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Daphnia: A Possible Way to Combat a Deadly Amphibian Pathogen

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Abstract

Globally there is a biodiversity crisis, with many groups of species threatened with extinction due to changes in the environment and human impacts. Amphibians are one such group and according to the IUCN, over 30% of amphibians are threatened by extinction. There are many factors have that can explain the decline of amphibians including pollution, habitat loss, climate change and disease. One factor is chytridiomycosis, an emerging infectious disease caused by the chytrid *Batrachochytrium dendrobatidis*. The chytrid enters the keratinized skin of the amphibian and asexually reproduces, where it disrupts host functions, often leading to host death.

Due to the severity of the disease, mitigation strategies are needed. Current strategies such as fungicides and "frog hotels" are not sufficient due to adverse effects and limitations. However, microorganisms recently have indicated potential for being used as a mitigation strategy. Some species of microorganisms have been observed to reduce the amount of viable *B. dendrobatidis* in freshwater environments and species can reduce infection strength. One promising group of microorganisms that can remove *B. dendrobatidis* are *Daphnia spp.*, planktonic crustaceans that live in freshwater and filter feed on unicellular organisms. We hypothesize that *B. dendrobatidis* is a viable food source for *Daphnia magna* and additionally will not be toxic to the *D. magna*.

In a laboratory experiment under controlled conditions, one set of *D. magna* was exposed to *B. dendrobatidis* as the only food source for seven days. Another set of *D. magna* was exposed to dechlorinated water as a control. Under a fluorescent microscope *B. dendrobatidis* was observed in the gut of *D. magna* that had been fed the *B. dendrobatidis* and had survived the course of seven days. The survival rates between the two experimental treatments did not differ,

however *B. dendrobatidis* was observed to not be toxic to *D. magna.* Although these results did not determine if *B. dendrobatidis* is a viable food source for *D. magna*, it does suggest *D. magna* as a potential mitigation strategy. Further experiments need to be conducted to determine to which extent *Daphnia* can be used as a mitigation strategy.

Introduction

The world is amidst a biodiversity crisis. Increasing numbers of species are threatened by extinction or are already extinct (Wake and Vredenburg, 2008). Changes within the environment, due to habitat loss, pollution, exploitation, climate change, invasive species and disease are creating surroundings that no longer support and suit many species. These multifaceted causes, often influenced or exacerbated by human interactions are contributing to an overall biodiversity loss (Wake and Vredenburg, 2008).

Of these groups of species experiencing biodiversity loss are amphibians, which are facing a plausible likelihood of extinction (Wake and Vredenburg, 2008). It has been observed that the current extinction of amphibians rate is roughly 211 times greater than the background extinction rate based upon the fossil record (McCallum, 2007). The acceleration in the extinction rate leaves it difficult to explain as a natural phenomenon (McCallum, 2007). There are multiple reasons for this increase in extinction risk, especially since a reliance on cutaneous respiration and reproduction in aquatic environments leaves amphibians already vulnerable to slight changes within their environment (Wake and Vredenburg, 2008). Plausible explanations for declines include competition with invasive species, over exploitation, habitat disruptions, increases in toxic chemicals and global warming (Collins and Storfer, 2003). However, recently it has been discovered that amphibian declines are part in due to an emerging infectious disease, called chytridiomycosis that has been found worldwide in amphibian populations (Fisher et al., 2012).

Chytridiomycosis is caused by the chytridiomycete fungus, *Batrachochytrium dendrobatidis* (Longcore et al., 1999). Multiple strains of *B. dendrobatidis* exist and differ in virulence when infecting amphibians (Fisher et al., 2009). Strains of *B. dendrobatidis* are currently found on every continent except Antarctica, indicating that most species of amphibians

are capable of being exposed to the disease (Fisher et al., 2009). Over 500 of 1240 known species of amphibians have been documented as prone to infection (Olson et al., 2013). However, not all amphibians that encounter *B. dendrobatidis* show the lethal symptoms of the disease. Some frog species are disease tolerant, enduring some level of infection without showing signs of the disease (Fisher et al., 2012; Woodhams et al., 2011). These tolerant or carrier species may have contributed to the spread of *B. dendrobatidis* through natural movement across a landscape or even by human translocation (Fisher et al., 2012). The likelihood of distribution to new environments by human interactions is increased by the saprobic nature of *B. dendrobatidis*, meaning that the chytrid is capable of living on substrates other than its amphibian host (Fisher et al., 2012; Longcore et al., 1999). The capability to survive on multiple substrates allows for *B. dendrobatidis* to persist in the environment, allowing it to continue its life cycle if nutrients are available even when susceptible amphibian hosts are not available.

