# The Effects of Motor Oil on Aquatic Insect Predation

**Abstract:** Modern technology and urbanization have brought about some important consequences for freshwater ecosystems. Urban run-off has carried a number of new pollutants, including motor oil and other petroleum products, into urban and suburban ponds, streams and rivers. To date, not much investigation has been carried out in regards to the effects of this oil pollution on freshwater organisms. In this experiment I investigated the effects of different concentrations of motor oil on dragonfly nymph activity and dragonfly consumption of damselfly nymphs in laboratory microcosms. The activity level and predation rate was monitored in four different concentrations of motor oil (.1%, .5%, 1%, 2%) as well as in a control. Results indicated a significant relationship between predation rate and oil concentration, but no correlation was found between dragonfly activity level and oil concentration. These results have implications for maintenance of aquatic species diversity as well as trophic interactions beyond the aquatic system, as both dragonfly and damselfly nymphs are important predator and prey items in aquatic food webs.

## Introduction

Pollution of aquatic environments by petroleum products is an issue of concern to many ecologists and environmentalists. While it receives a lot of attention after major commercial oil spills, this is not in fact the primary source of oil pollution. According to a report by the National Academies' National Research council, 29 million gallons of petroleum products enter North American waters annually from anthropogenic sources, 85% of which is from land runoff, oil-contaminated rivers, airplanes and small watercraft. All this is in addition to the 47 million gallons that naturally seep into waters through the ocean floor (Coleman, et al., 2003).

Petroleum products can have several negative consequences for aquatic ecosystems. The major constituents of petroleum products are complex hydrocarbons, comprising 80-90% of unused motor oil (Irwin, et al., 1997). Of all these hydrocarbon groups, polycylic aromatic hydrocarbons (PAHs) are thought have the most toxic effects, as they attach easily to sediment surfaces and are relatively resistant to degradation (Vo, et al., 2004). Oil slicks formed at the surface can potentially limit oxygen exchange at the water-air boundary, decreasing the levels of dissolved oxygen in the water. Oil at the surface also has the potential to coat the gills of aquatic organisms, adversely affecting respiration (Bhattacharyya, et al., 2003).

Not many studies have been done to investigate the effects of oil pollution on freshwater systems, as most large-scale oil spills occur in marine environments. However, studies on the effects of the more general category of freshwater pollutants, which include oils/grease, but also heavy metals and other toxic pollutants, have been done. In fact, aquatic insects are often used as an indicator of water quality, a concept known as biomonitoring. Benthic invertebrates are the most frequently used organisms in biomonitoring, as they are a diverse group of organisms which exhibit varying degrees of tolerance to environmental conditions (Merritt and Cummings, 1996). Insects of the order *Odonata*, which includes damselflies and dragonflies, are known to be moderately sensitive to environmental pollutants, and their absence from a particular habitat is frequently indicative of poor water quality. In an experiment investigating the effects of urban runoff on stream water quality, benthic organisms were surveyed upstream and downstream of an urban development. The prevalence of dragonflies was found to be significantly higher at upstream sites (22%) than at downstream sites (4%). It was concluded that this was a result of pollution and eutrophication decreasing the levels of dissolved oxygen in the stream sites downstream of the development (DeBarruel and West, 2003).

Odonates are particularly sensitive to dissolved oxygen levels. According to Merritt and Cummings (1996) "a major challenge for any aquatic insect is to obtain sufficient quantities of oxygen for its metabolic needs" (36). For dragonflies and damselflies this is particularly true due to their closed tracheal systems; rather than obtaining oxygen through direct contact with air, their tracheal gills uptake oxygen dissolved in the water (Merritt and Cummings, 1996). Therefore any circumstance which decreases dissolved oxygen levels has negative repercussions for dragonfly and damselfly respiration.

For this experiment, I set up a laboratory system to investigate the effects of different concentrations of motor oil on dragonfly nymphs and their predatory interactions with damselfly nymphs. The dependence of dragonflies and damselflies on relatively high levels of dissolved oxygen, and oil's effects on dissolved oxygen concentration led me to hypothesize that adding motor oil to simulated pond environments would affect activity levels in dragonflies. I predicted that an increase in concentration of motor oil would lead to lower activity levels in dragonflies.

