(2)

# Correlation of Behaviour and Reproductive Hormones in the Indian Rhinoceros (*Rhinoceros unicornis*) and the Sumatran Rhinoceros (*Dicerorhinus sumatrensis*)

**Merryn D. McInnch**
***Department of Biology***
***York University***

Draft Copy

**Supervisory Committee:**
**Dr. S. MacDonald**
**Dr. K. Goodrowe**
**Dr. C. Peng**
**Dr. B. Stutchbury**

development mentioned earlier is successful, the project will include the aforementioned group of Sumatran rhinoceri in Malaysia.

## Materials and Methods

### *Time Frame and Sample Size*

The results presented in this paper are from two Indian rhinoceri (one female and one male housed at the Metropolitan Toronto Zoo) and span a period of 87 days (July 9, 1996 to October 3, 1996). Hormonal and behavioural data were collected from these animals starting May 1, 1996 to December 28, 1996 and again from May 16, 1997 to the present, but the remaining data has not yet been processed. Urine samples were collected from the female and each animal was observed for a two-hour period, usually during the morning, an average of 3 to 4 times per week. Kasman et al. (1986) found that sampling urine three times per week was sufficient to construct a reliable profile of ovarian activity. Three samples a week is indeed sufficient for monitoring progesterone metabolites, whose peaks are long in duration, but estrogen peaks tend to be shorter in duration and so could be missed at only 3 samples per week.

The author has made arrangements to study the two Indian (1.1) and three Sumatran (1.2) rhinoceri at the Cincinnati Zoo and Botanical Garden in Ohio, and has also contacted the Fort Worth Zoo in Texas (1.1 Indian rhinos) and the Smithsonian Institution's National Zoological Park in Washington, D. C. (1.3 Indian rhinos). Several other zoos in North America have Indian rhinos, but there are no other Sumatran rhinos on the continent.

### *Behaviour Analysis*

Behaviours were classified as either continuous or discrete. Continuous behaviours (standing, lying down, walking and eating) were separated into three groups

**Introduction**

The Indian rhinoceros (*Rhinoceros unicornis*) and the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) are listed as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN). All species of rhinoceros are difficult to breed and have among the lowest reproductive rates known for mammals in captivity (Dinerstein et al., 1988; Goodwin and Holloway, 1972; Hindle et al., 1992; Reynolds, 1961). Long gestation periods and long inter-birth intervals are two reasons for low reproduction rates both in the wild and in captivity. In captive breeding two other factors have proven to be critical. The first is that, under natural conditions, Indian and Sumatran rhinoceri are solitary animals and generally associate only for breeding (Groves and Kurt, 1972; Laurie, 1982). If a pair is placed together when the female is not sexually receptive the resulting fight can be very vicious, especially in a captive situation where neither animal can run away. Tong (1961) reported that a pair of Indian rhinos at the Whipsnade Zoo in Great Britain fought until the female had to be removed while a pair at the Chicago Zoo could never be mated. In Malaysia a government-run breeding program for Sumatran rhinos has been ongoing for several years, but without any success. Several male-female introductions have resulted in fights and injuries (A. C. Bellem, National Zoological Park, pers. comm.; K. L. Goodrowe, Metropolitan Toronto Zoo, pers. comm.).

A second difficulty in captive breeding is that males and females do not necessarily come into their respective breeding states at the same time (Goodwin and Holloway, 1972). According to Tong (1961), after two years of breeding failure successful mating of a pair of Indian rhinoceri at the Whipsnade Zoo required hormone therapy for the male. During the two years before therapy the male appeared indifferent to the female when she was in heat, yet he seemed interested in breeding two days later. In a study at Dvur Kralove Zoo in the Czech Republic, Vahala et al. (1993) noted that in a population of Northern White rhinos (*Ceratotherium simum cottoni*) the males tended

to show sexual interest in the females 1 to 3 days before the females were sexually receptive. Keepers at the Metropolitan Toronto Zoo have reported (to the author) that the male Indian rhino tends to begin courtship displays a day or two before the female, and quiets down once the female begins to display. Very little behavioural work has been done with the Sumatran rhinoceros, and nothing is known about breeding synchrony in this species.

