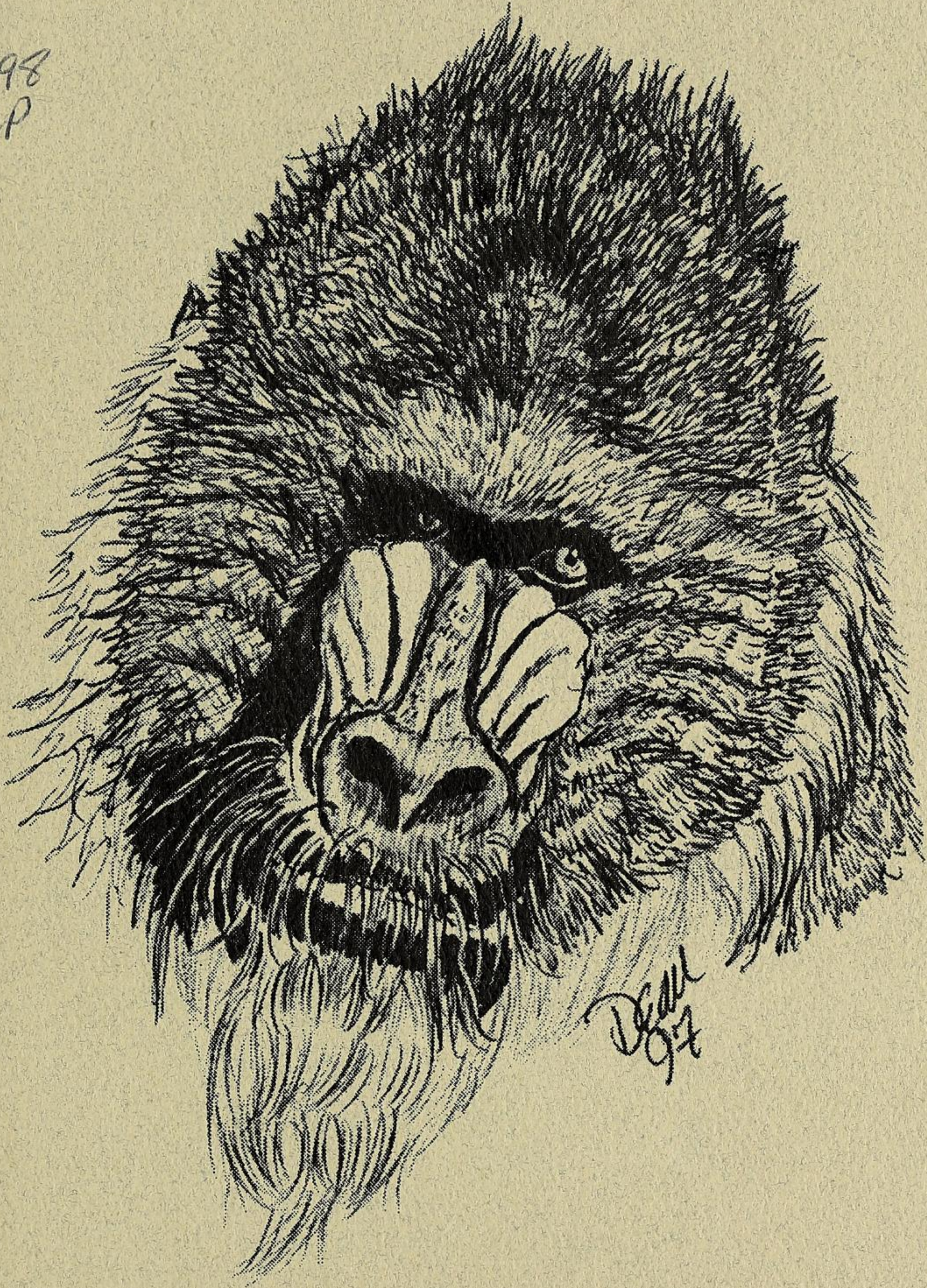


ANIMAL KEEPERS' FORUM

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Detecting Estrus In Captive Black Rhino (*Diceros bicornis*): An Example

PRELIMINARY RESULTS

By

*Farshid Mehrdadfar**

Metro Washington Park Zoo, Portland, Oregon U.S.A.

