Electronic Supplementary Information

Interacting Effects of Environmental Factors on *Daphnia magna* Removal of *Escherichia coli* from Wastewater

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Number of figures: 3

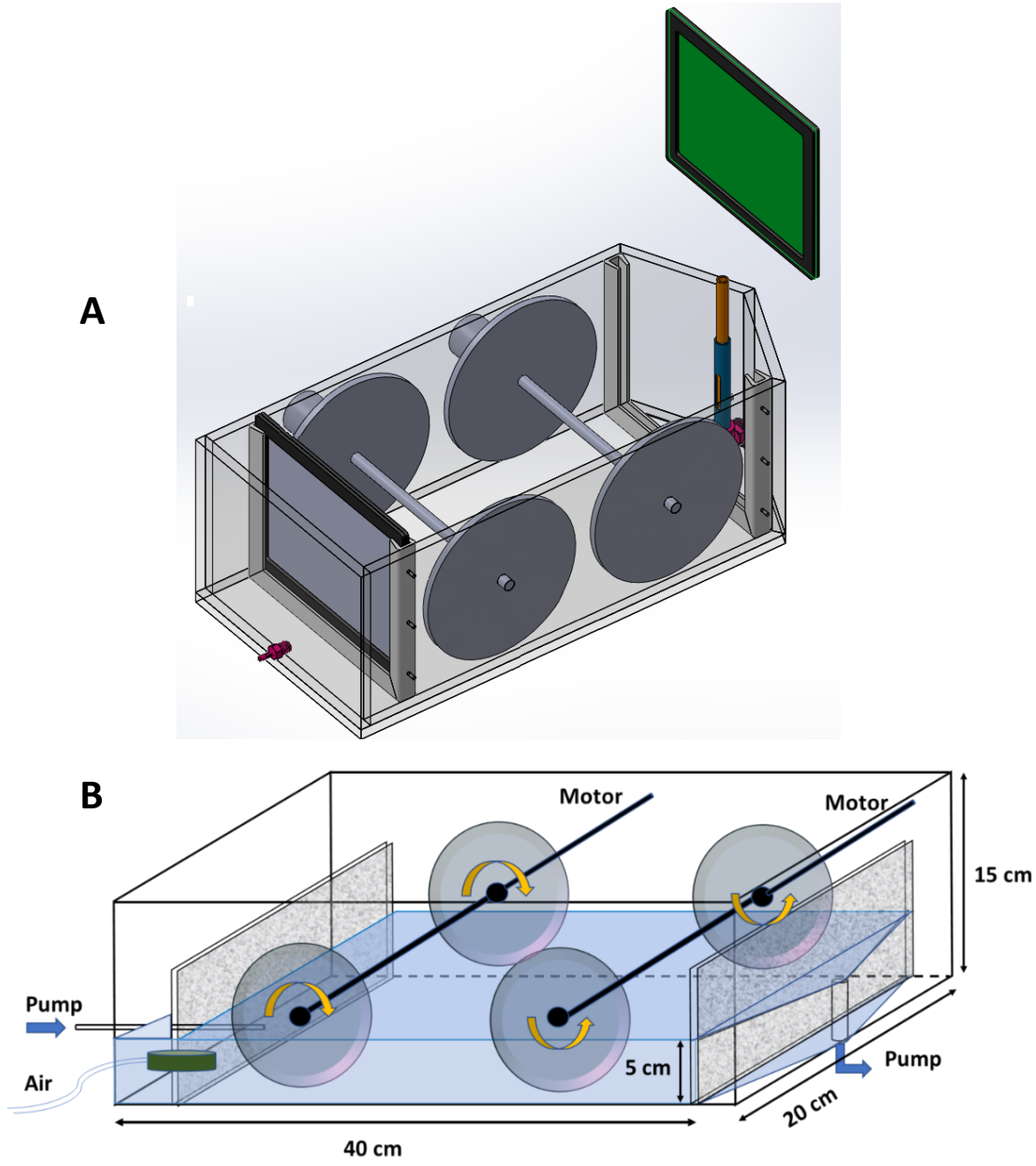

FIG S1 A) AutoCAD schematic of the experimental mesocosm design B) Overall dimensions and layout of mesocosm tank

