Supplementary Methods

Mock spike-in water baths (prepared as described in the main text) were rehydrated in 100 µl (i.e., concentrated samples) and were initially run in duplex qPCR reactions. An additional qPCR was run with a subset of mock spike water baths along with 6 field samples in both concentrated and standard form. Most samples were run in duplicate with the exception the 1 and 10 zoospore spike-in samples and the field concentrated samples which were run in triplicate. Quantitative PCR reactions were completed on a BioRAD CFX system. Data analysis and visualization was completed in R v4.2. Fisher Exact tests were used to analyze detection and Kruskal-Wallis tests were used to analyze estimated ITS copies.

Supplementary Results

The initial qPCR of concentrated mock samples resulted in atypical and spurious amplification curves for all but one replicate of the 10^5 level for Bd (Fig. S1A,B). Standards for both Bd and Bsal amplified. The subsequent qPCR with a subset of mock spike-in samples and field samples in both their concentrated and standard form further showed that concentrated samples inhibit or obscure detection while standard rehydration volumes allowed for successful amplification.

Bd detection was again not detected in concentrated samples and detection was significantly lower than that from standard samples. (Fisher: $p = 0.001$). While Bsal detection did occur and was not significantly different from standard samples (Fisher: $p = 0.18$), the amplification curves of all exhibited atypical patterns (Fig. S1C). More specifically, they exhibited the "hook" effect, which can be associated with inefficient hybridization of the probe to the single stranded DNA during annealing when there are high template amounts. ITS copy estimates were also significantly lower in concentrated samples compared to standard samples for both Bd (KW: $\chi^2 = 17.30$, $p < 0.001$) and Bsal (KW: $\chi^2 = 8.91$, $p = 0.003$). Furthermore, there was a significant correlation between standard load samples across the spike-in samples (Kendall: $R = 0.92$, $p < 0.001$), while there was no correlation among concentrated samples across pathogens (Kendall: $R = 0.39$, $p = 0.094$; Fig. S2A). Estimated Bd quantities from six field samples also show detection to be marginally more likely in standard samples (e.g. 1 ml rehydration) (Fisher's Exact (1t) $p = 0.09$; Fig. S2B). For field samples that showed amplification, curves exhibited expected patterns of exponential phase followed by plateauing.

