1 Introduction

An undeniable fact about the biological world is that things move around. Brine shrimp (AKA Sea-Monkeys) move by the rhythmic beating of their appendages acting in pairs. This method of locomotion makes it seem like the brine shrimp wiggle around. In this lab we will try to determine how well a random-walk movement model in space compares with observations of the brine shrimps’ movement.

If, on average, organisms make a ‘run’ of distance $\Delta s$ over a time interval of $\Delta t$, after which they re-orient and choose a new direction at random, it can be shown that the population density, $P(x,y,t)$, for a number of such organisms obeys the diffusion equation

$$\frac{\partial P}{\partial t} = D \left[ \frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2} \right] \quad (1)$$

The diffusion constant, $D$, is related to the random-walk parameters by $D = \frac{\Delta s^2}{4\Delta t}$.

The solution to (1) corresponding to predicted population densities following a point release of $N$ organisms at the origin at $t = 0$ is

$$P(r,t) = \frac{N}{4\pi Dt} e^{-\frac{r^2}{4Dt}} = N \cdot \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \cdot \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{y^2}{4Dt}} \quad (2)$$

where $r = \sqrt{x^2 + y^2}$ is the distance from the origin.

**Task:** With your group solve equation (1) to derive equation (2)

The mean square expectations, $\langle x^2 \rangle$ and $\langle y^2 \rangle$, for the two coordinates grow linearly with $t$, that is

$$\langle x^2 \rangle = \langle y^2 \rangle = 2Dt$$

because the density function can be written as the product of two independent Normal probability distributions. Since each coordinate is independent, we have

$$\langle r^2 \rangle = \langle x^2 \rangle + \langle y^2 \rangle = 2Dt + 2Dt = 4Dt \quad (3)$$

This gives a method for estimating the diffusion constant for a population which we will test in this lab using brine shrimp. Individuals can be tracked, with coordinates measured at several instants in time. The mean of the square displacement for all individuals can be calculated at each point in time and then fitted to a line. One quarter of the slope of that line is an estimate of the diffusion constant.

2 Procedure

We will be working with brine shrimp in a (nearly) two dimensional environment. There will be two experiments (one calibration and one validation) performed. You will work with your group to collect data, and then use that data to decide whether a random-walk movement model is appropriate for modeling brine shrimp.
2.1 Calibration Experiment

In this section of the lab you will measure the displacement of individuals from their initial point of release in time. The goal of the observation is to get data so that equation (3) can be used to estimate the diffusion constant for brine shrimp.

2.1.1 Materials

- Hatched brine shrimp
- Salt water
- Two small bowls
- An eye dropper
- A light table or overhead projector
- A large petri dish
- Transparent graph paper with 10 lines per inch
- A stop watch or timer

2.1.2 Setup

1. Indicate an origin on your transparent graph paper and relative to that origin annotate coordinates at one inch intervals to aid in position estimation on the fly.

2. Tape the graph paper to the light table.

3. Place the large petri dish on top and center it with the origin you previously indicated.

4. Place some salt water (about 50 ml) into the petri dish

5. Place some salt water into one of your small bowls; leave the other dry.

6. Suck up a number of brine shrimp and squirt them into the brined bowl.

2.1.3 Data Collection

To run the experiment, assign one group member to be your “Timekeeper,” one member to be the “Shrimp Watcher,” and one member to be the “Data Recorder.”

1. The “Shrimp Watcher” will suck up one shrimp and release it at the origin
   Be careful not to give the shrimp, or the water, a push upon release

2. When the shrimp is released the “Shrimp Watcher” will say “start” and the “Timekeeper” will start timing.

3. Every five seconds the “Timekeeper” will call out “mark” and the “Shrimp Watcher” will estimate the $x$ and $y$ location of the shrimp in tenths of inches. The “Data Recorder” will note the position and time.

4. Continue collecting data for 90 seconds or until the shrimp reaches the edge of the petri dish.
5. Suck the shrimp out of the petri dish, expel it into the second bowl and repeat the experiment with a new shrimp. You should repeat the experiment at least 10 times, switching roles so that every group member has a chance to observe.

### 2.1.4 Estimation of Diffusion Parameter

You have now completed the calibration process, however to proceed to validation we must parameterize the diffusion constant, $D$. From the data it should be possible to calculate the mean squared displacement of individuals at each time increment. That is, at each time, $t$, for each individual, $i$, calculate $r_{i,t}^2 = x_{i,t}^2 + y_{i,t}^2$, and from this calculate the mean square displacement at that time, $\langle r^2(t) \rangle = \frac{1}{N} \sum r_{i,t}^2$. The diffusion constant can now be estimated from the mean square displacement data using linear regression. Before the next lab (and for your write-up):

- Plot the mean square distance observations and the best-fit regression line (with zero intercept). You should plot the mean value of your data as well as an error bar.
- Determine from the slope of the regression line the predicted diffusion constant for this population using (3). **Bring this prediction and the graph of your regression line to the next class.**
- Explore some of the predictions from the diffusion equation using the diffusion constant you have estimated. For example:
  - Plot several of the Gaussian profiles predicted by the diffusion equation
  - Plot the predicted shape of arrival curves at distances of two and four centimeters from the origin.
2.2 Validation Experiment

In this section of the lab you will measure the number of individuals that reach a given distance from their initial point of release in time. The goal of the observation is to use our estimation of the diffusion constant, $D$, to decide how well the model captures the shrimp movement.

