Sterile Male Release Technique (SMRT) Using X-Ray Irradiation as a Potential Novel Method for Managing Invasive Quagga Mussels

– A Laboratory Experiment

A Report to Utah Division of Wildlife Resources

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ABSTRACT

A laboratory experiment was designed to evaluate the potential of X-Ray irradiation as a method to control invasive quagga mussels by producing sterile males. We used the range of exposures 0 (control), 600, 3000, and 5000 rads to determine an optimal level that damages the gametes without being lethal.

The results demonstrated that X-Ray treated quagga mussels had lower developmental success than the control. Adult quagga mussels were highly tolerant of X-Ray irradiation with high survival rates (> 95%) after treatment. Mussels from all treatments produced motile sperms and these sperms were able to bind and fuse with eggs. There was a decrease in sperm binding between treatments, which are most likely due to decreased motility and not an irradiation-induced deficiency of sperm binding mechanisms. Surprisingly, fertilized eggs in all treatments were able to divide and produce swimming trochophores. In terms of larval formation and developmental success, irradiation appears to decrease development between zygote and trochophore stage. However, a subpopulation of embryos successfully formed trochophores even at the highest X-Ray dosage. Therefore, the current experiment was not able to produce completely sterile males, even at the highest irradiation of 5000 rads in a single 15 minute session, which is at the prescribed level of human cancer radiation therapy spread over numerous treatments spanning a month. The current experiment did not find an X-Ray dose leading to generate 100% sterile males. Even a higher dose if found, may not be realistic for implementation. Therefore, irradiation may not be an effective tool to manage quagga mussels in open waters, and irradiation generates concerns, such as releasing potential mutant mussels into natural waters.

ACKNOWLDGEMENTS

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INTRODUCTION

The invasion of dreissena mussels (i.e., zebra (*D.polymorpha)* and quagga (*D. rostriformis bugensis*) mussels) to lakes and rivers of North America has already resulted in severe ecological and economical impacts (Nalepa and Schloesser 1993, Connelly et al. 2007). For example, following the 1988 invasion of the Great Lakes by zebra mussels, it is estimated that regional economic damages on the order of $4 billion were incurred in the first 10 years, largely from sport fishery losses (Roberts 1990). Before quagga mussels were found in western states, the economic loss due to the invasion of zebra and quagga mussels was already thought to be as high as $1 billion per year in the U.S. (Pimentel et al. 2005). It is estimated that, between 1989 and late 2004, approximately $267 million was spent on preventing dreissena mussel infestations of electric-generation and water-treatment facilities in North America (Connelly et al. 2007). Since 2007, dreissena mussels have been found in the western United States (Stokstad 2007), and significant amount of funds have been spent in dealing with these invasive pests. For example, the Metropolitan Water District of Southern California spent about $10-15 million a year on research, prevention, control, early detection, and education regarding its water systems (Fonseca 2009). The control and prevention of infestation by dreissena mussels are major concerns to managers of any type of water delivery system because of these impacts. Unfortunately, tools for effective, cost-efficient, and ecologically sound dreissena mussel control are limited. Many methods have been tested or are being developed for dreissena mussel control, such as chemical treatment, physical killing, mechanical removal, and biological control. These methods can be combined to more effectively control and manage the population size, depending on the development stage of the mussel and the specific situation of the lakes and rivers. More tests are needed to develop more effective ways to deal with invasive mussels.

Objective of Project

The object of this proposal was to examine a potential application of the Sterile Male Release Technique (SMRT) to the invasive quagga mussels. SMRT was initially developed to control invasive insects, thus it was initially termed the Sterile Insect Technique (SIT). SMRT has shown success to control the spread of several invasive species, primarily insects. The basic concept behind SMRT is to treat males with an external agent, typically irradiation, which renders them sterile. These sterile males are released into the wild and compete with normal, wild males for mates. If enough sterile males are released, female mating will occur predominately with sterile males leading to a decline in the population of the pest species. The objective of this project was to determine under laboratory conductions if irradiated quagga mussel males could prevent normal females from producing viable offspring.

Experimental Design

The basic design of this project was to collect adult quagga mussels from Lake Mead, Nevada and acclimate them in the lab. They were exposed to doses of irradiation ranging from 0 to 5000 rads from a linear X-Ray accelerator. Irradiated animals were returned to the lab and induced to spawn. The released gametes were tested to determine their ability to undergo normal development to the early larval stages. Specific focus was given to the cross between irradiated males and normal females as this is the fundamental cross behind SMRT.

Collection & Maintenance of Animals

Adult quagga mussels were collected from the Las Vegas Boat Harbor of Lake Mead. These mussels were placed in 10 gallon aquariums with circulating Lake Mead water for one week before X-Ray irradiation and spawning (see below). These mussels were fed with microalgae (*Isochrysis sp*) at 1×10^6 cells/ml daily. The water temperature was about 21^{o}C during the experiment.

Irradiation of Animals

The quagga mussels were exposed to different doses of X-Ray--600, 3000, 4000, and 5000 rads, respectively. For each dose, mussels were placed in 1000mL glass beaker filled with Lake Mead water, and exposed directly under an X-Ray accelerator (Fig. 1). The irradiator was a Clinac IX Varian 6 MV linear accelerator (Varian Medical Systems, Palo Alto, California). Mussels without X-Ray treatment were used as control. The shell length of selected mussels for different treatments was measured to the nearest 0.1 mm with a digital caliper (Table 1). The shell length of mussels in the treatment groups did not show significant differences with the control group (ANOVA, $P > 0.05$).