The life cycle of *B. dendrobatidis* consists of motile and sedentary stages, the first substrate independent and the latter is substrate dependent. The motile stage is defined as spherical zoospores that move using a flagellum until they encyst (Berger et al., 2005; Longcore et al., 1999). Once encysted on a substrate the sedentary stage begins. Several thread-like rhizoids grow from the encysted zoospore forming a zoosporangium surrounded by a chitin cell wall (Longcore et al., 1999). In the zoosporangium, zoospores develop and the chitin is broken down allowing for several zoospores to be released through a discharge papillae (Longcore et al., 1999). Zoospores are often motile for less than one day and can span a maximum distance of 2 cm before encysting, unless there is flowing water present (Piotrowski et al., 2004). This characteristic allows for dispersal to new areas as well as reinfection of the host.

B. dendrobatidis infects a host by entering and growing within the stratified epidermal cells of keratinizes skin, as an infectious zoospore (Berger et al., 2005; Longcore et al., 1999). Therefore, infections are limited to the mouthparts of tadpoles and the legs and body of adult frogs where keratinized skin can be found (Daszak et al., 1999; Van Rooij et al., 2015). The extent of infection can vary among adult and juvenile frogs, since susceptibility can vary across developmental stages (Scheele et al., 2014). In many species of susceptible amphibians, the development rate of *B. dendrobatidis* is similar to the shedding of the amphibian skin, allowing for the release of zoospores away from the body and reinfection into a nearby part of the host body (Berger et al., 2005). Clinical symptoms of adult and juvenile frogs include abnormal posture, lethargy, lesions and epidermal sloughing (Daszak et al., 1999). These clinical symptoms can leave frogs vulnerable to predation and starvation by making them less active. In addition to clinical symptoms, the pattern of *B. dendrobatidis* growth inhibits the salt absorption by the skin (Campbell et al., 2012). This inhibition leads to a depletion that disrupts the homeostasis of electrolytes, ultimately leading to cardiac arrest and death of the adults and juveniles (Campbell et al., 2012). Clinical symptoms in tadpoles often include a reduction in foraging behavior, resulting in reduced tadpole not growth rates (Venesky et al., 2009). Chytridiomycosis therefore, can be detrimental to frog populations.

The effects of *B. dendrobatidis* are severe on amphibian species, requiring intervention. Attempts to eradicate the disease appear futile, resulting in the need for mitigation strategies (Kriger and Hero, 2009; Scheele et al., 2014). One method is to reduce the likelihood of spreading the pathogen by regulating amphibians in the food trade, screening shipments of frogs and screening at zoos or laboratories to prevent the movement of infected individuals (Kriger and Hero, 2009). Proposed methods for mitigation in the field include treating environments with

fungicides, salts and antibiotics to limit infectious doses of *B. dendrobatidis* in the environment (Woodhams et al., 2011). Another option within the field is to create areas of warmer temperatures to allow for basking, to allow amphibians to increase internal temperatures to combat the disease (Scheele et al., 2014) . This can be achieved through shallow pockets of warm water, introducing warm rocks, canopy free zones and heating stations (Scheele et al., 2014; Woodhams et al., 2011). To reduce exposure of susceptible populations it has been suggested that infected individuals be translocated to eliminate a primary source of disease exposure, however the saprobic nature of *B. dendrobatidis* may allow for the pathogen to still persist (Kriger and Hero, 2009). Additional translocation strategies have been proposed such as adding frogs when they are less susceptible, such as adults instead of juveniles and ones clear of infection, to create a buffering effect for those in the environment (Scheele et al., 2014). Lastly, conservation methods for some species has been to remove individuals from the environment and allow them to live in captivity or "frog hotels" (Woodhams et al., 2011). All proposed methods often have limitations or adverse effects. For example, proposed methods of fungicidal treatments could lead to cumulative effects that harm to many organisms (McMahon et al., 2013). Removing species into captivity can also create situations of reducing genetic diversity and can be difficult to accomplish. Therefore, research for additional mitigation strategies in necessary.

