Toxicity of Road Runoff Entering the Waquoit Bay Estuarine System

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The Marjot Foundation • Marine Biological Laboratory • Citizens for the Protection of Waquoit Bay

Introduction

In this experiment I will determine the effect of road runoff which flows unimpeded into the Quashnet River in Waquoit, MA.

Phase 1

Using *Daphnia magna* and *Artemia salina* (brine shrimp) to assess the road runoff toxicity through a bioassay study of waters and sediments collected both from the street and from the Quashnet River. This study will assess the mortality and health of the organisms over a period of time. Population decrease and measurement of heart rate change likely will indicate toxins present in the stream.

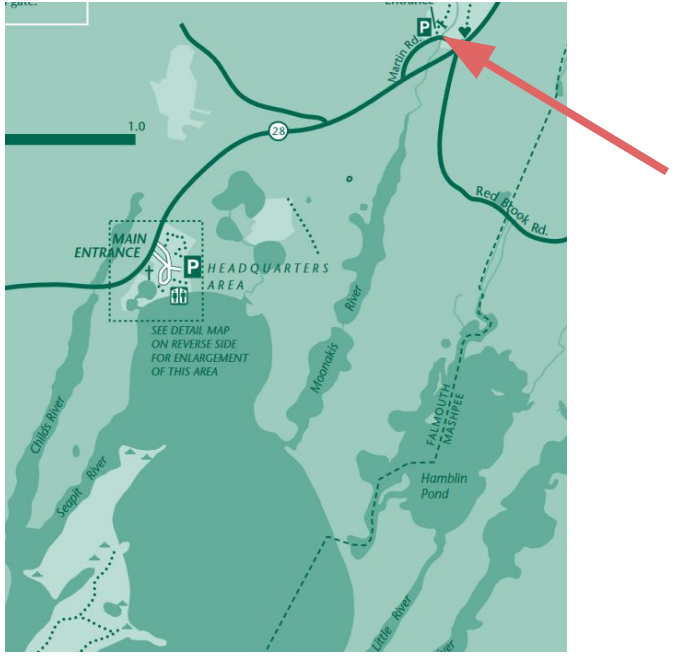
Phase 2

Chemical analyses on the water types which will determine potential toxins of concern related to the runoff and Phase 1 results. Bioassay and heartbeat studies with *Daphnia magna* will be conducted using solutions of specific toxins at various concentrations.

Site Details

This project investigates an area where water flows into the Waquoit Bay Estuary.

- Martin Rd in Waquoit MA crosses the Quashnet River.
- This road happens to be sloped
- This could lead to many toxicants being dumped into the stream.
- No catch basins are located on this street.



Hypotheses

- Toxins will likely be in higher concentrations in the road runoff sample, and result in the greatest mortality.
- There will be higher mortality in the downstream sample as compared to the upstream sample which will confirm the environmental impact from runoff.
- I anticipate similar results from the heart rate study which will show decrease heart rate in the impacted samples, a sign of toxins.
- Heavy metals and petroleum products from both asphalt and vehicles using the roadway will be identified as toxins in the second part of the study.
- Performing a bioassay of specific toxins should provide LD50 results to concur with Phase 1 mortality results and literature values.
- During the heart rate trials I anticipate to see similar results with a decrease in heart rate in all toxins.

Phase 1: Waquoit Water Bioassay and Heart Rate Study

During Phase 1 the following steps were performed:

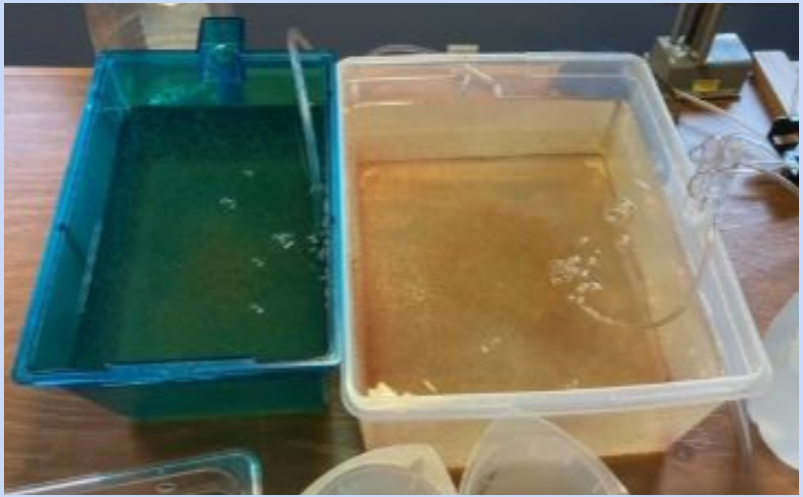
- Water and sediment collections from the Quashnet River and Martin Rd
- Create slurry samples using sediments mixed with water samples
- Culture *Daphnia magna* and brine shrimp for testing
- Prepare containers of each sample for bioassay study
- Place *Daphnia magna* and brine shrimp into associated containers
- Record deaths at even intervals over 1 week
- Perform study of heart rate using *Daphnia magna*
- Record individual heart beats in triplicate before and after exposure
- Evaluate results of bioassay and heart rate study for Phase 1

Sample Collection

- Water collection
  - 5 gallons at each location
- Sediment samples
  - 6 cores at a 6 inch depth
- Buckets for rainwater collection
- Strained water samples
- Slurry sample
  - 100 grams composited sediment with 4 liters of associated water
  - Slurry water was then filtered through strainer and cloth
  - These samples will better assess contaminants that enter and settle in the river bed.

