Fall 2016 Consumption X Parasitism X Temperature Experiment Protocol:

* Set up 3 dishes of eggs from the lab colony every 2-3 days with regular diet
  + Put one dish at each temperature--20C, 25C, 30C
  + Every new set of eggs is an “egg block”--give a sequential letter, and record the date that the eggs were set up in the data sheet--blocks are across all temperatures!
  + Stop this after ~ 2 weeks
* Check each dish for hatched individuals daily, separate individuals using a paintbrush into communal dishes by hatching date-- record hatching date and egg block
* Time points for weighing are **9 AM** and **3 PM**--be sure to do checks for new 3rds early enough that you’re weighing and parasitizing the caterpillars at 9am
* **BEFORE 9AM:** Check each dish (at each temperature) daily, replacing dried diet as needed. Also check each dish (at each temperature) daily for newly molted 3rd instars--this is when they enter the experiment
* Divide caterpillars at 3rd instar into either control (unparasitized) or treatment (parasitized) groups for each temperature
  + Check the counts sheet to see which treatments need filling
  + Try to have an equal number from each block go into the parasitized and unparasitized groups
* **Setting up the parasitized treatment:** Caterpillars that are destined for the parasitized treatment group will need to be parasitized **before\*** being weighed. (If necessary split up the jobs so that one person is checking/feeding, one person is parasitizing, one person is weighing, etc). Give the caterpillar a unique ID. Put the new 3rd on the parasitizing card, and either gently tape the head down, or carefully hold it it down. Stick the card and caterpillar into the wasp chamber, and try to attract a female to the caterpillar. Do your best to only have **ONE OVIPOSITION** per caterpillar. You can stick both hands into the wasp cage in order to brush off extra females. Once oviposition is complete, remove card and caterpillar (make sure all wasps are off the card and your hands!). Record the time, date, and number of ovipositions.
  + After oviposition, take caterpillars back to the lab room for weighing--do this in shifts if you have a bunch to oviposit
  + Give the caterpillar an individual small petri dish--label it with the caterpillar’s ID, temperature, treatment, block, and date of 3rd instar (record all this in the data sheet too!)
  + Weigh the caterpillar, and record the mass in the mass.T0 column. Record the time of weighing in the time.T0 column.
  + Cut the caterpillar a 6 hour chunk of food (check diet block chart!), weigh the chunk, and record the weight in the diet.cat.in.T0 column. Put the chunk in the petri dish with the caterpillar.
  + Cut a chunk of diet for a control (similar in size to the blocks the caterpillars are eating), weigh it, and record the mass in the diet.cont.in.T0 column. **REMEMBER:** You need a control block for EACH temperature for EACH time point--be very clear in your labelling, as we will have different caterpillars in lots of different time points! Be sure to label the date and the time point on the control diet dish, and try to put everything that’s at the same time point in the same tub
  + Set up in a bin with other caterpillars from that temperature that you set up that day (control and para). Label the bin with the next time point, weighing time, and date.
* **Setting up the control treatment:** It’s basically the same as the parasitized treatment, just don’t parasitize the caterpillars! Follow the steps below:
  + Give the caterpillar an individual small petri dish--label it with the caterpillar’s ID, temperature, treatment, block, and date of 3rd instar (record all this in the data sheet too!)
  + Weigh the caterpillar, and record the mass in the mass.T0 column. Record the time of weighing in the time.T0 column.
  + Cut the caterpillar a 6 hour chunk of food (check diet block chart!), weigh the chunk, and record the weight in the diet.cat.in.T0 column. Put the chunk in the petri dish with the caterpillar.
  + Cut a chunk of diet for a control (similar in size to the blocks the caterpillars are eating), weigh it, and record the mass in the diet.cont.in.T0 column. **REMEMBER:** You need a control block for EACH temperature for EACH time point--be very clear in your labelling, as we will have different caterpillars in lots of different time points! Be sure to label the date and the time point on the control diet dish, and try to put everything that’s at the same time point in the same tub
  + Set up in a bin with other caterpillars from that temperature that you set up that day (control and para). Label the bin with the next time point, weighing time, and date.
* **9 AM TIME POINTS:** All caterpillars will be weighed at the 9 am time point EVERY DAY that they’re in the experiment.
  + Weigh the caterpillar and record the mass in the mass.Tx column (for whatever time point you’re on). Record the time of weighing in the time.Tx column. Record the date in the date.Tx column. Record whether or not the caterpillar ate in the fed.Tx column (1 if it ate, 0 if it did not eat). If it’s hard to tell, a good indicator of feeding is frass production. So if there is no frass, the caterpillar probably didn’t eat.
  + Collect all the frass in the petri dish. Weigh the frass and record the mass in the frass.Tx column. Be sure to carefully check the petri dish for ALL frass. It can be stuck to the caterpillar or diet. Also be careful to not confuse crumbled diet with frass.
