ARTICLES

An Introduction to Biological Modeling Using Coin Flips to Predict the Outcome of a Diffusion Activity

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Abstract: In order to increase students' awareness for and comfort with mathematical modeling of biological processes, and increase their understanding of diffusion, the following lab was developed for use in 100-level, majors/non-majors biology and neuroscience courses. The activity begins with generation of a data set that uses coin-flips to replicate movement of dye molecules at an interface of a permeable gel. The class results are then collapsed into a single data set that is used to predict the movement of real dye molecules over time, which are then measured by students in a "wet-lab" activity.

Keywords: diffusion, predictive, modeling, interdisciplinary

INTRODUCTION

In 2011, The American Association for the Advancement of Science (AAAS) released a document entitled Vision and Change in Undergraduate Biology Education: A Call to Action. This document provides a blueprint for reforming biology education that outlines key concepts and skills that prepare biology students for 21st century biology. One recommendation is that students develop skills in modeling and simulations for biological discovery. Here we describe a laboratory activity that provides this experience while elucidating a concept difficult to grasp: diffusion.

All molecules and microscopic particles in suspension undergo diffusion. This phenomenon is driven by constant collisions with surrounding molecules, which causes them to undergo random and unpredictable changes in motion. How such motion is connected to another aspect of diffusion, where bodies move predictably towards regions of lower concentrations, is a concept difficult to get across in a biology curriculum, as has been documented by Meir et al. (2005) and Fisher et al. (2011).

The following activity was developed to increase students' awareness for and comfort with modeling of biological processes, and increase their conceptual understanding of diffusion. The activity has been tested in 100-level, majors/non-majors biology and neuroscience courses and a 100-level non-majors physics course. Our approach was to build on the pedagogical principle that active learning more deeply involves students in their own learning of physical processes (Meltzer and Thornton, 2012). To that end we had our students learn how random motion drives diffusion by (1) being actively involved in generating trajectories of several molecules undergoing random motion and (2) by analyzing those trajectories and relating them to motion towards regions of lower concentrations. Here the random motion that molecules undergo is simulated with coin tosses, each representing a random displacement that a dye molecule may undergo in a gelatinous medium. Students then complete a "wet-lab" in which they measure actual diffusion of food coloring into gelatin, across a water/gelatin interface, and compare the experimental results to the simulated data set. Good agreement was obtained between the actual experimental and predicted data sets. While students' predictions may deviate somewhat from the observable diffusion, these discrepancies allow for fruitful class discussions about the strengths and weaknesses of mathematical models and fundamental properties of diffusion. Finally, we assessed students' improved understanding of diffusion in all of our classes using a questionnaire that was administered before and after instruction on diffusion. Gains achieved by students exposed to the activity developed here were contrasted with those of control course sections that experienced a traditional classroom/laboratory introduction to diffusion.

MATERIALS AND METHODS
Participating institutions and courses

The diffusion laboratory activity we developed was implemented at three institutions, all

characterized as private, 4 year, liberal arts colleges, and in three disciplines. Those colleges (and disciplines) include Centenary College of Louisiana (Biology 101 and Neuroscience 101), Thiel College (Neuroscience 101), and Berea College (Physics 127). "Control" classes, i.e., classes in which students were exposed to a traditional diffusion laboratory activity, took place at Centenary (Biology 101 only). Classes in which students were engaged in the laboratory activity described in this paper are henceforth referred to as "experimental". With the exception of Berea's physics class, all remaining classes were populated with lower division life sciences majors and non-science majors. Most students enrolled in Berea's class were upper-level biology and chemistry majors. Despite the difference in population, we found the initial misconceptions and activity gains to be comparable.

Pre-laboratory activities

Classroom discussion

All students including those in control classes were exposed to classroom presentations describing the phenomenon of diffusion. These included presentations containing images of particles spreading into lower concentration regions along with a discussion of how this phenomenon accounts for the transport of gases and other molecules in biological tissues, as well as its connection to osmosis. Classroom discussions also covered visual and verbal explanations of how random particle motion accounts for diffusion.

