# Zooplankton Mortality Increases With Exposure to Dissolved Methane: Implications for Leaks From Hydraulically Fractured Gas Wells to Impact Freshwater Aquatic Communities

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**Abstract:**

Hydraulic fracturing has become the method of choice for extracting natural gas from shale deposits in the United States. As time has passed, environmental concerns have been raised as a result of research from the EPA and independent organizations, though most of it has focused strictly on the consequences this has for human health and land use, and little has focused on its effects on trophic cascades. The purpose of this investigation was to determine whether small leaks from hydraulically fractured gas wells can have definitive consequences on aquatic ecosystems by focusing on the lower levels of the trophic cascade. Methane-infused microcosms of a lake ecosystem did not demonstrate a significant increase in living zooplankton, though they did show a significant increase in dead zooplankton. More research on the mechanism behind the increase in mortality is needed to determine the overall consequences of small-scale leaks into aquatic communities before additional wells are drilled in areas that may be at risk.

**Introduction:**

Hydraulic fracturing (hydrofracking) is a process in which water and other chemicals are injected into deep shale formations which contain natural gas deposits. The natural gas then migrates upward through the fissures that are created and is captured by oil and gas companies and utilized as a carbon-based fuel source, much like fossil fuels extracted from tar sands, ocean floors and deserts. Hydraulic fracturing is currently the most widely used technique to extract natural gas from deep shale formations in the United States. Currently, shale gas deposits have been discovered in 38 states in the continental US, though a majority of the recent hydrofracking activity has taken place in the northeastern United States in the Marcellus and Utica shale deposits.

The environmental impact of this technique is currently a topic of hot debate; the oil and gas industry claims that it is completely safe, and that the chemicals it uses to open wells and extract the gas won't find their way into the aquifers (New York Times, 2011). Environmental groups are claiming the opposite, saying that these chemicals can migrate upwards, and a 1987 EPA report did find that an improperly sealed well lead to aquifer contamination on private

property that immediately bordered the drilling site (EPA report to Congress, 1987). The hydrofracking fluids and gels migrated into the aquifer via an improperly sealed abandoned well and deposited toxic material into the drinking water supply in the area. Moreover, recent studies have reached mixed conclusions, some claiming that methane is ubiquitous in groundwater (Molofsky et al.,2011); others claiming that methane concentrations in aquifers increase with proximity to hydraulically fractured wells (Osborn et. al., 2011). Clearly, more thorough, independent, and peer-reviewed studies are needed to settle this matter. It is worth noting that the studies in the former group were largely conducted by researchers affiliated with oil and gas companies, while those in the latter group were conducted by researchers at universities with no immediately clear conflicts of interest.

While it is already known that hydrofrack wells leak large quantities of methane into the atmosphere (Howarth et. al, 2011), little research has been conducted on the frequency and effects of natural seepage from wells into streams and the implications that this has for the wildlife communities in the surrounding areas. It is known that the fracturing process can produce fissures in addition to the main well bore that create a pathway to the earth's surface which the methane can then follow upwards, producing either water or air contamination in the vicinity of the crack. This is generally avoidable provided sound methods are used for testing the integrity of the rock beds above the shale formations prior to fracturing. Reports of streams with methane seeps from areas with high hydrofracking activity are becoming more common, and large scale fish and wildlife death are anecdotally associated with these (Gasland, 2010). However, the mechanism which causes the widespread death of wildlife in this area has yet to be empirically determined, and while it may seem quite obvious that it is polluted groundwater, it is also impossible to rule out other stochastic environmental events without any hard evidence to support this claim. The objective of this study was to simulate methane seepage into a freshwater aquatic community and then characterize the short-term results of this seepage on the zooplankton communities.

Zooplankton are located just above the base of most trophic cascades, feeding largely on phytoplankton and other productive bacteria (methanotrophs, chemotrophs, etc.). Since their population sizes are largely regulated by the amount of productivity in the ecosystem, the number of them present within a community can serve as a proxy for its overall productivity. Furthermore, they are the smallest organisms that, in a vast majority of species, are constitutively aerobic, making them a good proxy for aquatic oxygen levels. Lastly, they are easily viewed with light microscopes, making them ideal organisms to study when resources are limited.

**Methods and results:**

*Microcosms:* The microcosms of the communities were set up using water samples obtained from Lake Katherine in Winston-Salem, NC. The lake water was placed into large, ½ gallon mason jars and divided into two groups: control and methane-infused. Each group consisted of four separate microcosms; the methane-infused jars were linked to hoses bubbling methane gas

into them at a rate of ~20 bubbles/second (bubble diameter: ~.5 cm). The control jars were ventilated with aquarium pumps, so as to control for any effect bubble induced circulation might have. Each jar was filled with 1.5 liters of water from the lake, divided into the two groups, and then allowed to sit for 13 days with either the methane gas or ambient air bubbling through them. This amounted to approximately 1.7 million $cm^3$ of each type of gas bubbling through each jar. In order to facilitate the growth of phytoplankton communities, the microcosms were placed in a window sill so as to receive as much sunlight as possible.

