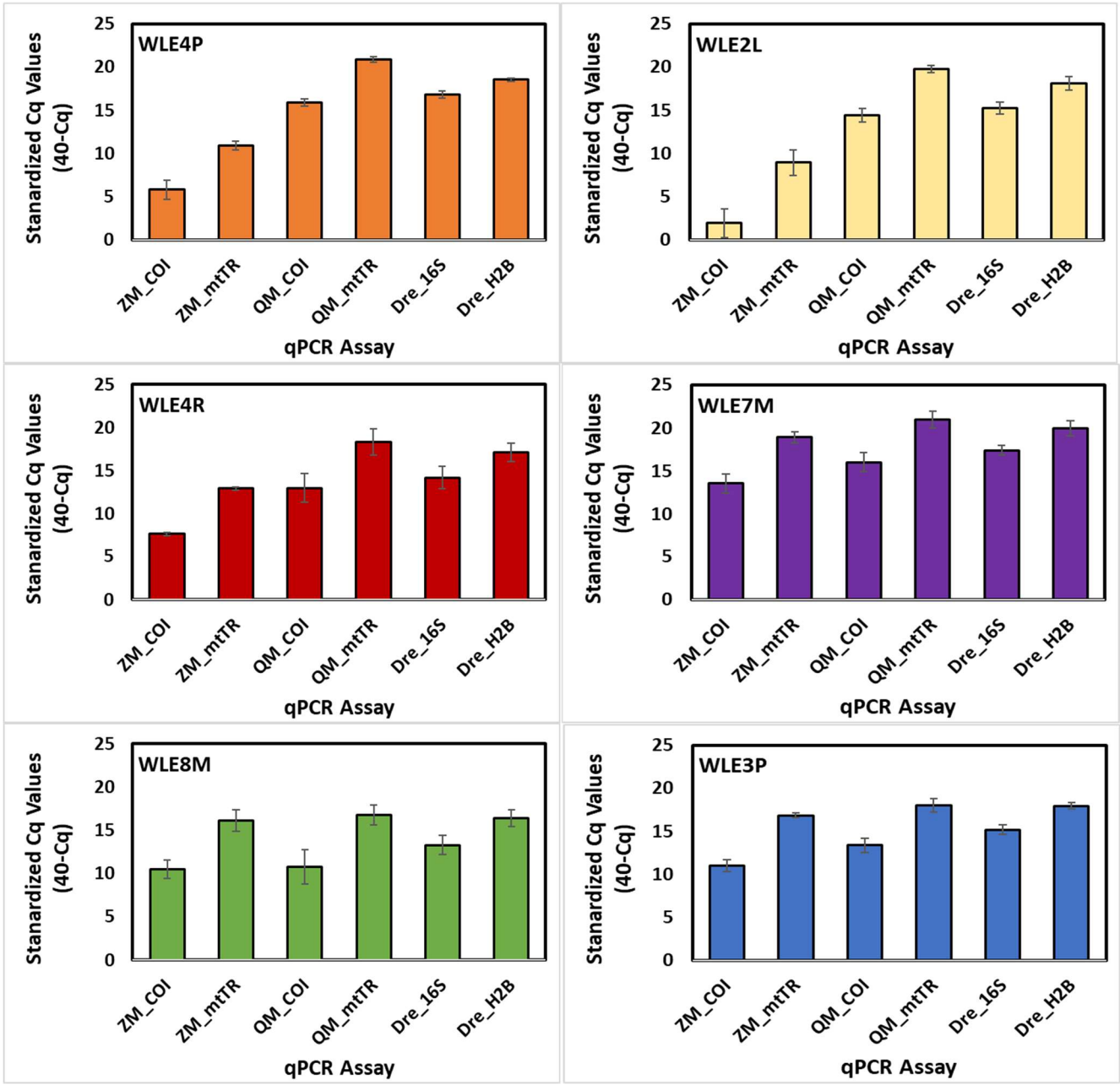

Figure S2. The Standardized Cq values (± standard deviation) for each of the six eDNA assays from triplicate water samples collected at WLE4P, WLE2L, WLE4R, WLE7M, WLE8M, and WLE3P.