2.2.1 Materials

- Hatched brine shrimp
- Salt Water
- An eye dropper
- A light table or overhead projector
- A large petri dish
- Transparent polar graph paper
- A stop watch or timer

2.2.2 Setup

1. Tape the graph paper to the light table
2. Place the large petri dish on top and center it with the origin
3. Place some salt water (about 50 ml) into the petri dish

2.2.3 Data Collection

To run the experiment, assign one group member to be your “Timekeeper,” one member to be the “Shrimp Watcher,” and one member to be the “Data Recorder.”

1. The “Shrimp Watcher” will suck up some shrimp and release them at the origin
   Be careful not to give the shrimp, or the water, a push upon release
2. When the shrimp is released the “Shrimp Watcher” will say “start” and the “Timekeeper” will start timing.
3. Every five seconds the “Timekeeper” will call out “mark” and the “Shrimp Watcher” will count how many shrimp are a given distance away from the center. The “Data Recorder” will note the population and time.
4. Continue collecting data for 20 seconds.
5. After you have finished find out how many total brine shrimp are in the petri dish.
3 Modeling

Now that you have performed both experiments it is time to decide how well a random-walk movement model captures the behavior of the brine shrimp. Equation (1) predicts the rate at which the population arrives at different spatial locations. For example, the predicted number of individuals arriving in a small box (of area $\Delta A$) at a distance $L$ away from the point of release is approximately

\[
N_{\text{box}} \approx \frac{N \Delta A}{2\pi(\sigma_0^2 + 2Dt)} e^{-\frac{L^2}{2(\sigma_0^2 + 2Dt)}}
\]

Here $N$ is the initial number released, $\sigma_0^2$ is the variance of the initial release profile and $D$ is the diffusion constant measured earlier. This prediction of the diffusion model can readily be used for validation.

Another prediction for a diffusing population regards the flux of individuals. In two dimensions the flux per length of circular arc is $-D \frac{\partial P}{\partial r}$, and therefore the total flux through a circle of radius $\rho$ surrounding the initial point of release is

\[
\Phi(r = \rho) = 2\pi \rho \left(-D \frac{\partial P}{\partial r} \right|_{r = \rho} = \frac{N \rho^2}{4Dt} \exp \left[ -\frac{\rho^2}{4Dt} \right] \]

Thus, one may count the net number of individuals crossing circles drawn around the initial point of release and compare with (5) to test the predictivity of a diffusion model. Alternatively, the cumulative number of individuals predicted to lie within a circle of radius $\rho$ around the initial point of release is

\[
C(\rho, t) = \int_0^\rho \frac{N}{4\pi Dt} \cdot e^{-\frac{r^2}{4Dt}} \cdot 2\pi r dr = N \left[ 1 - e^{-\frac{\rho^2}{4Dt}} \right]
\]

The number of individuals appearing in an annular region between circles of radius $\rho_1$ and $\rho_2$ is predicted to be

\[
N(\rho_1, \rho_2) = C(\rho_2, t) - C(\rho_1, t) = N \left[ e^{-\frac{\rho_1^2}{4Dt}} - e^{-\frac{\rho_2^2}{4Dt}} \right]
\]

Notice that the derivative in time of $N(\rho_1, \rho_2)$ relates the expected number in the annulus to the flux:

\[
\frac{d}{dt} N(\rho_1, \rho_2) = \Phi(r = \rho_1) - \Phi(r = \rho_2)
\]

By counting the number of individuals appearing in annular regions following the point release, predictions of the diffusion equation can be tested against data.

It is now up to your group to parameterize the random-walk model. You will report your findings so you should discuss:

- Is the random-walk model a good fit?
- What are the error sources?
- Is there anything poorly accounted for by the model?
4 Report

4.1 General Writeup Guidelines

Each writeup we will do in this class should have the following format:

- **Introduction** - Contains a general discussion of the problem
- **Methods** - Contains a summary of lab setup, how data was collected, description of model, and parameterization
- **Results** - Contains an analysis of the model
- **Discussion and Conclusion** - Contains an evaluation of results

4.2 Specific Lab Items

For this specific lab your writeup should include the following items in the indicated sections:

**Introduction** -
- A description of the lab.
- A description of the phenomenon you are trying to model, including 1-2 paragraphs containing background information about brine shrimp reproduction and locomotion.

**Methods** -
- Your work to solve equation (1) to derive equation (2)
- Estimation of the diffusion parameter.

**Results** -
- A graph of mean squared displacements and the regression line used to estimate $D$.
- Graphs of predicted brine shrimp diffusion and actual diffusion from the Validation Experiment in section 2.2

**Discussion and Conclusion** -
- Discussion (1-2 paragraphs) of whether or not the diffusion equation is an appropriate model for dispersal of brine shrimp, supported by your results.
- What did you learn from this experience?
Polar Graph Paper – For Arrival Rates Data Collection