The activity levels of both dragonflies and damselflies have implications for predation. Both dragonflies and damselflies are predatory insects, but presumably due to size advantage, dragonflies prey on damselflies and not vice-versa. Dragonflies are generally ambush predators, waiting for prey to approach before attacking, but will occasionally switch to a more active foraging strategy if food availability is low (Gullan and Cranston, 2005). They rely primarily on tactile cues in searching for prey but also take advantage of visual cues (Resh and Rosenberg, 1984).

As a corollary to my first hypothesis, I hypothesized that the addition of oil to the environments would have an effect on predation rates. A decrease in activity of damselflies (prey) would result in fewer visual cues for dragonflies and a decrease in activity level of dragonflies would lead to less active foraging behavior. Therefore, I predicted a negative correlation between oil concentration and number of prey eaten.

## Materials and Methods

I set up five clear plastic containers and filled each with 2 liters of pond water in an attempt to most effectively simulate a natural freshwater habitat. The first container was a control with no oil added, and the four successive containers were contaminated with increasing concentrations of unused motor oil (.1%, .5%, 1% and 2%). I collected dragonfly and damselfly nymphs from Willow Pond at Matthaei Botanical Gardens, and added one dragonfly predator to each container along with multiple damselfly prey. I performed two replicates of the experiment; two damselflies were placed with each dragonfly in the first replicate and six damselflies in the second. [Note: I misidentified several of the prey items I added to the containers in the $2^{nd}$ replicate, meaning that some containers contained a mixture of mayflies and damselflies. However, I don't believe this should have an effect on the experiment as both are preyed upon by dragonfly nymphs].

After placing the insects in the containers, I collected data on their activity level by tracking their movement at ten minute intervals. Every ten minutes I marked the approximate location of the dragonfly and then noted whether it moved from that spot in the following time interval. I collected activity data a second time after the insects had been exposed to the oil for five days. During each collection I recorded data for five time intervals.

In order to determine the predation rate, I counted the number of surviving damselflies after five days and then determined the number of prey eaten. I divided this by the initial number of prey items in order to determine the percentage of prey eaten. I used percentage of prey eaten rather than number of prey eaten to standardize the measurements for the two replicates, since they had different initial numbers of prey. Because I wanted to determine how a range of concentrations affected percentage of prey eaten, I performed a linear regression on the data.

## Results

The graph of the linear regression examining the relationship between oil concentration and dragonfly activity showed a very weak negative correlation between the two variables (Figure 2). The very low R squared value (.034) reveals that the best fit line is not an accurate reflection of the data at all. Furthermore, the p-value is well over .05 (.612) meaning that the slope is not significantly different from zero.

The graph of the linear regression relating percentage of prey eaten to oil concentration showed an overall negative correlation (slope = -.378) (Figure 1). The standard error measurement for the slope is relatively high as there are many data points which are quite far from the line. The R squared value of .527 indicates that the best fit line is a satisfactory representation of the data but is not exceptionally accurate. The low p-value (.017) denotes a significant relationship between the two variables.

## Discussion

The results do not support my first prediction of a negative correlation between oil concentration and dragonfly activity. The high p-value suggests that the activity data are a result of random chance and hence no significant relationship exists between the two variables. There are several possible explanations for this. First of all, more data would be necessary to make any solid conclusions about the activity level of dragonfly nymphs. With such a small sample size (four data points in two replicates) results are unlikely to show concrete trends, as any outliers have a significant effect. Secondly, dragonfly nymphs may not be particularly active organisms in general, as they are “sit and wait” predators and do not resort to active foraging unless it becomes necessary for survival (Gullan and Cranston, 2005). Lastly, activity measurements were taken during the day, while dragonflies tend to be more active at night as an avoidance mechanism from predation by fish and other aquatic organisms (Merritt and Cummins, 1996). Taking more activity data over a longer period of time, with some collections being done at night would likely provide a clearer picture of the relationship between oil pollution and activity levels.

