**Fecal testosterone monitoring in eastern diamondback rattlesnakes (*Crotalus adamanteus*).**

**Daly-Crews, K.B1., Metrione L.C.2, Penfold L.M.3**

1Affiliation, City, State, Country (USA), e-mail: [*email@hotmail.com*](mailto:email@hotmail.com)

2Affiliation, City, State, Country (USA)

3Affiliation, City, State, Country (USA)

Eastern diamondback rattlesnakes (EDB) are a species sparsely distributed in their native range across the southeastern United States. Wild populations would benefit immensely from reintroduction of captive-bred individuals into native habitats, but a better understanding of their reproductive biology is needed. In this study, fecal samples from captive adult males (n=3; 140 ± 3.7 cm) and captive adult females (n=2; 149 ± 3.8 cm) were collected over 11 months to investigate potential sex and seasonal differences. Fecal samples (0.5 g) were extracted using 90% ethanol, diluted 1:20 or 1:8 for males and females, respectively and fecal testosterone metabolites (FTM) measured using the R156/7 antibody and HRP (University of California, Davis, CA, USA). Parallelism between serially-diluted fecal extracts and standard testosterone hormone preparations was demonstrated for both sexes. FTM recovery for the males was 104% (r2=.99), and 84% (r2= 0.99) for females. Sample size currently precluded statistical analysis between sexes though mean FTM concentrations were 222 ± 83 ng/g for males and 78 ± 26 ng/g for females. For one adult male where 7 consecutive samples were collected 2-4 weeks apart, seasonal differences were measured with concentrations of 93 ± 9.7 during non-breeding season (Jan – April) and 412 ± 106 ng/g leading up to breeding season (May — Jun). It is important to note that hormone values for each fecal sample were not adjusted for number of days between defecation, and this is an area for further refinement for this species. These preliminary data indicate that fecal testosterone might be appropriate for measuring seasonality in adult male EDBs and has possible potential for distinguishing between adult males and females in this species.