Comparison of the Effectiveness of Formalin, Hydrogen Peroxide, and Ultra Violet Water Purification for the Control of Surface Pathogens on Fathead Minnow Eggs

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by

Jay B. Gulshen

Junior

Camdenton High School
P.O. Box 1409
Camdenton, MO 65020

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Mr. Chris Reeves
Science Research Instructor
Linn Creek, MO 65052
NAME: Jay Gulshen  
HOME ADDRESS: 256 Foxhead Shores  
Linn Creek, MO 65052  
SCHOOL: Camdenton R-III Schools  
SPONSER/TEACHER: Mr. Chris Reeves  
TITLE: Comparison of the Effectiveness of Formalin, Hydrogen Peroxide, and Ultra Violet Water Purification for the Control of Surface Pathogens on Fathead Minnow Eggs

Warm water fisheries throughout the United States have approximately a 50% mortality rate among fish before their eggs even hatch. This mortality is mainly due to pathogenic growth (particularly fungi) that suffocates and infects eggs. In order to counter pathogens, fisheries use chemical treatments, traditionally, formalin and hydrogen peroxide. However, with the environmental concerns surrounding these treatments, a new solution is needed; the alternative solution tested in this study was Ultraviolet (UV) water purification to determine if UV purification could be as effective at lowering the infection rate amongst eggs.

In order to compare the effectiveness of these three treatments, an original apparatus was constructed to simulate a commercial fishery. Fathead minnow (Pimphales promelas) eggs were used in four test groups (UV, formalin, hydrogen peroxide, and a control (no treatment)). Data on the condition of each egg (healthy, infected) was collected 24 hours after the last chemical treatment and 48 hours before the projected hatch time.

In both collections, UV purification was shown to significantly lower the infection rate amongst eggs in comparison to the formalin, hydrogen peroxide, and control groups.

The results from this study show that using UV water purification can decreases the infection rate, and thus the mortality rate amongst eggs in warm water fisheries. This improvement in the biological efficiency could significantly increase the profit yield of commercial fisheries. Not only that, but using UV purification would diminish the role of hazardous chemicals in fisheries, decreasing the danger these chemicals pose to the watershed through runoff.
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Purpose:

The purpose of this study was to determine:

1. How much, if at all, ultraviolet water treatment compares to traditional chemical treatments (formalin and hydrogen peroxide) for controlling the infection rate of warm-water fish eggs

In order to:

i. Reduce chemical damage to the watershed from run-off

ii. Increase the biological efficiency and, thus increasing the profit yield per hatch group by increasing the hatch size through the reduction of infections to eggs, and thus reducing mortality
Hypothesis:

It was hypothesized that:

1. The eggs in the Ultraviolet group will be less affected by surface pathogens than those of the Control group.

2. The eggs in the Ultraviolet group will be less affected by surface pathogens than those of the formalin group.

3. The eggs in the Ultraviolet group will be less affected by surface pathogens than those of the hydrogen peroxide group.
Independent and Dependent Variables:

Independent Variable: Type of water treatment

Dependent Variable: The infection rate among the eggs in each group

Constants: Temperature, light source and intensity, source of water, and genetic similarity of species of egg donors


