

**Low Level Contaminant Effects on Benthic Macroinvertebrates
in Strawberry Creek, California**

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ABSTRACT

Chemical spills negatively impact the surrounding ecosystem when they occur. Biomonitoring is a method of study to quantify these impacts. To investigate how a spill would affect an urban stream, I conducted a laboratory experiment to study the impacts of low level contaminants on benthic macroinvertebrates from Strawberry Creek, California. I collected sediment from the stream and mixed in vegetable oil as the contaminant to create four treatment groups (control, low, medium, high). Various invertebrates were collected using a kicknet along the banks of the stream and individuals were placed into 16 microcosms. Each microcosm was a stoppered funnel, containing 1-3 centimeters of sediment, 1 liter of stream water, and an airstone connected to an airpump. 2-5 invertebrates were added to each funnel. Funnels were loosely covered with mesh. The experiment ran for one week and was replicated three times. Odonates had the highest 7-day survival rate (53.85%) and survived the highest average number of days (8.54 ± 6.80) across all treatment levels. Caddisflies had the lowest 7-day survival rate (18.19%) while mayflies and stoneflies survived the lowest average number of days (4.34 ± 5.13) across all treatment levels. Emergence and escape from the funnels occurred for mayflies and odonates. Natural resilience of the odonates may have played a role in their high survival rates while the morphology of the mayflies, stoneflies, and caddisflies may explain their low survival rates. Toxicity studies should be conducted then to assess impacts of chemical spills and results can help shape remediation efforts.

KEYWORDS

biomonitoring, vegetable oil, bioassay, toxicity study, microcosm

INTRODUCTION

When chemical spills occur, there is always a negative impact on the surrounding ecosystem. However, an ecosystem's response can differ depending on the source of the pollutant. There are two major sources of pollution: point source and non-point source. Point source pollution is defined as pollutants that originate from a single identifiable source, like a pipe or discharge from a factory (NOAA 2008a). Non-point source pollution is defined as originating from multiple sources and locations (NOAA 2008b) and can be much more diffuse in the environment. Chemical spills can fall under both categories; for example, an oil spill would be considered point source pollution whereas urban runoff would be considered non-point source pollution. Time can also play a role in how an ecosystem is affected by a chemical spill. Immediate impacts include organism mortality and loss of species diversity (Guzman et al. 1991) while long term impacts include delayed recovery and compromises to reproduction and health of organisms (Peterson et al. 2003). One aspect in these long term impacts is when the contaminants settle into the sediment and persist longer in the environment.

Because chemicals from spills settle into the sediment, benthic macroinvertebrates, aquatic insects, and other freshwater invertebrates can serve as tools for biological monitoring. Biological monitoring is a method to determine how human impacts can affect a living system. Benthic macroinvertebrates are typically used for biological monitoring of aquatic systems due to the ease of collecting and identifying them, their trophic level as the base of food webs, and community level studies of macroinvertebrates can determine the health of the system (Cairns, Jr. and Pratt 1993). It is important to conduct biological monitoring to identify ecological risks for all organisms (Karr and Chu 1997). Most biological monitoring studies are done at the community level as the presence of certain families of organisms can indicate toxicity levels (Lytle and Peckarsky 2001).

Conducting toxicity studies allows the determination of a contaminant's effect on organisms. However, most studies focus on the community level and use an assortment of organisms in order to draw a wider inference about a contaminant's effect (Cairns, Jr. and Pratt 1993). Inferences drawn from these studies can vary based on the length of the study or on the organisms used. Moreover, spills of large scales tend to garner more focus and attention due to their immediate severity on an ecosystem. A study assessing the impacts of a 26,500 liter diesel spill in New York revealed that invertebrate density at the spill locations were significantly lower

than the reference density. A year after this spill, invertebrate density was similar between the reference and spill locations (Lytle and Peckarsky 2001). Because of the focus on community level studies, there have not been many studies conducted where the focus is on specific organismal responses.

The objective of this study is to determine the effect of low level contaminants on benthic macroinvertebrates in Strawberry Creek. I examined the effect of vegetable oil as a contaminant on mayflies, odonates, stoneflies, and caddisflies in terms of their short-term survival, longevity, and behavioral responses. I hypothesize that low level contaminants will differentially and negatively impact the benthic macroinvertebrates from Strawberry Creek.

METHODS

Study site

Strawberry Creek is an urban stream in Berkeley, CA. The stream originates in Strawberry Canyon and flows through the University of California, Berkeley campus, after which the stream is culverted to flow under the city of Berkeley to the San Francisco Bay. The stream has always faced water quality issues and only within the past 30 years has water quality improved. In 1987 when the University of California, Berkeley created the Strawberry Creek Management Plan, water quality and ecological health improved through various restoration projects and other management practices. However, situated in an urban area the creek still faces some water quality issues. Current impacts to water quality in the stream include urban runoff and chemical spills. The most recent spill occurred in December 2011 when an emergency generator spilled approximately 1,650 gallons of diesel fuel into the basement of UC Berkeley's Stanley Hall; while most of the spill was contained within the building, a portion did enter the stream near the Wickson Bridge through a storm drain (Karlman 2011).

Study organisms

To determine the toxicity of low level contaminants in Strawberry Creek to benthic macroinvertebrates, I conducted bioassays using benthic invertebrates collected from the Faculty Glade portion of Strawberry Creek on the UC Berkeley campus (Figure 1). I used a kicknet to collect the macroinvertebrates from Strawberry Creek and collected water and sediment for the microcosms experiment. At the University of California, Berkeley’s Resh Lab, I sorted the collected organisms into families. The families used in this experiment varied because of seasonal availability.

