Investigating Ulcerative Lesions in Captive Black Rhinoceros, *Diceros bicornis*

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By

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ABSTRACT

INVESTIGATING ULCERATIVE LESIONS IN CAPTIVE BLACK RHINOCEROS, *DICEROS BICORNIS*

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The sustainability of the captive black rhino (*Diceros bicornis*) population is compromised by problems of high morbidity and mortality, skewed sex ratios and several poorly understood disease syndromes. Due to the prevalence and severity of ulcerative oral and skin lesions, a disease often referred to as superficial necrolytic dermatitis (SND) has become of increasing concern for the health management of this species. One symptom in dogs with SND is severe hypoaminoacidemia (decreased plasma amino acid concentrations), and nearly all cases are fatal. Plasma was collected monthly for 1 year and amino acid concentrations were measured in captive black rhinos with (n = 4.0) and without (n = 20.14) lesions clinically consistent with SND. None of the affected black rhinos exhibited hypoaminoacidemia for any of the amino acids evaluated, or for total amino acid concentrations ($P > 0.05$). Based on the lack of plasma hypoaminoacidemia and comparatively low mortality rate in captive rhinos with lesions, this study concluded that this syndrome is not classical SND. Previous studies identified captive variables that

related to decreased health in black rhino. To examine whether captive variables were associated with lesions in black rhinos, facility and socio-environmental surveys were completed for 25.20 black rhinos from 18 AZA accredited U.S. institutions between 2005 and 2007. Of the 45 total rhinos, 40 were of the eastern subspecies, *D. b. michaeli*, and five were of the southern subspecies, *D. b. minor*. Forty-two were captive born, three were wild caught, and the mean age was 14.6 (range: 2 – 38) years. None of the variables measured were associated ($P > 0.05$) with black rhinos having lesions. To examine the relationship between adrenal activity and lesion status, twice-weekly fecal samples were collected from these same 25.20 black rhinos for 1 year. During the collection period, 5.1 rhinos exhibited skin lesions, 1.0 had oral lesions only and 1.0 had both (age 2 – 25 y). Baseline mean (± SEM) corticoid metabolite concentrations were lower ($P < 0.05$) in rhinos with (n = 5.1, baseline mean = 29.9 ± 3.3 ng/g) than without lesions (n = 19.19, baseline mean = 40.0 ± 2.4 ng/g). For a single male rhino that developed skin lesions during the study, the mean corticoid concentrations were lower ($P < 0.01$) when lesions were present (overall = 30.1 ± 2.4 ng/g, baseline = 28.7 ± 2.2 ng/g) than absent (overall = 36.5 ± 1.0 ng/g, baseline 35.3 ± 0.8 ng/g). These data suggest that ulcerative skin and oral lesions may be associated with suppressed adrenal activity, although it is not known if this is a precursor or a by-product of lesion manifestation. More research examining symptoms of stress, such as abnormal behavior, adrenal hyper or hypoplasia and measures of immunosuppression, would help determine if black rhino health is truly compromised by captivity related stress. Future studies should also examine other common physiological measurements, such as gonadal hormones and

serum chemistry, among rhinos with all disease manifestations, lesions, idiopathic hemorrhagic vasculopathy, anemias and hepatopathys. These types of studies may reveal additional underlying physiological similarities lead to identifying common factors that negatively impact black rhino population health.

CHAPTER 1

Introduction and Background Information

There are five extant species of rhinoceros living in Asia and Africa. The greater one horned or Indian (*Rhinoceros unicornis*), the Sumatran (*Dicerorhinos sumatrensis*) and the Javan (*Rhinoceros sondaicus*) rhinoceros live in Asia, and the white (*Ceratotherum simum*) and black (*Diceros bicornis)* rhinoceros live in Africa. All species of rhinos are classified as endangered on the IUCN Red List and are (with the exception of the southern white rhino) listed in Appendix I of CITES (Emslie and Brooks, 1999).

The white rhino (also called the square lipped rhino) is native to central and southern African long and short grass savannahs. There are two subspecies: the Northern white (*C. s. cottoni,* n = ~ 9) and the Southern white (*C. s. simum*, n = ~14,500). In the 1960s there about 2,500 free range Northern white rhinos. However, it is believed that in 2008 the last wild Northern white rhinos were poached, leaving only a small number in captivity. The remaining Southern subspecies populations are doing very well as the result of successful conservation efforts after their population was reduced to less than 20 individuals in 1885. The success of the population is mirrored by a CITES down listing to Appendix II, however there are still strong regulations on trade. White rhinos

are grazers that feed primarily on short grasses. They are semi-social and territorial; frequently several females will live in the same area (Emslie and Brooks, 1999).

Black rhinos (also called hook or prehensile lipped rhinos) are native to sub-Saharan regions of Africa, in tropical bush lands and savannahs. Compared to white rhinos, they are solitary, although some females share overlapping territory. In the 1960s, more than 100,000 black rhinoceros were living free-range in Africa. In the last 50 years they have suffered a 97% population decrease, leaving only about 2,500 individuals in the wild in the early 1990's (Emslie and Brooks, 1999). There are currently approximately 3,600 black rhinos living in the wild. These are separated into four subspecies: the Eastern (*D. b. michaeli,* n = 520*)*, the Southwestern (*D. b. bicornis,* n = 1,310*)*, the Southern (*D. b. minor*, n = 1,770*)* and the Western (*D. b. longpipes*). The last of the Western subspecies were scattered in Cameroon and are believed to have been poached to extinction in 2005 (Internal Rhino Foundation, rhinos-irf.org).

All rhino populations experience both internal and external threats to their sustainability. Externally, the international illegal trade of rhino horn for medicinal and ornamental purposes continues to be the dominant threat to their survival. Rhino horn is a keratin based material similar to human fingernails that grows continuously throughout their life at a rate of 3-5 cm/year (Pienaar et al., 1991). Traditional Chinese Medicine (TCM) uses rhino horn for its supposed healing properties, primarily as a fever reducer. Rhino horn has also been used to treat ailments including influenza, poisoning, jaundice, swelling, convulsions, typhoid, epilepsy, restlessness, abscesses, hepatitis, leukemia, hemorrhages, rhinitis, cerebro-vascular diseases, external burns and as a general tonic

(Nowell et al., 1992). Although no conclusive evidence supports these claims, many believe there is no medicinal substitute for these healing properties (Ching-Hwei, 1991). Despite strong regulations against its sale and consumption, the underground trade is still thriving in Asia and beyond (Emslie and Brooks, 1999). Rhino horn is also valued ornamentally and the most valuable daggers (jambiyas) are those with handles of rhino horn (Martin, 1985). In Middle Eastern countries, such as Yemen and Oman, these daggers carry the most cultural significance and are great status symbols (Martin et al., 1997). Due to its translucence, rhino horn (over other potential materials) is considered awe inspiring and beautiful. Although Yemen has recently joined as a CITES party nation, authorities have not strictly enforced bans on carving rhino horns once the material is in the country (Martin et al., 1997).

Internal threats are not ones that directly "kill" rhinos, but they compromise conservation efforts aimed at protecting them. The stability of established rhino populations is threatened by civil war, political instability, poverty, corruption and land tenures within their range countries (Emslie and Brooks, 1999). Currently, a number of conservation strategies designed to protect and increase wild black rhino populations exist, but these are difficult to maintain when other areas of governance take priority. Compounding this situation, unstable job markets, declining prosperity and apathy can lead to financial desperation. This, in combination with decreased program effectiveness, results in increased poaching opportunities. Continual conservation efforts include extensive surveys and monitoring, increasing protected areas and perhaps most

importantly, strengthening anti-poaching rhino protection units (RPU's) that constantly patrol rhino habitat.

Black rhinoceros in captivity

Given the increased threats to the population viability of wild black rhinos, a stable captive population is necessary to act as a hedge against extinction (Smith and Reed, 1992). Of the three extant black rhino subspecies, two are currently managed in captivity; the eastern black rhino, *D. b. michaeli* (n = 175), and the southern black rhino, *D. b. minor* (n = 60) (Emslie and Brooks, 1999). Unfortunately**,** zoo populations of black rhinos are not self-sustaining due to high mortality and morbidity, skewed sex ratios, mysterious disease syndromes and suboptimal reproduction (Smith and Read, 1992; Dennis et al., 2007). Lacy (1987) estimated that fewer than half of the black rhinos in captivity have reproduced, resulting in a birth rate that is lower than the death rate. The high death rate is due in part to a handful of poorly understood health issues, the majority of which have not been observed in free-range black rhinos, or other rhino species. Taken together, these unusual disease syndromes are severely decreasing the population health and sustainability of captive black rhinos. According to the Association of Zoos and Aquariums (AZA) 2004 Rhino Research Master Plan, investigating the pathophysiology and environmental correlates of black rhino diseases is a high priority in the quest of creating a self-sustaining captive population to protect this endangered species from extinction.

Idiopathic hemorrhagic vasculopathy syndrome (IHVS) is a syndrome where severe swelling of the neck and limbs eventually leads to lameness, oral ulcerations,

anorexia and non-hemolytic anemia (Murray et al., 1999; Murray et al., 2000). The swelling results from large quantities of blood pooling in the subcutaneous and soft tissues of affected areas. The pathogenesis of IHVS is unknown and there are no apparent sex or subspecies predilections. Case studies suggest it may be related to either an autoimmune disorder or an immune complex disease. Determining the underlying cause of IHVS is also complicated by a lack of species specific diagnostic tests and the innate complexity of immune diseases (Dennis et al., 2007).

Hemolytic anemia has been cited as one of the most frequent causes of black rhino death in captivity, due to a 75% mortality rate (Miller et al., 1982; Paglia and Dennis, 1999; Dennis et al., 2007). The etiology of hemolytic anemia is unknown; studies attempting to determine the cause of hemolysis have found no metabolic abnormalities (Gray and Hunter, 1986; Fairbanks and Miller, 1990). Given the discrepancies between free-range and captive black rhino diets, it has been suggested that there may be a link between nutrition and anemia (Dierenfeld et al., 1988; Ghebremeskel et al., 1988). In contrast to white rhinos, black rhinos browse over 200 species of plants, most of which are difficult to grow in the US, or are too expensive to import (Dierenfeld et al., 1988; Emslie and Brooks, 1999). Therefore, black rhinos may not adapt well to captive diets, which is primarily composed of hays and local sources of browse. Studies have suggested that components of the captive diet are lower in Vitamin E and may result in higher iron absorption than wild diets (Dierenfeld et al., 1995). Therefore, several current studies are evaluating whether there is an association between black rhino disease and hypophosphatemia, hypovitaminosis and other nutritional derangements (Dierenfeld

et al., 1995; Dennis et al., 2007). Compared to individuals in the wild and to white rhinos, black rhinos have higher levels of serum ferritin, which increase as rhinos age and as time in captivity increases. This finding suggests that iron overload may be a by-product of captivity (Kock et al., 1992; Smith et al., 1995). In general, mammals lack an effective method for excreting excess iron and if faced with excess iron or reduced levels of iron competitors in the captive diet, black rhinos appear to deposit iron into tissues. This iron overload (or hemochromatosis) could play a significant role in the underlying mechanisms and pathophysiology of the constellation of black rhino disease syndromes (Paglia and Dennis, 1999).

Leukoencephalomalacia, a disease diagnosed post-mortem by subcorticol necrosis of the cerebral white matter, has occurred in four captive females. None of the known causes of leukoencephalomalacia (e.g. trauma, encephalitis, toxins) were evident in these cases. Case studies do not point to any common cause and the disease is not believed to be congenital as onsets suddenly with rhinos exhibiting normal behavior up until that point (Dennis et al., 2007).

Several reports contribute black rhino mortality to toxic hepatopathy (Schmidt et al., 1982; Kock et al., 1994; Dennis et al., 2007). In some cases, these hepatopathys are observed in conjunction with other previously mentioned diseases syndromes, such as anemias, ulcerative lesions (to be discussed below) and IHVS. Other cases were in association with creosote poisoning, a condition presumed as currently controlled by eliminating the material used to seal telephone poles in facility enclosures. As with other

syndromes, the disease mechanisms and pathophysiology of hepatopathys are poorly understood.

Ulcerative skin and oral lesions in black rhinos

The focus of this dissertation is the debilitating syndrome of ulcerative epidermal and oral lesions in black rhinos. Munson et al. (1998) first described ulcerative lesions in black rhinos and suggested that they exhibited clinical similarities to superficial necrolytic dermatitis (SND) in domestic dogs. In rhinos, lesions begin as raised plaques, progressively leading to vesicles and erosions, and finally ulcers. Chronic ulcers are usually bilaterally symmetrical and tend to expand peripherally, with most lesions presenting on pressure points, such as the back, feet, hocks, lateral body wall, tail, head, ears and vulva/prepuce. Lesions can occur suddenly and may progress slowly, with most cases having prolonged coursers of eruptions at multiple sites. Ulcerative lesions in black rhinos contain no viral, bacterial or fungal components, show minimal inflammation and often occur in conjunction with other clinical pathologies. In a report by Munson et al. (1998), 23 of 34 affected rhinos had concurrent health conditions, including liver disease, anemia, gastrointestinal diseases, respiratory tract infections and urinary tract disease. Other associated symptoms included weight loss, lameness, abnormal behavior, anorexia, weakness, pregnancy and estrus/breeding. Approximately 50% of the captive population has been affected by either cutaneous or oral mucosal manifestations of skin lesions. Almost half of these (42%) had recurrent episodes; however over half of the animals with only a single episode died with unresolved lesions. No sex, age, subspecies, geographic or temporal trends are apparent. There have been incidences of lesions occurring after

"stressful" situations; six of the documented cases occurred after transportation or introduction (Munson et al., 1998). Lesions often resolve themselves (and possibly return) and are generally variable in response to treatments.

An epidemiologic study of the captive black rhinoceros population in the United States (296 of 334 animals, 88.6%, historically) indicated that black rhinos with skin lesions were twice as likely to die within a given time period than those animals without lesions (Dennis et al., unpubl.). This survival model was designed to include the following health conditions: hypercalcemia, hypophosphatemia, jaundice, diarrhea of unknown etiology, epitaxis, dental calculus, loose teeth, anemia, tail sloughing, loose horns, neurologic abnormalities, ataxia, tremors, luxating patella, lameness, swelling of limbs and shoulders, IHVS and skin lesions. Certain issues positively affect rhino survival (number of offspring, being captive or wild born), while others have a negative affect (birth year, number of institutions rhino has lived). Several health issues increase the likelihood that a rhino will die in a given time period: skin lesions (2X), confusion, ataxia and tremors (2.3X), IHVS (4X), muscle necrosis (5X), and jaundice (75X). Taken together, investigating and determining the pathophysiology of mysterious black rhino disease syndromes is a priority in creating a self-sustaining captive population.

Captivity and black rhino health, welfare and behavior

Captive variables have been shown to adversely affect well-being, altering both behavioral and physiological responses in several species (Carlstead et al., 1993; Wielebnowski, 2003; Freeman et al., 2008). Several captive variables (e.g., sound, odor, light, substrate, diet, space, relationship with humans and other animals) vary between

facilities and native habitats; thus, the impact of these on animal health should be addressed to maximize welfare (See Morgan and Tromborg, 2006, for review). While many wild animals successfully cope with captivity, an inadequate captive environment may make it difficult for some species to "thrive" compared to their wild counterparts (Lindburg and Fitch-Synder, 1994; Carlstead, 1996; Wielebnowski, 2003). Aspects of a species life history (genetics, behavior, nutritional requirements, population structure and physiology) also must be considered when designing captive management programs. When a captive population fails to thrive or reproduce, it is often due to the effects of species-inappropriate social and/or environmental parameters of captivity (Lindburg and Fitch-Synder, 1994; Clubb and Mason, 2003). In recent years, addressing potential shortcomings in the captive environment and their affect on animal health, behavior and welfare has become a high priority in animal management.

Carlstead et al. (1999 a,b) surveyed several North American zoos to describe the environment and social structure of captive black rhino populations and how these affected their success in captivity. High black rhino mortality was correlated with the degree to which their enclosure perimeters were exposed to the public; the more exposed the rhinos were to the public, the higher their mortality rate. Female breeding success was associated with increased enclosure area and breeding pairs were most successful when the female was rated as the dominant partner. Certain behaviors (e.g. aggression) were negatively correlated with reproductive success, a variable often used as an indicator of good health and fitness. Other behaviors (e.g. stereotypies) decreased the reproductive performance of females. These data suggest that black rhino behavior and

temperament may be a good primary indicator of compromised health in captivity, and that certain aspects of the captive environment may be reducing their fitness.

Stress and animal health

Analyses examining the role that stress plays on animal health may be a useful management tool in that it can identify potential causes of compromised health before other physiological symptoms may occur (Trindle et al., 1978; Carlstead et al., 1992; Wielebnowski, 2003). Hans Selye first studied the effects of glucocorticoids (GCs) and the stress response in the 1930's. Stress is defined as a state of threatened (or perceived threatened) homeostasis, and generally beneficial to individual fitness (Munck et al., 1984; Sapolsky et al., 2000). During a stress response, a repertoire of physiological and behavioral responses work together to increase fitness by allowing the individual to cope with changes in its environment (See Sapolsky et al., 2000 for review). GCs have both permissive and suppressive effects that act as compliments to each other. Permissive levels (or basal levels) act as 'primers' to prepare the homeostatic defense mechanisms to facilitate the increase in catecholamines and glucocorticoids upon activation of the stress response. Following acute stress, the suppressive effects of GCs allow the individual to cope, adapt and recover from the episode. Corticoids limit the extended actions of the stress response and contribute to the individual's return to basal levels. This relationship is vital in times of courtship, parturition, copulation and hunting when GCs are a vital component. GCs are also a key factor of the short term "fight or flight" response; a process that improves fitness by mobilizing energy reserves to priority tissues. In contrast to this short term (adaptive) response, a long term or chronic (maladaptive) stress

response may decrease individual fitness by compromising overall health (Sapolsky et al., 2000; Mostl and Palme, 2002; Carlstead and Brown, 2005).

The HPA axis and glucocorticoids

The cascade of hormones released during the stress response is regulated by the hypothalamic – pituitary – adrenal (HPA) axis. The first wave response to a perceived stressor is by the neuro-endocrine system and occurs within seconds of stress detection (See Matteri et al., 2000 for review). The response involves increased secretion of catecholamines (epinephrine and norepinephrine) by the sympathetic nervous system and adrenal medulla. Arginine vasopressin and corticotrophin releasing hormone (CRH) are released into portal circulation from the para-ventricular nucleus (PVN) of the hypothalamus. This subsequently stimulates the synthesis adrenocorticotropin hormone (ACTH) from pre-opiomelanocortin (POMC), which is released from the anterior pituitary gland.

ACTH triggers corticosteroid production in the zona fasiculata of the adrenal cortex. Cholesterol, from LDL or synthesized from acetate, is converted to cholesterol esters and stored in lipid molecules until activated by ACTH. Once ACTH binds to adrenocortical receptors, adenyl cyclase converts ATP to cAMP, which activates protein kinase A, which phosphorylates cholesterol ester hydrolase. In the cytoplasm, this molecule catalyzes the formation of free cholesterol that is then transported into the mitochondria. Once in the mitochondrial membrane, ACTH acts on 2-melanocortin to cause cleavage by P450scc (STAR) to cholesterol desmolase which converts cholesterol to pregnalone. Pregnalone travels to the smooth endoplasmic reticulum, where 3β-

hydroxysteroid dehydrogenase (HSD) converts pregnalone to progesterone (the delta 4 pathway) or converts it to 17α-hydroxypregnenolone (delta 5 pathway). In the mitochondria, 17α-hydroxyprogesterone can be converted to 11–deoxycortisol and then converted to cortisol. Alternatively, 21OH can convert progesterone to 11-deoxycorticosterone, which is converted by 11OH to corticosterone.

Circulating GCs primarily refer to the hormones cortisol, corticosterone, or a mix of the two. Once released, 95% of hormones bind to the carrier protein corticosteroid binding globulin (CBG). While bound, GCs are biologically inactive and serve only as a reservoir. Free, or unbound, steroids are available to bind to target receptors and continue the stress response. Free steroids are regulated by 11β–hydroxysteroid dehydrogenase, which will convert them to their inactive forms, hydrocortisone and cortisone. Glucocorticoid release is regulated by negative feedback mechanisms that signal the HPA axis to terminate the stress response. Increased levels of GCs inhibit either anterior pituitary release of ACTH or hypothalamic release of CRH (the long loop response); increases in ACTH levels inhibit release of CRH (short loop) and CRH can inhibit its own release (ultra - short loop).

The physiological effects of stress

The acute or adaptive stress response combines a variety of cognitive, emotional, neurological and somatic signals. These behavioral and physical reactions are adaptive because they are time limited and improve the individual's chances for survival, and are often referred to as the "fight or flight" response. Behavioral adaptation includes increased arousal, alertness, cognition, vigilance and focused attention, heightened

analgesia, increased body temperature, suppression of appetite and suppression of the reproductive (HPG) axis (Matteri et al., 2000).

Physical adaptation primarily consists of the mobilization of energy to target tissues, and the inhibition of other systems not needed at the moment. This process includes shunting available oxygen and nutrients to the CNS and stressed tissues, increased blood pressure, heart rate and respiration, increased gluconeogenesis and lipolysis, inhibited growth and reproduction and increased detoxification. This short term response also down regulates the inflammatory and immune responses which prevents them from overshooting, potentially leading to autoimmune diseases. These combined physiological responses allow the individual to respond and cope with the external stimuli (or stressor), then return to a balanced homeostasis. In general, the stress response is intended to be short term; a physiological response that helps the individual to cope with a distinct external stressor. However, chronic stimulation of the HPA axis, and the subsequent release of glucocorticoids, may result in a litany of disorders that lead to decreased health and fitness. Both the acute stress response and the effects of long term chronic stress are discussed below.

The central nervous system is the first to respond to a perceived stressor. The parasympathetic nervous system is responsible for growth and digestion, and is the "calming" response. The complimentary sympathetic system is responsible for arousal and vigilance. Catecholamines are released by the adrenal medulla, causing the sympathetic nervous system to suppress the parasympathetic nervous system. The effects of this include inhibited salivation, inhibited digestion, increased heart rate, dilated

pupils, increased oxygen supply, decreased clotting time, increased platelet adhesion and adrenaline is stimulated. Increased GCs decrease REM sleep, increase slow wave sleep and increase wake time. Acutely, these actions prepare the individual for fight or slight, however over time these types of stimulation can result in mood changes (e.g. depression) and neuro-degeneration (e.g. stereotypies, fear, aggression) (Carlstead and Shepherdson, 2000).

As with the central nervous system, increased levels of GCs heighten the actions of the cardiovascular system. Increased GCs result in faster mobilization of glucose and oxygen to target tissues, and reduced blood flow to other organs. A concurrent increase in vasopressin increases water reabsorption by kidneys, thus increasing blood volume. Increases in blood pressure (vasoconstriction) result from the mineralcorticoid-like actions of GCs (as well as actions of catecholamines) which increase sodium and water retention, and decrease the vasodilators prostacyclin and bradykinin. Heart and respiratory rates also increase. Chronically, these affects would likely result in damage to the heart muscle, weakened vessels, high cholesterol deposition and hypertension (Sapolsky et al., 2000).

Some of the most pronounced and investigated effects of long term stress are the impacts on immune responses. In an acute stress response, GCs prevent the immune system from overshooting in response to a stressor by suppressing vasodilatation, infiltration of leukocytes and pain (analgesia). Further, GCs inhibit cell-mediated (T-cell dependant) and modulate humoral (B cell dependant) responses. This response opposes changes in vascular permeability, which helps to decrease inflammation and edema. GCs

inhibit the release of cytokines and decrease the number of receptors, block maturation of lymphocytes, marginate the lymphocytes and monocytes in the bone marrow and induce T cell apoptosis. In an acute reaction, these combined effects help the individual return to baseline; immune actions happen quickly and GCs help prevent autoimmune disease by cutting off the response. In chronic stress, these levels may remain below baseline (immunosuppression), increasing the susceptibility to pathogens, disease and decreasing wound healing. During times of hemorrhage, the acute response involves vasoconstriction and an increase in catecholamines and rennin to reduce blood loss. Over time, these processes cause sodium and water retention, leading to hypokalemia (high potassium). The long term anti-inflammatory actions suppress histamine and serotonin, inhibit interleukin-2 and induce lipocortin. Lipocortin inhibits lipase A, which is the precursor for prostaglandins that mediate the immune response.

Glucocorticoids have principally catabolic actions on metabolism. The acute response to a stressor is to mobilize energy stores toward target tissues where they are most needed. In carbohydrate metabolism, GCs largely oppose the actions of insulin (rapid insulin resistance) which facilitates glucose uptake into muscle and adipose, fatty acid storage, blocking the breakdown of triglycerides, conversion of glucose to glycogen and the inhibition of gluconeogenesis. High levels of glucocorticoids lead to the catabolism of stored glycogen to glucose (gluconeogenesis), the catabolism of proteins to amino acids and the breakdown of triglycerides to fatty acids and glycerol (lipolysis). By increasing the amount of glucose, amino acids and fatty acids in blood, maximum release of energy is available for use by target tissues. In the short term, this is a very effective

way to exploit energy reserves to address an immediate problem. However, in the long term, this can cause fatigue, muscle wasting, diabetes, increased cholesterol, centripedal obesity and myopathy (Sapolsky et al., 2000).

Increased concentrations of GCs affect the gastrointestinal actions; in times of stress digestion is inhibited in order to address more life threatening situations. As mentioned previously, these effects include decreased saliva (dry mouth) and decreased blood flow to digestive organs. Over time, decreased blood flow allows bacteria to flourish and can increase the risk of bacterial damage that causes stress ulcers. Compounding this, GCs also decrease the prostaglandins (via immunosuppression) that protect the stomach from ulcers.

Chronic stress can adversely affect individual growth and development. Prolonged GC secretion leads to a decrease in growth hormone (GH) and inhibits insulin-like growth factor I (IGF-I) effects on target tissues. This may result in delayed or arrested growth and puberty onset. CRH also increases somatostatin secretion, which further inhibits growth hormone, thus causing long term suppression of its effects on target tissues. Chronic GC release may also inhibit thyroid function. Stimulation of the HPA axis suppresses the production of thyroid stimulating hormone (TSH) and inhibits of the thyroxine to triiodothyronine conversion. Chronic stress also impacts an individual's connective tissues, such as bone and cartilage. High levels of GCs inhibit the proliferation and differentiation of osteoblasts, the production of collagen, osteocalcin and other matrix components. Over time, calcium stores are reduced by ultimate reductions in intestinal calcium absorption, increases in renal calcium excretion and

increases in serum parathyroid hormone (PTH) levels. High levels of GC's also increase the number of osteoclasts, which can lead to osteoporosis and decreased linear growth. Prolonged elevated GCs can result in poor wound healing and tissue repair by decreasing fibroblast proliferation and function and inhibiting production of matrix proteins.

During an acute stress response reproductive activity is secondary, thus many aspects of this system are suppressed to benefit the long term fitness of the individual. However, chronic stress negatively impacts an individual's long term reproductive abilities through its actions at multiple locations on the hypothalamic – pituitary - gonadal (HPG) axis. CRH and β-endorphins suppress the secretion of gonadotropin releasing hormone (GnRH) from the anterior pituitary by stimulating pre-opiomelanocortin (POMC) peptide secreting neurons, which act as the precursors to ACTH. The pituitary's response to GnRH is reduced, which decreases the amounts of follicle stimulating hormone (FSH) and leutenizing hormone (LH) that are released to the gonads.

These hormones are also directly inhibited by GC activity. In females, GCs inhibit ovarian sensitivity to LH, resulting in reduced folliculogenesis, estrogen and progesterone secretion, rendering egg release unlikely. GCs decrease the secretion of progesterone and increase prolactin. Combined together, this can disrupt uterine wall maturation, decreasing the likelihood of proper fetal implantation. Additionally, low follicular phase estrogen will delay or prevent ovulation. Androgens secreted from the adrenals cannot be converted to estrogen if fat cells are diminished by lipolysis. This can result in too many androgens and not enough estrogen. Chronic stress can also have very deleterious effects

on a pregnancy and fetal development. The increase in catecholamines decreases blood to the uterus, thus decreasing maternal-fetal nutrient exchange. High concentrations of ACTH may directly impair fetal neural development, and the decreased immune function could even result in fetal expulsion. Prostaglandins (which experience suppressed production) are needed to properly time a birth, and increases in oxytocin and catecholamines may lead to early birth.

In males, elevated GCs can inhibit testicular sensitivity to LH and FSH, reducing testosterone production, and therefore spermatogenesis. Chronic elevations in GCs may also inhibit the actions of the parasympathetic nervous system, decreasing the ability to achieve an erection and/or ejaculate, ultimately causing impotence.

Biological and laboratory validation of glucocorticoid measures

GCs are metabolized in the liver (also some in kidneys), then reduced and conjugated with glucuronic acid to make hydrophilic molecules. The now inactivated hormones can be excreted in the urine, or deconjugated by intestinal flora and excreted in feces (although some exceptions exist). High interspecies variation exists regarding the percentage of GC's excreted in each material, their primary metabolites and whether they are conjugated or deconjugated (Palme and Mostl, 1997; Mostl and Palme, 2002). Therefore, species specific validation must be performed in order to establish the best methods of GC measurement. There are two components to this: laboratory and biological validation (Wasser et al., 2000).

Laboratory validation ensures that the techniques used to measure hormones are appropriate. The first step in validation is a parallelism which determines if the assay is

measuring what it should be measuring, as well as the ideal dilution factor for the assay. Radioactively labeled and unlabeled (sample) antigens should have an equal affinity to the assay's antibody, and therefore bind competitively. If the sample curve resembles the pattern of the standard curve, the assay is measuring the preferred hormone of the sample accurately. The most accurate dilution factor to use will be where the samples are at 50% of maximum binding. The second step to laboratory validation is an accuracy/recovery check, which tests for any potential interference that other substances in your samples may have on the assay. This is done by adding known quantities of hormone and running the assay. The percent recovery must be between 85 and 115%, as there should be little difference between the expected and the observed assay results. Recoveries outside of this range indicate that components of the sample are interfering with the assays accuracy, causing either an under or over-estimation of hormone concentrations.

Biological validation is important to ensure that the metabolites measured are accurately reflecting the physiological processes you want to examine. The first step is to determine what material (feces or urine) is best for recovering hormone, and whether the hormone in this material accurately reflects the hormone activity in question. By tracking radioactively labeled corticosterone in the feces it is determined that feces are a suitable material for measuring adrenal corticoid activity (Palme and Mostl, 1997; Wasser et al., 2000). An ACTH challenge will assess whether the corticoids found in feces accurately reflect fluctuations in adrenal activity. In a challenge, baseline concentrations followed a spike in corticoids a day or two after ACTH administration would be expected, and followed by a return to baseline concentrations. This would show that the material is

efficient for measuring the desired hormone metabolites, as well as determine the lag time between increased adrenal activity and excreted fecal corticoids. Another validation step often involves high performance liquid chromatography (HPLC), a technique that can help identify excreted metabolite forms in a given sample. HPLC separates a sample into fractions that are analyzed for cross-reactivity in an immunoassay. This determines whether the selected assay quantifies significant amounts of biological metabolic forms (see Brown et al., 2001 for black rhinoceros validation).

Longitudinal hormone monitoring is a valuable tool that describes the hormone cascade through the HPA axis. Several different biological materials have been used to measure hormone concentrations: blood, saliva, urine, milk, feces. Previously, blood was predominantly used to examine hormone activity; however this material is not ideal for a study examining corticoids. Corticoids have a diurnal peak (meaning they peak once a day) and a pulsatile release (as opposed to continuous) so care must be taken as to the timing of extraction and the interpretation of its results. Collecting blood in itself may act as a stressor, especially if you have to restrain or anesthetize the animal, therefore complicating data (Millspaugh and Washburn, 2004). For these reasons, non-invasive monitoring using urine, saliva, milk and/or feces has become more popular (Wasser et al., 2000). However, obtaining milk, saliva and urine still require individual manipulation, some training and possible contamination, leaving feces the ideal material for analysis of corticoids. Feces are a preferred non-invasive material because samples can be collected without stressing the animal in for both wild and captive populations (Mostl and Palme, 2002). Feces are also a more appropriate material for assessing adrenal activity because

the sample represents a hormone "pool" that reflects an average concentration of circulating GCs over time, whereas sampling blood will only give a hormone "snapshot" of a particular point in time (Wasser et al., 1997). Therefore, there is less need for a strictly timed collection protocol; feces need only be fresh, well mixed and from an easily identifiable source. To avoid any further degradation to corticoid metabolites, feces must be frozen or processed (freeze dried) while the sample is still fresh (Mostl and Palme, 2002).