**Farshid is currently employed at
Disney's Animal Kingdom, Lake Buena Vista, FL*

The black rhinoceros (*Diceros bicornis*) is one of the most endangered of the large mammalian species. Formerly it was the most widespread and numerous rhino and notably successful member of the herbivore community. The range and number of *Diceros* dwindled steadily with the increasing human population. Its decline has been tremendously accelerated in the last decade by poaching to meet the demand for rhino horn from Arab and Asian countries, and it is rapidly approaching extinction (Estes, 1992). The numbers of the free ranging population of this rhinoceros species have plummeted, dropping from an estimated 65,000 in 1970 to less than 4,00 today, due to severe poaching (Martin, 1993).

Further, captive populations are not self-sustaining. The captive black rhino population is decreasing by approximately 7% per year. With the continuation of this trend a heavy population reduction will result. Fertility and a better chance for survival of each individual are factors to be stressed. Reproduction rate need to be doubled to keep up with mortality. The total loss of captive born calves is 20% during their first year, and mortality is also high in two to three-year old calves (Klos, 1983).

As with many species, knowledge about the reproductive behaviors of rhinos can facilitate their propagation in captivity as well as provide needed information about conservation in the wild. The stability of the African black rhino population in the wild and in captivity is a matter of international concern. The captive population (210 animals), while secure from poaching, suffers from a low birthrate. While most captive mature female black rhinoceroses have reproduced in the past, approximately 85% have produced only one offspring while in captivity (Foose, 1994). This suggests a possible unknown cause of subfertility in the captive population (Czekala and Callison, 1996). Efficient reproductive management in captivity requires clear understanding of the timing and occurrence of several reproductive events. Currently, some animal managers can identify events such as estrus through changes in a rhino's behavior (Fouraker and Wagner, 1996).

In writing this article, it is my intent to present a husbandry management routine that may have facilitated our female black rhino to her first confirmed pregnancy.

Study Animals

This study examined the olfactory behaviors exhibited by the male black rhino toward the female black rhino's urine and feces. Metro Washington Park Zoo (Portland, OR) currently houses 1.1 black rhinos (Studbook #376 & 396). Our male black rhino (house name "Pete"), was born in 7 May 1987 at the Denver Zoo and arrived to our zoo on 25 June, 1988. Our female black rhino (house name "Miadi"), was born in 4 November, 1988 at the Brookfield Zoo and arrived to our zoo on 15 March, 1990 (Figure 1). They were introduced to each other in 16 October, 1990.