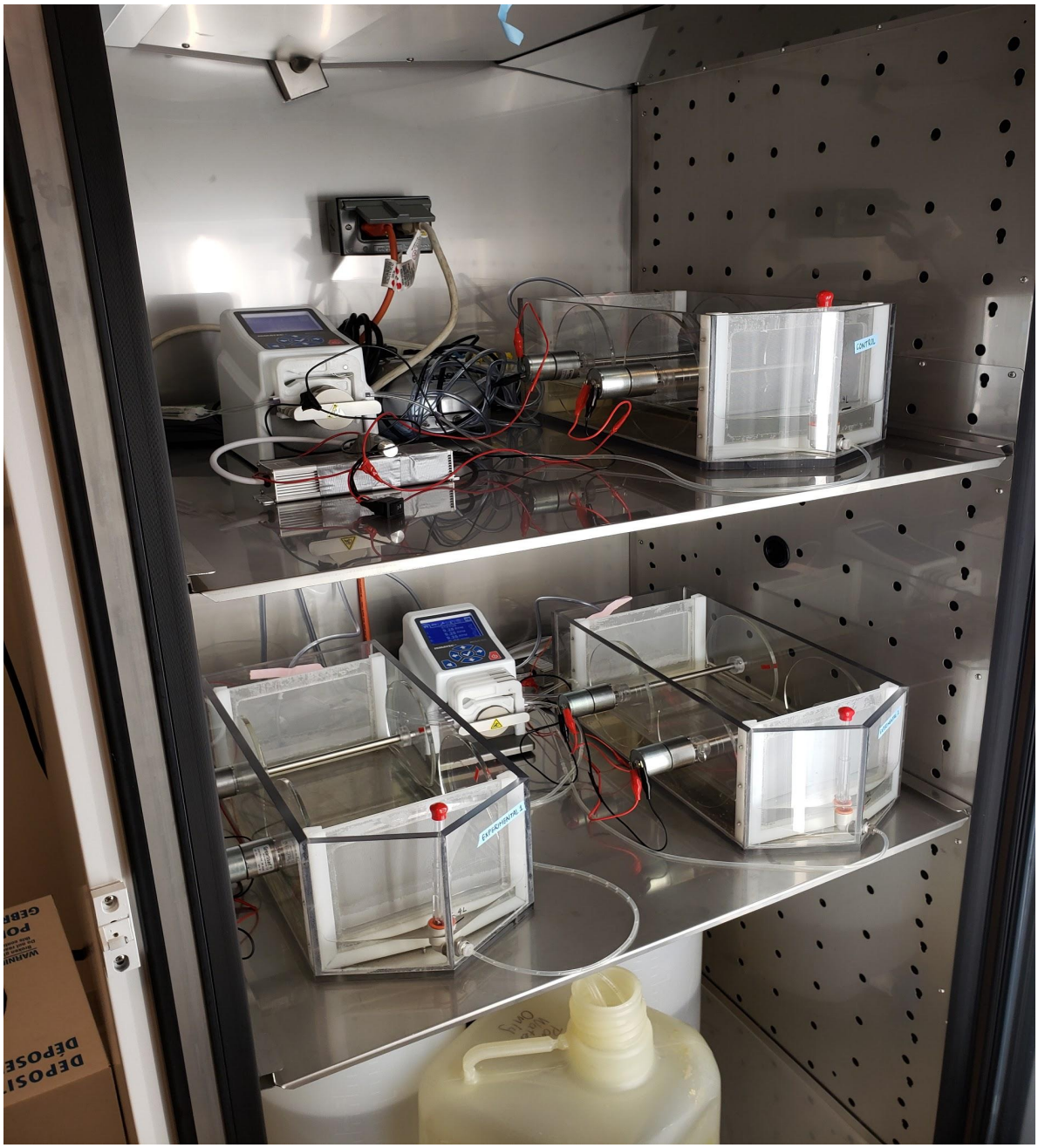

FIG S2 Image of experimental equipment setup inside the temperature controlled environmental chamber