Introduction

Located in Stoutland, Missouri, Ozark Fisheries is a provider of goldfish and koi for customers all over North America and Western Europe. Ozark Fisheries produces a large variety of goldfish, including three different types of koi, and twelve different types of goldfish, including a variety of Common goldfish, Telescopes, and Fantails. Ozark Fisheries is responsible for the goldfish through producing the fertilized eggs, hatching the eggs, raising the fish, and finally, shipping the fish to their clients. During the hatching process, the eggs are laid on nests in a large container filled with water directly from No. 1 Spring, along with a few lesser springs which all flow into the East Osage watershed and into the Lake of the Ozarks, with oxygen being pumped into the nesting tanks using an aspirator. The eggs stay like this until they hatch (usually within five to seven days), but there are many dilemmas to overcome in this process. There is a large mortality among eggs over the course of hatching. The main cause of mortality is pathogenic growth on dead eggs, breaking them down, and then spreading to live eggs; this can happen quite easily and rapidly not only due to the density of egg clusters in a limited amount of space, but, in an aquaculture environment, the lack of a current increases the ability for pathogens to spread to other egg clusters. The majority of pathogenic development on eggs is from aquatic fungi known as Saprolegniales, and they are found in all natural water supplies (Rach et. al. 2005). Fungal infections can develop through colonizing on dead eggs, and then either suffocating or invading live eggs (Rach et. al. 2005). This problem is a serious financial burden for the fishery. In order to combat these problems, a variety of chemical treatments have been developed. Historically, formaldehyde and formalin has been the principal treatment; however, with a growing concern of the health and environmental consequences of
using these treatments, fisheries are looking at other treatment methods (Rach et. al. 2005). The most accepted successor of formalin is hydrogen peroxide ($\text{H}_2\text{O}_2$) (Pers. Com. Mr. Stan Smith). Hydrogen peroxide is considered substantially more environmentally attuned than formalin (Marking et al. 1994); but, the efficiency of $\text{H}_2\text{O}_2$ has been inconsistently reported; while one study may report $\text{H}_2\text{O}_2$ as a significantly worse pathogenic controller, others may report it significantly better, or that there is no difference at all (Rach et. al. 2005, and Rach et. al. 2005). While another solution could be the use of Ultraviolet purification, this is an idea that has been over-looked by commercial fisheries.

The current status quo treatment, and the one Ozark Fisheries had used until last spring, is the use of formalin to destroy fungal growth and break down dead or infected eggs before the Saprolegniales or other pathogens can colonize and spread to other eggs. Ozark Fisheries applies formalin to the holding water in order to control fungal, bacterial, or parasitic growth on the eggs. However, while formalin is not known to have a direct impact on the eggs themselves, there are some problems with its use (Rach et. al. 2005). Ozark Fisheries maintains a dissolved oxygen rate of 110%-120% saturation (S. Smith, Pers. Com., February 3, 2009). But, formalin depletes the dissolved oxygen (DO) levels in the water, lowering it down to 60% (S. Smith, Pers. Com., February 3, 2009). As a result, the fishery struggles to maintain the appropriate amount of DO in the water to keep all of the eggs alive. Environmentally, formalin poses a potentially dangerous threat once it makes its way into the local streams. Formalin is very chemically close to formaldehyde, which is a know carcinogen, so there are also human health concerns associated with formalin (International Agency for Research on Cancer 2004). Formalin has been determined to be very persistent in ecosystems and is considered to be toxic at even very low levels of concentrations (Rach et. al. 2005); thus, formalin can have substantial negative
environmental impacts if even small amounts are able to enter the watershed. So, formalin as an agent to minimize egg mortality during hatching is not without its problems.

One of the newest methods to control surface pathogens on warm water aquaculture eggs involves treating the water in which eggs are submersed with hydrogen peroxide. Ozark Fisheries has recently begun the process of switching over to the use of hydrogen peroxide. But, when directly compared, *Salvelinus namaycush* (Lake Trout) eggs treated with hydrogen peroxide had a 5% lower survival rate than *S. namaycush* eggs treated with formalin (Rach *et al.* 2005). Also, when hydrogen peroxide is added to water, the peroxide reacts with the water and breaks down to oxygen; thus, the use of hydrogen peroxide, unlike formalin, does not deplete the DO levels. It has, contrary to formalin, the side effect of actually increasing dissolved oxygen up to 150%. There are, however, some positive aspects to using hydrogen peroxide. For example, since hydrogen peroxide so readily breaks down, it is considered by some to be significantly less of a threat to aquatic ecosystems. But, it has been suggested that if too much hydrogen peroxide is allowed into the watershed, it is possible that the drastic increase in DO could harm the ecosystem by creating an effluent zone that creates bloom of aerobic microorganisms (R. Warbritton, Pers. Com., January 7, 2009). Therefore, while hydrogen peroxide is considered healthier for the environment than formalin, there is still a possibility for run-off to hurt the local aquatic ecosystems.