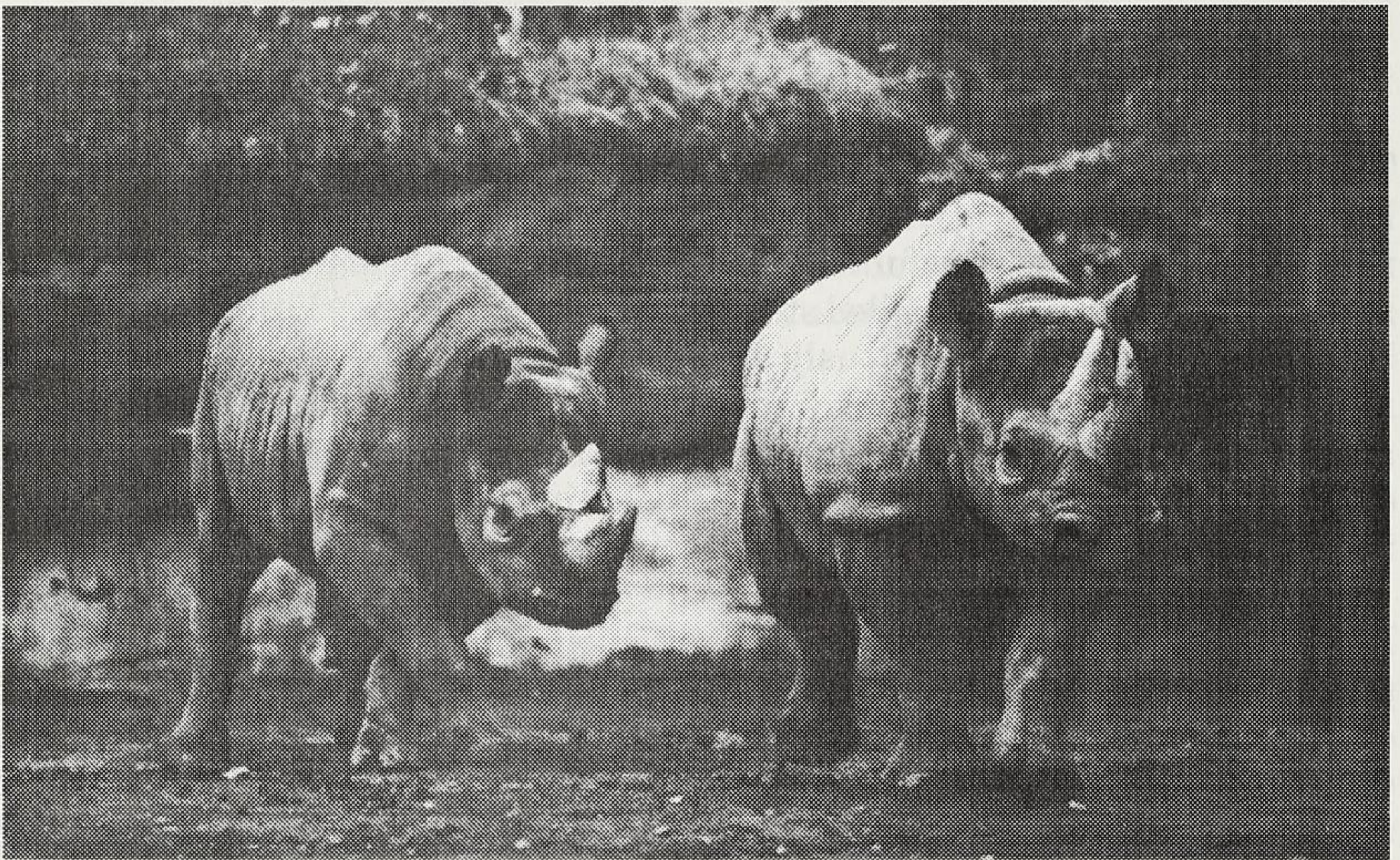


Figure 1: 1.1 black rhino currently cared for at Metro Washington Park Zoo, Portland, OR. (Photo by Farshid Mehrdadfar)

Holding Area

The holding area was designed to allow an olfactory and limited tactile contact between the male and the female. The boundaries and the dividing walls were constructed with 17 cm. (diameter) (6.69 in) metal bars spaced 20.5 cm. (8.07 in) apart.

During the past three years I have been the primary trainer for our male black rhino to cooperate with routine medical procedures (e.g., blood drawing, temperature collection, physical check-ups). along with the above assignment, I have been actively involved with observing and recording the breeding

behaviors of both black rhinos. The daily husbandry routine was designed to allow the pair access to each other in their exhibit from approximately 0900 hrs. to 1800 hrs. every day. At around 1800 hrs. the animals were brought back to their holdings and separated until the following morning.

The combination of this juncture gave me an insight to olfactory behaviors exhibited by these animals. I soon realized that a reliable indicator of the female's estrous period could be the reaction of the male toward the urine and feces of the female. I began monitoring and recording the behaviors exhibited by Pete toward Miadi's urine and feces inside the rhino barn.

Ethogram

The ethogram used for this study was established specifically for detecting estrus in the female by observing the behaviors of the male:

- **Urine/Feces Investigation:** focal animal participates in smelling (nostrils close or contacting) the sample.
- **Testing:** focal animal smears the sample by dragging his upper lip in the sample.
- **Licking:** focal animal extends his tongue contacting the sample (urine) while actively moving tongue in and out of his mouth.
- **Flehmen:** head raised, underside of upper lip curled up (Figure 2).
- **Penis Unsheathed:** partial erection of the penis, facing anterior (not in association with urination).
- **Pacing:** repetitive locomotion in a specific area.



