Laboratory Exercise in Behavioral Genetics Using Team-based Learning Strategies

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Abstract: In this paper, we describe a two-week learning module where students tested the role of the *fruitless* gene on aggression and courtship in *Drosophila melanogaster* via team-based learning (TBL) strategies. The purpose of this module was to determine if TBL could be used in the future as a platform to implement the course goals and teach scientific skills in two sections of a junior/senior-level college Behavioral Genetics laboratory. We utilized the TBL format: pre-class preparation, readiness assurance, and concept application. The first week students learned the concepts necessary to understand the role of the *fruitless* gene on behavior and were tested as individuals and teams during the Readiness Assurance Test (RAT). They practiced working with the organisms and observing their behavior before developing novel research experiments and engaging in an extensive peer review process of their experimental designs. The following week, each group re-designed and implemented their experiments. Student performance improved during the team RAT, they preferred TBL, and were more prepared for their final research projects. Therefore, we found that incorporating TBL in this laboratory module was a successful tool toward encouraging the development of scientific skills in this laboratory.

Key words: Team-based learning, student-centered learning, teacher-centered classroom

INTRODUCTION

Pedagogical research has shown that team-based learning (TBL) is an effective student-centered learning strategy for teaching in undergraduate science lectures (Metoyer et al., 2014; Nieder et al., 2005). TBL transforms teacher-centered classrooms with pre-class preparation, group work, and peer teaching to strengthen knowledge acquisition and application (Michaelsen & Sweet, 2008). This provides a learning environment that allows instructors to focus on the core competencies and disciplinary practices, as outlined by the American Association for the Advancement of Science Vision and Change Report (see Figure 1; Bauerle et al., 2009). McInerney & Fink (2003) found that the combination of challenging student projects and TBL improved student recall, as exemplified by increased final exam scores. Therefore, TBL may provide the opportunity for students to practice science authentically in laboratory settings, in the way that scientists implement research.

In this paper, we describe our implementation of TBL in one learning module over a two-week period in two sections of a majors only, junior/senior-level college Behavioral Genetics laboratory. Given that animal behavior requires a deep understanding of several disciplines of biology and that phenotypes can be widely variable, animal behavior can be difficult for students to grasp and test in laboratory settings. Therefore, we aimed to determine if TBL was suitable format to teach undergraduate students behavioral genetics. TBL was employed in an effort to reach our learning objectives for the course (see Table 1) in a learning module looking at the role of the *fruitless* gene on courtship and aggression in *Drosophila melanogaster*.

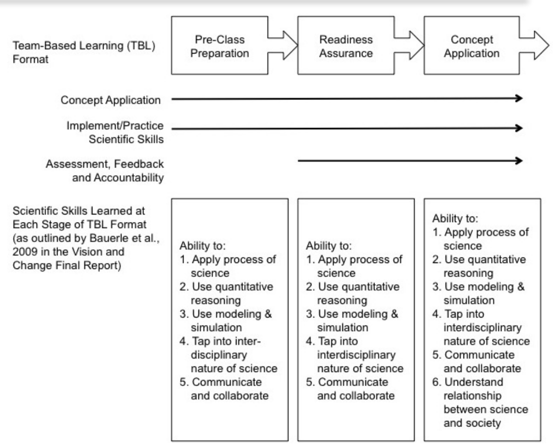

Fig. 1. Diagrammed representation of the potential for the team-based learning format to incorporate the core competencies and disciplinary practices (i.e. scientific skills) as outlined by the National Science Foundation Vision and Change Report (Bauerle et al., 2009).

General Theoretical Background

Drosophila melanogaster are an ideal model system to study intersexual and intrasexual selection in the classroom or laboratory. Many studies have found a range of aggressive behaviors in males, such as lunging and boxing (Yurkovic et al., 2006; Baier et al., 2002), and that males will establish hierarchal relationships and territories to attract females (Yurkovic et al., 2006; Hoffmann, 1987; Dow & von

Table 1. Learning objectives of the module and our application through the TBL format.

