Preliminary Assessment of Fuel Spill Effects Utilizing *Daphnia similis* as a Test Organism

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Human interference in the aquatic ecosystem has created an unideal environment fpo-ooor aquatic creatures, emphasizing accident fuel and oil leaks from offshore cargo ships and offshore oil drilling, respectively. In this context, a preliminary study was conducted to analyze further the effects that a fuel spill causes in a water environment. The methodology used the standard method of conducting ecotoxicological essays using *Daphnia similis* but was adapted for a preliminary context, using fewer resources to obtain a primary analysis. The results indicate that fuel spills are still dangerous to underwater life, even in low concentrations. This study showcases the immediate effects of fuel spills and is still open for further experimentation.
Keywords: *Daphnia*. Ecotoxicology. Fuel. Assessment.

Introduction

There is a crescent number of studies utilizing *Daphnia* as a stakeholder for their essays in Ecotoxicology. The organisms are more sensitive and allow for a finer analysis of chemical compounds and effluents [1,2]. In this fashion, *Daphnia* are the organisms used to monitor the quality of freshwater ecosystems. It has become a staple, and its application has grown exponentially in ecotoxicology studies. The *Daphnia* used in this essay belongs to *Daphnia similis*. Its anatomy is very similar to other *Daphnia* species, such as *Daphnia magna* and *Daphnia pulex*. In tropical climates, it is more common the use of *Daphnia similis* because these species are more adapted to living in tropical conditions [3].

Daphnia Anatomy and Life Cycle

The anatomy of *Daphnia similis* (Figure 1) is very similar to those of other *Daphnia* species, although other species may present more eggs per organism and a higher sensibility [4-6]. The organism has a pretty simple structure and is easy to visualize due to its transparent body. Also, they are microscopic organisms (about 0.5 to 5.0 nm), being easy to manipulate in a laboratory setting.

A *Daphnia*'s life cycle is concise. For laboratory uses, the cycle goes for 28 days. Up until the first 24 hours of life, the organisms are called neonates or newborns; from the first day to the seventh day, they become younglings, and from the seventh day forward, they enter the reproductive stage, ending on the 28th day, when the organisms are discarded [8]. Although every organism cultivated in a lab is a female, there is some chance of males appearing in the environment. It is commonly found in nonformidable settings for cultivating *Daphnia* species or when there is any stress caused by external means to the organisms, such as loud noises or shaking. All of this will result in deceased and/or paralyzed organisms, together with ephippium (Figure 2), that are always discarded.

Fuel Contamination Context

The aquatic ecosystem has been suffering from the impacts caused by human intervention in nature, which increases the amount of spill from numerous chemical compounds. This causes a decrease in the quality of life of the species that inhabit the water [9]. Water contamination through accidents of cargo transport ships leaking

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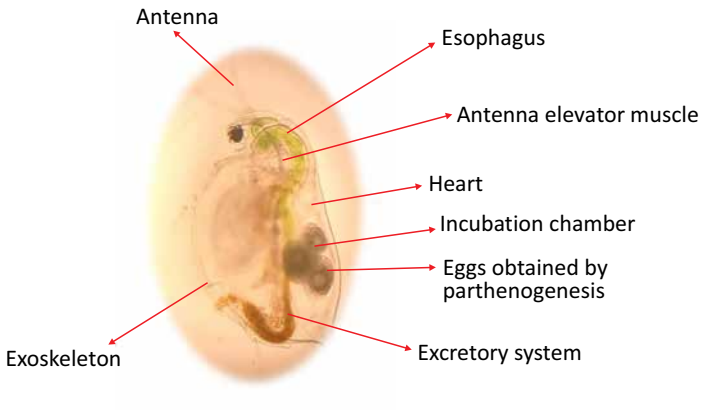
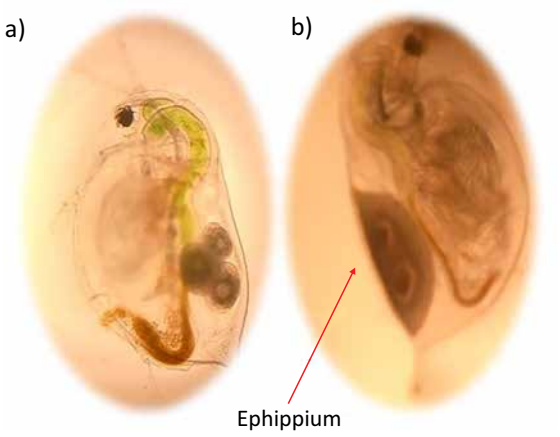
Figure 1. *Daphnia similis* anatomy.