Figure 2: Flehmen, this behavior was closely observed and recorded during this study. (Photo by Farshid Mehdadfar)

Data Collection Methods

The male's response to the female's urine and feces were compared to progesterone values from weekly blood samples collected from the female. We have been collecting blood assays from our female black rhino for the past three and half years and collecting behavioral data on their breeding behaviors/copulation. From these blood samples (Michel et al., in prep), it was established that Miadi was cycling in a regular pattern (Table 1).

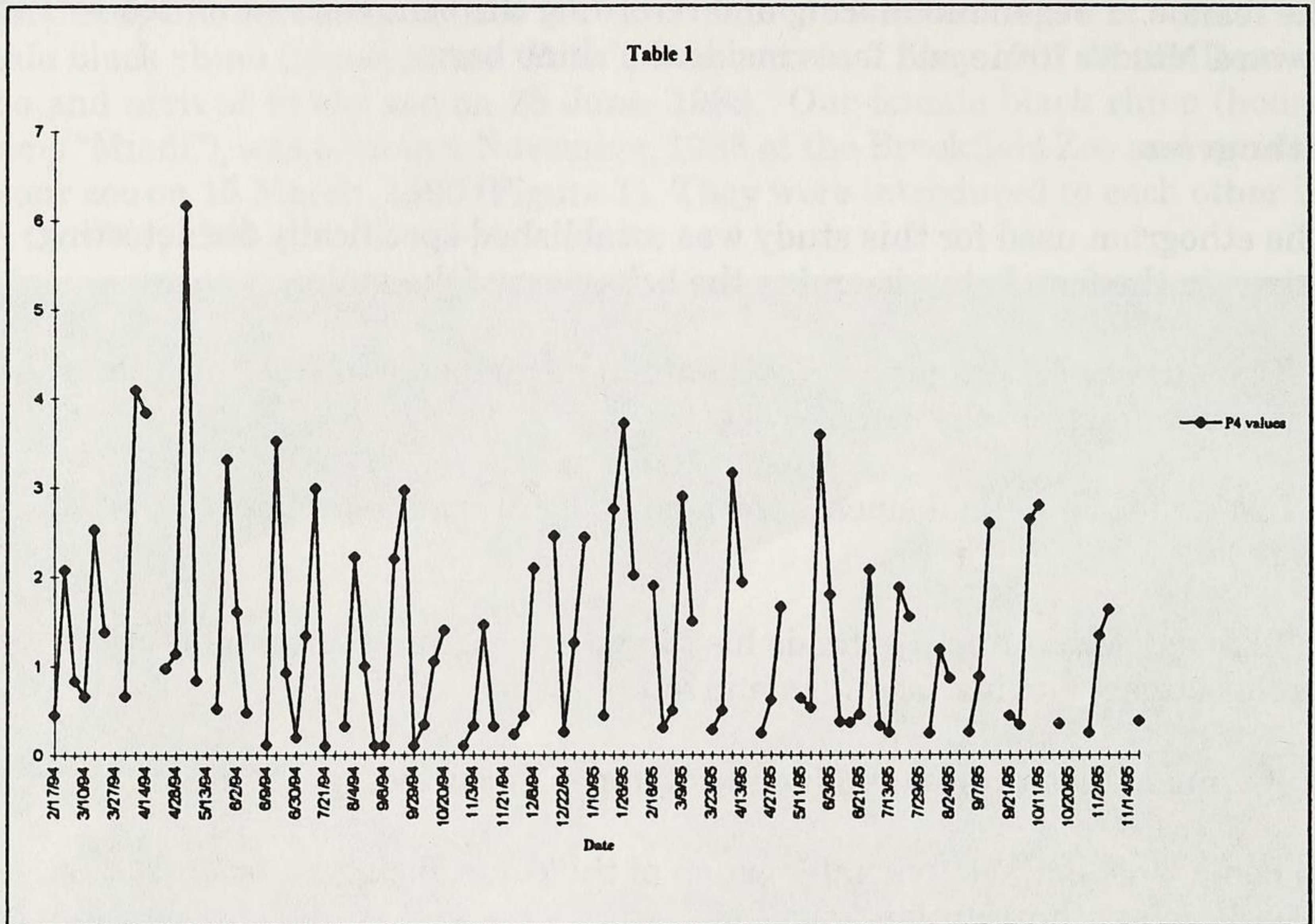


Table 1: Progesterone levels driven from weekly blood samples (female black rhino "Miadi")