The hormonal cycle of female Indian rhinoceri can easily be monitored by measuring urinary steroids, namely estrogen and progesterone metabolites (Bellem et al., 1998; Kassam and Lasley, 1981; Kaaman et al., 1986). The cycle of female Sumatran rhinos poses more of a problem, but efforts to develop suitable assays are ongoing (refer to Materials and Methods).

The purpose of this project is to determine whether a correlation exists between the female estrus cycle and the behaviour of female and male Indian and Sumatran rhinoceri, the goal being to create a behavioural chart which would enable zookeepers to predict when their animals are ready to breed. Such a chart could replace, or at least reduce the need for, expensive, time-consuming and labour-intensive hormone analysis, while improving reproduction rates and lessening the chance of injury to the animals. Previous studies have focused on reproductive physiology (Hindle and Hodges, 1990; Hindle et al., 1992; Kaaman et al., 1986) or on behaviour (Dinerstein et al., 1988; Laurie, 1982) but few researchers have attempted to combine the two approaches. Bellem et al. (1998) have done some behavioural work with three Indian females, but the primary focus of their paper was hormonal cycles. Some behavioural work has been done with three Sumatran rhinos (1.2) at the Cincinnati Zoo, but again the focus was on hormones (A. C. Bellem, National Zoological Park, pers. comm.; T. Roth, Cincinnati Zoo, pers. comm.). The author hopes to conduct in depth studies of both species with larger sample sizes and with the primary focus on behaviour. Male Indian rhinoceri will be studied as well as female, something which has not been done to date. Assuming that the assay

based on the percentage of the observation period they consumed: 0-25% = 0, 26-50% = 1 and 51+% = 2. Discrete behaviours such as flehmen (curling of the upper lip), vocalization, trotting/cantering and urine spraying were separated into three groups based on the number of times they occurred during the observation period: 0 occurrences = 0, 1-3 occurrences = 1 and 4+ occurrences = 2. These categories were chosen, after several weeks of behavioural observations, to reflect overall frequency of the behaviours. Category 0 indicates the usual frequency, category 1 indicates an increase in the behaviour which may or may not be meaningful and category 2 indicates an exceptionally high frequency of the behaviour.

*Hormone Analysis*

Measurement of urinary estrogen metabolites (estrone conjugates or EC) and progesterone metabolites (pregnanediol-glucuronide or PdG) has proven useful in monitoring the reproductive cycle of the female Indian rhinoceros (Bellem et al., 1998; Kasman et al., 1986; Kassam and Lasley, 1981). EC levels peak immediately before ovulation and then decline rapidly. Estrus (the time of maximum fertility) coincides with the EC peak. The PdG level begins to rise up to 5 days before the EC peak and remains elevated for 1 to 17 days thereafter (Kasman et al., 1986). In this study the female's cycle length was defined as the interval between two successive increases in EC concentration (to levels two or more standard deviations above baseline) which were followed by a sustained elevation in PdG (again to levels two or more standard deviations above baseline). Baseline concentrations were calculated as the mean EC and PdG values below the arbitrarily chosen cutoff points of 200 ng/mg Cr and 300 ng/mg Cr, respectively. Creatinine is a substance which is excreted in the urine and is used as an index for water content.

The EC concentration was separated into three groups: 0-200 ng/mg Cr = 0,

201-500 ng/mg Cr = 1 and >500 ng/mg Cr = 2. These categories were chosen after inspection of the data using the same rationale as was used in choosing the behaviour categories. An EC rate of 2 which was followed by a prolonged rise in PdG indicates that the female was in estrus on that particular day.

Radioimmunoassay (RIA) for progesterone metabolites in the feces of female Sumatran rhinos has proven useful in monitoring estrus cycles (A. C. Bellem, National Zoological Park, pers. comm.), but few labs are equipped to process assays involving radioactivity. The Sumatran rhinoceri at the Cincinnati Zoo are currently being monitored by this method, but RIA cannot be used at the Malaysian field site. At this time the author is working with A. C. Bellem at the National Zoological Park's Conservation and Research Center (Front Royal, Virginia) to develop an enzyme immunoassay (EIA) protocol analogous to the current RIA. The lab in Malaysia is already equipped to run this type of EIA, but if we are not successful there is another EIA protocol which has already proven successful in preliminary tests but which would require time and money in order to equip the lab with the necessary chemicals, etc..