Learning Objectives	TBL Process Applicable
1. Understand the role of genetics on complex behavioral phenotypes in *D. melanogaster*.	Pre-class Preparation Readiness Assurance Concept Application
2. Identify, measure, and quantify specific behavioral phenotypes in *D. melanogaster*.	Pre-class Preparation Readiness Assurance Concept Application
3. Formulate hypotheses, design, and perform experiments following the scientific process.	Pre-class Preparation Concept Application
4. Detect and resolve procedural problems.	Readiness Assurance Concept Application
5. Develop and implement independent novel research.	Concept Application

Schilcher, 1975). Females, on the other hand, exhibit less aggressive behaviors and share resources, rather than commandeering them like males (Vrontou et al., 2006).

In an effort to mate with the female, *D. melanogaster* males perform an elaborate courtship dance for females (see Yamamoto and Koganezawa [2013] for a visual representation of the display). The courtship dance is a succession of genetically predetermined behaviors that generally follow the same order: 1) orientation: the male quickly "orients" himself in front of the female, 2) tapping: the male taps the female on her abdomen, 3) song: the male performs a courtship song by extending and vibrating his wing, 4) licking: the male licks the female's genitals, 5) mounting: the male attempts to mount the female to copulate (also called attempted copulation), and 6) successful copulation: the male successfully mounts the female (Yamamoto and Koganezawa, 2013). During courtship, both males and females emit pheromones to detect suitable and viable females (Dickson, 2008; Everaerts et al., 2010). Several genes, including the *fruitless* (*fru*) gene, regulate the neural loci influencing the individual stages of the courtship dance and aggression (Lee & Hall, 2000). The *fru* gene, which encompasses approximately 130 kb, encodes for 18 variable isoforms all belonging to the family of BTB-ZnF (Broad-complex tramtrack and bab zinc finger) transcriptional factors (Ito et al., 1996; Ryner et al., 1996). The different isoforms arise through initiation of transcription from one of four different promoters and the alternative splicing of the 5' and 3' ends (Heinrichs et al., 1998; Goodwin et al., 2000).

By modifying *fru*, male patterns of aggression can be feminized and female patterns made more masculine (Vrontou et al., 2006; Demir & Dickson, 2005). For example, males expressing the feminizing fru^F isoform will orient away from females, they will indiscriminately court males and females, or courtship can be completely blocked (Demir & Dickson, 2005). fru^F males left on food plates for several hours or days begin to form courtship chains in which each male courts the one ahead of him (Hall, 1978; Demir and Dickson, 2005). Females expressing the fru^M variant also exhibit significant changes in courtship behavior. fru^M females display male sexual instincts; they will court wild-type females, and when placed together on food plates, will also form courtship chains similar to the chains formed by fru^F males (Demir and Dickson, 2005).

PROCEDURE

This learning module was implemented in two sections over a two-week period. Each laboratory was 4 hours long; if needed, this laboratory could be condensed into one 4-hour long laboratory. Each section consisted of approximately 21-24 enrolled students. Because we utilized the team-based learning format, students worked both prior to lab as well as during lab, in large teams (7-8 students per group), small groups (2-3 students), and individually.

The laboratory exercise tested the role of the *fru* gene on aggression and courtship in *D. melanogaster*. A list of materials to implement this experiment can be found in the Lesson Plan, supplied in the Supplemental Materials. We used Canton-S wild type and *fruitless* mutants in this laboratory exercise. Canton-S is one wild type strain commonly used in *Drosophila* research laboratories. Wild type strains are ideal for both teaching and research purposes because they are genetically and phenotypically variable in their population, but Canton-S is not easily accessible for teaching. Another wild type strain, Oregon-R, is readily available via Carolina Biological (Item #172100). Alternative mutants, to address similar research questions for aggression and courtship, can be purchased through Carolina Biological (see Table 2).

Table 2. Additional strains readily available through Carolina Biological Supply Company to test similar behavioral genetics questions.

Strain	Supplier	Item No.	Behavioral Phenotype To Test
Ebony	Carolina Biological	172500	Disrupted circadian rhythm (Newby & Jackson, 1991) and courtship (Wang et al., 2008), and increased aggression (Jacobs, 1978)
Sepia	Carolina Biological	172575	Mating success (Stanić & Pavković-Lucic, 2005)
Wrinkled	Carolina Biological	172600	Mate choice and courtship song
Flightless	Carolina Biological	144455	Mate choice, courtship dance, and courtship song
Black	Carolina Biological	172330	Decreased aggression (Jacobs, 1978)