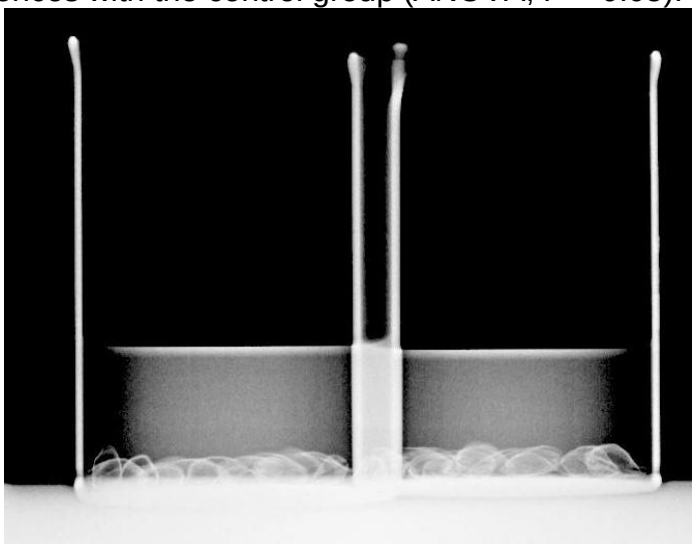

Figure 1. Image for quagga mussels in beakers treated with X-Ray. The mussels were placed in 4 cm of Lake Mead water.

Table 1. Shell length (mm) of mussels used in the experiment

Treatment	Mean	Minimum	Maximum	N
Control	18.7	15.5	23.9	41
600 rads	18.1	15.1	22.1	24

3000 rads	19.4	16.0	25.4	39
4000 rads	17.6	15.1	22.5	27
5000 rads	18.1	14.6	24.4	28

RESULTS

Spawning of Quagga Mussels

Spawning protocols were adapted from McAnlis et al. 2010. Animals were isolated overnight in 120-mL specimen cups containing filtered Lake Mead water (LMW). All experiments were carried out at room temperature (~20-22ºC). Immediately prior to spawning individual animals were rinsed with Lake Mead water and transferred to 25 ml flat-bottom tubes. Animals were induced to spawn by submergence in 1 mM 5-hydroxytryptamine (serotonin) for 30 minutes. Animals were washed twice to remove serotonin and re-suspended in Lake Mead water. Males and females typically began spawning within 15-30 miniutes or 60-70 minutes, respectively, following serotonin treatment. Once females began spawning they were transferred to 50 ml crystallizing dishes to complete spawning to minimize oocyte damage. Sperm concentration was determined by hemocytometer counts. It is important to note that we had very poor spawning levels particularly among irradiated females. Only one or two females spawned from the irradiated pool of animals. This may be attributed to the irradiation inhibiting female spawning greater than male spawning. Or it may be linked to a possible lower reproductive state for females collected from Lake Mead at a specific time and depth. Since the ultimate application of SMRT focuses on releasing males, we focused our efforts on crosses involving irradiated males.

Fertilization Series & Sample Collection for Assessment of Sperm Motility

Eggs from individual females were collected and transferred into 10 ml beakers containing Lake Mead water. Sperm from irradiated males where added to give a final sperm concentration of 10^4 sperm/ml. Samples were fixed at 0, 5, 20, 35, 80 minutes and at approximately 24 hours post insemination. Samples (500 ul) of inseminated eggs were fixed 1:1 with 4% paraformaldehyde in Mussel Buffer (McAnlis et al. 2010).

Potential changes in sperm motility due to irradiation treatment were performed. Briefly, a subsample of sperm was placed on slides and viewed under a phase-contrast microscope. General sperm motility was recorded as well as qualitative observations on sperm swimming pattern. Video recordings of sperm motility were made.

Somewhat surprisingly, sperm from all irradiation levels exhibited motility. In general the control (0 rad), 600, and 3000 rads treated sperm exhibited relatively indistinguishable sperm motility. Sperm were highly motile and exhibited the typical sinusoidal flagellar beating. Sperm from 5000-rad males also exhibited motile sperm. While active and having a relatively uniform linear movement, 5000-rad sperm flagellar beating appeared more irregularly jerky in nature

relative to the highly uniform sinusoidal beat of the control and lower dosage treatments.

Assessment of Sperm Binding

Critical to the development of a SMRT technique for quagga mussels is the requirement that irradiated sperm be able to bind to normal eggs. To assess sperm binding, the 5-minute post insemination time points were examined for numbers of bound sperm. The experimental analysis assessed 50 eggs for three independent crosses between irradiated males and normal females. Using phase contrast microscopy the number of bound sperm on each egg was determined. Sperm in the jelly layer surrounding the egg were not counted. Only sperm bound perpendicular to the egg surface were scored.

Sperm binding occurred in all treatments (Fig. 2). Even the 5000-rad treatment, which showed altered sperm motility, had bound sperm. There was a statistically significant difference in bound sperm between treatments with the 5000-rad treatment being significantly lower than the controls (Fig. 3). These data suggest that sperm are not sufficiently damaged by the irradiation to prevent them from binding to the egg. The lower binding rates, especially in the higher irradiation treatments, may be due to decreased sperm motility or possible damage to the cellular sperm binding machinery such as the sperm acrosome.

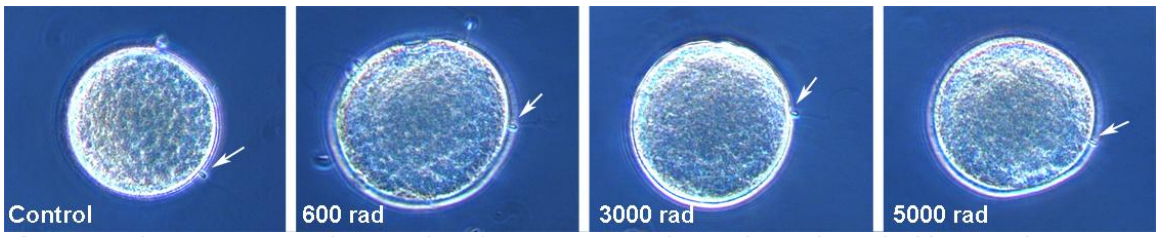

Figure 2. Phase contrast micrographs of quagga mussel eggs inseminated with control or irradiated sperm. At 5 minutes post insemination, sperm from all four treatments were able to bind to the surface of untreated eggs. Arrow indicates bound sperm.

