**Methods**

Field Collection

*Sarracenia purpurea* leaf water samples were collected from 97 plants across three sites in the Apalachicola National Forest in northern Florida in July of 2024. These sites were chosen for the presence of known *S. purpurea* populations. Fifty samples were collected from Site 1 (Crystal Bog), 38 samples were collected from Site 2 (Pleaphase Savanna), and eight samples were collected from Site 3 (Twisted Cyprus Bog). At each site, leaves were chosen haphazardly for sampling with a minimum of ~1 m distance between each leaf. No two leaves were sampled from the same plant. Samples were collected using clean plastic transfer pipettes with ~5 cm of the tip cut away to avoid getting clogged with leaf water detritus. Before collection, the leaf water was mixed to suspend detritus by repeatedly pipetting up and down. Leaf contents were transferred to 50 ml conical tubes. The entire volume of leaf water was collected when possible; if the leaf volume exceeded 50 ml, only 50 ml was collected. Samples were then transported in Styrofoam trays to Forida State University.

Within 24 h of collection, samples were visually inspected for the presence of late-instar mosquito larvae (*Wyeomyia smithii*), which if present were removed using a plastic transfer pipette. The excess leaf water discarded during the process of mosquito larvae removal was filtered using a xx µm mesh sieve and frozen for later use as a seed community of bacteria to help mimic the conditions of natural pitcher plant leaf water.

Sample tube lids were sealed with parafilm and transported via commercial flight in a checked bag to California State University, Northridge. Sample tubes were then stored in a growth chamber (growth chamber company) with a 12/12 h light/dark cycle, at 25C during the day and 18C at night. Using custom Palmer cell-style counting slides, 100 µl samples from each tube were scored for the presence of *Habrotrocha rosa*, other species of rotifers, and ciliates. Samples were monitored daily for the presence of developing mosquito larvae, which were removed promptly upon detection.

Rotifer Isolation and Rearing

In order to test for diversity in response to temperature, clonal cultures of *H. rosa* were first established by isolating individuals from the field-collected leaf water samples. To be selected for use in isolations, leaf water samples had to meet two criteria: the absence of an overwhelming abundance of ciliates, and an estimated rotifer density of >2 individuals per 100 µl. Meeting these criteria were seven samples from Site 1 (Crystal Bog), six samples from Site 2 (Pleaphase Savanna), and one sample from Site 3 (Twisted Cyprus Bog).

To isolate rotifers, 0.5 ml of leaf water was transferred into a 12-well tissue culture plate. Individual rotifers were transferred with the smallest volume of leaf water possible using a clean, pulled Pasteur pipette into another well filled with 0.5 ml of sterile spring water. This washing step was repeated a second time into another well of sterile spring water, and a third time into an unoccupied well in a 24-well tissue culture plate that contained 0.3 ml of a modified version of the rotifer media described by Plasota and Plasota, 1980 (1% sodium chloride, 0.06% ammonium chloride, 0.05% dipotassium phosphate, 0.02% monopotassium phosphate, 0.002% magnesium sulfate dissolved in distilled water). To serve as a basal resource for the bacteria carried over during isolation, xx mg of fish food flakes (TetraColor Tropical Fish Flakes, Tetra) were added to each isolation well.

Preliminary experiments using this isolation method indicate that the likelihood of a haphazardly-chosen individual of *H. rosa* proliferating into a culture of clones is only xx%. This is consistent with expectations, because the reproductive period of *H. rosa* lasts only 5-7 d. With a lifespan of 12-24 d, some individuals have been observed to live for nearly two weeks after losing the ability to reproduce (Ellison and Bledzki, xxxx). Therefore, in order to reach the goal of five clonal cultures per leaf, 32 individuals were isolated from each selected leaf water sample, for a total of 448 isolated individuals of *H. rosa* across 20 24-well tissue culture plates.

At 14 and 28 days post-isolation, every well in each 24-well tissue culture plate was observed, and if more than 20 individuals were present, the entire contents of the well was transferred to a sterile 125 ml flask filled with 100 ml of rotifer media and containing one sterile wheat seed as a resource for bacteria. Of the 448 attempted isolations, 85 were successful, representing x leaves from x site, and x leaves from x site. *Habrotrocha rosa* was only present in two leaf water samples from Twisted Cyprus Bog at the time of isolation, and fewer than five isolations from these samples were successful. For these reasons, Twisted Cyprus Bog was excluded from these experiments.

Temperature Response Experiment

To assess the effect of increased temperature on growth rate and dry mass, clonal cultures of *Habrotrocha rosa* were left to grow at two temperatures for 39 d. The clones used in this experiment were randomly selected from available clonal stock cultures in such a way that they represented three individuals isolated from each of three leaves across two sites located >5 km apart. This design allowed for the analysis of variation in response to temperature within leaves, among leaves, and between sites.

To prepare for this experiment, clonal stocks were first concentrated and washed. To concentrate stocks, between 20 and 60 ml of the 100 ml stock (dependent on initial estimates of stock concentration) was passed through a xx µm filter (filter company). To wash away excess debris from the stock flasks, 5 ml of sterile media was pipetted into the filter and allowed to drain away. Rotifers captured by the filter remained submerged throughout the duration of these steps.

Evolution Experiment

Estimating Growth Rate

Rotifers in a 0.3 ml sample were counted using a compound microscope and custom-made counting slides every 3 d during each experiment. Counting continued until ~90% of replicates appeared to reach carrying capacity. These data were fit to a logistic growth model using the R package ‘growthrates’ (R 4.4.3).

Estimating Dry Mass

For each replicate, a total of 80 ul was transferred to a custom counting slide (20 ul in each corner to ensure an even distribution of rotifers). Videos of each replicate were then recorded using a compound microscope and a Sony a7ii mirrorless camera. To avoid imaging the same individual twice, these videos traced the path of the maze-like pattern on the counting slide (inspired by [type of counting plate thing]). When possible, at least 20 images from each video featuring a unique and fully-extended rotifer were exported using Adobe Premier Pro. Rotifer images were downscaled by 50% and converted to 8-bit before batch processing using a custom trained pixel classifier in Ilastik to produce segmented images differentiating rotifers from the background (Ilastik). Segmented images were converted to binary and further separated into individual regions of interest (ROIs) using the particle analysis features in Fiji, a distribution of ImageJ. The resulting ROI masks that met a minimum aspect ratio of 2.5, that is, at least 2.5x longer than they were wide, were further analyzed using a custom Python script: First, the raw masks were rescaled back to their original resolution and cleaned (gaps and holes filled). Then, a midline was estimated using the skeletonize function in the skimage library. This midline was then compared to the original binary mask to produce width estimates along the length of the rotifer. These width estimates were integrated over the length of the rotifer, and using the sum of resulting frustum segments, an estimate of volume was derived. Consistent with [paper that talks about vol > mass], volume was converted to dry mass.