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APES Topic 9 - Toxicology Mr. Romano

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by Kyla Dunn

In Florida's Lake Apopka, size does matter. Yet it took two years for University of Florida zoologist Lou Guillette to believe his own research findings. His data showed disturbing trends in the male alligator population of this lake, located just outside of Orlando. The most striking finding was the alligators' small penis size: 25% smaller than in normal males. Furthermore, these males had testosterone levels as low as a typical female's--a serious threat to their fertility. How had these "gender-bending" defects occurred? Could manmade chemicals in the waters of Lake Apopka be responsible? Concerned about the reproductive health of both wildlife and humans, scientists everywhere began taking notice.

Catching alligators is no small feat. Alligators are nocturnal, and must be studied at night. To find his alligators, Guillette takes to Lake Apopka in an airboat and scans the darkened water with a high-powered flashlight. An alligator's eyes reflect back the light, glowing red in the darkness. While the airboat slowly approaches the animal, one of Guillette's intrepid graduate students reaches out to grab it behind the head and hoist it onboard. Larger animals are pulled alongside the boat with a noose. The team then takes blood samples from each alligator and a variety of body measurements, including phallus size.

Guillette uses phallus size as an indicator of proper hormone signaling. His early research showed that alligators at Lake Apopka have abnormal levels of the sex hormones estrogen and testosterone. What's more, he suspected that contaminants in this pesticide-ridden lake might be blocking the male alligators' response to their own testosterone. Guillette knew that penis growth, whether in an alligator or a human, is dependent on testosterone--and for normal alligators more testosterone means more growth. If chemicals were blocking the effects of testosterone in these males, Guillette reasoned, he should see it very clearly: Apopka's males would have shorter penises. Not only that, but there would be no simple relationship between penis size and the amount of testosterone in an alligator's system. Guillette tested this new theory in the field.

What Guillette found in 1994-1995 was a 25% reduction in phallus size in both juvenile and adult males from Lake Apopka. These animals had very low levels of testosterone when compared to alligators from a healthy lake, Lake Woodruff. This most likely contributed to their reduced penis size. But more importantly, a Lake Apopka alligator's penis size did not faithfully reflect the amount of testosterone in its blood. Something was preventing that testosterone from having the intended effect.

By contrast, the alligators of Lake Woodruff developed normally. For them, more testosterone meant larger phallus size. Lake Woodruff is located in a national wildlife refuge on the St. Johns River, about 50 miles north of Orlando, and is similar to Apopka in climate and food availability. In fact, it is similar in all aspects save one: it has no history of pollution. And significantly, Guillette found that alligator penises were shortest in that part of Lake Apopka near the former Tower Chemical Company.

Chemical Culprits

Guillette wondered if pollution could be causing the strange defects he found at Lake Apopka. In 1980, Lake Apopka had been the site of a severe chemical spill that left it one of Florida's most polluted lakes. A waste pond at the Tower Chemical Company overflowed, spilling large amounts of the pesticides DDT and another pesticide, dicofol, into the lake. Soon afterward, 90% of Apopka's alligators disappeared. Tissue samples from the remaining alligators showed high levels of p,p'-DDE, a breakdown product of DDT, along with a host of other contaminants. p,p'-DDE was the most common contaminant in the alligators' eggs. In addition, Apopka suffered chemical runoff from agricultural areas around the lake, and from a nearby sewage treatment plant. Could this be the difference between Lake Apopka and Lake Woodruff? Could a spill that took place over a decade earlier still be hurting the alligators of Lake Apopka?

Guillette still remembers when the pieces of this puzzle fell into place. It happened when a colleague told Guillette what he had learned at a recent meeting organized by Theo Colborn. Environmental contaminants, the colleague said, could act like hormones. For example, p,p'-DDE, the major contaminant in Lake Apopka, was known to block the action of testosterone. Suddenly, what Guillette was seeing in Lake Apopka made sense; chemicals were disrupting hormones in these animals.