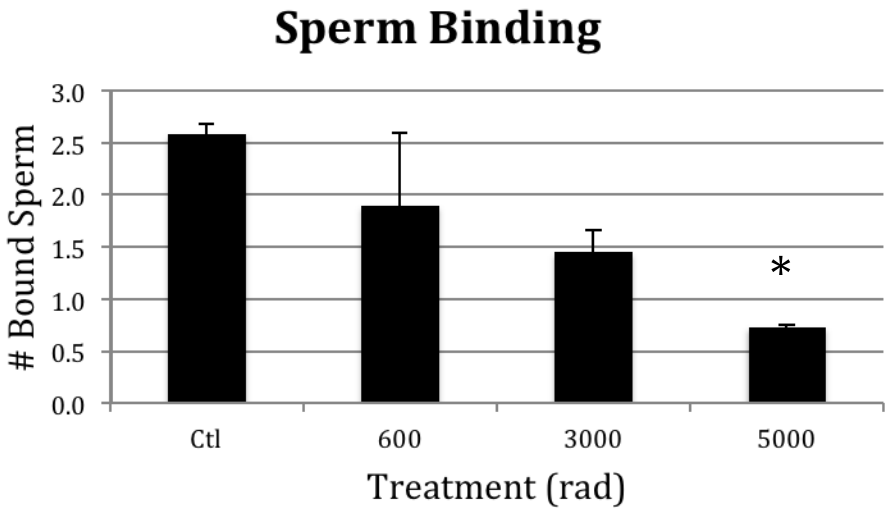

Figure 3. Mean number of bound sperm per egg at 5 minutes post insemination based on three independent crosses of untreated eggs and irradiated sperm. Fifty eggs per trial were counted. Treatment indicates the level of male irradiation. Bars = standard error of mean. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$).

Assessment of Egg Activation

The next step in the fertilization process is activation of the egg. Sperm binding and/or fusion with the egg typically induces egg activation. This activation initiates the completion of meiosis that prepares the egg's DNA for recombination with the sperm's DNA. This resumption of meiosis can be visualized by the presence of polar bodies, which is the excess DNA not used by the egg. To determine if irradiated sperm were capable of activating eggs, the numbers of eggs that produced polar bodies was determined between the four treatments. Fifty eggs from the 20-minute time points for the three independent crosses between normal females and irradiated males were examined for polar body presence.

Sperm in all treatments were able to initiate egg activation as indicated by polar body formation. Eggs were evaluated for the presence or absence of polar bodies. Eggs that were either not activated where distinguished by the female DNA in meiotic arrest (Fig. 4a). Eggs that where activated resumed meiosis and produced the first polar body (Fig. 4b). Polar body formation was similar in morphology between controls and all irradiation levels (Fig. 4c-e). There was a significant decrease in numbers of activated eggs between the control and the 3000-rad and 5000-rad treatments (Fig. 5). Egg activation is significant because it typically leads to later egg events including the activation of any polyspermy blocks. These blocks are the basic concept underlying SMRT's adaptation to quagga mussels.

