Part XIV

Regulating the Animal Body

Are Pollutants Affecting the Sexual Development of Florida's Alligators?

Alligators are among the most interesting of animals for a biologist to study. Their ecology is closely tied to the environment, and their reptilian biology offers an interesting contrast to that of mammals like ourselves. Studies of alligator development offer powerful general lessons well worthy of our attention.

In no area of biology is this more true than in investigation of alligator sexual development. This importance is not because sexual development in alligators is unusual. It is not. As with all vertebrates, sexual development in alligator males—particularly development of their external sexual organs—is largely dependent on the androgen sex hormone testosterone and its derivatives. In the alligator embryo, these steroid hormones are responsible for the differentiation of the male internal duct system, as well as the formation of the external genitalia. After the alligator's birth, androgen hormones are essential for normal maturation and growth of the juvenile male reproductive system, particularly during puberty.

The strong dependence of a male alligator's sexual development on androgens is not unusual—mammals show the same strong dependence. So why should researchers be interested in alligators? In a nutshell, we humans don't spend our lives sloshing around in an aquatic environment, and alligators do. Florida alligators live in the many lakes that pepper the state, and, living in these lakes, they are exposed all their lives to whatever chemicals happen to be added to the lakewater by chemical spills, industrial wastes, and agricultural runoff.

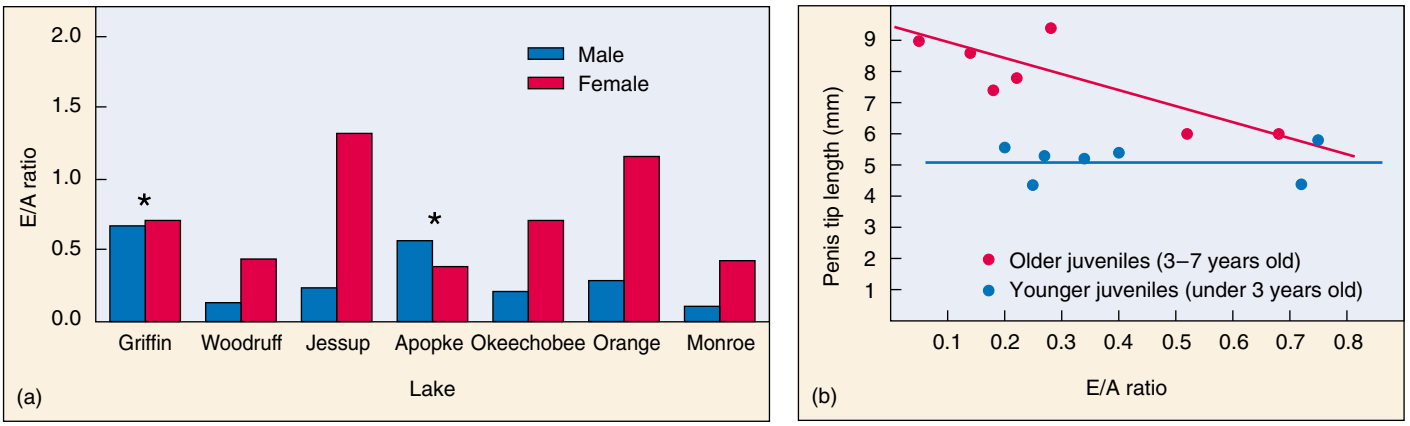
The androgen-dependent sexual development of alligators provides a sensitive barometer to environmental pollution, because the androgen response can be blocked by a class of pollutant chemicals called endocrine disrupters. When endocrine-disrupting pollutants contaminate Florida lakes, their presence can be detected by its impact on the sexual development of the lakes' resident alligators. Just as the death of coal miners' canaries warn of the buildup of dangerous gas within the shaft of coal mines, so disruption of the sexual development of alligators can warn us of dangerous chemicals in the environment around us.

Catching alligators is a job best done at night. Alligators in Florida lakes, like the one shown here in the hands of Professor Guillette, seem to be experiencing developmental abnormalities, perhaps due to pollution of many of Florida's lakes by endocrine-disrupting chemicals.

One of the great joys of biological research is being able to choose research that is fun to do. Few research projects offer the particular joys of studying alligators. With State Game Commission permits, researchers go to lakes in central Florida, wait till after dark, then spend the night on the lake in small boats hand-capturing the animals. As you might guess, researchers mostly choose juvenile individuals. The captured animals are confined in cloth bags until sex can be determined, body measurements made, and blood samples collected, and then released.