It would have been helpful to measure the dissolved oxygen levels in the different environments, as the whole notion of the decreased activity levels was predicated on the notion that surface oil inhibits diffusion of oxygen through the water. Some studies suggest that it takes several months of exposure to oil before a measurable difference in oxygen concentration is detectable. Harrel (1985) studied the effects of an oil spill in a Texas stream, comparing the water quality of the contaminated stream with that of a nearby control stream. Four days after the initial oil spill, oil was visible on the water surface and there was a strong smell of hydrocarbons, but measurements of water quality did not differ much between the two streams. Not until six months later were decreases in dissolved oxygen concentration apparent (Harrel, 1985). If this were the case in our system, then no difference in activity levels would be expected after only five days.

Though prediction 1 was not supported, prediction 2, regarding the relationship between oil concentration and predation rate, was supported. The regression line showed a statistically significant negative correlation between the two variables. The reasons for these results are not entirely clear, however, nor are the results free of statistical flaws. One of the biggest statistical problems I encountered was due to the fact that I had different numbers of initial prey items in the two replications. While I tried to standardize the predation measurements by examining the percentage of prey eaten as opposed to the number of prey eaten, this did not completely solve the problem, because there was not a continuous range of possible values for the percentage. For the first replicate, because there were only two prey, there were only three incremental possibilities for predation percentage, 0%, 50% and 100% while in the 2$^{nd}$ replicate there was a broader yet still incremental range (0, 1/6, 1/3, ½, etc). Statistically it would have been far better to have six initial prey items in both replicates.

While my initial hypothesis that decreased oxygen levels would lead to less active foraging behavior from the dragonflies and fewer movements (i.e. visual cues) by the damselfly may still hold, it is called into question by the failure of prediction 1. An alternative possibility is that the thick, opaque film formed on the top of the water blocked a large portion of the incoming light and made it more difficult for the dragonflies to clearly see prey. It is also possible that ingestion of toxic water-soluble components of oil led to changes in the predator's ability to search for or attack prey.

There is a compelling possibility that the number of prey eaten was more strongly correlated to predator size than to concentration of oil. I did not take quantitative data on predator size, but based on my observations, those predators that ate the largest percentage of prey also tended to be the largest of the predator group. This makes logical sense as large predators have higher energy needs and need to consume more prey in order to satisfy those needs. The size of predators and prey was not a factor that was controlled for and may have had a significant impact on the results.

A second factor which was not controlled for but could have affected the results was the presence of secondary food sources. It was assumed that in this environment dragonfly nymphs would prey exclusively upon damselfly nymphs; however, dragonflies are generalists and will consume anything small enough for them to handle. Since pond water was used in the experiment, small prey items such as Daphnia and midges were present in unknown quantities, because their small size made them impossible to filter out. Smaller dragonflies may have preferred to go after these smaller prey items in lieu of damselflies, violating the underlying assumption of the experiment.

Future versions of this experiment could be improved by using larger sample sizes and taking more measurements over a longer time period. It would be interesting to include the size of the predator as one experimental factor by measuring either the weight or length of the dragonflies, and examine the interactions between size and oil concentration, and how that effects predation rate, determining which factor seems to have the greatest effect. Also, future experimenters would ideally find a way to ensure that damselflies were the only food resource available to dragonflies.

The results of this experiment have implications for those trying to maintain a species balance within aquatic ecosystems. If oil concentration does directly affect predation (in addition to other factors like size) it is important to put forth greater efforts to reduce oil pollution coming into ponds and rivers from everyday sources such as road run-off. Dragonfly and damselfly nymphs both play key roles in aquatic food webs both as predators and as prey. By changing predatory behavior and species composition at one trophic level, oil pollution has the potential to radiate throughout the food web, causing indirect effects up to the human level.