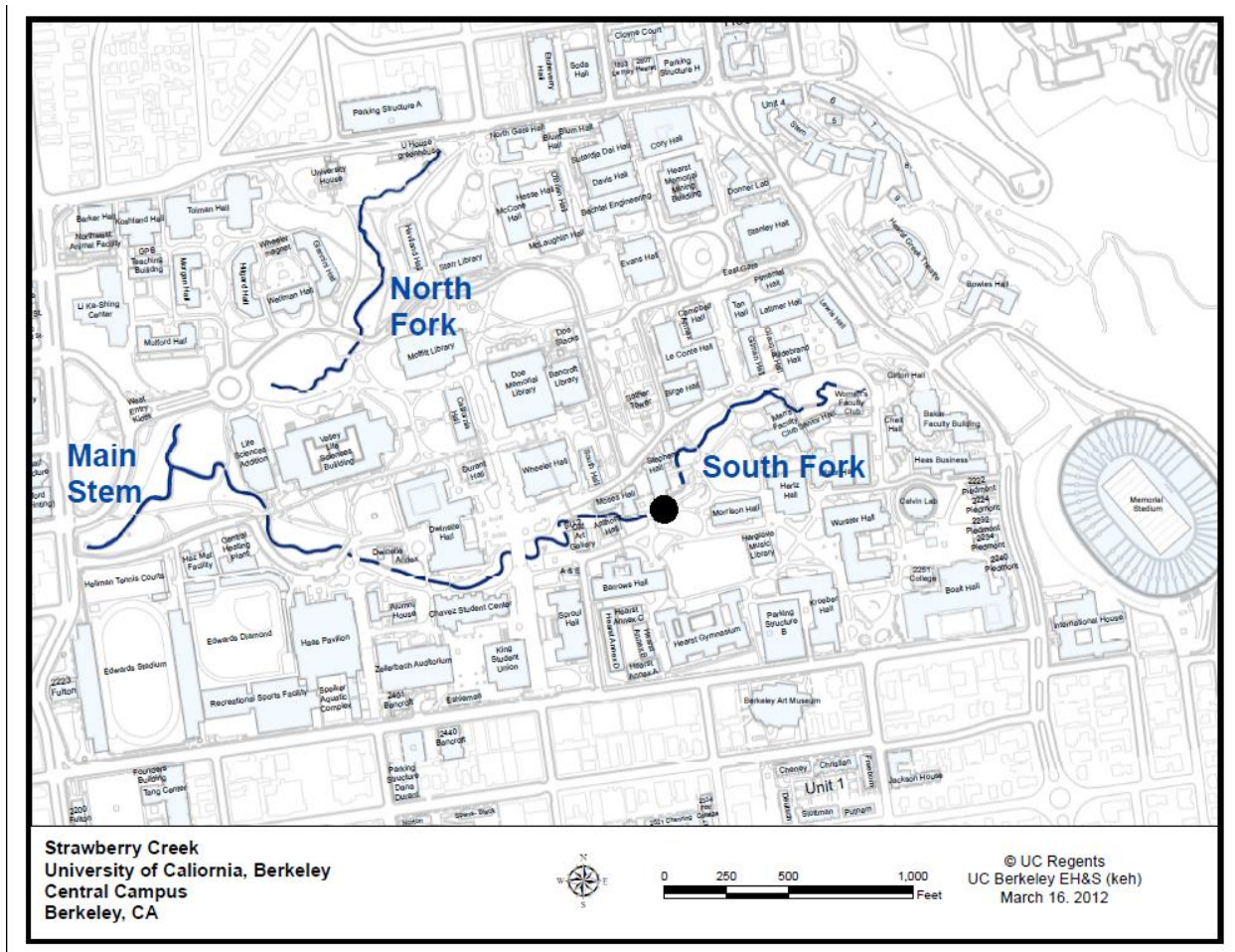


Figure 1. A map of Strawberry Creek as it runs through the University of California, Berkeley Campus. The creek starts in Strawberry Canyon (east of campus) and runs through campus before being culverted under the city of Berkeley (west of campus). The black circle denotes the location of sample collection, Faculty Glade.

Experimental design

To determine the effect of sediment contamination on benthic macroinvertebrates, I adapted a toxicity assay from an amphipod assay by Borgmann, Norwood, and Nowierski (Blaise and Ferard 2005). The experimental design consists of sixteen 48 oz. funnels in a Styrofoam stand (Figure 2) under four treatment levels (control, low concentration, medium concentration, and high concentration). I stoppered the narrow end of each funnel with a cork and used silicone based adhesive sealant to prevent leaks.



Figure 2. A picture of the experimental design. 16 funnels were spread over five rectangular pieces of Styrofoam.

Using vegetable oil as the contaminant, I separated the collected sediment into four plastic containers and added the necessary amount of oil to create my treatment groups. For the control sediment, no amount of oil was added. To create the low concentration sediment, I mixed 100 mL of oil into the sediment. I created the medium concentration and high concentration sediment groups by mixing 300 mL of oil and 500 mL of oil, respectively, into the respective container. The containers of sediment were mixed and then left to sit for at least three days. After creating the contaminated sediment, I put approximately 2 cm of “low” sediment into four funnels, 2 cm of

“medium” sediment into another four funnels, 2 cm of “high” sediment into another four funnels, and 2 cm of “control” sediment into the last four funnels. After all the funnels had sediment, I carefully pipetted approximately 1 liter of stream water into each funnel to reduce the chance of resuspension of the sediments. After the addition of water, I placed an air pump and air stone into each funnel. I added in two to five organisms per funnel (Figure 3). I ran the bioassay for a seven day period. I performed three trials (Blaise and Ferard 2005).