Culturing *Daphnia* and *Artemia*

Artemia salina (Brine Shrimp)



Daphnia magna

Bioassay Setup

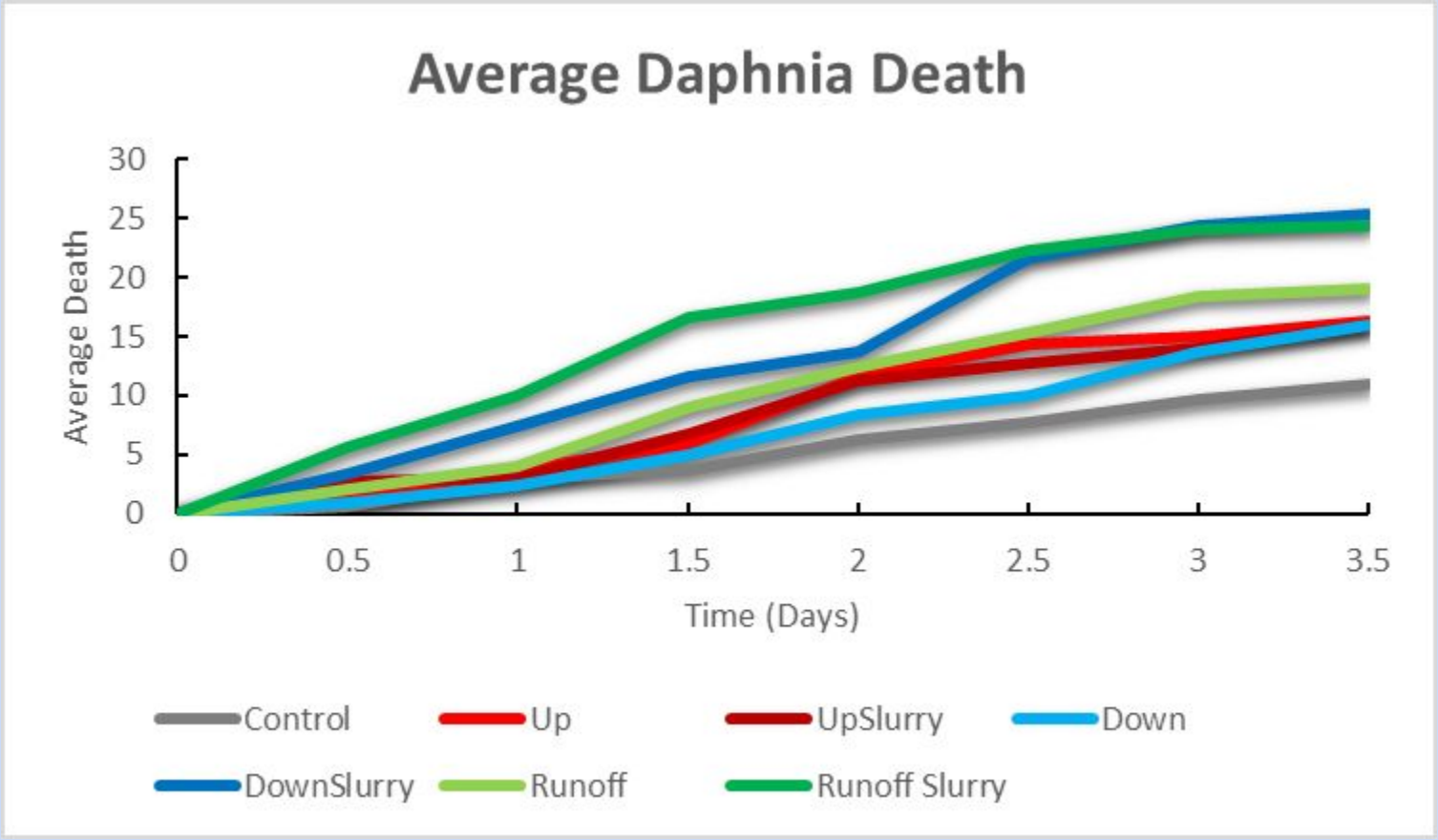
After all the samples were collected and processed, 250mL of samples were placed into labeled polystyrene containers.