Source: adapted from Oliveira and colleagues [7].

Figure 2. Comparison between a healthy organism with parthenogenesis eggs and an ephippium.



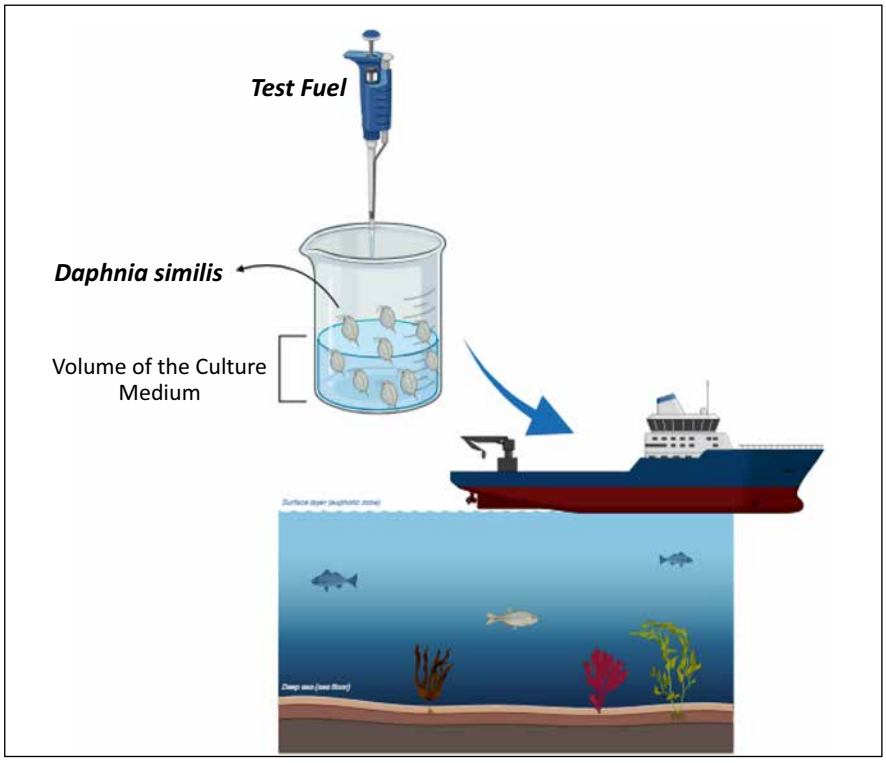
or offshore oil drilling has aroused some concern in the world [10]. In this manner, this study aims to show the immediate preliminary effect of a fuel spill in a freshwater environment using four different compounds and *Daphnia similis* as a test organism.

Materials and Methods

The tests aimed to simulate a fuel spill, with 10 organisms (*Daphnia similis*) per replica mixed with fuel in 50 mL of culture medium (Figure 3). The volume of fuel needed for each replica was the dilution factor, which consists of dividing the total volume by a certain number to obtain a percentage that determines the fuel concentration in correlation to the total volume of the test solution. In this fashion, the percentages chosen for the essay were 0.1%, 0.05%, 0.024%, 0.012%, and 0.006%. Nevertheless, as this was only a preliminary test to evaluate the instant effects, only the most negligible concentration (0.006%) was used.

The organisms were distributed in 5 replicas (10 organisms in each one), one replica for each fuel, and a control (containing only the culture medium).

Figure 3. Illustration representing the method used for the preliminary tests.



The fuels used for this study are showcased in the following table (Table 1) and their specifications.

Results and Discussion

After adding the fuel in each replica, it was noted that the replicas containing both the HVO and the S10 diesel had an immediate effect of immobilization at the surface of the test solution with 100% efficiency. However, some organisms were still moving with restrictions in the S10 replica, but in the HVO, there was complete paralysis. E1G and E2G had the opposite effect; the organisms were not immobilized nor paralyzed and kept moving like normal. More tests must be conducted to establish a final result using the higher fuel concentrations chosen for the final essay. The volume of the test solution could also be changed for a more realistic approach since there is more water than animals in freshwater and saltwater ecosystems.

Table 1. Fuels used for this essay.

Test Fuels Used	Specific Mass (Kg/m³)	Water Content (%/M/m)	Viscosity (40°C)
S10 Diesel	835.7	0.11	3.48
HVO	774.07	0.10	3.89
Ethanol 1 G (E1G)	809.8	7.24	Not detected
Ethanol 2 G (E2G)	795.4	1.83	Not detected

Conclusion

This study is open for future work. Those tests must be done with higher concentrations to measure the full effects of contamination through fuel spill in a water environment, emphasizing S10 diesel and HVO since those had the more immediate effects in the *Daphnia*.

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