

Langerhans Lab Protocols

NC STATE UNIVERSITY

Daily Steps for Live Fish Care in BRF 221 and 223

Morning Feeding:

Summary:

- Monday-Friday (7:00am - 10:00am):
 - *Artemia* (i.e. brine shrimp) for all *Gambusia* and NC Creek Chub **juveniles**.
 - Flakes and/or bloodworms for the rest (**see feeding calendar for food types used during the recent past**).
- Saturday, Sunday, and Holidays:
 - Feed all *Gambusia* **adults** and NC Creek Chub **adults** flakes and/or bloodworms once/day (preferably mid-day if possible; **see feeding calendar for food types used during the recent past**).
 - Feed all *Gambusia* **juveniles** *Artemia* once/day (preferably mid-day if possible).
 - Feed NC Creek Chub **juveniles** *Artemia* for the first feeding of the day.

Feeding Steps:

1. Harvest the hatched *Artemia* from the aerated solution in the hatchery cone in BRF 221 and start another batch for tomorrow
 - a. See the “*Artemia* (brine shrimp) Culture and Harvest” protocol.
2. Transfer *Artemia* into each inhabited tank in the Aquatic Eco-Systems rack in BRF 223 and any other tanks in both rooms.
 - a. Use the large pipette to **gently** transfer the *Artemia* solution into each tank. Stir the solution frequently to make the *Artemia* uniformly distributed before drawing solution into the pipette.
 - **Male *Gambusia*:**
 - Transfer 1 full uptake squeeze (~3mL) into tanks with **one adult male**.
 - Transfer 1½ uptake squeeze (~4.5mL) into tanks with **two adult males**.
 - Transfer 2 full uptake squeezes (~6mL) into tanks with **three adult males**.
 - **Female *Gambusia*:**
 - Transfer ~1 uptake squeeze (~3 mL) per **each adult female**.
 - Transfer 2 full uptake squeezes (~6mL) per **each pregnant female**.
 - **Juvenile *Gambusia*:**
 - Transfer 1 full uptake squeeze (~3mL) per **4 juveniles** depending on their size.
 - b. On each side of the BRF 223 rack, feed the bottom rows first moving upwards (may reduce disturbance to the fish after introducing the food).
 - c. During the feeding process, make note of any dead fish and plan to remove/preserve them after all fish have been fed.
 - See the instructions in step 6 and 7 below for the proper procedure.
 - d. **Keep a close watch for any mature *Gambusia* males (i.e. visible gonopodium) in juvenile tanks and move them to a separate tank as soon as possible!**
 - e. If you have any *Artemia* remaining in the beaker after following the feeding amounts above, distribute the rest among the 10-20 gallon tanks, depending on the number of inhabitants.
 - f. If you run out of *Artemia* before all fish have been fed, feed the remaining fish crushed flakes using a blue straw and plan to modify the amount of cultured *Artemia* (and resulting volume of water/*Artemia* solution) to suit the number of fish in your care.
3. Feed any fish that require flakes and/or bloodworms (**see feeding calendar for food types used during the recent past**).
 - a. Feed each **adult male *Gambusia*** flakes only (tiny pile of flakes at the end of the thin end of the blue straw per each male), no bloodworms.
 - a. Feed each **adult female *Gambusia*** approximately twice the amount of flakes males are fed depending on their size.
 - b. Feed each NC Creek Chub **adult** flakes and/or bloodworms as appropriate.
4. Record feeding on the calendar in the binder for each room.
5. Thoroughly rinse the pipette and plastic beaker.

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Morning Feeding Steps continued:

6. If you observed any dead NC Creek Chub, individually record the date, species, any possible reasons for death, and brood/sex (if applicable/known) and remove them from the tank(s).
 - a. Preserve them as groups in an appropriately labeled jar (by population) containing 95% ethanol.
 - These jars *may* be kept in the BRF fish rooms (i.e. jar storage location can be variable).
7. If you observed any dead *Gambusia*, individually record the date, species, brood, sex, and any possible reasons for death on C:\Users\LangerhansLab\Dropbox\FishCare\TankMaintenance_ChemLog.xlsx and remove them from the tank(s).
 - a. Take dead fish to the lab in DCL 382, keeping track of who is who if there are multiple mortalities.
 - b. Preserve each fish separately in an appropriately sized sample tube or vial (in cupboard below the microwave).
 - Submerge in 95% ethanol (in the fume hood).
 - c. Include a small piece of rite in the rain paper (top drawer left of computer G) inside the sample tube or vial indicating the species, brood, "died in lab", and date (e.g. *G. hubbsi*, Cousteau brood B, died in lab, 5/6/14).
 - d. Place the tightly sealed container in an appropriate area in the lab for DRILL (database) entry.