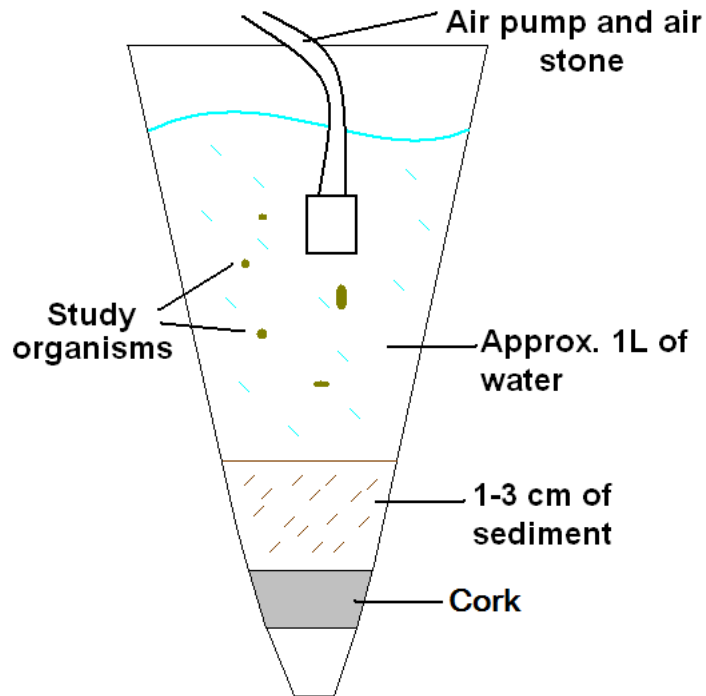


Figure 3. A schematic of the funnel microcosm used in the experiment.

Data collection

To determine how the invertebrates were affected by the presence of contaminants in their environment, I made several observations related to mortality, longevity, and behavioral responses. I recorded how long each individual lived and how many individuals died during the one week experimental period. I also recorded how many days each individual lived after the one week experimental period. Once an individual was discovered to be dead, it was removed from the microcosm and placed into a vial for identification purposes. I also observed the behaviors of

treatment and control individuals to determine if any differences were present. Only individuals that could be conclusively identified to the family level were used for data computation.

RESULTS

The study organisms were identified to belong to the following families: Baetidae, Leptophlebiidae, Heptageniidae, Nemouridae, Lepidostomatidae, Polycentropodidae, and Coenagrionidae.

Macroinvertebrate assays

7-day survival rate

Over the seven day experimental period, survival rate across all study organisms and treatment groups was highest in the odonates and lowest in the caddisflies, with the survival rate of mayflies and stoneflies in the middle (Figure 4). Mayflies and stoneflies had a survival rate of 20.69%, caddisflies had a survival rate of 18.19%, and odonates had a survival rate of 53.85%.

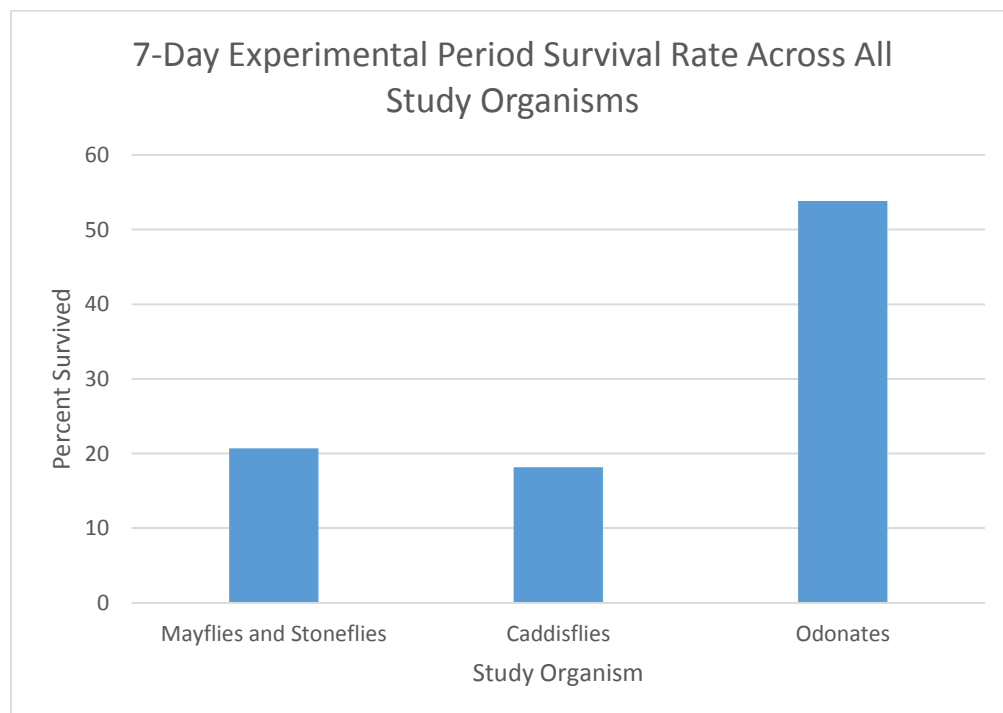


Figure 4. 7-Day Experimental Period Survival Rate across All Study Organisms and Treatment Groups. A bar chart depicting the survival rate of each study organism over the seven day experimental period. The bars were generated by aggregating all treatment groups under a study organism. Mayflies and stoneflies totaled 29 individuals, caddisflies totaled 11 individuals, and odonates totaled 13 individuals.

Within the mayflies and stoneflies study group, the medium treatment group had the highest 7-day survival rate while low and high treatment groups had the lowest survival rate (Figure 5). Control had a survival rate of 37.5%, low had a survival rate of 0%, medium had a survival rate of 60%, and high had a survival rate of 0%.

