# Diet and Metabolism in Bean Beetles

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## Abstract

Although bean beetle adults (*Collosobruchus maculatus*) do not require additional nutritional intake following pupation, they are capable of ingestion. Here we describe a lab exercise where students conducted an experiment to determine which (if any) potentially nutritive molecules influence adult bean beetle metabolism. Metabolic activity was measured indirectly via $CO_2$ output and $O_2$ consumption. Following the completion of the experiment, data from all groups were analyzed to produce class conclusions that served as background for additional experimentation. The project as described requires approximately two hours for setup and one, two-three hour lab session for introduction, experimentation, discussion, and analysis. Primary objectives of the project were to have students test hypotheses related to the influence of different nutritive molecules on $CO_2$ production in bean beetles and use the resulting data to generate a novel follow-up experiment(s). In *BIOL 101: Principles and Methods* (a majors/non-majors course), this activity precedes the development of group projects designed by the students themselves. Therefore we use this activity in part to help students develop familiarity with the bean beetle model, experimental design and the scientific method in general. However, the activity could easily be modified for other situations or used as a stand-alone exercise. Ideally, this lab would follow lectures in which students had learned about different macromolecules, their constituents, and the primary metabolic functions of each. Additionally, students should have a working knowledge of the scientific method and a general understanding of the bean beetle life cycle prior to this lab.

## Background

In 2000 we designed and implemented a studio-format course to introduce majors and nonmajors to biological concepts. We abandoned the standard lecture-laboratory format and replaced our introductory biology course (BIOL 101) with a studio-format course in which material is presented by an instructor then immediately followed by an experiment, observation of a microscope slide, or participation in some other experiential activity by students.

## Format of Course

- Teach biology as a process
- Use hypothesis-driven, guided-inquiry laboratory experiments
- Two 165 minutes session per week
- Maximum of 24 students/classroom; room designed for lecture, discussion and group laboratory work (Figures 1 and 2)

Although the lab described here was designed to fit within this structure, it could easily be modified to fit within a standard 2-3 hour lab, or expanded to a multi-week project. The latter would allow for additional rounds of student-generated hypotheses, or follow-up experiments.

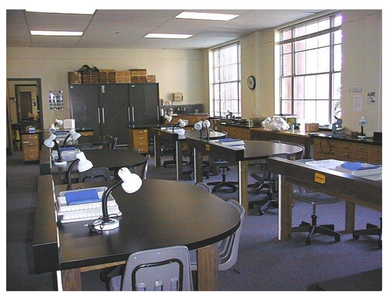

Figure 1. Room layout for studio format courses. Lab tables face front of the room but are designed for cooperative laboratory work. Each of six tables seats four students and are typically assigned to a single group for the duration of a semester.

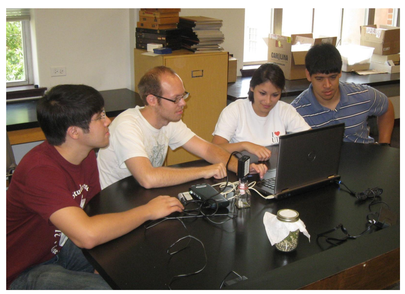

Figure 2. Students observing data collection for the bean beetle lab. As described above, the classroom was redesigned such that all lab work is performed in groups of four students. In this case, the table is equipped with a computer running LoggerPro, adaptor hardware, $CO_2$ probe and container with beetles.

## Experimental Design

General information related to housing and culturing beetles may be found in *A Handbook on Bean Beetles* (Beck and Blumer, 2011) and is not described here. You will want to allow plenty of time (likely several months from the time a colony is established) to culture an adequate number of beetles for your class. As described, this lab requires 40-50 beetles per group.

In our course, this lab follows lectures on both cellular respiration and an introduction to bean beetles. The former introduces students to the LoggerPro hardware/software and gas analysis. In the latter, students discuss the life cycle of bean beetles and are assigned portions of *A Handbook on Bean Beetles* as background reading. As most students have never worked with an insect model, it is important to spend adequate time introducing the bean beetles. Following this introduction, students were presented with the following framing questions:

- Will adult bean beetles consume liquid nutritive solutions?
- If so, how could you determine (directly and indirectly) the effect on metabolic activity?
- Which solution will have the greatest effect on $CO_2$ production and/or $O_2$ consumption?

The class broke into small groups to discuss which nutritive molecules are likely relevant given the bean beetles' life cycle. We then asked the groups to share their comments and talk about considerations in experimental design (duration of data collection, replication, number of specimens per container, and appropriate control group(s)). Once the class decided on the specific nutritive solutions to be tested, each group generated hypotheses that were tested in the subsequent lab. Following this session, feeding containers (Figure 3) were prepared as described below.