Prior to Experimentation

Each line was reared on standard fly medium and incubated at room temperature (~25º C) and a 12:12 light:dark photoperiod. Three to five days prior to experimentation, adults (Canton-S females and males, fru^C males and females, fru^F males, and fru^M females [identified using the serration on their wings]) were anesthetized using carbon dioxide, sexed by the presence of sex combs, and painted on their dorsal thorax with non-toxic acrylic paint to identify between sex and strains (see Figure 2). An alternative to painting is to rear newly-eclosed adults on medium dyed with food coloring; this will turn their abdomens the color of the food coloring (R. Yukilevich, personal communication, 19 September 2014). Each individual was housed separately in 23-mL plastic vials (capped with cotton balls) until testing. To optimize aggression, adults should be collected at eclosion and kept in isolation until testing following the methods of Vrontou et al. (2006).

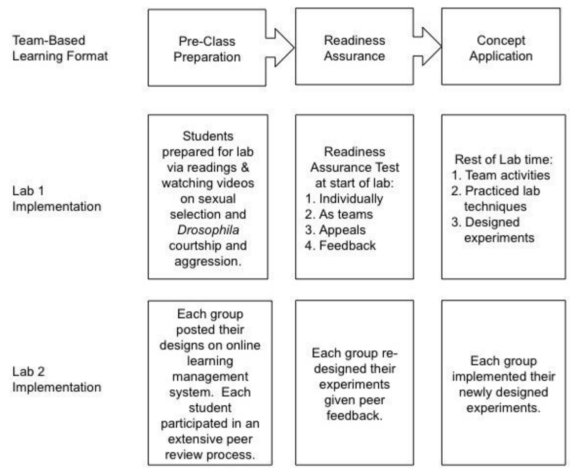

Fig. 3. A diagram representing our implementation of the team-based learning format during week one (Lab 1 Implementation) and week two (Lab 2 Implementation) of this laboratory module.

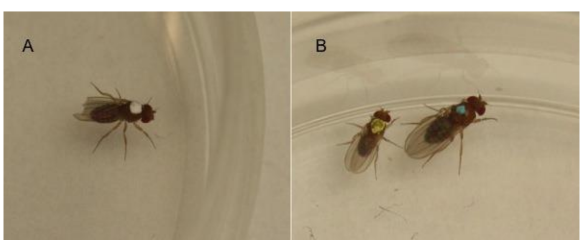

Fig. 2. A) A male painted with white non-toxic acrylic paint on its dorsal thorax. B) A male (left) painted yellow and a female (right) painted blue on their dorsal thorax with non-toxic acrylic paint.

Week 1: Learning to Work with Drosophila and Observe Behavior

To simulate TBL in the laboratory, we followed the format for team-based learning in the first laboratory session (see Figure 3). The TBL format includes opportunities for pre-class preparation, readiness assurance, and concept application in this sequence (Michaelsen & Sweet, 2008). For pre-class preparation, we posted the lab worksheet, three readings (previously published journal articles), and eight videos on *Drosophila* courtship and aggression on the online learning management system (see Lab Worksheet in Supplemental Materials). Students individually prepared for the first week's laboratory by watching the videos and reading the materials supplied online.

At the beginning of the laboratory, students were individually given a Readiness Assessment Test (RAT); this is a short, but challenging, multiple choice assessment designed to test students' comprehension of the readings and critical thinking abilities (two versions are supplied in Supplemental Materials). Students were divided into teams of seven to eight individuals prior to the laboratory by distributing member resources equally and avoiding previously formed coalitions (see Michaelsen & Sweet, 2008 for further information). Each team took the same RAT again in their teams using test cards, called the Immediate Feedback Assessment Technique or IF-AT. They were encouraged to discuss the possible answers at length and come to a consensus on an answer before proceeding. One student in the team scratched the card to reveal a "*" if the answer was correct. If they were incorrect, they were given the opportunity to change their answer for a diminishing score. At the end of the RAT, each team was allowed to write and submit an appeal if they felt that any of the questions were unfair, incorrect, or too ambiguous. Through discussion of the questions immediately following the RAT, we were able to identify and resolve students' misconceptions and answer any remaining questions.