*Zooplankton counting:* At the end of the 13 days, the methane and air hoses were disconnected and about half the water in each jar was examined underneath a light microscope. Sampling was conducted so as to ensure that both the upper and lower layers of water within the jar were examined, thus avoiding any errors that may have arisen from sampling from strictly one or the other. The numbers of living and dead zooplankton were then measured via light microscope and recorded. Since only half of the water in each microcosm could be analyzed, each value for living and dead zooplankton was then multiplied by two. Since the jars were sampled to ensure a uniform representation of each layer within the water column, it stands to reason that they represented a uniform sample of the population. Thus, simply doubling each value created an accurate estimate of the total zooplankton, living and dead, within each microcosm. The controls contained less life on average than the methane infused jars, though the differences were not significant (Figure 1). However, the methane infused jars contained significantly more dead zooplankton than the control jars (Figure 2*)*. Significance was determined using an ANOVA: single factor test.

**Discussion:** The methane-infused microcosms displayed a larger amount of productivity than the control microcosms, as evidenced by the increase in total number of zooplankton species and individuals. It is important to note that while differences between the numbers of living zooplankton in each community are not significantly different, 75% of the methane-infused microcosms show an increase in living zooplankton with respect to their control counterparts. The significant increase in dead zooplankton in the methane-infused microcosms is rather alarming. It appears that the infusion of methane into the water allowed methanotrophic bacteria to rapidly increase in number, providing more food for the zooplankton. However, this most likely caused the subsequent death of the zooplankton through a combination of two factors which caused the water to become anoxic. First, methanotrophic bacteria utilize oxygen and methane to form formaldehyde which is then converted into various organic compounds that the bacteria uses to survive (Hanson and Hanson, 1996). This depletes the environment of dissolved oxygen, and increases the amount of dissolved carbon dioxide. Secondly, increasing the concentration of methane necessarily lowers the solubility of oxygen in water, resulting in further depletion of oxygen, and this likely lead to the widespread mortality within the zooplankton community. Further studies in which the concentration of dissolved oxygen can be empirically determined are needed.

The mass death of the zooplankton as a result of methane seepage into their community is cause for grave concern, as they represent a significant portion of the lower rungs of most trophic cascades. Additionally, the increase in living zooplankton should not be taken to mean that all is well; the problem of biomagnification of methane and other chemicals not tested in this study but still commonly found in streams near hydrofrack wells could become a large issue warranting our concern. Furthermore, anoxic waters are known to cause widespread death of other aquatic organism, such as fish and larger crustaceans, such as crayfish. Obviously, this is a situation that anyone concerned with the environment would like to avoid. Another particularly alarming aspect of these results is that each methane-infused microcosm was exposed to far less methane for a much shorter duration than streams near hydrofrack operations typically are. Anecdotal evidence suggests that millions of cubic meters of methane and other long chain hydrocarbons are released in seeps, and these can last for months, if not years (Gasland, 2010). However, it is important to note that even relatively small, short-term leaks can have a pronounced effect on the trophic cascade within an ecosystem.

Lastly, this study was restricted to studying the effects of only methane; however, a true seep from a hydrofrack well would contain methane in addition to many other types of long chain hydrocarbons whose negative effects could be observed much more immediately. Additionally, drill pad construction in forested areas can create large quantities of sediment runoff into streams, which alters the nutrient content of the water. It is estimated that between 15 and 40 tons per hectare per year of sediment is generated due to drill pads at well sites (Groat and Grimshaw, 2012), producing further alterations to the aquatic ecosystem.

**Conclusion:**

The widespread increase in both living and dead zooplankton is cause for concern, and blindly rushing to hydraulically fracture every shale deposit we can without further knowledge of the environmental consequences seems to be a foolhardy plan. However, it would also be obtuse to suggest that we avoid hydrofracking altogether, as it is a more reliable source of relatively clean energy than other forms of alternative clean energy. Clearly, natural gas, particularly the large deposits in the Marcellus and Utica shale, represent a potentially excellent economic and energetic alternative to imported fossil fuels for the United States. However, the current lack of regulation of the industry, combined with corporate greed and a lack of environmental responsibility has created a situation in which the extraction of these gases puts the environment at enormous risk. Anecdotal reports of spills and seepages of “produced water”, fracking fluid that comes back out of the well after fracturing, from the pits that it is stored in before treatment and disposal also factors into the environmental impact, though studies on the effects of this have not yet been completed. Clearly, further research on the environmental consequences of this process is needed so as to implement more effective regulations on an industry that is, currently, virtually unregulated. Economic benefits alone do not justify the widespread destruction of our planet.