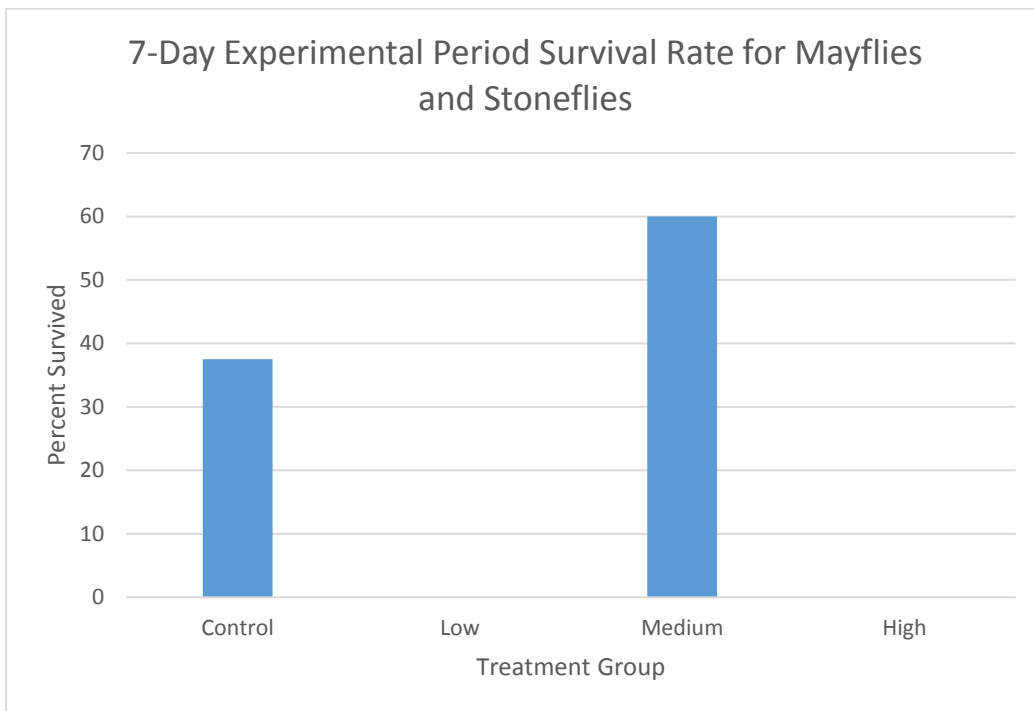


Figure 5. 7-Day Experimental Period Survival Rate for Mayflies and Stoneflies. A bar chart depicting the survival rate for mayflies and stoneflies over the seven day experimental period. The control group had 8 individuals, the low group had 9 individuals, the medium group had 5 individuals, and the high group had 7 individuals.

Within the caddisflies study group, the low treatment group had the highest 7-day survival rate while medium and high treatment groups had the lowest survival rate (Figure 6). Control had a survival rate of 25%, low had a survival rate of 33.33%, medium had a survival rate of 0%, and high had a survival rate of 0%.

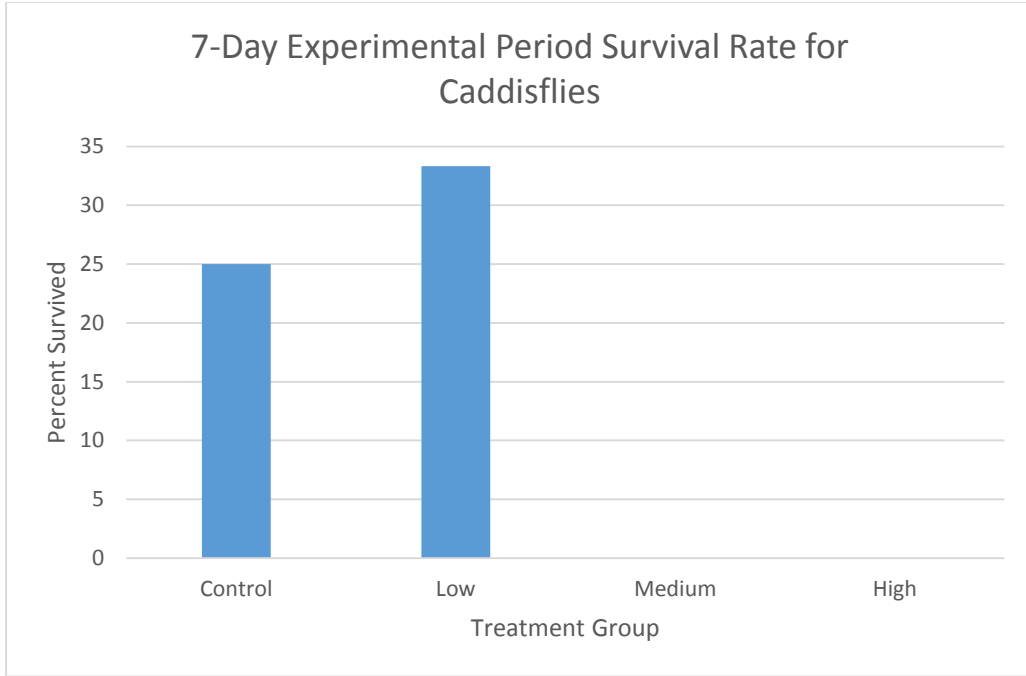


Figure 6. 7-Day Experimental Period Survival Rate for Caddisflies. A bar chart depicting the survival rate for caddisflies over the seven day experimental period. The control group had 4 individuals, the low group had 3 individuals, the medium group had 1 individual, and the high group had 3 individuals.

Within the odonates study group, the low treatment group had the highest 7-day survival rate while the high treatment group had the lowest survival rate (Figure 7). Control had a survival rate of 50%, low had a survival rate of 80%, medium had a survival rate of 50%, and high had a survival rate of 0%.