**Results**

Behavioural changes, in particular increased flehmen, vocalizations, locomotion (trot/canter) and urine spraying, were noted in the Metro Toronto Zoo rhinos from August 23 to August 30, 1996 (Days 114 to 121 in Figs. 1-5). An EC peak on August 27, 1996 (Day 118) indicates that the female was in estrus at this time, as this peak was followed by the required increase in PdG (not shown). Two other EC peaks (Days 108 and 110) rose above 500 ng/mg Cr but they were not followed by PdG peaks. It is not surprising that a solitary animal would begin courtship displays just before the onset of estrus, thus allowing time to locate a mate, nor is it surprising that this display would continue for a day or two after estrus when the egg might still be viable.

Fig. 1 shows the relationship between EC and flehmen in the female. She only performed flehmen on three days, just before and on the day of her estrus, while flehmen and estrus show less correlation in the case of the male. EC and vocalization are strongly correlated in both animals (Figs. 2 and 3). The male trotted and cantered only around the time of the female's estrus (Fig. 4), while she was seen to trot on two other occasions. The female only sprayed urine around the time of her estrus (Fig. 5), while the male sprayed regularly.

**Discussion**

According to Eisenberg and Kleiman (1977) behaviour studies such as the one described in this paper are critical for planning long-term captive breeding programs. Ethological studies have often elucidated the essential requirements for the maintenance and propagation of exotic species. The main importance of behavioural studies is their predictive value - they offer a norm from which deviations may be measured. These deviations may allow zookeepers to keep track of normal physiological changes and may also give early warning of pathological abnormalities. Eisenberg and Kleiman (1977) noted that estrus behaviour in the Indian rhinoceros appears to be more subtle than in many other mammals, requiring the evaluation of numerous behaviour patterns discerned after long-term observations if a pair are to be placed together for breeding.

Although hormone analysis has been used successfully to assist in the breeding of Indian rhinoceri and to monitor reproductive cyclicity in Sumatran rhinoceri (not, however, to assist in their breeding), there are disadvantages to its use. The collection and processing of samples requires time and money. Urinary and fecal EC concentrations peak immediately before or on the day of ovulation, and by the time the samples have been analyzed it may be too late to put the animals together. Construction of hormone profiles for each individual animal is essential for evaluating fertility potential and estimating the dates of future estrus, but intra-animal variation in