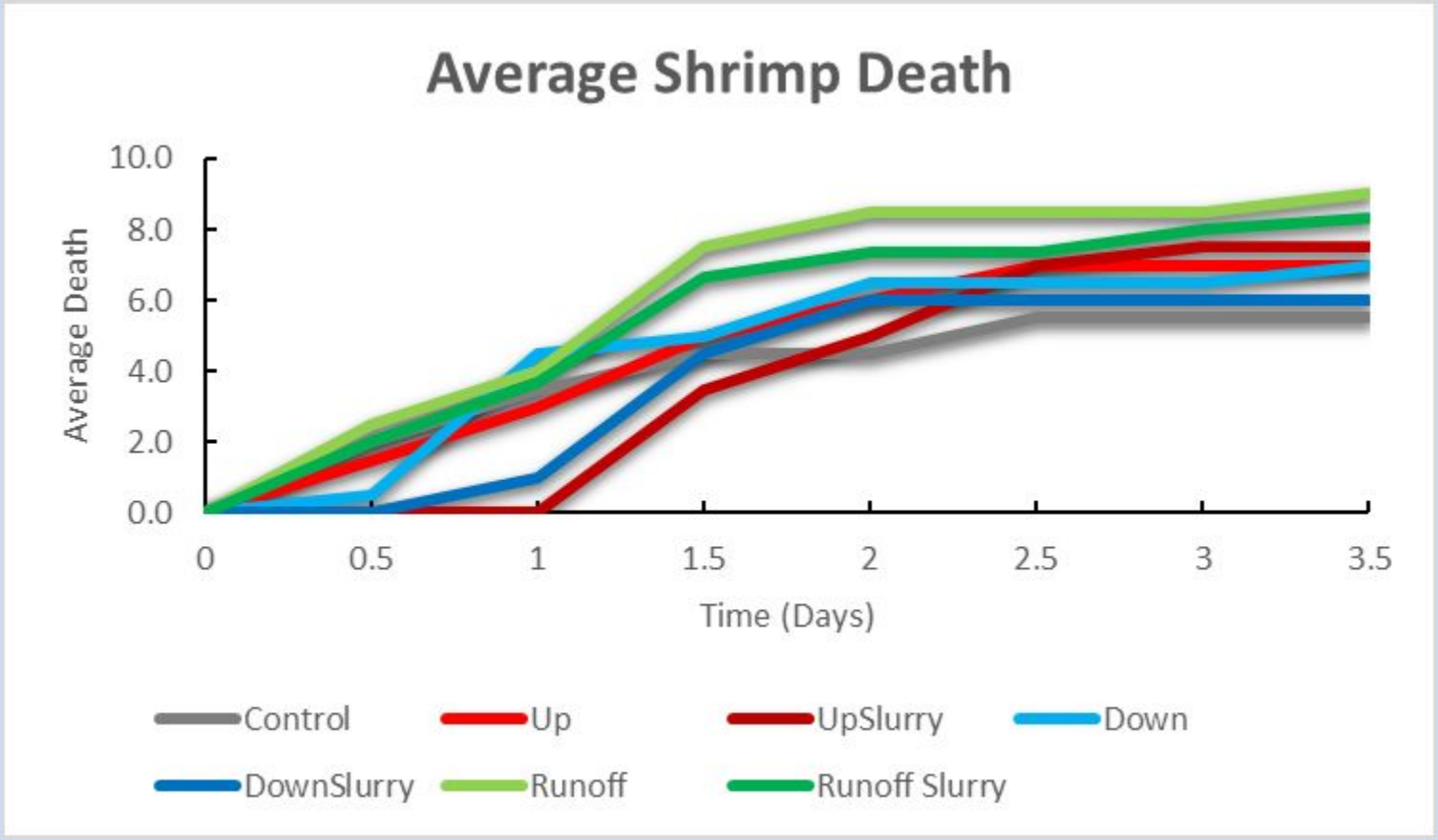
- Upstream
- Downstream
- Runoff
- Control
- Upstream Slurry
- Downstream Slurry
- Runoff Slurry

Six containers for each water location or slurry were prepared. Three to be used for the *Daphnia magna* and the other three for the brine shrimp.

Bioassay Testing

- 42 Total Containers
  - 21 *Daphnia magna*
  - 21 *Artemia salina*
- Equal specimens in each container
  - 30 *Daphnia magna*
  - 10 *Artemia salina*
- Observed over about a week’s time
- Checking for deaths about every 12 hours
- Dead were removed by plastic pipette
- Counting was done by hand with magnifier


Average Daphnia Death
Average Death
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
Time (Days)
Control
Up
UpSlurry
Down
DownSlurry
Runoff
Runoff Slurry