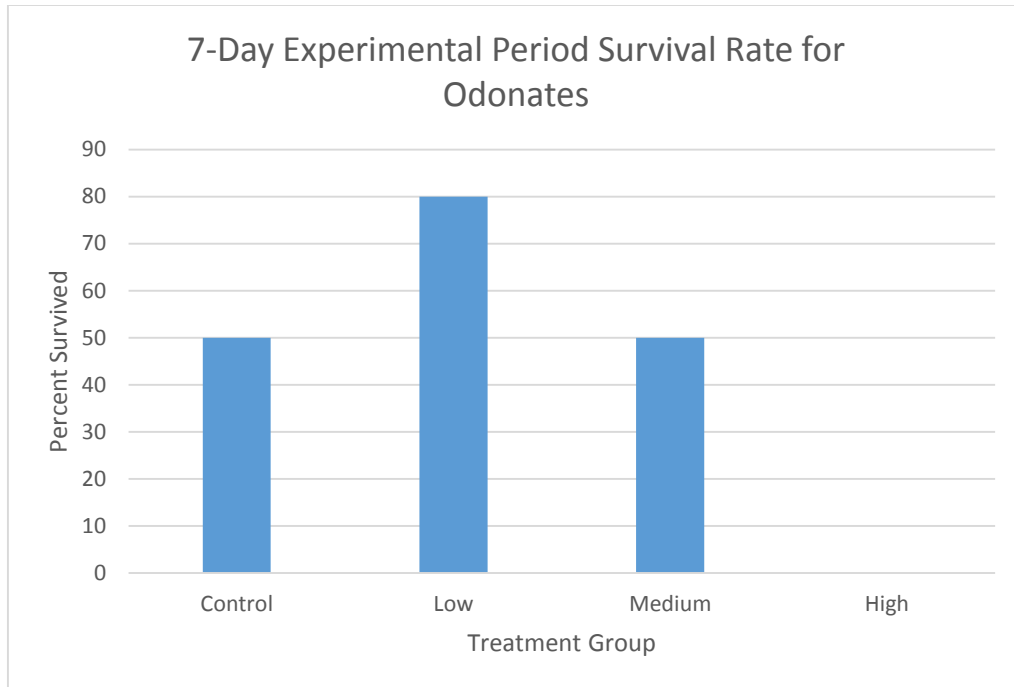


Figure 7. 7-Day Experimental Period Survival Rate for Odonates. A bar chart depicting the survival rate for odonates over the seven day experimental period. The control group had 4 individuals, the low group had 5 individuals, the medium group had 2 individual, and the high group had 2 individuals.

Longevity

Across all study organisms and treatment groups, long term survival was highest in the odonates and lowest in the mayflies and stoneflies, with the number of days survived by caddisflies in the middle (Figure 8). The average number of days survived and their respective standard deviations are reported in Table 1.

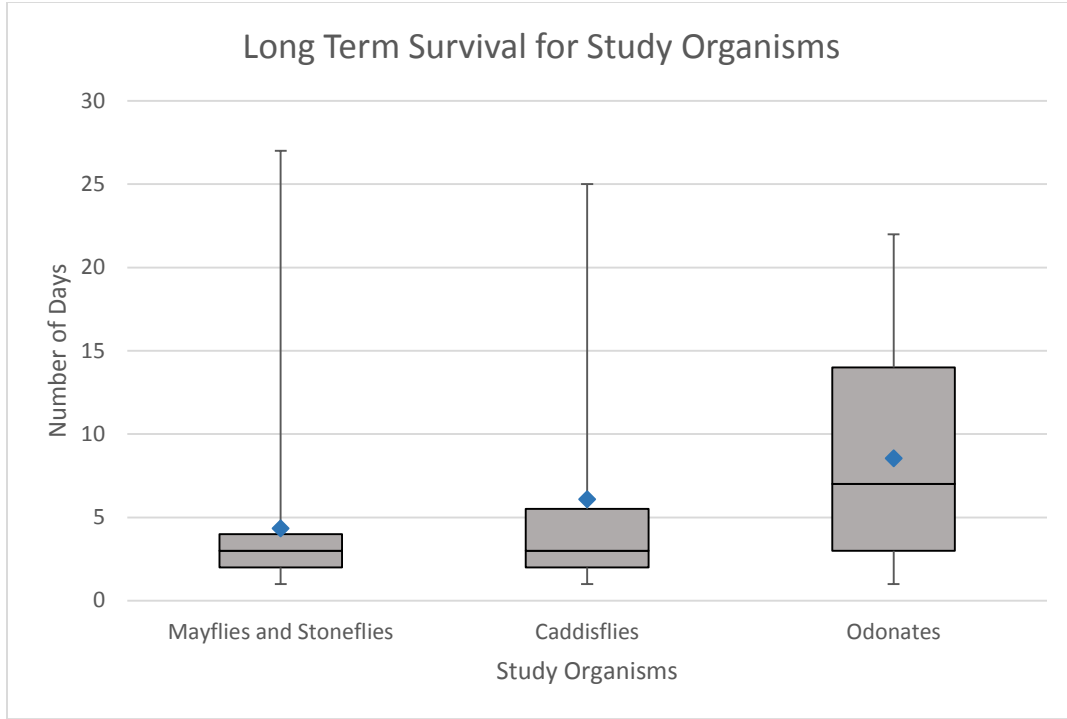


Figure 8. Long Term Survival for All Study Organisms and Treatment Groups. A boxplot depicting the long term survival of study organisms. The boxes were generated by aggregating all treatment groups under a study organism. The average number of days survived (blue marker) by mayflies and stoneflies, caddisflies, and odonates are 4.34 ± 5.13 days, 6.09 ± 7.37 days, and 8.54 ± 6.80 days, respectively. Mayflies and stoneflies totaled 29 individuals, caddisflies totaled 11 individuals, and odonates totaled 13 individuals.

Table 1. Average number of days survived by each group of study organisms. A table summary of the average number of days survived by each group of study organisms. The data was generated by aggregating all treatment groups under a study organism. Mayflies and stoneflies totaled 29 individuals, caddisflies totaled 11 individuals, and odonates totaled 13 individuals.

Study Organism	Average Number of Days Survived
Mayflies and Stoneflies	4.34 ± 5.13
Caddisflies	6.09 ± 7.37
Odonates	8.54 ± 6.80

Within the mayflies and stoneflies study group, long term survival was highest in the control group and lowest in the high group (Figure 9). The average number of days survived and their respective standard deviations are reported in Table 2.