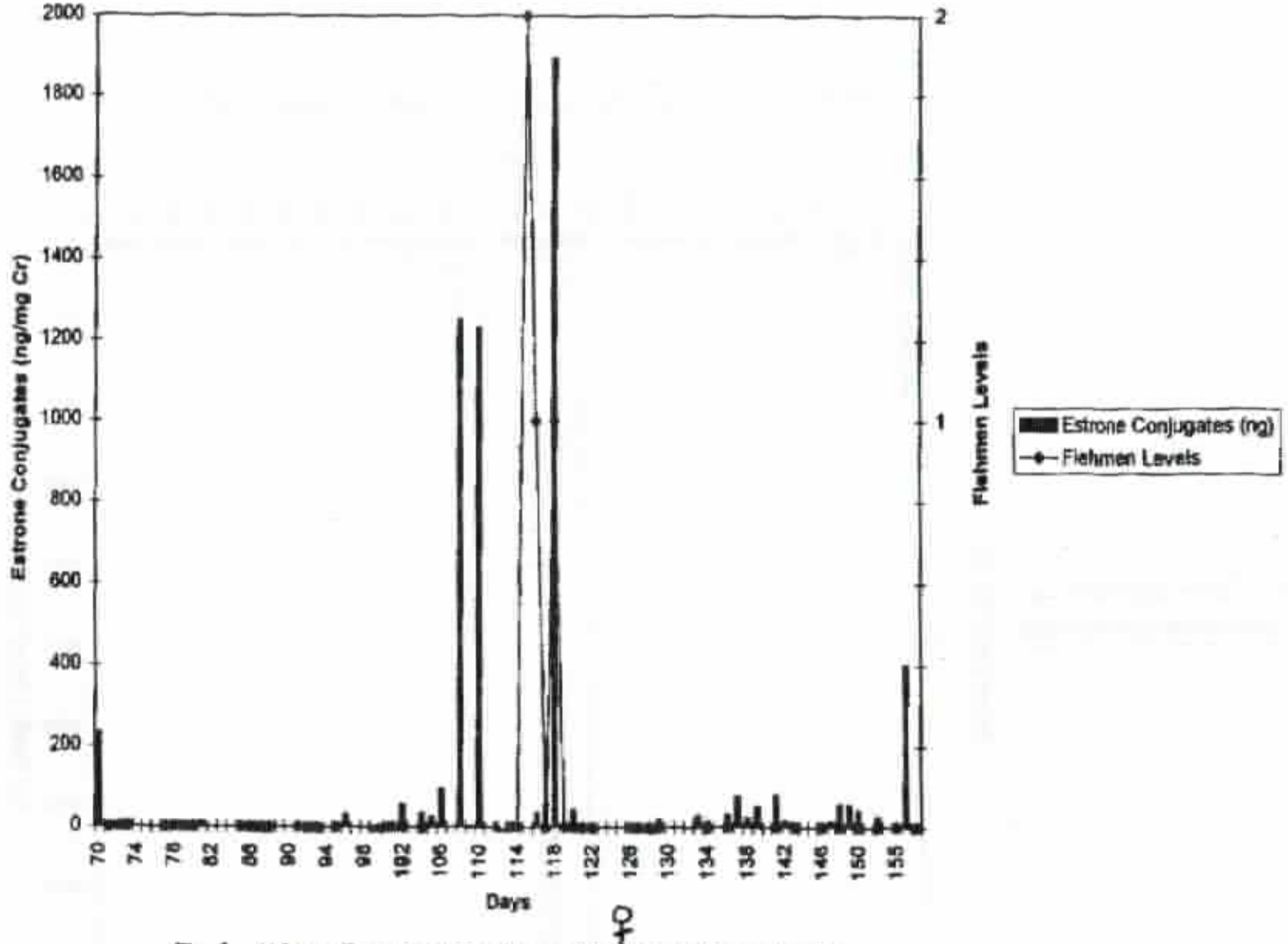


Fig. 1 Urinary Estrone Conjugates and Indira's Flehmen Levels.