  + Weigh the old block of diet that the caterpillar was eating and record the mass in the diet.cat.out.Tx column (the # will be the previous time point). Throw away the old diet block
  + Weigh the old control diet and record the mass in the diet.cont.out.Tx column. (the # will be the previous time point). Check the Control Diet count--we need 10 blocks from each time period (6, 18 and 24 hours), for each treatment. If that time period/temperature needs more control diet blocks, put the block in a plastic bag, label everything that was on the petri dish, and put it in the freezer. Update the count sheet.
  + Cut a new block of diet for either 6 hours or 24 hours, depending on the time point (check the diet block chart for size!). Weigh the new diet block and record the mass in the diet.cat.in.Tx column (the # will be the current time point)
  + Cut a new control block of diet for that time point/temperature that is a similar size and dimension to the blocks the caterpillars are eating. Weigh the block and record the mass in the diet.cont.in.Tx (the # will be the current time point)
  + Check the sacrifice count--if that time point/temperature is empty, remove 1-2 PARASITIZED caterpillars and freeze them. Record a 1 in the sacrifice.Tx column (everyone else will have 0’s). Do this AFTER you weigh the caterpillar and their old food, but before you give them new food. Record the data for that caterpillar in the sacrifices data sheet.
  + If any of the caterpillars have molted, record the date of the new instar, and the TIME POINT when you found the newly molted caterpillar
  + Update the label on the bin with the next time point, time to be weighed, and date of the next time point (EX: T3, 3pm, 11/4)
* **3PM TIME POINTS:** Caterpillars will be weighed at 3pm for the first ~7 days after they molt into the 3rd instar.
  + Weigh the caterpillar and record the mass in the mass.Tx column (for whatever time point you’re on). Record the time of weighing in the time.Tx column. Record the date in the date.Tx column. Record whether or not the caterpillar ate in the fed.Tx column (1 if it ate, 0 if it did not eat). If it’s hard to tell, a good indicator of feeding is frass production. So if there is no frass, the caterpillar probably didn’t eat.
  + Collect all the frass in the petri dish. Weigh the frass and record the mass in the frass.Tx column. Be sure to carefully check the petri dish for ALL frass. It can be stuck to the caterpillar or diet. Also be careful to not confuse crumbled diet with frass.
  + Weigh the old block of diet that the caterpillar was eating and record the mass in the diet.cat.out.Tx column (the # will be the previous time point). Throw away the old diet block
  + Weigh the old control diet and record the mass in the diet.cont.out.Tx column. (the # will be the previous time point). Check the Control Diet count--we need 10 blocks from each time period (6, 18 and 24 hours), for each treatment. If that time period/temperature needs more control diet blocks, put the block in a plastic bag, label everything that was on the petri dish, and put it in the freezer. Update the count sheet.
  + Cut a new block of diet for 18 hours (check the diet block chart for size!). Weigh the new diet block and record the mass in the diet.cat.in.Tx column (the # will be the current time point)
  + Cut a new control block of diet for that time point/temperature that is a similar size and dimension to the blocks the caterpillars are eating. Weigh the block and record the mass in the diet.cont.in.Tx (the # will be the current time point)
  + Check the sacrifice count--if that time point/temperature is empty, remove 1-2 PARASITIZED caterpillars and freeze them. Record a 1 in the sacrifice.Tx column (everyone else will have 0’s). Do this AFTER you weigh the caterpillar and their old food, but before you give them new food. Record the data for that caterpillar in the sacrifices data sheet.
  + If any of the caterpillars have molted, record the date of the new instar, and the TIME POINT when you found the newly molted caterpillar
  + Update the label on the bin with the next time point, time to be weighed, and date of the next time point (EX: T3, 3pm, 11/4)
* Continue measuring consumption until wasp emergence.
  + Once wasps begin emerging, place parasitized caterpillar into a new, clean petri dish (copy all info!). Be sure to transfer all wasps that may have fallen off the cuticle of the caterpillar. Use soft, flat forceps and be gentle! We don’t want to injure the wasp larvae and therefore skew our metric of wasp performance (# that spin cocoons). Record the date, time point and instar of emergence in the appropriate column of the spread sheet (near the beginning). Write “Count Cocoons \_\_\_\_\_” on the lid, with the date 48 hours after emergence. (Ex: if the wasps emerge on 11/16, you’ll count the cocoons on 11/18).
  + Don’t weigh the caterpillar once the wasps start emerging, to avoid damaging the wasps. You also don’t need to weigh the food or any frass at the emergence time point. Do record the old control diet weight (diet.cont.out.Tx) for that time point, but not the new control diet (diet.cont.in.Tx)
  + 48 hours after wasp emergence, count the number of cocoons in the petri dish (use a counter). Be sure to check carefully that you counted all of them--sometimes they’re hidden under the caterpillar. Record the number of cocoons in the num.coc column.
  + Count the number of wasp larvae that did not spin cocoons (use a counter). These are easily distinguishable from the cocoons. The cocoons are white, spherical, and sometimes fuzzy (they’re made of silk). The wasps that failed to spin will look like little yellowish grubs. Be sure to count accurately! Record this number in the num.fail.spin column.
  + Once both the number of cocoons and the number of larvae that failed to spin are recorded in the data sheet, the column num.em should automatically fill out with the sum of the previous values. Double check to make sure this number is correct.
  + asdf