Post-morning feeding:

- Check the water level in the reservoir for the BRF 223 rack.
 - Should have ≥ 20 gallons.
 - If refilling is necessary, refer to instructions in the BRF 223 rack basic maintenance protocol.
- Check solution levels in the white buckets near the top of the BRF 223 rack above the controls and filters.
 - If refilling is necessary, refer to instructions in the BRF 223 rack basic maintenance protocol.
- Check the pressure gauges on the left side of the BRF 223 rack.
 - If the pressure differential between the two gauges is between 15 and 20psi, filter replacement is required. Refer to the rack system main manual for replacement instructions.
- Check the filter pad underneath the Plexiglas panel near the square recirculation channel leading into the grey reservoir on the bottom right side of the BRF 223 rack to ensure water is easily flowing through the pad and not across the pad and into the main reservoir.
 - Replacement may be necessary ~every 4 weeks; refer to the log sheet in the binder for the last date it was replaced. Refer to instructions in the BRF 223 rack basic maintenance protocol for proper replacement procedure.
- Fill any 10-20 gallon tanks with low water in both rooms using water from the BRF 223 rack by means of the clear tube coiled up at the upper right of the rack as you walk in the BRF 223 door.
- Change water in 10-20 gallon tanks as needed also using BRF 223 rack water.
 - See the fish tank water changing protocol.
 - Make sure the fish have had enough time to eat (at least 10 min) before siphoning water out for cleaning.
- Change 4-5 gallons of water in the recirculating tank system in BRF 221 daily if the system is housing many fish in all tanks; less frequent water changes may be more appropriate if the system is only housing a few fish.
 - Refer to C:\Users\LangerhansLab\Dropbox\FishCare\TankMaintenance_ChemLog.xlsx for the date of last water change.
 - Ammonia test values ≥ 1 suggest that water needs to be changed.
 - Refer to the Small Recirculating System Basic Maintenance protocol in the binder for the proper procedure.
- Check the Water Chemistry Target Ranges protocol (one in the binder for each room) for testing procedures, required frequency, and value ranges for water in tanks housing blue hole and NC Creek Chub.
 - Perform the tests and record the results on C:\Users\LangerhansLab\Dropbox\FishCare\TankMaintenance_ChemLog.xlsx if testing is required on a particular day.
 - The rack system in BRF 223 monitors and records the necessary water chemistry values automatically.
 - This information can be accessed at C:\Users\LangerhansLab\Dropbox\BRFRACK223.
- Record all tank cleanings, water changes, treatments (e.g. stress zyme or fungus clear), and filter pad replacements, etc. on C:\Users\LangerhansLab\Dropbox\FishCare\TankMaintenance_ChemLog.xlsx.

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Afternoon Feeding:

Summary:

- Monday-Friday (1:00pm – 5:00pm):
 - Flakes and/or bloodworms for all (**see feeding calendar for food types used during the recent past**).

Feeding Steps:

1. Feed flakes and/or bloodworms for all in BRF 221 and 223 as appropriate (**see feeding calendar for food types used during the recent past**).
 - a. Feed each **adult male** *Gambusia* flakes only (tiny pile of flakes at the end of the thin end of the blue straw per each male), no bloodworms.
 - b. Feed **adult female** *Gambusia* flakes and/or bloodworms as appropriate (**see feeding calendar for food types used during the recent past**).
 - Feed each **female** approximately twice the amount of flakes males are fed depending on their size.
 - c. Feed each NC Creek Chub **juvenile** flakes only (tiny pile of flakes at the end of the thin end of the blue straw), no bloodworms.
 - d. Feed each NC Creek Chub **adult** flakes and/or bloodworms as appropriate (**see feeding calendar for food types used during the recent past**).
 - Feed a similar amount of food as is fed to *Gambusia* **females** depending on their size and eating habits (e.g. if a substantial amount of food is left uneaten each day, decrease the amount for subsequent feedings).
 - e. Record feeding on the calendar in the binder for each room.
2. Securely close the tan black-out curtain in BRF 221 to block light from the *Artemia* hatchery during the night.