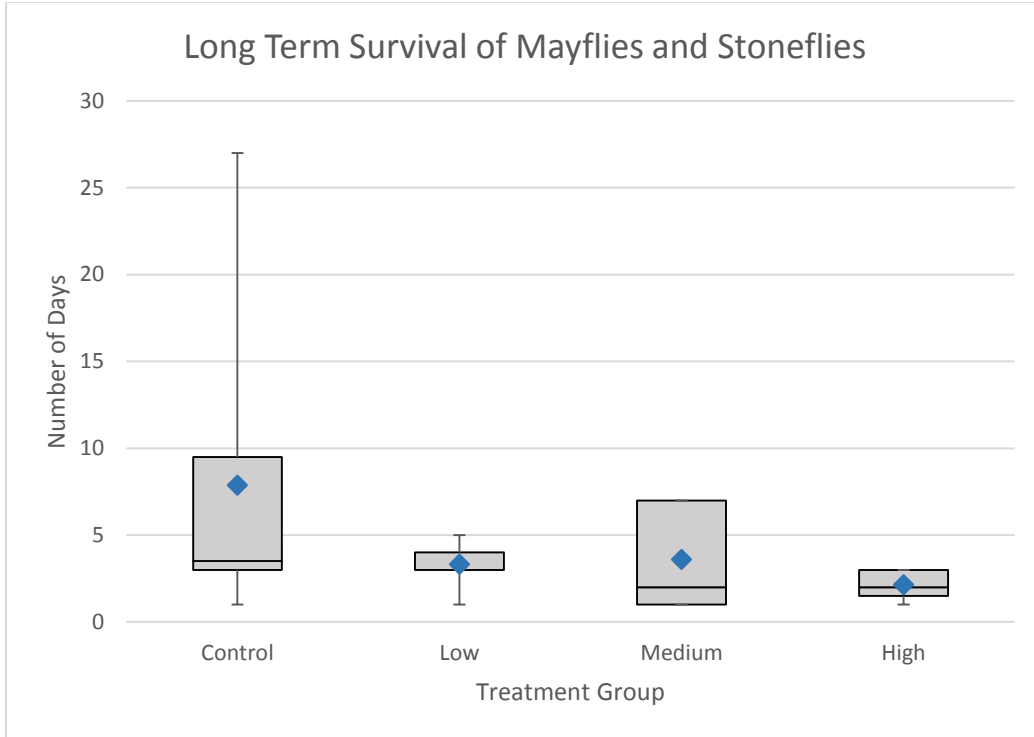


Figure 9. Long Term Survival for Mayflies and Stoneflies. A boxplot depicting the long term survival of mayflies and stoneflies by treatment groups. The average number of days survived (blue marker) by control, low, medium, and high are 7.88 ± 8.76 days, 3.33 ± 1.12 days, 3.60 ± 3.13 days, and 2.14 ± 0.90 days, respectively. The control group totaled 8 individuals, the low group totaled 9 individuals, the medium group totaled 5 individuals, and the high group totaled 7 individuals.

Table 2. Average number of days survived by mayflies and stoneflies in each treatment group. A table summary of the average number of days survived by mayflies and stoneflies by treatments groups. The control group totaled 8 individuals, the low group totaled 9 individuals, the medium group totaled 5 individuals, and the high group totaled 7 individuals.

Concentration	Average Number of Days Survived
Control	7.88 ± 8.76
Low	3.33 ± 1.12
Medium	3.60 ± 3.13
High	2.14 ± 0.90

Within the caddisflies study group, long term survival was highest in the control group and lowest in the high group (Figure 10). The average number of days survived and their respective standard deviations are reported in Table 3.

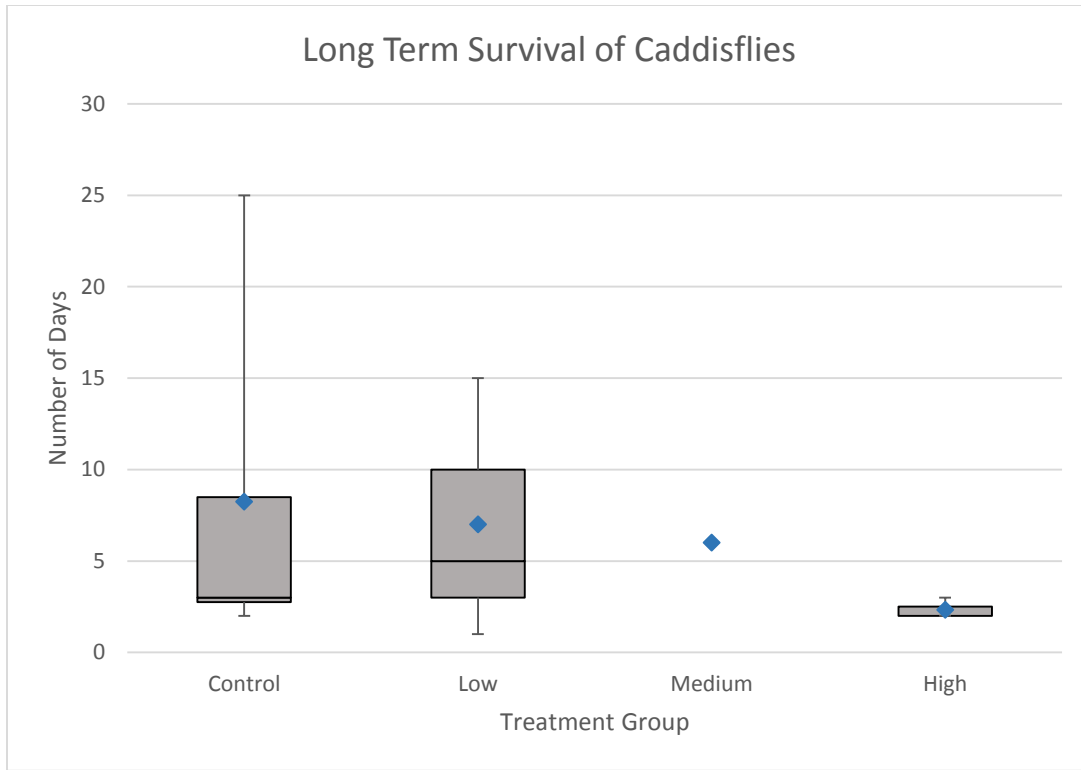


Figure 10. Long Term Survival for Caddisflies. A boxplot depicting the long term survival of caddisflies by treatment groups. The average number of days survived (blue marker) by control, low, medium, and high are 8.25 ± 11.18 days, 7.00 ± 7.21 days, $6.00 \pm *$ days, and 2.33 ± 0.58 days, respectively. The control group had 4 individuals, the low group had 3 individuals, the medium group had 1 individual, and the high group had 3 individuals.

Table 3. Average number of days survived by caddisflies in each treatment group. A table summary of the average number of days survived by caddisflies by treatment groups. *: There is no standard deviation for caddisflies in medium treatment because there was only one individual that could be conclusively identified. The control group had 4 individuals, the low group had 3 individuals, the medium group had 1 individual, and the high group had 3 individuals.

