Day -1:

Coat temporary holding tubes with gelatin. Start natural pitcher plant bacteria culture. Autoclave finely-ground fish food.

Day 0:

Select the 25C replicate with the highest count for each clone. Mix by inverting six times, then count three, 0.1 ml samples using palmer counting slides.

Calculate volume needed to transfer into the temporary holding tubes and verify source culture volume is sufficient.

Prepare a 20 ml temporary culture for each clone at a density of 50% of K (.5 x 743 rotifers per ml) in gelatin coated tube.

Evolution experiment:

Transfer 7.5 ml from well-mixed temporary culture into high and low experimental microcosms, top off with 7.5 ml sterile media. Add 0.1 ml of natural pitcher plant bacteria concentrate (1 mL frozen natural bacteria to 100 mL media and 0.06 g sterile ground fish food) and 0.2 ml fish food solution (0.25 g finely-ground sterile fish food flakes dissolved in 25 mL of sterile DI water)

Feed every 9 d, and 50% water change every 36 days.

Initial r & K estimates:

Transfer 0.8 ml of the temporary culture into 15 ml tubes, ten for each high and low temperature treatments. Top off with 9.2 ml sterile media. Add 0.05 ml natural pitcher plant bacteria concentrate and 0.1 ml fish food solution. (with a final volume of 10 ml, these cultures will start at 2% of the average K)

Count 0.1 ml using a palmer slide every 2 d. Feed every 9 d.

Notes:

How do I choose which of the current experimental cultures to take from for each clone?

* Whichever 25C replicate has the highest count on Day 0

Does their current temperature treatment matter? Should I put them all in 25 C now?

* Current temp does matter, only in that additions to mixed cultures from all 17 clones original from the same temperature, thus preventing leaching of effects of phenotypic plasticity into the all-important early days of clonal selection under the two temperatures. Clones from one temperature would have an early advantage over those randomly selected from a different temperature.

Need to calculate average k and aim for that for with equal part from each clone.

* The average K across all 17 clones differs based on temperature. At lower temperatures, K = 85.4 +/- 4.34. At high temperatures, K = 63.2 +/-5.23.

Autoclave fish food? Calculate mass of fish food needed.

* Normal amount of fish food is needed. Best to mix up a new batch of fish food solution each feeding day to avoid bacteria growing in the solution.