REPRODUCTIVE LIFE HISTORY AND THE EFFECTS OF SEX AND SEASON ON MORPHOLOGY IN *CROTALUS OREGANUS* (NORTHERN PACIFIC RATTLESNAKES)

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ABSTRACT

Over continuing long term data collection, two trends of reproductive ecology of a population of Northern Pacific Rattlesnakes (*Crotalus oreganus*) in the Carrizo Plain of central California have been discovered using radiotelemetry, snout-vent length and mass data. Using the co-occurrence of male and female snakes as a proxy for courtship, we have also discovered that the snakes mate in the spring and fall. Using snout-vent length and mass, male snakes are larger than female

snakes. The team has also begun to collect blood samples along with all of the other data for future analysis. We hope to use the blood samples collected to determine the effects of sex and season on immune function with methods such as flow cytometry.

INTRODUCTION

Rattlesnakes are carnivorous ectothermic heterotherms like all snakes and most reptiles. Around fifty species exist in the Americas today. Their name is derived from the rattle located at the tip of their tails. The rattle is used as a warning device when the snakes feel threatened. The scientific name *Crotalus* is derived from the Greek word for “castanet”.

Some species of rattlesnakes mate only once per year (spring or fall), while others mate at both times (Beaupre et al.,1998), (Duvall 1998). All species are viviparous and young are self-sufficient from birth (Klauber, 1997).

Dr. Emily N. Taylor began researching the Northern Pacific Rattlesnake, *Crotalus oreganus*, at Cal Poly San Luis Obispo where she is a biology professor. In the fall of the 2006, Taylor began researching a rattlesnake population in the Chimineas Ranch in the Carrizo Plain of California. The ranch is located about 60 miles east of Cal Poly.

Crotalus oreganus is the only species of rattlesnake to inhabit the Carrizo Plain. Taylor hopes to better understand reproductive behaviors of this species. Reproductive activity is to be studied extensively. It is hypothesized that the majority of the snakes’ movements and location choices during breeding seasons are dependent on optimal breeding conditions.

Because Taylor’s research concerns population trends, much time is needed to collect data. The data to be further augmented are seasonal snake location changes and qualitative data from observation of behavior and reproductive interaction. I intend to switch my focus from initially collecting data in the field to analyzing leukocytes from blood sample obtained in the field by other team members with flow cytometry. The project is a work in progress.

BACKGROUND

Many species exhibit sexual dimorphism: phenotypic differences between sexes. Most snake species, including rattlesnakes, exhibit size dimorphism. Size dimorphism has been documented in Western Diamond-Backed Rattlesnakes (*Crotalus atrox*). Adult male snakes were larger in size than adult females (Beaupre et al., 1998). The efficacy of increased male size may be in reproductive success (Brown, 2000). Until now, size dimorphism has not been studied in *C. oreganus*.

Crotalus oreganus populations in Idaho have been found to mate biannually; in fall and spring (Diller and Wallace, 2002). Reproduction occurs biennially; every other year. Females usually skip years because it takes a while to gain enough fat to support a litter. The same temporal trend in reproduction appears to prevail in *C. oreganus* populations in the Carrizo Plain.

Because little is known about *C. oreganus*, more research must be conducted to determine the role the species plays in the ecosystem. Before we can understand this role, it is necessary to characterize intra-specific interactions.

METHODS

In the fall through spring of 2006-07, radio transmitters were surgically inserted (see Figure 1) into the abdomens of 20 snakes (10 male and 10 female) in order to gain data regarding their movements. The sexes of the snakes were noted during their original capture. The snout-vent lengths and masses of each snake were also recorded. Snakes were assigned numbers (1-20) with different radio frequencies. The snakes were placed at the exact locations of their original capture. All of the snakes are within the close range of one another (approximately 0.25 miles). Location data are collected weekly during the active months of spring through early fall. Location data are collected using a radiotelemetry receiver and antenna (Figure 2). Once the data collector locates the snake, a description of the behavior of the snake if it is visible and a qualitative description of the snake's location are recorded. Quantitative data concerning the snake's location are noted as well using a Global Positioning Device (GPS). Latitude, longitude and elevation are gained from the GPS. Beginning Spring Quarter 2008, monthly blood samples were collected (Figure 3), and blood smears were made to obtain immunological data (Figure 4).

Figure 1. Surgical procedure for radio transmitter implantation.

Figure 2. The author using radiotelemetry to locate snakes.

Figure 3. Blood is collected from the snake for lab analysis.