Another approach to discovering mitigation strategies has been to observe the differences of *B. dendrobatidis* among environments. It has been noted that the prevalence of *B. dendrobatidis* in different locations varies, locally and regionally even if altitude and temperature – major factors in prevalence – are similar (Schmeller et al., 2014). Upon investigating different aquatic environments, those with greater prevalence of microorganisms had less *B.*

dendrobatidis, highlighting a potential use as a mitigation strategy (Schmeller et al., 2014). In laboratory experiments, microorganisms including rotifers, ciliates and species of *Daphnia* can reduce *B. dendrobatidis* by predation or passive filtration (Buck et al., 2011; Schmeller et al., 2014). The presence of microorganisms also reduced infections of tadpoles and the infection strength when exposed to *B. dendrobatidis* (Schmeller et al., 2014). However, reduction of *B. dendrobatidis* in the field can be limited due to complex interactions between the hosts, fungi and aquatic microorganisms, providing the need for more research (Buck et al., 2015).

Daphnia is a genus of small planktonic crustaceans that inhabit freshwater environments. *Daphnia* are grazers that consume nanoplanktonic algae, bacteria, fungi, protozoa and detritus of 1-100 µm in size (Thorp and Covich, 2010). Species of chytrids can be filtered by grazing *Daphnia*, and digested due to the lack of a thick cell wall (Kagami et al., 2004). For example, the chytrid *Zygorhizidium planktonicium* is rich in supplementary nutrients and can serve as a food source for *Daphnia* (Kagami et al., 2007*). Daphnia pulex* have been observed having *B. dendrobatidis* zoospores in their guts, highlighting a possibility of reduction of prevalence and disease (Buck et al., 2011). *Rana aurora* tadpoles exposed to *Daphnia* and *B. dendrobatidis* in mesocosms, resulted in an increase in survival among tadpoles and decrease in zoospores when compared to mesocosms without the zooplankton, indicating an immediate effect on the amphibians (Hamilton et al., 2012). *Daphnia magna*, a larger species that can grow to 5 mm effectively removed *B. dendrobatidis* from the environment while smaller *Daphnia* did not suggesting that *Daphnia* size may influence the potential as a mitigation strategy (Searle et al., 2013).

Whereas studies have demonstrated the ingestion of *B. dendrobatidis* by different species of *Daphnia,* little is still known about the relationship. It is possible that *B. dendrobatidis* harms

Daphnia, an effect that has not been shown, most likely because most studies last only a few hours or a day. It is also unknown if *B. dendrobatidis* is a viable food source for *Daphnia.* It is debated whether *Daphnia* selectively or passively graze when feeding (Butler et al., 1989; Thorp and Covich, 2010). For these reasons *Daphnia* could either inconsequentially ingesting *B. dendrobatidis* zoospores or may select to feed on other more nutritious organisms when given the opportunity. Both possibilities would limit the potential use of *Daphnia* as a mitigation strategy, presenting the need to determine if *B. dendrobatidis* is a food source. Due to the research indicating that other chytrids can be nutritious to *Daphnia*, our hypothesis was that *B. dendrobatidis* is a food source and nontoxic for *Daphnia magna*, further highlighting its potential use as a mitigation strategy.

Methods

Modified from the protocol used be Buck et al. 2011, a stock solution of Nile Red dye was produced by adding 0.0054 g of Nile Red dye to 20 mL of dimethyl sulfoxide (DSMO). The Nile Red dye stock was added to a1% tryptone 1% agar solution at a concentration of 500 µg/L. This mixed solution was then used to pour multiple plates. Plates were inoculated with 2 mL of *B. dendrobatidis* strain 274. Inoculation of new plates occurred every two days. After nine days, plates were used for the experiment.