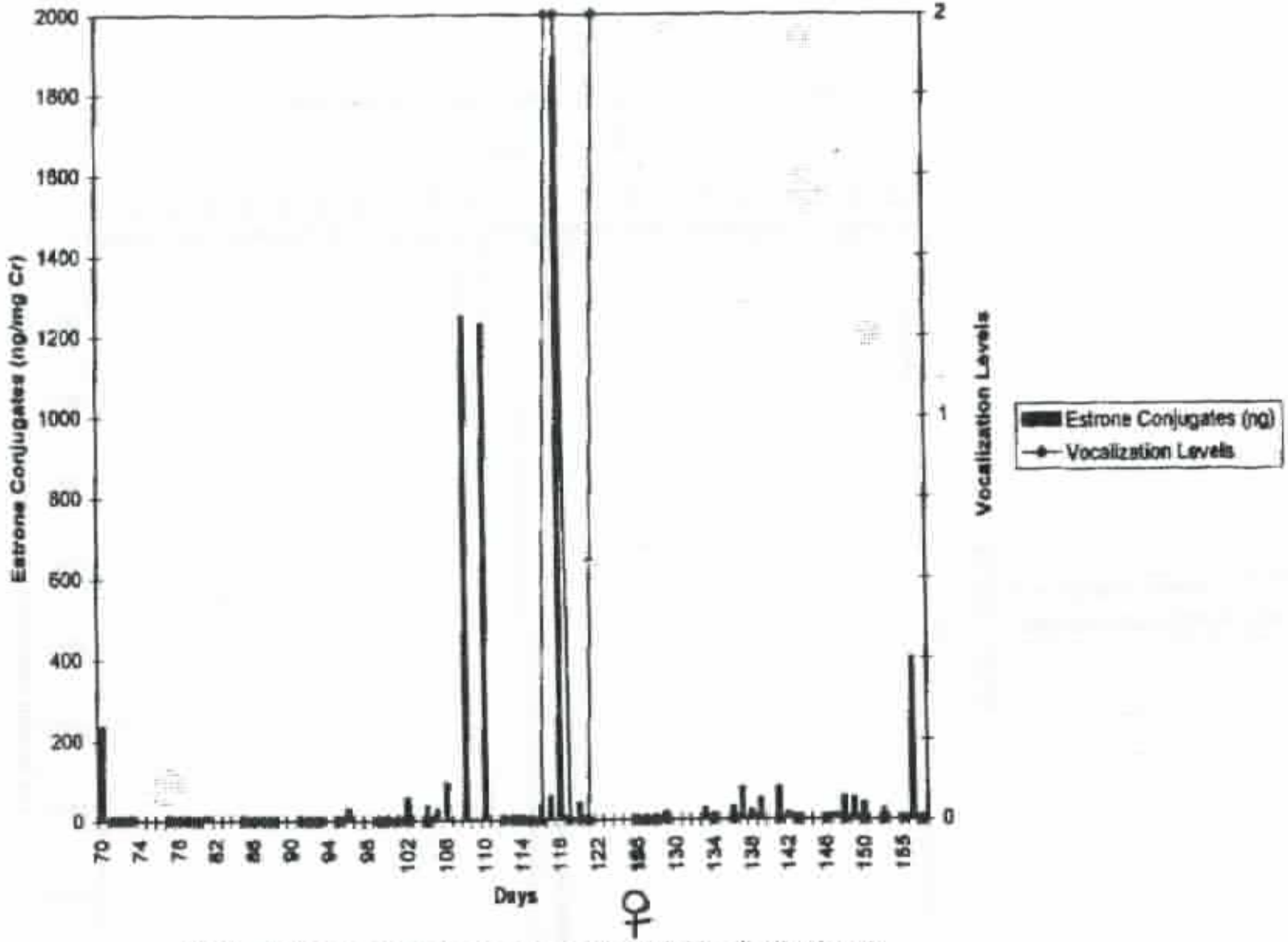


Fig. 2 Urinary Estrone Conjugates and Indira's Vocalization Levels.

