Abstinence Makes the Heart Beat Longer: A Statistical Analysis on the Consequences of Mating and Predation Risk in Longevity for Freshwater Snails

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Introduction:

In this work, we look into the effects of mating and predation risk on the survivability of the simultaneously hermaphroditic freshwater snail *Physa acuta*. Earlier, an experiment was performed on 30 families of *Physa acuta* snails under four different conditions: Snails were either exposed to non-lethal predation cues in their water or not, and were either exposed to other snails with which to mate or not. The snails which were not exposed to potential mates were capable of self-fertilizing. Throughout the experiment, data was collected recording when each snail undergoing each treatment died. We estimated the survivor function, which predicts the probability that a snail under any of the environmental conditions lives to a given age, and we estimated the hazard ratios with respect to mating status and predation. Our analysis predicts how much more likely a snail who has mated or who is living in the presence of predator cues is to die on a given day than one who isn't. We plan to use the survival function in a predictive model which will determine the optimal mating strategies for *Physa acuta* and other simultaneous hermaphrodites.

The Survivor Function

We estimated survivor functions for each of the four snail groups using Kaplan-Meyer tables. This method calculates the probability of a particular failure event occurring at a given point in time, and is updated whenever a a failure occurs. In our case, the failure event is the death of a snail. The following formula is used to determine the survival probability after the n+1th failure time:

$$S_{n+1} = S_n \frac{(N_{n+1} - D_{n+1})}{N_{n+1}}$$

Where S_n is the survival probability at the nth failure time, N_{n+1} is the number of snails alive at the n+1th failure time, and D_{n+1} is the number of snails which died during the n+1th failure time.

A plot of our survival curves for each of the environmental treatments is shown in Fig 1.

Hazard Ratios

Now that we have survival curves for each of the four environments, we can run a Cox Proportional Hazards regression analysis. This method evaluates the risk of failure rather than the probability of survival, and so it requires a conversion of survival functions to hazard functions. To do so, we find the number of observed failures over each failure period, and from that we can calculate the expected number of failures over each failure period. Then the hazard ratio is just the ratio of the total number of observed to expected events in two separate environments:

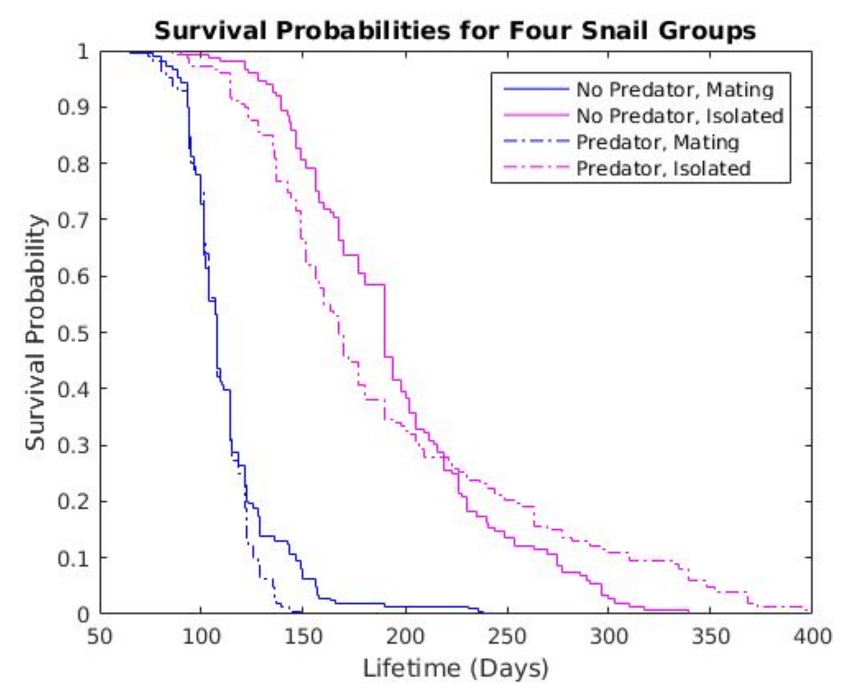
$$HR = \frac{\left(\frac{\sum O_1}{\sum E_1}\right)}{\left(\frac{\sum O_2}{\sum E_2}\right)}$$