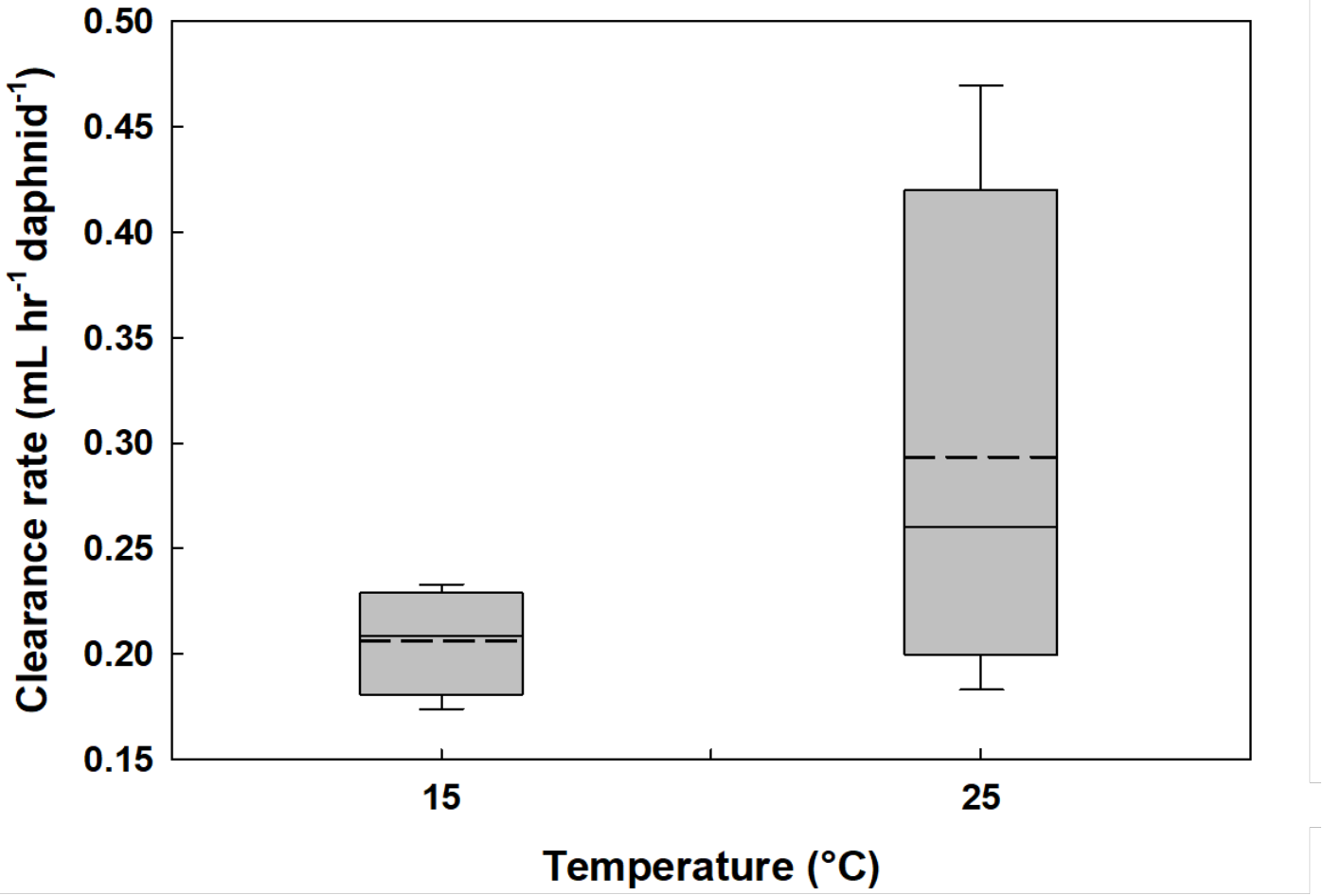

FIG S3 Box plots comparison of clearance rate (CR) values at 15 vs 25 °C. The mean is represented as a dashed line and the median as a solid line.