Homework assignment in experimental classes

Following classroom discussions, each student in an experimental class was given as a homework assignment the task of simulating the motion of 10 molecules undergoing diffusion, all starting at the boundary between two media (see Fig. 1). The exercise is designed to simulate the same situation that students would encounter later in the lab, i.e., the diffusion of food coloring molecules into gelatin. Instructions for this exercise are described below.
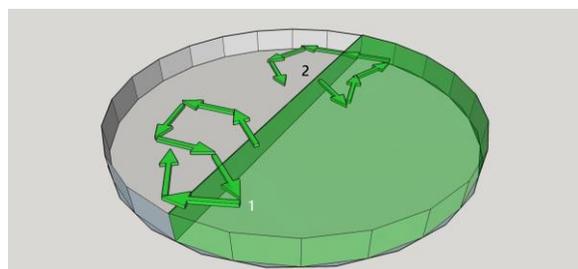
Figure 1. A petri dish is half filled with gelatin and the other half filled with a dilute aqueous solution of food coloring. The arrows illustrate exaggerated random movement of two dye molecules, both located initially near the gel-dye interface. Each student is asked to simulate the motion of 10 dye molecules over 24 time steps, according to instructions described in Figure 2 and in the procedure narrative.

Each student was assigned the task of simulating the trajectories of 10 dye molecules starting near the gel-dye interface. To illustrate how the simulation works, students conducted a practice run in class using the instructions shown in Fig. 2. The procedure chosen was designed to reproduce the main features of diffusion as they relate to random motion, but without the complexity of accounting for every possible direction or step length that a molecule can undertake. For example, each molecule was assumed to move a distance of 0.5 mm every 5 minutes, which corresponds to the root-mean-square distance obtained for a small dye molecule in gelatin[1]. For simplicity, we limited the random movements of dye molecules to one of three possible directions: a positive 0.5 mm movement into the gel, a negative 0.5 mm movement away from the gel, or no net movement (i.e., the dye moved parallel to the interface). This simplifies the modeling activity as it only keeps track of the dye movement away from the interface. Students are asked to simulate the movement of 10 molecules over a period of 4 hours at 5-minute intervals, for a total of 240 data points. The handout for the homework assignment we used is available online at http://bit.ly/butcherdiffusion.
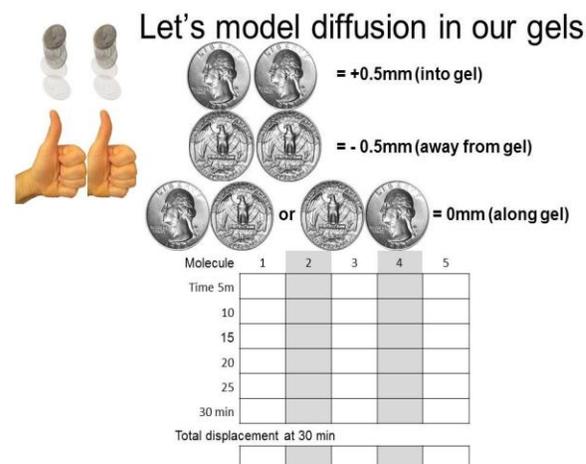
Figure 2. Graphic used to illustrate the rules of the coin flip model. Note in this illustration, each student is only asked to generate 30 data points. In the actual homework assignment they are asked to generate 240.

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[1] The 0.5 mm step size corresponds to the theoretical diffusion length obtained for a dye in a gel over a period of 5 minutes. To obtained this distance we assumed that green food coloring has a diffusion coefficient similar to a common dye such as R6G in a 1.5% agarose gel, which has a coefficient in gel similar to that in water, $D = 2.8 \times 10^{-6}$ cm^2/s (Fattin-Rouge et al., 2004).

Laboratory activities

Homework analysis

At the start of the lab period we ask students to set up the "wet lab" portion of the project, which includes adding food coloring to each dish. These activities are detailed in the next section. Once started, and while students wait for dye molecules to diffuse, the analysis of the simulated data takes place.

First, we ask students to submit the summary data from the table in the handout to the instructor who then generates a graph of the locations of the modeled dye molecules. This graph serves as a prediction for where dye molecules will be located at a given time point (see Fig. 3). Once the class graphs are generated, we ask students to discuss what the data are telling them. This can be accomplished either as a single group session, or in a think-pair-share format. Students are often confused by what the model shows them about the leading edge of the dye. For example, in the 30 min graph (Fig. 3), a small number of molecules are predicted to reach the 2mm point. Some students will claim that this means the leading edge will be clearly discernable at that point in the gel. In reality, such a small number of molecules will likely be invisible. The graded green bars on the top of each graph are used to help students appreciate that a gradient of dye molecules will occur rather than a sharp edge. We ask each group to use the graphs to predict where the visible leading edge of the dye will be for each time point. We then discuss with the students how these predictions are really hypotheses that the model allows us to generate.
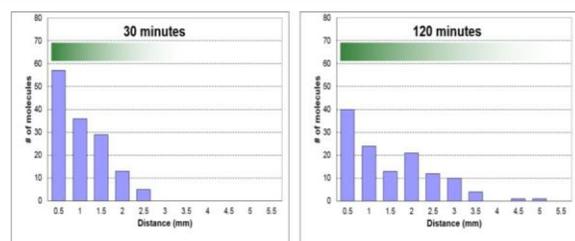
Figure 3. Representative data for two time points (30- and 120-minutes) obtained from simulated motion of 240 molecules. As noted in the panel at left, by the 30 minute time point, most of the simulated molecules are still located at or near the interface. By 120 minutes, they have diffused several millimeters into the gel. The graded bars above each graph represent how the data might be visualized in terms of concentration. Only positive movement (into the gel) is displayed in these graphs.