To apply concept application, as well as practice laboratory skills and behavioral observations, students engaged in a series of activities as teams with the instructors (see Lesson Presentation in Supplemental Materials). These served to further engage students in understanding the role of the *fru* gene on behavioral phenotypes in *Drosophila*. These activities incorporated the 4S's: the students were asked a series of multiple-choice, specific questions addressing a significant problem (Parmelee and Michaelsen, 2010). The students were required to come to a team consensus on the answer in the allotted time and simultaneously report their answers. At this point, the instructors were able to address any additional misconceptions or gaps in understanding of the material.

The activities were followed by a short lecture and demonstrations (see Lesson Presentation in Supplemental Materials) to teach students how to handle, sex, and observe *Drosophila* behaviors. Teams were divided into smaller groups of two to three students to practice scientific skills and run experiments. Dividing the teams provided students with the opportunity for greater participation in application of the concepts and scientific skills, which would not be feasible with large teams in a laboratory setting.

In their smaller groups, students practiced aspirating, anesthetizing, and identifying phenotypes and sexes. Once students felt comfortable and were successfully performing the lab techniques and working with flies, they placed individual adults together in petri dishes to practice observing male courtship and female and male aggressive behaviors. We focused on male courtship behaviors because they are easier for students to observe and quantify without the use of expensive laboratory equipment.

While observing these behaviors, each group applied their novel observations and knowledge gained in the readings to formulate a unique research question, hypothesis and prediction. For the remainder of the lab period, each group developed hypotheses-driven experimental designs to independently test a novel research question, which uploaded onto the online learning management system's discussion board before leaving.

Week 2: Independent Experimentation

To employ the TBL format in the second laboratory (see Figure 3), students prepared for the laboratory by participating in an extensive peer review process of their peer's experimental designs. Each student provided feedback to at least one group using the online learning management system discussion board. In addition, the instructor provided additional feedback as support, where necessary. Prior to the laboratory, each group was responsible for reviewing their peers' and instructor's feedback.

At the beginning of the laboratory, each group re-designed their experiments given the provided feedback and independently carried out their experiments. Most experiments required trouble-shooting and each group was provided ample time and supplies to do so. If needed, students were allowed the entire lab period to fix problems with the experiment and obtain enough data to make logical, evidence-driven conclusions concerning their hypotheses. When the experiments were completed, each group analyzed and recorded their results in their lab notebooks, which were handed in and graded at the end of the semester.

RESULTS AND DISCUSSION

Results from Student Experiments

During Week 2, each group of students proposed novel hypotheses and tested independent research projects of one aspect of the role of the *fru* gene on either aggression or courtship. Our objective was to prepare students to independently run their final research projects at the end of the semester. Because of this, each group of students pursued different projects depending upon their interest.

The peer review process was a strong component of the TBL approach in this learning module. The students provided insightful feedback and reasoning using their understanding of animal behavior, behavioral genetics, the scientific process, and their readings. The peer review process was an opportunity for reinforcement of conceptual learning and procedural knowledge learned during the first week of this laboratory module.

Educational Outcomes of the Learning Module

Given that animal behavior is an interdisciplinary science that can be challenging to teach and for students to fully understand, we used TBL with this module to determine if this teaching format would enhance student comprehension of both the concepts and the techniques necessary to test behavioral genetics questions using *D. melanogaster*. Students were formally assessed at the beginning of week 1 with the Readiness Assessment Test (RAT) and were polled at the end of the semester with a survey created by Mennenga (2010) to determine the effectiveness of the team-based learning method.

Results from the Readiness Assessment Test

The Readiness Assessment Test (RAT) was used to encourage student preparedness, reinforce the concepts learned in the readings, and provide an opportunity for discussion, argumentation and peer instruction for students to work through their misconceptions of the material. To test whether the RAT improved overall student performance, we compiled the scores for both sections and compared the averages of the individual RAT scores to the team RAT scores using a Paired-Samples T-Test (SPSS v. 22.0). We found that students performed significantly higher ($p < 0.0001$, see Figure 4A) on the team RAT, in comparison to the individual portion; students scored 24.9% higher on the team portion.

