Appendix D. CFC Express Operating Steps and Helpful Hints

The following are step-by-step instructions for operating the CFC Express centrifuge connected to a peristaltic pump by inflow tubing in a settling basin of water.

Start-up

- To open the centrifuge, turn the knob on the top of the unit to the "unlocked" position, then push the knob down.
- The glass doors should pop open, then lift the doors to the upright position.
- Push the centrifuge bowl into the rotor. The inflow port (high port) is on the left; the outflow port (low port) is on the right. (Recommend attaching the inflow and outflow tubing to the centrifuge bowl ports prior to placing the bowl in the centrifuge.)
- **IMPORTANT:** The bowl must be pushed all the way down; double check it is properly seated by pressing the bowl down with careful force a few times.
- Lower the glass doors and secure them around the top of the centrifuge bowl. The doors will only close if the bowl is positioned correctly. If the door does not close, reposition the bowl (both left to right and by pushing it down).
- Once the glass doors are shut, turn the knob to the "locked" position.
- Plug in the centrifuge. Flip the switch on bottom left side of unit (by plug) to turn unit on (fan will start running).
- Press the round black button on the front of the unit to start the rotor. It will light up blue once it is on.
- **IMPORTANT:** If you hear a screeching, grinding noise, press the round button again immediately to turn off the unit. The bowl was not seated correctly (probably not down far enough). Open the unit. Inspect the bowl. If there was significant damage (for example, plastic flakes), clean the unit and start over with a new bowl. If the unit does not power up after a grinding event, the fuse may have blown. The fuse and a spare are located in a small black drawer above the plug. Replace the blown fuse with the spare, and remember to replace the now-empty spare slot with a new fuse.
- The unit will get up to 10,000 rpm within 5 seconds. Once it is up to speed, the noise will reduce a bit. It is okay to run the centrifuge dry.
- Once the centrifuge is running at 10,000 rpm, turn on the peristaltic pump and set it to the desired flow rate (for example, 600 mL/min). Reposition the tubing in the pump head as needed until flow begins.
- Double check that the inflow tubing is below the water surface (use a float system as needed).
- After the bowl fills (about 300 mL in 30 seconds), water will start flowing from the outflow line. Check the flow rate of the peristaltic pump by measuring the outflow with a graduated cylinder (for example, 300 mL in 30 seconds.

Periodic Bowl Emptying

When the centrifuge bowl nears sediment capacity (about 15 g), the running volume increases. The run time until this happens depends on the SSC. For SSC < 100 mg/L, empty the bowl(s) into the sample jar a minimum of every 12 hours. For higher sediment waters, empty every 4–8 hours. To do this:

- Turn off the peristaltic pump.
- Stop the centrifuge rotor (push round black button on the front of the centrifuge).
- Once the rotor has come to a complete stop, turn the top knob from "locked" to "unlocked" and push to open the glass doors.
- Gently remove the outflow tubing from the outflow port, replace the port cap, and bag the tip of the outflow tubing in a clean bag.
- Gently remove the inflow tubing from the inflow port, replace the port cap, and bag the tip of the inflow tubing in a clean bag.
- Remove the bowl. Sometimes the bowl resists removal, especially if the sample is warm or the bowl is heavy with sediment. Tug and jiggle upward. If necessary, the suction between the bowl and the rotor can be broken by forcing a little water between the bowl and the rotor.
- Vortex and (or) manually agitate the sediment in the bowl so it is in suspension rather than adhered to the sides of the bowl. Repeatedly striking the bowl with the palm of the hand may be effective in loosening adhered sediment.
- Take both port caps off and invert the bowl over the sample jar.
- Pour the water out of the outflow port into the sample jar. (If a wet sample weight is desired, pre-weigh the empty sample container.)
- Periodically resuspend the sediment in the bowl to transfer as much sediment as possible during the first 300-mL pour.
- Add a small volume (20 mL) of native water from the centrifuge outflow or appropriate unreactive blank water to the bowl through the inflow port using a squirt bottle. Use a squirt bottle of appropriate material for the analyses.
- Cap the ports and repeat the agitation and pouring steps. If centrifuging will continue with the same bowl, there is no need to get every last particle out of the bowl the first time. Be mindful of the rinse water volume—use enough to efficiently transfer the bowl sediment to the sample jar without overfilling the sample jar.
- Inspect the bowl. If the integrity is intact, put it back in the centrifuge to continue centrifuging. If it is worn, replace it with a new bowl. One common location for bowl wear is the top of the bowl where the spinning plastic top can grind into the top of the bowl and create a groove (fig. 6*B*). There also may be bits of plastic dust in and around the centrifuge to indicate grinding is occurring. The other common location for bowl wear is at the horizontal groove near the bottom of the bowl (fig. 6*B*). If too much sediment accumulates in the groove, the plastic pushes out and weakens the bowl at that point. This increases the difficulty in seating the bowl correctly in the centrifuge, and removing it. Additionally, ensure that the bowl exterior is clean and dry if reusing.
- Reattach the inflow and outflow tubing to the bowl, reseat the bowl in the centrifuge, lock the glass doors, and restart the centrifuge.
- Once the centrifuge is up to speed, restart the peristaltic pump and complete other checks (for example, tubing is below water surface, flow rate is correct, outflow tubing is directed to drain, and sufficient ice to keep settling basin water cool).
- Cap, label, and store sample jar quiescently at 4 °C (in laboratory refrigerator or field cooler).
- **NOTE:** If the centrifuges will be running for multiple days, decant the overlying water in the sample jar from the previous day just before emptying the current day's bowl into the jar (otherwise the jar will overflow). Use a clean pipette or carefully pour the overlying water from the jar while retaining the settled sediment. It is recommended to decant the overlying water back into the settling basin to avoid losses of fine colloidal sediment.

Settling Basin Sediment Transfer

As a settling basin is drained of water, transfer the settled sediment following these steps:

- If large settling basins are being used (110-L capacity), it is helpful to transfer the Teflon™ liner with sample to a smaller container, such as a 5-gal bucket once there is less than 20 L of water remaining. During this transfer, rinse the sides of the bag with blank water or sample water to rinse sediment from the sides of the bag into the remaining water. The slippery Teflon™ surface reduces sediment adhesion.
- Pump down the remaining water until about 100 mL remains with the settled sediment.
- Move the tubing/float system to a new full settling basin.
- Carefully transfer the bag sediment into the sample jar. For large bags, this requires some care. The suggested technique is to contain all the water and sediment in one bottom corner of the bag, then roll down the bag to a smaller size. Crease a line from the corner of the bag to transfer as much sediment along the crease into the sample jar as possible. Add small volumes of blank water and repeat the suspending and pouring process until bag is empty. A Teflon™ spatula could also be used to scoop and scrape sediment from the bag. As this is not a quantitative sediment transfer, it is okay to leave some sand particles in the bag.
- Repeat the process for each settling basin until all are empty. Just prior to emptying the next bowl or bag into the sample jar, decant the overlying water in the jar back into a full settling basin to avoid overfilling the sample jar.
- It is recommended that the basin draining and bowl emptying be staggered so there is time for sediment to settle in the sample jar prior to decanting the overlying water back into the settling basin.
- This is an iterative process of reducing the original large volume of water to about 300–600 mL of water mixed with the sediment sample.
- Cap, label, and store sample jar quiescently at 4 °C overnight.

Remove Overlying Water in Final Sample

After the sample has settled quiescently for about 24 hours, remove the overlying water as follows:

- Use a clean (methanol-rinsed) glass pipette (50- or 100-mL) and bulb to pipette the overlying water from the jar to clean glass centrifuge tubes that fit the floor centrifuge, for example, IEC Model K floor centrifuge (Needham Heights, Massachusetts) with 100-mL tube rotor and glass centrifuge tubes.
- Once the overlying water is equally distributed in the tubes, spin the tubes (for example, for 30 minutes at 5,000 rpm).
- Remove (by pipette) and discard the overlying water in the centrifuge tubes.
- Transfer the sediment pellet at the bottom of each tube to the sample jar (by scoop, or vortexing and transferring with a few milliliters of water).