D. magna were kept in a 10 L aquarium as well as four 1 L beakers containing dechlorinated water prior to the experiment. Every other day the *D. magna* were fed with filtered and diluted green water obtained from the local area that depended on the evaporation rate. Enough green water was added to keep the tanks a green, yet clear color. The tanks had bubblers for aeriation and were placed next to a light with a timer, giving cycles of 14 hours of light and 10 hours of dark to the *D. magna.*

Prior to the seven-day experiment, *D. magna* were added to a 250-mL beaker containing dechlorinated water. One inoculated Nile Red plate was flooded with 15 mL of dechlorinated water. After fifteen minutes the plates were scrapped, and 8 mL of the water containing *B. dendrobatidis* was added to the 250-mL beaker. Additional *D. magna* were placed in a 250-mL beaker containing dechlorinated water and 8 mL of water from a scraped uninoculated Nile Red plate. After three hours of exposure, *D. magna* were sacrificed in 75% ethanol. The *D. magna* were viewed under a fluorescent microscope to determine if *B. dendrobatidis* was present in the gut and determine if the Nile Red leaches into the flooded water.

Twelve *D. magna;* four small, four medium and four large, were added to ten beakers filled with a 142 mL of dechlorinated water. The beakers were randomly assigned to either the "Control" or "Bd" treatments. *D. magna* were left untouched in "Control" and "Bd" beakers for thirty minutes prior to the application of treatment. For the "Control" treatment, five uninoculated plates containing Nile Red dye were flooded with 15 mL of dechlorinated water. After, ten minutes the plates were scraped into one beaker. From this beaker, 8 mL of the scraped dechlorinated water was added to the "Control" treatment beakers. Five inoculated plates that were nine days old were flooded with 15 mL of dechlorinated water. After ten minutes the inoculated plates were scraped and pooled into one beaker. From this beaker, the "Bd" treatment beakers received 8 mL of dechlorinated water containing *B. dendrobatidis.* Prior to adding the 8 mL to the "Bd" treatment, the concentration of *B. dendrobatidis* was observed in the pooled beaker to verify that the concentrations were similar, indicating that during water changes the "Bd" treatments were within a reasonable range (Table 1). Additional dechlorinated water was added by pipette to the beakers to bring the volume to 150 mL. *D. magna* deaths were recorded daily for each beaker in both treatments for seven days.

Water changes were done every two days by filling clean 250 mL beakers with 140 mL dechlorinated and transferring individual live *D. magna* with a pipette. Depending on the treatment, 8 mL of dechlorinated water from a scraped inoculated ("Bd") or uninoculated Nile Red plate ("Control") was added to the new beakers at each water change. After seven days, the experiment was ended. On the seventh day surviving *D. magna* were sacrificed in 75% ethanol. A fluorescent microscope was used to view the *D. magna* to determine if *B. dendrobatidis* was present in the gut.

Results

Under green light from a fluorescent microscope, *D. magna* exposed to *B. dendrobatidis* were observed to have zoospores within the gut after three hours of exposure (Figure 1). The *D. magna* exposed to dechlorinated water from an uninoculated plate for three hours did not fluoresce. Seven of the ten surviving *D. magna* that were exposed to *B. dendrobatidis* fluoresced after the seven-day experiment (Table 2). The seven surviving *D. magna* were in "Bd" beakers 1, 2, 4 and 5. All twelve *D. magna* in "Bd" beaker 3 died by the end of the seven-day experiment. The surviving eight *D. magna* from the "Control" group did not fluoresce.

A Cox Proportional Hazards test was preformed between the two treatments. A p-value of 0.67 was obtained, indicating that the was no significant difference between "Control" and "Bd" treatments. On second day of the seven-day experiment, there was a mass die off in both treatments. The proportion surviving in the "Control" treatment dropped from 0.9 to 0.3 and the proportion surviving in the "Bd" treatment dropped from 0.95 to 0.17 on day two. However, after day two there were no additional deaths in the "Bd" treatment, the surviving proportion was maintained at 0.17 until the end of the experiment. The "Control" treatment continued to

experience death, with the surviving proportion being 0.13 on the last day of the experiment (Figure 2).

