

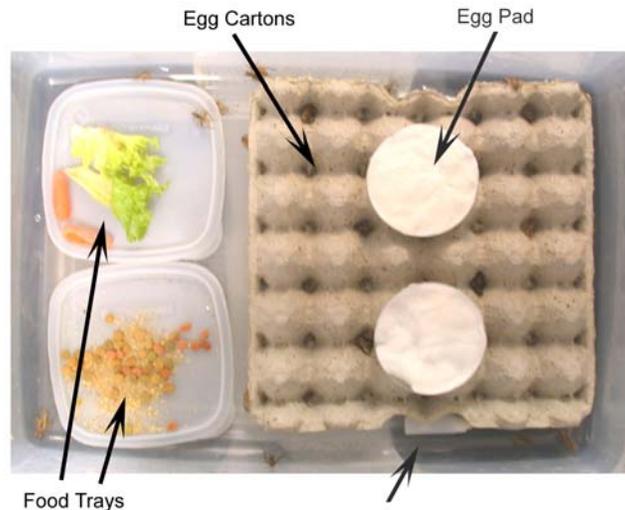
# ***Manual for Rearing Crickets, Flies and Mealworms***



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## 1.1. Culturing crickets

*Step 1:* Cricket tubs should be set up as shown in Figure 1. Every tub has two square egg cartons and two plastic food trays. Our food trays are lids of containers that we already had in the lab. One food tray holds cricket and cat food and the other, one piece of washed organic lettuce ( $\approx 3 \text{ inches}^2$ ) and two small organic carrots. Tubs with small juveniles should have some cricket food placed upon the ground under the egg carton. For adults and subadults, replace food trays at every feeding; for juveniles, replace food trays only as necessary.



**Figure 1: Layout for each cricket tub.**



**Figure 2: Water feeder**

All tubs have two water feeders (Figure 2) placed at the opposite end to the feeding trays (Figure 1). Water feeders should be checked at every feeding and replaced when they are less than half full of water. Water feeders are made by filling a small plastic vial with water and stopping the end with wet cotton wool. It is a good idea on quiet days to spend  $\frac{1}{2}$  an hour making up fresh water feeders. They can be stored in the fridge.

*Step 2:* Adult tubs have two egg pads which are positioned as shown in Figure 1. Adults have fully developed wings and juveniles do not. Egg pads are replaced three times per week and are set up by placing wet cotton into a large Petri-dish. Egg-pads should be damp, but should not have free sitting water. When the cotton wool is first placed in the Petri-dish, turn it upside down and squeeze out the excess water by squeezing the cotton wool between your fingers and the bottom of the Petri-dish. The cotton wool should also cover the entire inside of the Petri-dish. Each used pad should be removed from its Petri-dish and housed in a plastic container with holes (Figure 3) for hatching. Write the date on the plastic tub so that we can monitor hatching time.



**Figure 3: Housing of used egg-pads**

*Step 3:* Every weekday 1/5<sup>th</sup> of the cricket tubs should be replaced by a fresh one. This way, every tub is washed once every week (juvenile tubs are replaced only as necessary). To change the tubs, gently lift the egg cartons and hold in the dirty tub until the crickets have settled down. With the two tubs placed next to each other, lift the egg cartons and place them into the clean tub. Replace or swap over the food trays as necessary and gently tip the remaining crickets into the clean tub. Care should be



**Figure 4:** Tubs set up with binder clips to confirm that they have been cleaned for the week.

taken to not get dead crickets into the clean tub. Replace or swap over the water feeders and egg-pads as necessary. A binder clip should be attached to each changed tub so that you know which tubs have been changed and which still need to be done (Figure 4). At the end of the week you can remove all of the clips and start again in the proceeding week. Store tubs with the food trays facing outwards so that you can easily see whether the food is low etc. If tubs are unacceptably dirty, if they are

becoming damp inside, or if food is eaten too quickly, the cricket density in the tub may be too high. In such a case, divide the crickets into two tubs.