Stress in captivity

Several studies have examined relationships between adrenal activity (as measured by GC concentrations) and various indicators of animal health (Carlstead et al., 1993b, leopard cats; Whitten et al., 1997, chimpanzees; Graham and Brown, 1996, domestic cat; Owen et al., 2005, giant pandas). Stoinski et al. (2002) showed that Western lowland gorillas had higher urinary corticoids when they were housed alone rather than in social groups. A study by Weilebnowski et al. (2002) revealed several captive variables that were associated with increased corticoids in the clouded leopard. These variables included the number of keepers, display status, location of predators, self-injuring behaviors and enclosure parameters. Increasing the vertical length of the clouded leopard enclosures decreased corticoid levels, hence increasing animal health. Terio et al. (2004) demonstrated that captive cheetahs had higher baseline corticoid concentrations than free-range cheetahs, that cheetahs on exhibit had higher corticoid concentrations than cheetahs off exhibit and that hand reared cheetahs had lower corticoid concentrations than mother reared cheetahs. Adrenal hyperplasia, an indication of chronic stress, was exhibited by

several captive individuals. Owen et al. (2004) and Powell and Carlstead (2006) showed that giant pandas had higher urinary cortisol concentrations during times of increased noise and construction. Young et al. (2004) demonstrated that carnivores in stressful situations, such as restraint, blood sampling and introductions, had higher cortisol concentrations than animals in controlled settings.

Several studies have examined adrenal corticoid activity in black rhinos. Turner et al. (2002) monitored fecal corticoids in four white rhinos and five black rhinos during capture, translocation and release into a wildlife preserve in southern Africa. Fecal samples were obtained for six weeks after translocation into the preserve. Black rhinos showed higher corticoid activity than whites during translocation, as well as during the first week after their release. Additionally, compared to white rhinos, black rhinos still had higher corticoid levels 4-6 weeks after translocation, suggesting that black rhinos take longer to acclimate to and cope with new environments.

Carlstead and Brown (2005) demonstrated the validity of fecal corticoid monitoring for assessing adrenal activity in rhinos by examining adrenal activity in 10.16 black rhino and 6.13 white rhino across 14 U.S. facilities. Higher baseline mean concentrations were found in black compared to white rhinos (also see Brown et al., 2001). It may be significant that black rhinos are considered more stress-sensitive and have higher mortality rates compared to white rhinos. Among black rhinos, data indicate that stress-related mortality may be environmentally induced, either by social conflicts with other rhinos and/or by aspects of the facility design. For example, too much exposure to the public around the perimeter of an enclosure appears to be a variable that elicits elevations

in corticoids and possibly has long-term deleterious effects on health. In terms of social stress, black rhinos are considered less "sociable" to each other than white rhinos because they are solitary in the wild, whereas white rhinos live in groups. Black rhinos in zoos with more variable fecal corticoid levels (as measured by the variance, range and max values) were shown to spend significantly more "hours together per day with conspecifics," and do more "chase" and "fight." Therefore, it may be that some of the greater variation in fecal corticoid levels in black rhinos is explained, in part, by negative social interactions with conspecifics.

Purpose of study

Captive black rhino populations around the world are not self-sustaining, in part due to high mortality and morbidity, skewed sex ratios, suboptimal reproductive success and poorly understood disease syndromes. A current priority of the Rhino Species Survival Plan (SSP) is to investigate stress and health factors as they relate to socio-environmental variables in captivity. This study focused on two specific priority areas of black rhino research as stated in the Rhinoceros SSP Five-Year Plan: "investigating the pathophysiology and environmental correlates to black rhino diseases," and "investigating relationships between measurable indicators of stress, such as corticoid concentrations, and environmental correlates and health problems." Data provided in previous studies suggest that black rhino health is compromised by environmental-husbandry conditions (Carlstead et al. 1999a,b; Carlstead and Brown, 2005). However, those data do not reflect a complete representation of the physiological responses of black rhinos to the wide range of variables present in captivity. The objective of this study was

to examine potential relationships between captive variables, amino acid metabolism and adrenal activity and the presence of lesions in captive black rhinos. Recent studies suggested that lesions may be related to metabolic changes resulting from captivity "stress." We used fecal corticoid analyses as an index of adrenal status, and plasma amino acid assessments to compare rhinos with and without lesions. The purpose of this comprehensive, multi-disciplinary study was to provide potential information useful for developing species appropriate management strategies as it pertains to a specific disease syndrome characterized by ulcerative skin and oral lesions (often referred to as superficial necrolytic dermatitis or SND).

CHAPTER 2

Hypoaminoacidemia Does Not Occur in Black Rhinoceros (*Diceros bicornis)* Exhibiting Ulcerative Lesions Resembling Superficial Necrolytic Dermatitis.

ABSTRACT

Several unusual disease syndromes have compromised the sustainability of the captive black rhinoceros (*Diceros bicornis*) population. One of these, ulcerative oral and skin lesions, has become of increasing concern for the health management of this species. Lesions exhibited by black rhinos are clinically similar to those observed in other species with superficial necrolytic dermatitis (SND). One biochemical alteration in dogs with SND is a severe hypoaminoacidemia (decreased plasma amino acid concentrations), and nearly all cases are fatal. The objective of this study was to determine if black rhinos with analogous lesions exhibit a similar hypoaminoacidemia. Amino acid concentrations were measured in monthly plasma samples collected for 1 year from captive black rhinos with (n = 4) and without (n = 34) lesions clinically consistent with SND. The rhinos with skin and/or oral lesions were captive born males, ages 2, 6, 17 and 23 years, from four different facilities. Three rhinos recovered from skin (n = 2) and oral lesions (n = 1). However, the one male with both skin and oral lesions died with the disease. None of the affected black rhino exhibited hypoaminoacidemia for any of the amino acids evaluated, or for total amino acid concentrations ($P > 0.05$). Based on the lack of

hypoaminoacidemia and the comparatively low mortality rate in captive rhinos with lesions, we conclude that this syndrome is not entirely consistent with SND observed in other species. This is the first time plasma amino acid concentrations for any rhinoceros species have been reported and these data will be useful for future assessments of captive rhino nutritional status and other potential metabolic diseases.

INTRODUCTION

Superficial necrolytic dermatitis (SND) (also called metabolic epidermal necrosis, hepatocutaneous syndrome or necrolytic migratory erythema) belongs to a group of syndromes in which cutaneous lesions indicate the presence of a glucagon-secreting pancreatic tumor or hepatic disease. In the domestic dog, it is a particularly severe disorder that has been associated with a variety of concurrent health problems, including glucagonomas (Gross et al., 1993), diabetes mellitus (Walton et al., 1986), hepatic pathologies (Jacobson et al., 1995; Miller et al., 1990), hyperadrenocorticism and hypothyroidism (Outerbridge et al., 2002), dietary and a possible association with phenobarbital administration (March et al., 2004). Lesions associated with SND also have been reported in the cat (Kimmel et al., 2003), red fox (Van Pouke et al., 2005), and human (Marinkovich et al. 1995), but at much lower incidences.

A decade ago, Munson et al. (1998) described ulcerative lesions in black rhino and suggested they exhibited clinical similarities to SND in domestic dogs. The lesions begin as raised plaques, progressively leading to vesicles and erosions, and finally ulcers. Ulcers are usually bilaterally symmetrical and tend to expand peripherally, with most lesions presenting on pressure points, such as the back, feet, lateral body wall, tail, head,

ears and vulva/prepuce. Lesions can occur suddenly with remissions, or progress slowly as prolonged eruptions over time. Oral lesions generally are more persistent than skin lesions. Ulcerative lesions in black rhinos contain no viral, bacterial or fungal components and show minimal inflammation, and often occur in conjunction with other clinical pathologies. In a report, 23 of 34 affected rhinos had concurrent health conditions, including liver disease, anemia, gastrointestinal diseases, respiratory tract infections and urinary tract disease (Munson et al., 1998). Other associated symptoms can include weight loss, lameness, depression, anorexia, weakness, pregnancy and estrus/breeding. A recent retrospective, epidemiologic study of the captive black rhino population in the United States (1930 - 2001) found that 296 of 334 animals (88.6%) with skin lesions were twice as likely to die as compared to those without lesions (Dennis et al., unpubl.). The frequent occurrence of these lesions, compounded with an unknown etiology and absence of effective treatments, makes this syndrome a serious and urgent health concern for the captive black rhino population.

In the dog (Outerbridge et al., 2002), human (Marinkovich et al., 1995) and cat (Kimmel et al., 2003), hypoaminoacidemia (i.e., reduced plasma amino acid concentrations) often is associated with SND outbreaks. Low amino acid concentrations may contribute to disrupted epidermal homeostasis, which results in ulcerative lesions forming at the joints, footpads and other locations susceptible to stress and injury (Outerbridge et al., 2002). Normal levels of amino acids are essential for the pliability, strength and hydrophobic nature of the epidermis. By contrast, hypoaminoacidemia can lead to dermal spongiosis, which increases epidermal fragility. The cause of

hypoaminoacidemia in animals afflicted with SND is unknown; however, in dogs a retrospective study concluded it was not related to malnutrition, but rather to a metabolic hepatopathy that caused an unexpected, sudden increase in the hepatic catabolism of amino acids (Outerbridge et al., 2002).

Because of the clinical and histopathological similarities between the cutaneous lesions observed in black rhinos and those observed in dogs diagnosed with SNDs, the objective of this study was to determine if affected rhinos had the same dramatic reductions in plasma amino acid concentrations. If they are not significantly lower in rhino exhibiting lesions, it would suggest the syndrome is distinct from SND in dogs and other species.

METHODS

Sample collection

Plasma samples were collected approximately monthly for 1 year under normal dietary conditions (no fasting or dietary restrictions) from 23 male and 15 female black rhinos at 16 AZA accredited zoos. Blood was collected into plastic heparinized tubes, centrifuged and the plasma (2-4 mL) stored frozen (-20º C) for a maximum of 6 months in 1 mL aliquots for amino acid analysis. Personal communication with zoo veterinarians at each facility determined whether a rhino exhibited ulcerative skin and oral lesions consistent with those previously described (Munson et al., 1998).

Sample collection and analysis

Plasma amino acid concentrations were measured by an automated analyzer (Biochrom 30, Biochrom, Ltd., Cambridge, UK) using cation-exchange chromatography and spectroscopic determination of a ninhydrin reaction with amino acids. Analyses were conducted at the University of California Amino Acid Laboratory (Department of Molecular Biosciences, School of Veterinary Medicine, Davis, CA). Norleucine was used as an internal standard to standardize amino acid concentrations across time.

Data analysis

Statistical analyses were conducted using Intercooled Stata (v. 9.0, StataCorp, LP. College Station, Texas). Monthly plasma amino acid values were averaged for each individual for each amino acid and total amino acids. A molar ratio of branched chain amino acids (BCAA) to aromatic amino acids (AAA) was calculated for each rhino using the formula (valine + leucine + isoleucine) / (phenylalanine + tyrosine) and a mean ratio was calculated for each rhino. The mean ± standard deviation was calculated for rhinos with and without lesions, and T-tests used determined if there were statistical differences between the groups.

RESULTS

Details on the captive black rhinos evaluated in this study are presented in Table 2.1. Four rhinos developed ulcerative lesions during the collection period; two had skin lesions, one had oral lesions and one had both types of lesions. All rhinos with lesions were captive born males from different institutions. Three facilities had rhinos with and

without lesions housed in the same or adjacent enclosures. Three of the rhinos with skin lesions had a history of lesion eruption prior to this study.

The mean concentrations (± SEM) and ranges for all plasma amino acids for rhinos with lesions, without lesions and for the rhino that had the most severe case of skin

Table 2.1. Summary of the captive black rhinos, with and without skin and/or oral lesions, evaluated in this study.

	No lesions	Lesions
Eastern subspecies	25	4
Southern subspecies	9	0
Number of males.females	19.14	4.0
Number of zoos	14	4
Number captive born	30	4
Number of wild caught	4	0
Mean age (range) in years	16 (2-38)	12 (2-23)
Total number of rhinos	34	4

and oral lesions are presented in Table 2.2. There were no differences in concentrations of individual plasma amino acids, or total amino acids between rhinos with and without lesions ($P > 0.05$), or between the rhino with the most severe case of ulcerative lesions and those without lesions ($P > 0.05$). There also was no difference ($P > 0.05$) in the BCAA:AAA molar ratio between rhinos with (4.4 ± 0.7 SD) and without (4.5 ± 0.6 SD)

lesions (See Appendix I for individual rhino amino acid concentrations). In dogs with SND, the most severe reductions in amino acid concentrations are reported for arginine, glutamine, proline and threonine (< 20% of normal) (Outerbridge et al., 2002). However, neither temporal patterns nor mean concentrations of these amino acids differed between rhinos with and without lesions ($P > 0.05$) (Fig.2.1).

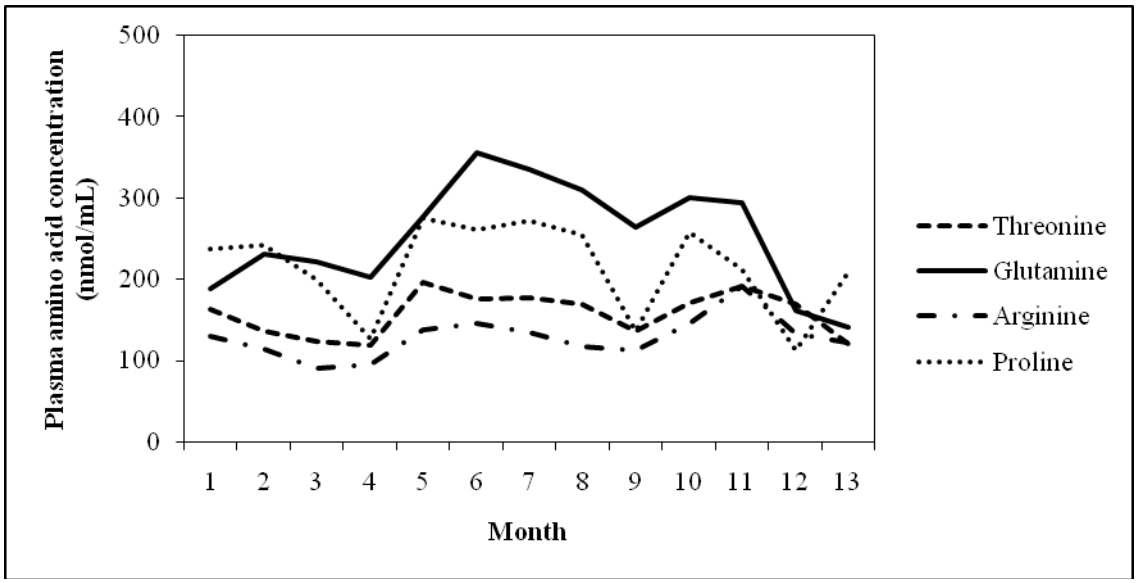
Table 2.2. Mean plasma amino acid concentrations (nmol/mL) ± standard error (range) in black rhino with and without ulcerative skin and/or oral lesions, and in the rhino that died and exhibited the most severe skin and oral lesions. Plasma samples were collected approximately monthly for one year.

Amino Acid	Rhino without lesions (n = 34)	Rhino with lesions (n = 4)	Severe lesions
Alanine	262.9 ± 9.9 (148.0 - 386.3)	264.0 ± 33.4 (163.8 - 301.1)	290.9 ± 11.1 (269.0 - 333.7)
Arginine	142.4 ± 7.4 (93.6 - 311.2)	134.5 ± 13.78 (101.0 - 158.7)	158.7 ± 9.7 (140.8 - 189.4)
Asparagine	46.2 ± 4.5 (6.8 - 101.6)	79.8 ± 20.2 (35.6 - 117.7)	109.8 ± 9.0 (84.6 - 133.4)
Aspartic acid	24.4 ± 2.7 (2.5 - 67.1)	23.8 ± 7.7 (6.3 - 40.6)	40.6 ± 4.4 (31.2 - 54.5)
Citrulline	37.1 ± 1.9 (20.4 - 63.0)	37.8 ± 12.6 (15.6 - 72.2)	15.6 ± 1.7 (11.1 - 19.7)
Glutamic acid	149.6 ± 5.1 (89.4 - 205.0)	132.5 ± 20.4 (72.4 - 160.4)	152.5 ± 7.2 (131.3 - 173.6)
Glutamine	265.4 ± 8.8 (162.4 - 352.5)	339.5 ± 38.1 (258.6 - 435.7)	304.0 ± 15.8 (275.0 - 361.1)
Glycine	462.8 ± 12.9 (333.7 - 608.9)	427.3 ± 34.4 (364.3 - 522.5)	395.2 ± 18.9 (353.5 - 448.5)
1-Methyl histidine	6.2 ± 0.3 (2.6 - 9.7)	7.3 ± 0.9 (5.3 - 8.6)	8.4 ± 1.2 (5.8 - 11.4)
3-Methyl histidine	29.6 ± 18.0 (10.2 - 91.5)	26.5 ± 17.6 (10.2 - 50.3)	25.0 ± 4.5 (14.1 - 38.4)
Histidine	92.8 ± 2.4 (63.3 - 120.2)	87.0 ± 5.3 (78.0 - 102.0)	102.0 ± 4.3 (90.0 - 113.6)
Isoleucine	121.3 ± 3.9 (83.7 - 172.3)	120.3 ± 16.1 (95.5 - 138.0)	138.0 ± 5.9 (119.5 - 154.8)
Leucine	206.9 ± 38.6 (145.6 - 312.6)	204.0 ± 32.2 (156.2 - 223.8)	223.8 ± 24.7 (188.1 - 321.3)
Lysine	132.8 ± 5.7 (83.7 - 255.3)	125.5 ± 12.7 (98.9 - 158.4)	158.4 ± 9.3 (134.9 - 189.3)

Methionine	27.0 ± 1.0 (15.6 - 38.6)	29.0 ± 3.8 (22.3 - 36.1)	36.1 ± 2.9 (30.8 - 46.7)
Ornithine	115.2 ± 11.8 (47.3 - 466.7)	110.5 ± 11.4 (90.3 - 142.3)	111.2 ± 5.9 (97.0 - 130.1)
Phenylalanine	103.2 ± 4.5 (62.3 - 178.4)	99.5 ± 7.1 (84.5 - 115.7)	115.7 ± 9.5 (100.8 - 150.6)
Proline	199.5 ± 10.6 (105.8 - 336.7)	220.5 ± 16.8 (175.8 - 254.8)	254.8 ± 15.9 (221.5 - 313.5)
Serine	147.5 ± 5.5 (74.7 - 229.3)	175.0 ± 18.8 (125.2 - 206.4)	202.2 ± 9.3 (180.3 - 233.4)
Taurine	39.4 ± 2.8 (20.0 - 97.2)	26.3 ± 3.3 (18.0 - 33.7)	28.0 ± 6.7 (16.7 - 51.8)
Threonine	163.9 ± 5.4 (93.6 - 224.7)	162.5 ± 17.0 (128.1 - 208.5)	208.5 ± 7.8 (194.8 - 237.3)
Tryptophan	82.6 ± 3.0 (42.4 - 111.2)	81.5 ± 6.1 (67.3 - 94.6)	87.0 ± 2.9 (81.8 - 98.5)
Tyrosine	65.3 ± 2.6 (41.8 - 103.7)	68.5 ± 7.9 (59.0 - 92.2)	92.2 ± 6.7 (73.1 - 109.8)
Valine	411.9 ± 12.1 (276.7 - 552.3)	395.8 ± 27.0 (329.1 - 459.3)	410.3 ± 9.0 (394.8 - 442.7)
Total amino acid values	3344.1 ± 70.9 (2624.6 - 4465.3)	3381.0 ± 153.8 (2962.6 - 3670.6)	3670.6 ± 154.4 (3368.4 - 4252.6)

P > 0.05 for all amino acids

a.



b.

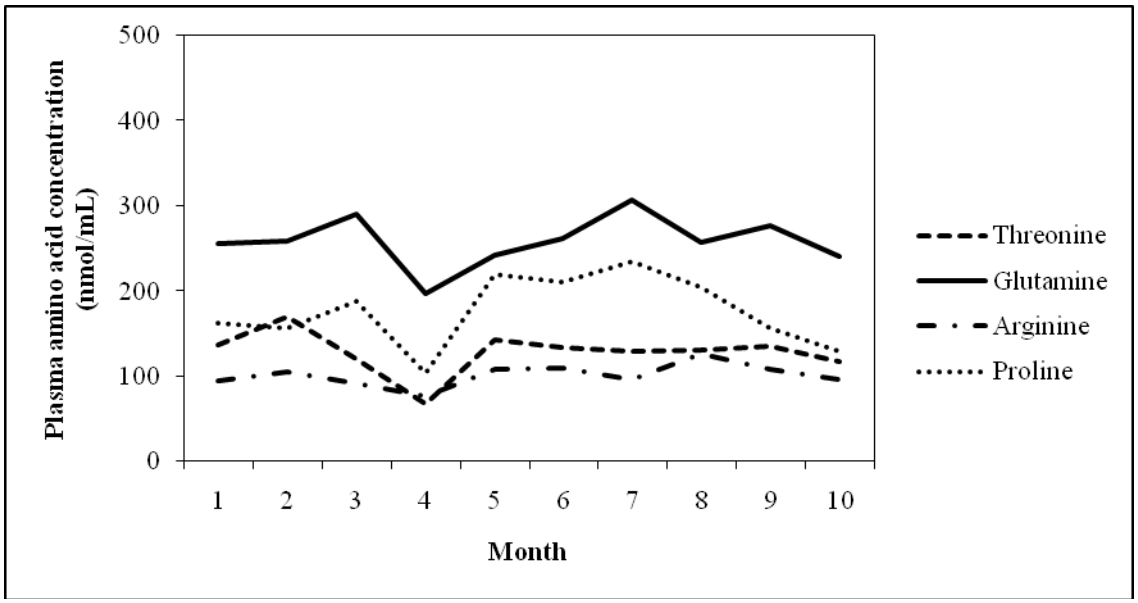


Figure 2.1. Profiles of the four amino acids that exhibit the greatest hypoaminoacidemia in dogs with SND (Outerbridge et al., 2002) for (a) a black rhino that never exhibited lesions and (b) a black rhino with lesions.

DISCUSSION

Hypoaminoacidemia was not observed in any of the black rhinos that exhibited ulcerative skin and/or oral lesions during the study period. These results are in stark contrast to findings in dogs, where animals diagnosed with SND exhibit marked reductions (≤ 60% of normal) in all but four measured amino acids (glutamic acid, phenylalanine, tryptophan and ornithine), and in the total amino acid concentration (only ~30% of normal) compared to healthy individuals (Outerbridge et al., 2002; March et al., 2004). The four amino acids associated with the most severe hypoaminoacidemia in dogs (arginine, glutamine, proline and threonine) also showed no quantitative or qualitative differences between rhinos with and without lesions, further emphasizing the lack of a relationship between amino acid metabolism and the outbreak of ulcerative lesions in rhinos. Afflicted rhinos exhibited lesions intermittently throughout the collection period, with manifestations ranging from 1 month to 1 year with no changes in amino acid values as symptoms progressed.

One major difference between rhinos and dogs diagnosed with lesions, is that SND in dogs is nearly always fatal (mean survival: 6.43 months, range: 2 - 32 months) (Outerbridge et al., 2002). Partial recovery and short-term survivability was achieved only in dogs that were fed additional protein or amino acid supplements, or given parenteral amino acid infusions (Outerbridge et al., 2002). By contrast, many rhinos with either type of lesion frequently recover from episodes without treatment, although recurrence is common (Munson et al., 1998). Of the four affected black rhinos in this study, three had had lesions historically, and three have since entered remission. The

male with oral lesions exhibited concurrent severe weight loss, fatigue and anorexia before recovering. Unfortunately, the rhino with both skin and oral lesions died during the study. Prior to his death, the animal displayed neurological symptoms, including abnormal behavior and confusion. He had intermittent ulcerative lesions on his pressure points, tail and tongue, alternating foreleg lameness of unknown etiology, arrested growth for his age, and bouts of lethargy and mild anorexia. This rhino had persistent jaundice with increases in serum bilirubin, total protein, SGOT, alkaline phosphatase, iron and copper concentrations. Post-mortem examination revealed extensive subcutaneous hemorrhage and edema. The adrenal glands had a focus of subcortical mineralization, with a small number of lymphocytes and plasma cells. Extensive areas of coagulative necrosis in the deep adrenal cortex were surrounded by a rim of congestion, hemorrhage and degenerate neutrophils and cortical epithelial cells. Upon liver examination, the hepatic cords were disrupted and the hepatocytes were swollen with marked amounts of perinuclear brown pigment determined to be lipofusion. Several small hematomas were found in the brain and pituitary tissues. These were attributed to the rhino falling repeatedly prior to his final collapse. The kidney and heart tissues were unremarkable (K. Nelson, pathology report).

Another difference between rhinos and dogs is the age of lesion onset. In dogs, it is primarily older individuals that are afflicted with SND. Thus, age-related hepatic pathologies may be responsible for the observed hypoaminoacidemia (March et al., 2004). By contrast, skin and oral lesions occur in black rhinos of all ages (Munson et al., 1998), and in this study animals were 2, 6, 17 and 23 years of age. Many dogs with SND,

and humans with necrolytic migratory erythema (NME), a syndrome clinically similar to SND, have hyperglucagonemia secondary to a glucagon secreting pancreatic tumor (Allenspach et al., 2000; Bond et al., 1995; Torres et al., 1997). Increased glucagon can stimulate gluconeogenesis and result in increased amino acid catabolism. Plasma glucagon has not been measured in black rhinos, but the lack of hypoaminoacidemia observed in this study suggests that pancreatic tumors are not a likely cause of these lesions. Taken together, the absence of hypoaminoacidemia, a lower mortality rate and the different array of concurrent health conditions suggests the constellation of clinical and biochemical signs observed in black rhinos are not identical to SND as described in dogs.

To the best of our knowledge, this is the first study to publish plasma amino acid concentrations in any rhinoceros species. Individual amino acid concentrations vary greatly among species, making direct comparisons difficult. Amino acids in the cat and dog are two to five times higher for some amino acids and two to three times lower for others (Outerbridge et al., 2002; Kimmel et al., 2003). In the horse (Order: *Perissodactyla*), a more closely related species to the rhinoceros with similar nutritional requirements and digestive system, concentrations of some amino acids (e.g., asparagine, citrulline, isoleucine, glycine and theorine) were comparable between the species, but not all (Amino Acid Laboratory, Department of Veterinary Molecular Biosciences at the University of California, Davis webpage; http://www.vetmed.ucdavis.edu/vmb/aal/aal.html). For example, glutamine and serine were approximately double in the horse, while glutamic acid and phenylalanine were

higher in black rhinos. Diet, season, age and sex differences can all impact amino acid metabolism and circulating concentrations (Zicker et al., 1990). Thus, monthly samples were collected over the course of a year to provide a more reliable estimate of baseline amino acid values. In addition, rhinos were not subjected to fasting or any kind of specialized or restricted diets.

In other species, these data can be a valuable diagnostic tool for monitoring or studying liver or renal diseases (Gulick et al., 1980), nutritional imbalances (McLaughlan et al., 1974) and genetic defects affecting amino acid metabolism (Bremer et al., 1981). Branched chain amino acids are catabolized in a variety of tissues, while the aromatic amino acids are catabolized solely in the liver. As a result, the BCAA:AAA ratio has been used in research and clinical patients to evaluate and monitor some hepatic pathologies with the ratio decreasing as the severity of hepatic dysfunction or portal-systemic shunting increases (Gulick et al., 1980; Rutgers et al., 1987). There were no differences in the mean concentrations of the branched chain amino acids (BCAA) to aromatic amino acid (AAA) ratio between the normal and affected animals in this study. Similarly, the mean value for the molar ratio in dogs with SND was 2.6, which did not indicate severe hepatic dysfunction (normal: 3.0 – 4.0) (Outerbridge et al., 2002). Although there are no previous data for rhinos, the lack of a difference between the molar ratios of rhino with and without lesions suggests that the lesions were not caused by hepatic pathologies.

In conclusion, building a sustainable captive black rhinoceros population has proven difficult because of high mortality, morbidity, skewed sex ratios and suboptimal reproductive success. Identifying the etiology and developing treatments for the unusual

disease syndromes observed in this species (e.g., idiopathic hemorrhagic vasculopathy syndrome (IHVS), hepatopathy, iron overload, hemolytic anemia and ulcerative lesions) (Dennis et al., 2007) is currently a high priority of the Rhinoceros Species Survival Plan (SSP). Based on the results of this study, ulcerative lesions observed in black rhinos are not consistent with SND described for other species. Thus, further research is needed to elucidate the physiological mechanism(s) and pathogenesis of this disease.

CHAPTER 3

Decreased Fecal Corticoid Concentrations Are Associated with Skin and Oral Lesions in the Captive Black Rhinoceros, *Diceros bicornis*

ABSTRACT

Captive black rhino populations around the world are not self-sustaining, due in part to compromised health caused by debilitating skin and oral lesions. Facility and socio-environmental keeper surveys were completed and twice-weekly fecal samples were collected from 25.20 black rhinos from 18 U.S. zoos for 1 year to examine the relationship between captive variables and adrenal activity and the presence of ulcerative lesions. There were no relationships ($P > 0.05$) between facility and socio-environmental variables or rhino behavior indices and lesion onset. During the collection period, 5.1 rhinos had skin lesions, 1.0 had oral lesions and 1.0 had both. Of these eight rhinos, all recovered from ulcerative lesions, except one rhino with oral and skin lesions that died shortly after the end of the study. There were no differences ($P > 0.05$) in fecal corticoid variability (± SEM) between rhinos with (CV = 57.1 ± 7.2 ng/g) and without (53.8 ± 2.3 ng/g) lesions. Baseline mean (± SEM) corticoid metabolite concentrations were lower ($P < 0.05$) in rhinos with ulcerative lesions (n = 5.1, baseline mean = 29.9 ± 3.3 ng/g) as compared to those without (n = 19.19 baseline mean = 40.0 ± 2.4 ng/g). For a single

male rhino that developed skin lesions during the study, the mean corticoid concentrations were lower ($P < 0.01$) when lesions were present (overall = 30.1 ± 2.4 ng/g, baseline = 28.7 ± 2.2 ng/g) than absent (overall = 36.5 ± 1.0 ng/g, baseline 35.3 ± 0.8 ng/g). These data suggest that ulcerative skin and oral lesions may be associated with compromised adrenal activity, although the significance of this relationship needs further investigation.

INTRODUCTION

The North American zoo black rhino population is not self-sustaining, in part because of high mortality and morbidity rates, skewed sex ratios and suboptimal reproductive success. A recent survey analyzing the survivability of the captive U.S. black rhino population (296 of 334 rhinos, 88.6%) found several health issues impacted the species: hypercalcemia, hypophosphatemia, jaundice, diarrhea, epitaxis, dental calculus, loose teeth, anemia, tail sloughing, loose horns, neurological abnormalities, ataxia, tremors, luxating patella, lameness, and swelling of the limbs and shoulders (Dennis et al., unpubl). Furthermore, the species exhibits an array of disease syndromes, such as idiopathic hemorrhagic vasculopathy syndrome (IHVS), hepatopathy, iron overload, hemolytic anemia and ulcerative lesions (Dennis et al., 2007). Understanding the etiology of these health problems, and developing effective treatments or preventative strategies is a high priority of the Rhinoceros Research Advisory Group (RAG). One particularly debilitating disease syndrome, ulcerative skin and oral lesions, is the focus of this study.

Lesions begin as raised plaques, progressively leading to vesicles and erosions and finally ulcers. Munson et al. (1998) has found that, like SND in dogs, lesions contain no viral, bacterial or fungal components and show minimal inflammation. In rhinos, chronic ulcers are usually bilaterally symmetrical and tend to expand peripherally, with most lesions presenting on pressure points, the back, hoof coronary bands, lateral body wall, tail, head, ears and vulva/prepuce. Lesions can occur suddenly and may progress slowly, with most cases having prolonged eruptions at multiple sites. Munson et al. (1998) found that as much as 50% of the captive population had been affected by lesions, with no age, sex or subspecies predilection. Almost half of these individuals (42%) had recurrent episodes, and a majority with only a single episode died with unresolved lesions. A survey by Dennis et al. (unpubl.) found that rhinos with lesions were more than twice as likely to die as those without. Assigning mortality rates to lesions in black rhinos can be difficult, however, because they often occur in conjunction with other clinical pathologies (e.g., reductions in hematocrit, serum albumin and cholesterol concentrations). In the study of Dennis et al. (unpubl.), 23 of 34 affected rhinos had other health problems, such as iron overload, anemia, and liver, gastrointestinal and urinary tract diseases (Munson et al., 1998). Other concurrent problems included weight loss, lameness, depression, anorexia, and weakness. Rhinos are also known to exhibit lesions in association with pregnancy, estrus/breeding and occasionally after traumatic events, such as translocation and death of a conspecific (Munson et al., 1998). These findings led clinicians to suspect that ulcerative lesions in black rhino may, in part, be stress related (Munson et al., 1998).