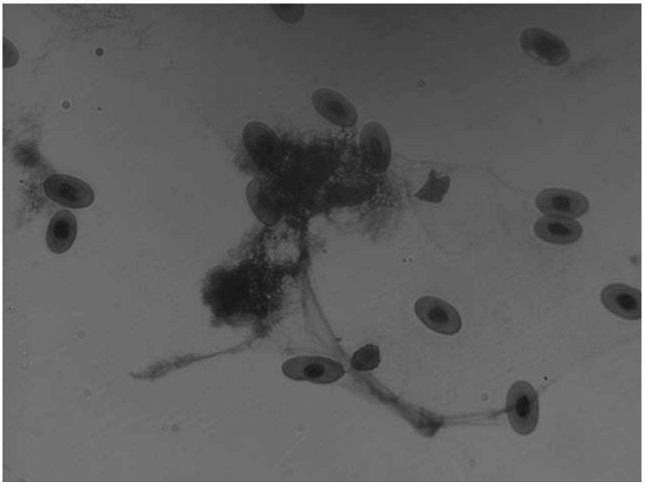

Figure 4. Blood smears are made with parts of the blood samples collected in the field.

All snake handling is done by the experienced professors and graduate students for safety reasons. After snakes to be captured are located, tongs are used to hold the snake in one place while a clear plastic tube is placed over the snake from the head end. Once this process is completed, the capturer holds the snakes body and tube with one hand and the caudal portion of the snake with the other for data collection (Figure 5). A blood sample is collected intravenously via the caudal vein on the ventral side of the snake just superior to the rattle (Figure 3). The snout-vent length is then measured and after the snake is weighed in a bag. The rattlesnake is promptly and cautiously released by holding the bag with the opening side down.

Figure 5. A rattlesnake is "tubed" for safe administration of anesthesia and data collection

I will be using flow cytometry of the Animal Science Department to analyze the blood cells. Flow cytometry is one of the cutting edge technologies for biological analysis of small organisms such as bacteria or cells. With this technology I will be able to quantify various aspects of the snake blood cells that I would not be able to do with a microscope alone. I am hoping to search for the presence of particular identifying cell receptors. I will specifically be looking for immune cell receptors. Once this task is complete, I will be able to observe seasonal and sexual differences in immune function.

RESULTS AND DISCUSSION

I went to the study site to collect data twice during the 2008 Winter Quarter. The snakes are known to hibernate during the winter, therefore none were seen in the open until a survey during the beginning of March. Each data collection took roughly one day. At times of great activity such as March 8 through March 9, data were collected twice (e.g., on a Saturday and Sunday). Data collection takes a great amount of time because each snake needs to be located and time is necessary to travel to the site. Also, the number of snakes is continuously climbing.

One of the snakes was killed and consumed by a predator. This was determined because the radio transmitter was found on the ground covered in tooth marks and the most probable way for this to occur would be the natural predation of the snake. A new snake will need to have a radio transmitter implanted in place of the lost snake. The vast majority of the snakes were underground. Again this relatively inactive behavior can be accounted for by the weather conditions.

From data collected throughout the study, size dimorphism is apparent in *C. oreganus*. Males have a greater snout-vent length than females. Because the two-tailed P value was less than 0.001, the difference is statistically significant (Figure 6). The males are also more massive than females. Again the difference is statistically significant with the two-tailed P value less than 0.001 (Figure 7).

Because copulation events are difficult to observe, males found with females was used as a proxy for reproduction events. This is an accepted practice in herpetology because rattlesnakes are otherwise solitary and male-female consortships tend to occur only in the breeding seasons. From the data collected over that past year, mating in *C. oreganus* seems to be biannual, occurring in the fall months and more so in spring (Figure 8). This mating season allows for breeding to be more flexible in Northern Pacific Rattlesnakes than other species which only have one reproductive period each year. A Northern Pacific Rattlesnake female may mate with a male in the fall, store his sperm through the winter and mate with another male (or more) in the spring. Then when she ovulates in late spring her clutch could be fathered by multiple males. This fertilization by multiple males offers a greater chance that more offspring will survive and also lends itself to more variation in the gene pool. It also allows females to offer their young the best chance for survival. If a female were forced to give birth at the same time every year, she may be forced to place her newborn offspring in an unfavorable environment. With the spring/fall choice, the female is able to censor the environment of her offspring.

