How Female Preference of Red Coloration in Male Three-spined Sticklebacks Unexpectedly Causes Oxidative DNA Damage

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Male three-spined sticklebacks display red coloration on their ventral side in order to attract females during mating season. The brighter the color, the more attractive they are to females. A consequence of this is that the brighter coloration expends energy to display, causing damage to the fish. Sin-Yeon Kim looks at how different intensities of red coloration shapes mating and courtship behavior in these fish. She also looks at the consequences of brighter coloration by looking at the oxidative DNA damage of different shades of male sticklebacks, as well as how global warming creates this damaging affect.

Studies have been done to show that the brighter red coloration on male sticklebacks attracts females and shows dominance over other males, as these fish are territorial. These fish tend to be brighter when in the presence of multiple males and have duller coloration where there is less competition. Kim uses red colored and non-colored dummies during mating season to see if there is any change in the intensity of the red coloration when a female is present.

Immature three-spined sticklebacks were collected and raised until they reached sexual maturity: development of blue eye coloration. Males were then placed in separate tanks where no other males could be seen and were given materials to build a nest. The males were split into two groups; one group was presented with a male dummy stickleback that displayed a red coloration on its cheeks and belly, where the other group was presented with a colorless male dummy. The red dummy was a brighter red than the male sticklebacks to imply competition. The number of times the stickleback attacked the dummy was recorded for 5 minutes. Female dummies were then introduced, and mating behavior was observed. They looked for fanning (actions displayed to signal the nest location) biting, and a zig-zag dance. The number of fanning displays waere counted. Males were then photographed. The area of where the red coloration was found as well as the intensity of the color was recorded. This was repeated 10 days after the first trial.

Kim found that both fish groups were more aggressive during the second trial but found that the fish with the red colored dummy was more aggressive. They also found that larger males were less aggressive than the smaller males. Fanning was also found to increase during the second trial and the group presented with the colored dummy was found to engage in more fanning behavior. Larger males also tended to fan more often. The area of the red coloration was found larger in males with the red dummy. In addition, the color intensity decreased from trial one to trial two in the colored group, whereas, the intensity increased from trial one to trail two

in the males presented with the colorless dummy. This confirms Kim's hypothesis that males become more competitive and increase their coloration when presented with a rival male.

Other studies have been done to show the negative affects of this coloration. For example, males that display a brighter coloration tend to die younger because of the energy spent maintaining this coloration as opposed to spending energy on protecting and maintaining healthy cells. The brighter coloration attracts females because it implies that the male will produce healthy offspring because the red coloration is a sign that the male is healthy, however, it can also harm the male and possibly the offspring. This paper focuses on the relationship between brighter coloration and how it affects the oxidative DNA in somatic and germline cells. Kim also looked to see if less damage is done later in the breeding season (February through August) than early in the season by changing the photoperiod. They hypothesize that males who reproduced later in the breeding season would grow faster and have less DNA damage then those who bred right away.

100 juvenile three-spined sticklebacks were collected and kept in a tank with a schedule of 15 hours of light to 9 hours of dark. Two groups were formed: the "early" and the "late." The "early" male fish were presented with nest building material and a female for five minutes twice a week starting early March where the "late" group started early April. The fish were examined and found to display their peak red coloration in May and June. In early June, muscle, teste, and sperm samples were collected from the males. The level of oxidative DNA damage was measured in each male fish.

They found that the "late males" had a larger red area than the "early" males in April and May, whereas growth rates were similar in both groups. They believe this may be due to the "late" fish compensating for delayed breeding activity. It could also be because the late breeding season allowed for more carotenoids to be stored and allowed them to invest in coloration while they were not busy breeding. During the peak breeding season, more oxidative DNA damage was found in brighter males. This may be because carotenoids are important in processes such as antioxidant protection and immunostimulation and investing them in coloration is a trade-off. The results also show that colorful males are not actually as good when it comes to reproduction because of the increased DNA damage to the sperm. In fact, males with less coloration where found to have higher fertilization and a higher hatch rate then the more colorful males, although they attracted more females. This suggests that sneaker males are successful in reproducing

because their duller coloration allows for more energy to be invested in reproduction. Genetic variation has also been found throughout the population and is suggested to be maintained in the population because of DNA damage and female preference.

Kim furthered studied the oxidative DNA damage by looking at how global warming affects these cells. Heat has been found to damage germ and soma cells, which affects reproduction. Since this damage can occur before the breeding season, sperm may be damaged, and the individual may not be able to reproduce. Kim looks at how the increasing temperatures due to global warming affects the three-spined sticklebacks, specifically looking at reproduction by observing the oxidative DNA damage.