Munson et al. (1998) first described ulcerative lesions in black rhinos, suggesting there were clinical similarities to superficial necrolytic dermatitis (SND) observed in domestic dogs. However, one of the major symptoms of SND in dogs, hypoaminoacidemia, was not observed in affected black rhinos (Dorsey et al., unpubl.). This finding led to the speculation that lesions in black rhino are not classic SND. In support of this assertion are other examples of major symptomatic differences between SND in the dog and ulcerative lesions in black rhino. For one, the mortality rate in dogs is almost 100%, whereas rhinos with lesions frequently recover from episodes without treatment, although recurrence is common (Outerbridge et al., 2002; Munson et al., 1998; Dennis et al., 2007). Another difference is the age of lesion onset. In dogs, it is primarily older individuals that are afflicted with SND. By contrast, skin and oral lesions in black rhinos occur in animals of all ages. Taken together, these observations suggest that despite the visual similarities, this disease syndrome in black rhino is not consistent with SND in other species.

The hypothalamic–pituitary–adrenal (HPA) axis controls an individual's adaptive stress response. In reaction to stimuli, hypothalamic corticotropin-releasing hormone (CRH) stimulates pituitary adrenocorticotropin hormone (ACTH), which causes a surge in cortisol secretion from the adrenal cortex. By contrast, a compromised HPA axis can negatively impact the ability of an individual to maintain homeostasis and successfully cope with stressors in its environment. Assessing potential species-specific sources of stress in captivity is critical, especially given how chronic stress can severely affect animal health and fitness (Weilebnowski, 1998). Several studies have addressed the

relationship between captive variables and species' health and/or adrenal activity. For example, a study by Wielebnowski et al. (2002) found that clouded leopards kept on public display, housed near large predator felid species or in enclosures with less vertical space had higher fecal corticoid concentrations. Modifying one of the environmental variables, the vertical height of the enclosure, decreased corticoid concentrations in individual clouded leopards.

Assessments of adrenal corticoid activity in association with management and environmental factors also have been conducted in black rhinos. Turner et al. (2002) monitored fecal corticoids in four white and five black rhinos captured and translocated to a wildlife preserve in southern Africa**.** Black rhinos showed higher corticoid activity than white rhinos in response to capture, and for the first week after release. Additionally, compared to white rhinos, black rhinos still exhibited higher corticoid levels 4-6 weeks after translocation, suggesting they take longer to acclimate to new environments. Carlstead and Brown (2005) examined fecal corticoid patterns in 10.16 black rhinos and 6.13 white rhinos across 14 U.S. facilities and found higher baseline mean concentrations in black compared to white rhinos (also see Brown et al., 2001). Anecdotally, black rhinos often are considered more 'stress-sensitive' than white rhinos, and may therefore be more reactive to stressors in captivity (Carlstead and Brown, 2005).

Many aspects of the captive environment can be potentially stressful and adversely affect individual well-being (Carlstead et al., 1993; Wielebnowski et al., 2002). While many animals successfully adapt to captivity, others fail to thrive when compared to wild counterparts. This may be due, in part, to species-inappropriate management practices,

either social and/or environmental (Lindburg and Fitch-Synder, 1994; Carlstead, 1996; Clubb and Mason, 2003; Wielebnowski, 2003). Objectively assessing how individuals respond to captive conditions is undeniably one of the greatest challenges facing species conservationists. Fecal corticoid metabolite monitoring is one way to assess physiological responses; facility surveys are another tool that can provide insight into how captive variables (environment, management, temperament and social) impact animal health and behavior (e.g. Gold and Maple, 1994; gorillas; Wielebnowski and Brown, 1998; cheetahs; Wielebnowski et al., 2002, clouded leopards; Freeman et al., 2004, African elephants; Owen et al. 2004, giant pandas). These types of studies are increasingly relevant, but to date have not been used extensively in the study of wildlife disease.

A current priority of the Rhino TAG is to investigate how socio-environmental variables in captivity relate to stress and health factors (AZA Rhino Research Advisory Group, 2004). The frequent manifestation of ulcerative lesions, compounded with an unknown etiology and lack of effective treatment, make this a severe health concern for the captive black rhino population. If this disease syndrome is related to captivity stress, then it may be associated with changes in adrenal steroidogenic activity. Using fecal corticoid assessments and facility surveys, this study examined the relationship between adrenal function and captive variables with ulcerative lesions in black rhinos.

METHODS

Facility and socio-environmental survey

Keepers from 18 AZA accredited zoos, who had at least 6 months experience working with the study animals, completed facility and socio-environmental surveys (See Appendix II and III). The facility survey consisted of 34 questions about the physical characteristics of the rhino enclosure (e.g., square footage, amount of public exposure, construction material, substrates) at each facility. The socio-environmental survey consisted of 43 questions related to general husbandry procedures (e.g., diet, social management, types of enrichment and training). Keepers also completed temperament and behavior questions about each rhino, which were later developed into behavioral indices. Additional life history information (e.g., age, sex, number of zoo transfers, wild caught/captive born) was obtained from the North American Regional Studbooks for Eastern and Southern black rhinoceros (Foose, 2006). Climate data (e.g., average temperature, average precipitation, elevation) were obtained from the NOAA National Climatic Data Center (NCDC) website http://www.ncdc.noaa.gov/oa/ncdc.html.

Fecal sample collection

Fresh, well mixed fecal samples were collected twice weekly for about 1 year from each rhino and stored frozen until processing and analysis. Collection began in 2005 and continued through the winter of 2007, depending on the individual rhino's starting date. In 2007, 1.0 rhino evaluated in 2005-2006 developed skin lesions, so fecal samples were collected for an additional 3 weeks to compare with samples collected when the rhino was healthy. Veterinary records were used to classify rhino health and if

they exhibited ulcerative skin and oral lesions consistent with those described by Munson et al. (1998).

Fecal corticoid analysis

Fecal samples were processed and analyzed using the methods described by Brown et al. (2001), except that shaking, rather than boiling, was used to extract steroids. Frozen feces were lyophilized, pulverized and 0.2 g of well-mixed powder shaken (70 pulses/min; Large Capacity Mixer, Glas-Col Terre Haute, USA) in 5 mL of 90% ethanol:10% distilled water for 40 min. After centrifuging at 500g for 20 min, the supernatant was recovered and the pellet re-suspended in 90% ethanol, vortexed for 1 min and re-centrifuged for 20 minutes. The resulting combined supernatants were dried under air and re-dissolved in 3 mL ethanol, sonicated for 20 min and dried again. Extractant was re-suspended in 1 mL ethanol, sonicated for 20 minutes, and dried completely under air. The extractant was re-suspended in 1 mL of phosphate buffered saline (PBS; 0.01 M $NaPO_4$, 0.14 M NaCl, 0.5% BSA, 0.01% NaN_3), vortexed for 1 min and sonicated for 20 min. Extraction recovery efficiency was >75% for ^{3}H-corticosterone added to dried fecal samples before extraction.

A double-antibody ^{125}I corticosterone radioimmunoassay (MP Biomedical, LLC., Irvine, CA), previously validated for black rhinos (Brown *et al*., 2001), was used to quantify corticoid metabolite concentrations in feces. Extractants were diluted (1:10) in phosphate-buffered saline before analysis. For all assays, the intra and inter-assay coefficients of variation were <10%.

Data Analysis

All statistical analyses were conducted using Intercooled Stata (v. 9.0, StataCorp, LP. College Station, Texas). Survey variables were tested for normality and highly skewed variables were removed from the analysis. Variables with little or no variation among individuals were removed from the analysis. Principal components factor analysis with varimax rotation of 20 behavior and temperament questions was used to create indices to describe the different behaviors that rhinos exhibited. Logistical regression assessed causal relationships between rhino behavior indices, facility parameters, life history or socio-environmental variables and the development of lesions.

The overall mean, minimum, maximum and mean elevated (values 1.5 standard deviations, SD, above the mean corticoid concentration) concentrations, and corticoid coefficients of variation (CV) were calculated for each individual (Brown et al., 2001, Wielebnowski et al., 2002, Carlstead and Brown, 2005). Baseline fecal corticoid concentrations were determined by an iterative process where all values above the mean plus 1.5 times the SD were removed, the mean recalculated and the process repeated until no values exceeded the mean + 1.5 SD (Brown et al., 2001). T-tests determined if there were significant differences in hormone concentrations between rhinos with and without lesions. Significance was set at $P < 0.05$.

RESULTS

Surveys were completed and fecal samples collected for glucocorticoid analysis from 25.20 black rhinos from 18 AZA-accredited U.S. zoos. Twelve facilities were located in an urban setting and eight were suburban. One zoo housed only one rhino, six

zoos had two, eight zoos had three, one zoo had four and two zoos housed five rhinos. The mean exhibit age was 18.3 years (range: 2 – 73 years), and consisted of one to six indoor/outdoor stalls. Seventeen facilities used chlorine bleach an average of once a week. The mean estimated amount of public exposed perimeter in the indoor enclosure was 31.7 (range: 0 - 200) feet (due to inconsistent responses, outdoor perimeter was unavailable). The mean estimated inside enclosure area was 250 (range: 80 - 540) square feet, and mean estimated outdoor enclosure area was 12,677 (range: 0 – 65,000) square feet.

Of the 45 rhinos in this study, 40 were of the eastern subspecies, *D. b. michaeli*, and five were of the southern subspecies, *D. b. minor*. These numbers reflected the proportion of each subspecies in captivity. Forty-two were captive born and three were wild caught. The mean age was 14.6 (range: 2 – 38) years. Rhinos lived in an average of 2.2 (range: 1 - 6) zoos in their lifetime. Rhinos spent a mean 7.5 (range: 0.5 - 31) years at the current facility, and were managed by an average of 1.6 (range: 1 - 8) keepers at any one time. Rhinos spent an estimated average of 10 (range: 0 - 24) hours on display, rotating stalls 0 - 2 times each day in the warmer months. On an average day, keepers spent about 1.5 (range: 0.5 – 6.0) hours directly interacting with the rhinos, using an average of 5.2 (range: 1 - 7) different kinds of enrichment on a daily basis (e.g., movable logs, mud wallows, pools, rubbing posts and sprinklers). Although kinds and amounts of dietary supplements varied greatly, a combination of hay, legumes and grain was the primary component of rhino diets; all received some type of browse. Eighteen rhinos were part of an intended breeding pair, but only four pairs were housed together

full time. The remaining pairs were put together for an average of 9.6 (range: 5.5 - 24) hours a day when the female exhibited signs of estrus.

Principal components factor analysis of the behavior survey data identified five distinct factors with eigenvalues > 1.1. These factors were categorized into behavioral sub-dimensions: aggressive, friendly, fearful, cautious and timid (Table 3.1). Items loading > 0.40 on each factor were used to create an average summative index for the behavior sub-dimension. For each index, a Cronbach's alpha was calculated to test for internal reliability. This procedure produced alpha values ranging from 0.72 - 0.86. Logistical regression revealed no relationships ($P > 0.05$) between the development of skin lesions and the rhino temperament sub-dimensions, facility, life history or socio-environmental variables of captivity (See Appendix IV).

Seven rhinos (age 2 – 25 years) from five facilities developed ulcerative lesions during the study period. Of these, 4.1 rhino had skin lesions, 1.0 had oral lesions and 1.0 had both. Four of these rhinos had verified lesions in the past, one had an unknown health history and one had never had them before. All affected rhinos were captive born, *D. b. michaeli.*

Four rhinos with a history of lesions exhibited them consistently or intermittently throughout the collection period with no documented start and stop dates, and so were considered to have lesions throughout the study. A 2-year old male rhino developed oral

Table 3.1. Results of principal components factor analysis of behavior survey questions. All answer options were a ranking of 1 – 5; 1 meaning the rhino does not exhibit this behavior and 5 meaning the rhino always exhibits this behavior.

Behavior	Survey Questions
Aggressive	Is this rhino aggressive towards the keeper?
	Is this rhino aggressive towards the zoo patrons?
	Is this rhino aggressive towards others?
Cautious	Does this rhino act cautious of the keeper?
	Does this rhino act aloof towards zoo patrons?
	Does this rhino act cautious of zoo patrons?
	Does this rhino act cautious of others?
Fearful	Does this rhino act fearful of the keeper?
	Does this rhino act fearful of zoo patrons?
	Does this rhino act fearful of others?
Friendly	Does this rhino usually allow touching by zoo patrons?
	Does this rhino approach easily when called?
	Is this rhino friendly towards others?
Timid	Does this rhino act timid of the keeper?
	Does this rhino act timid toward zoo patrons?
	Does this rhino act timid toward others?
	Does this rhino act fearful towards others?
	Does this rhino act aloof towards others?

lesions 5 months into the study, which lasted for about 3 months. This individual also exhibited concurrent severe weight loss, fatigue and anorexia, and was still underweight at study conclusion. Another male developed lesions immediately after sample collection and these lasted for approximately 2 months. However, there were no differences in corticoid concentrations between the time with and without lesions ($P > 0.05$). The rhino in Fig 2a had severe skin lesions and died at 6-years of age, about 6 months after the study ended. Lesions were present when the study began and over the course of the year got progressively worse. This rhino exhibited ulcerative lesions on the elbows, hocks, back, tail and tongue, alternating foreleg lameness of unknown etiology, arrested growth for age and bouts of lethargy and mild anorexia. He also had persistent jaundice with increases in serum bilirubin, total protein, SGOT, alkaline phosphatase, iron and copper concentrations. Before death, the rhino was severely underweight and displayed neurological symptoms, including abnormal behaviors, such as confusion and aggression. Throughout the collection period, concentrations of fecal corticoids were consistently low (overall mean = 19.9 ± 1.2ng/g, baseline mean 17.0 ± 0.1ng/g). Post-mortem examination revealed extensive subcutaneous hemorrhage and edema. Upon liver examination, the hepatic cords were disrupted and the hepatocytes were swollen with marked amounts of perinuclear brown pigment determined to be lipofusion. Several small hematomas were found in the brain and pituitary tissues. The kidney and heart tissues were unremarkable. The adrenal glands had a focus of subcortical mineralization, with a small number of lymphocytes and plasma cells. Extensive areas of coagulative

necrosis in the deep adrenal cortex were surrounded by a rim of congestion, hemorrhage and degenerate neutrophils and cortical epithelial cells (K. Nelson, pathology report).

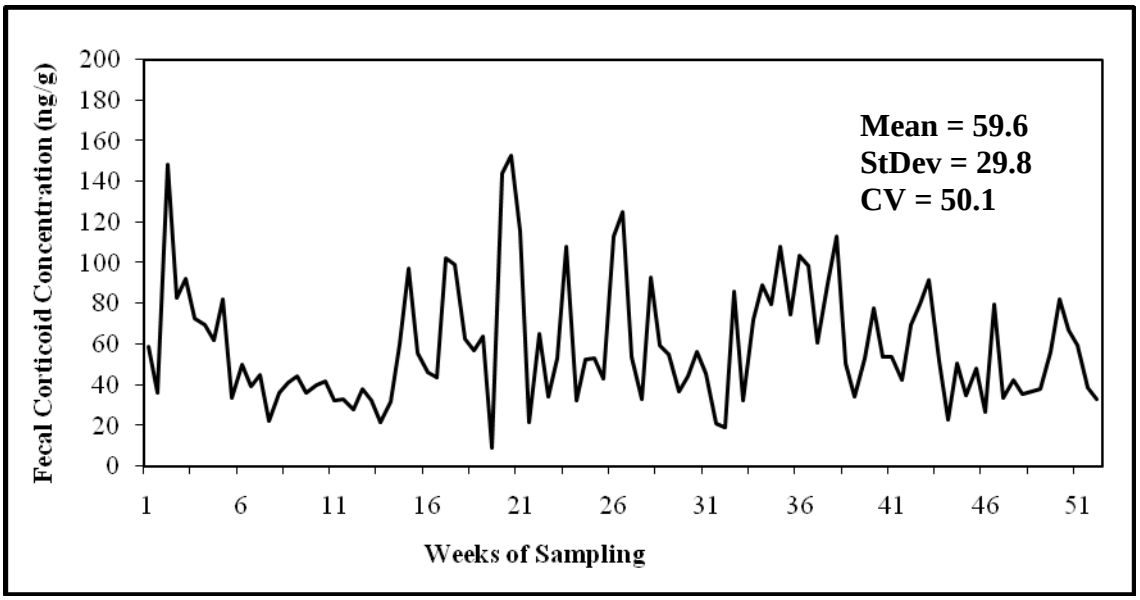
See Appendix V and VI for individual corticoid profiles and individual corticoid measures, respectively. As shown in Table 3.2, there were no differences ($P > 0.05$) in fecal corticoid concentration between rhinos with and without lesions when evaluated as overall, elevated, CV, minimum and maximum means. By contrast, baseline mean concentrations were lower ($P < 0.05$) in rhinos with lesions compared with those without. Representative individual fecal corticoid metabolite excretion profiles for two rhinos without and two rhinos with lesions are presented in Figures 3.1 and 3.2, respectively. Fecal corticoids exhibited high variability around the mean within individual rhinos regardless of disease status. The rhino that developed skin lesions after the initial collection period had ended exhibited lower overall and baseline corticoid concentrations ($P < 0.01$) during the subsequent 3 weeks of collection when lesions were present (Table 3.3). The time span between the end of the first and beginning of the second collection period was 17 months.

Table 3.2. Fecal corticoid metabolite concentrations (mean ± SEM ng/g and range) in black rhinos with lesions and without lesions.

	Overall	Baseline	Elevated	CV	Max	Min
Lesions absent	45.1 ± 4.0	40.0 ± 2.4[a]	108.6 ± 7.0	53.8 ± 2.3	143 ± 9.5	14.6 ± 1.2
(n = 38)*	(24.3 - 84.9)	(20.1 - 72.0)	(49.3 - 203.1)	(19.9 - 83.1)	(62.8 - 282.3)	(2.7 - 40.1)
Lesions present	34.6 ± 2.8	29.9 ± 3.3[b]	93.1 ± 15.2	57.1 ± 7.2	125.7 ± 24.6	13.6 ± 1.6
(n = 7)	(19.4 - 50.8)	(17.0 - 43.9)	(34.6 - 143.8)	(20.9 - 78.7)	(37.6 - 219.5)	(8.6 - 19.6)

[a,b]Values within columns with different superscripts differ ($P < 0.05$)
*Indicates number of individual rhinos

a.



b.

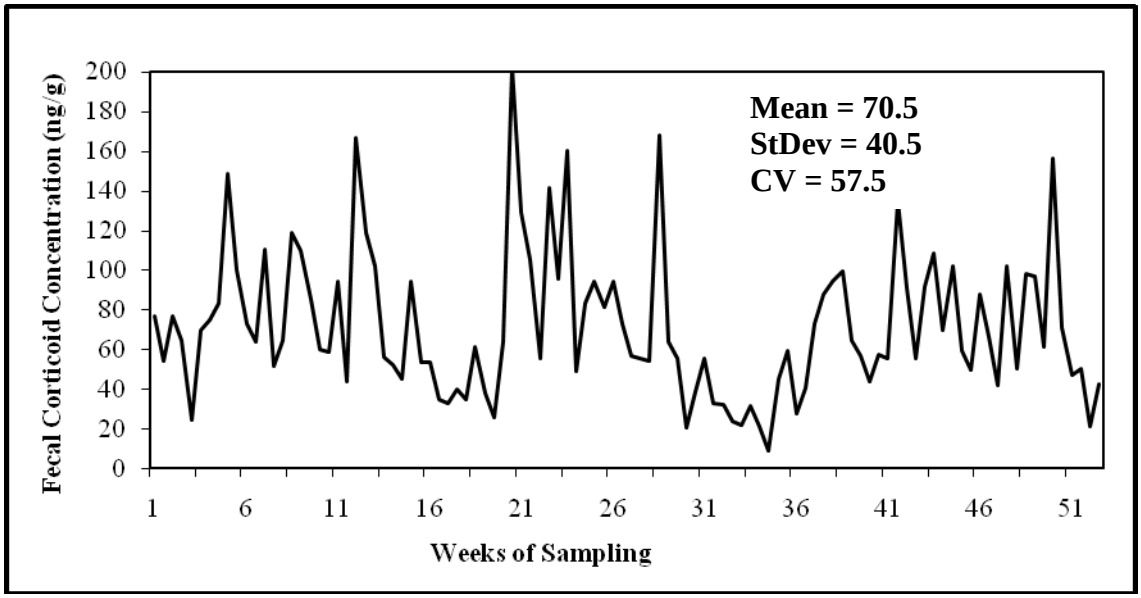
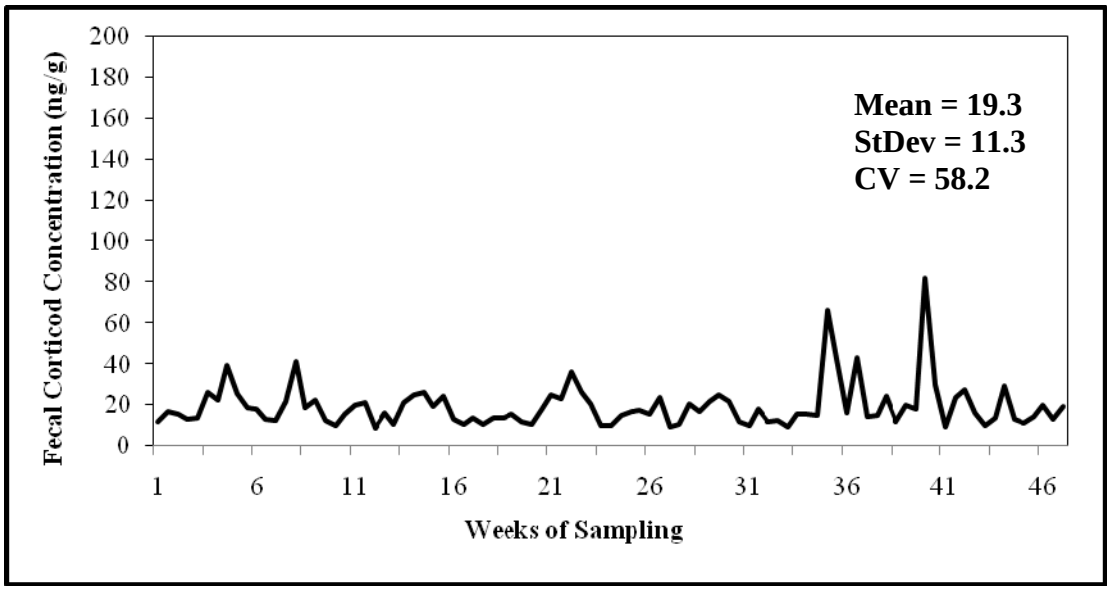


Figure 3.1. Representative fecal corticoid profiles for a male (a) and female (b) black rhino without skin lesions.

a.



b.

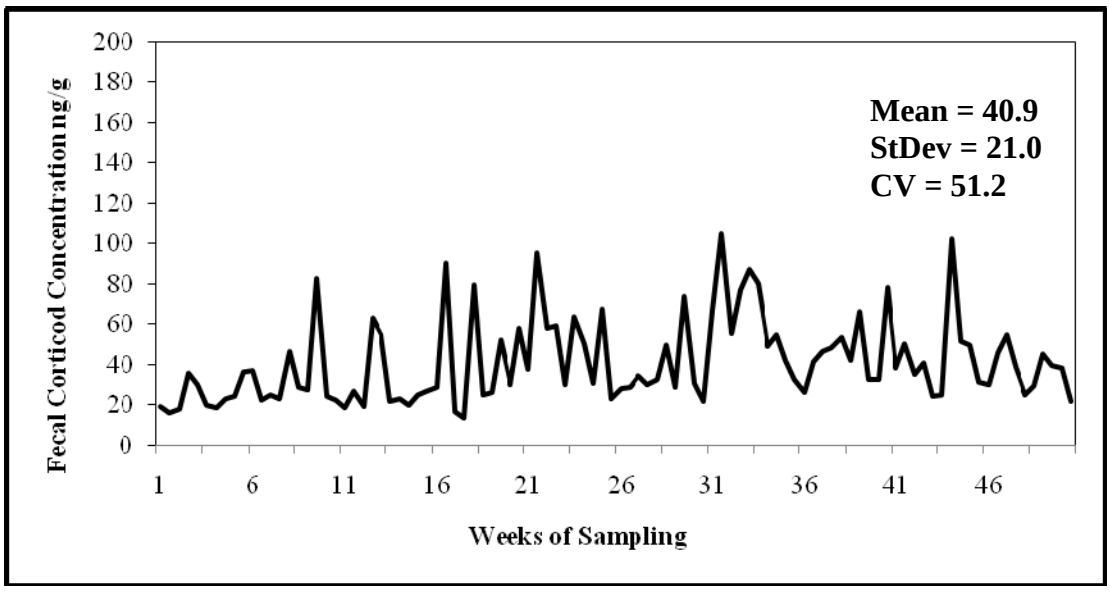


Figure 3.2. Fecal corticoid profiles in two male black rhinos exhibiting (a) skin and oral lesions and (b) skin lesions only.

Table 3.3. Fecal corticoid metabolite concentrations (mean ± SEM ng/g and range) in a single black rhino (age 10) before and after development of skin lesions.

	Overall	Baseline	Elevated	CV	Max	Min
No lesions	36.5 ± 1.0[a]	35. 3 ± 0.8[a]	54.9 ± 2.4	50.2	66.1	20.2
(n=75)*	(20.3 - 66.1)	(24.7 - 48.8)	(49.8 - 66.1)			
With lesions	30.1 ± 2.4[b]	28.7 ± 2.2[b]	45.2 ± 0.1	28.0	45.2	19.6
(n=12)	(19.6 - 45.2)	(19.6 - 39.9)	(45.2)			

[a,b]Values within columns with different superscripts differ ($P < 0.01$)
*n indicates number of fecal samples

DISCUSSION

This is the first study to identify a physiological factor associated with skin and oral lesions in black rhinos; animals with ulcerative lesions exhibited lower mean baseline fecal corticoid concentrations compared to those without. Although the reduction was moderate, a 25% decrease, this finding suggests that compromised adrenal function may play a role in the manifestation of this disease. In other species, chronic illnesses such as pneumonia, sepsis, pulmonary disease, heart disease can cause adrenal exhaustion in humans (Ligtenberg et al., 1999; Zaloga and Marik, 2001; Wu et al., 2008). However, before it can be determined whether reduced adrenal function is a cause or effect of ulcerative lesions in black rhinos, consecutive longitudinal data before and after disease onset must be obtained. However, in the one rhino that was evaluated twice, before and after development of lesions, fecal corticoid concentrations were reduced by nearly 20% during the time lesions were present as compared to when they were not.

Unfortunately, there was a 17-month gap in fecal collections, so the temporal relationship between lesion development and changes in adrenal activity could not be determined.

Corticoid concentration in rhinos without lesions were similar to those reported previously (Brown et al., 2001). Furthermore, other measures of adrenal activity (e.g. elevated, minimum, maximum and CV means) were similar across all rhinos, both with and without lesions. High intra- and inter individual corticoid variability is common in black rhinos. This finding led Carlstead and Brown (2005) to speculate that measures of variability, SD or CV, might be better indicators of chronic stress, at least under some circumstances, because they reflect changes in adrenal reactivity to stimuli, in addition to overall secretory rates. In their study, results did suggest that higher corticoid variability was related to several behavioral and social factors: e.g., higher levels of fighting and increased time pairs were together (black rhinos), and ovarian acyclicity and stereotypic pacing (white rhinos). Higher 'mortality rate at zoo' also was associated with increased corticoid CV. By contrast, elevated mean corticoid concentrations were associated with adverse responses to humans: e.g., greater exposure to zoo visitors (black rhino) and antagonistic behavior towards keepers (white rhino). Thus, depending on the category of stressor, alterations in adrenal function might involve changes in cortisol secretory reactivity or basal release.

Identifying possible links between lesions and captive variables was a major goal of this study; however, no significant relationships between rhino behavioral scores, facility parameters or socio-environmental factors and lesion status were found. Instead the one significant finding was lower overall mean fecal corticoid concentrations in black

rhinos with ulcerative lesions compared to those without; corticoid variability was the same. This appears to contrast with the findings of Carlstead and Brown (2005) where higher, rather than lower adrenal activity was associated with presumed stress-associated factors, including a higher mortality rate at zoo. This finding may reflect a fundamental difference in the biological cost associated with ulcerative lesions as compared to other physiological or psychological stressors in the captive environment, or it may represent one response among a continuum of responses leading up to the development of this disease.

Under normal circumstances, the stress response is a short term adaptive coping mechanism. However, chronic activation of the HPA axis and subsequent continuous release of glucocorticoids can result in a litany of disorders that decreases animal health and fitness. Persistent release of ACTH causes initial hypertrophy and hyperplasia of the adrenal glands, most notably the zona fasiculata. The cortex undergoes hypervascularization, increasing the number of mitochondria, smooth endoplasmic reticula and filopodia (Dallman, 1984). Therefore, adrenal hyperplasia can indicate a chronic stress situation (Estivariz et al., 1992), which has been documented through post-mortem examinations in several captive species (McColl, 1983, platypus; Rideout et al., 1985, armadillos; Suleman et al., 2000, African green monkeys; Terio et al., 2004, cheetahs). Munson et al. (1998) observed several cases of adrenal hyperplasia in captive black rhinos. The design of this study did not permit determining if adrenal hyperplasia preceded reduced excretion of corticoid metabolites in rhinos with lesions. However, the post-mortem report for the rhino with the most severe case of lesions that died did

indicate a morphological degeneration of the adrenal cortex; this male also had the lowest overall and baseline mean corticoid concentrations of all the study animals. Thus, adrenal exhaustion and/or hypoplasia appear to have been an end result of ulcerative lesions, at least in this individual. Past anecdotal evidence also has suggested that lesion onset is associated with "stressful" events (Munson et al., 1998). Of the eight afflicted rhinos in this study, four developed lesions after a "stressful" event: mating, translocation to a different zoo, Hurricane Katrina and the loss of a bonded conspecific.

In conclusion, no facility, management or behavioral variables were related to the development of ulcerative lesions in black rhinos; however, a significant finding was the observation of reduced fecal corticoid concentrations in those with lesions. The reason for this apparent decrease in adrenal steroidogenic activity is unknown, but it may reflect an inability of these rhinos to mount an appropriate stress response to the cause and/or effect of this condition. If affected black rhino are chronically stressed to the point of adrenal exhaustion, then it could account for some of the other clinical symptoms often associated with skin lesions: severe weight loss, muscle wasting, loss of appetite, IHVS, iron overload, anemias, hepatopathys and lethargy (Jubb et al., 1985; Munson et al., 1998; Dennis et al., 2007; Molenaar et al., 2008). In the broader sense, occurrence of lesions may only be one manifestation of an underlying health problem; thus, it might provide more insight to consider this problem in conjunction with the other disease syndromes that afflict captive black rhinos. Such a meta-analysis might reveal physiological and/or socio-environmental variables that are common among rhinos with varying health issues. Future studies should involve analyses of nutritional status, dietary

needs, serum chemistry profiles, other endocrine parameters (e.g., insulin and thyroid hormones) and immune function in the context of disease status. Additionally, necropsies of rhinos that die must include evaluations of adrenal gland pathology, especially the adrenal cortex, to determine if altered adrenal function, perhaps a consequence of captivity stress, is associated with any of these syndromes. Clearly, creating a healthy, self-sustaining *ex situ* population of black rhinos is going to require reducing the current high mortality and morbidity rates. To that end, a better understanding of what causes debilitating disease syndromes in black rhinos is needed so that effective mitigating strategies can be developed, whether they be medicinal treatments or alterations in management practices, or both.

CHAPTER 4

CONCLUSIONS

The population health of captive black rhinos is compromised in part by several poorly understood disease syndromes. The goal of several recent and/or current research studies (Paglia and Miller, 1993; Munson et al., 1998; Paglia and Dennis, 1999; Pessier et al., 2004; Dennis et al., unpubl.) has been to better understand the mechanisms and pathophysiology of these various diseases, and determine why they are only exhibited by the captive population (i.e., not other rhino species or free-range black rhinos). Although the etiologies of these syndromes are still unknown, data generated from each study will be used to build an information database to facilitate identifying future research needs, not only in regard to ulcerative lesions, but for the other disease syndromes currently affecting captive black rhino population health.

Prior to this dissertation, ulcerative lesions in black rhinos were largely assumed to be superficial necrolytic dermatitis (SND), a syndrome observed in the domestic dog, cat and few other species (Outerbridge et al., 2002; Kimmel et al., 2003). This was primarily due to the clinical and histological similarities of the lesions in each species. Results of the plasma amino acid study (Chapter 2) suggested ulcerative lesions in rhinos are not the same disease as SND. This is a pivotal discovery as it refutes a long held theory surrounding the etiology of lesions in black rhino. Ruling out hypoaminoacidemia

as a cause of lesions means that alternative hypotheses need to be developed and tested, preferebly in the context of other disease syndromes to determine if any have a common etiology.