Wet lab procedure

The following is a list of supplies and preparation steps needed prior to lab meeting time:

• One petri dish filled with 2% clear gelatin solution (prepared the day before the lab, then ½ of gel removed just before start of lab; see Fig. 4)
• Fifty-ml colored water made with up to a 1:1 solution of food coloring and water (we used green, any dark color should work, however all groups should use the same color or the size of the dye molecules will influence the rate of diffusion producing more variability)
• Transfer pipette
• Printed ruler (see Fig. 4)

Once set, the gelatin can be easily removed from the petri dish using a scalpel to cut a straight interface, then carving out the gel using a flat spatula. This process works best if the gels are cooled in a refrigerator for several hours as the 2% solution is fairly soft at room temperature.

Students begin the lab period by being instructed to place the printed ruler under each petri dish with the zero mark precisely under the cut edge of the gel. Then they are asked to fill the ½ empty portions of the petri dishes with food coloring mixture (http://bit.ly/butcherdiffusion). Finally, students are cautioned not the bump the dishes (or the tables if they are not anchored) since doing so can cause the dye mixture to spill over the top of the gel.

Figure 4. Representative image of gel after 120 minutes. By this point, the green dye has diffused into the gel several millimeters. The enlarged image at right illustrates how the leading edge of the dye is not sharply delineated. Each group of students is asked to discuss where they believe the leading edge is located and then reach a consensus measurement. This variability provides a point of discussion when compared to the model.

At the 30-, 60- and 120- time points, students are asked to observe their dishes and note where the visible leading edge is actually located. We found that students often have difficulty visually determining where the interface of the dye is located. To help visualize the edge, we ask each group to capture an image using their cell phone cameras. While not every student will have such a device, at least one per group is virtually guaranteed. These

images can then be enlarged on their cell phones to help with this determination (see Fig. 4). Students should be repeatedly cautioned to avoid bumping the dish (or table) during this process.

After all data points have been observed, we ask students to compare their predictions from the model (e.g., Fig. 3) and the actual observed measurements. To determine the diffusion leading edge for the simulated data, we look for the distance where the number of molecules dropped to a low value such as "10". Figure 5 illustrates how the simulated and gel data compare. The results indicate that the coin-flip activity accurately models the diffusion of dye molecules through the gel.
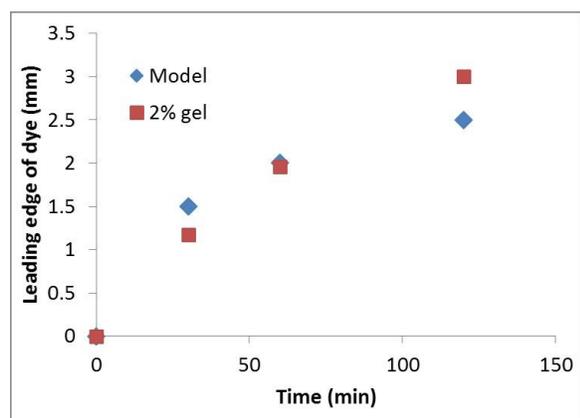
Fig. 5. Comparison of modeled and experimental diffusion through gelatin. Here diffusion is measured as the distance traveled by the "leading edge" of diffusing molecules.

The choice of "10" as a threshold value for the leading edge in the simulations was determined as follows. First we examined one of the images we captured of the gels (e.g., Fig. 4) and analyzed it using Microsoft Paint. Paint allows one to determine the luminance of a single pixel on an image with the aid of the Color Picker function under Tools. Once a pixel is selected with this tool, its color parameters can be determined with Edit Colors. The "Lum" number, or luminance, is a measure of brightness. From this we determined that what we perceive as the leading edge is the spot where the luminance of the dye increases by a factor of four relative to the luminance near the gel interface.

In terms of dye concentration, a luminance four times higher corresponds to a dye concentration four times smaller. Comparing this to the simulated data, given that the number of dye molecules typically average around "40" near the gel interface, the edge should be found where the concentration is "10". Whatever number is chosen, in this case "10", it should be used to identify the diffusion edge for all graphs.