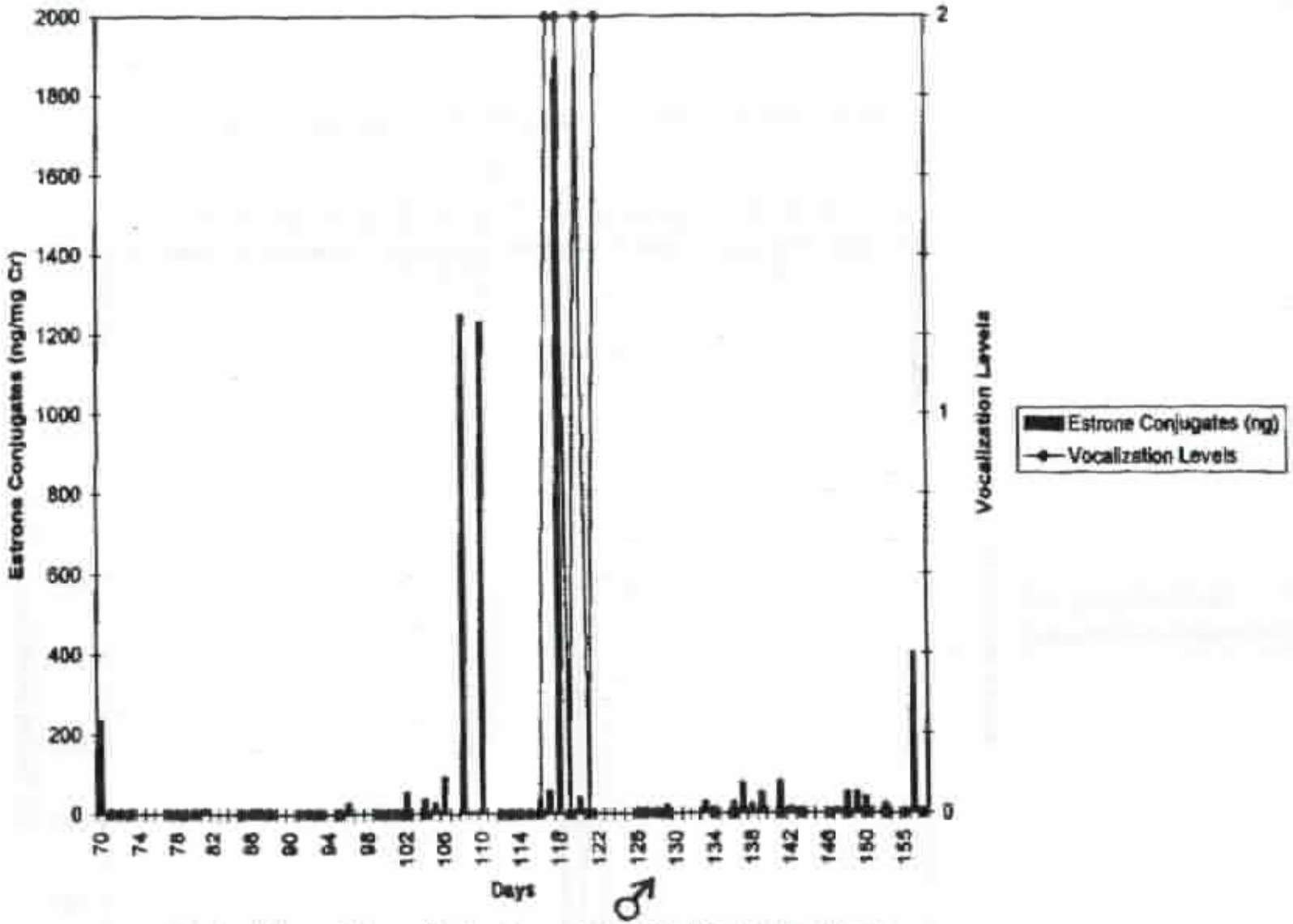


Fig. 3 Urinary Estrone Conjugates and Patrick's Vocalization Levels