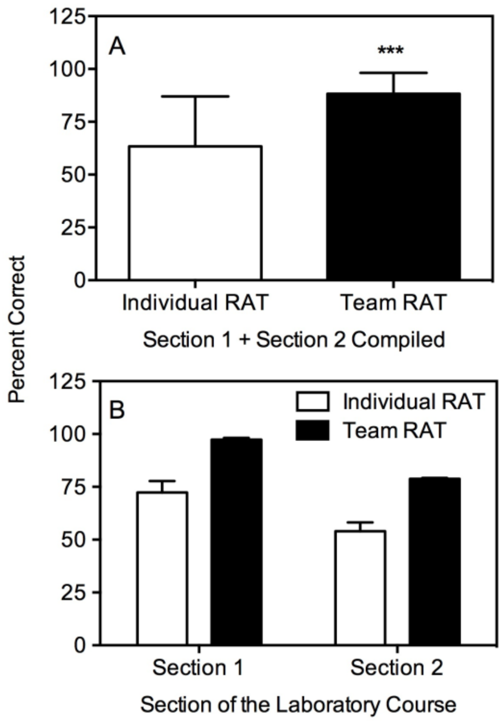

Fig. 4. Effect of the Readiness Assessment Test (RAT) on student performance. When scores were compiled for both sections of the course (A), the level of performance increased significantly (Paired-Samples T-Test, $p < 0.0001$) by 24.878%. Both sections showed considerable improvement in the team portion of the RAT. Means ± SEM in (A) and (B). *** = $p < 0.0001$.

Since students in the first section were provided the answers using the IF-AT cards and were allowed to take the RAT home, the second section of the course was given a different RAT that tested the same concepts. Therefore, we compared differences in scores between the two sections using Repeated Measures ANOVA (SPSS v. 22.0). We found a significant difference ($p < 0.0001$, see Figure 4B) between the two sections. However, each section followed the same trend: the students performed higher on the team portion of the RAT than the individual RAT. The difference between the sections is due to lower scores on both the individual RAT and team RAT; the trend that this section scored lower than the other was observed throughout the semester.

We found that the use of the RAT in this module provided students with the opportunity to be responsible and accountable for preparing for the laboratory and to work with the content at a higher level. By organizing the students into teams and giving them the same RAT to complete as a team, they were able to reinforce their knowledge via argumentation and peer teaching, as well identify and change their misconceptions of the content. We found that student misconceptions during the RAT were centralized to how the genotypes related to phenotypic outcomes in behavior, given that these strains exhibit complex behaviors controlled by a spliced variant. We recommend incorporating practical and procedural knowledge into the RAT (which we did not do in this module) because we noted that students struggled initially with sexing the flies, using the dissection microscopes to observe the behaviors, and incorporating environmental (i.e. sound, light, size of chambers, etc.) variables that alter behavior into their designs.

Our results are corroborated by other studies (Beatty et al., 2009; Wiener et al., 2009) that have found that either the RAT or similar classroom assessment techniques improve student performance. The success of the RAT is dependent on the incorporation of student preparedness with peer instruction and collaborative learning in the teams. Both peer instruction (Rao and DiCarlo, 2000; Ramaswamy et al., 2001) and collaborative learning (Tao and Gunstone, 1999; Springer et al., 1999) have been established as successful methods for fostering a deeper understanding of both conceptual and procedural knowledge in science education (Simon and Cutts, 2012).

Results of Experiments and Final Projects

We found that, although they gathered data for their experiments, the majority of students were not confident with their results because they either were unable to solicit the behaviors or their results were unexpected. This is because, until this laboratory, we used a more traditional "cookbook" laboratory format where students were given protocols to perform during the laboratory. This was the first inquiry-based laboratory where the students designed and implemented their own experiments. As a result, they were able to apply the concepts and use problem-solving skills developed in the module to see the flaws in their experimental designs. Although their experiments did not necessarily work, these lessons are the basis of scientific procedural learning.

As a result of this laboratory module, students were better prepared at the end of the semester to develop independent research projects. Instead of a final examination, students worked in their small groups to design and implement projects that addressed a novel research question in behavioral genetics using either *D. melanogaster* or another invertebrate system used in the course. We found that students not only retained the conceptual knowledge learned in the module, but that they were practicing science, designing their experiments, problem-solving, and working with the behaviors and animals with more precision and confidence than before.

Results from a Student Survey

At the end of the course, students were polled using a survey established previously by Dr. Heidi Mennenga (Mennenga, 2010) to test the efficacy of team-based learning in nursing courses. The surveys consisted of three categories (called "subscales") to determine whether the implementation of TBL was effective in the course. These categories included a series of questions that measured if student accountability, their preference for TBL over traditional teaching methods, and their satisfaction were higher than neutral scores. The higher the score is over the neutral score indicates higher levels of student accountability, preference, or satisfaction.