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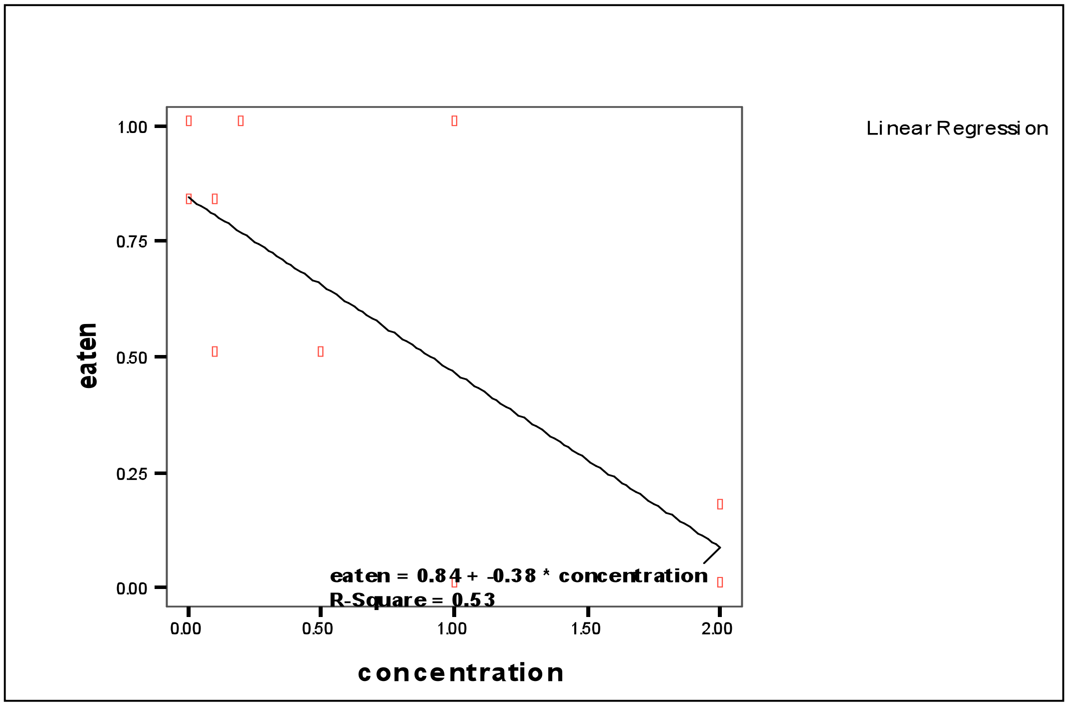


Figure 1: Linear relationship between percent concentration of motor oil and percent prey eaten. The negative slope shows a negative correlation between the variables.

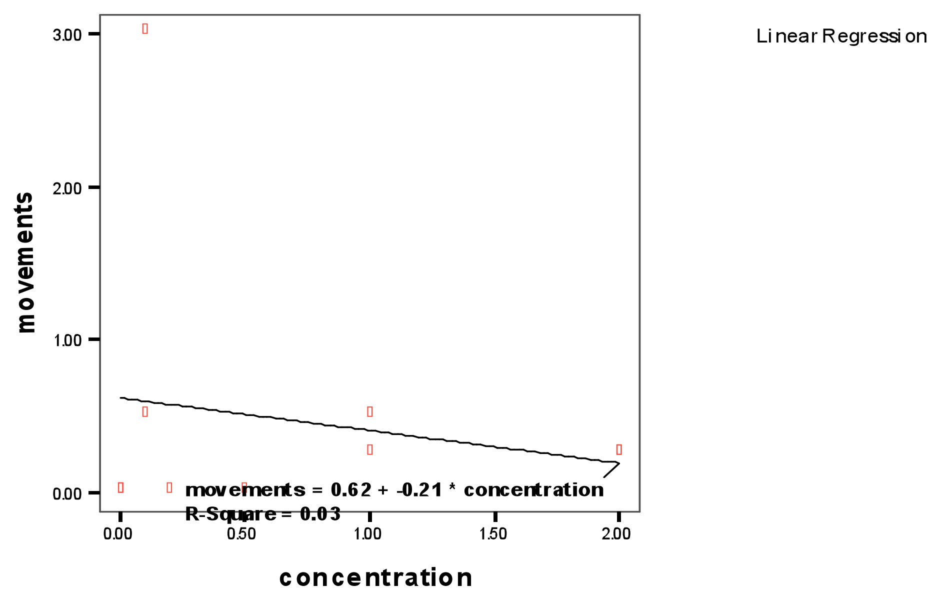


Figure 2: Linear relationship between percent concentration of motor oil and number of dragonfly movements. Slope of the line indicates a slight negative correlation, but a high p-value makes the data insignificant.