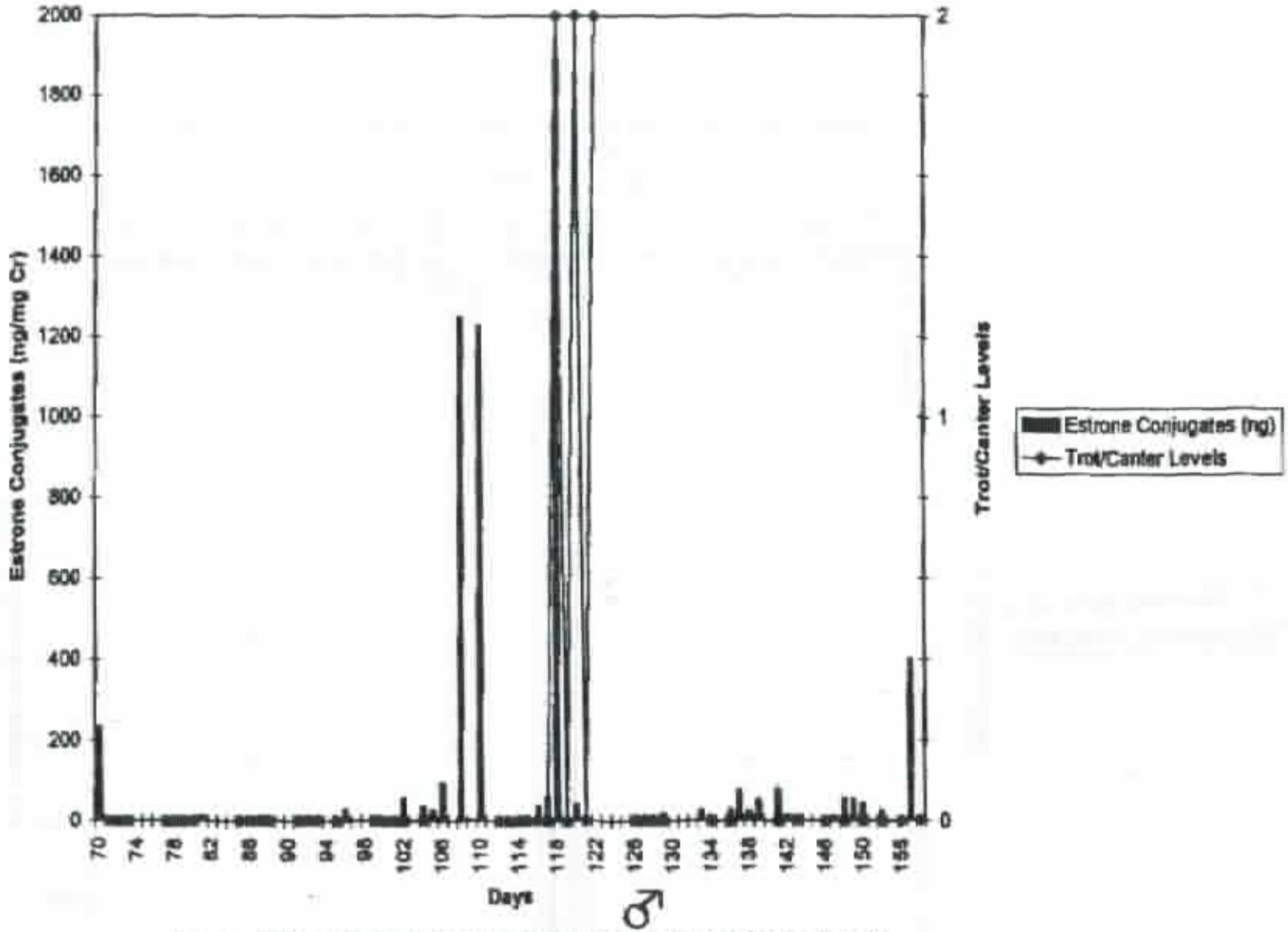


Fig. 4 Urinary Estrone Conjugates and Patrick's Trot/Canter Levels.