As mentioned in Chapter 3**,** previous studies suggested that variables present in the captive environment may contribute to decreased black rhino population health (Carlstead et al., 1999a,b; Carlstead and Brown, 2005). However, no surveyed variables of captivity, or rhino behavioral scores, were significantly associated with rhinos having lesions. This does not necessarily suggest that black rhino health is unaffected by captive variables, but rather there may not be specific variables related to lesions. Or, individual rhinos may react differently to variables in the captive environment, which subsequently dictates the impact on individual rhino health. One recommendation is to use these survey results, in addition to fecal corticoid data, to re-evaluate relationships with respect to all of the primary black rhino disease syndromes (IHVS, iron overload, anemias, hepatopathys), rather than only lesions. A thorough analysis of the health records for all 45 individuals in this study would allow evaluating relationships among rhinos with each disease syndrome separately, as well as by overall healthy (no symptoms of disease) versus unhealthy (lesions, IHVS, iron overload) groups. Examining each disease syndrome separately could reveal associations between the distinct diseases and specific variables, or differing physiological measures. The different disease manifestations of black rhinos are frequently exhibited simultaneously (Munson et al., 1998; Dennis et al., 2007; Molenaar et al., 2008) and therefore may be different manifestations of a common metabolic derangement. Considering lesions in conjunction with other disease

syndromes afflicting captive black rhinos, i.e., conducting a meta-analysis, could provide more insight into what is having such a negative impact on animal health. If different syndromes share a common captivity-related etiology regardless of the manifestation, certain shared variables may be associated with decreased rhino health. Defining these variables would help rhino population managers identify potential "at-risk" individuals that may be experiencing sub-optimal species-specific conditions.

Previous research, both behavioral and physiological, has suggested that captive black rhino health may be compromised by chronic stress (Munson et al., 1998; Carlstead et al., 1999 a,b; Carlstead and Brown, 2005). Fecal glucocorticoid analyses demonstrated that rhinos with lesions had significantly lower baseline concentrations of corticoid metabolites than rhinos without lesions. It is difficult to interpret these results as it is uncertain whether compromised adrenal activity is a precursor or an effect of lesions. As mentioned in Chapter 3, cortical shunting occurs during adrenal exhaustion. Preganalone, a precursor molecule for hormone synthesis, is primarily converted to cortisol, resulting in decreased production of other steroids, such as DHEA and reproductive hormones. Therefore, samples used in this study could be used to compare reproductive hormones between rhinos with and without lesions, or between healthy and unhealthy rhinos to determine if these hormones follow similar secretory or excretory patterns as corticoids. If cortical shunting is taking place in unhealthy rhinos, then perhaps adrenal exhaustion is a true possibility. Corticoid data alone cannot define whether a population is experiencing stress; additional measures are necessary. Examining alternate indices of stress, such as adrenal hypo- and hyperplasia, abnormal

behaviors, complete blood counts (CBC), immunological values and biochemical profiles, could be used to support corticoid data and help elucidate if the captive population is truly experiencing chronic stress**.** Additionally, if banked serum samples were available from individuals during the study timeframe, a retrospective analysis might reveal whether altered blood chemistry is associated with reduced fecal corticoids and disease status. Analyzing more diverse physiological data from the same individuals, especially within the same time period, would elucidate more associations between disease mechanisms and possible associations with "stress."

Longitudinal corticoid monitoring of animals before, during and after disease onset would help ascertain the relationship between physiological measures and disease onset. The male rhino in this study that developed lesions in association with decreased corticoids had a time lapse in sample collection; therefore, it was not possible to determine whether a decrease in corticoid concentrations occurred first. Individuals could be selected according to certain "at-risk" characteristics: specific environmental variables (perhaps determined through survey meta-analyses), adrenal hypo- and hyperplasia, rhinos that will soon be confronted by potential stressors and rhinos that exhibit other disease syndromes. If resources are used to monitor adrenal activity (and possibly additional physiological parameters) in these key rhinos, temporal changes in corticoids in relation to lesion outbreak (or other manifestations of disease) could be determined.

Supplementing data generated in this study with biological data from wild black rhinos would be invaluable. There currently are no baseline fecal corticoid data for wild

populations, although some studies are looking at some individuals now. Assuming that data are obtained through extraction and assay methods similar to ours, corticoid concentrations from wild and captive black rhinos could be compared. If corticoids are elevated in the captive population, then there is more support for the hypothesis that diseases may ultimately manifest secondary to adrenal exhaustion caused by chronic stress. For example, if our "healthy" rhino sample exhibits higher corticoids than the wild population, this may suggest that the captive population as a whole is experiencing chronic stress. However, diseases may not manifest until adrenal exhaustion occurs, as exhibited by decreased corticoid concentrations. If the rhinos had chronically elevated corticoids which lead to adrenal exhaustion preceding disease onset, the data collected would only reflect corticoid activity after adrenal exhaustion has set in. These data would need to be substantiated by the other measures of stress (mentioned above) before further suggesting that black rhinos are experiencing chronic stress. Conversely, if rhino corticoids are not significantly elevated in captivity, then stress may not be a factor; it may be that chronic illness is causing reduced corticoid concentrations.

Lastly, a thorough analysis of necropsy reports of all black rhinos that die of disease might reveal more specific causes of lesions or other diseases. The one rhino that died during this study exhibited almost every disease syndrome known to affect rhinos (lesions, IHVS, anemia, iron overload, hepatopathys) as well as associated concurrent symptoms (blood pooling, lameness, weight loss and abnormal behavior). Many of these pathologies were only evident upon most-mortem examination. This male also had the lowest baseline corticoid concentrations of the entire study. No other rhinos died during

the study; thus, it was not possible to assess the complete health status of each individual and determine if other unseen problems were present. There can be no doubt that complete post-mortem analyses are critical to efforts to understand the causes of disease in the black rhino.

In conclusion, it is hoped that information generated by studies within this dissertation will advance research on the mechanisms of black rhino disease manifestations. Even if there is not a common etiology among the different disease syndromes, additional information garnered from other stress indices, analyses of health indicators in banked serum samples and comparative evaluations of wild populations might facilitate a better understanding of the pathophysiology of black rhino disease. These data would then provide support to assist and direct changes to future management practices with the goal of maximizing captive black rhino health and welfare and creating self-sustaining populations.

APPENDIX I

The following tables show the average amino acid concentrations (nmol/mL) for each black rhinoceros. The first table lists data for rhinos that do not have lesions. The second table lists data for rhinos that had lesions.

Amino acid concentrations of black rhinos without lesions (part 1 of 3)

	Amino Acid								
Black Rhino	**Alanine**	**Arginine**	**Asparagine**	**Aspartic acid**	**Citrulline**	**Glutamic acid**	**Glutamine**	**Glycine**	**1-Methyl histidine**
Azizi	311.6	142.0	95.0	15.8	23.0	146.6	270.6	454.7	6.8
Badru	321.3	116.4	38.7	20.1	31.1	156.6	232.9	554.2	6.5
Betsy	183.6	104.1	31.7	12.5	56.4	156.7	253.2	498.9	7.9
Bomani	350.9	127.9	29.3	20.3	26.0	198.8	232.0	550.9	5.6
Brewster	233.0	126.9	40.9	7.1	53.8	105.7	193.4	543.6	7.4
Chai	202.3	96.5	54.8	9.9	29.4	178.4	277.6	476.1	4.6
Chula	267.8	143.8	62.1	10.0	46.3	149.3	266.4	368.1	6.1
Ebony	208.6	93.6	6.8	34.9	46.5	159.8	249.0	405.0	4.4
Imara	229.7	128.3	19.9	52.1	27.8	139.6	215.5	405.8	7.9
Indy	281.9	121.3	88.9	12.5	41.7	157.8	330.2	519.6	8.7
Inge	297.3	180.2	30.3	47.8	36.2	124.8	317.1	464.7	4.2
Jimma	332.9	311.2	32.6	55.5	56.2	163.7	339.8	450.1	6.3

Jimma	209.8	141.4	25.6	32.8	48.9	165.6	162.4	402.6	7.3
Jody	277.2	127.3	67.4	10.8	33.3	187.0	260.9	438.5	3.8
Jomo	274.0	147.0	101.5	11.9	28.4	137.3	352.5	403.5	6.5
Julie	234.5	108.6	52.0	16.9	63.0	100.8	263.9	423.0	8.5
Juma	265.1	104.1	33.2	2.5	26.9	167.4	285.6	538.1	4.3
Kabisa	170.5	108.8	30.8	10.0	53.6	145.5	277.0	379.8	5.6
Kibibbi	337.1	267.8	28.0	67.1	42.7	140.4	326.1	555.2	5.9
Kipenzi	211.5	143.0	15.3	40.9	20.4	114.1	208.0	385.8	4.9
Kit	366.5	207.9	32.9	31.3	32.8	191.4	254.6	570.1	5.1
Luyisha	217.8	125.4	21.5	41.6	33.7	117.3	191.7	452.0	4.4
Marshall	288.1	134.4	82.3	30.6	34.0	89.4	260.3	352.0	7.9
Mojo	386.3	146.4	79.3	15.4	36.4	163.5	323.1	547.8	7.8
Mshindi	283.8	155.1	80.7	13.9	42.7	170.2	316.2	382.8	9.7
Nakili	221.6	143.5	62.5	14.5	31.6	154.4	313.3	382.7	6.9
Niki	298.3	117.0	70.4	11.9	39.3	152.4	323.6	572.1	7.1
Rosie	208.9	139.7	63.2	11.3	22.0	102.3	276.9	479.2	2.6

Rudy	148.0	125.6	11.0	17.7	36.3	108.4	184.7	333.6	6.5
Sababu	300.0	141.0	84.5	28.3	29.9	171.2	247.0	473.6	7.3
Toshi	230.2	140.4	16.5	23.4	27.8	155.4	288.6	554.8	5.6
Toto	259.3	137.0	24.9	24.1	55.9	205.0	297.3	608.9	6.6
Travis	325.9	182.8	26.5	27.2	26.0	190.2	227.4	505.9	6.5
Tucker	189.8	122.4	21.0	49.7	37.0	99.8	217.1	361.9	6.3
Werikhe	273.9	128.5	54.8	22.0	21.9	167.6	252.7	397.3	4.0

Amino acid concentrations of black rhinos without skin lesions (part 2 of 3)

	Amino Acid							
Black Rhino	**3-Methyl histidine**	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methionine**	**Ornithine**	**Phenylalanine**
Azizi	44.0	91.2	99.2	154.9	128.9	28.8	72.3	82.6
Badru	51.5	86.3	85.2	145.6	92.1	25.3	52.3	66.3
Betsy	27.4	69.9	100.6	174.0	99.1	21.6	104.1	81.3
Bomani	34.4	90.7	87.1	147.0	113.4	23.0	55.3	70.9
Brewster	16.1	85.2	138.7	224.5	119.3	24.8	128.2	88.5
Chai	33.2	97.6	100.8	168.6	118.4	21.3	103.2	81.6
Chula	28.5	93.9	116.7	180.9	121.4	29.3	136.8	83.4
Ebony	34.7	92.0	101.2	206.4	89.9	18.4	120.6	89.3
Imara	11.0	87.8	114.9	199.2	123.7	28.6	76.1	106.8
Indy	11.3	101.3	126.9	216.1	119.8	33.6	101.6	178.4
Inge	33.4	105.4	126.5	231.5	151.4	27.8	73.7	114.2
Jimma	16.6	120.2	172.3	312.6	255.3	38.5	150.1	163.0
Jimma	31.2	96.9	128.0	215.6	120.5	26.8	133.3	101.6

Jody	28.5	90.0	127.8	202.9	149.8	24.6	147.5	86.4
Jomo	91.5	93.6	110.0	174.9	137.1	28.5	91.1	82.8
Julie	11.5	70.6	144.4	238.7	105.5	23.4	112.8	95.2
Juma	36.2	88.9	83.7	151.9	105.9	24.4	65.9	62.3
Kabisa	23.7	80.3	92.0	157.4	83.7	21.0	88.6	89.6
Kibibbi	23.1	115.5	162.8	287.9	210.4	36.5	83.0	149.7
Kipenzi	18.3	100.7	109.5	186.9	146.4	29.2	69.5	87.2
Kit	45.8	119.4	121.3	199.3	172.7	29.1	72.8	95.0
Luyisha	21.7	91.1	113.9	198.2	118.1	21.4	107.5	83.4
Marshall	18.0	73.0	140.8	227.9	126.0	23.4	142.4	107.8
Mojo	13.8	91.9	132.0	234.7	156.3	38.6	132.0	131.5
Mshindi	30.9	100.6	154.8	251.9	184.0	33.1	205.8	133.9
Nakili	27.3	97.0	140.3	237.8	127.4	26.3	119.1	111.2
Niki	10.2	97.4	152.0	246.1	141.9	37.0	94.7	139.2
Rosie	73.3	71.7	108.5	194.6	102.8	25.0	466.7	124.1
Rudy	15.5	75.6	120.8	207.3	115.4	15.6	89.2	106.7

Sababu	50.8	111.0	154.2	241.0	134.9	31.7	123.4	113.9
Toshi	35.7	93.3	116.8	224.1	145.2	27.9	91.0	101.5
Toto	12.8	87.2	110.0	195.1	123.1	21.0	156.3	99.9
Travis	47.0	99.8	90.0	158.0	158.9	30.7	47.3	81.5
Tucker	15.1	63.3	134.3	220.9	118.4	22.2	112.4	104.3
Werikhe	13.3	117.1	125.5	225.3	134.2	26.9	105.1	118.3

Amino acid concentrations of black rhinos without skin lesions (part 3 of 3)

	Amino Acid							
Black Rhino	**Proline**	**Serine**	**Taurine**	**Threonine**	**Tryptophan**	**Tyrosine**	**Valine**	**All**
Azizi	246.0	142.9	27.8	164.4	89.7	53.7	339.4	3240.0
Badru	172.7	136.9	35.1	93.6	87.5	42.3	294.9	2951.4
Betsy	151.2	124.6	47.7	170.5	82.2	50.9	359.6	2978.3
Bomani	191.1	141.4	58.1	110.5	80.0	41.8	276.7	3067.3
Brewster	154.3	129.8	24.2	123.9	90.7	60.1	471.2	3206.4
Chai	146.6	127.7	80.0	152.5	72.2	63.1	346.4	3052.5
Chula	180.8	167.9	29.3	160.3	42.4	49.1	391.7	3156.8
Ebony	261.8	93.4	97.2	127.4	77.8	103.7	386.0	3114.6
Imara	179.9	133.9	37.1	172.0	110.0	65.3	387.5	3069.4
Indy	318.8	176.7	37.5	200.3	102.5	92.0	431.3	3817.5
Inge	219.2	198.4	34.2	196.4	85.0	56.7	423.9	3588.9
Jimma	299.3	195.0	41.3	224.7	78.9	91.6	552.3	4465.3
Jimma	180.1	118.8	28.0	160.3	84.8	76.6	415.8	3117.7

Jody	164.3	156.9	46.8	169.8	76.2	63.5	354.0	3308.7
Jomo	233.1	144.6	29.6	177.1	85.6	65.3	364.8	3378.8
Julie	205.3	95.2	40.7	172.3	62.0	50.7	519.5	3227.6
Juma	149.3	119.4	27.7	146.8	53.1	44.3	304.3	2906.8
Kabisa	105.8	74.7	31.8	119.5	74.3	53.6	336.3	2624.6
Kibibbi	281.5	229.3	42.0	217.0	110.3	70.3	516.3	4310.0
Kipenzi	117.0	128.4	31.4	145.9	110.8	69.3	354.2	2850.7
Kit	197.3	175.6	33.8	110.9	76.4	49.2	377.1	3576.2
Luyisha	115.5	137.9	25.3	160.7	97.9	48.0	419.5	2975.9
Marshall	336.6	149.1	40.1	210.6	46.0	79.3	461.8	3470.7
Mojo	313.3	191.0	38.9	216.9	111.2	76.6	444.1	4034.0
Mshindi	248.7	151.3	24.5	189.9	98.5	75.9	510.2	3854.9
Nakili	202.4	111.6	39.7	168.9	87.1	83.5	491.4	3414.8
Niki	267.3	168.0	35.3	194.7	70.2	81.9	488.2	3829.2
Rosie	173.5	125.3	23.6	150.3	60.1	66.1	415.0	3493.5
Rudy	116.6	125.9	26.8	151.1	94.4	76.6	432.4	2758.1

Sababu	244.4	185.9	60.4	173.2	67.9	67.7	491.0	3740.7
Toshi	117.8	142.4	23.0	162.3	92.7	65.8	421.8	3305.6
Toto	155.8	178.6	59.5	186.4	71.0	59.4	384.5	3527.5
Travis	166.7	171.4	36.5	125.3	68.8	50.2	310.4	3164.9
Tucker	151.4	148.5	20.0	176.1	95.8	72.1	508.7	3071.1
Werikhe	215.5	164.0	63.3	157.6	95.4	69.4	439.1	3394.4

Amino acid concentrations of black rhinos with lesions (1 of 3).

	Amino Acid								
Black Rhino	**Alanine**	**Arginine**	**Asparagine**	**Aspartic acid**	**Citrulline**	**Glutamic acid**	**Glutamine**	**Glycine**	**1-Methyl histidine**
Ahadi	301	155	118	17	72	72	436	427	5
George	164	101	55	6	22	146	259	364	9
Jello	300	123	36	31	41	160	359	523	7
Moja	291	159	110	41	16	152	304	395	8

Amino acid concentrations for black rhinos with skin lesions (2 of 3)

	Amino Acid							
Black Rhino	**3-Methyl histidine**	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methionine**	**Ornithine**	**Phenylalanine**
Ahadi	50	81	96	156	99	23	142	84
George	10	78	135	216	131	22	99	92
Jello	17	87	112	220	114	35	90	106
Moja	25	102	138	224	158	36	111	116

Amino acid concentrations of black rhinos with skin lesions (3 of 3).

	Amino Acid							
Black Rhino	**Proline**	**Serine**	**Taurine**	**Threonine**	**Tryptophan**	**Tyrosine**	**Valine**	**All**
Ahadi	217	167	18	152	67	64	329	3355
George	176	125	25	128	77	59	459	2963
Jello	234	206	34	161	95	59	385	3536
Moja	255	202	28	209	87	92	410	3671

APPENDIX II

The Facility Survey completed by each institution participating in this study.

Zoo_____

Rhino names and SSP's# _____ Date_____

Please select the position that best describes your relationship with the rhinos at your facility:

Curator Manager Veterinarian Keeper Other

How many years have you worked with black rhinos at this facility? _____

Institution information

Please describe the location of your facility

urban suburban rural other

Please describe the scale of *ambient* noise.

very low low medium high very high

Please indicate the common cause of noise at your facility:

highway traffic air-traffic trains subway other

Please indicate the common odors surrounding the enclosure.

food exhaust chemicals other

Do you use chlorine or other cleaners? ___yes ___no

If yes, what is the frequency of use _____/weekly

What is the concentration? _____

Please list species that are located in adjacent enclosures:

Facility Description

Exhibit Statistics

What is the age of the exhibit (yrs) _____

Please describe any recent structural changes.

How many enclosures/stalls are in exhibit? _____ Please fill out a description for each stall/enclosure the rhino uses

Enclosure statistics – Indoor

Approximate dimensions of stall

(ft)_____

What is the amount of public-exposed perimeter (ft) _____

Is the public view above, below or equal level with the rhino? _____

What types of barriers exist between rhino and public?

concrete fence dry moat water moat other

What type of barrier between exists between adjacent rhinos?

concrete fence other

What kind of visual barriers allow the rhino to hide from keeper, conspecifics or public.

None deadfall boulders foliage dirt mounds walls other

What kind of substrate is in the exhibit?

grass concrete dirt mulch limestone sand rock straw other

Is the indoor stall heated in winter? ___yes ___no

If so, at what temperature (◦F)? _____

Do you use: forced air radiant floor heat other

Is the air circulated in summer? ___yes ___no

Enclosure statistics – Outdoor

Approximate dimensions of stall (ft)_____

Approximate square -footage of enclosure _____

Please estimate the percent of shaded area_____

What is the amount of public-exposed perimeter (ft)_____

Is the public view – above, below or equal level to

rhino_____

What types of barriers exist between rhino and public?

concrete fence dry moat water moat glass wall other

What type of barrier exists between adjacent rhinos?

concrete fence glass wall other

What visual barriers allow the rhino to hide from keeper, conspecifics or public.

None deadfall boulders foliage dirt mounds walls other

What kinds of substrate are in the enclosure?

grass concrete dirt mulch limestone sand rock other

APPENDIX III

The socio-environmental/behavior survey completed for each individual black rhino in each institution participating in this study.

Zoo_____ Rhino's name _____

Rhino SSP# _____ Date_____

Please select the position that best describes your relationship with the rhinos at your facility:

Curator Manager Veterinarian Keeper Other

How many years have you worked with black rhinos at this facility? _____

Sociological - Environmental Survey

Please fill out one per rhino at the beginning of study

Represents the normal daily environment and temperament for each rhino

Life History Information

What best describes this rhino:

Wild caught Captive born Conceived in wild but born in captivity Hand reared

Institution History

How many years has this rhino been at this institution? _____

This rhino has been housed alone in this institution for _____ years

This rhino has been housed with other rhinos in this institution for _____ years

Keeper Information and practices

On average, how many keepers are there at one time? _____

What is the total number of keepers for this animal? _____

When in close proximity to the animal, is the interaction *primarily*:

_____protected _____unprotected

On average, how many hours of interaction with the rhino do you have in one day?

_____

Is the rhino conditioned for husbandry/medical treatments? ___yes ___no

Is the rhino trained? ___yes ___no. How often?

Several X daily daily several times weekly weekly other

Please describe the daily interactions with this rhino.

Please describe, in a *typical* day, how many:

Hours the rhino is inside? _____. Outside? _____

Hours are spent with conspecifics? male _____hrs. female _____hrs.

Do these hours change according to weather/season? ___yes ___no

If yes, please explain:

How often does the animal rotate between stalls? _____

Please describe the primary types of enrichment?

sprinklers logs suspended on chains yards connected by gates rubbing posts

pools mud wallows movable items (logs etc) other (please explain)

In general, how many hours is the rhino on display per day? _____

Is the animal sprayed with water or bathed? _____ How often? _____

Breeding Pairs (if applicable)

How many hours/day is the rhino with its potential mate during breeding attempts?

_____. How many hours/day when not breeding? _____

Diet

Please describe the usual daily diet, including quantities?

This rhino is fed _____ times each day

Is the food shared between rhinos? _____

Where is food located? _____

Is water provided ad lib? _____ yes _____ no

Is browse provided? ____yes _____no

If yes, what type(s)?

Are daily vitamins given? ___ yes ___no

If yes, please describe kinds and dosages:

Are any other supplements provided? ___ yes ___no If yes, please describe kinds and dosages:

General Rhino Behavior

How many male rhinos share this enclosure with the described rhino? _____

How many female rhinos share this enclosure with the described rhino?_____

Interactions with conspecifics if applicable

What is the relationship of this rhino to others in enclosure:

____breeding pair ____parent/offspring ____siblings ____no relation NA

Does this rhino act more aggressively or more submissively towards other male rhinos?

always aggressive towards all males usually aggressive towards some or all

equally aggressive and submissive usually submissive to all males

always submissive to all males neither aggressive nor submissive

not applicable

Does this rhino act more aggressively or more submissively towards other female rhinos?

always aggressive towards all females usually aggressive towards some or all

equally aggressive and submissive usually submissive to all females

always submissive to all females neither aggressive nor submissive

not applicable

In relation to others, how solicitious is this rhino with male rhinos?

solicits the most interactions | solicits more interactions than receives

solicits and receives interactions equally | usually receives more interactions

only receives interactions | neither solicits nor receives interactions

depends on the other rhino | not applicable

In relation to others, how solicitious is this rhino with female rhinos?

solicits the most interactions | solicits more interactions than receives

solicits and receives interactions equally | usually receives more interactions

only receives interactions | neither solicits nor receives interactions

depends on the other rhino | not applicable

<u>Interactions with People</u>

How does animal behave toward *you*?

aggressive friendly timid/shy aloof cautious/wary likes touching

approaches spontaneously approaches you when called other

Please describe this rhino's interactions with other keepers.

aggressive friendly timid/shy aloof cautious/wary likes touching

approaches spontaneously approaches when called other

In general, how does this rhino respond to novel objects?

extremely inquisitive inquisitive and curious typical/interested response

interested, but timid extremely timid/fearful shows no interest

not applicable

Please rate this rhino's obedience to commands:

___Very low ___Low ___Medium ___High ___Very high

Please describe this rhino's behavior towards maintenance people, tour groups, etc…

aggressive friendly timid/shy aloof cautious/wary approaches

spontaneously other

Has the personality/temperament of this rhino changed within the past 5 years? If so, please explain.

Please provide any additional comments which may give extra insight into the temperament and social activities of this rhino.

APPENDIX IV

Logistical regression analysis for socio-environmental, facility and behavior indices in zoos.

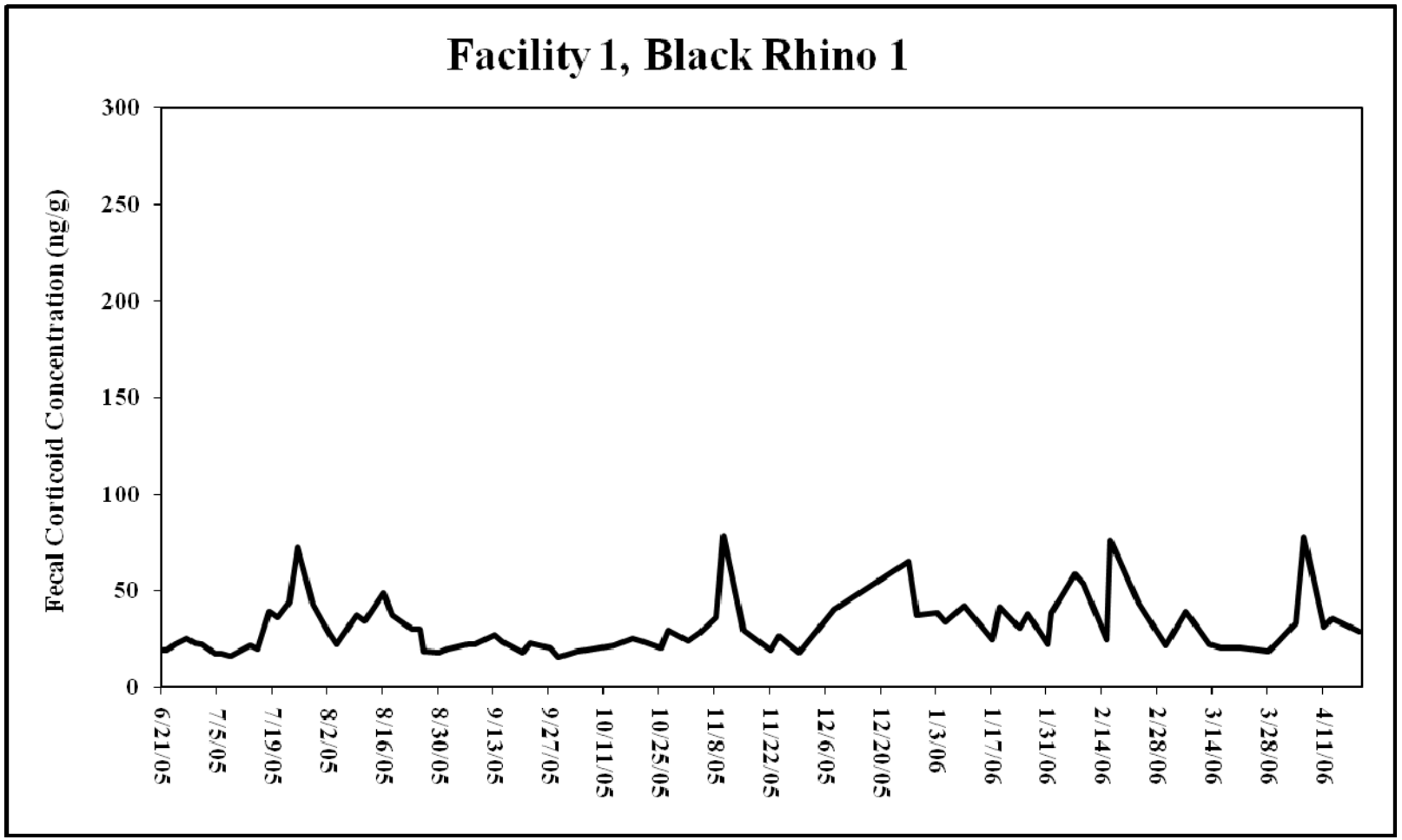
Variable	Skin Lesions	
Socio-Environmental Variables	**Slope ± SEM**	**R^2**
Subspecies	Dropped	
Latitude	-0.03 ± 0.07	0.01
Average temperature	-0.01 ± 0.04	0.00
Average precipitation	-0.02 ± 0.03	0.01
Sex	-1.70 ± 1.10	0.01
Age	-0.01 ± 0.05	0.00
Number of facilities rhino has lived	0.35 ± 0.35	0.02
Rhino rearing	-0.01 ± 0.45	0.00
Number of years rhino at current facility	-0.01 ± 0.06	0.00
Number of years rhino has lived alone	0.08 ± 0.07	0.03
Number of years rhino has lived with conspecifics	0.04 ± 0.05	0.01
Number of black rhino at facility	-0.25 ± 0.37	0.01
Number keepers working with rhino at a time	-0.43 ± 0.52	0.03
Total number of people who ever work with rhino	-0.62 ± 0.37	0.18
Number of hours a day rhino interacts with keepers	-0.35 ± 0.40	0.02
Number of different kinds of keeper-rhino interaction	0.68 ± 0.62	0.04
Training frequency	-0.10 ± 0.61	0.00
Rhino changes routine seasonally	-0.25 ± 1.17	0.00
Number of hours a day rhino is inside	0.10 ± 0.06	0.08
Number of hours a day rhino is outside	0.03 ± 0.07	0.01
Number of times a day rhino rotates stalls	-0.22 ± 0.54	0.01
Number of hours a day rhino is on public display	-0.06 ± 0.10	0.01
Rhino is sprayed with water	0.25 ± 1.17	0.00
Hours rhino is with conspecific for mating purposes	-0.05 ± 0.10	0.02
Number of times a day rhino is fed	-0.40 ± 0.88	0.01
Rhino food is shared	0.00 ± 0.50	0.00
Food is shared with a male	-0.56 ± 0.10	0.03
Food is shared with a female	-1.33 ± 1.05	0.08
Rhino is housed with breeding partner	-1.50 ± 1.15	0.06

Rhino is housed with parent	Dropped	
Rhino is housed with a sibling	Dropped	
Rhino is housed with a non-relative	-0.79 ± 1.15	0.02
Rhino is obedient to commands	0.19 ± 0.73	0.00
Rhino is inquisitive of novel items	0.04 ± 0.35	0.00
Facility Variables		
Noise level at facility	0.16 ± 0.46	0.00
Bleach is used at facility	Dropped	
Frequency of bleach use	-0.09 ± 0.37	0.00
Exhibit age	-0.01 ± 0.02	0.00
Number of stalls in exhibit	0.23 ± 0.32	0.01
Inside exhibit square footage	-0.00 ± 0.00	0.00
Inside public exposed perimeter	0.02 ± 0.01	0.09
Type of inside public view of rhino	-0.41 ± 0.35	0.03
Inside concrete barrier between rhino and public	0.57 ± 0.84	0.01
Inside fence barrier between rhino and public	1.53 ± 0.90	0.08
Inside dry moat barrier between rhino and public	-0.53 ± 0.90	0.01
Inside water moat barrier between rhino and public	2.67 ± 1.31	0.11
Inside glass wall barrier between rhino and public	0.32 ± 1.20	0.00
Inside concrete barrier between rhinos	0.37 ± 0.90	0.00
Inside fence barrier between rhinos	0.43 ± 0.83	0.01
Inside dry moat barrier between rhinos	0.85 ± 0.95	0.02
Inside water moat barrier between rhinos	Dropped	
Inside glass wall barrier between rhinos	0.18 ± 1.18	0.00
Inside no visual barriers	0.87 ± 0.89	0.03
Inside deadfall visual barrier	Dropped	
Inside boulder visual barrier	0.09 ± 0.91	0.00
Inside foliage visual barrier	Dropped	
Inside dirt mound visual barrier	Dropped	
Inside wall visual barrier	-2.34 ± 1.13	0.16
Inside concrete substrate	Dropped	
Inside dirt substrate	Dropped	
Inside sand substrate	Dropped	
Inside rock substrate	Dropped	
Inside straw substrate	0.32 ± 1.20	0.00
Inside rubber substrate	Dropped	
Kind of heat	-0.16 ± 0.26	0.01
Temperature heat is set to	-0.26 ± 0.34	0.02

Air is circulated	-0.35 ± 0.92	0.00
Outside square footage	-0.00 ± 0.00	0.05
Percant of shade outside	0.01 ± 0.02	0.01
Type of outside public view of rhino	0.16 ± 0.30	0.01
Distance rhino can see out of enclosure	-0.00 ± 0.00	0.05
Outside concrete barrier between rhino and public	-0.15 ± 0.90	0.00
Outside fence barrier between rhino and public	1.35 ± 1.13	0.05
Outside dry moat barrier between rhino and public	0.37 ± 0.90	0.00
Outside wet moat barrier between rhino and public	-1.02 ± 1.13	0.02
Outside wall barrier between rhino and public	-1.45 ± 1.13	0.05
Outside concrete barrier between rhinos	-0.57 ± 0.89	0.01
Outside fence barrier between rhinos	-0.14 ± 0.83	0.00
Outside dry moat barrier between rhinos	0.35 ± 0.92	0.00
Outside wet moat barrier between rhinos	Dropped	
Outside wall barrier between rhinos	1.06 ± 0.96	0.03
Outside no barrier between rhinos	0.50 ± 0.92	0.01
Outside no visual barriers	Dropped	
Outside deadfall visual barrier	-0.37 ± 0.90	0.00
Outside boulder visual barrier	0.57 ± 0.89	0.00
Outside foliage visual barrier	1.29 ± 0.84	0.06
Outside dirt mound visual barrier	-0.88 ± 1.14	0.01
Outside wall visual barrier	Dropped	
Outside grass substrate	0.12 ± 0.82	0.00
Outside concrete substrate	Dropped	
Outside dirt substrate	0.025 ± 0.01	0.01
Outside mulch substrate	Dropped	
Outside limestone substrate	Dropped	
Outside sand substrate	Dropped	
Outside rock substrate	0.21 ± 1.12	0.02
Kind of outside topography	0.26 ± 0.40	0.01
Number of different kinds of enrichment	-0.98 ± 0.59	0.01
Behavior Indices		
Aggressive Behavior	0.88 ± 1.03	0.02
Fearful behavior	-1.87 ± 1.84	0.04
Friendly behavior	Dropped	
Cautious behavior	0.14 ± 1.11	0.00
Timid Behavior	-1.60 ± 1.48	0.04

APPENDIX V

Fecal corticoid profiles for individual black rhinos. Profile indicated by * are rhinos that had ulcerative lesions during the study.