Using a One-Sample T-Test (SPSS v. 22.0) the average for each category was compared to the neutral score for each category (as established in Mennenga, 2010). Results from the survey (see Figure 5) showed that the use of TBL in this laboratory module resulted in significantly higher: 1) accountability ($p < 0.0001$), 2) preference for TBL over our previous "cookbook" approach ($p < 0.001$), 3) student satisfaction ($p < 0.0001$), and 4) total, a.k.a. the sum of all three categories ($p < 0.0001$). In addition, students commented in the survey that TBL increased both their preparation and conceptual understanding of the material. In particular, students said team-based learning helped: "better prepare us in understanding the experiment before hand," and "[me] understand the material during class."

Regardless of our short implementation of TBL (over two weeks instead of the entire course), these results indicate that students felt that TBL improved their personal accountability during pre-class preparation and group work, they preferred TBL to our traditional laboratory format, and that their overall satisfaction improved. To improve student performance and "buy in" to the TBL approach, Michaelsen and Sweet (2008) suggest the implementation of TBL throughout the entire semester. This is important because team cohesion, cooperative learning, and student responsibility require extended periods of time for development.

Benefits of Approach to Students

Improved student achievement in this learning module coordinated with overall student satisfaction as seen in the student evaluations. We observed that students' understanding of animal behavior and the role of genetics on behavioral phenotypes, ability to design hypothesis driven experiments, and engagement in the class was heightened using this format. Student groups developed unique research questions, clear hypotheses, strong peer reviews, and were better at collaborating with their peers. Several students developed strong innovative final research projects using these experiments as a basis. In addition, this approach increased innovation, creativity in future research, problem solving, and practice with experimental design.

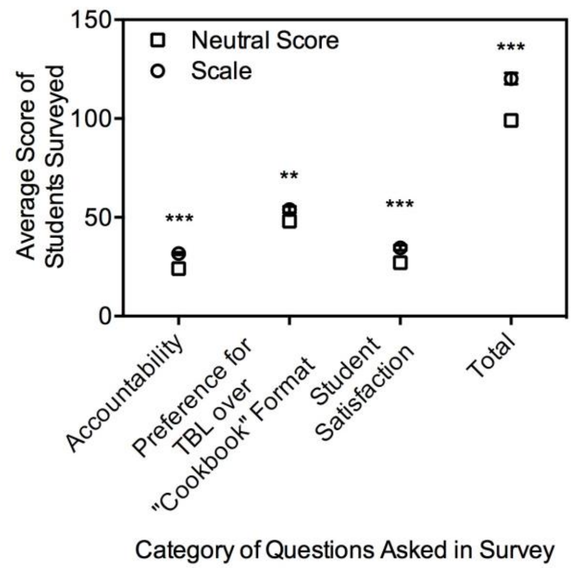

Fig. 5. Results from a student survey to determine the efficacy of the implementation of team-based learning (TBL). Student preference for TBL over traditional methods was significantly higher across all three categories: accountability ($p < 0.0001$), preference for TBL over traditional "cookbook" format ($p < 0.001$), satisfaction ($p < 0.0001$), and the total of all three scales ($p < 0.0001$). Means ± SEM. ** = $p \leq 0.001$, *** = $p < 0.0001$.

CONCLUSION

Many undergraduate laboratories employ "cookbook" laboratory exercises; this approach does not provide students with an authentic, research-driven experience. Educational outcomes of "cookbook" laboratories are limited because students do not develop the scientific or laboratory skills as intended. By combining the laboratory module with TBL, we were able to provide students with an inquiry-based, research experience in the laboratory. In addition, we successfully addressed the learning objectives of the laboratory module: both conceptual knowledge (Table 1 Objective 1) and scientific skills (see Table 1 Objectives 2-5) improved, as well as overall performance, both within the laboratory module and after. We have shown that combining TBL in a biology laboratory course may be an effective strategy in increasing student conceptual and procedural knowledge of science. Therefore, TBL has the potential to transform undergraduate laboratories from the traditional "cookbook" model to a student-centered inquiry-driven model focused on acquisition of scientific skills. However, implementation of TBL in larger, controlled studies is necessary to determine if TBL is an option for inquiry-based teaching in biology laboratories.

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