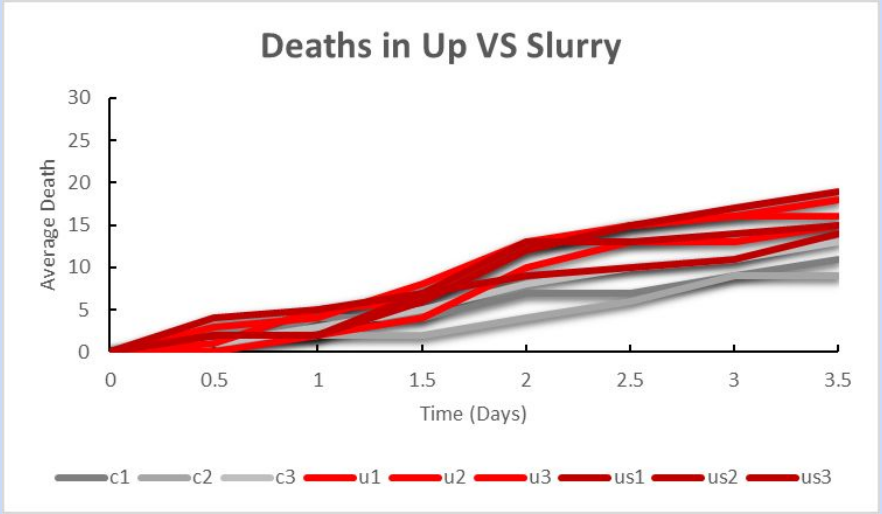

Average Shrimp Death
Average Death
10.0
8.0
6.0
4.0
2.0
0.0
0
0.5
1
1.5
2
2.5
3
3.5
Time (Days)
Control
Up
UpSlurry
Down
DownSlurry
Runoff
Runoff Slurry

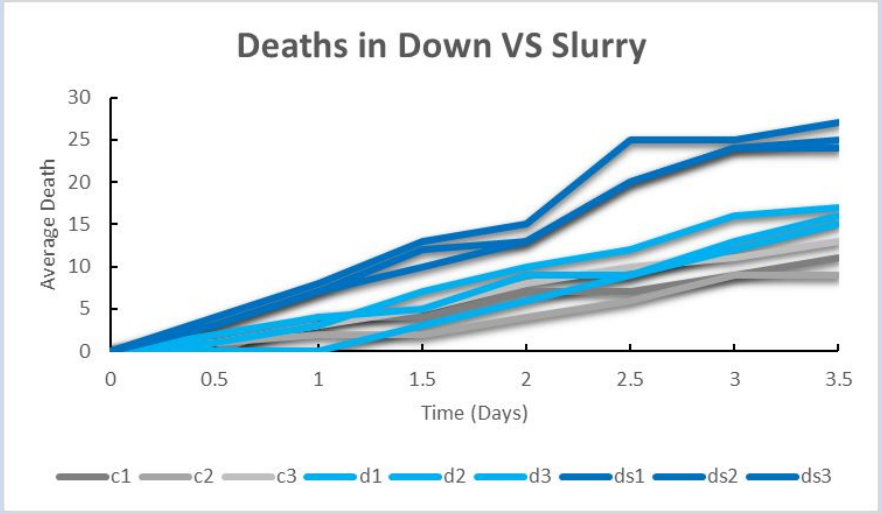
Daphnia Slurry Discussion

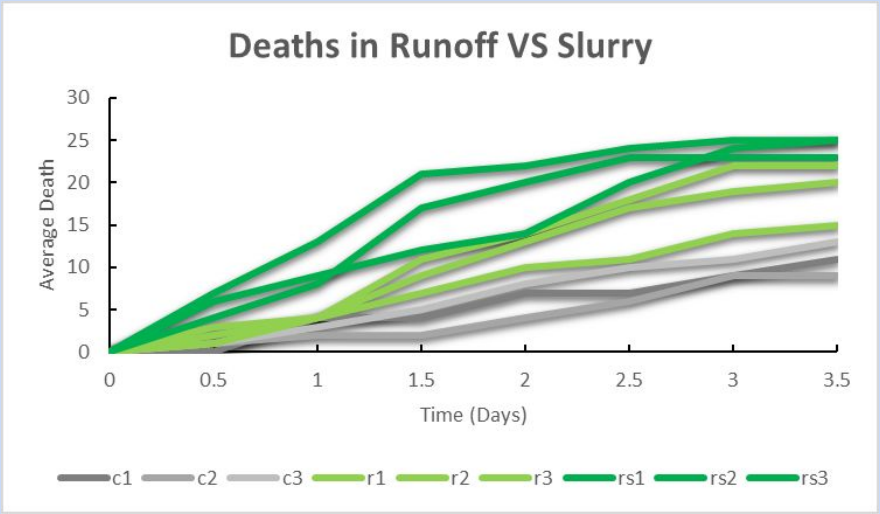
Something very interesting to note is the significant increase in mortality with the downstream and runoff slurry samples in each of the trials.

Of course in any body of water, in particular this case of a moving stream, runoff solids (including toxicants) will frequently sink to the bottom, so the sediment itself leads to the toxicity effect in the water.

Due to this fact I figured it would be significant to show the two in comparison with the results from the previous test but with more clear comparisons.