Facility 1, Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
6/21/05
7/5/05
7/19/05
8/2/05
8/16/05
8/30/05
9/13/05
9/27/05
10/11/05
10/25/05
11/8/05
11/22/05
12/6/05
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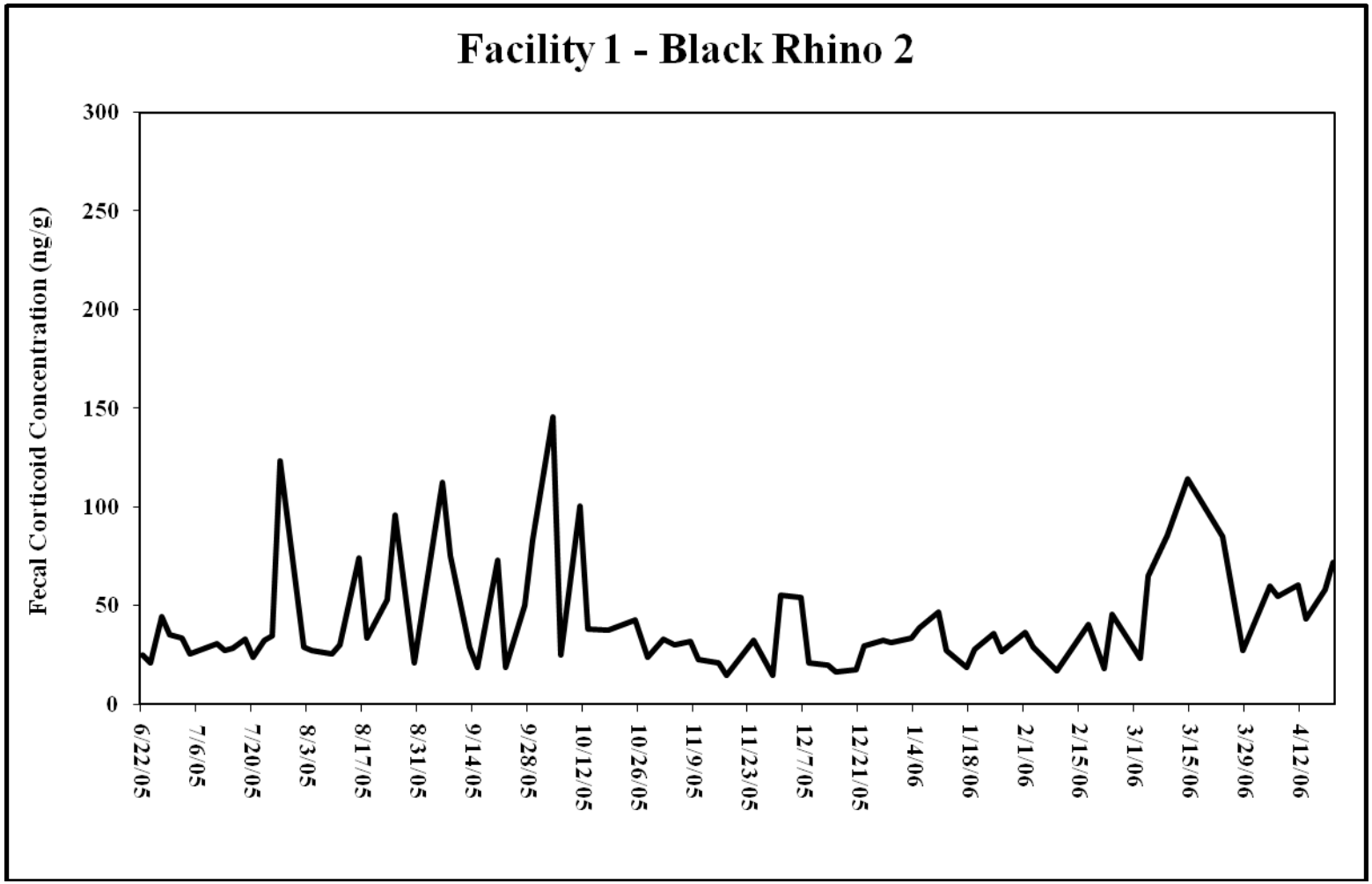
2/28/06
3/14/06
3/28/06
4/11/06


Facility 1 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
6/22/05
7/6/05
7/20/05
8/3/05
8/17/05
8/31/05
9/14/05
9/28/05
10/12/05
10/26/05
11/9/05
11/23/05
12/7/05
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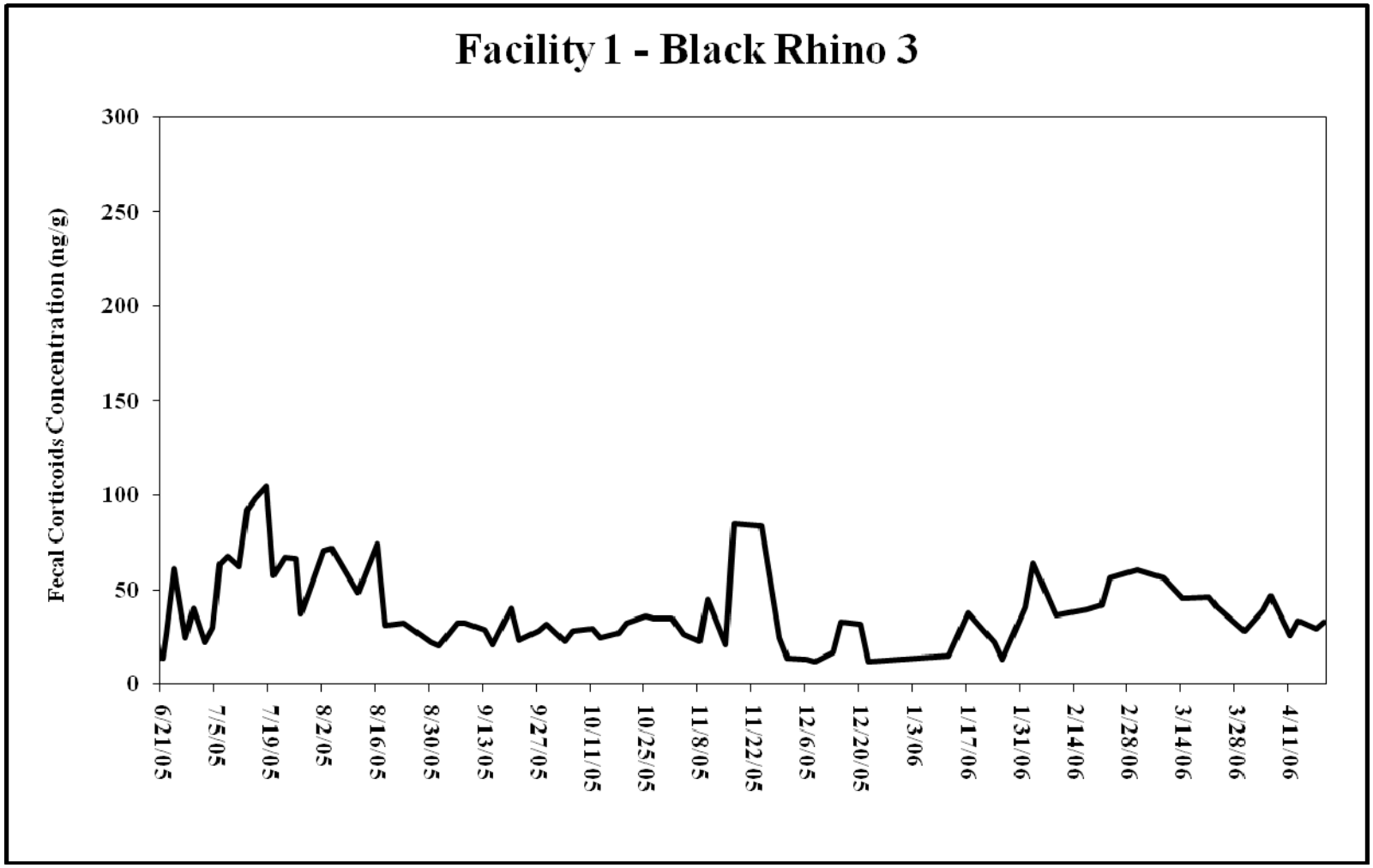
3/1/06
3/15/06
3/29/06
4/12/06


Facility 1 - Black Rhino 3
Fecal Corticoids Concentration (ng/g)
300
250
200
150
100
50
0
6/21/05
7/5/05
7/19/05
8/2/05
8/16/05
8/30/05
9/13/05
9/27/05
10/11/05
10/25/05
11/8/05
11/22/05
12/6/05
12/20/05
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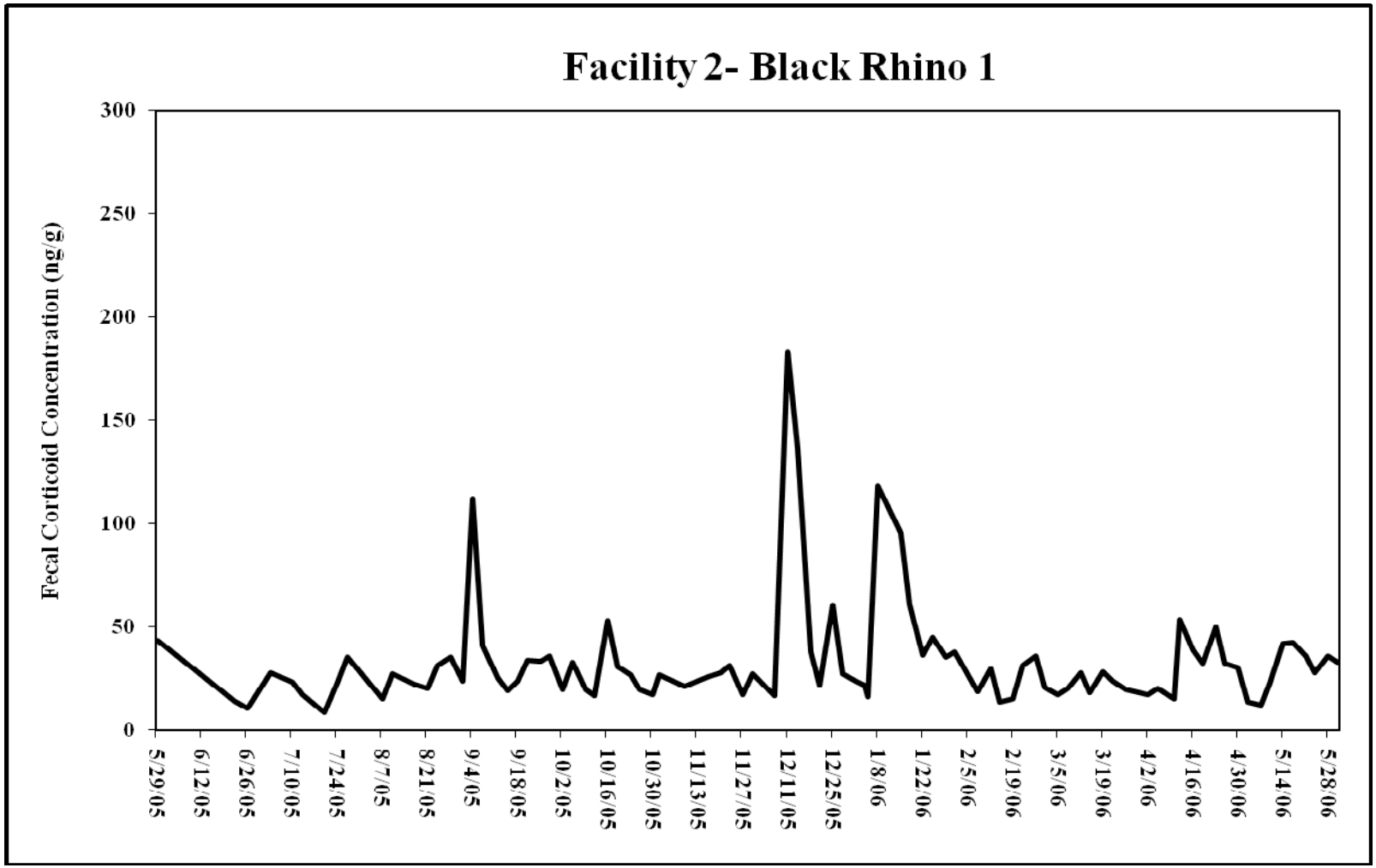
2/28/06
3/14/06
3/28/06
4/11/06


Facility 2- Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/29/05
6/12/05
6/26/05
7/10/05
7/24/05
8/7/05
8/21/05
9/4/05
9/18/05
10/2/05
10/16/05
10/30/05
11/13/05
11/27/05
12/11/05
12/25/05
1/8/06
1/22/06
2/5/06
2/19/06
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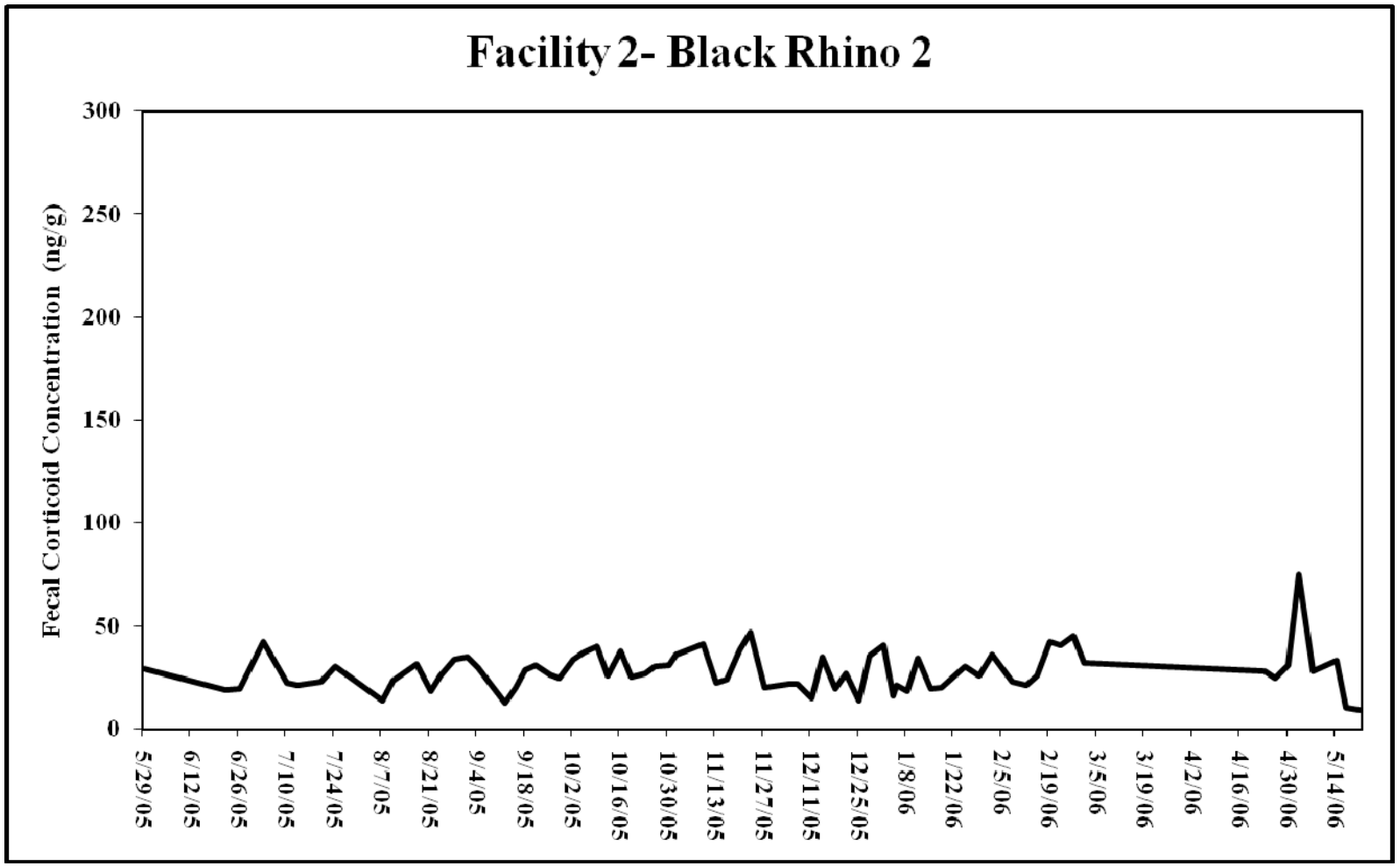
4/2/06
4/16/06
4/30/06
5/14/06
5/28/06


Facility 2- Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/29/05
6/12/05
6/26/05
7/10/05
7/24/05
8/7/05
8/21/05
9/4/05
9/18/05
10/2/05
10/16/05
10/30/05
11/13/05
11/27/05
12/11/05
12/25/05
1/8/06
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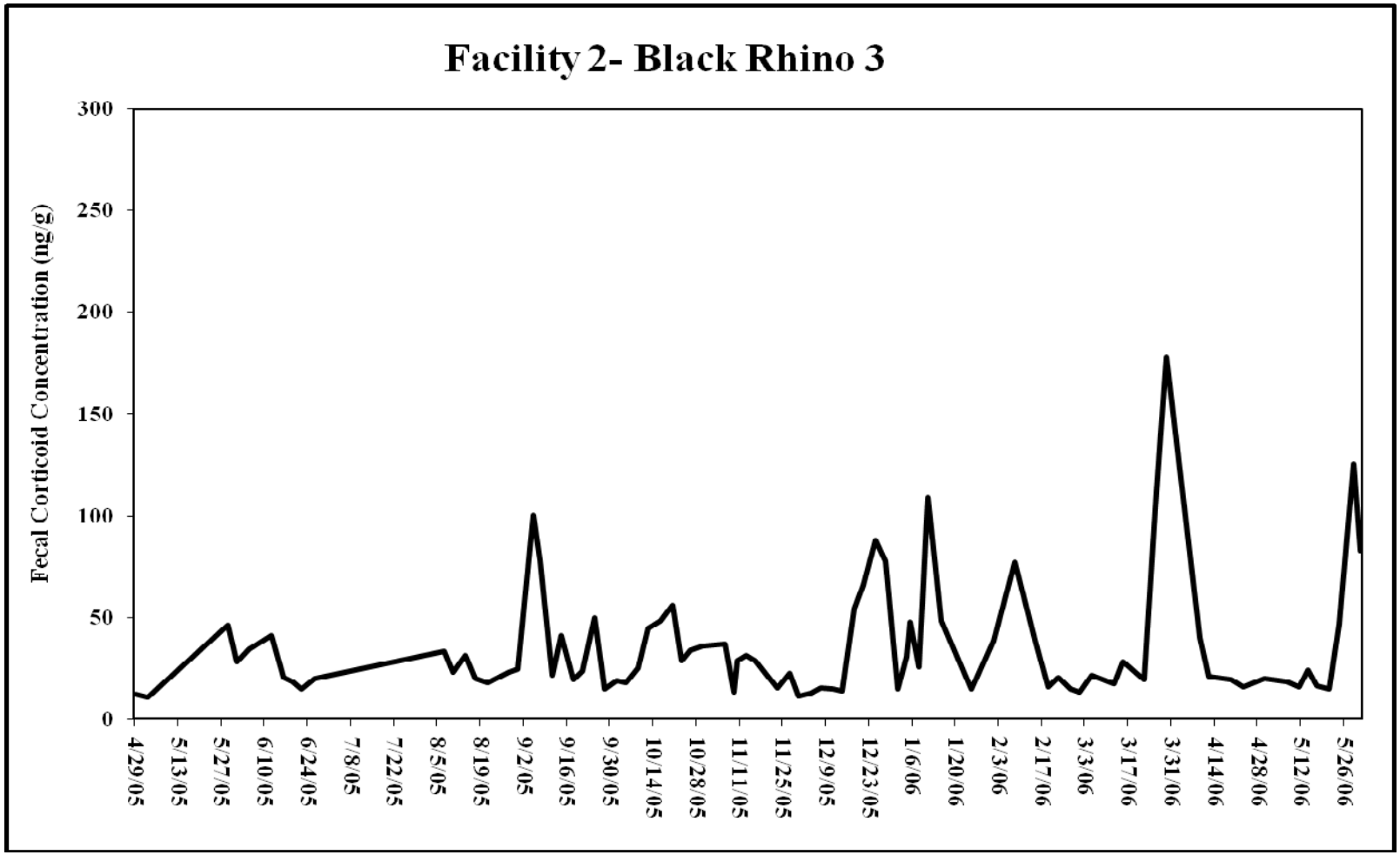
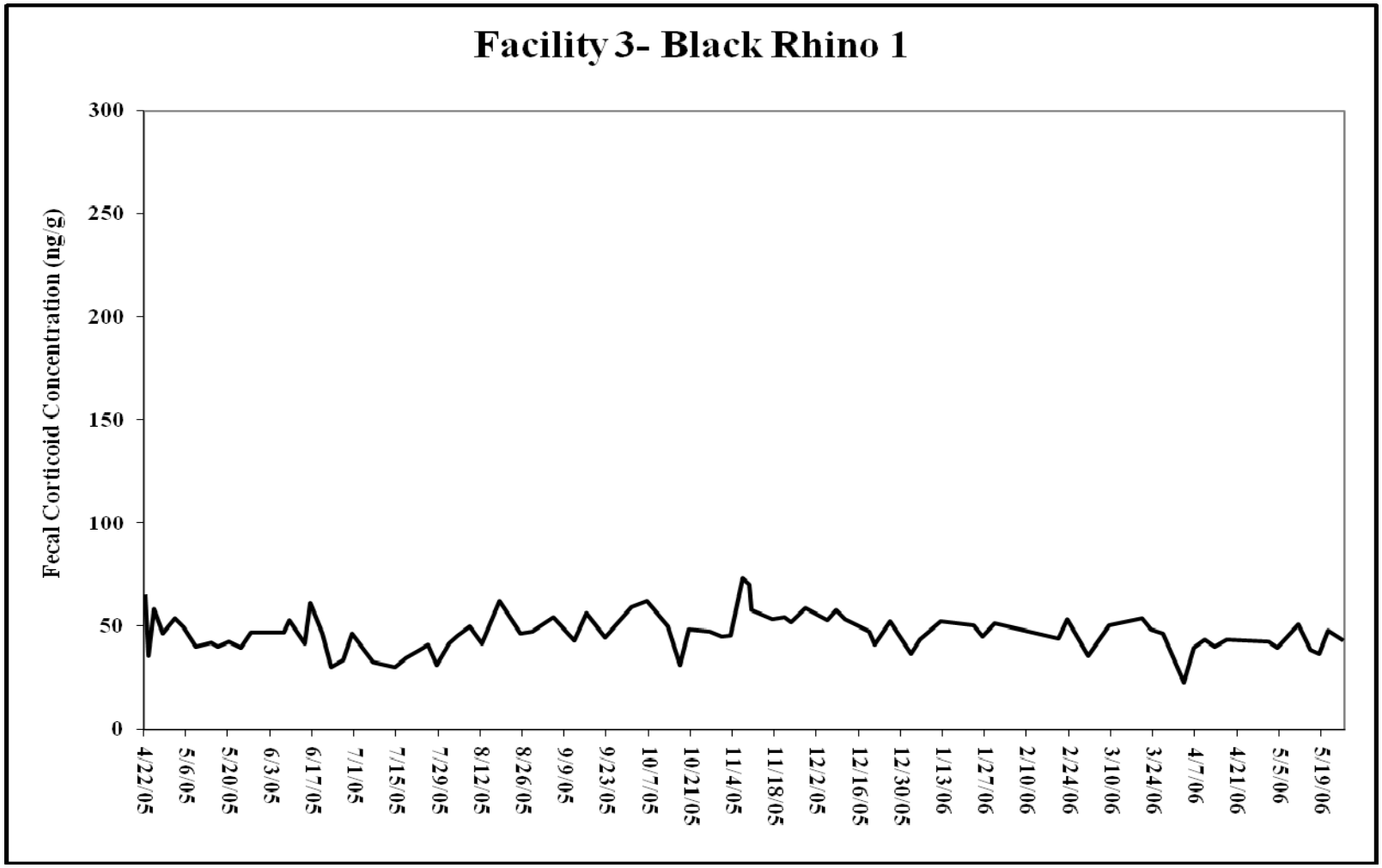
4/2/06
4/16/06
4/30/06
5/14/06


Facility 2- Black Rhino 3
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
4/29/05
5/13/05
5/27/05
6/10/05
6/24/05
7/8/05
7/22/05
8/5/05
8/19/05
9/2/05
9/16/05
9/30/05
10/14/05
10/28/05
11/11/05
11/25/05
12/9/05
12/23/05
1/6/06
1/20/06
2/3/06
2/17/06
3/3/06
3/17/06
3/31/06
4/14/06
4/28/06
5/12/06
5/26/06


Facility 3- Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
4/22/05
5/6/05
5/20/05
6/3/05
6/17/05
7/1/05
7/15/05
7/29/05
8/12/05
8/26/05
9/9/05
9/23/05
10/7/05
10/21/05
11/4/05
11/18/05
12/2/05
12/16/05
12/30/05
1/13/06
1/27/06
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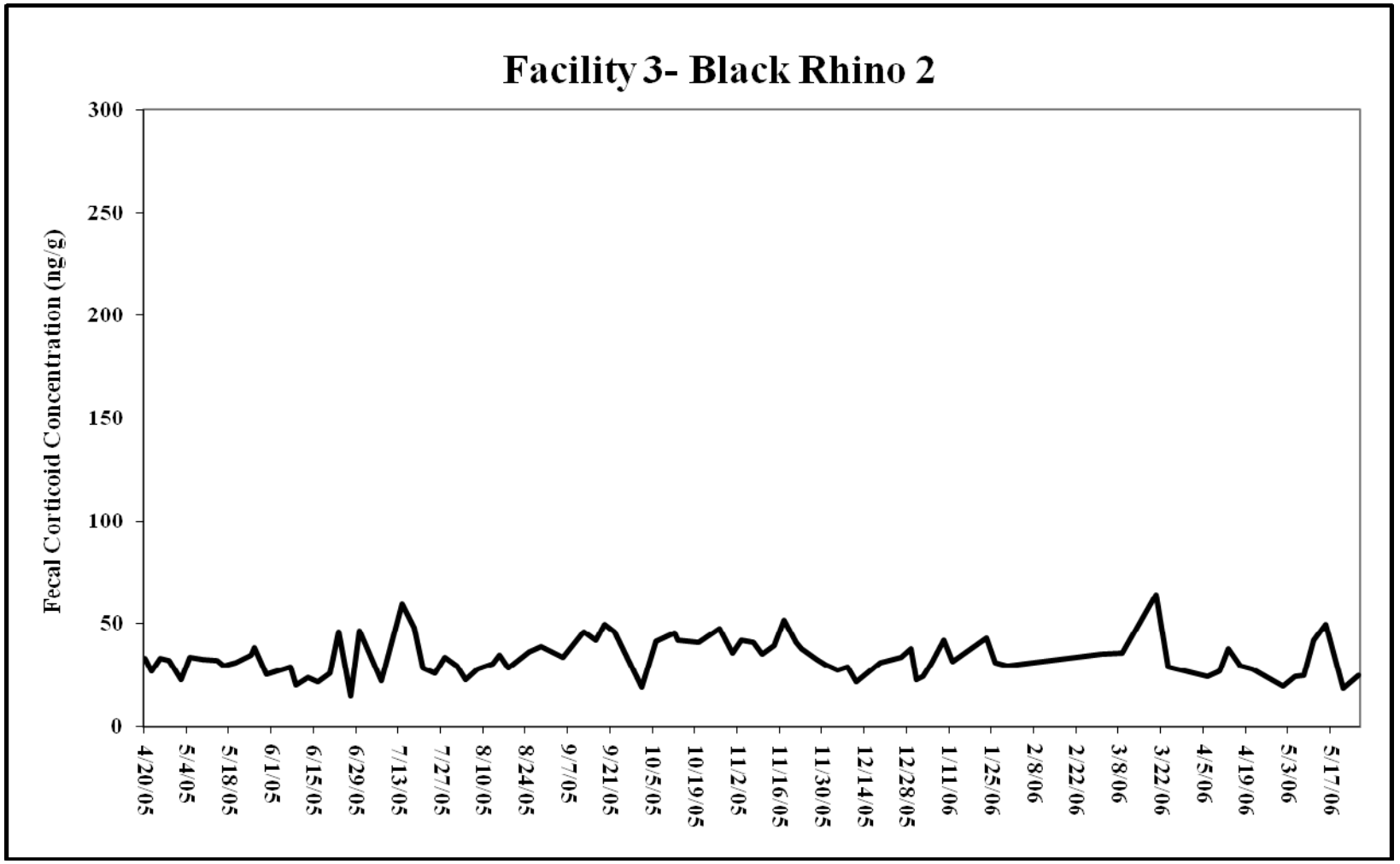
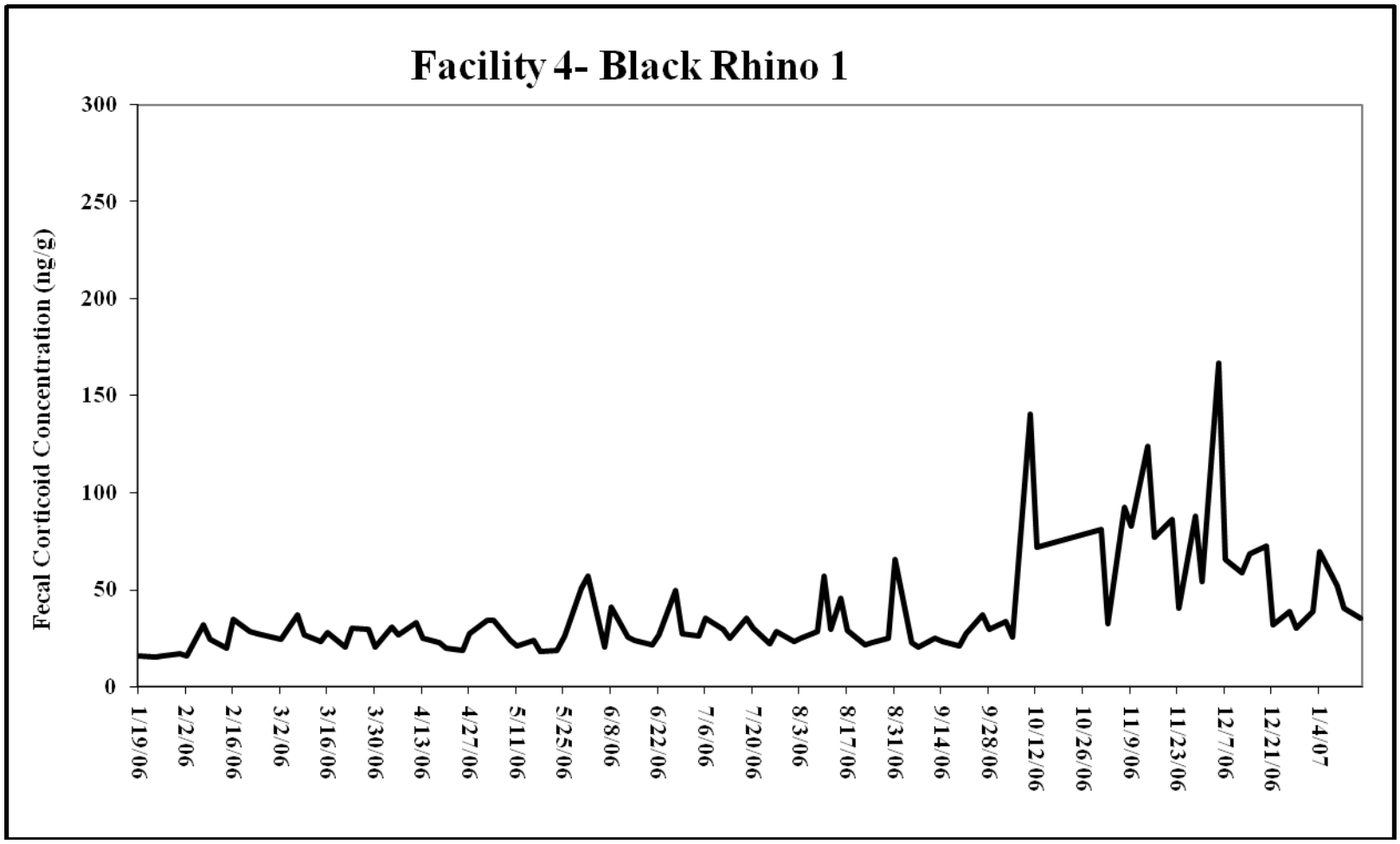
3/10/06
3/24/06
4/7/06
4/21/06
5/5/06
5/19/06