Discussion

The fluorescent microscopy demonstrated that *D. magna* can remove infectious *B. dendrobatidis* zoospores from the environment after three hours of exposure to the chytrid (Figure 1). *D. magna* also remove *B. dendrobatidis* from the environment after seven-day exposure (Table 2). While only seven out of the ten surviving *D. magna* fluoresced, two were molting which could have affected their eating behaviors and the last one had visible *B. dendrobatidis* on the exterior under a light microscope. Since there was visible *B. dendrobatidis* that did not fluoresce, there may have been an issue with the Nile Red or the fluorescent microscope. The wide range of fluorescence intensity across the seven surviving *D. magna* could additionally be explained by an issue with the Nile Red dye or the fluorescent microscope. Thus, the three non-fluorescing *D. magna* may not have had enough Nile Red present to indicate that the chytrid was present in the gut despite the presence of the chytrid. Conducting a PCR to determine if *B. dendrobatidis* genetic material was present among the *D. magna* would further demonstrate the ability to ingest zoospores. The ability to remove zoospores supports the potential use of *Daphnia* as a mitigation strategy for the deadly amphibian pathogen as discussed in previous studies. This finding is helpful since current mitigation strategies such as, fungicides and captivation programs as seen in "Frog Hotels" are not ideal (Scheele et al., 2014; Woodhams et al., 2011).

The hypothesis that *B. dendrobatidis* would be nontoxic to *D. magna* was supported by the observation of death in both treatments regardless if the chytrid was present. We verified that the *D. magna* in the "Control" treatment were not exposed to *B. dendrobatidis* as none of the *D.*

magna or water in the treatment fluoresced and no zoospores were observed under a light microscope. Also, all the remaining *D. magna* exposed to *B. dendrobatidis* survived after the mass die off on day two. Therefore, the mass die off on the second would have to be explained by other factors.

Possible explanations for the mass die off on the second day include: contamination and/or shock. Even though an acclimation time was allotted, the switch from the holding tank and beakers may have shocked the *D. magna.* Additionally, the physical movement of the *D. magna* from one beaker to another with each water change may have been a stressor. *D. magna* were transferred from the holding tank to the treatment beakers using a pipette and protocol was established to ensure that air pockets would not form. While this method was done slowly, the currents created by the pipette could have stressed the *D. magna*. Alternative methods for transferring the *D. magna* should be tested to find an ideal one that reduces or removes this stress. Studies have shown that *D. magna* when exposed to different salinities than their original environment do not thrive as well as those placed in same salinity (Thorp and Covich, 2010). However, it was assumed that the temperature, ion levels and pH were the same across all beakers, tanks, and containers utilized in this study because the dechlorinated water in the treatment beakers and holding tanks were from the same source and located in the same room. For these reasons, it is assumed that the water did not shock the *D. magna,* but in further studies measuring the salinity and other water chemistry variables would be beneficial.

It is inconclusive if shock occurred to the *D. magna* during the experiment, but there was evidence of possible contamination. The dechlorinated water containing *B. dendrobatidis* from the first day was examined under a microscope and was contaminated with unknown bacteria. It is unknown when this contamination occurred, however, it could have contributed to the death

observed since several known bacterial secretions are toxic to species of *Daphnia* (Martin-Creuzburg et al., 2011). A film had also formed on the beakers which was not present in the holding tank and beakers. The film could have resulted from not having a bubbler to aerate the water. Aeration could eliminate films but, *D. magna* can easily adapted to different oxygen concentrations and will turn a redder hue when exposed to low oxygen (Heisey and Porter, 1977). Since the *D. magna* in both treatments were a transparent color, they were not exposed to low oxygen concentrations, removing a hypoxic environment as a possible stressor. Therefore, aeration is only needed to reduce films.