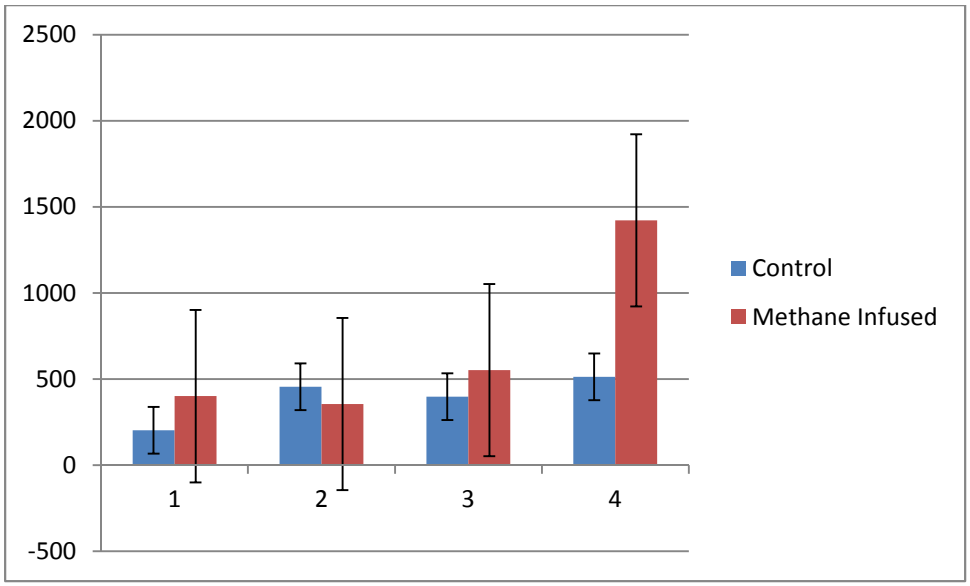


Figure 1: Numbers of living zooplankton individuals in each microcosm with error bars representing one standard deviation from the mean. Differences between these microcosms were found to be insignificant ($p=.3$).

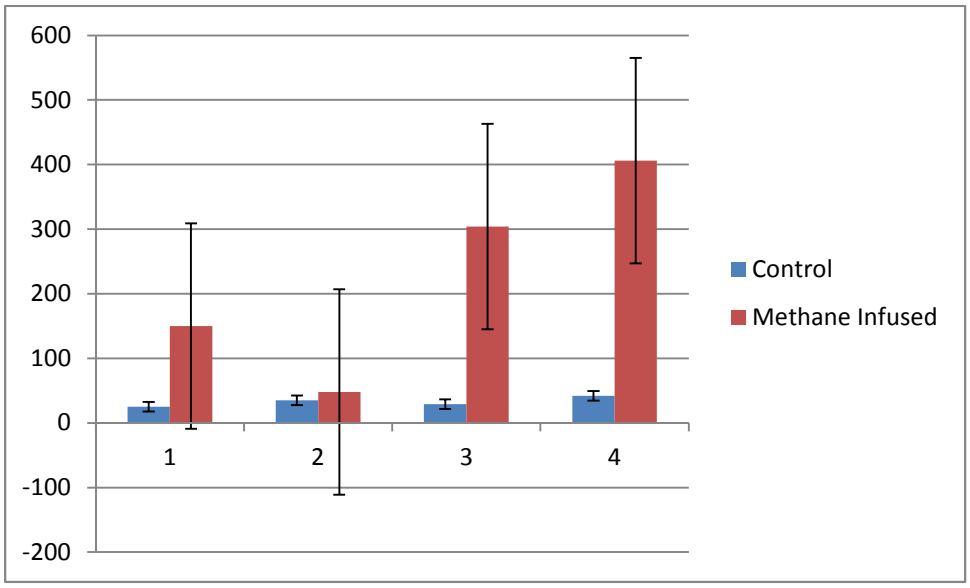


Figure 2: Numbers of dead zooplankton individuals in each community with error bars representing one standard deviation from the mean. Differences in these microcosms were significant ($p=.05$), indicating that the increase in mortality observed in these communities were likely due to methane infusion.

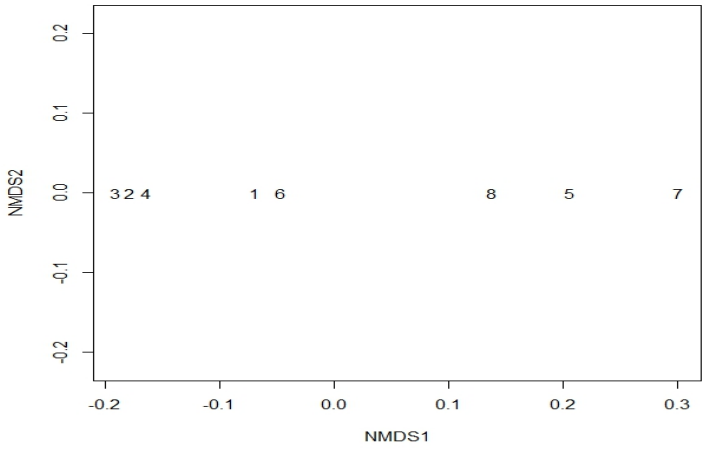


Figure 3: Results of the Bray-Curtis ordination represented with non-metric multidimensional scaling. Numbers 1-4 represent control microcosms, while 5-8 represent the methane infused microcosms. Axes show relative distance of each plot from the other in terms of living and dead zooplankton individuals. NMDS plot created using meta-MDS in the "vegan" package in R 2.14.1.

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