Deaths in Up VS Slurry
Average Death
Time (Days)
c1 c2 c3 u1 u2 u3 us1 us2 us3

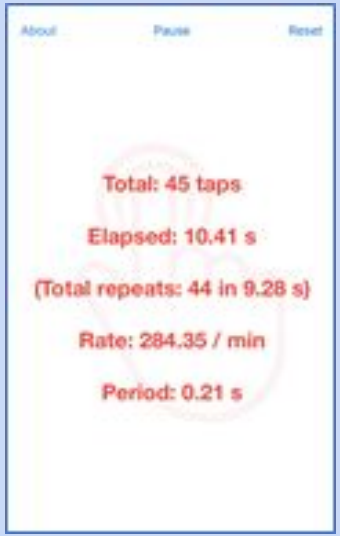

Deaths in Down VS Slurry
Average Death
Time (Days)
c1 c2 c3 d1 d2 d3 ds1 ds2 ds3

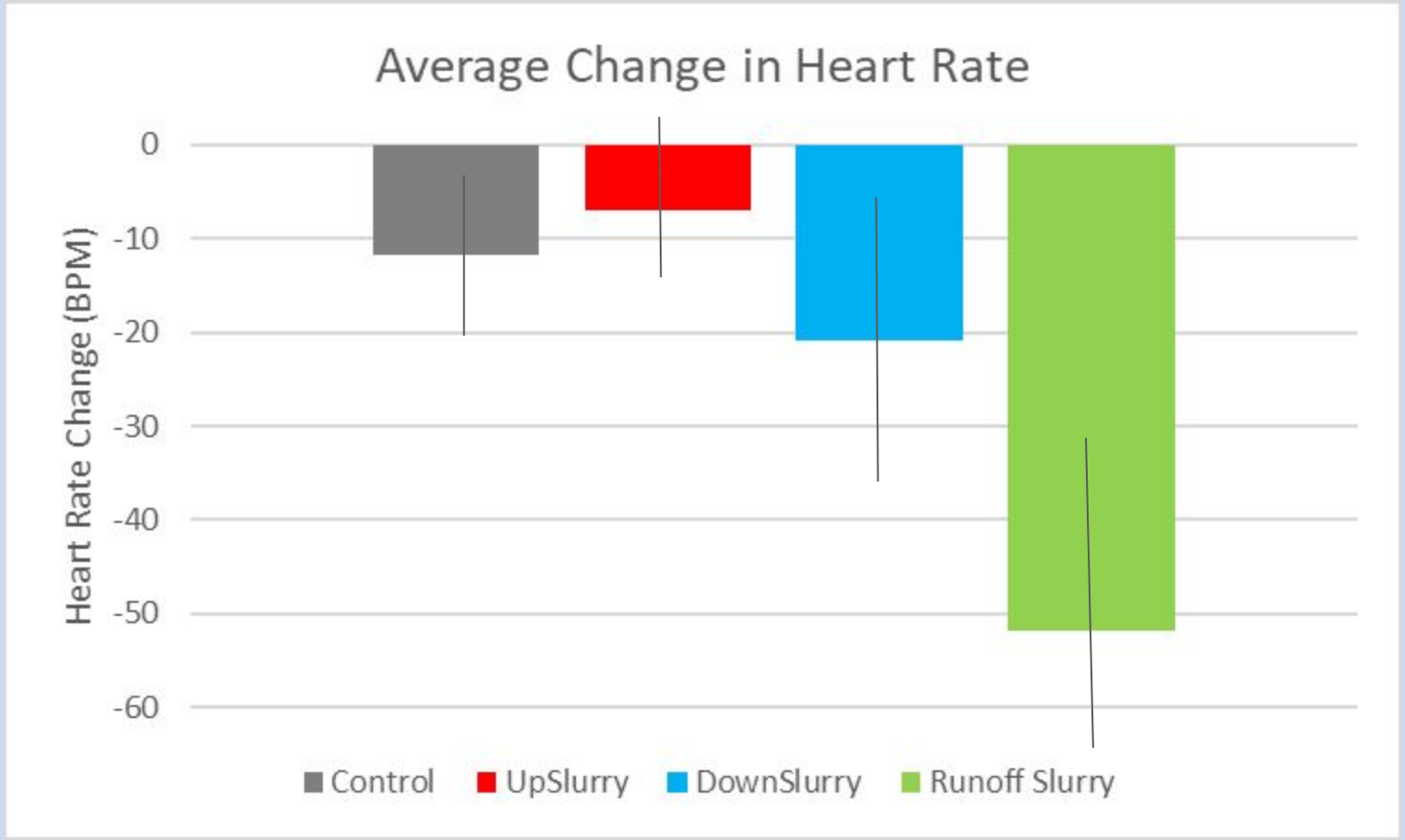

Deaths in Runoff VS Slurry
Average Death
Time (Days)
c1 c2 c3 r1 r2 r3 rs1 rs2 rs3

Daphnia Heart Rate Discussion

Looking at changes in heart rate due to exposure to toxins could also be a significant way measure health

- Individual daphnia on a slide
  - with minimal water to keep *Daphnia* still
  - *Daphnia*s are translucent
- Check heart rate
- Place *Daphnia* in solution
- Wait 10 minutes for them to acclimate
- Check heart rate for a second time
- Each heart rate measurement was taken three times before and after
  - Tapping app was used for accuracy
  - *Daphnia* heart rate is very fast – over 200 beats per min




Average Change in Heart Rate
Heart Rate Change (BPM)
0
-10
-20
-30
-40
-50
-60
Control
UpSlurry
DownSlurry
Runoff Slurry

Phase 2: Chemical Toxin Testing

During this second phase the following steps were performed. This was fairly similar process to Phase 1 but with a focus on specific toxins.