Concentration	Average Number of Days Survived
Control	8.25 ± 11.18
Low	7.00 ± 7.21
Medium	$6.00 \pm *$
High	2.33 ± 0.58

Within the odonates study group, long term survival was highest in the control group and lowest in the high group (Figure 11). The average number of days survived and their respective standard deviations are reported in Table 4.

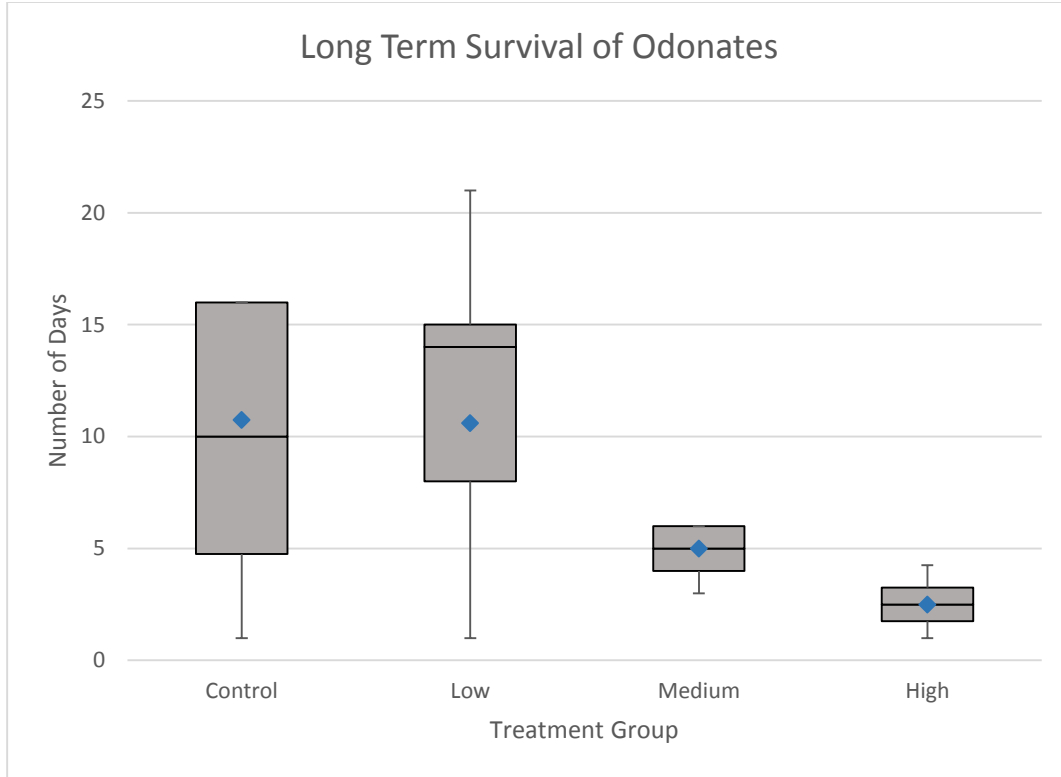


Figure 11. Long Term Survival for Odonates. A boxplot depicting the long term survival of odonates by treatment groups. The average number of days survived (blue marker) by control, low, medium, and high are 10.75 ± 9.22 days, 10.60 ± 6.11 days, 5.00 ± 2.83 days, and 2.50 ± 2.12 days, respectively. The control group had 4 individuals, the low group had 5 individuals, the medium group had 2 individual, and the high group had 2 individuals.

Table 4. Average number of days survived by odonates in each treatment group. A table summary of the average number of days survived by odonates by treatment groups. The control group had 4 individuals, the low group had 5 individuals, the medium group had 2 individual, and the high group had 2 individuals.

Concentration	Average Number of Days Survived
Control	10.75 ± 9.22
Low	10.60 ± 6.11
Medium	5.00 ± 2.83
High	2.50 ± 2.12

Behavioral responses

Each group of study organism exhibited different behavioral responses to the experiment. Molting was observed during the experiment in the mayflies and stoneflies and the odonates. Furthermore, some of the mayflies that molted had emerged to their subimago stage but were

unable to escape from the funnels and instead became stuck in the oil. However, it was observed that some individuals from the mayflies and stoneflies and odonates groups did manage to escape as some bodies were not found.

DISCUSSION

Results from biological monitoring studies of urban streams can provide insight on how to remediate freshwater environments after chemical spills. The results gained from this experiment support my hypothesis that benthic macroinvertebrates of Strawberry Creek were differentially and negatively impacted by low-level contaminants in the sediment. Although a majority of the replicates did not survive the one week experimental period, odonates stood out in their high number of days survived across the treatment groups. Moreover, it was observed that each group of organisms displayed different behavioral responses to the treatment groups, with odonates and mayflies attempting to leave the microcosms with some success. Overall, the high concentration treatment group was most toxic to all four groups of study organisms.

Short-term survival

Across all treatment groups, the odonates had the highest survival rate while caddisflies had the lowest. The odonates' high survival rate suggests that out of the four groups of study organisms, they were the least impacted by low level contaminants. The odonates' high survival rate observed in this experiment can possibly be attributed to their natural resilience related to their position in freshwater food webs. Larval odonates are oftentimes the most conspicuous predators of other macroinvertebrates (Wissinger 1988). Due to biomagnification, predators will accumulate higher amounts of contaminants in their systems as they mature. A higher resilience to these contaminants would ensure survival of the species. This resilience likely played a role in why the experiment's odonates survived the short-term period; the level of contamination in the experiment was within a tolerable range. Similar reasoning likely explains the low survival rate of the caddisflies. Caddisflies are both a low trophic level food source and decomposition facilitators and also rely heavily on their surrounding environments to build their cases and nets in their larval

stages (Whiles et al. 1999). Therefore, caddisflies interact directly with the environment and any contaminants present would have a large impact on their survival.