Table S1: Algal clearance rates with reported standard errors of *D. magna* in the presence and absence of an *E. coli* spike

Environmental conditions		**Clearance rates of Algae (mL hr^{-1} $daphnid^{-1}$)**	
Temp (°C)	*E. coli* spike	Replicate 1	Replicate 2
15	Spike	0.10 ± 0.04	0.11 ± 0.04
	No spike	0.14 ± 0.06	0.15 ± 0.05
25	Spike	0.17 ± 0.06	0.17 ± 0.03
	No spike	0.24 ± 0.06	0.29 ± 0.03

Calculation of *E. coli* removal after a storm event in a hypothetical treatment wetland

Experimental clearance rates from the flow-through system mesocosms were utilized to estimate the removal of *E. coli* that could be achieved following a large storm event resulting in a surge of *E. coli* entering a treatment wetland. The calculations are completed for the following scenarios: A) the two temperatures tested in our studies that may occur due to seasonal changes in different geographic regions B) the presence or absence of an algal bloom. We use the first order rate equation to estimate the removal of *E. coli* expected based on each scenario:

$$\frac{C_t}{C_o} = e^{-kt}$$

The parameters used for the calculation were:

C_0 = *E. coli* concentration due to stormwater runoff = 10^5 CFU/100 ml
C_t=*E. coli* concentration at a given time t in wetland in CFU/100 ml
k= the removal rate in hr^{-1} which is obtained from the experimental clearance rate values
t= the residence time of the hypothetical treatment wetland = 4 days

The density of daphnids used in the calculations was 100 daphnids L^{-1} which represents a zooplankton density that can be experienced during seasonal peaks.

The log removal values were subsequently reported after solving for C_t

Scenario 1: 25 °C, 10^5 CFU/100 ml *E. coli*

With additional algal biomass

$$C_t = 10^5 \exp\left(-0.25 \frac{ml}{hr\ daphnid} \times \frac{24h}{day} \times \frac{100\ daphnids}{1000\ ml} \times 4days\right) = 9.1 \times 10^3 \frac{CFU}{100ml}$$

$$-log\left(\frac{C_t}{C_o}\right) = 1.0$$

Without additional algal biomass

$$C_t = 10^5 \exp\left(-0.47 \frac{ml}{hr\ daphnid} \times \frac{24h}{day} \times \frac{100\ daphnids}{1000\ ml} \times 4days\right) = 1.1 \times 10^3 \frac{CFU}{100ml}$$

$$-log\left(\frac{C_t}{C_o}\right) = 1.9$$

Scenario 2: 15 °C, 10^5 CFU/100 ml *E. coli*

With additional algal biomass

$$C_t = 10^5 \exp\left(-0.23 \frac{ml}{hr.daphnid} \times \frac{24h}{day} \times \frac{100\ daphnids}{1000\ ml} \times 4days\right) = 1.1 \times 10^4 \frac{CFU}{100ml}$$

$$-log\left(\frac{C_t}{C_o}\right) = 1.0$$

Without additional algal biomass

$$C_t = 10^5 \exp\left(-0.22 \frac{ml}{hr.daphnia} \times \frac{24h}{day} \times \frac{100\ daphnids}{1000\ ml} \times 4days\right) = 1.2 \times 10^4 \frac{CFU}{100ml}$$

$$-log\left(\frac{C_t}{C_o}\right) = 0.9$$

The estimated log removal values only serve as a preliminary estimate. Additional system parameters are needed to test these simplified estimates.