Although many confirmed intromissions were observed during the past three and half years, progesterone values indicated that Miadi had not conceived. Further, it seemed that the intensity of the interactions, as it related to breeding behavior of our pair, was decreasing over time.

Following my visits to different facilities that have had numerous successes in breeding of this species, the idea of separating the male and the female from each other for four consecutive cycles was presented to the Animal Management staff at our zoo. The purpose of the separation was to increase the level of breeding activity once we re-introduced the animals at the onset of Miadi's fifth cycle from the separation period. Although we were seeing regular cycles in our blood assays we quickly discovered that, we could not pinpoint the exact onset of each estrus. After suggesting and presenting the idea behind detecting estrus from behaviors exhibited by male's response to female's urine and feces, I was given the opportunity and the chance to predict and set forth the re-introduction

date. Although development of noninvasive urinary, fecal and salivary assays for monitoring progesterone and estrogen metabolites in rhinos has been both exciting and encouraging (Schwarzenberger et al 1993, Hindle et al 1992, Czekala et al 1996), to date no study has been conducted on the olfaction behaviors of the male toward the female's urine and feces during the estrous cycle.

We felt that it is of the utmost importance to establish a primary caregiver (keeper) for conducting this project and following the husbandry routine in the rhino barn. This element reduces the variables and insures the continuity of husbandry regime. For the purpose of this study, I was given the above assignment.

The following is a brief outline of this noninvasive project:

- Urine and feces collection and presentation of the assays to male rhino: On 23 January, 1996 we began to manage these animals separately, i.e., we no longer gave them access to their exhibit at the same time (our male rhino was given access to the exhibit every other day of the week). Both urine and feces of the female were collected daily from inside the barn. I utilized two test sites for presenting the urine and feces to Pete (test site A was located in the exhibit and test site B was located in a holding area, separate from the stalls that the animals were housed in). The test sites were routinely cleaned (by fresh water only) before the presentation of the assays to insure the least amount of contamination.
- The freshly collected urine and fecal samples were placed at the test site less than five minutes before giving Pete access to the site and his responses were taped for a period of 20 minutes. It must be noted that during each presentation, it was important to keep the environment as calm as possible; distractions presented during testing seemed to influence the reaction of the animal toward the samples.
- In order to better study and score the behaviors exhibited by the male at the test sites, the male's responses in both test sites were videotaped.
- Each 20-minute segment of this tape was analyzed and scored daily (Table 2).