*Step 4:* Egg pads should be checked for hatchlings three times per week. Tape over the holes on containers housing the egg pads a couple of days before hatching is expected. Hatchlings should be transferred to a tub set up as described above (section 1.1), mixing offspring from several different egg pads to minimize inbreeding. After hatchling production has been minimal for at least three days, egg pads may be discarded, and the plastic containers cleaned. Any containers/egg pads that become infested with flies should be frozen for at least one day to kill the flies, then discarded/cleaned. Many of the smaller crickets will be used for food for the frogs, so it is important to maintain numbers such that there are enough adults and intermediate stages for the colony to be maintained.

To keep fruit flies under control, use fly traps made by combining a large pinch of yeast and a pinch of sugar with water in a small plastic cup. Cover the cup with plastic wrap and secure with a rubber band; poke a pencil-sized hole in the plastic wrap. Flies will be attracted to the yeast, get trapped in the cup, and eventually drown. (You can help ensure that the flies stay trapped by swirling the liquid to immerse them.) Rinse out weekly. Keep fly traps wherever flies are a problem. You can also use sticky fly traps where flies are a problem.

*Step 5:* Report the starting and finishing date of consumables on the appropriate form (see Section 1.7) and make sure that you wash up at the end of the day so that tubs and feeding trays are ready for the next day. It is important to monitor the use of consumables so that we can make sure that they are always available. Please notify the appropriate person when any consumable or supply is running low. Delays in consumable availability could cause serious problems. There is limited space for washing to dry, so remove all washed equipment at the start of each day and store it in

the correct place. Finally, always use the filtered water for the egg-pads, washing of the food and for the water feeders. When washing up the tubs and food trays wash in hot tap water and then rinse in the filtered water.

## 1.2. Culturing fruit flies



Figure 5: Example of fly jar

*Step 1:* Flies are kept in one quart wide-mouth glass jars. Jars need to be sterilized in the autoclave before use. Place the jars into the autoclave and ensure that they have aluminum foil over the mouth. Make sure that you've been trained to use the autoclave before beginning! Adjust the "Minutes Steam Sterilize" to 25 minutes and the "Minutes Steam Dry" to 7-10 minutes. If there is a problem, press the reset button and start again. When ready press the "Gravity: cycle select" button. Record your name and date on the autoclave log sheet. Everything is very hot, so wear autoclave gloves and take care not to touch the sides of the machine while loading and unloading the jars. The metal measuring cups and washed cotton should also be sterilized in the autoclave.

*Step 2:* Each jar contains a medium of dehydrated mashed potato, sugar (sugar:potato ratio 1 tablespoon:1 cup), water and yeast. First place  $\frac{2}{3}$  cup dehydrated mashed potato and 2 teaspoons sugar in the bottom of the jar and swirl to mix (Figure 5). The mixture should be about 1-inch deep. Then slowly pour in about  $\frac{1}{2}$  cup filtered water, allow the water to soak into the potato mix, and sprinkle a small pinch of yeast over the top of the mixture. Be careful not to add too much water, as it will result in runny medium that slides out of the jar when trying to remove the flies.

*Step 3:* Once the jars have the medium in them, transfer adults from at least two different jars (to minimize inbreeding) into each new jar. Each new jar should only have about 100 flies, so you may need to divide the flies from a jar into two or more new jars. After flies have been added, cover the top of the jar with a square of cotton fabric and seal with the ring part of the lid (Figure 6). Label each new jar with the date it was started and the strain of fly. Label old jars with "H" (for harvested), the date flies were most recently removed, and the strain of fly. Store jars in the appropriate place.



Figure 6: Lid of fly jar

New jars will start producing flies in 10 days to 2 weeks. Old jars can be rechecked for flies up to once per week. As with the crickets, flies will be needed for feeding the frogs, so it is important to maintain enough individuals from each generation to sustain numbers.

*Step 4:* Any jars in which flies have regained flight should be placed in the freezer for at least one day in order to kill all flies and prevent the flight back-mutation from invading the colony. Any jars that have medium that is too runny to use, or that are producing few enough flies that the jars could be better used to start new cultures can also be placed in the freezer for a day. Jars that are no longer producing flies can be cleaned without being placed in the freezer. Jars must be cleaned and autoclaved before using for new cultures. Cotton fabric squares should also be washed with hot water and then autoclaved and reused.