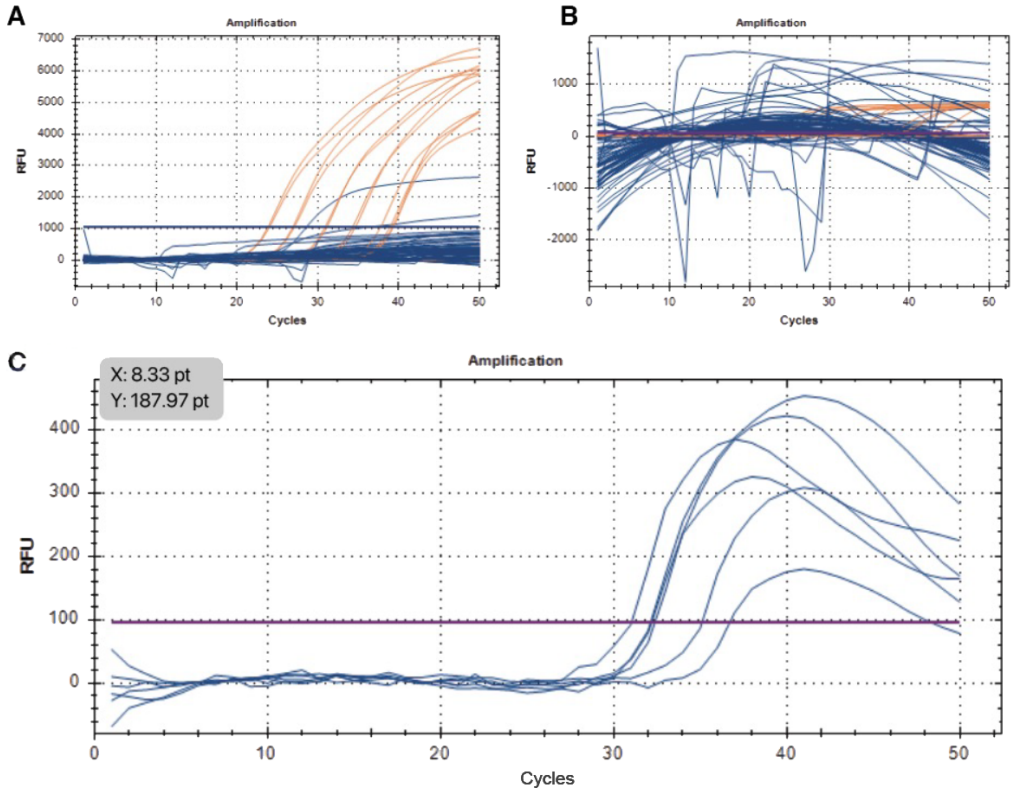

Figure S1. Amplification curves from concentrated samples. (A) Bd qPCR amplification curves (FAM labelled), (B) Bsal qPCR amplification curves (Cy5 labelled) from 1st qPCR and (C) Bsal qPCR of "amplifying" concentrated samples from 2nd qPCR that exhibited the hook effect. Blue curves represent 'unknown' samples and orange curves represent standards.

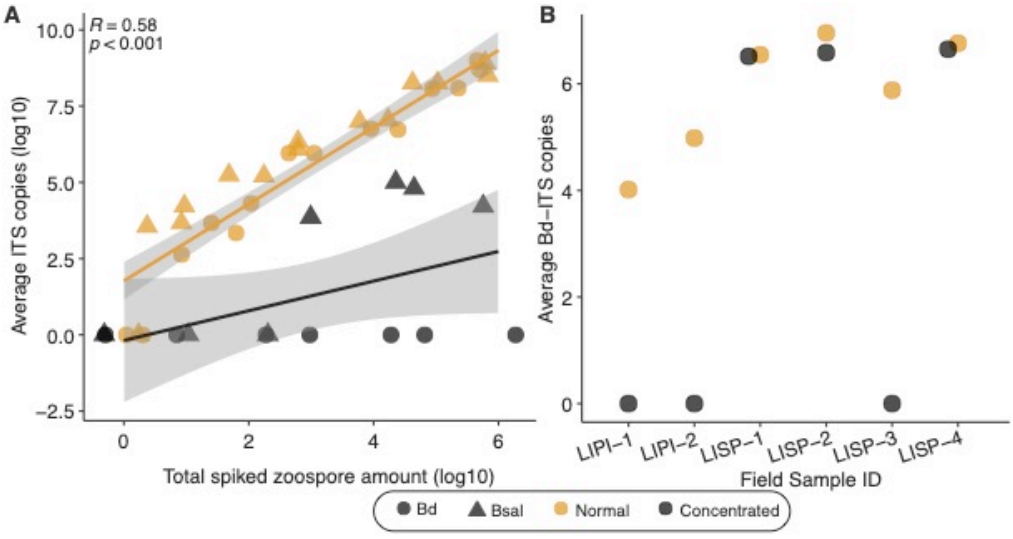

Figure S2. Concentrated samples exhibit reduced detection and pathogen load estimates. Average ITS copy estimates from standard (1 ml rehydration) and concentrated (100 µl) samples from mock spike-in samples with Bd and Bsal (A), and (B) field samples from leopard frogs for Bd.

Table S1. Detection rates of Bd across species for skin wash and swab samples. ITS copy ranges are provided in parentheses.

Species	n	Detection rate	
		Wash	Swab
L. blairi	41	0.927 (0 to 2.7×10^4)	0.561 (0 to 1.1×10^5)
L. pipiens	105	0.848 (0 to 6.4×10^7)	0.752 (0 to 2.9×10^7)
L. sphenocephalus	167	0.904 (0 to 3.6×10^5)	0.886 (0 to 9.1×10^4)