Preparing Petri Dishes:

Control Group:

- 1. Put spring water to the 10 mL line of the graduated cylinder
- 2. Using the pipette, add at least 10 Daphnia to the cylinder
- 3. Gently pour contents into petri dish
- 4. Seal petri dish with two layers of tape
- 5. Write on the tape, labeling that it has 0 mL of caffeine

2 mL of Caffeine:

- 1. Put spring water to the 8 mL line of the graduated cylinder
- 2. Using pipette, add 2 mL of pure caffeine to the graduated cylinder
- 3. Using pipette, add at least 10 Daphnia to the cylinder
- 4. pour contents into petri dish
- 5. Seal petri dish with two layers of tape
- 6. Write on the tape, labeling that it has 2 mL of caffeine

1 ½ mL of Caffeine:

1. Put spring water to the 8 ½ mL line of the graduated cylinder

- 2. Using pipette, add 1 ½ mL of pure caffeine to the graduated cylinder
- 3. Using pipette, ad at least 10 Daphnia to the cylinder
- 4. Gently pour contents into petri dish
- 5. Seal petri dish with two layers of tape
- 6. Write on the tape, labeling that it has 1 ½ mL of caffeine

1 mL of Caffeine:

- 1. Put spring water to the 9mL line of the graduated cylinder
- 2. pipette, add 1 mL of pure caffeine to the graduated cylinder
- 3. Using pipette, add at least 10 Daphnia to the cylinder
- 4. Gently pour contents into petri dish
- 5. Seal petri dish with two layers of tape
- 6. Write on the tape, labeling that it has 1 mL of caffeine

Preparing to Record Data:

- 1. Take compound microscope and turn it on
- 2. Set up tripod and camera so that capturing heart of magnified Daphnia is possible

Recording Data:

- 1. Take petri dish marked without any caffeine and place under microscope
- 2. Never turn on light because temperature has an effect on heart rates of Daphnia
- 3. Make sure the camera can record all of the Daphnia
- 4. Start recording + stop after one minute
- 5. Remove petri dish and dispose of the solution with Daphnia
- 6. Repeat steps 1-5, changing the petri dish to the one marked with 1 mL of caffeine
- 7. Repeat steps 1-5, changing the petri dish to the one marked with 1 ½ mL of caffeine
- 8. Repeat steps 1-5, changing the petri dish to the one marked with 2 mL of caffeine
- 9. Take videos from camera and slow them down
- 10. Record the heart rate of each Daphnia
- 11. Only keep 10 of the results for each petri dish