While factors of the experimental set-up could have contributed to the mass death, it is beneficial to note that *B. dendrobatidis* is not harmful to *D. magna*, since the zooplankton is important within freshwater ecosystems. *Daphnia* are key members of freshwater communities helping maintain water quality, serving as a food source for fish and indicators for metal contamination (Dodson and Hanazato, 1995). This finding further highlights the potential use of *D. magna* as a mitigation strategy.

Overall, the data from the seven-day experiment suggests that it is inconclusive if *B. dendrobatidis* is a viable food source for *D. magna*. The mass death on day two, removed many of the *D. magna* from the experiment, limiting the opportunity to find possible differences in survival proportions if they existed (Figure 2). Although trends for higher survival proportions in the "Bd" treatment existed at the beginning and end of the experiment, they were insignificant. However, the existence of these trends highlights the need for more experiments.

Repeating the experiment again with modifications and an established colony of *D. magna* could reveal conclusive results and further insights to *B. dendrobatidis* being a viable food source for the zooplankton. As mentioned above providing additional steps to eliminate

shock such as, better transferring methods, monitoring the water conditions and adding aeration need to be included in the set up. A longer acclimation period of twenty-four hours would allow for the *D. magna* to adjust to the new environment and provide a starving protocol. After the twenty-four-hour acclimation, any shocked *D. magna* could be removed to ensure that the experiment starts with a healthy sample size. Likewise, we would suggest that the pooled water from scraped plates should be examined under a microscope for any possible contaminates before use as treatment. While this protocol was included after the mass death, it may have eliminated the observed contamination prior to the initiation of the protocol. In addition to measures to reduce shock and contamination, more replicates per treatment are needed to obtain more data. The larger sample size could also serve as a buffer if unusual deaths were to occur again. The experiment should also be carried out for a longer time, such as several weeks. This should allow for differences in the *D. magna* survival to be observed if differences in survival between the two treatments do indeed exist. Additional observations could be made to note the health of the *D. magna* in the treatments. Stress due to poor food quality, is indicated by a reduction in offspring, an increase in asexual reproduction of "resting eggs" as well as reduced growth in *Daphnia* (Steinberg et al., 2010). If the treatment with *B. dendrobatidis* were to exhibit *D. magna* that did reproduce live offspring it could indicate that the zoospores are providing quality nutrition. Therefore, monitoring reproduction of live offspring, growth and the amount of asexual reproduction could give insight to the nutritional value.

Additional experiments would be necessary to have a more complete understanding of the interactions of *Daphnia* with its environment. The interactions between *Daphnia* and other species have intricate and not well known consequences, requiring further research (Woodhams et al., 2011). Some *Daphnia.* are known to prey upon other known consumers of *B.*

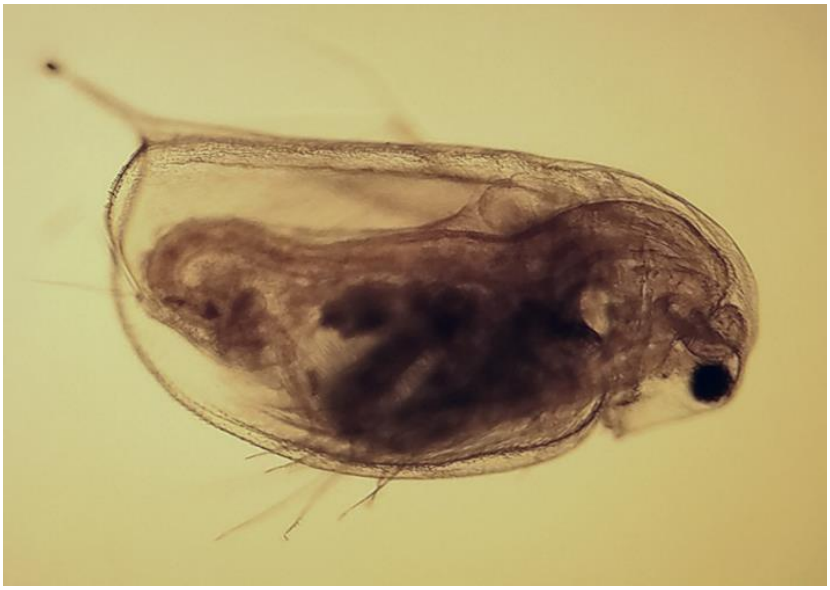
dendrobatidis such as ciliates and may compete with these consumers as well (Schmeller et al., 2014). Experiments with different ecological assemblages of these other known *B. dendrobatidis* consumers would give insight on the cumulative effect and if it would alter the filtration done by *Daphnia.* Field experiments would give insights into understanding if *Daphnia.* will selectively graze on other known food sources or if it is a selective grazer. For example, when *Daphnia* were placed in water that contained large amounts of algae, a main food source, the amount of *B. dendrobatidis* did not reduce significantly, suggesting that *Daphnia* may selectively graze for algae over zoospores (Searle et al., 2013). If this trend were to be observed in future experiments it would limit its potential use as a mitigation strategy.