Table 2

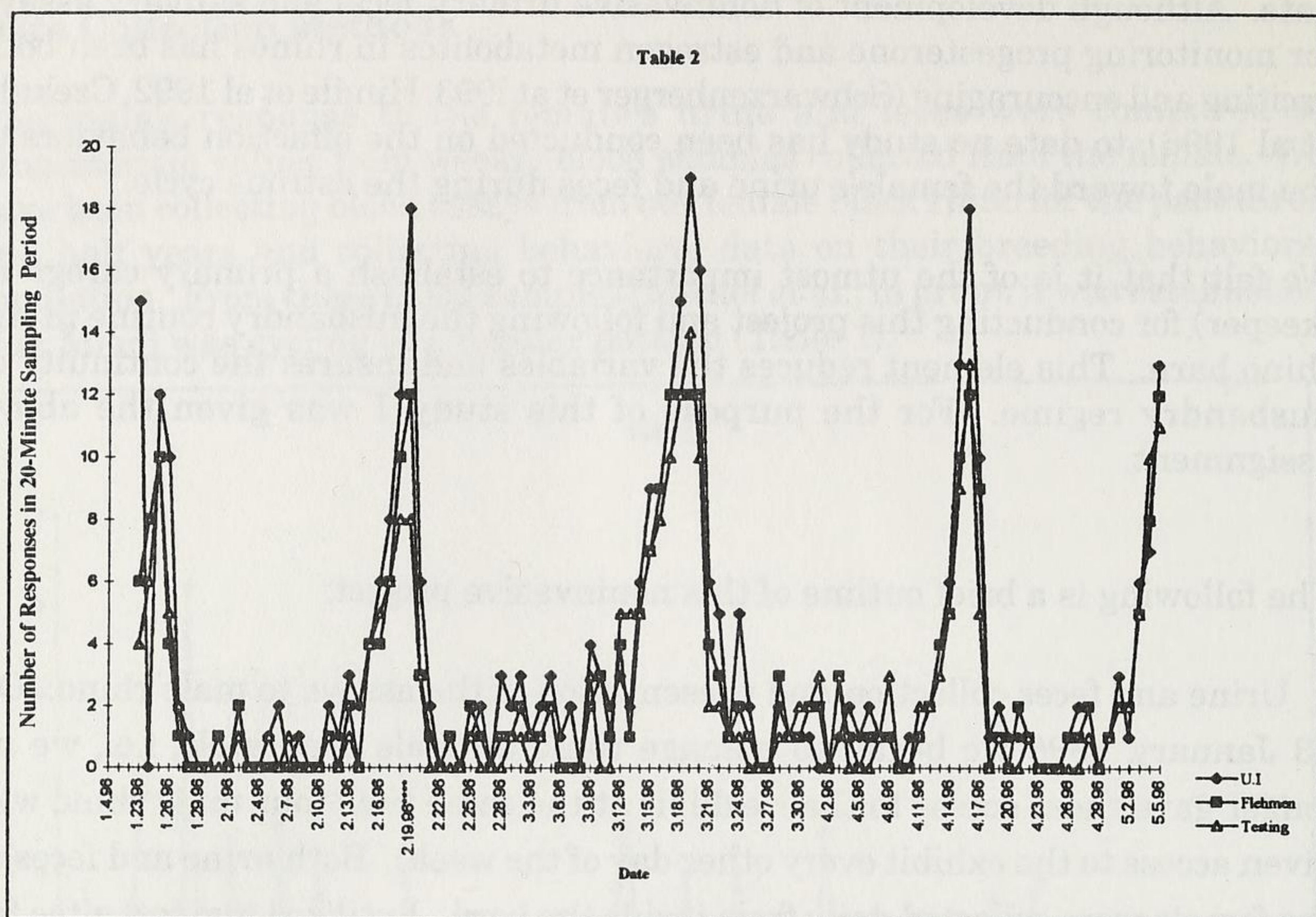


Table 2: Selected behaviors of male black rhino toward urine & feces of female black rhino

Upon presentation of the daily assays, I was interested in the amount of time Pete spent investigating the samples, the specific behaviors (described in ethogram) exhibited during each investigation and the intensity of these behaviors, I was also interested in the behaviors exhibited between the animals inside the barn (they were still housed in adjacent stalls at night). It seemed that during the peak estrus, the duration, frequency and intensity of, the selected behaviors established in the ethogram increased (Table 3). Whereupon, the normal husbandry routine, Pete exhibits readiness and compliance toward the commands given to him, during the peak estrus cycle, Pete exhibited hesitation to shift from the barn and into the exhibit and his attention span decreased. Both animals exhibited restlessness and the rate of pacing increased during the same period. While beyond visual contact with Miadi, the rate of Pete's vocalization ("meowing" sound) increased.