- Determine the toxicants to test from Chemical Analysis results
- Order or create toxicants for those identified
- Make varying concentrations for each toxicant for bioassay
- Place *Daphnia magna* in each container
- Record deaths at even intervals over a weeks time
- Perform study of heart rates using daphnia magna at specific tox levels
- Record individual heart beats in triplicate before and after exposure
- Evaluate results of bioassay and heart rate study for Phase 2

Chemical Testing Results

Samples were submitted to RI Analytical in Warwick RI for Chemical Testing.

- Petroleum Hydrocarbons
- Semivolatile Analysis
- Metal analysis
- Phosphorus
- Nitrogen
- Bacteria

For metal analyses, Aluminum and Iron were chosen for Phase 2 testing due to them being very common pollutants from the road and easily purchasable.

Sample Identification:	UP	UP SLURRY	DOWN	DOWN SLURRY	RUNOFF	RUNOFF SLURRY
Sample Date:	7/23/2021	8/1/2021	7/23/2021	8/1/2021	7/29/2021	8/1/2021
Aluminum mg/L	<0.050	0.417	<0.0500	0.31	0.67	11
Iron mg/L	0.16	8.4	0.16	6.9	0.72	17

Chemical Testing Results Continued

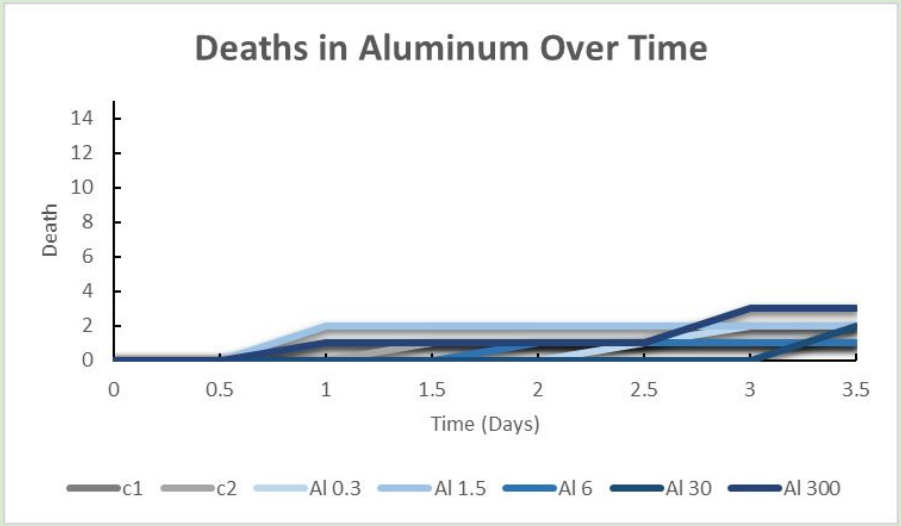
In further review of the analytical data, one of the most interesting observations was the presence of Polyaromatic Hydrocarbons (PAHs) in the Runoff Dirt sample and in the Down Sediment sample with no concentrations in the Up Sediment. PAHs are comprised of many chemicals and can be highly toxic. PAHs are commonly found in asphalt material and other petroleum products such as used motor oil.

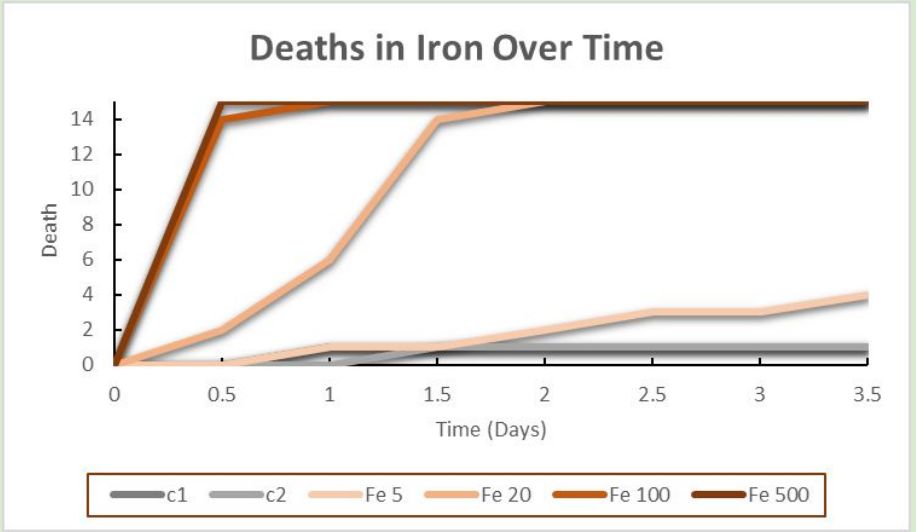
Sample Identification:	UP SEDIMENT	DOWN SEDIMENT	RUNOFF DIRT
Sample Date:	7/23/2021	7/23/2021	7/29/2021
Benzo(a)anthracene mg/kg	<0.096	0.4	0.86
Benzo(b)fluoranthene mg/kg	<0.096	0.3	0.94
Benzo(k)fluoranthene mg/kg	<0.096	0.37	1.1
Benzo(g,h,i)perylene mg/kg	<0.096	0.23	0.8
Benzo(a)pyrene mg/kg	<0.096	0.37	1
Chrysene mg/kg	<0.096	0.46	1.4
Fluoranthene mg/kg	<0.096	1	2.2
Phenanthrene mg/kg	<0.096	0.75	1.1
Pyrene mg/kg	<0.096	0.95	2.1

