1. **Supplementary Figures:**

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| **Supplementary Figure 1. Conditions within sampled research aquariums.** The acidity (pH; measured in pH units) and concentration of ammonia (NH4+), nitrite (NH2-) and nitrate (NH3-) in parts per million (ppm) were measured weekly in both the freshwater and saltwater research aquariums used to simulate environmental samples. The phosphate (PO4; ppm), general mineral hardness (degrees of general hardness; °dkH), calcium (Calcium; ppm) and salt (Salinity; ppm) concentrations were recorded weekly for the saltwater research aquarium. Missing data points were typically due to global shortages of reagents |

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| **Supplementary Figure 2. Optimization of nucleic acid isolation kit for eDNA and eRNA microorganism detection:**106 *S. aureus* colony forming units (CFU) or 1ng of nucleic acids (NA) were spiked into 500mL of freshwater or salt water, filtered with borosilicate glass filtration (GF/F) filters, and extracted with one of either DNeasy PowerWater kit (white bars), RNeasy PowerWater kit (white/spotted bars), MagMAX™ Total Nucleic Acid Isolation Kit (grey bars) or MagMAX™ *mirvanna* RNA Isolation Kit (black bars), then reverse-transcribed with Superscript IV (Invitrogen) and tested with *16S* species-specific qPCR, alongside a sterile filtered water (MILIQ), and background (H2O) negative controls. Samples were either reverse-transcriptase negative representative of genomic DNA (DNA) or reverse-transcriptase positive representative of genomic DNA and *16S* RNA expression (DNA+RNA). qPCR signal cycle threshold (Ct Value; Log2 scale,) shown. *Undefined* results were given a value of 40. The no-template control (NTC) of the PCR was *undefined.* Data were compared with a two-way ANOVA with *post-hoc* Bonferroni’s multiple-comparisons test; (NS *P* > 0.05; \*\*\* *P* ≤ 0.001; \*\*\*\* *P* ≤ 0.0001).. Triplicate replicate extractions with single reverse transcription reactions per extraction were performed Mean ± SEM of three technical replicates shown. |

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| **A** |
| **B** |
| **Supplementary Figure 3. Gram-stain of bacteria from freshwater.** Representative images of Gram-stained bacteria isolated from 500 mL of water from sampled freshwater research aquarium. The specimen was concentrated to 100 μL by centrifugation, dried, Gram-stained and visualized by light microscopy. Gram-negative bacteria are shown in red, while Gram-positive bacteria are shown in purple. (**A**) Representative micrograph displaying abundant Gram-positive bacteria and (**B**) co-localized Gram-positive and Gram-negative bacteria were found in equal abundance. |