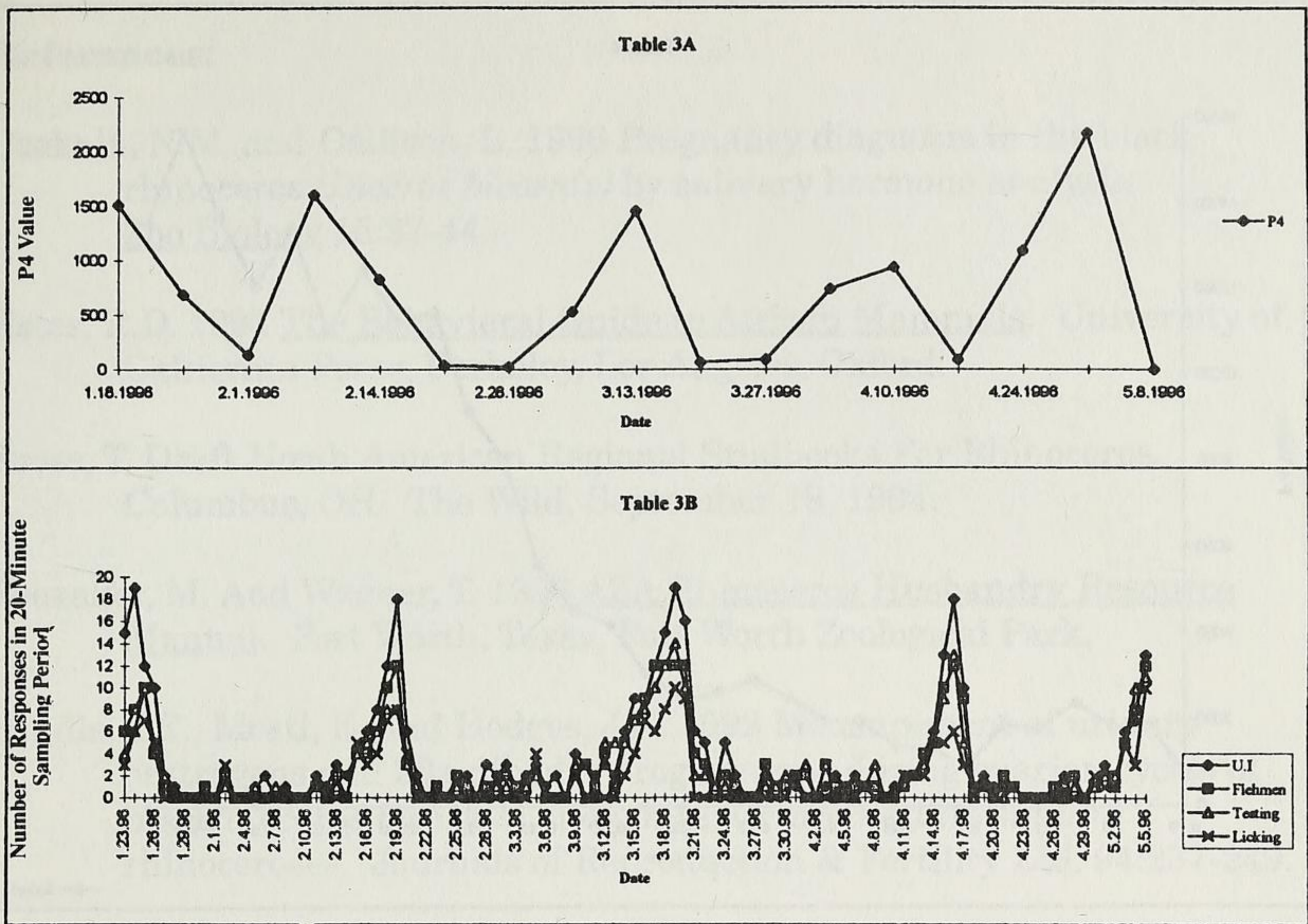


Table 3A & 3B: Progesterone levels (driven from weekly blood assays) during this study and the olfactory behaviors of male black rhino toward urine and feces of female black rhino.

Results and Discussion

Based on these observation I was able to predict the onset of estrus in the female and then we decided to reintroduce the animals together for breeding. Our rhinos were re-introduced on exhibit in 6 May 1996. The pair was observed copulating on this day and again on 7 May 1996. Prior to re-introduction, it was decided to keep the animals on exhibit overnight as long as weather permitted. Since June of 1996 analyses of progesterone levels in the female's blood show a stable rise indicative of pregnancy (Table 4). To date these progesterone levels remain elevated. The preliminary results of this study indicate that olfactory observation of an chemosensory behaviors can provide vital clues in detecting estrus in black rhinos.