Figure 3. Representative equipment. A) Feeding chamber consisting of a small canning jar (pint) and Kimwipe lid. Cotton pad was saturated with nutrient solution and placed in jar prior to introduction of 25 adult beetles. B and C) Two different styles of Vernier Carbon Dioxide probes used for metabolic assessment. Gas ports (indicated by red arrow in C) were covered with cheesecloth (shown in B) prior to insertion in collection chamber. We used 50ml Falcon tubes and 125ml Nalgene bottles (shown in C). The set up for oxygen detection was similar.

## Equipment and Supplies

Feeding setup was assembled prior to the student lab. One feeding pad and culture container were used for each solution to be tested. We used small canning jars capped with dry baby wipes or Kimwipes (Figure 3A). Regardless of the container, you will want to provide adequate dry space for the beetles or they will likely drown in the nutrient solutions.

Nutrient solutions (protein, starch, amino acids, sugars, lipids, etc.) and concentrations can vary depending on the specific research questions raised by the students. We used 5% (w/v) solutions in distilled water in the example data (Figure 4 and 5).

Bean beetle characteristics (sex, age, mated or virgin) are all dependent on the specific hypotheses generated by the students. For a 50ml Falcon tube or 125ml Nalgene bottle (Fig 3C), we obtained consistent data with 20-25 beetles per tube. As mentioned above, plan ahead to have sufficient specimens. For a class of 24-30 students, you may easily need several hundred beetles if testing $CO_2$ and $O_2$ separately with multiple solutions.

## Equipment and Supplies cont.

Each group of students will need:

- Several pairs of forceps, access to a freezer to anesthetize the beetles, two containers (one for each sensor; Falcon tubes or Nalgene bottles), a test-tube rack to hold the assembled probes and containers.
- A computer with Logger Pro software, Vernier computer interface, and $CO_2$ / $O_2$ sensors,
- Bean beetles, approximately 20-25 beetles per container

## Data Collection

In our course, each group of students (six groups per class section) was given a single sample to test. Therefore, within a single section, each sample was only tested once (Figure 4). However, as we have six sections of the class running in parallel, it was possible to duplicate tests across sections, compile the course data, and analyze various hypotheses using the course data set. For example, as each section met at slightly different time points, the effect of this timing was assessed (Figure 5). Alternatively, as data collection required approximately 70 minutes (including setup time), it would be possible to run multiple trials within a single three hour lab. This would allow for collection of replicate data within a single class section.

We collected data every three minutes for one hour. Following data collection, we asked students to create a graph of their data using Excel and then calculate the mean and rate of change for each gas. This allowed us to discuss various ways to analyze the data. If the class has experience with more sophisticated statistical tests, the data could easily be analyzed more thoroughly using ANOVAs.

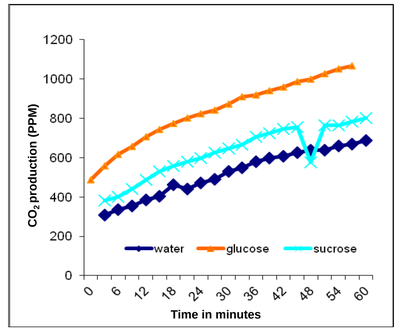


**Figure 4: Representative metabolic data collected from three student groups each testing a different nutritional supplement.** $CO_2$ concentration was assessed at three minute intervals for 60 minutes. The initial time point (time=0) served as a probe calibration point.

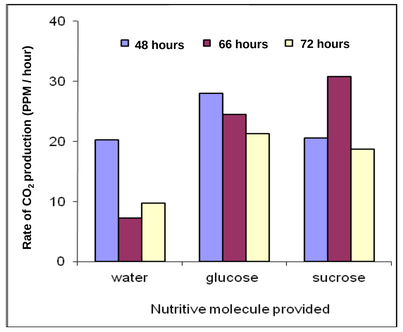


**Figure 5: Composite metabolic rate data as a function of nutritional supplements** Metabolic response to three nutritive supplements (water, glucose, and sucrose) were tested at these times: immediately following a 48 hour feeding window (blue bars, 48 hours), 18 (red, 66 hours), or 24 hours after feeding (yellow, 72 hours).

## Conclusions

- Students gain a better understanding of the scientific method in general as well as this model organism in specific through multiple in-class experiences
- Students were more engaged with the material because of their interest in model organism
- The project allowed students to become more confident with experimental design as reflected in the quality of the end of semester group projects
- The project provides instructors with the opportunity to discuss different approaches to statistical analyses

## References

Beck CW and Blumer LS 2011. *A handbook on bean beetles, Callosobruchus maculatus.* http://www.beanbeetles.org/handbook/

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