Harvesting Algae V.2

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This is a protocol to harvest and maintain algae from a chemostat. In the Duffy Lab, the algae used is *Ankistrodesmus falcatus.*

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Harvesting Algae: Written by JMHO 1/4/13
Updated by IAO 05/23/13

1. **On the day prior to harvesting, add vitamins**. The ratio is **1.0 mL vitamin solution/0.5L** new media added. This allows algae in the exponential growth phase to take up vitamins. These vitamins are indirectly given to Daphnia to supplement their diet of algae.
2. **Take out enough algae to make an even number of runs in the centrifuge** (via the spout on the bottom of the chemostat). For example, if your centrifuge holds sixteen 50mL centrifuge tubes, you would want to take off the algae mixture in multiples of 800mL to prevent having to balance the centrifuge with blanks.
3. **Fill up tubes with 50 mL algae**. Be careful to get it close to 50mL (although you don't need to waste your time measuring with a graduated cylinder because the centrifuge probably isn't that

sensitive).

4. **CAP TIGHTLY!** A loose cap can really be bad for all involved!
5. **Spin the tubes at 3000rpm for 5-15 minutes**. In the past when we were running tight on time, I frequently stopped the cycle after 5 minutes and got most of the cells out of the suspension. I would say if you're food limited (new chemostats etc) spin it for **20**, but if you're sitting around waiting for it to finish I usually do 5 minutes.
6. **Pour off supernatant asap and KEEP the pellet.** Try to do this as soon as the spinning cycle stops. Letting the spun down tubes sit only helps break up the pellet = bad for retaining algae.
7. **Resuspend algae in filtered lake water or ADaM and place in pitcher.** Gently tap the tubes (after pouring off the liquid) to break up the algae pellets. Then fill a tube ~ half full with filtered lake water and pour back and forth between tubes to resuspend the algae in new media. You can also use a squirt bottle filled with filtered lake water or ADaM to resuspend algae that is stuck on the bottom of the tube. We have to do this because the Daphnia can't be exposed to too much media in which the algae grow.
8. **Mix the resuspended algae for at least 5 minutes on a stir plate and count using a hemocytometer.** I would recommend starting the algae stirring on a magnetic plate asap to help break up any clumps you couldn't get out yourself. Count the cells on a hemocytometer. Sometimes the algae "soup" in the pitcher is pretty dense if you've harvested a lot, and you might need to carefully dilute the mixture by 10x to make it easier to count.
9. **Calculate the amount you need to use to get 1 L of 1,000,000cells/mL.** Generally the equation looks like this:

X mL * current density cells/mL = 1,000 mL * 1,000,000 cells/mL

Where: you calculate your current density using the hemocytometer and X is the amount you need to measure out and dilute up to 1000mL to get 1,000,000cells/mL.

1. **Try to keep food for only up to ~7 days**.If you keep it much longer than that, it starts smelling funny. You'll have to adjust the amount of food needed based on how things are going in the lab. You can probably get by most of the time making food once a week.

Additional notes about algae chemostats/troubleshooting:

-Choose to harvest the chemostats that are most dense (dark green)

-If you see buildup inside the chemostat or if spun down algae has yellow layer on top of green: Older chemostats (or those getting shaded out by accumulation of algae on the inside of the glass) yield pellets that have a yellow-ish layer of cells on the top. I'm pretty sure they are either dead or unhealthy, so I always take this as a cue to take it down and start using another one. We've never had a problem using that pellet for our animals, though. I think there are enough healthy cells to compensate for any nutritional difference.

-Freezing left-over food: If you have too much resuspended food, we generally freeze it at -20 C for times of emergency when there's not enough fresh food. We've never seen any difference in nutrition, although I haven't tested that directly. The algae-sicles have saved us in a few tight spots before.

-If a chemostat suddenly dies, autoclave it and THROW it away. This happened only 1 time in 3 years, and I never figured out what happened. Also, it's not worth trying to use that algae.

-If the chemostats aren't growing well:

- Check to see if the air tubing is attached properly.
- Check the air filter in between the tubing attachments. This will clog over time. Replace if you can see a color instead of white.
- Check the filters under the air pumps and replace if too gross.
- Check glass tubing with air flow to see if it's clogged (which occurs with increasing age of the chemostat)

- Consider if the media was made properly.

Hemocytometer counts example: 201, 209 (REMEMBER to compensate if you diluted your original sample)
Average = 205 cells/25 large squares
Multiply by 10,000 to get cell density = 2,050,000 cells/mL (NOTE: this conversion factor if given by the papers that come with the hemocytometer)
Calculate your volume:
(1,000mL * 1,000,000 cells/mL)/(2,050,000 cells/mL) = 487.8mL If you dilute this to 1000mL, you'll get a mixture that's 1,000,000cells/mL.
IE, add 487.8mL of algae, and 512.2mL of filtered lake water to each 1L bottle.