Another possible solution to surface pathogens is the use of Ultraviolet (UV) water purification. UV treatment is a developing technology that is just of late beginning to be used for water purification. However, UV purification has only been introduced to the aquaculture industry on a small scale; most users of UV water sterilization are research oriented institutions rather than private-sector businesses. This is largely due to the cost of UV technologies; but as
the cost of UV units decreases, UV purification is becoming a reasonable option for the private sector. Yet, companies can look at the possibility of using UV purification and see that UV purification will not distort the DO levels, and UV purification would eliminate the use of harmful chemicals that could end up in the local watershed, but because there is not sufficient research proving weather or not UV purification makes a significant impact on the survival rate of their fish, commercial fisheries have not considered ultraviolet purification as a possible option to control pathogenic growth. If UV purification can produce results equal or better than formalin and hydrogen peroxide, then the UV purification market could expand into aquaculture, and there would be less chemical discharge into the watershed.

Commercial fisheries have recognized the environmental and health concerns associated with formalin, and some have begun to convert to the use of hydrogen peroxide; but an innovative solution to completely eliminate chemical run-off is the use of ultraviolet light. Fisheries like Ozark Fisheries are looking at new methods of controlling Saprolegniales or other pathogens; however, any new method would need to be at least as efficient as formalin. Because of these two conditions in the commercial fisheries market, whichever treatment is most successful at controlling these harmful pathogens has a chance to revolutionize the way fisheries everywhere protect their eggs and transform the entire fisheries industry.
**Method**

**Apparatus Design**

The apparatus used in this experiment was a home-made design consisting of the Penguin® 1140, a submersible water pump with a 300 gallons per hour pump capacity, four plastic 12 quart containers which served as holding tanks, ¾” vinyl tubing, and a flow diverter designed specifically for this research made of 3” PVC capped on both ends with ¾ “ holes bored into both sides (one hole serving as the inflow on one side and three outflow holes on the other). A picture of the apparatus is provided (Attachment 3).

**Fish Eggs**

In this experiment, *Pimephales promelas*, or flathead minnow, eggs were used as an alternative to goldfish eggs due to the minnow’s availability as well as more preferential breeding and lab conditions. However, flathead minnow eggs are often used in the place of other fish species in experimentation, so the results of this experiment will be applicable to other species of fish, primarily goldfish. The Flathead minnow eggs used in this experiment are from a group of multi-generational lab minnows from the United States Geological Survey (USGS) fisheries lab in Columbia, Missouri. Dr. Ryan Warbritton, a fisheries biologist for the USGS fisheries lab was contacted and served as the qualified scientist for the duration of experimentation. A technique used by Dr. Warbritton to stimulate spawning was used to gather approximately 7,000 eggs to be used in this experiment. Male *P. promelas* were given access to 3” PVC pipes that were cut
longitudinally and served as spawning chambers (called ‘tiles’). Dr. Warbritton used a commonly used technique to estimate the number of eggs on each tile and those estimations were used to equally distribute the 40 tiles used in the study between three treatment groups (Hydrogen Peroxide, Formalin, and UV) and the control. The tiles were then divided into the twelve holding tanks as evenly as possible according to the estimated number of eggs on each tile. All twelve holding tanks were kept in a locked room at Camdenton High School (Camdenton, MO) with the only light source being a 100-watt floodlight providing only indirect light. These variables were kept constant due to the propensity of fathead minnow embryos to prematurely hatch if a sudden change in light, sound, or vibration is detected. Water temperature for the duration of the experiment was kept constant within a range of 18 and 22 °Celsius.