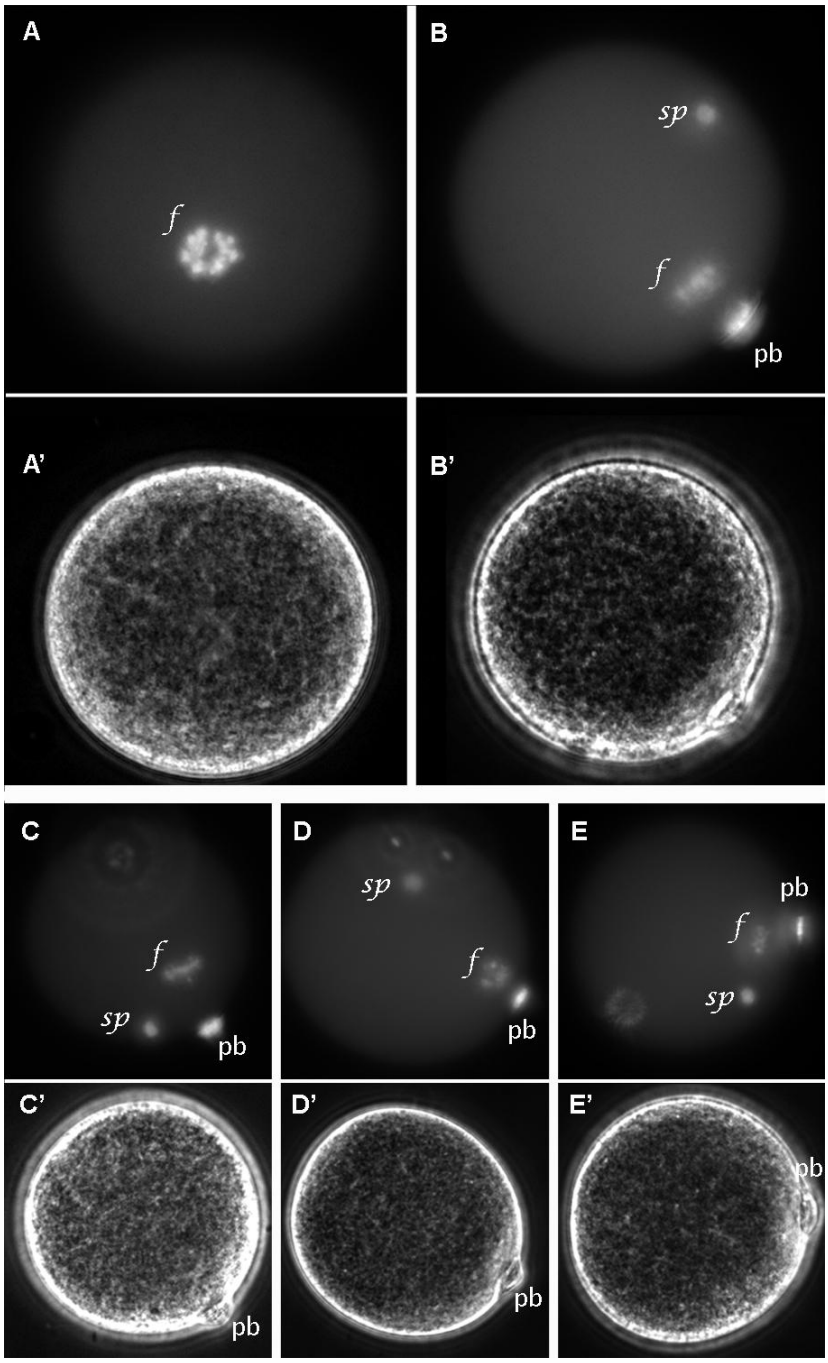

Figure 4. Fluorescent (A-E) and corresponding phase (A'-E') images at 20 minutes post insemination of eggs fertilized with treated sperm. (A,A') Unfertilized egg showing only the female nucleus (f) in the egg cytoplasm. (B,B') Egg fertilized with control sperm. Egg has resumed meiosis resulting the 1st polar body (pb) and the haploid female nucleus (f). Sperm nucleus (sp) has entered the egg cytoplasm and begun to decondense. (C-E) Eggs fertilized with irradiated sperm at (C,C') 600 rad, (D,D') 3000 rad, and (E,E') 5000 rad. Irradiated sperm from all treatments were able to enter the egg cytoplasm and begin to decondense(sp) and resumption of oocyte meiosis producing the haploid female nucleus (f) and first polar body (pb).

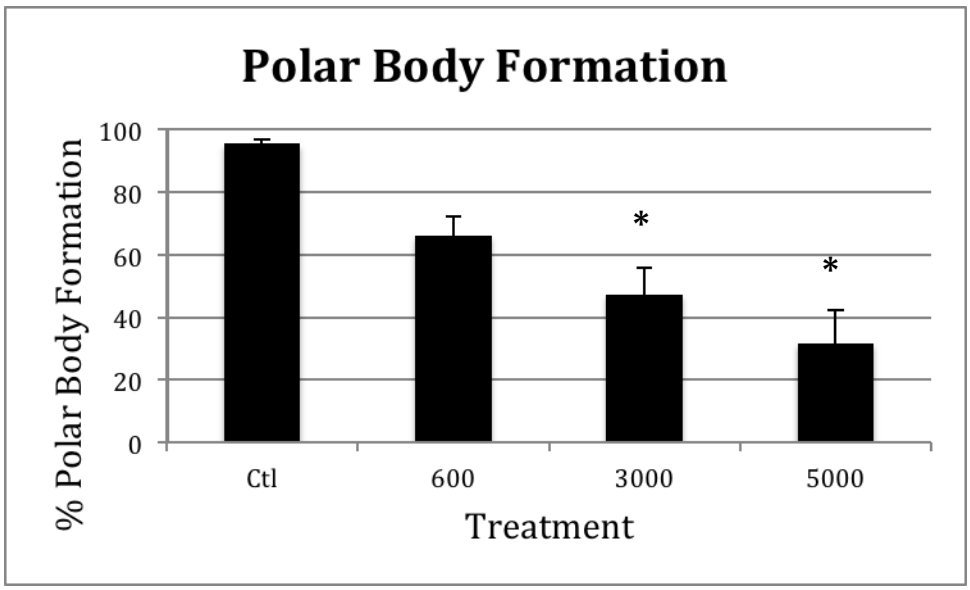

Figure 5. Evaluation of egg activation based on the presence of polar bodies. Mean number of eggs with 1st polar body was determined at 20 minutes post insemination. Fifty eggs from three independent crosses of untreated eggs and irradiated sperm were counted. Treatment indicates the level of male irradiation. Bars = standard error of mean. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$).

Assessment of Fertilization Success

The next step was to determine if irradiated sperm had the ability for fusion with the egg plasma membrane and to enter into the egg's cytoplasm. Fertilization is defined as the incorporation of the sperm nucleus into the egg. In most animal models, either sperm binding or sperm fusion is the key to activating the block to polyspermy preventing other sperm from fertilizing the egg. Sperm nuclei incorporated into the egg's cytoplasm begin to decondense in preparation for combining with the female's chromosomes. These decondensing sperm nuclei can be identified when labeled with the DNA specific dye (Hoechst 3334) and viewed under a fluorescent microscope (Fig. 4b).

Sperm from all treatments were able to fertilize normal eggs (Fig. 4c-e). There was a significant decrease in mean number of eggs between the controls and the 3000-rad and 5000-rad treatments. This decline mirrors the changes observed in egg activation and is most likely attributed to the lower numbers of bound sperm able to bind to eggs in the higher irradiation treatments. However, it cannot be completely ruled out that there is some effect of irradiation on sperm that decreases sperm incorporation success. This appears unlikely, as few sperm were found still bound to the egg, which would be expected if they could not enter into the egg's cytoplasm.