Facility 3- Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
4/20/05
5/4/05
5/18/05
6/1/05
6/15/05
6/29/05
7/13/05
7/27/05
8/10/05
8/24/05
9/7/05
9/21/05
10/5/05
10/19/05
11/2/05
11/16/05
11/30/05
12/14/05
12/28/05
1/11/06
1/25/06
2/8/06
2/22/06
3/8/06
3/22/06
4/5/06
4/19/06
5/3/06
5/17/06


Facility 4- Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
1/19/06
2/2/06
2/16/06
3/2/06
3/16/06
3/30/06
4/13/06
4/27/06
5/11/06
5/25/06
6/8/06
6/22/06
7/6/06
7/20/06
8/3/06
8/17/06
8/31/06
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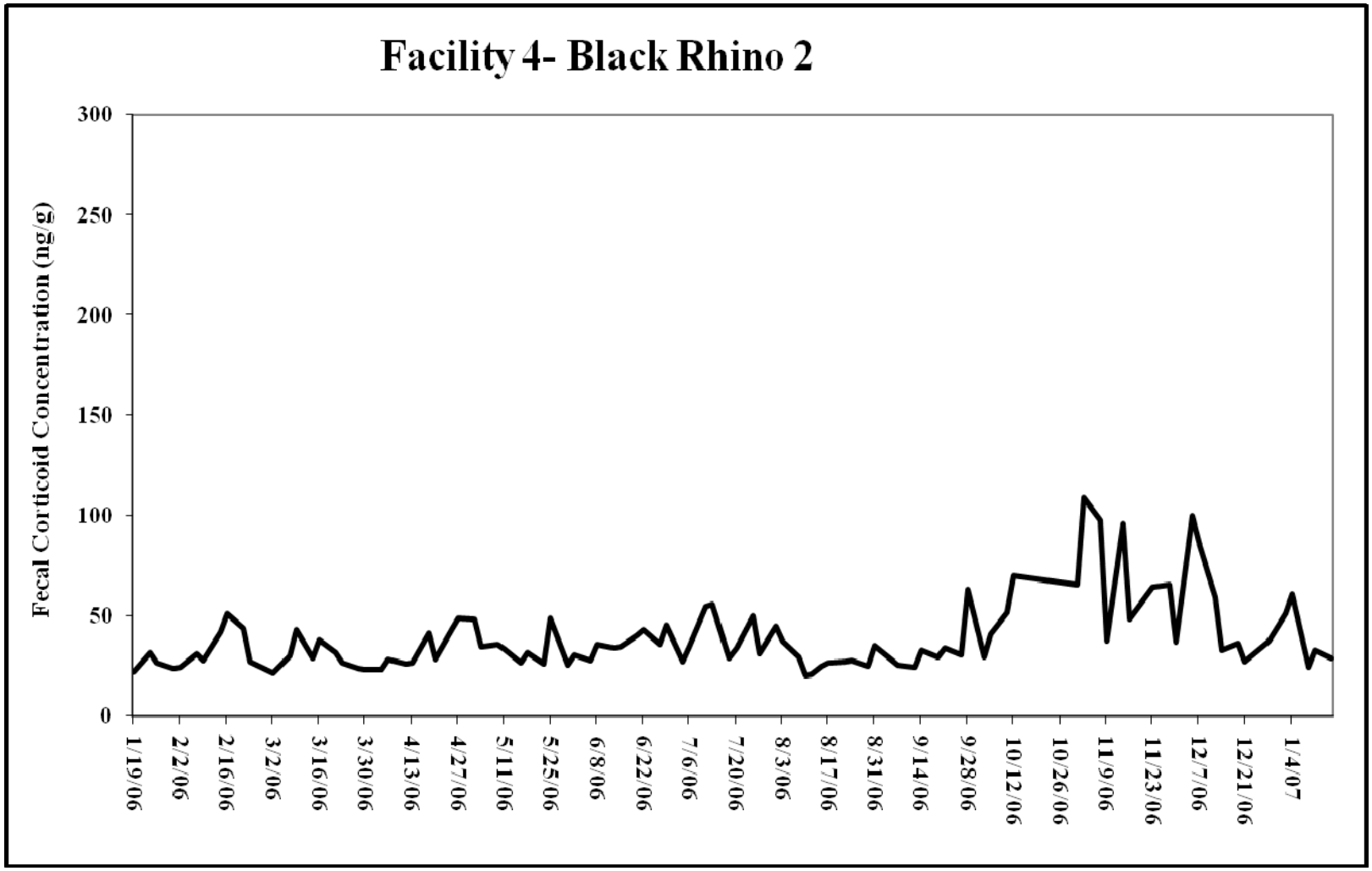
11/23/06
12/7/06
12/21/06
1/4/07


Facility 4- Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
1/19/06
2/2/06
2/16/06
3/2/06
3/16/06
3/30/06
4/13/06
4/27/06
5/11/06
5/25/06
6/8/06
6/22/06
7/6/06
7/20/06
8/3/06
8/17/06
8/31/06
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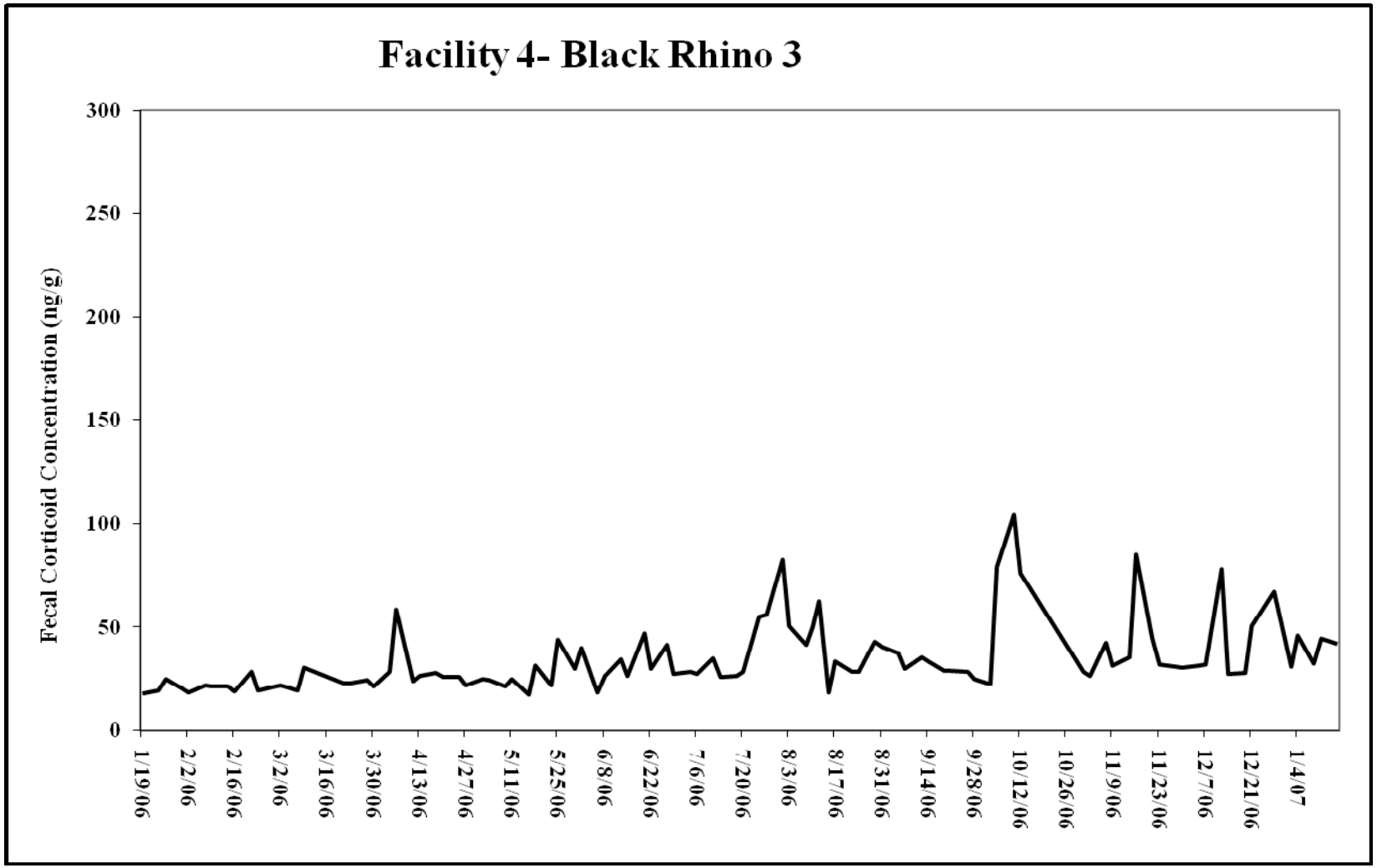
11/23/06
12/7/06
12/21/06
1/4/07


Facility 4- Black Rhino 3
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
1/19/06
2/2/06
2/16/06
3/2/06
3/16/06
3/30/06
4/13/06
4/27/06
5/11/06
5/25/06
6/8/06
6/22/06
7/6/06
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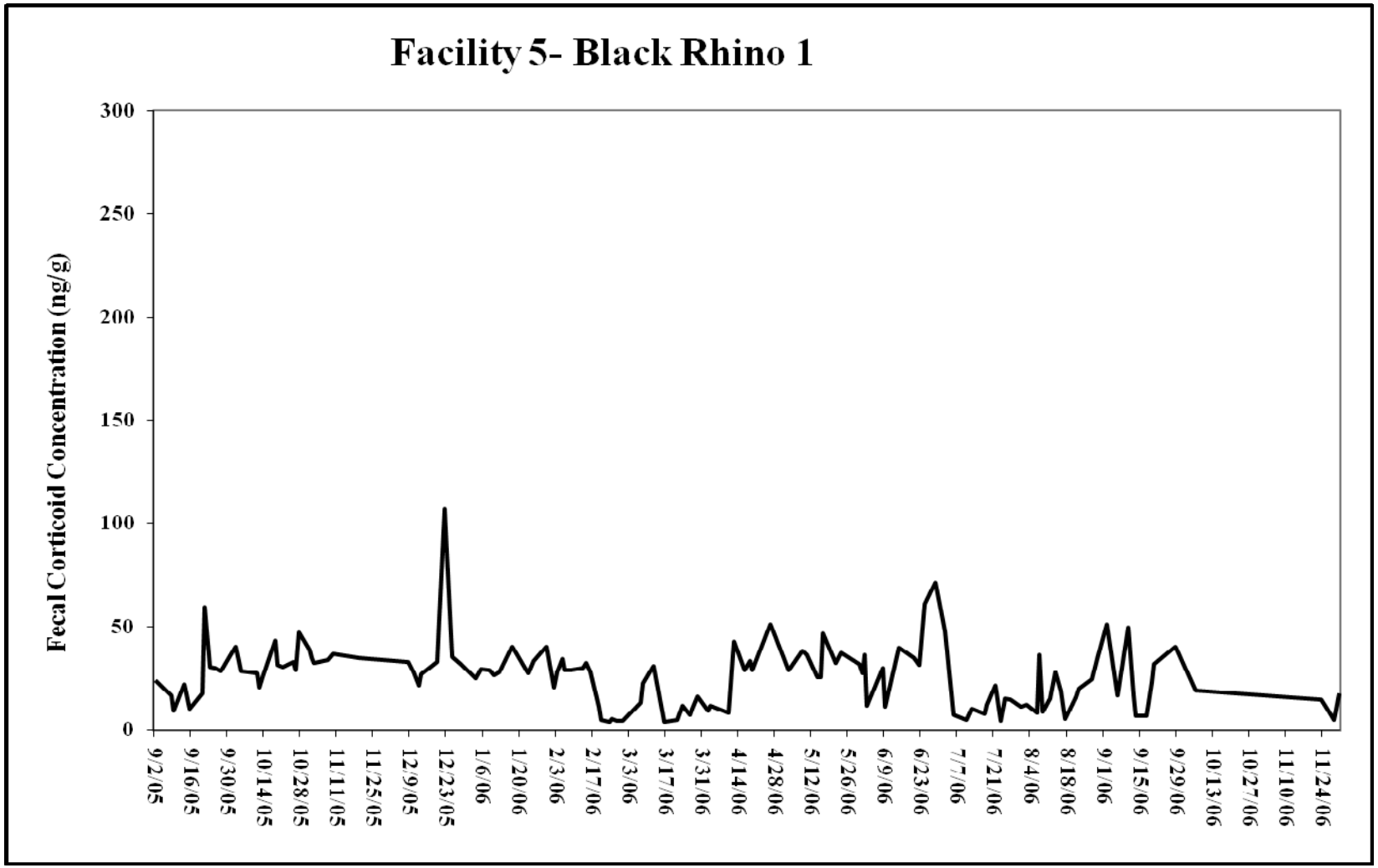
11/23/06
12/7/06
12/21/06
1/4/07


Facility 5- Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
9/2/05
9/16/05
9/30/05
10/14/05
10/28/05
11/11/05
11/25/05
12/9/05
12/23/05
1/6/06
1/20/06
2/3/06
2/17/06
3/3/06
3/17/06
3/31/06
4/14/06
4/28/06
5/12/06
5/26/06
6/9/06
6/23/06
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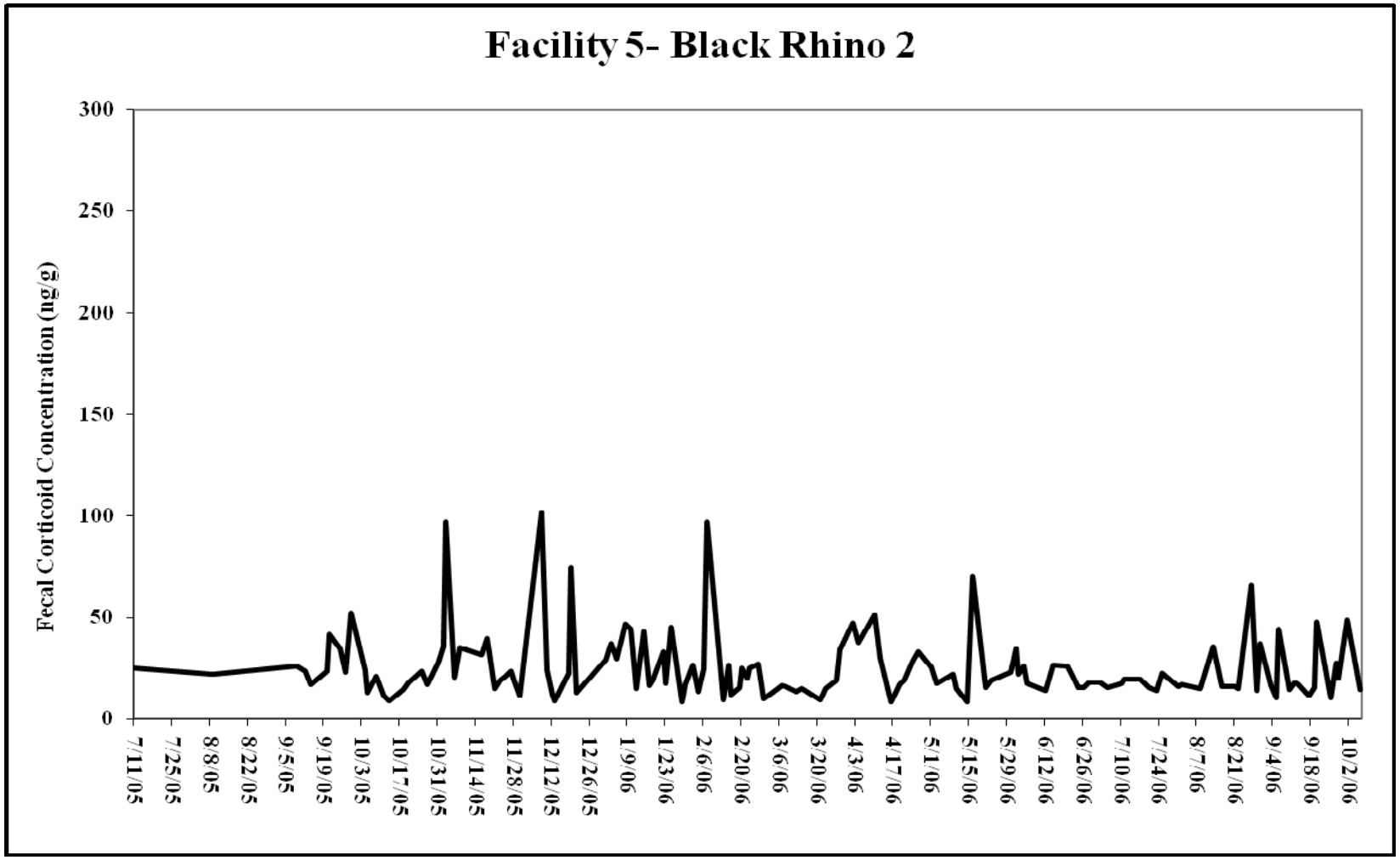
11/24/06


Facility 5- Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
7/11/05
7/25/05
8/8/05
8/22/05
9/5/05
9/19/05
10/3/05
10/17/05
10/31/05
11/14/05
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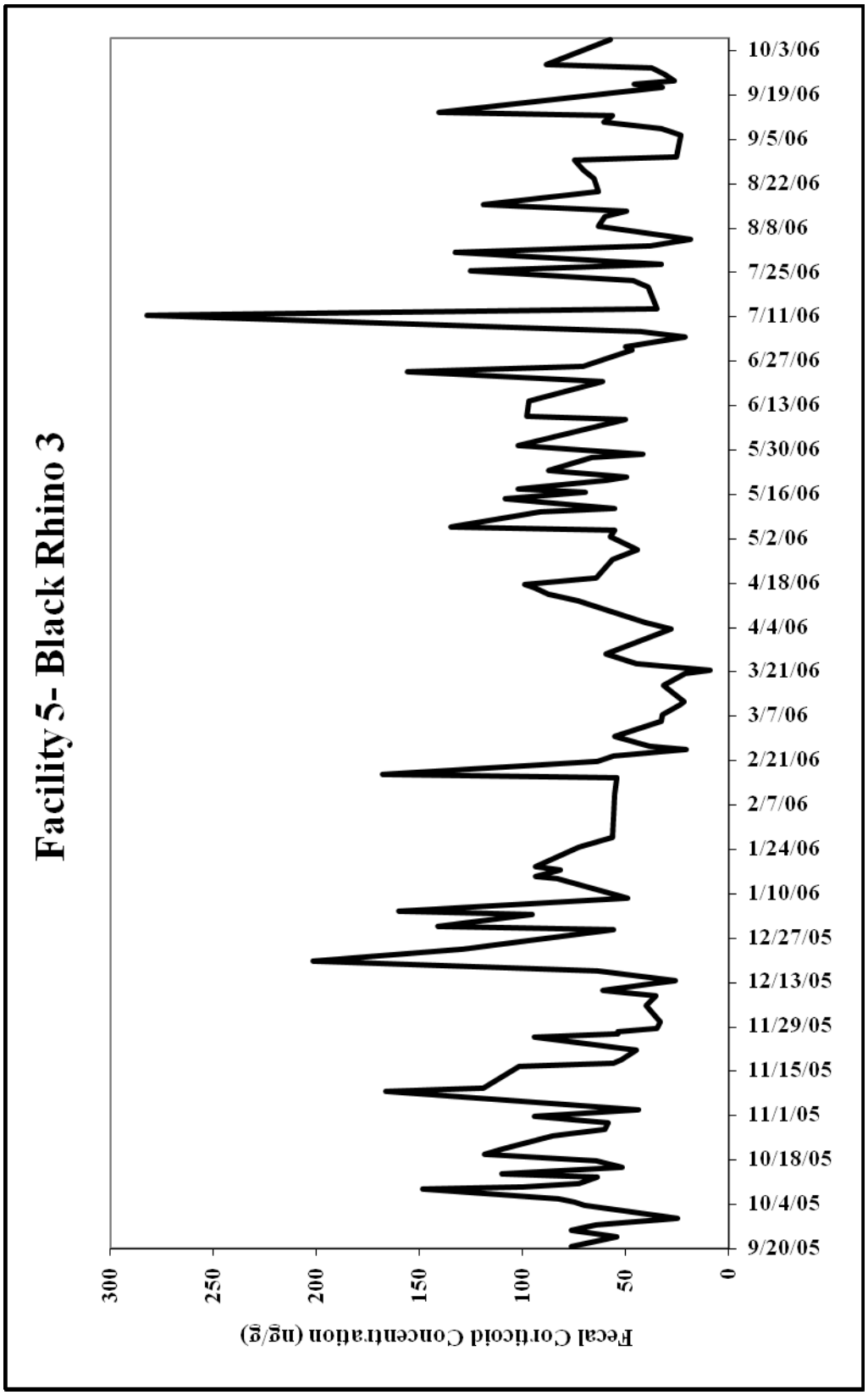
10/2/06


Facility 5- Black Rhino 3
Fecal Corticoid Concentration (ng/g)
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250
200
150
100
50
0
10/3/06
9/19/06
9/5/06
8/22/06
8/8/06
7/25/06
7/11/06
6/27/06
6/13/06
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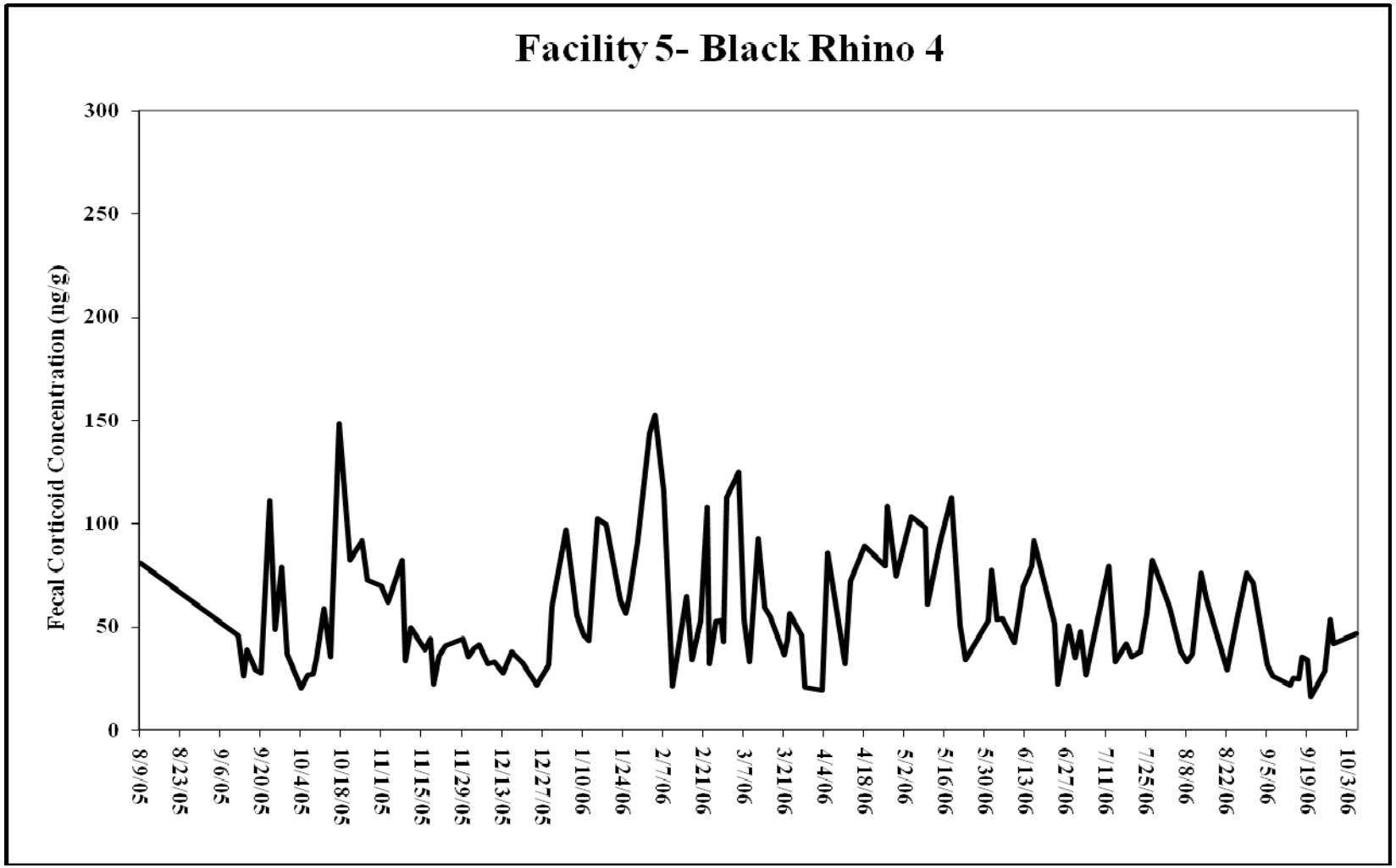
9/20/05


Facility 5- Black Rhino 4
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
8/9/05
8/23/05
9/6/05
9/20/05
10/4/05
10/18/05
11/1/05
11/15/05
11/29/05
12/13/05
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9/5/06
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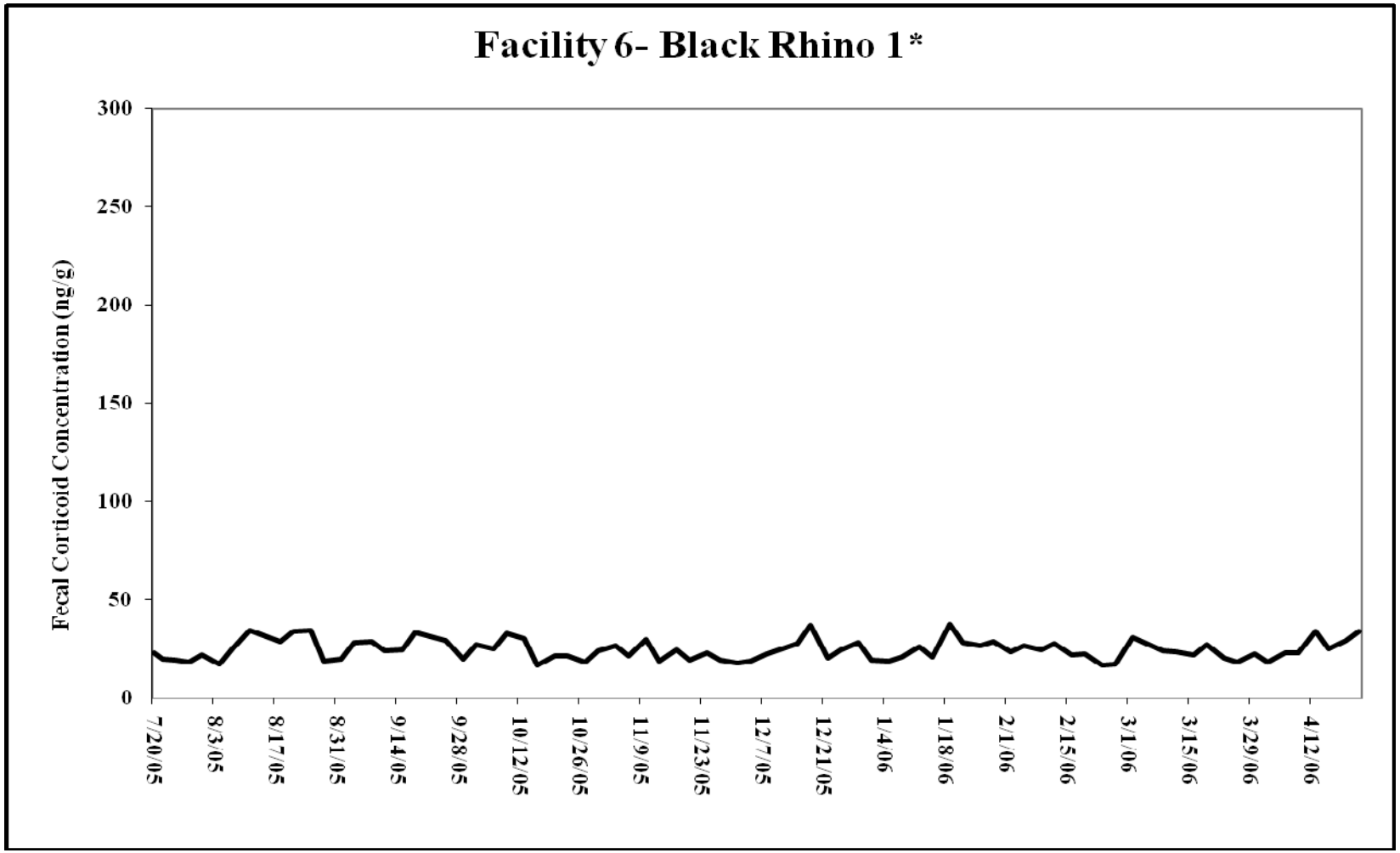
10/3/06