**Water Treatments**

Three experimental groups were established to test for the effectiveness of surface pathogen prevention (Hydrogen Peroxide, Formalin, and UV). A control group was also added which had no treatment during the duration of the experiment. Each group (experimental and control) had three separate tanks which each contained between four and five tiles. Each tile contained between 150 and 750 eggs.

In order to accurately replicate the procedures used by Ozark Hatcheries, the concentrations of Formalin and 30% Hydrogen Peroxide (H₂O₂) used by the hatchery, 16 parts per million, were scaled down for the purposes of this experiment. Because Formalin and H₂O₂ can have negative side-effects if used later during the gestation period, the hatchery adds both chemicals to the water in the bottom tank every six hours for the only the first 24 hours after hatch. No chemicals
are added after that time by the hatchery and were also not added for the purpose of this experiment.

The non-chemical treatment regime used for this experiment involved the use of a model number sp-1, 120-volts, 60 hertz, .16 amps, 14 watt-lamp Trojan Technologies™ UV water Filtration system which has a market value of around $1,000 (provided at no charge from Aquafine corporation™) (Aquafine). This UV water filter was attached as an inline system in which the filter treated the water after being pumped from the reservoir but before the water reached the holding tanks.

Pre-eye Data Collection

Approximately twenty-four hours after the last chemical treatments were applied, each tile was taken out individually, photographed using a Sigma™ 3.5-5.6/18-70 macro lens attached to a 10.2 mega pixel Sony™ digital SLR α200 camera and placed back in the holding tank it came from. Every effort was made to expedite this portion of the procedure so as to reduce any chance of desiccation to the eggs. The pictures were then digitally examined through Windows Photo Gallery to determine an exact count of infected and non-infected eggs.

Late Stage Eyed Data Collection

The development of the eggs was carefully monitored in order to avoid premature egg hatch. Both Dr. Warbritton of USGS and Mr. Stan Smith of Ozark Fisheries gave us an estimated time frame for hatching. Once the eggs were within a day of hatching, the tiles were placed in a refrigerator in order to drastically minimize the possibility of heat and light to cause premature
hatching, while not yet eradicating the eggs. The tiles were then examined under a Swift SM-80 stereomicroscope, with 20x magnification, to determine the number of living, blank (unfertilized), and infected eggs.


**Discussion of Results**

Pre-eyed Data Collection:

In the first collection of data, many of the eggs were not developed enough to make a clear, adequate distinction between the non-infected fertilized, and the non-infected unfertilized. Thus, it was necessary to forego a count of perceived blank count, and only make a count of infected and non-infected eggs.

*Control*

The control group contained a total of 792 non-infected eggs and a total of 573 infected eggs. There were a total of 1,365 eggs in the control group. This means that after only twenty-four hours from the addition of the last chemical treatment, there was a mere 42% infection rate among the control eggs. The control group showed the highest infection rate in comparison to the other three treatments. The data collected from this group is illustrated in Figure 1.

*Ultraviolet*

The UV group contained a total of 1,905 non-infected eggs and a total of 132 infected eggs. This means there was a total of 2,037 eggs in the UV group. So, at the time of the first data collection there was only a 6% infection rate for the UV group. This group showed the lowest infection rate by a significant margin. Once statistics were run on this data, the UV group had an exceptionally noteworthy ln ration, showing that this data is highly significant. The data collected from this group is illustrated in Figure 2.
**Hydrogen Peroxide**

The hydrogen peroxide group had 1,168 non-infected eggs and 350 infected eggs. The hydrogen peroxide group contained a total of 1,518 eggs. This equates to a 23% infection rate in the hydrogen peroxide group. The infection rate of hydrogen peroxide ranked third among treatments in the first collection. The data collected from this group is illustrated in Figure 3.