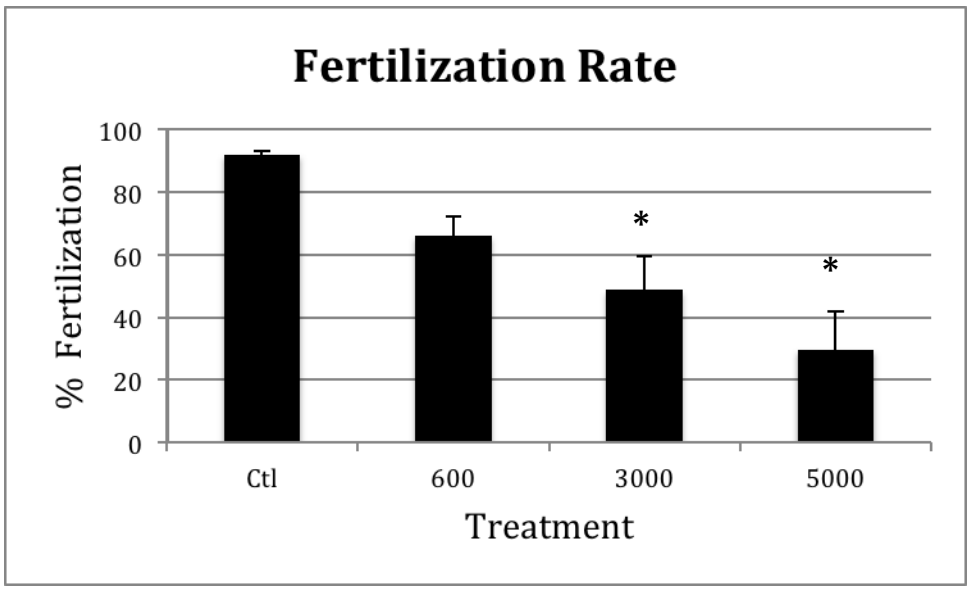

Figure 6. Fertilization rate based on numbers of eggs with incorporated sperm. Mean number of eggs with a fertilizing sperm in the egg's cytoplasm was determined at 20 minutes post insemination. Fifty eggs from three independent crosses of untreated eggs and irradiated sperm were counted. Treatment indicates the level of male irradiation. Bars = standard error of mean. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$).

Assessment of Development to First Cleavage

In order for zygote (a fertilized egg) development to continue, the zygotes must be able to undergo first cleavage. The ability for zygotes to undergo normal first cleavage is indicated by several factors. First, sperm must be able to generate the cellular machinery needed for cell division. In particular, the sperm must contribute its centrioles to the egg's cytoplasm, directing the formation of the mitotic spindle. This spindle is responsible for separating the chromosomes into the two daughter cells that will form from this first cell division. The spindle also creates part of the driving mechanism that physically divides the cells (cytokinesis). The second important factor indicated by successful first cleavage is the separation of chromosomes (karyokinesis). Again the spindle is responsible for correctly separating the chromosomes into the two genetically identical daughter cells. If the spindle is not functioning correctly, karyokinesis may be disrupted.

Zygotes were able to divide in all treatments (Fig. 7). However, there was a significant difference between the treatments. Over 90% of the eggs in the control treatment had divided to form two-cell embryos (Fig. 8). Conversely less than 25% of the 3000-rad and 5000-rad treatment zygotes had divided. There are two possible explanations for the decrease in first cleavage. First, the lower fertilization rates in the higher irradiated treatments accounts for much of the decrease. Secondly, there may be a decreased ability for the zygotes in the higher irradiated treatments to divide. Possibly, any direct damage to the sperm's DNA during irradiation could have hindered karyokinesis. It is important to notice

that over 20% of the eggs were able to divide to form a two-cell embryo even at the highest level of irradiation.

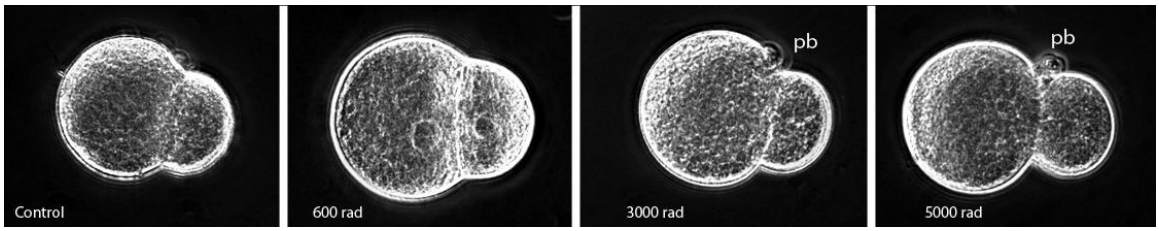

Figure 7. Phase micrographs of 2-cell embryos at 80 minutes post insemination. Zygotes from each of the treatments were able to divide (1st cleavage) resulting in 2- embryos. Pb- polar body visible in some images due to angle of image**.**

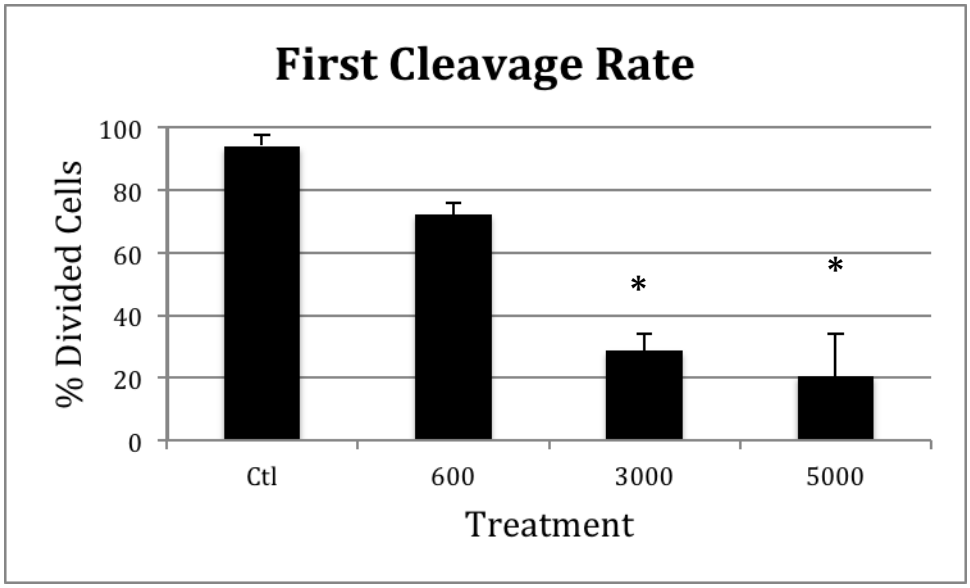

Figure 8. Mean number of eggs that underwent first cleavage producing a two-cell embryo was determined at 80 minutes post insemination. Fifty eggs from three independent crosses of untreated eggs and irradiated sperm were counted. Treatment indicates the level of male irradiation. Bars = standard error of mean. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$).