Other chytrids have zoospores that provide nutrients such as nitrogen, vitamins and fatty acids that contribute to the growth and reproduction of the zooplankton (Gleason and Lilje, 2009). As a chytrid, *B. dendrobatidis* shares similar chemical makeup to the other known nutritional chytrids, suggesting that it could contain nutritional value if *Daphnia* is capable of digesting. Further research determining the nutritional value and chemical make-up of *B. dendrobatidis* would be useful in addressing this possibility.

This study focused on elucidating one of the complex interactions that occur in a natural aquatic environment, between *D. magna* and *B. dendrobatidis*. Although the results were inconclusive if *B. dendrobatidis* is a viable food source for *D. magna* it did indicate that the zoospores can be ingested and that *B. dendrobatidis* is nontoxic to the *D. magna* zooplankton. These observations highlight the possibility of using *D magna* and potentially other *Daphnia* as a mitigation strategy for combating infections of amphibians by this deadly pathogen. However, limitations are also present since *Daphnia* are difficult to keep alive and struggle to survive in environments different from their own. Utilizing an organism naturally found in these

ecosystems would be an ideal mitigation strategy, since current strategies like fungicides can cause harm to the surrounding ecosystems. Fungicides currently proposed have been observed to have adverse effects on tadpoles, frogs, zooplankton like *Daphnia* as well as other organisms (McMahon et al., 2013). These adverse effects include death or shorten life spans in some cases, potentially aiding in the prevalence of *B. dendrobatidis*. Other strategies such as removing amphibians from the environment and placing in captivity have drawbacks, such as the effort to move and treat as well as removing essential components of the food web. Looking for other options that do less harm, such as adding organisms in that are already present may have better long term and short-term implications. These mitigation strategies will give further insight into the complex interactions of *B. dendrobatidis* and its environment, hopefully leading to a resolution for the declining amphibian populations, diminishing the biodiversity loss.

A.



B.

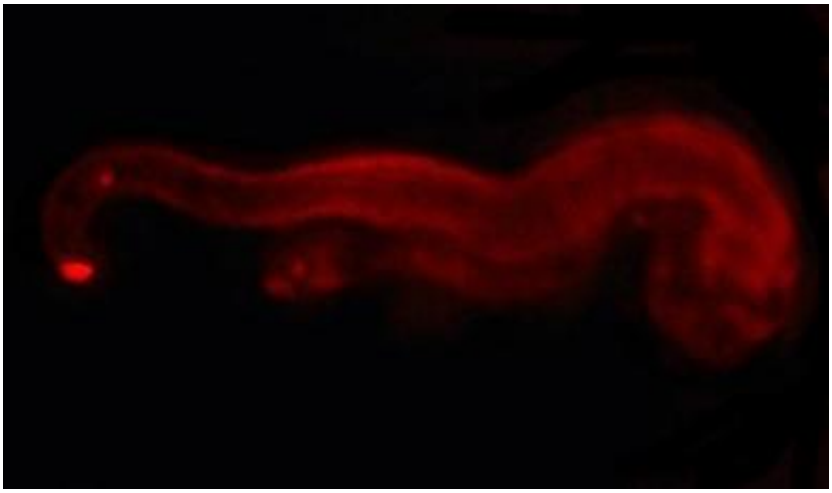


Figure 1. (A) Anatomy of *D. magna* under bright field microscope at 40x. (B) *D. magna* shown with excited Nile Red, stained *B. dendrobatidis* in the gut when viewed under green light at 40x.