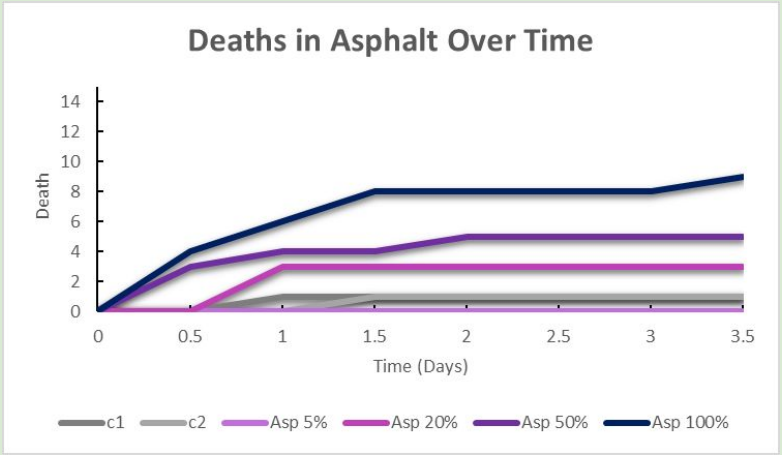
Chemical Standard Preparation and Bioassay

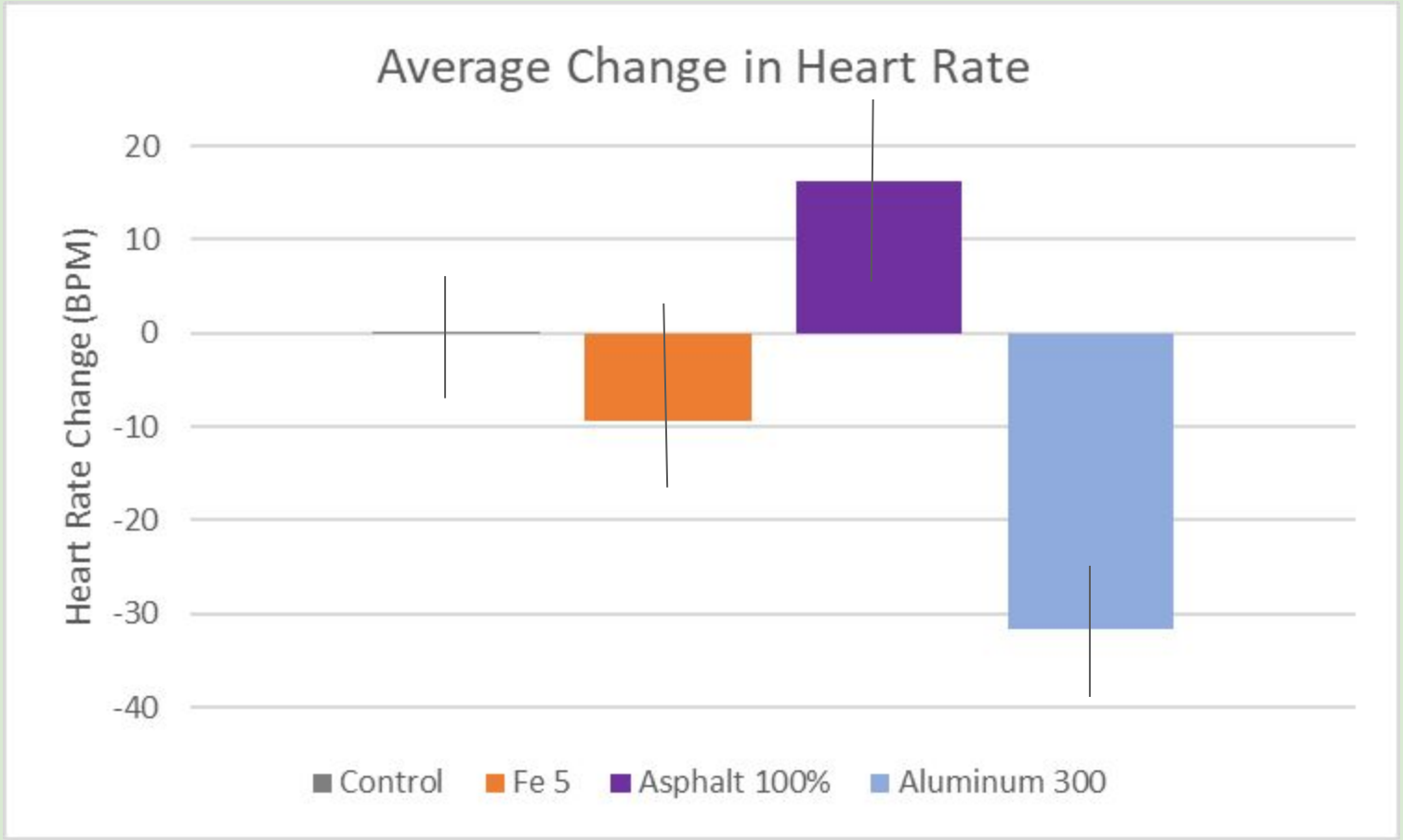
- Aluminum (aluminum oxide)
  - Ordered online then diluted
  - 0.3, 1.5, 6.0 and 30 mg/L
- Iron (ferrous iron)
  - Ordered online then diluted
  - 5.0, 20, 100, 500 mg/L
- Asphalt for a PAH samples
  - Collected from pothole
  - The material was screened and then mixed with spring water (50 grams to 1L) consistent with environmental testing protocols
  - 5%, 20%, 50% and 100% of mixture