Assessment of Development to Ciliated Larval Stage

To see if development of ciliated larvae (trochophores) varied between treatments, we allowed the insemination crosses to develop for approximately 24 hours. Briefly, additional Lake Mead water was added to each insemination beaker and allowed to develop overnight at room temperature. The following day, the percentage of ciliated embryos was determined by scoring the embryos as either a (1) ciliated embryo or (2) Arrested embryo/Uncleaved Zygote/Unfertilized egg. All eggs and embryos were counted in each dish and percentages determined.

All treatments were able to produce ciliated larvae (Fig. 9). As somewhat expected based on fertilization rates, the controls had the highest percentage of eggs develop into ciliated larvae. Almost 60% of the control eggs developed into ciliated larvae while less than 10% of the 3000-rad treatment and less than 5% of the 5000-rad treatment developed. There was a statistically significant difference in larval formation between the control/600-rad treatment and the 3000/5000-rad treatment. Two things are significant with these data. First, nearly 60% of the control eggs developed into ciliated larvae under In vitro culture conditions. This was higher than expected given the difficulty of maintaining larval development in the lab. Second, while only 5% of the 5000-rad eggs developed into ciliated larvae, we were hoping to see no larval development at all. Given a low estimation of 30,000 eggs released per spawn based on zebra mussel spawns (Ackerman et al. 1994), each mating involving a 5000-rad irradiated male could result in 1,500 larvae.

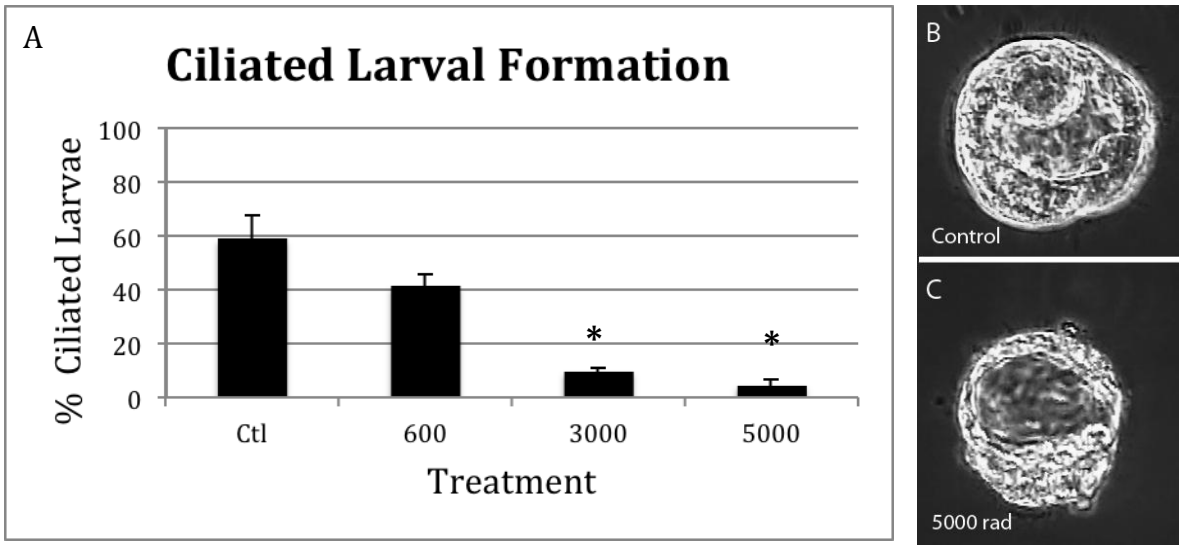

Figure 9. (A) Percentage of eggs that developed into ciliated larvae 24h post insemination. All eggs and larvae from the three independent crosses for each treatment where counted and percentage of larvae determined. Bars = standard error of mean. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$). (B,C) Captured images from video microscopy of swimming larvae resulting from crosses involving control sperm (B) or 5000-rad sperm (C). Cilia visible on the surface of the larvae. Similar larvae were observed for 600-rad and 3000-rad irradiations.

When we look at development success, as calculated by the % larval formation/fertilization rate, we see the following trend (Fig. 10). The controls and 600-rad treatment had a greater than 60% developmental success indicating that approximately 60% of the fertilized eggs developed into ciliated larva. The 3000-rad and 5000-rad treatments had much lower developmental success. Only 20% (3000-rad) and 10% (5000-rad) of the fertilized eggs developing into ciliated larvae. This would indicate that under laboratory conditions these zygotes were more developmentally challenged than controls.