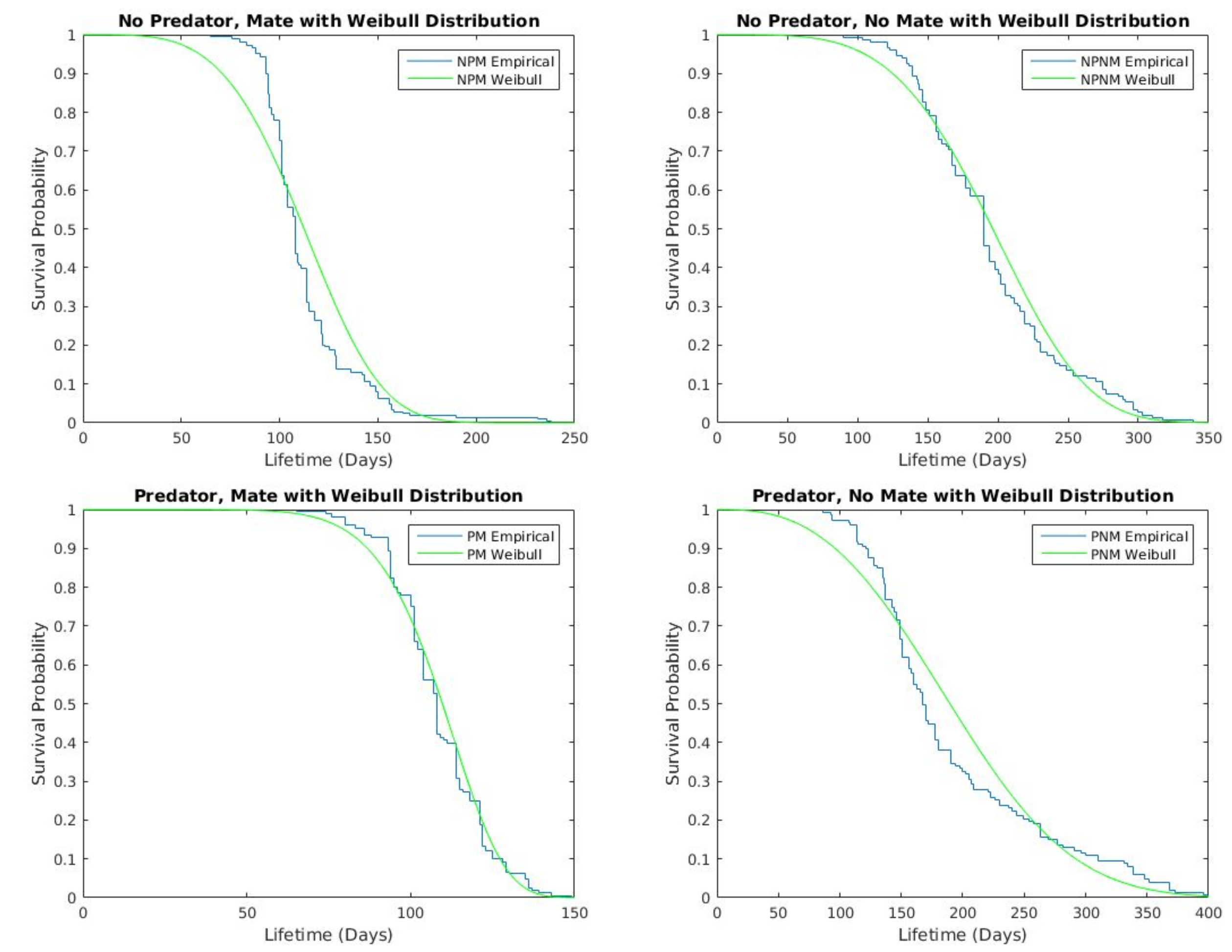
Where O_1 and E_1 are the observed and expected deaths under environmental condition 1 and O_2 and E_2 are the observed and expected deaths under environmental condition 2