### **1.3. Culturing mealworms**

*Step 1:* Mealworm larvae are set up in medium-sized plastic containers. Fill each container to about one third with mealworm mixture (see below). Place four small organic carrots and a piece of egg-carton on top of the mixture. Pupae (Figure 7) and adult beetles should be collected every two days from the larval containers (be sure to check them under the egg carton) and set up in a new container (set up as described above for the larvae) to lay eggs. Place about 50 pupae/adults in each adult container. Once the adults die and the eggs hatch, continue the cycle by collecting newly emerged adults and placing them in a new adult container and so on. Remember to mix individuals from different containers to minimize inbreeding.



**Figure 7: Mealworm pupae**

*Step 2:* Mealworm feed consists of 1 bag of cornmeal, ½ cup of cricket food and 1 tablespoon of bone meal. Sift the individual ingredients before adding them to the container to remove large objects. Sifting can be done in a fume hood or outdoors (to avoid breathing the dust).

## 1.4. Appendix 1: Sources for supplies

Organism	Item	Procedure
<b>General use</b>	Latex gloves	<a href="http://www.mugeneralstores.com">http://www.mugeneralstores.com</a> or scientific supplier
	Paper towels	<a href="http://www.mugeneralstores.com">http://www.mugeneralstores.com</a> or scientific supplier
<b>Crickets</b>	Organic carrots & organic green leaf lettuce	local grocery
	Cotton roll	<a href="http://www.jorvet.com/pricelist.cgorder_form.php">http://www.jorvet.com/pricelist.cgorder_form.php</a> product number = J197; discount if order 90 rolls
	Cat food	local grocery or Wal-Mart
	Cricket food	<a href="http://www.flukerfarms.com">http://www.flukerfarms.com</a> ; high calcium cricket food, product 71106
	Egg cartons	<a href="http://eggcarton.com/">http://eggcarton.com/</a> ; product #ETP-30
	Petri-dishes	scientific supplier
	Crickets	<a href="http://www.reptilefood.com">http://www.reptilefood.com</a> ; ¾ inch crickets are subadults appropriate for starting or supplementing laboratory colony
	Large plastic tubs	Wal Mart
	Small plastic tubs	Wal Mart
	Solo 16oz deli cups (with holes)	The Bean Farm, 425-861-7964 or <a href="http://www.beanfarm.com/">http://www.beanfarm.com/</a>
<b>Flies</b>	Sugar	local grocery
	Yeast	local grocery. (not rapid-rise or bread machine yeast)
	Plain dehydrated mashed potato	local grocery.
	Cotton fabric	Wal-Mart – thin, white and 100% cotton (e.g., muslin)
	Aluminum foil	local grocery.
	Jars (quart-sized, wide mouth, with ring)	WalMart (in season)
<b>Mealworms</b>	Bone meal	local feed store (Bourne Feed – 573-474-4113; 4011 I-70 Dr. SE)
	Corn meal	local grocery.
	Cricket food	<a href="http://www.flukerfarms.com">http://www.flukerfarms.com</a> ; high calcium cricket food; product 71106
	Small plastic tubs	Wal Mart

### 1.5. Appendix 2: Cricket consumables check list

Cotton Wool		Lettuce		Carrots		Cat food		Cricket food		Egg Cartons	
Start	Finish	Start	Finish	Start	Finish	Start	Finish	Start	Finish	Start	Finish

### 1.6. Appendix 3: Mealworm consumables check list

Bone meal		Carrots		Corn meal		Cricket food	
Start	Finish	Start	Finish	Start	Finish	Start	Finish

### 1.7. Appendix 4: Fly consumables check list

Mashed potatoes		Yeast		Sugar		Cotton fabric		Aluminum foil	
Start	Finish	Start	Finish	Start	Finish	Start	Finish	Start	Finish