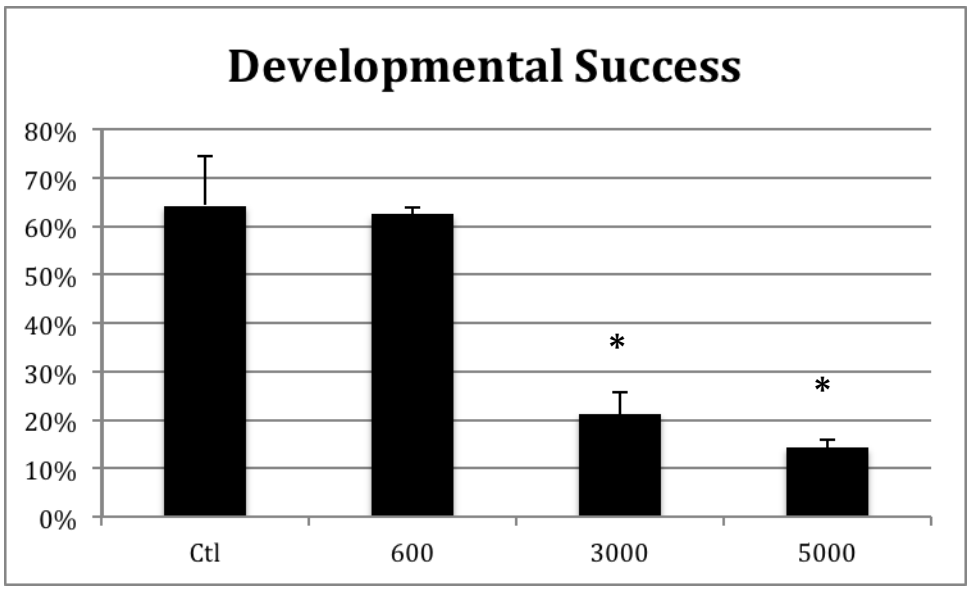

Figure 10. Developmental success (% ciliated larvae/% fertilization) was calculated for each independent cross of the various treatments. This is an indicator of what portion of the fertilized eggs ultimately developed into ciliated larvae. An asterisk (*) indicates significant difference based on 1-way ANOVA and Tukey-Kramer multiple comparisons ($p<0.05$).

Assessment of Irradiated Mussel Mortality

X-Ray irradiated mussels were brought back to the glass aquarium with circulating Lake Mead water. Totally 36, 26, 40, 33, and 29 mussels were used for the control, 600, 3000, and 5000-rad treatments, respectively. All mussels were alive. The mortality rates of mussels with different treatments were examined at day 15, 30, and 45, respectively. The viability of mussels was evaluated using a dissecting needle between the posterior margins of the mussels' shell valves (Comeau et al. 2011).

During the 45 day observation period, mussels from all treatments showed high survival rates (Fig. 11). At the end of the study, the survival rates of the control, 600, 3000, and 5000-rad treatments were 94.4%, 96.2%, 97.5%, and 96.6%, respectively.

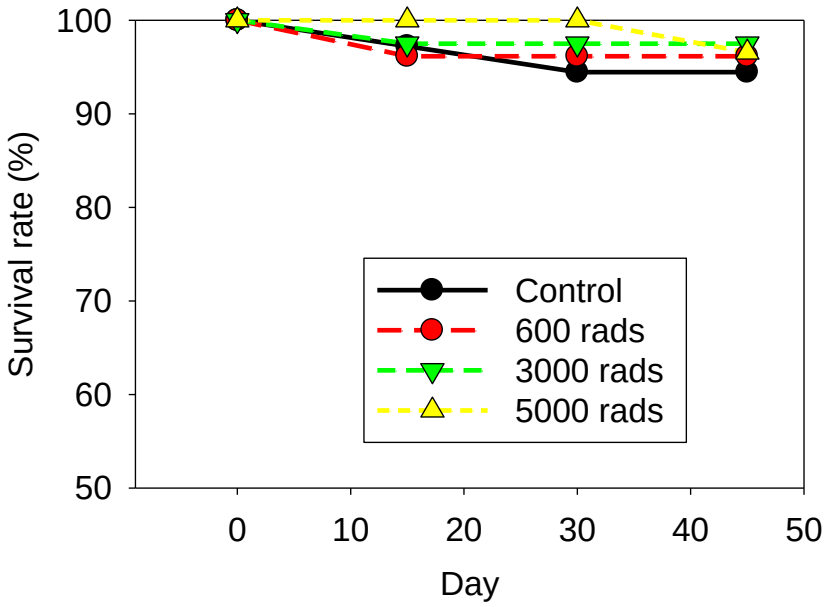

Figure 11. Survival of X-Ray treated quagga mussels.

CONCLUSION

Quagga mussels were surprisingly tolerant of X-ray irradiation. Less than 10% mortality was observed after exposure even in the highest dosage. Given that the 5000-rad dosage was 2.5x the level used on crayfish, we were surprised that the highest dosage was not 100% lethal (Aquiloni et al. 2009). For comparison, the 5000-rad exposure level is at the prescribed level of human cancer radiation therapy, which is spread out over numerous treatments spanning a month. Whereas the quagga mussels received this dosage in a single, 15 minute exposure.

Within 1-2 days of exposure, mussels from all treatments were able to produce motile sperm. While motility appears slightly impaired in the 5000-rad dosage, even these sperm were able to contact and bind to eggs. This is significant since sperm motility is essential for sperm to reach the egg as well as the actual events of fertilization. The latter has even more significance in broadcast spawning species such as dreissena mussels.

All treatments of sperm were able to bind to the egg. This suggests that the sperm's acrosome remains intact. The acrosome is a vesicle on the tip of the sperm required for sperm-egg binding and fusion; it is particularly sensitive to environmental changes. We hypothesized that disruption of the sperm's acrosome would occur in higher radiation levels and might alter fertilization success. This appears not to be the case although direct analysis for acrosomal

integrity was not performed. There was a decrease in sperm binding between treatments. This is most likely due to decreased motility and not an irradiation-induced deficiency of sperm binding mechanisms. If binding and not motility were affected, we would expect to find higher concentrations of sperm in the jelly layer surround the egg. This egg jelly attracts sperm to the egg. If binding were inhibited, we would expect unbound sperm to remain in the jelly layer, while in the control the sperm would pass through the layer and bind to the eggs. We did not observe any difference in unbound sperm associated with the jelly layer between treatments.

Irradiation did not appear to affect the sperm's ability to fuse with the egg and decondense in preparation for fusion with the female genome. We hypothesized that potential DNA damage caused by irradiation might stop the fertilization process at this point. That does not appear to be the case as development continues after the sperm entered the egg. Likewise, irradiated sperm were able to activate the egg as indicated by meiotic resumption and polar body formation. Presumably this also activates the eggs slower block to polyspermy as evident by sperm detaching from the egg surface 15 minutes post insemination.

