**Rotifer trait response to temperature**

*Day -X*

1. Count stock cultures to determine viable clones (density of > 5 rotifers per mL)
2. Prepare cross-linked gelatin tubes (see below) (need to know date of this, notebook)
3. Prepare natural pitcher plant bacteria sample by adding 1 mL frozen natural bacteria to 100 mL media (Table 1) and 0.06 g sterile ground fish food, then prepare the fish food solution (0.25 g finely-ground sterile fish food flakes dissolved in 25 mL of sterile DI water).

*Day 0*

1. Concentrate stock cultures using a filter (xx um, new and sterile)
2. Wash two times with 5 ml sterile DI water, then once with sterile media
3. Transfer washed rotifers to sterile media in gelatin-coated 15 ml tubes
4. Mix well (cap and gently invert 3 times) and transfer three 300 uL samples into wells in a 48-well plate for counting
5. Count rotifers. Calculate volume needed to reach target density in microcosms (20 rotifers per mL in a 10 mL volume). If concentration not achieved, filter more of the stock culture.
6. Transfer calculated volume of cleaned rotifers into microcosms (15-mL conical tubes), top off to 10 mL with media
7. Add 50 uL of natural pitcher plant bacteria
8. Feed with 100 uL of fish food solution every 9 days (starting on day 0). Count 100 uL samples using palmer cells every 3 days for xx days. To count, fully invert tube six times, then collect sample by dipping the pipette tip in to the same depth (2/3 the pipette tip length).

Table 1: Artificial media, modified from Plasota & Plasota 1980.

|  |  |  |
| --- | --- | --- |
| **Artificial Media** |  |  |
| Ammonium Chloride | NH4Cl | 60 mg |
| Magnesium Sulfate | MgSO4 | 2 mg |
| Monopotassium Phosphate | KH2PO4 | 20 mg |
| Dipotassium Phosphate | K2HPO4 | 50 mg |
| Sodium Chloride | NaCl | 1000 mg |
| Distilled Water | H2O | 1000 mL |

Table 2: Experimental design, 108 15 mL clonal microcosms from 2 sites, 3 leaves per site, 3 clones per leaf, 3 replicates per clone grown at two temperatures (ambient, 25C; stressful, 30C).

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Site** | **Leaf** | **Clones** |
| Ambient (25C) | Crystal Bog | Leaf 1 | 3x Clones (n = 3) |
|  |  | Leaf 2 | 3x Clones (n = 3) |
|  |  | Leaf 3 | 3x Clones (n = 3) |
|  | Pleaphase | Leaf 1 | 3x Clones (n = 3) |
|  |  | Leaf 2 | 3x Clones (n = 3) |
|  |  | Leaf 3 | 3x Clones (n = 3) |
| Stressful (30C) | Crystal Bog | Leaf 1 | 3x Clones (n = 3) |
|  |  | Leaf 2 | 3x Clones (n = 3) |
|  |  | Leaf 3 | 3x Clones (n = 3) |
|  | Pleaphase | Leaf 1 | 3x Clones (n = 3) |
|  |  | Leaf 2 | 3x Clones (n = 3) |
|  |  | Leaf 3 | 3x Clones (n = 3) |

**Gelatin non-stick treatment**

Dissolve 0.1 g store-bought gelatin in 50 mL DI water by heating the water up in a microwave for a few seconds. Add 0.1 mL 100% formalin and stir. Pour the solution into each tube over a spill dish, then pour back out into beaker to reuse. While pouring the solution back, twirl the tube so the entire interior is coated. Tubes will be stored on their side to dry air overnight. Before adding media and rotifers, tubes should be gently rinsed with DI water to remove any residual formalin.