Facility 6- Black Rhino 1*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
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8/3/05
8/17/05
8/31/05
9/14/05
9/28/05
10/12/05
10/26/05
11/9/05
11/23/05
12/7/05
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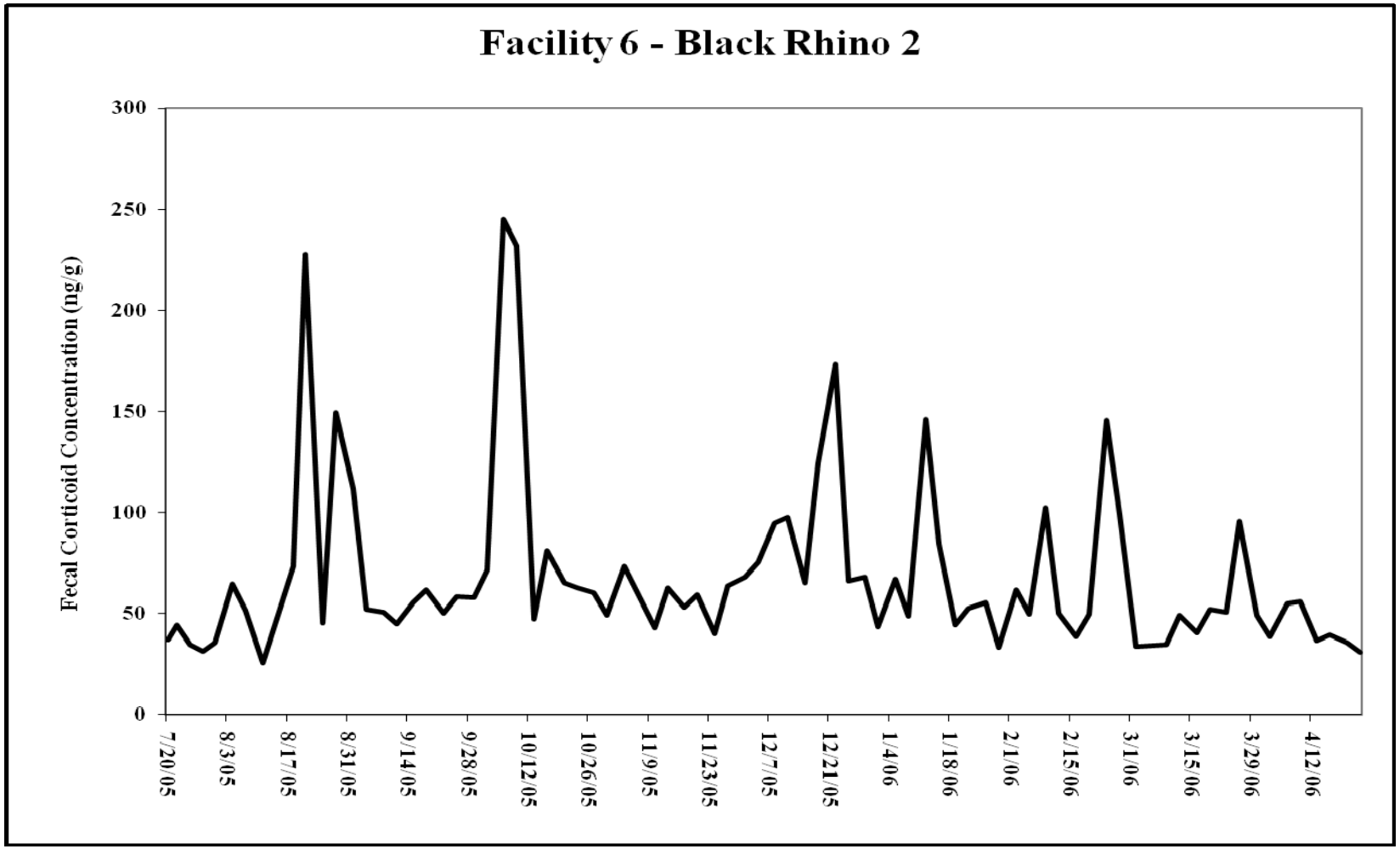
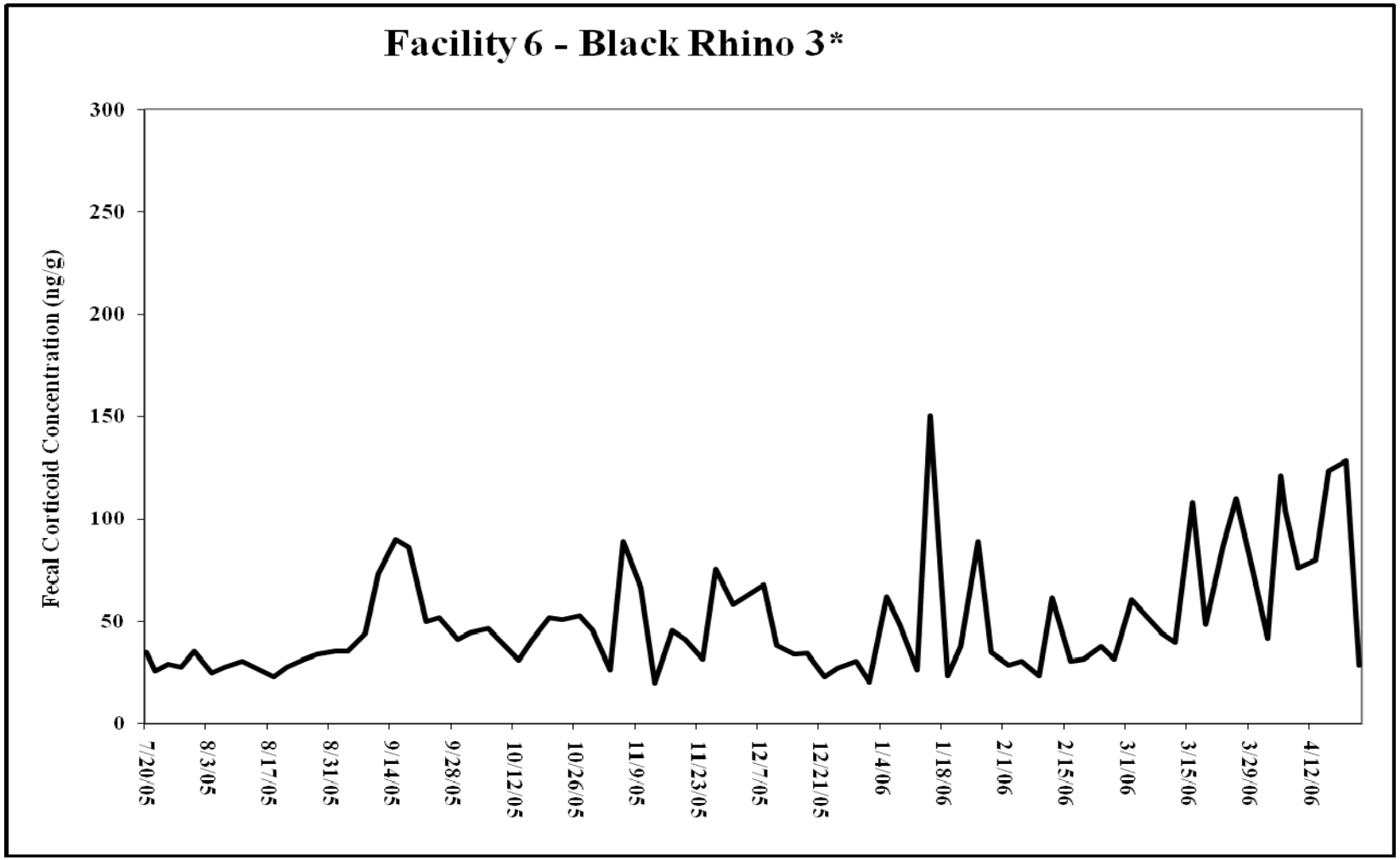
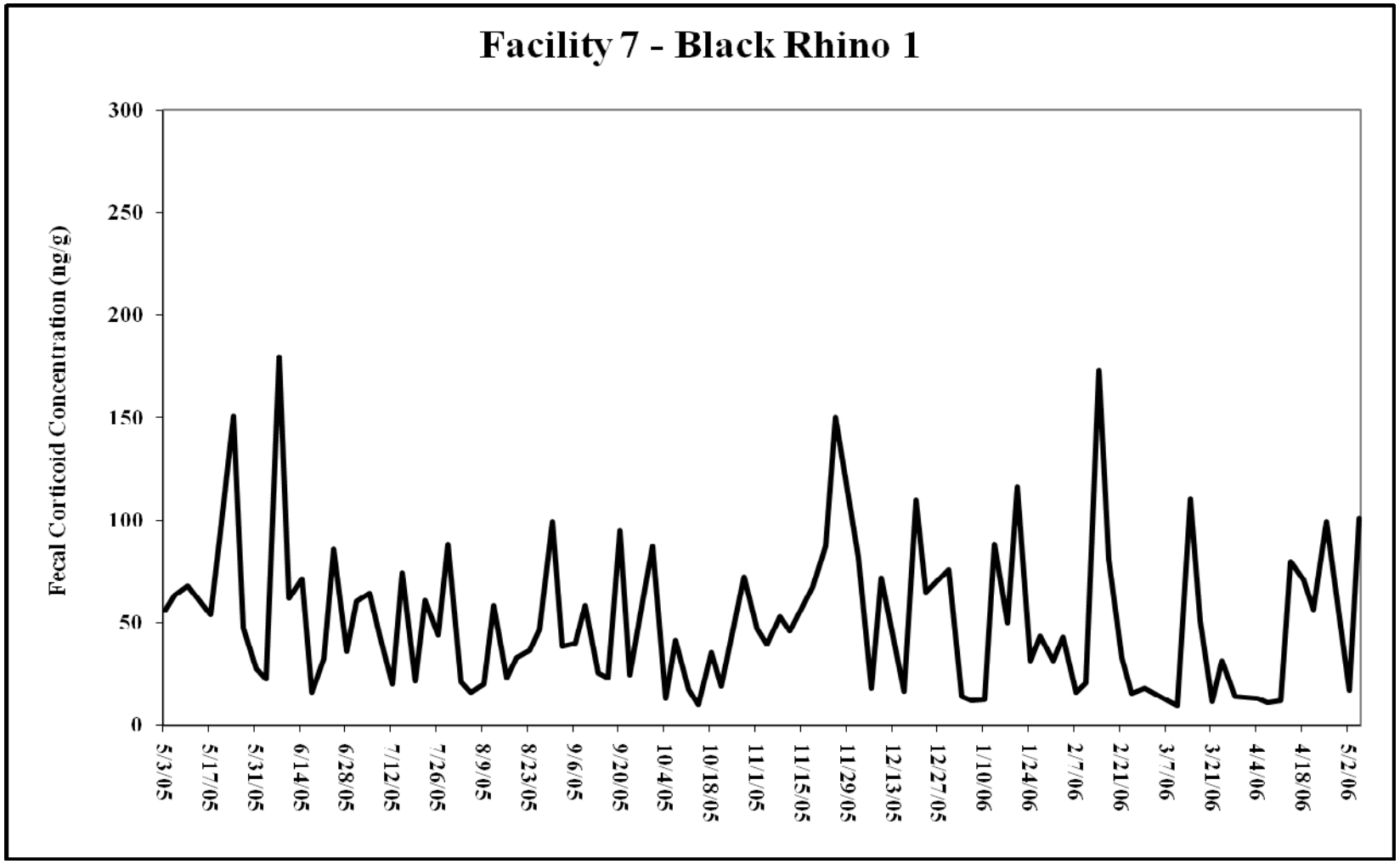
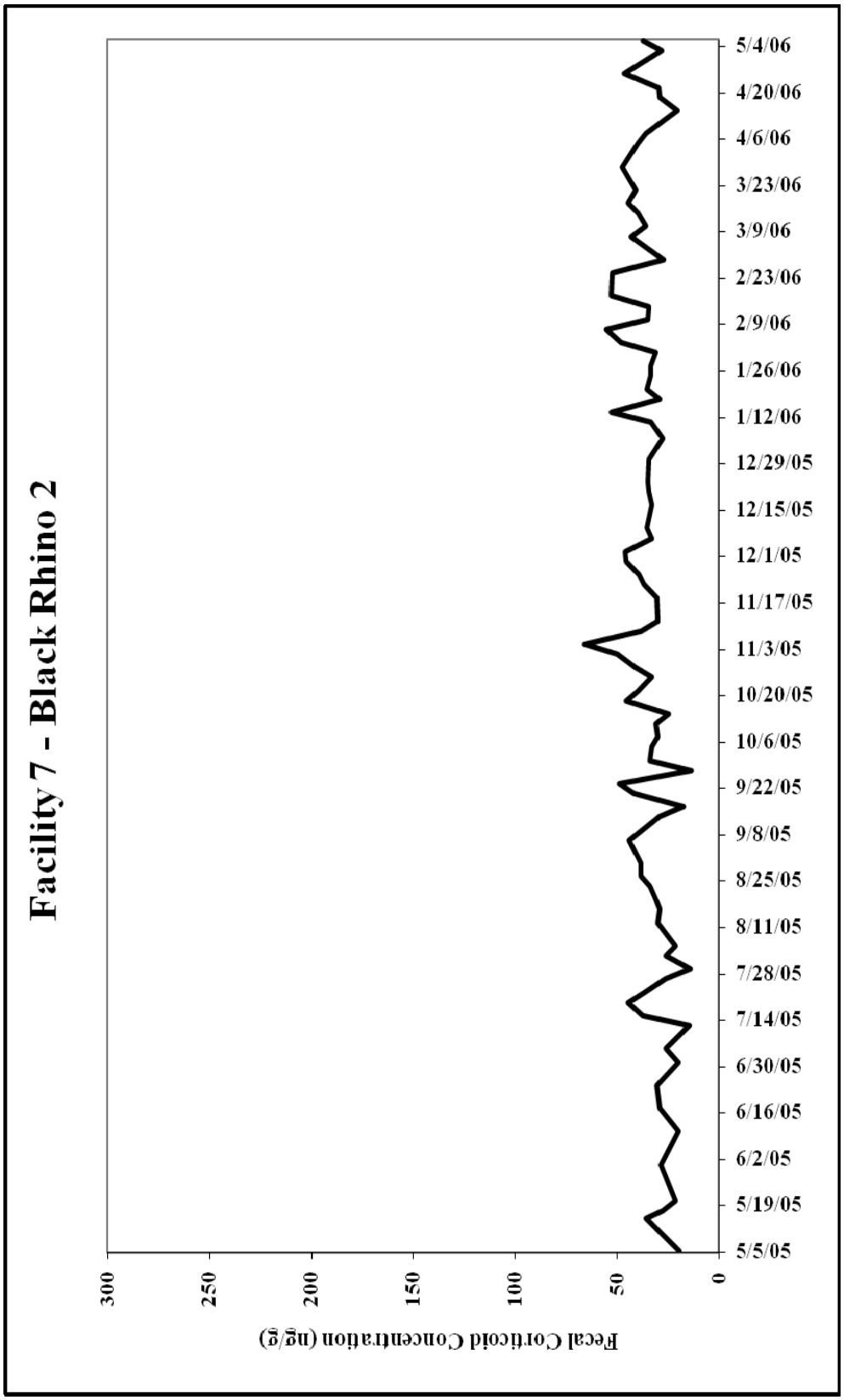
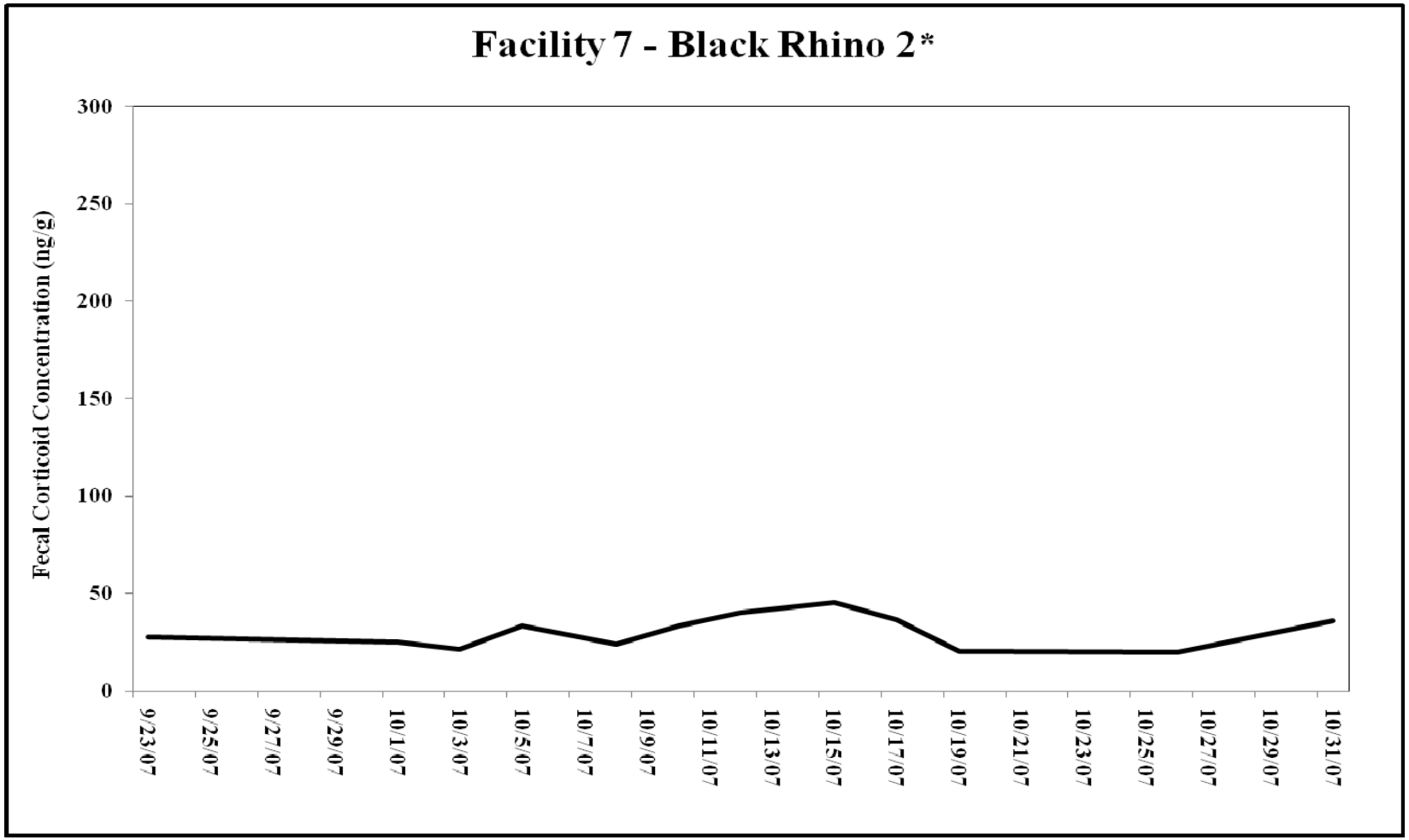
3/15/06
3/29/06
4/12/06


Facility 6 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
7/20/05
8/3/05
8/17/05
8/31/05
9/14/05
9/28/05
10/12/05
10/26/05
11/9/05
11/23/05
12/7/05
12/21/05
1/4/06
1/18/06
2/1/06
2/15/06
3/1/06
3/15/06
3/29/06
4/12/06


Facility 6 - Black Rhino 3*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
7/20/05
8/3/05
8/17/05
8/31/05
9/14/05
9/28/05
10/12/05
10/26/05
11/9/05
11/23/05
12/7/05
12/21/05
1/4/06
1/18/06
2/1/06
2/15/06
3/1/06
3/15/06
3/29/06
4/12/06


Facility 7 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/3/05
5/17/05
5/31/05
6/14/05
6/28/05
7/12/05
7/26/05
8/9/05
8/23/05
9/6/05
9/20/05
10/4/05
10/18/05
11/1/05
11/15/05
11/29/05
12/13/05
12/27/05
1/10/06
1/24/06
2/7/06
2/21/06
3/7/06
3/21/06
4/4/06
4/18/06
5/2/06


Facility 7 - Black Rhino 2
Fecal Cortiocid Concentration (ng/g)
300
250
200
150
100
50
0
5/4/06
4/20/06
4/6/06
3/23/06
3/9/06
2/23/06
2/9/06
1/26/06
1/12/06
12/29/05
12/15/05
12/1/05
11/17/05
11/3/05
10/20/05
10/6/05
9/22/05
9/8/05
8/25/05
8/11/05
7/28/05
7/14/05
6/30/05
6/16/05
6/2/05
5/19/05
5/5/05


Facility 7 - Black Rhino 2*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
9/23/07
9/25/07
9/27/07
9/29/07
10/1/07
10/3/07
10/5/07
10/7/07
10/9/07
10/11/07
10/13/07
10/15/07
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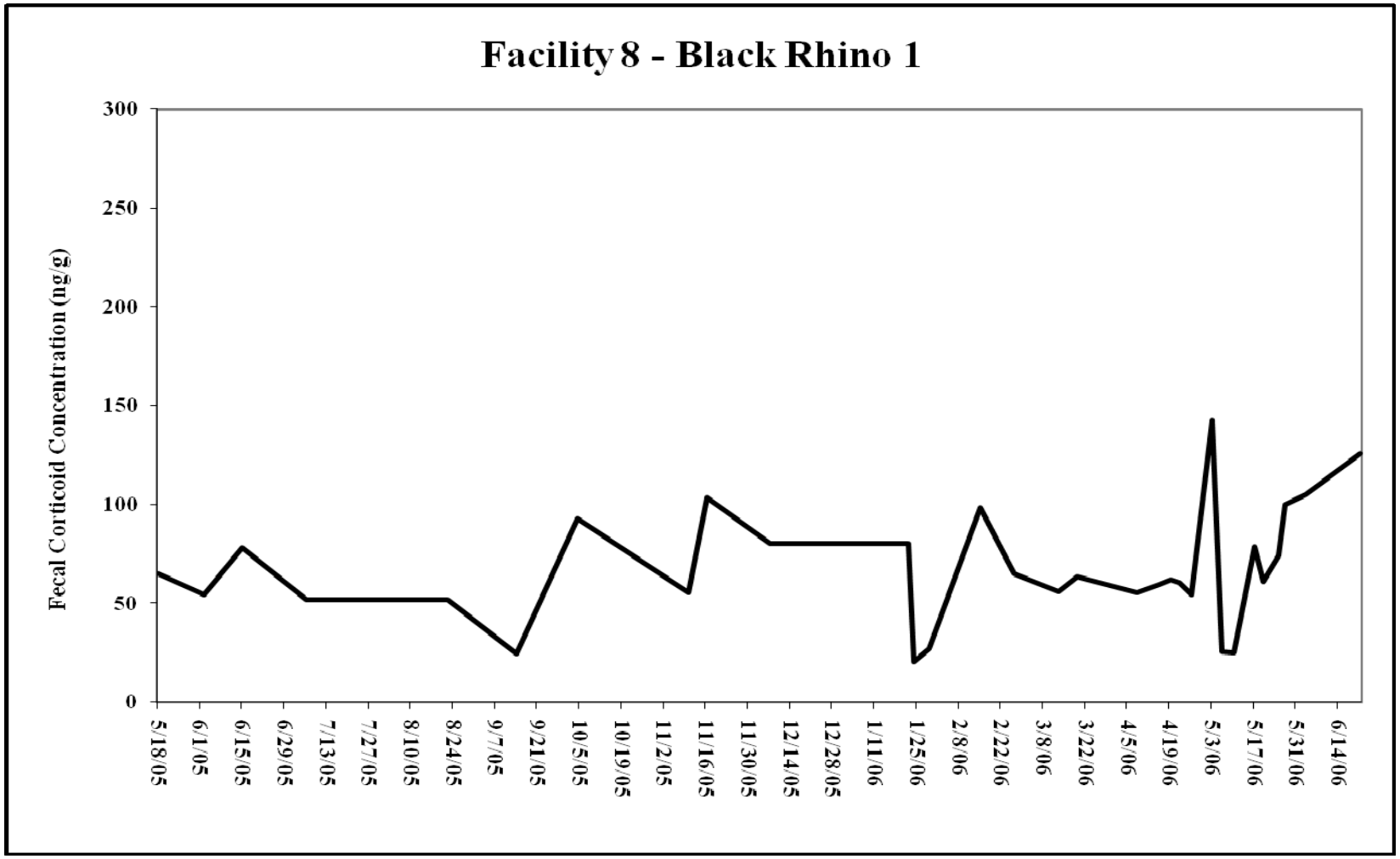
10/25/07
10/27/07
10/29/07
10/31/07


Facility 8 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/18/05
6/1/05
6/15/05
6/29/05
7/13/05
7/27/05
8/10/05
8/24/05
9/7/05
9/21/05
10/5/05
10/19/05
11/2/05
11/16/05
11/30/05
12/14/05
12/28/05
1/11/06
1/25/06
2/8/06
2/22/06
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3/22/06
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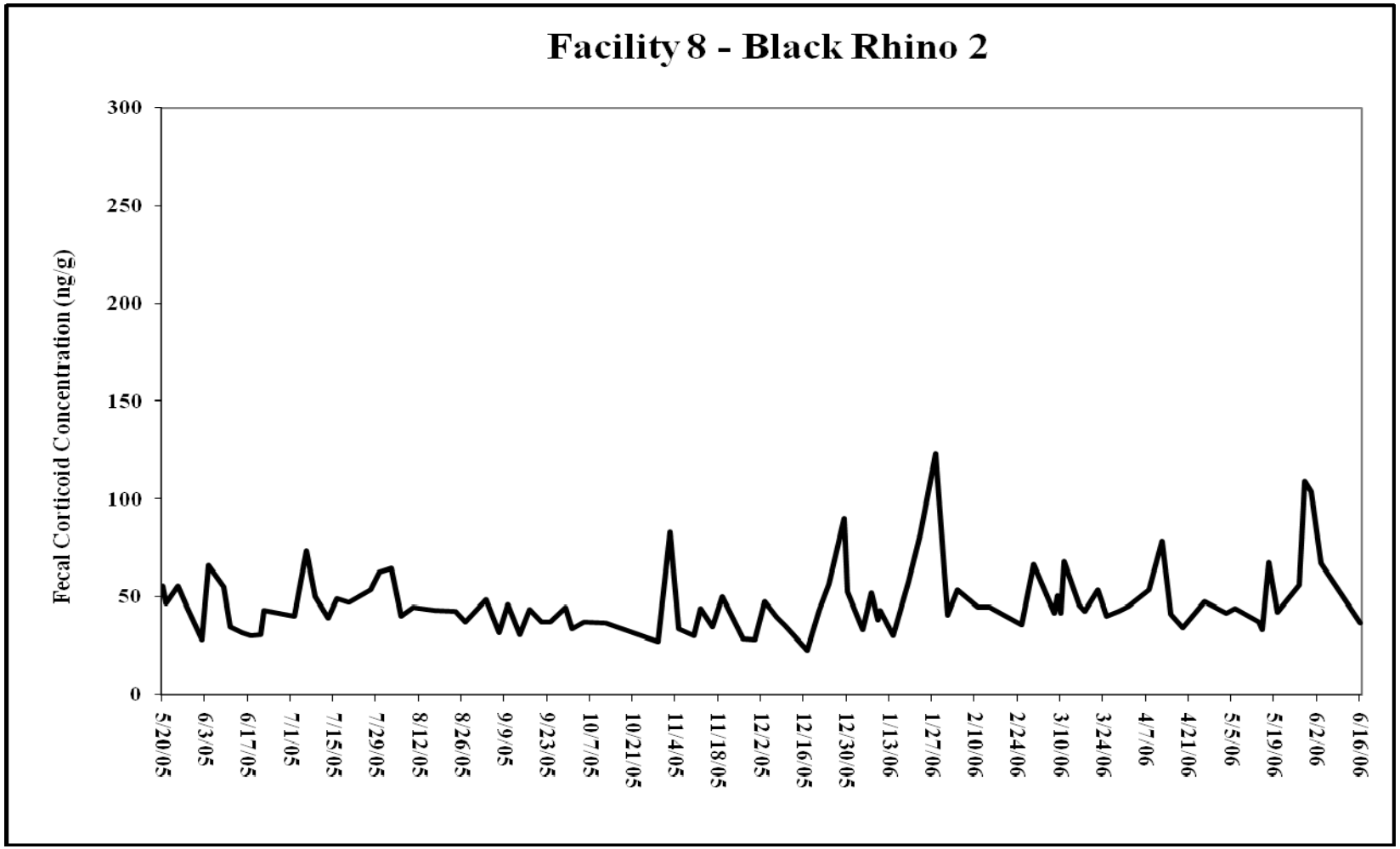
4/19/06
5/3/06
5/17/06
5/31/06
6/14/06


Facility 8 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/20/05
6/3/05
6/17/05
7/1/05
7/15/05
7/29/05
8/12/05
8/26/05
9/9/05
9/23/05
10/7/05
10/21/05
11/4/05
11/18/05
12/2/05
12/16/05
12/30/05
1/13/06
1/27/06
2/10/06
2/24/06
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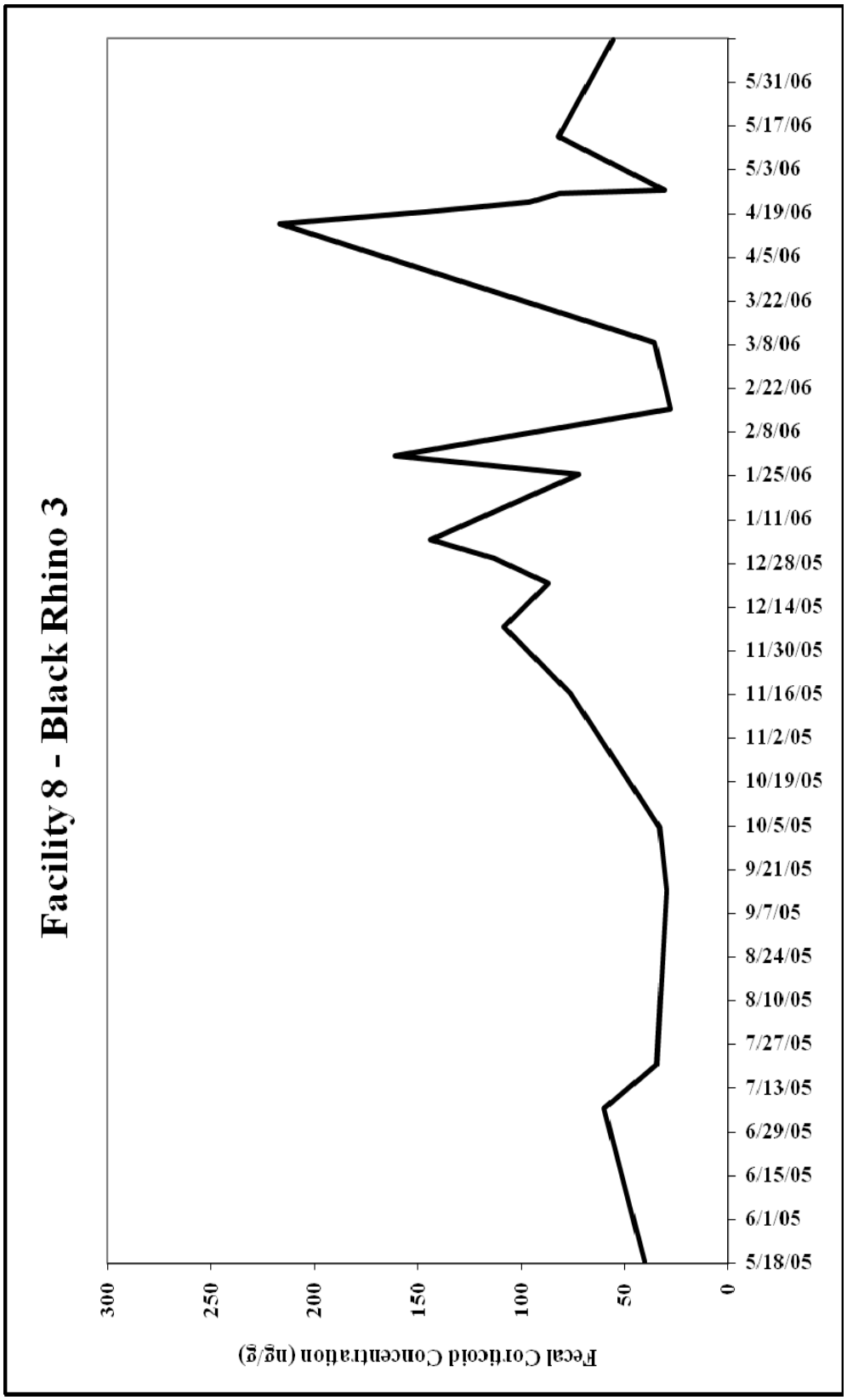
3/24/06
4/7/06
4/21/06
5/5/06
5/19/06
6/2/06
6/16/06