Typically, comparisons between modeled and experimental data will produce some discrepancies.

Common discrepancies include apparent outliers in the simulated data, due to statistical noise, or offset experimental data, due to poor estimation of leading edge location. These can be used as discussion points of how to improve on the experiment. More advanced classes can then follow up the lab with lecture materials on the actual equations that are used to model diffusion. In our case, the latter was left for inclusion in a course in physics many of the students would take in subsequent years.

Assessment instruments used

To assess the effectiveness of the lab activity we developed a questionnaire that probes students' conceptual understanding of how diffusion is related to random motion of every particle embedded in a medium. The full questionnaire is viewable online at http://bit.ly/butcherdiffusion. In brief the questions ascertain whether students understand that

1) Particles in suspension move randomly.
2) Solvent molecules also move randomly.
3) At equilibrium concentrations are the same in gelatin as in water.
4) Diffusing particles require no added energy to sustain their motion.
5) Diffusion is a slow process sometimes requiring hours to move distances of a few millimeters.

The questionnaire was administered before and after the lab activity, and at the end of the course as part of the final. To compare our results with a previously published report on students' conceptual understanding of diffusion and osmosis (Fisher et al., 2011), we included two of the Fisher et al. questions that pertained to diffusion in our final assessment (see http://bit.ly/butcherdiffusion).

RESULTS

Responses to assessment questions devised in this study are shown in Figure 6. The results correspond to the average responses of all students from all participating institutions. The left panel summarizes the conceptual gains achieved by students exposed to the diffusion activity we created. The results are in sharp contrast with those achieved in control classes, where students achieved modest to no gains.
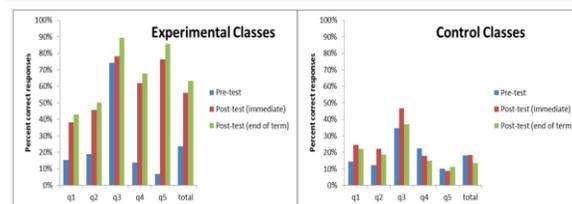
Figure 6. Summary of responses to assessment questions revised in this study.

We also broke down the responses in experimental classes according to disciplines and institutions in which the activity was carried out (data not shown). The results demonstrate remarkable consistency in post-test responses to all questions. The consistency is especially striking given the range of disciplines in which the activity was taught and of faculty backgrounds in diffusion phenomena.

Finally we compare the response of students in our experimental classes to questions derived from Fisher et al.'s survey (2011), which assesses students' conceptual understanding of diffusion and osmosis. As expected, most students responded correctly that molecules move towards regions of lower concentrations. Students from our experimental classes, however, identified the correct reason at a considerably higher rate (35%) than those surveyed by Fisher et al. (2011) (22%).

DISCUSSION

The present work was aimed at developing a laboratory activity that introduces students to the use of modeling in biology and elucidates the concept of diffusion. Our results demonstrate that the activity succeeded on both fronts.

In terms of modeling, the use of coin flips allowed students to arrive at quantitative predictions of diffusion distances for different times. Those results were consistently in fair to good agreement with experimental results. The modeling activity also demonstrated that diffusion is expected to generate gradients rather than movement with a sharply defined edge. The difficulty in defining a diffusion edge may in fact account for the difficulty that some students experienced in obtaining good results. Importantly, the predictions made about diffusion distances and gradients were achieved without mathematical manipulations, thus making the modeling activity accessible to both science and non-science majors.

In terms of conceptual understanding, our assessment tools demonstrate robust gains in the experimental classes compared to those in control counterparts. The gains cut across all concepts tested. Furthermore, those gains seem to be long lasting judging by the fact that students responded equally well on the surveys administered during finals. Importantly, our experimental classes demonstrated an improved performance over those documented by Fisher et al. (2011) for students at a large public university.

While our laboratory activity led to significant gains in conceptual understanding across all questions asked, the final score achieved for questions 1 and 2 (Fig.6) seem distinctly lower than the remaining ones in our assessment tool. We speculate that those lower gains could be accounted for by the way those assessment questions could have been interpreted. The answers to those questions were intended to be "d. All of the above" in both cases, signifying that dye or water molecules can move in any of the directions indicated in answers a, b, and c. However, one can argue that answer "e, "It is impossible to predict the movement of molecules" since the movement of the molecules is intrinsically random. In fact, a large fraction of students responded in this way. If we pooled answers "d" and "e" together, the gains achieved in the first two questions would be significantly higher than reported in Fig. 6.

ACKNOWLEDGEMENTS

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