Three-spined stickleback fish were captured and bred in order to produce juvenile fish. Two groups were created: one with normal temperatures and one with warmer temperatures. The water was reduced in one tank to stimulate winter temperatures (14°C in November to 9°C in January and then increased to 14°C in March), whereas the other tank was kept warm (temperatures ranging from 14°C in March to 18°C in June). Fish were kept from February until June when sperm samples were taken. Oxidative DNA damage in sperm and muscle cells was recorded.

Both sperm and muscle cells in the warmer temperature fish were found to have more oxidative DNA damage especially during the reproductive season. The warmer temperature fish also showed slower growth rates during the winter, but then had similar growth to the controlled fish when returned to spring temperatures. The control group also gained more mass during the winter, however, the warm-treated adult fish that had low body mass in the spring gained more mass during the winter months then the control fish. That being said, more DNA damage was found in the warm-temperature fish as opposed to reduced DNA damaged in the control fish. Mass gain and DNA damage in sperm was found to be statistically significant but not in the muscle. Since faster growth in the warm temperature fish occurred during breeding season, it not only damaged the DNA, but used up energy for growth as opposed to reproduction. Kim concluded that warmer temperatures are affecting the reproduction of three-spined sticklebacks reducing fertility and survival of the adult, which ultimately will reduce their population overtime.

Kim mentioned that there have been other studies that show no damage is done to oxidative DNA over long periods of warm temperatures. She suggested that this was because the

temperature stress activates endogenous antioxidant defenses for homeostasis. This should be further researched in order to see if that is a factor in order to reduce the DNA damage. In addition, seeing the affects warmer temperatures has on homeostasis could also help us understand the affects temperature has on other aspects of this fish species. That being said, a way to improve this study would be to perform it on a longer time scale. Since other studies looked at the long-term affects of temperature, it might be beneficial to do this study over a few years in order to see the long-term effects. As mass is strongly correlated with DNA damage, it would be useful to observe the DNA damage when the fish slows or stops growing to see if there is more or less DNA damage overtime or if most of the damage is done during the rapid growth period, also suggesting that this study could be repeated with adult sticklebacks as opposed to juvenile fish.

The importance of this research not only creates opportunity for further research, but it helps us understand the long-lasting impacts on not only the three-spined stickleback, but on other species as well. The fact that two factors are damaging the DNA of male sticklebacks and females are still choosing these males creates less offspring being produced which will ultimately reduce their populations. Them being small fish make them prey for many organisms such as birds and other fishes, so a reduction in their populations would also decrease the larger predator populations. In addition, since global warming affects specifically sperm in these fishes, it most likely affects the sperm in other species as well. Since not all fishes are putting energy into creating pigment, they aren't damaging their sperm DNA, meaning they may not be able to tolerate as much damage as the sticklebacks, which could quickly cause a decline in their populations. Further research could be done on other fishes as well to see if global warming affects other fish species before they are unable to maintain a stable population.

This research is also important to study because it is interesting that females prefer brighter males despite the reproductive disadvantages. It may be important to understand why this is and if there is a factor that we don't know about that may be affecting their decision. Further research should be done to investigate this reason; an idea is that this is because of pre-sensory bias in female fishes. For example, in the trinidadian guppy, females eat orange berries and are attracted to the orange coloration. Overtime males displayed orange spots and the females, being already attracted to the orange coloration, chose the males with the orange spots. Overtime, the males displayed brighter orange spots and females choose them over the duller

colored males. Further research could be done with the three-spined stickleback to see if this is the case or if there may be another factor affecting female mate choice. Another reason why females may refer brighter males is that this increases the amount of sneaker males in the population. The fewer colored males were found to produce more offspring, so the increased amount of sneaker males may help increase the population. Further research could be done to test this effect.

Another way to improve the research is by retesting male's ability to change intensities of red coloration when presented with rival males. Kim tested whether males are able to change their coloration and courtship behavior when presented with a dummy male and female. Her study focused on creating two groups and comparing them. Instead of making two groups, she could have used one male and did two trails like before but instead of repeating the trial, have one trial with a colorless dummy and one trial with the colored dummy and see if the individual male reacts differently. This would better fit her research topic as it would show if the individual is able to quickly react to different situations. It would also show differences in the male's courtship display. This could help in the future if we are able to monitor the populations of these fishes. For example, if many brightly colored fish are seen in an area, it may be beneficial to try and control their populations in order to reduce the oxidative DNA damage being done and if individual males can quickly adapt to this change, it will allow for healthier offspring to be produced.

In conclusion, Kim's research not only looks at how three-spined sticklebacks use coloration as a main technique but also the damage it may cause as well as how global warming is affecting this fish. This research allows us to look at more fish species of similar behaviors as well as creates new research opportunities to see how global warming is negatively affecting reproductive rates, which is imperative to maintain a healthy population. If we are able to further study and understand what is happening in these fish, we can better help protect them in the future.

Works Cited

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