Log Rank Test

We also used a log rank test to verify the validity of our hazard ratios. The log rank test tests the null hypothesis of no difference in survival between two independent groups. Running this analysis for mating/isolated and predator/no predator had the following results:

H_0 := The two survival curves are the same
H_a := The two survival curves are different
Rejection region: We can reject the null hypothesis if our calculated test statistic is > 3.84
(The rejection region was determined based on a chi-square test statistic with 1 degree of freedom)





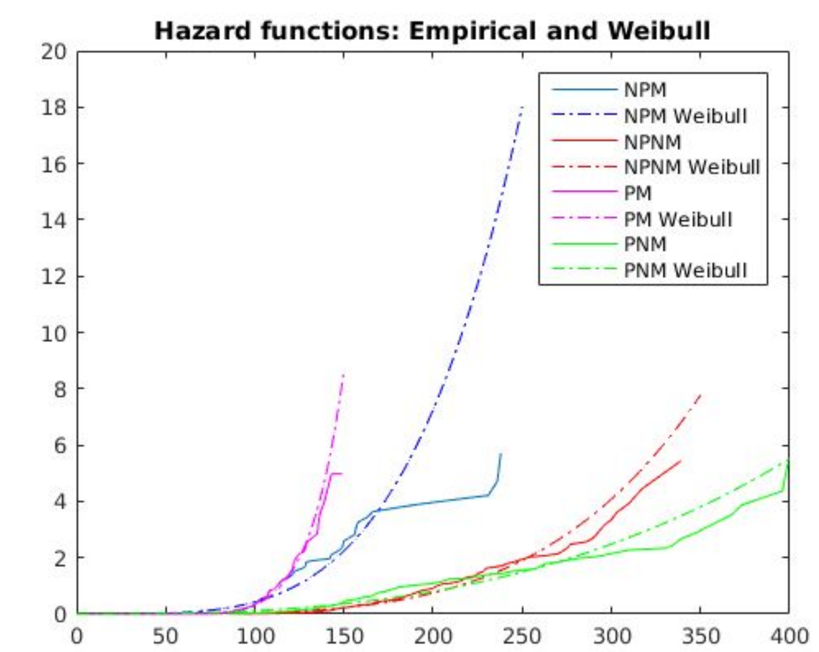


Fig 1. (Top left) The survival curves for the four environmental treatments.
Fig 2. (Above) Survival curves and their Weibull distribution fits for each of the environmental conditions
Fig 3. (Left) Cumulative hazard curves and their Weibull distribution fits, to be implemented in future models

Results:

In running the Cox Proportional Hazards analysis, we found the following hazard ratios:
Predator/No Predator
HR = 1.041
Mating/Isolated:
HR = 4.021

These findings are supported by our Log Rank tests, which gave test statistics as follows:
Mating/Isolated:
Test statistic: 393.271
Predator/No Predator:
Test statistic: 0.299

Based on this analysis, we conclude that *Physa acuta* snails which mate with other snails are at a 4 times greater risk for death at any given time than those snails which refrain from mating. However, exposure to non lethal predator cues have no statistically significant impact on survivability.

Further Research:

This analysis was part of an ongoing project to formulate a predictive model to determine the optimal mating strategy for *Physa acuta* snails. In order to use this analysis in that model, we need to fit the survivor data to a probability distribution, and figures 2 and 3 show a two parameter Weibull distribution fitted over each of the survival curves, as well as the resulting cumulative hazard functions, which will ultimately be used in our model.

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