**Formalin**

The formalin group had 1,732 non-infected eggs and 499 infected eggs. The Formalin group contained a total of 2,231 eggs. The infection rate for the formalin group was 22%. The formalin group had the second lowest survival rate. The data collected from this group is illustrated in Figure 4.

The photographs of the eggs allowed for the hasty return of the eggs back in the water in order to prevent mortalities from them spending extended periods of time spent out of the water, and also allowed for the tiles to be, while still manually, counted using digital advantages to make a more accurate count (adjusting the brightness and contrast, zoom, etc.). Despite these advantages, all 7,151 eggs were manually counted. In the pre-eyed data collection, the UV group had the lowest infection rate followed by the formalin group with the second lowest, the hydrogen peroxide group in third, and the control group with the highest infection rate. The results of this comparison are illustrated in Figure 10. The results from this collection were significant with a p value of p<0.001.
Late Stage Eyed Data Collection:

In the second data collection, which occurred after 11 days of incubation, living eggs were determined to have a visible heartbeat, blank eggs were eggs that were never fertilized (which have no noticeable developing embryo inside the egg), but were not contaminated by pathogenic growth, and infected eggs were any egg that exhibited fungal growth (or contamination from some other surface pathogen).

Control

The control group contained a total of 704 living eggs, 18 blank eggs, and a total of 643 fungal eggs. This means there was a total of 1,365 eggs in the control group by the second collection. These numbers show an infection rate of 42% to 21%. However, these numbers are not correct because of the number of eggs broken down between the first and second collections (See Eggs Unaccounted For, page 15). The control group showed the second lowest infection rate in comparison to the other three treatments during collection two. The data collected from this group is illustrated in Figure 5.

Ultraviolet

The UV group contained a total of 1,514 living eggs, 17 blank eggs, and a total of 209 infected eggs, which totals to 1,740 eggs. So, at the time of the first data collection there was a 13% infection rate for the UV group. This group, again, showed the lowest infection rate. Also, as with the first collection, the UV data was highly significant with a notable natural log (ln) ratio. The data collected from this group is illustrated in Figure 6.
**Hydrogen Peroxide**

The hydrogen peroxide group had 929 living eggs, 10 blank eggs, and 264 infected eggs. This totals to 1,203 eggs. The infection rate among the hydrogen peroxide group remained constant at 23%. The infection rate of hydrogen peroxide ranked third among treatments. The data collected from this group is illustrated in Figure 7.

**Formalin**

The formalin group had 1,075 living eggs, 31 blank eggs, and 729 infected eggs. This totals to 1,835 eggs accounted for in the second count. The survival rate for the formalin group was 41%. The formalin group had the highest infection rate in the second collection. The data collected from this group is illustrated in Figure 8.

In the late stage eyed data collection, the UV group had the lowest infection rate followed by the control group with the second lowest, the hydrogen peroxide group in third, and the formalin group with the highest infection rate. The results of this collection also had a p value of p<0.001. These results are illustrated in Figure 10.

**Eggs Unaccounted For Between Data Collections**

While calculating the results of the second data collection, it was noticed that the figures of total eggs in collections one and two were not equivalent. Each test group had between 300 and 400 fewer eggs in the second collection than in the first collection. This event was especially
noticeable in the control group where the total eggs decreased from 1,365 in the first collection, to 890 in the second collection. This represented a -34.8% reduction of eggs between the two counts. However, it was noticed during the second collection, that in nearly every tile in every group, there was evidence of heavy fungal growth without any remains of eggs. Therefore, the conclusion was drawn that the deficit of eggs in the second collection can most likely be attributed to the pathogen, most likely a fungus, completely breaking down the eggs that were unaccounted for in the final collection. The fact that the control group exhibited the highest proportion of ‘missing eggs’ only decreases the credence to this claim of the second lowest infection rate in the second data set. Figure 11 illustrates this occurrence among each test group.