These solutions along with 2 controls were placed into 250ml containers. Then 15 daphnia were added to each container for the bioassay. Deaths were counted every 12 hours.


Deaths in Aluminum Over Time
Death
Time (Days)
c1
c2
Al 0.3
Al 1.5
Al 6
Al 30
Al 300


Deaths in Iron Over Time
Death
Time (Days)
c1
c2
Fe 5
Fe 20
Fe 100
Fe 500


Deaths in Asphalt Over Time
Death
Time (Days)
c1
c2
Asp 5%
Asp 20%
Asp 50%
Asp 100%


Average Change in Heart Rate
Heart Rate Change (BPM)
20
10
0
-10
-20
-30
-40
Control
Fe 5
Asphalt 100%
Aluminum 300

Chemical Discussions

The chemical toxicity for Iron was inline with expected mortality at higher concentrations with the LD50 at 20 mg/L after 48 hrs. While the aluminum LD50 was greater then the high 300 mg/L solution which contradicted literature LD50 at 2.3 mg/L at 48 hrs. Interestingly the Daphnia had an unpredicted color change to a Whitish look at the higher Aluminum solutions. The Asphalt with an LD50 at 100% solution supports my hypothesis causing the toxin effect.

The Heart Rate data for both the Iron and Aluminum both had a decreased heart rate which I had expected, showing toxicity. However the Asphalt showed an increased heart rate suggesting a sort of adrenaline boost.

Conclusions

The Phase 1 data, both the heart rate and mortality testing, concurred with my hypotheses showing the effects of toxicity from greatest in the road runoff and downstream collections. In particular where results from the slurries were better representative due to the fact that more toxins will be settled there.

If I were to do this project again it might be interesting to look at shorter core depths when taking the sediment samples.

There was potential for human error during many processes which could have affected data slightly.

The Phase 2 data was also fairly inline with my hypothesis, although there were certain incongruences in the results. The most notable being how the Asphalt caused an increase in heart rate instead of a decrease and with the Aluminum turning the daphnia a whitish color. Further research and investigation would need to be done in order to determine the cause of these phenomenons.

If I were to do this project again I would look at other potential toxins I was not able to test in order to determine all factors in the system.

Additionally looking at more specific PAH's instead of just Asphalt would be interesting.

Project Significance

The Waquoit Estuary has great ecological value with marshes and wetlands, and runoff can have catastrophic effects to the entire ecosystem.

Determining toxicants in road runoff and realizing their effect to the ecosystem, and more specifically organisms, can educate the towns on the importance of protecting the streams and bays in our area.

This study points out that locations such as Martin Rd, which do not have collection basins, need attention and resources to remedy runoff issues.

Trial and Error

Collection of a rainfall event was challenging. First two collection attempts no rain occurred at location. However the third attempt was a success!

The first ever actual bioassay test of this was a complete catastrophe that lead to the death of pretty much all the daphnia fairly instantly. I believe this was most likely due to a spike of a high temperature experienced in room during this summer testing. I attempted to create a constant environment with the retest and all future bioassays.

During Phase 2, I washed some containers that were not dried properly and still contained detergent which led to another small round of deaths.

Sometimes this stuff happens and just goes to show that sometimes science can be very unpredictable.

Thank You to Marjot Foundation, CPWB, Kelsey Chenoweth, and Marine Biological Laboratory

- Marjot Foundation
  - One of four Awarded the Marjot Foundation grant for New England
  - Award provided funding for material costs and chemical testing to make project feasible
- Citizens for the Protection of Waquoit Bay - CPWB
  - Matt Patrick and Michael Bingham who learned of prior year study
  - Instigating the project with concern for the bay
- Kelsey Chenoweth
  - Project Mentor with extensive experience of Waquoit Bay estuary
  - Researcher at the Marine Biological Laboratory in Woods Hole
- Marine Biological Laboratory
  - Providing use of light microscope to use at home
  - Sophia Kelley for providing algae food for the *Daphnia magna*

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