For over six years, Louis Guillette of the University of Florida, Gainesville, and his students have been carrying out just this sort of research. Their goal has been to assess the degree to which agricultural and other chemicals have polluted the lakes of central Florida, using as their gauge the disruption of normal sexual development in alligators.

To assess hormonal changes that might be expected to inhibit male sexual development, Guillette's team looked at the relative ratio of androgens (which promote male development) to estrogens (which promote female development) in each captured alligator. Some male endocrine disrupters act like estrogens, while others decrease native androgen levels. In either case, the ratio of estrogen to androgen (the E/A ratio) increases, producing a more estrogenic environment and so retarding male sexual development. Particularly after puberty, the growth of male alligators' external sexual organs is very dependent upon a high-androgen environment. Any pollutant that raises the E/A ratio would be expected to markedly inhibit this development.



Alligator sexual development inhibited by contamination. (*a*) Ratio of estrogen/androgen (E/A) plasma concentrations in large juvenile alligators 3-7 years old. A relatively larger ratio in males is atypical and indicates an estrogenic hormonal environment, as opposed to the expected androgenic hormonal environment. (*b*) Sexual development in male alligators, measured by penis length as a function of E/A ratio. In small juveniles under 3 years old, there is no apparent influence. In older juveniles 3-7 years old, there is a pronounced effect, higher E/A ratios retarding sexual development.

The Experiment

Guillette's team first looked at animals in two lakes and then expanded the research to look at animals from several other lakes. Alligators were initially collected from Lake Woodruff National Wildlife Refuge and from Lake Apopka. Lake Woodruff is a relatively pristine lake with no agricultural or industrial runoff. Lake Apopka, on the other hand, is a large eutrophic lake exposed to various agricultural and municipal contaminants. In 1980, the lake experienced a sulfuric acid spill from a chemical company, and has a history of pesticide contamination by DDT.

Clear comparisons of alligators collected from different lakes required that animals be captured as nearly as possible at the same time, to minimize possible variation due to photoperiod, temperature, and nutrition. This experimental requirement led to truly prodigious feats of alligator catching by the research team. On a single night in 1994, 40 male alligators were hand-captured from Lake Woodruff. The following night, 54 males were captured from Lake Apopka. In a broader study of seven lakes carried out the following year, 528 animals were captured during a 17-day period.

The external genitalia and total body length were measured on captured animals. Body-length is a good indicator of the age of the alligator. Alligators reach puberty at about 3 years of age, and this must be taken into account when making comparisons.

Blood samples were taken from each animal in order to determine the plasma levels of estrogen and testosterone. Investigators measured plasma concentrations of estradiol-17β and testosterone. By comparing the ratio of the two values, the researchers estimated the E/A ratio, and thus if the internal environment was androgenic or estrogenic.

The Results

In most of the seven lakes studied, female alligators showed a much higher E/A ratio than males (graph *a* above), a normal result. The exceptions are Lake Griffin and Lake Apopka, the most polluted of the lakes. The larger E/A ratio observed in male alligators caught from these two lakes indicates an estrogenic hormonal environment in these animals rather than the normal androgenic one.

Does this estrogenic environment have an impact on juvenile sexual development? Yes. Researchers observed that postpuberty juvenile males from Lake Apopka and Lake Griffith (where E/A ratios were elevated) exhibited stunted reproductive organs compared to those found in Lake Woodruff and other lakes (graph *b* above).

Prepuberty males did not show this effect, exhibiting the same size external reproductive organs whatever the E/A ratio. This is as you would expect, as organ growth occurs primarily after puberty, in response to androgen hormones released from the testes.

A primary contaminant found in alligators' eggs in Lake Apopka is *p,p*'-DDE, a major metabolite of DDT. *p,p*'-DDE has been shown to bind to androgen receptors, and functions as an antiandrogen. The presence of *p,p*'-DDE reduces the androgen effect in cells, creating a more estrogenic environment.

The researchers also measured levels of plasma testosterone. The plasma levels of testosterone were significantly reduced in alligators from Lake Apopka compared to the control animals removed from Lake Woodruff. These reduced levels of plasma testosterone from the Lake Apopka alligators also act to reduce the E/A ratio, and so to produce the observed abnormalities in reproductive structures.

To explore this experiment further, go to the Virtual Lab at www.mhhe.com/raven6/vlab14.mhtml