Most surprising and disconcerting was that fertilized eggs in all treatments were able to divide and produce swimming (trochophore) larvae 24 hours later. We expected that the higher dosages would damage the sperm DNA sufficiently to hopefully prevent early cleavages and certainly block development well before the trochophore stage. As evidenced by the decrease in larval formation (Fig. 9) and developmental success (Fig. 10), irradiation appears to decrease development between the zygote and trochophore stage. However, there is still a subpopulation of embryos that successfully formed trochophores even at the highest dosage.

Implications for application of SMRT on quagga mussels

The fundamental concept behind the success of SMRT is that sterile males can prevent production of viable offspring in females. In many of the successful cases, mating occurs between a single female and a single, or very limited, number of males. For example, in the screw-worm fly (*Cochliomyia hominivorax*) and the tsetse fly (*Glossina austeni*), females mate only once. If this single mating occurs with a sterile male, the female will never produce viable offspring and is essentially removed from the reproductive population. For aquatic species, SMRT has been used to manage sea lamprey, *Petromyzon marinus* (Twohey et al. 2003), and X-Ray irradiation has been shown to be effective to reduce male fertility in the perspective of adopting the SMRT technique to control invasive crayfish (*Procambarus clarkii*) populations (Aquiloni et al. 2009).

There are essentially two stages at which SMRT inhibits the production of offspring. The first is at the level of mating between individuals. If a female mates with a single male, the ideal SMRT technique would be to have a completely sterile male with no gamete production. This is the basis of the success seen in

the screw-worm fly and tsetse fly. How the male gametes interact with the egg is irrelevant as long as no offspring are produced. A key requirement is that irradiated males are able to compete against normal males for mates.

The second stage where SMRT prevents offspring production is at the level of fertilization. Females would mate with one or more males and sperm competition for eggs would dictate the success of SMRT. For example, a female would mate with multiple males and sperm from each male would compete for who gets to fertilize the egg resulting in the next offspring. For successful SMRT, sterile male gametes would either need a competitive advantage to reach and fertilize the eggs first, or they must be able to directly compete with normal males.

The application of SMRT to dreissena mussels would rely on sperm competition for the prevention of reproduction. *Dreissena* mussels do not exhibit monogamous mating; presumably multiple males and females simultaneously spawn. This separates them from many of the other species for which SMRT has been successful. Several steps as follows need to be addressed to develop successful SMRT for *dreissena* mussels.

(1) Generation of sterile males. Even at the highest level of irradiation we were unable to produce completely sterile males. The reason behind this is unclear. The most obvious difference between mussels and other SMRT species is the calcium carbonate shell encasing mussels. However, we would expect the irradiation to travel through the relatively thin shell when compared to the thickness of the calcium phosphate human bone traditionally targeted by our irradiation equipment. Higher levels of irradiation are impractical using standard medical irradiation equipment.

(2) **Production of competitive sperm.** Irradiated sperm were able to bind to eggs. While binding rates were lower, there was no apparent disruption in the binding mechanism. Except for the 5000-rad dosage, there was no obvious difference in sperm motility. However, there was a difference in numbers of sperm reaching the eggs. Subtle differences may be present that limit the sperm's ability to reach the egg. Differences between normal and irradiated sperm in reaching and binding to eggs may be exacerbated in the wild. Gamete release and water currents may have a more dramatic impact on irradiated sperm relative to normal sperm, and reduce irradiated sperm binding rates to insignificant levels.

(3) **Bound sperm would have to fail to produce viable offspring.** As mentioned, all treatment levels were able to produce motile trochophore larvae under laboratory conditions. This creates two concerns. First, irradiated, released males would still be contributing offspring each spawning season. While numbers may drop off, there are still low levels of reproduction. Second, and of greater concern, is the genetic composition of these offspring. UV radiation was historically used to generate mutant strains of organisms used in genetic

research. The UV induced damage would alter gene and gene expression, changing the behavior (or phenotype) of the irradiated individual. It would be important to fully understand the viability to adulthood of those offspring generated from irradiated sperm to insure no genetically modified organisms were being indirectly introduced.

(4) **The sterile sperm would render an egg not viable.** Due in part to low spawning rates, and not identifying an irradiation level that produced high sperm binding but not fertility, we have not yet directly tested competition between irradiated and normal sperm. Some irradiated sperm did bind and develop into ciliated larvae, but this rate was lower than in controls. This would suggest that irradiated sperm might be limiting further development of eggs, which is an essential feature of SMRT as mentioned above.

(5) **Sterility in males needs to target the gamete stem.** Although not part of this project, this is an essential feature needed for implementation of SMRT in *dreissena*. This projected focused on immediate, short-term effects of irradiation on quagga mussels. It is essential for SMRT released males to be permanently sterile. This can be achieved in one of two ways. Either irradiation must be sufficient to permanently damage the stem cells in the testis responsible for spermatogenesis, or irradiated males that are reintroduced into the wild should have other chronic effects that lead to their demise prior to subsequent rounds of spermatogenesis. This is an inherent feature of SIT successfully applied to insects.

Several aspects of this project suggest that SMRT has potential application for quagga mussel control. These include irradiated sperm binding, inducing egg activation and lower developmental success. However, several findings raise greater concerns about potential application of SMRT. The first concern is that no level of radiation tested produced complete sterility, even at levels approaching logistically impractical dosages. Secondarily, the concerns regarding potential survivability and impact of offspring resulting from crosses involving irradiated sperm need to be addressed. A third concern is the reduced ability of irradiated sperm binding, and how successful would these sperm be in reaching and binding with eggs in a natural spawning bed. Lastly, it would be important to show that irradiation treatments would permanently sterilize males destined to be released into the wild. We feel that these concerns greatly outweigh the potential positive findings for SMRT application to dreissena mussels.

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