Results

To get the best picture of the final results of each group the amount of would have to be the total number of eggs present in the first collection divided by living eggs in the second collection. Through this equation the results are quite different, they provide an overall efficiency for each treatment. The UV group still has the lowest infection rate with 26%, but the hydrogen peroxide group had the next lowest with 39%. Then, with the third lowest infection rate was the control group with 48%, followed by the formalin group with a 52% rate of infection. These results are illustrated in Figure 12.
Tables & Figures

Table #1
Results of the first data collection for each test group with the calculated infection rate (p>0.001).

**Pre-Eyed Data Collection Results**

<table>
<thead>
<tr>
<th></th>
<th>Not Infected</th>
<th>Infected</th>
<th>Total</th>
<th>Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>792</td>
<td>573</td>
<td>1365</td>
<td>42%</td>
</tr>
<tr>
<td>UV</td>
<td>1905</td>
<td>132</td>
<td>2037</td>
<td>6%</td>
</tr>
<tr>
<td>Formalin</td>
<td>1732</td>
<td>499</td>
<td>2231</td>
<td>22%</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>1168</td>
<td>350</td>
<td>1518</td>
<td>23%</td>
</tr>
</tbody>
</table>

Table #2
Results of the second data collection for each test group with the calculated infection rate (p>0.001).

**Late Stage Eyed Data Collection Results**

<table>
<thead>
<tr>
<th></th>
<th>Living</th>
<th>Blank</th>
<th>Infected</th>
<th>Total</th>
<th>Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>704</td>
<td>18</td>
<td>168</td>
<td>890</td>
<td>21%</td>
</tr>
<tr>
<td>UV</td>
<td>1514</td>
<td>17</td>
<td>209</td>
<td>1740</td>
<td>13%</td>
</tr>
<tr>
<td>Formalin</td>
<td>1075</td>
<td>31</td>
<td>729</td>
<td>1835</td>
<td>41%</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>929</td>
<td>10</td>
<td>264</td>
<td>1203</td>
<td>23%</td>
</tr>
</tbody>
</table>
Figure 1
Results of the control group in the pre-eyed collection.

Figure 2
Results of the ultraviolet group in the pre-eyed collection.
Figure 3
Results of the hydrogen peroxide group in the pre-eyed collection.

Figure 4
Results of the formalin group in the pre-eyed collection.
Figure 5
Results of the control group in the second collection.

![Control Results from Late Stage Eyed Data Collection](image1)

Figure 6
Results of the ultraviolet group in the second collection.

![Ultraviolet Results from Late Stage Eyed Data Collection](image2)
Figure 7
Results of the hydrogen peroxide group in the second collection.

![Hydrogen Peroxide Results from Late Stage Eyed Data Collection](image)

Figure 8
Results of the formalin group in the second collection.

![Formalin Results from Late Stage Eyed Data Collection](image)
Figure 9
Comparison of the infection rates of each test group from the first collection (p<0.001).
Figure 10
Comparison of the infection rates of each test group from the second collection (p<0.001).
Figure 11
Comparison of the total eggs of each test group recorded from both collections. This shows the amount of eggs that were completely broken down between the first and second collections.

Number of Eggs for Each Data Collection

<table>
<thead>
<tr>
<th>Water Treatment Groups</th>
<th>Pre-Eyed Data Collection</th>
<th>Late Stage Eyed Data Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1365 ± 49</td>
<td>890 ± 49</td>
</tr>
<tr>
<td>UV</td>
<td>2037 ± 52</td>
<td>1740 ± 52</td>
</tr>
<tr>
<td>Formalin</td>
<td>2231 ± 54</td>
<td>1835 ± 54</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>1518 ± 48</td>
<td>1203 ± 46</td>
</tr>
</tbody>
</table>
Figure 12
Comparison of the overall efficiency of the all of the groups by finding a percentage of the living eggs in the second collection and the total eggs in the first collection.