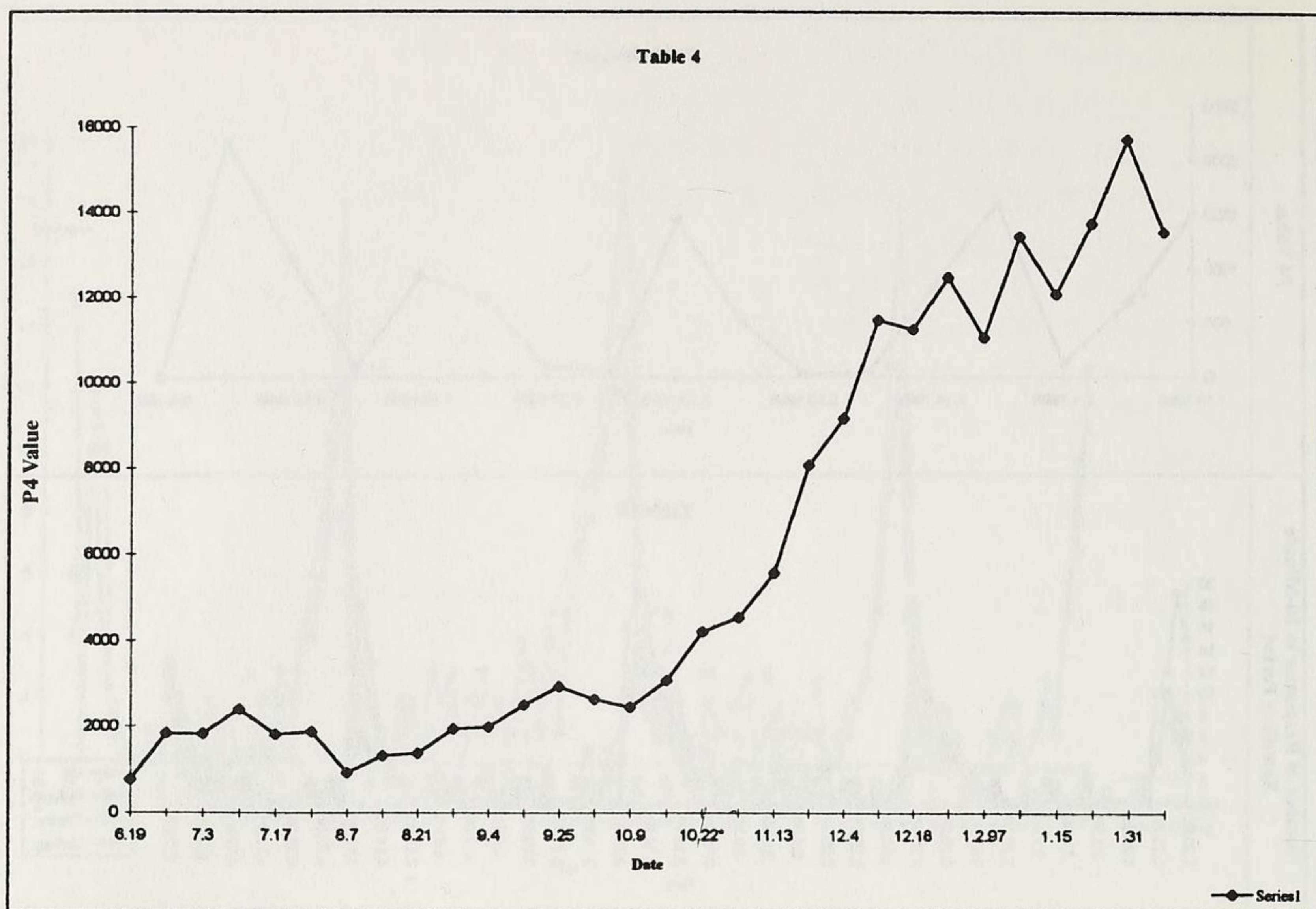


Table 4: Rise of progesterone level, indicative of pregnancy. Female black rhino “Miadi”.

Acknowledgment:

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