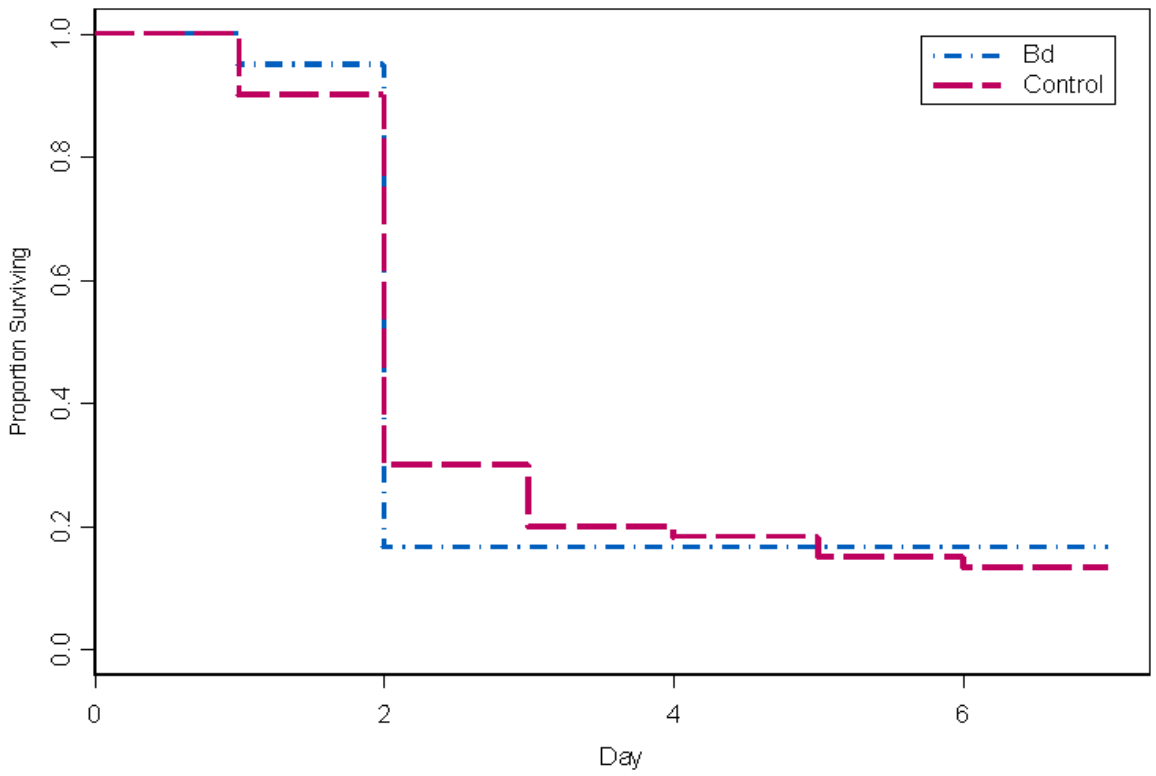


Figure 2. *D. magna* survival per treatment over the course of the seven-day experiment with a p-value of 0.67.

Table 2. Concentrations of *B. dendrobatidis* for the "Bd" treatment water changes

Day	*B. dendrobatidis* zoospores/mL
0	62,000
2	116,000
4	62,000

Table 2. Fluorescent microscopy of surviving *D. magna* exposed to *B. dendrobatidis*

Beaker Number	Individual	Fluoresced	Description
1	2-small	Yes	Dim glowing
	3-small	Yes	Also had globular growths
	4-small	No	Molting
2	2-small	Yes	Some *B. dendrobatidis* on exterior
	3-small	Yes	*B. dendrobatidis* on exterior
	4-small	No	*B. dendrobatidis* on exterior
4	2-small	No	Molting
	3-small	Yes	Dim glowing
	4-small	Yes	Bright glowing
5	4-small	Yes	Glowed through out

*All *D. magna* died in beaker number 3 after the seven-day experiment

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