Morphology and physiology may also play a role in why odonates had the highest survival rate during the one week experimental period. The study organisms had different mechanisms for respiration and their respiratory organs (gills) are also in different locations (Eriksen, Resh, and Lamberti 1996). In mayflies, stoneflies, and caddisflies, the respiratory organs are located on the ventral side, usually along their abdomen or near the neck (Edmunds, Jr. and Waltz 1996, Stewart and Harper 1996, Wiggins 1996). Because of their location and the benthic macroinvertebrates' preferred habitat at the bottom of streams, if the sediment is contaminated it is highly likely for the gills to be affected due to contact with the contaminants. Furthermore, these groups' gills are tracheal and tracheal gills are most sensitive to contaminants; toxicants can damage the respiratory surface and cause death through respiratory stress (Eriksen, Resh, and Lamberti 1996). While the gills of odonates are also tracheal (Eriksen, Resh, and Lamberti 1996), their location at the ends of their tails limits the exposure to contaminated sediments. Furthermore, North American species of odonates are able to shed their gills and grow new ones (Westfall and Tennessen 1996). This suggests the possibility that the odonates in my experiment could have shed their contaminated gills, which would increase their survival rate by reducing their respiratory stress.

Longevity

Odonates had the highest average number of days survived across all treatment groups and study organisms which suggests that it may be possible for them to survive in a contaminated environment until the system recovers. Bioassessment by the U.S. Environmental Protection Agency has shown that odonates are relatively more tolerant than the other study organisms (mayflies, stoneflies, and caddisflies). Odonates are reported to have a northwest regional tolerance value of 9 out of 10, while mayflies, stoneflies, and caddisflies have a northwest regional tolerance value ranging from 2-4 (Barbour et al. 1999). A community level toxicity study has also shown that more tolerant taxa tend to dominate a region that has been affected by a chemical spill (Lytle and Peckarsky 2001). As odonates were the most tolerant taxa in my study, it is within natural trends for them to have survived a higher average number of days compared to the mayflies, stoneflies, and caddisflies.

Behavioral responses

Both mayflies and odonates attempted escape from the microcosms with some success which suggests the possibility that the two families were replicating a natural response. Within the experimental group of mayflies, some individuals emerged and managed to escape from the microcosm while some became stuck in the oil that had risen to the surface. With the odonates, individuals likely escaped from the microcosms by crawling out of the funnels due to the loose covering of mesh. This escape behavior has been observed in another study where invertebrates were exposed to a contaminant (Beyers 1998). The observed escape behavior is important as it gives organisms the possibility to leave contaminated areas for cleaner environments and return when the contaminated environment has recovered to normal conditions. The shift in species composition of a contaminated area has been noted in a study where less tolerant taxa were not very present in the area compared to more tolerant taxa (Gerhardt, Janssens de Bisthoven, and Soares 2004). In another study where the contaminated area was studied again a year later after recovery of the system, it was noted that composition of the species between the previously contaminated locations and the reference locations were similar (Lytle and Peckarsky 2001). These reinforces the possibility that the study organisms exhibited the escape behavior in an attempt to leave the contaminated environment until the environment had recovered.

Effects of oil

The negative impact of oil in the sediment concurs with results from another vegetable oil study. A study in a freshwater wetland that was impacted by a vegetable oil spill yielded similar results where more tolerant taxa in the environment were favored (Selala et al. 2013). The authors concluded that these more tolerant taxa can be used as indicators specifically for vegetable oil spills. In Strawberry Creek, odonates may be better suited for being indicators in vegetable oil spills than mayflies, stoneflies, and caddisflies. Although in the long term, the impact of vegetable oil would gradually decrease as they biodegrade faster compared to other oils (Anand and Chhibber 2006). However, vegetable oil may remain in the environment for up to six years (Selala et al. 2013) and relative to the lifecycles of benthic macroinvertebrates (1-2 years), this length of

time can still have a significant effect on the organisms. Clay may reduce the concentration of vegetable oil as it would sequester the oil into the sediment, allowing for anaerobic bacteria to begin its decomposition (Selala et al. 2013). During my experiment, occasionally oil from the sediment would float up to the surface and solidify. Using clay to sequester the oil would likely reduce this phenomenon from occurring.

Limitations and future directions

Contaminants can range from heavy metals to oils to agricultural runoff and thus results from one spill study will likely not predict exactly what would happen after a different spill. My experiment used vegetable oil as its contaminant, thus my conclusions are not as applicable for how benthic macroinvertebrates may be affected by other contaminants. My experimental design adequately addressed my hypothesis as it allowed me to achieve low levels of contaminant through the high water-low sediment ratio. However, the quality of my data was compromised due to escaped individuals. Because their escape was not directly inferred and only assumed, my data was not as accurate as possible. Furthermore, the conclusions from this study would also not apply to all streams, as urban streams respond differently compared to other types of streams due to their higher loads of nutrients from runoff and increased impervious cover (Paul and Meyer 2001).

Further studies in a similar area as this project could investigate the effect of a different contaminant on Strawberry Creek. Because Strawberry Creek is an urban stream, pharmaceuticals and even personal care products can end up in the water. A study was recently conducted to determine the impacts of such contaminants on algae (Wilson et al. 2013). Diesel is also a possible contaminant to research as a diesel spill has occurred in the stream in the past. Another approach would be to investigate the effects of vegetable oil over a longer period of time, possibly one year as most of the study organisms used in this project have a one year life cycle. It is also possible to replicate this study with a different stream to determine if there would be differences in results. To develop a more focused study, the same experiment can be conducted on only a single group of organisms to observe more detailed responses.

CONCLUSIONS

The results of this study show that benthic macroinvertebrates in Strawberry Creek are affected negatively and in different ways by low-level contaminants. The results fill in the knowledge gap in benthic macroinvertebrate toxicity studies as most studies focus on the community level but not on specific bioassay responses. The study also highlights the importance of toxicity studies as such studies can be used to determine the health of a stream. The presence of more tolerant taxa can indicate that the environment is of poor quality. This study has also raised the suggestion that clay can be used a remediation tool to reduce the impact of vegetable oil spills.

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