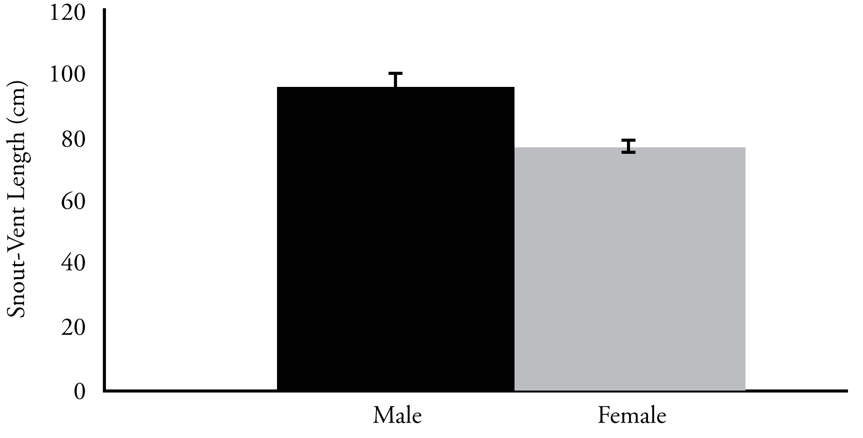

Figure 6. The lengths from snout to vent (cloacae) were measured and averaged for ten male and ten female *C. oreganus*. Males are significantly longer than females (p<0.001).

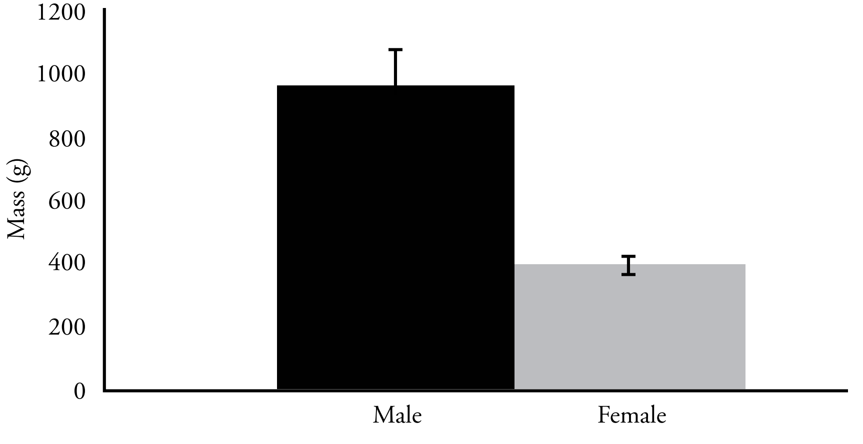

Figure 7. The masses were measured and averaged for ten male and ten female *C. oreganus*. Males are significantly more massive than females (p<0.001).

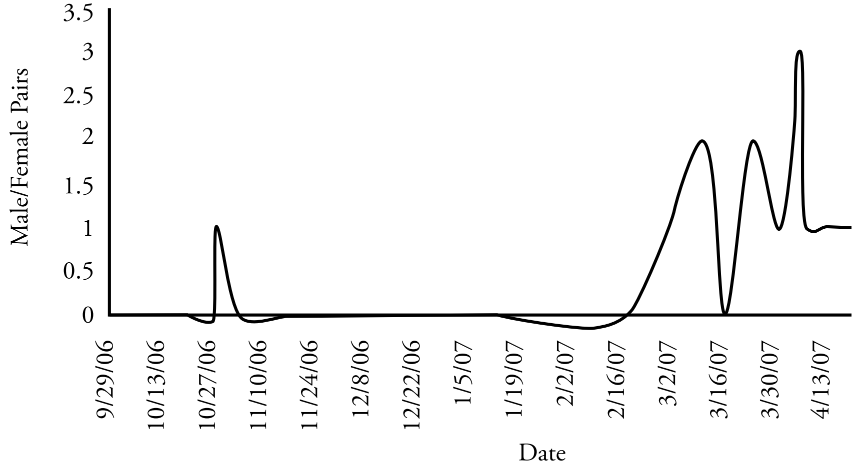

Figure 8. On each date of observation from September 29, 2006 to May 24, 2007 the number of male female pairs observed is recorded.

CONCLUSIONS

Radiotelemetry has been and will continue to be used to collect data regarding reproductive behavior and movements of C. oreganus. Sexual dimorphism has been found to occur; males are larger than females in snout-vent length and mass. There is little data collection during the winter because the snakes are in hibernation. Data collection commences each spring when the snakes are most active.

I have mastered the radiotelemetry technique, use of global positioning systems (GPS). I am looking forward to moving the research forward with the use of flow cytometry. I would recommend this research to anyone who enjoys adventure and unique taboo fauna.

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