Phase I: Characterization: Triglycerides and Storage proteins.

* This phase of experimentation will be used as a reference for further experimentation. I will be probing the lipid and protein content of wandering larvae. My question is simple, under optimal conditions that either induce continuous development or programmed diapause, how does this colony of insects perform? To do this I will sample late 5th instar larvae that have begun the wandering stage of their life history, and test for triglyceride content (type and quantity) and storage protein content (type and quantity). The following tables and graphs illustrate my intended treatments and experimental expectations:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| UZ 12:12 days | Larva | Larva Spiked | Blank | Blank Spiked |
| UZ 16:8 days | Larva | Larva Spiked | Blank | Blank Spiked |
| BE 12:12 days | Larva | Larva Spiked | Blank | Blank Spiked |
| BE 16:8 days | Larva | Larva Spiked | Blank | Blank Spiked |

|  |  |  |
| --- | --- | --- |
| UZ 12:12 days | Standard Curve | Larva Lymph |
| UZ 16:8 days | Standard Curve | Larva Lymph |
| BE 12:12 days | Standard Curve | Larva Lymph |
| BE 16:8 days | Standard Curve | Larva Lymph |

* Data collected for each sample: To analyze the lipid and protein content the following data points will be collected and used to compare the difference between each of the associated phenotypes and used to make assumptions about the metabolic requirements and activities of each of these types.

**Lipids**

Dry larva weight

Total lipid weight/ “Clean” lipid weight

GC FID (lipid retention time and abundance)

**Proteins**

Bradford Assay (concentration)

SDS Page Gel (protein kDa)

ICBR (protein sequence and abundance)

Phase II: Lipid Depletion Assays

* Using lipid depleting compounds I would like to take the information from Phase I and determine if it is possible to merge phenotypes. Specifically, I want to deplete the lipid content in one phenotype to determine if it is possible to force the specimen to present the phenotype of a larvae that it has not been primed to become. My question is, does depleting the resources of a diapause primed larvae lead an altered phenotype that resembles a nondiapause larvae? Again using a similar treatment scheme as the characterization experiments I will sample late 5th instar larvae that have begun the wandering phase of their life history, and test for triglyceride content (type and quantity) and storage protein content (type and quantity). Additionally I will include a cohort of individuals that have been exposed to a lipid depletion compound. Using the table and graphs below I will illustrate;

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| UZ 12:12 days | Larva | Larva Spiked | Blank | Blank Spiked |
| UZ 12:12 days | Larva w/ lipase | Larva w/ lipase Spiked | Blank w/ lipase | Blank w/ lipase Spiked |
| UZ 16:8 days | Larva | Larva Spiked | Blank | Blank Spiked |
| UZ 16:8 days | Larva w/ lipase | Larva w/ lipase Spiked | Blank w/ lipase | Blank w/ lipase Spiked |
| BE 12:12 days | Larva | Larva Spiked | Blank | Blank Spiked |
| BE 12:12 days | Larva w/ lipase | Larva w/ lipase Spiked | Blank w/ lipase | Blank w/ lipase Spiked |
| BE 16:8 days | Larva | Larva Spiked | Blank | Blank Spiked |
| BE 16:8 days | Larva w/ lipase | Larva w/ lipase Spiked | Blank w/ lipase | Blank w/ lipase Spiked |