![Bar chart showing overall efficiency](chart.png)

- **Control**: 48%
- **UV**: 26%
- **Formalin**: 52%
- **Hydrogen Peroxide**: 39%

**Water Treatment**

**Infection Rate**

- 0%
- 10%
- 20%
- 30%
- 40%
- 50%
- 60%
Attachments:

Attachment 1
This is a picture of one section of a UV tile in the Pre-Eyed Data Collection. This picture provides a good representation of each possible egg condition.
Attachment 2
This is a picture of one section of a formalin tile in the Late Stage Eyed Data Collection. This picture provides a distinct representation of infected and non-infected eggs.
Attachment 3
This is a picture of the apparatus.
Statistical Analysis:

A G-test (variation of a Chi-square analysis) was conducted using Microsoft Excel to determine the significance of the reported results. The G-test did show that both collections of results were significant, both with a p-value of $p < 0.001$.

**G-Test Analysis**

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>Expected Ratios</th>
<th>Expected Frequencies</th>
<th>Ratio</th>
<th>In Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>42</td>
<td>23.25</td>
<td>23.25</td>
<td>1.80645</td>
<td>24.83730842</td>
</tr>
<tr>
<td><strong>UV</strong></td>
<td>6</td>
<td>23.25</td>
<td>23.25</td>
<td>0.25806</td>
<td>-8.127273977</td>
</tr>
<tr>
<td><strong>Formalin</strong></td>
<td>22</td>
<td>23.25</td>
<td>23.25</td>
<td>0.94624</td>
<td>-1.215778931</td>
</tr>
<tr>
<td><strong>Hydrogen Peroxide</strong></td>
<td>23</td>
<td>23.25</td>
<td>23.25</td>
<td>0.98925</td>
<td>-0.24865107</td>
</tr>
</tbody>
</table>

**p value =** $7.49484E-15$

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>Expected Ratios</th>
<th>Expected Frequencies</th>
<th>Ratio</th>
<th>In Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>21</td>
<td>24.5</td>
<td>24.5</td>
<td>0.85714</td>
<td>-3.237164276</td>
</tr>
<tr>
<td><strong>UV</strong></td>
<td>13</td>
<td>24.5</td>
<td>24.5</td>
<td>0.53061</td>
<td>-8.23840881</td>
</tr>
<tr>
<td><strong>Formalin</strong></td>
<td>41</td>
<td>24.5</td>
<td>24.5</td>
<td>1.67347</td>
<td>21.11085692</td>
</tr>
<tr>
<td><strong>Hydrogen Peroxide</strong></td>
<td>23</td>
<td>24.5</td>
<td>24.5</td>
<td>0.93878</td>
<td>-1.453114737</td>
</tr>
</tbody>
</table>

**p value =** $1.10028E-14$
Conclusions:

Based on the results of this study the following conclusions may be made:

1. The first hypothesis which stated that the ultraviolet group would be less affected by pathogens than the control was supported (p<0.001)

2. The second hypothesis which stated that eggs in the Ultraviolet group will be less affected by surface pathogens than those of the formalin group was supported; (p<0.001)

3. The third hypothesis which stated that eggs in the Ultraviolet group will be less affected by surface pathogens than those of the hydrogen peroxide group was also supported; (p<0.001)
Future Studies

Future studies could include:

- The environmental impact of Formalin and hydrogen peroxide could be examined; including the chemicals persistence in the watershed and the impact those chemicals have on the ecosystem with macro-invertebrates, sensitive fish, and aquatic plants.
- Varying the intensity of the ultraviolet light or the concentration of the chemicals
- The use of eggs from different species of fish such as goldfish could be used to validate this studies application to commercial fish.
- A study to determine if using Ultraviolet light to sterilize the water before introducing the eggs could further reduce the infection rates among eggs could help expand the usefulness of Ultraviolet purification systems.
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Bibliography:

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