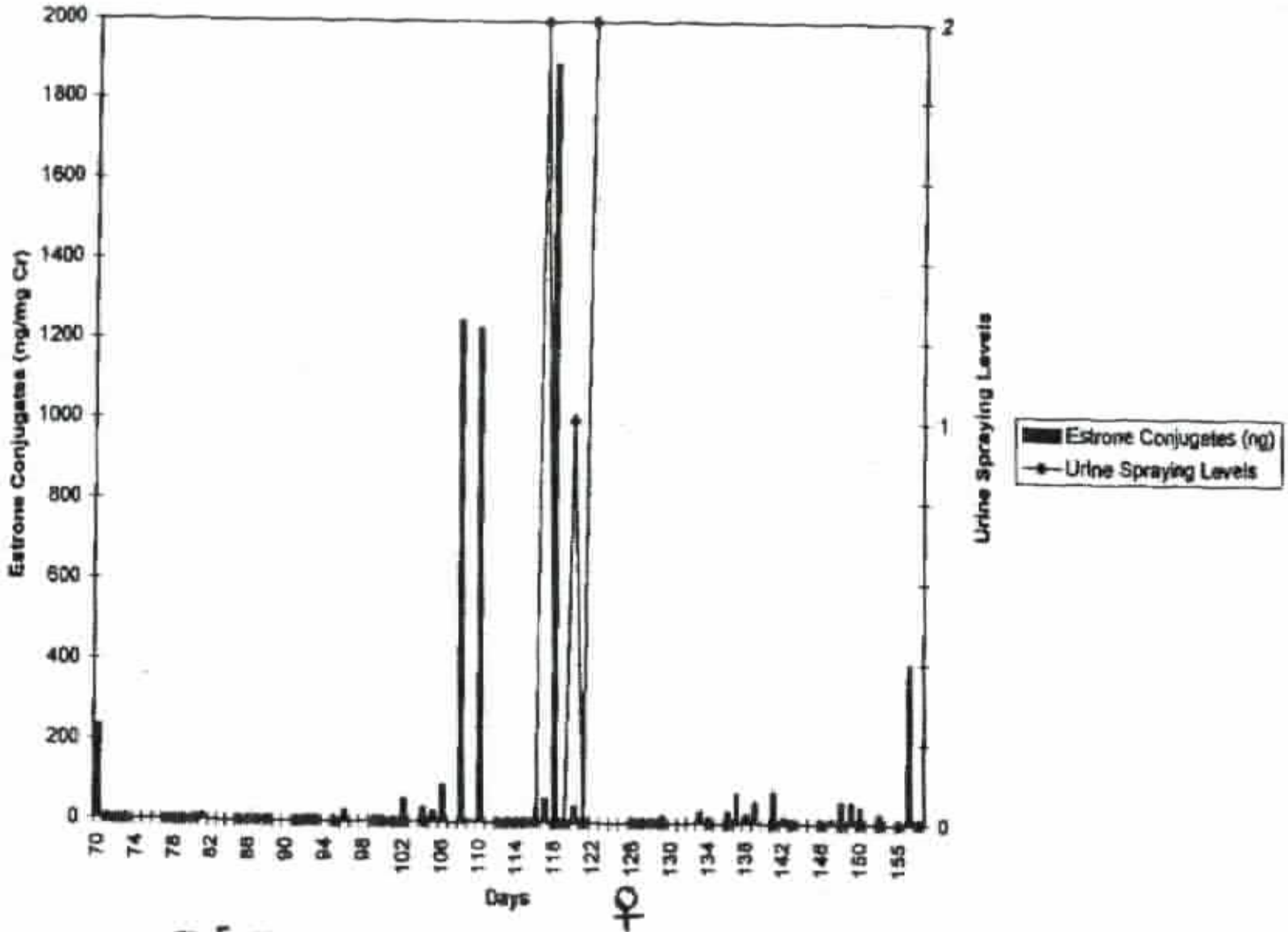


Fig. 5 Urinary Estrone Conjugates and Indira's Urine Spraying Levels.

reproductive cycles makes it impossible to predict exactly when estrus will occur. As the female rhino is the only one who knows exactly when she is sexually receptive an understanding of her behaviour is critical, and understanding the behaviour of the male may help us to interpret the female's signals.

A behavioural chart such as the one being attempted by this study could not only assist in more precisely identifying the female receptive period but could potentially replace hormone analysis in situations where time, labour and/or money considerations make regular sample collection and processing impractical. Bellem et al. (1998) suggested that longer assessment of individual females and comparison to a larger database might be useful in evaluating the fertility potential of captive rhinoceri. Hopefully the data collected during this study can help to build such a database.

**References**


Bellem, A. C., M. Dee and K. L. Goodrowe (1998). Hormonal Events During Estrous Cyclicity and Pregnancy in the Greater One-Horned Asian Rhinoceros (*Rhinoceros unicornis*). Unpublished paper.

Dinerstein, E., C. Wemmer and H. Mishra (1988). Adoption in Greater One-Horned Rhinoceros (*Rhinoceros unicornis*). *J. Mamm.*, **69(4)**: 813-814.

Eisenberg, J. F. and D. G. Kleiman (1977). The Usefulness of Behaviour Studies in Developing Captive Breeding Programmes for Mammals. *Int. Zoo Yb.*, **17**: 81-89.

Goodwin, H. A. and C. W. Holloway (1972). IUCN Red Data Book - Volume 1: Mammalia. International Union for Conservation of Nature and Natural Resources, Switzerland.

Groves, C. P. and F. Kurt (1972). Dicerorhinus sumatrensis. *Mamm. Species* **21**: 1-6.

Hindle, J. E. and J. K. Hodges (1990). Metabolism of Oestradiol-17β and Progesterone in the White Rhinoceros (*Ceratotherium simum simum*). *J. Reprod. Fert.*, **90**: 571-580.

Hindle, J. E., E. Möstl and J. K. Hodges (1992). Measurement of Urinary Oestrogens and 20α-Dihydroprogesterone During Ovarian Cycles of Black (*Diceros bicornis*) and White (*Ceratotherium simum*) Rhinoceroses. *J. Reprod. Fert.*, **94**: 237-249.

Kasman, L. H., E. C. Ramsay and B. L. Lasley (1986). Urinary Steroid Evaluations to Monitor Ovarian Function in Exotic Ungulates: III. Estrone Sulfate and Pregnanediol-3-Glucuronide Excretion in the Indian Rhinoceros (*Rhinoceros unicornis*). *Zoo Biol.*, **5**: 355-361.

Kassam, A. A. H. and B. J. Lasley (1981). Estrogen Excretory Patterns in the Indian Rhinoceros (*Rhinoceros unicornis*), Determined by Simplified Urinary Analysis. *Am. J. Vet. Res.*, **42(1)**: 251-255.

Laurie, A. (1982). Behavioural Ecology of the Greater One-Horned Rhinoceros (*Rhinoceros unicornis*). *J. Zool., Lond.*, **196**: 301-341.

Reynolds, R. J. (1961). Asian Rhinos in Captivity. *Int. Zoo Yb.*, **2**: 17-42.

Tong, E. H. (1961). The Breeding of the Great Indian Rhinoceros at Whipsnade Park. *Int. Zoo Yb.*, 2: 12-15.

Vahala, J., P. Spala and M. Svitalsky (1993). Maintaining and Breeding the Northern White Rhinoceros at Dvur Kralove Zoo. *Int. Zoo Yb.*, 32: 16-20.