While the waters of the lake were relatively clean, "gender-bending" pesticides from the spill had moved into the food chain. Alligators were at the top of that food chain, and accumulated contaminants like p,p'-DDE through the fish that they ate. Females then deposited the chemicals in their eggs where they could influence development of the embryos. It all made sense. The population decline, the abnormal hormone levels, the strange structures Guillette had found in the alligators' testes and ovaries...they were signs of scrambled hormone signaling during development. Guillette was convinced that manmade chemicals were the culprits.

Proving the Link

For a scientist, however, correlation is not enough. Contaminants were present in alligators with reduced penis size, but were they causing it? To prove that contaminants could cause the problem, Guillette had to move his experiments into the lab. If he could treat eggs in the laboratory with p,p'-DDE and show the same abnormalities as in the wild, he would show that the chemical can actually cause the problems. Guillette collected "clean" eggs from Lake Woodruff for this experiment, and added contaminants to see what effect they would have. Sure enough, a mixture of manmade chemicals could reproduce the effects he saw in the wild. A mixture of the two most common contaminants in Lake Apopka, DDD and DDE, did the trick. These chemicals caused depressed testosterone, smaller penis size, and anatomical defects in male alligators that would have otherwise hatched normally. In females, the chemicals caused elevated estrogen levels. This was a clear indictment of manmade chemicals, and Guillette's most provocative experiment to date.

Early Indications

When Guillette began studying Florida's alligators in 1985, he had no idea how explosive his work would become. He had a simple goal: to survey the health of the alligators. Alligator ranchers wanted to know how many eggs they could collect and raise for hides without harming the population. Guillette and his colleagues had identified lush Lake Apopka, Florida's fourth largest lake, as a likely source. They soon discovered, however, that Apopka's alligator eggs were in short supply. The alligator population was 90% smaller than it had been a decade earlier, and the eggs there were three times as likely to die before hatching as eggs from other lakes. Why were female alligators having problems making good eggs?

To find out what was wrong, Guillette decided to raise some of these eggs in the lab. In 1992, his research team collected eggs not only from this troubled lake but also from Lake Woodruff where the alligator population was thriving. The healthy eggs from Woodruff would be a "control" group, used for comparison. That comparison proved informative. When baby alligators from each lake finally hatched, the most obvious difference was in their viability: many of the Lake Apopka hatchlings died within the first 10 days. But even more significant differences emerged when Guillette began to measure hormones in older alligators.

Guillette found that Apopka's alligators had atypical hormone levels. Males had the low testosterone levels of a female: over three times lower than in normal males from Lake Woodruff. Apopka's females, for their part, had twice the normal amount of estrogen. It appeared that the entire population had been "feminized" during embryonic development. When Guillette looked closer, he found abnormalities in the reproductive organs of these alligators as well. Under a microscope, he could see that the males had poorly developed testes that started to produce sperm unusually early in life. The females, too, had unusual structures in their ovaries. Normally the ovaries contain many units called follicles, each housing a single egg. In Lake Apopka females the follicles housed up to three or four eggs, and those eggs had many nuclei instead of just one. None of the Lake Woodruff alligators had these defects. Convinced he must have done something wrong to get these results, Guillette repeated his research the next year. The results were the same. It was then that Guillette began to look at phallus size in these animals.

An Unsettling Update

Recently, Guillette made another disturbing discovery. He found shorter penises and abnormal hormone levels in alligators from other lakes, including Lakes Okeechobee and Griffin. These are average lakes in the state of Florida, not ones adjacent to a Superfund site like Lake Apopka. The finding has scientists concerned. It suggests that background levels of contamination, levels that humans are exposed to every day, could cause permanent changes in developing young--at least in alligators. Guillette's biggest concern is that these changes will reduce the ability of the alligator population to respond to events like hurricanes and disease outbreaks. With their fertility on the brink of disaster, an unwelcome challenge could still push them over the edge.

KEY POINTS / SUPPLEMENTAL INFORMATION:

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