Facility 8 - Black Rhino 3
Fecal Cortiecld Concentration (ng/g)
300
250
200
150
100
50
0
5/31/06
5/17/06
5/3/06
4/19/06
4/5/06
3/22/06
3/8/06
2/22/06
2/8/06
1/25/06
1/11/06
12/28/05
12/14/05
11/30/05
11/16/05
11/2/05
10/19/05
10/5/05
9/21/05
9/7/05
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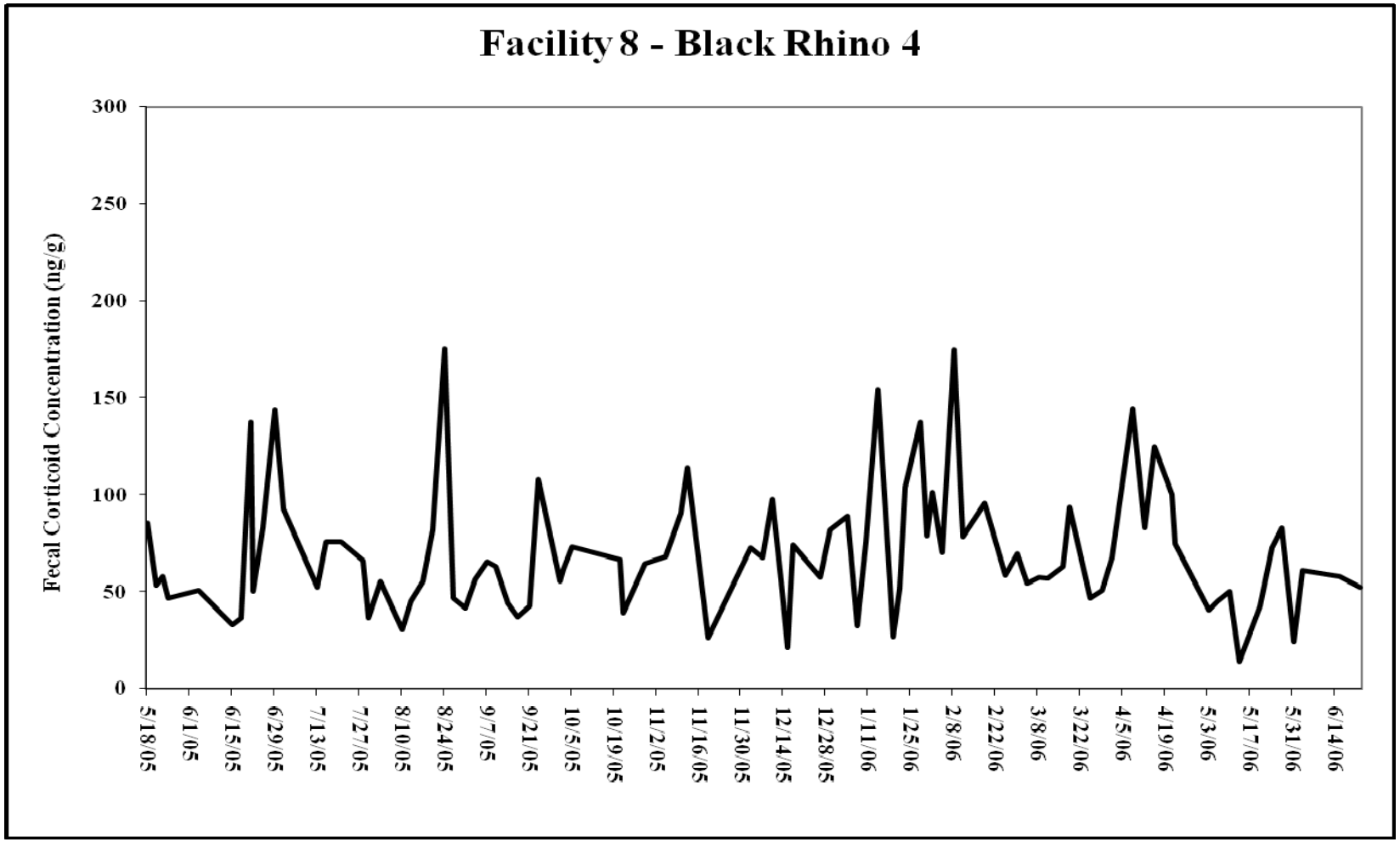
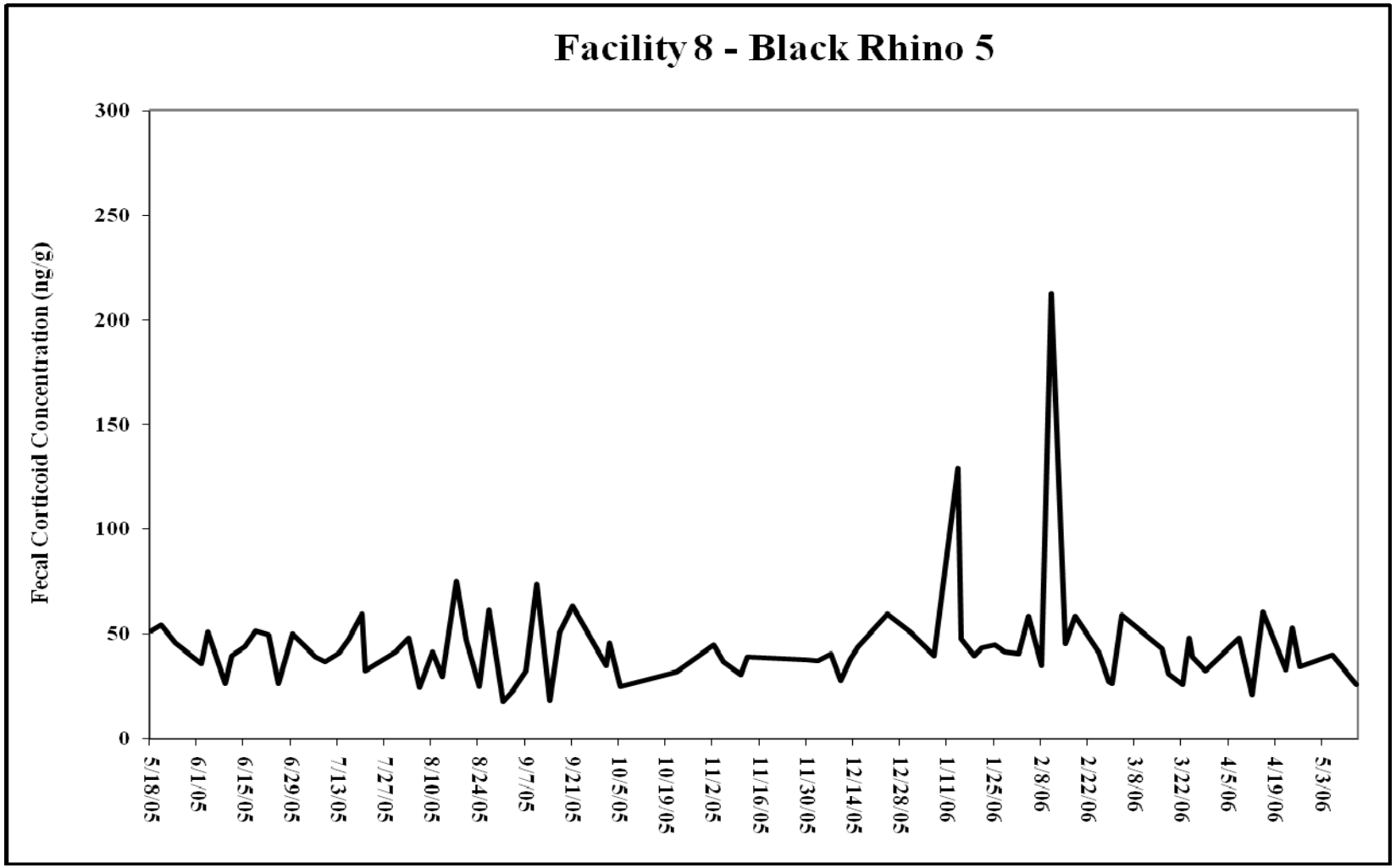
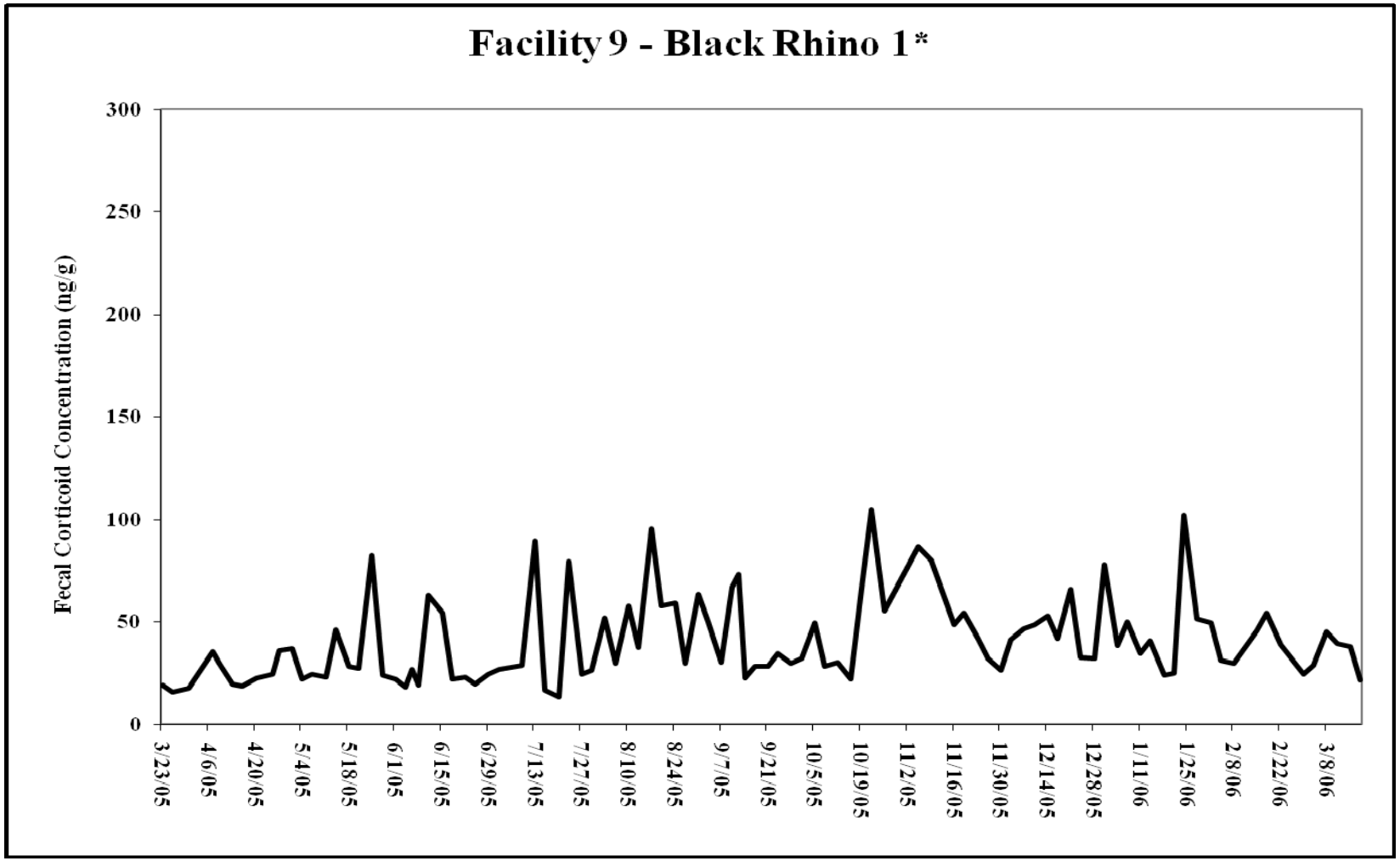
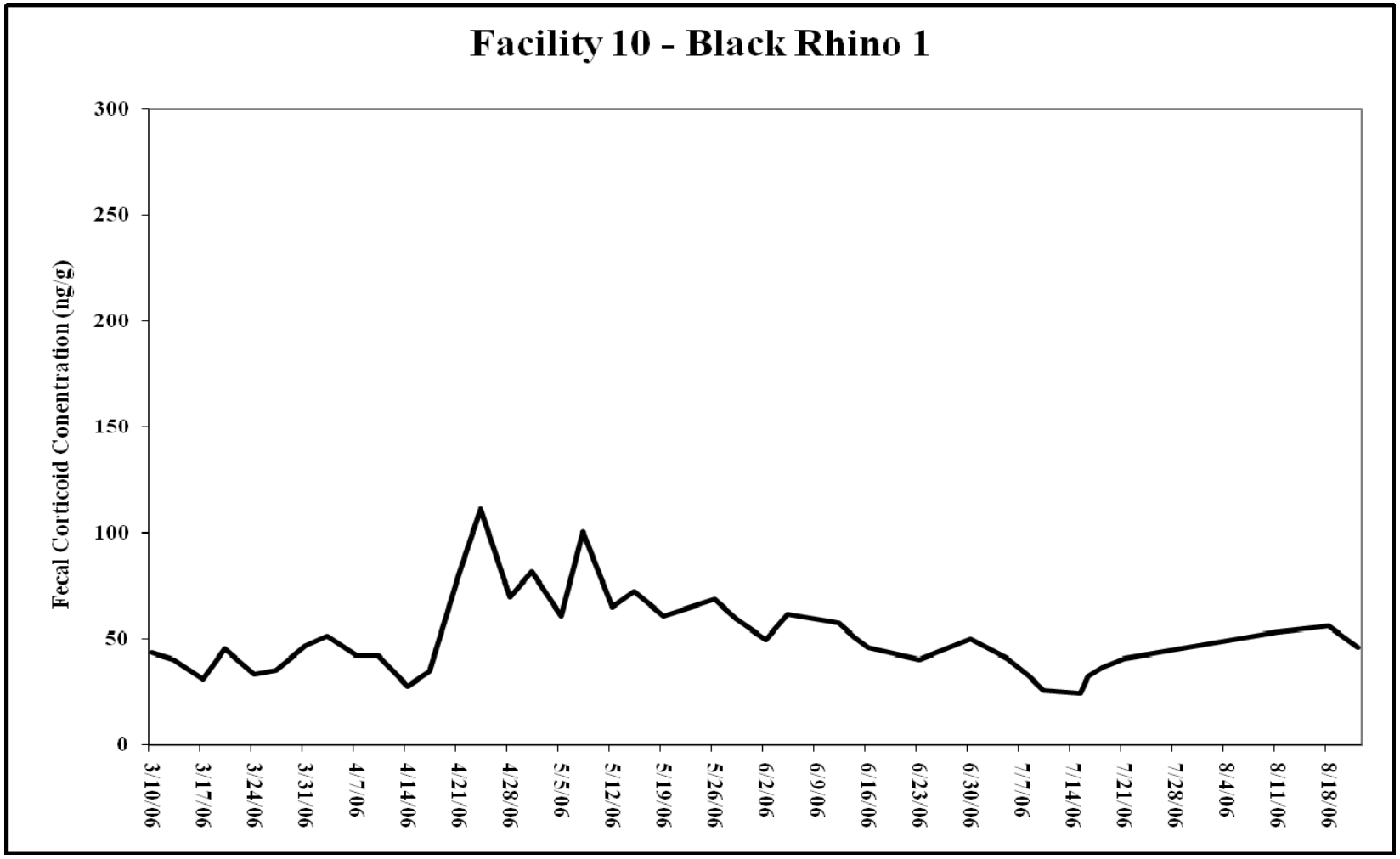
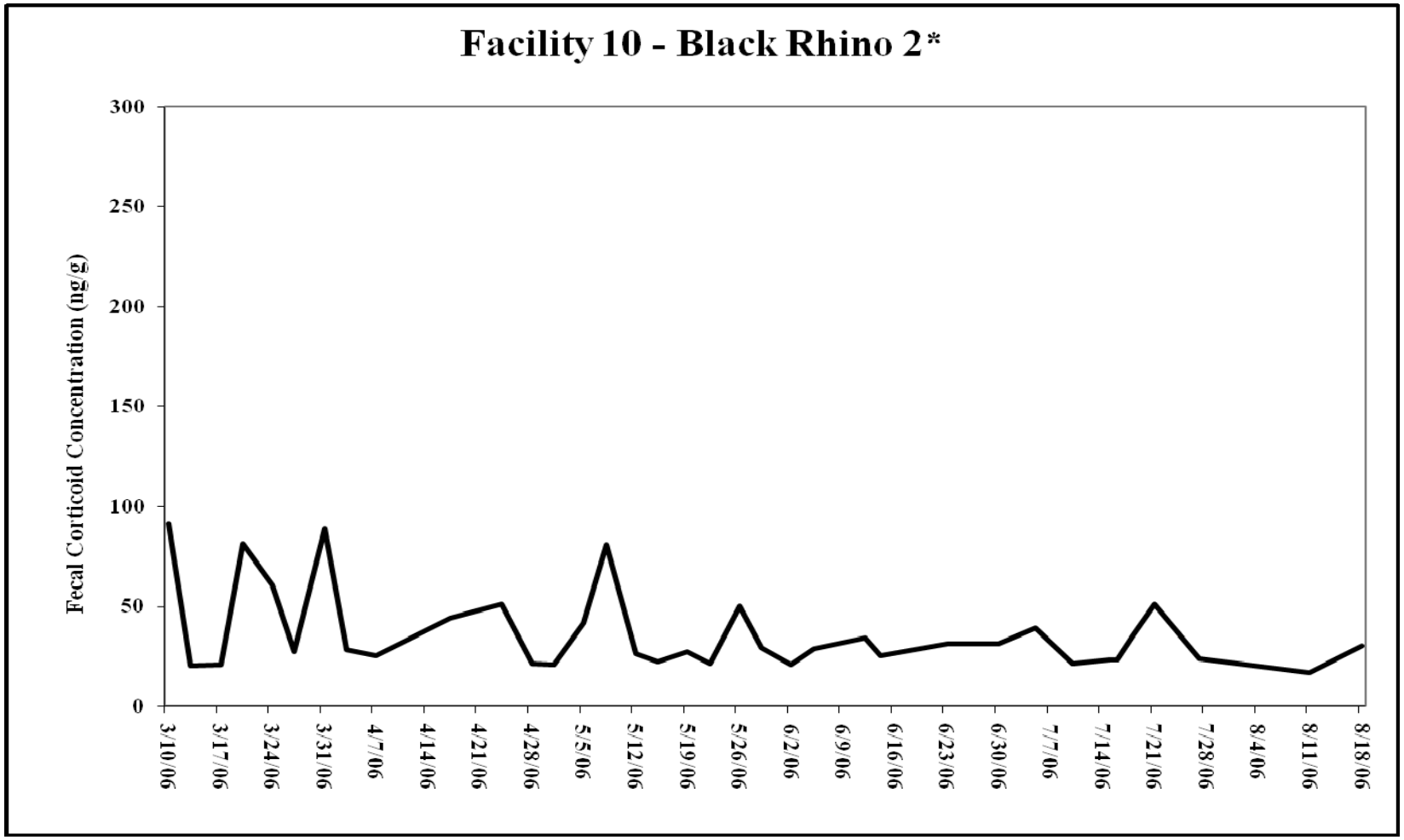
8/10/05
7/27/05
7/13/05
6/29/05
6/15/05
6/1/05
5/18/05

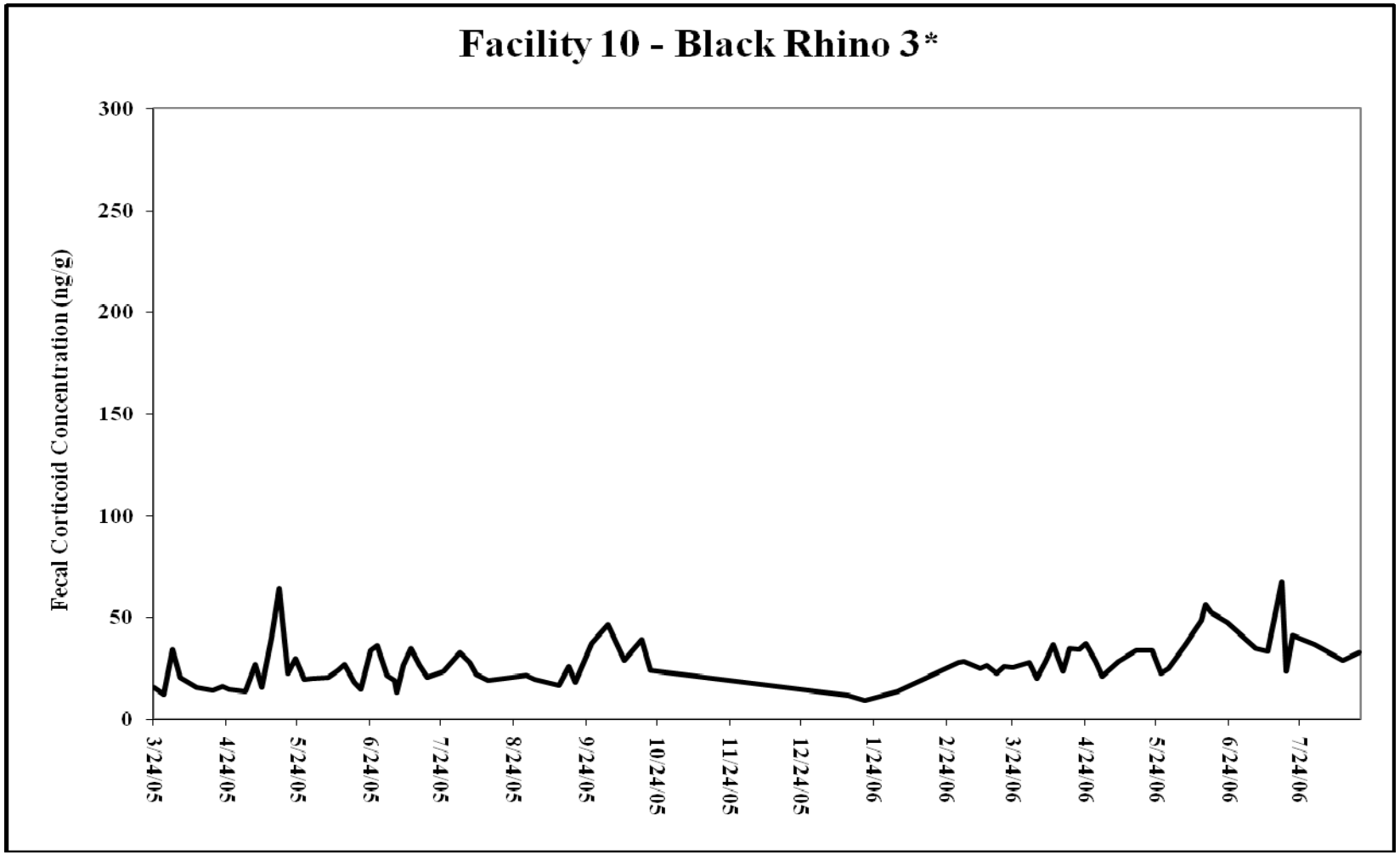

Facility 8 - Black Rhino 4
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/18/05
6/1/05
6/15/05
6/29/05
7/13/05
7/27/05
8/10/05
8/24/05
9/7/05
9/21/05
10/5/05
10/19/05
11/2/05
11/16/05
11/30/05
12/14/05
12/28/05
1/11/06
1/25/06
2/8/06
2/22/06
3/8/06
3/22/06
4/5/06
4/19/06
5/3/06
5/17/06
5/31/06
6/14/06

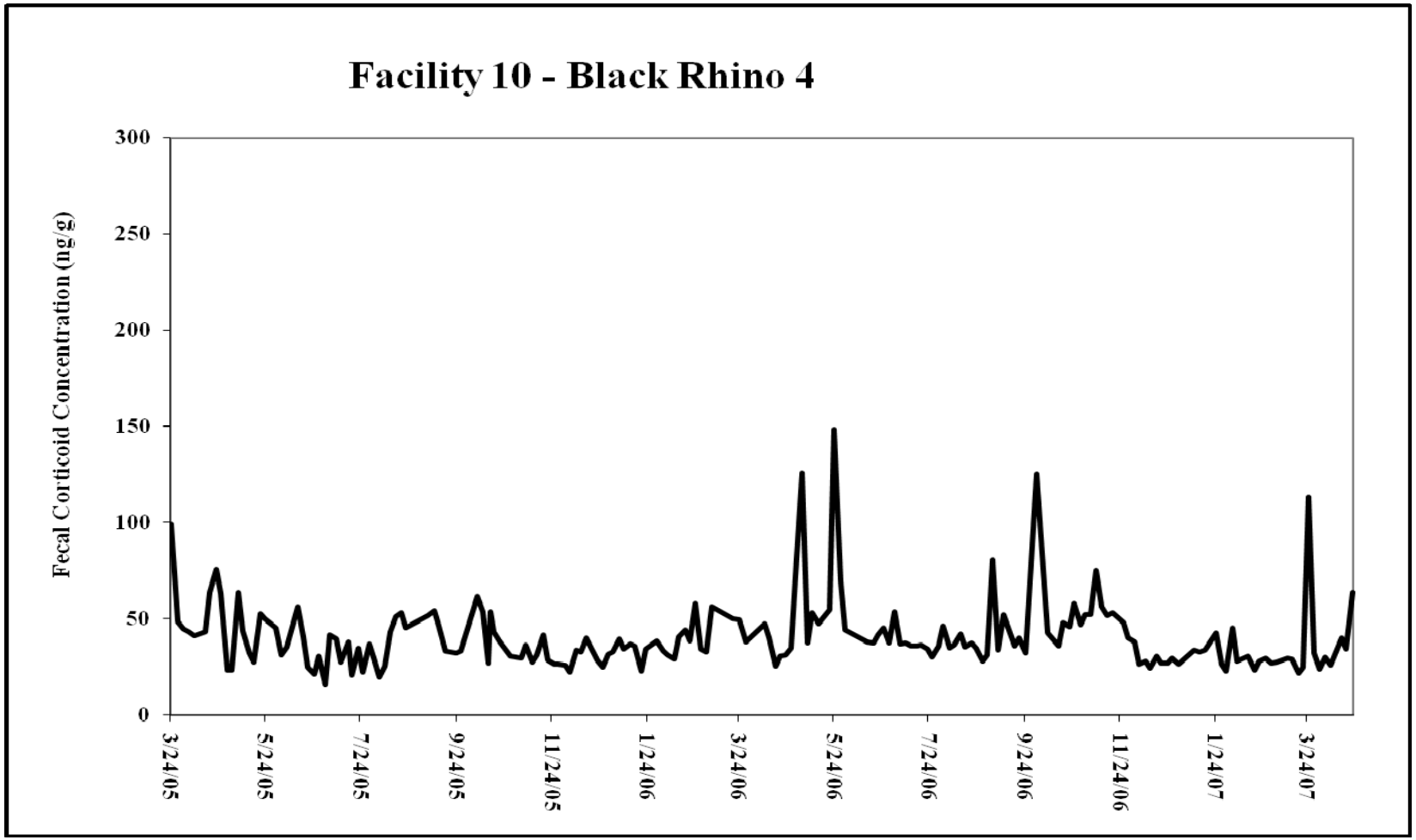

Facility 8 - Black Rhino 5
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
5/18/05
6/1/05
6/15/05
6/29/05
7/13/05
7/27/05
8/10/05
8/24/05
9/7/05
9/21/05
10/5/05
10/19/05
11/2/05
11/16/05
11/30/05
12/14/05
12/28/05
1/11/06
1/25/06
2/8/06
2/22/06
3/8/06
3/22/06
4/5/06
4/19/06
5/3/06

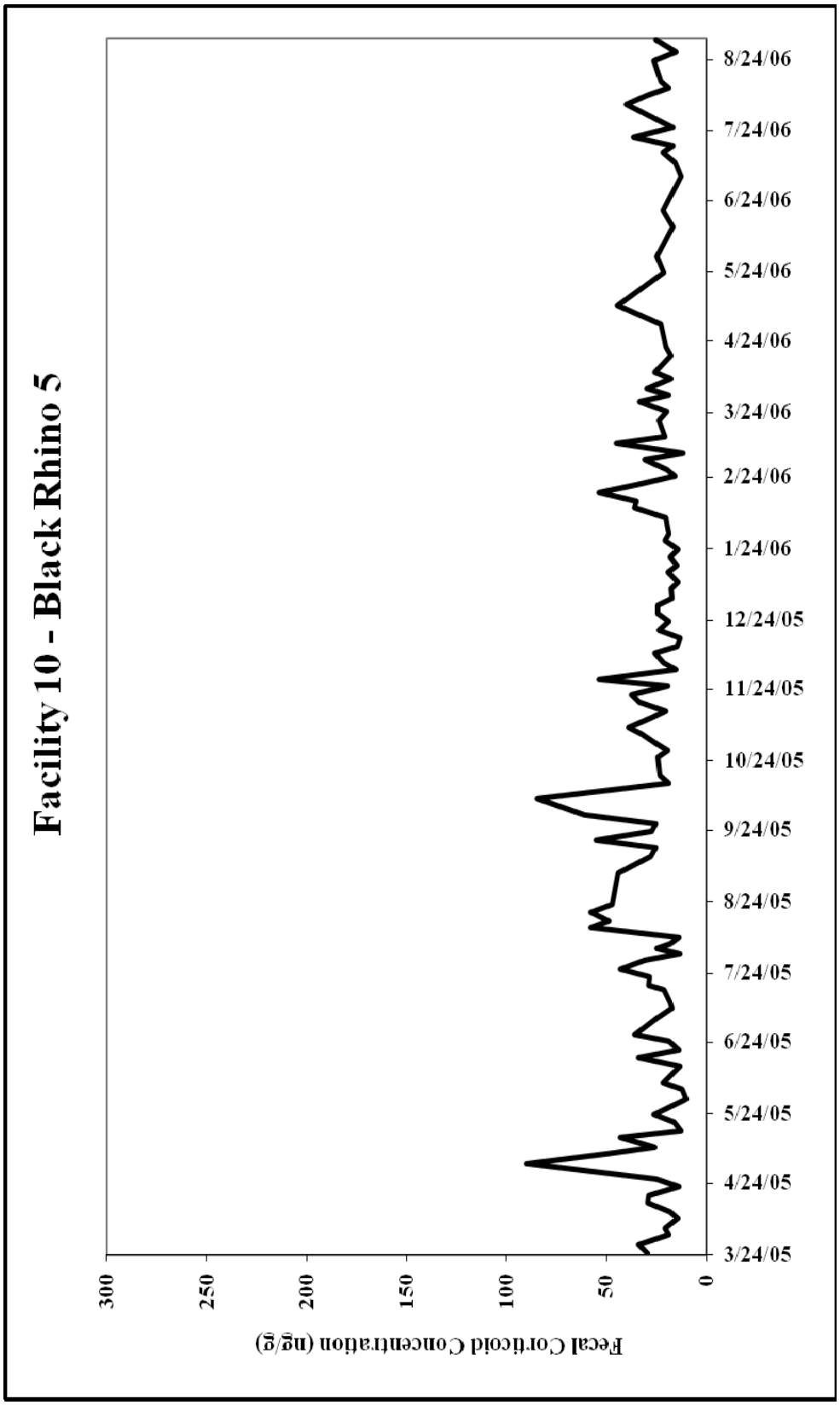

Facility 9 - Black Rhino 1*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
3/23/05
4/6/05
4/20/05
5/4/05
5/18/05
6/1/05
6/15/05
6/29/05
7/13/05
7/27/05
8/10/05
8/24/05
9/7/05
9/21/05
10/5/05
10/19/05
11/2/05
11/16/05
11/30/05
12/14/05
12/28/05
1/11/06
1/25/06
2/8/06
2/22/06
3/8/06

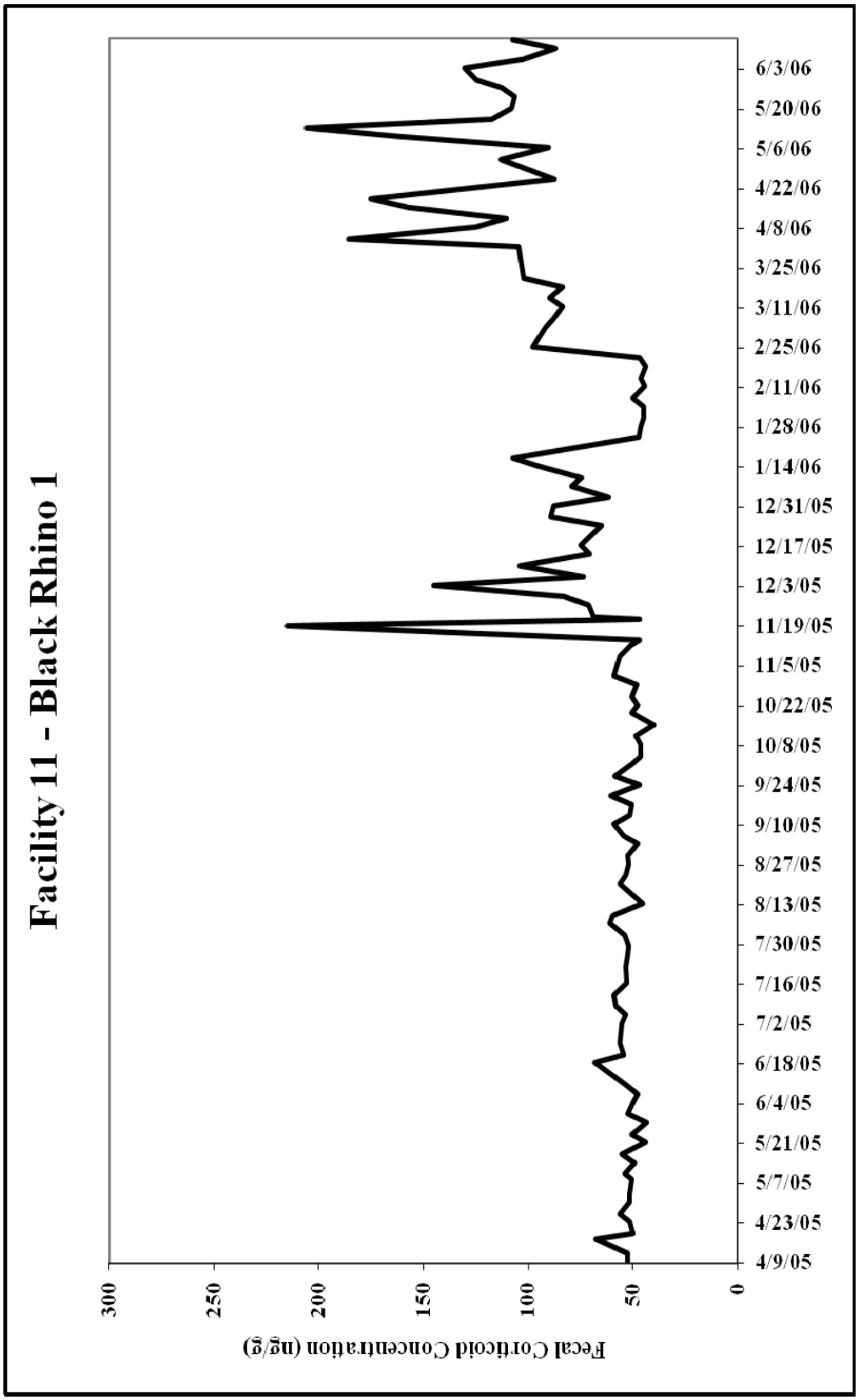

Facility 10 - Black Rhino 1
Fecal Corticoid Conentration (ng/g)
300
250
200
150
100
50
0
3/10/06
3/17/06
3/24/06
3/31/06
4/7/06
4/14/06
4/21/06
4/28/06
5/5/06
5/12/06
5/19/06
5/26/06
6/2/06
6/9/06
6/16/06
6/23/06
6/30/06
7/7/06
7/14/06
7/21/06
7/28/06
8/4/06
8/11/06
8/18/06

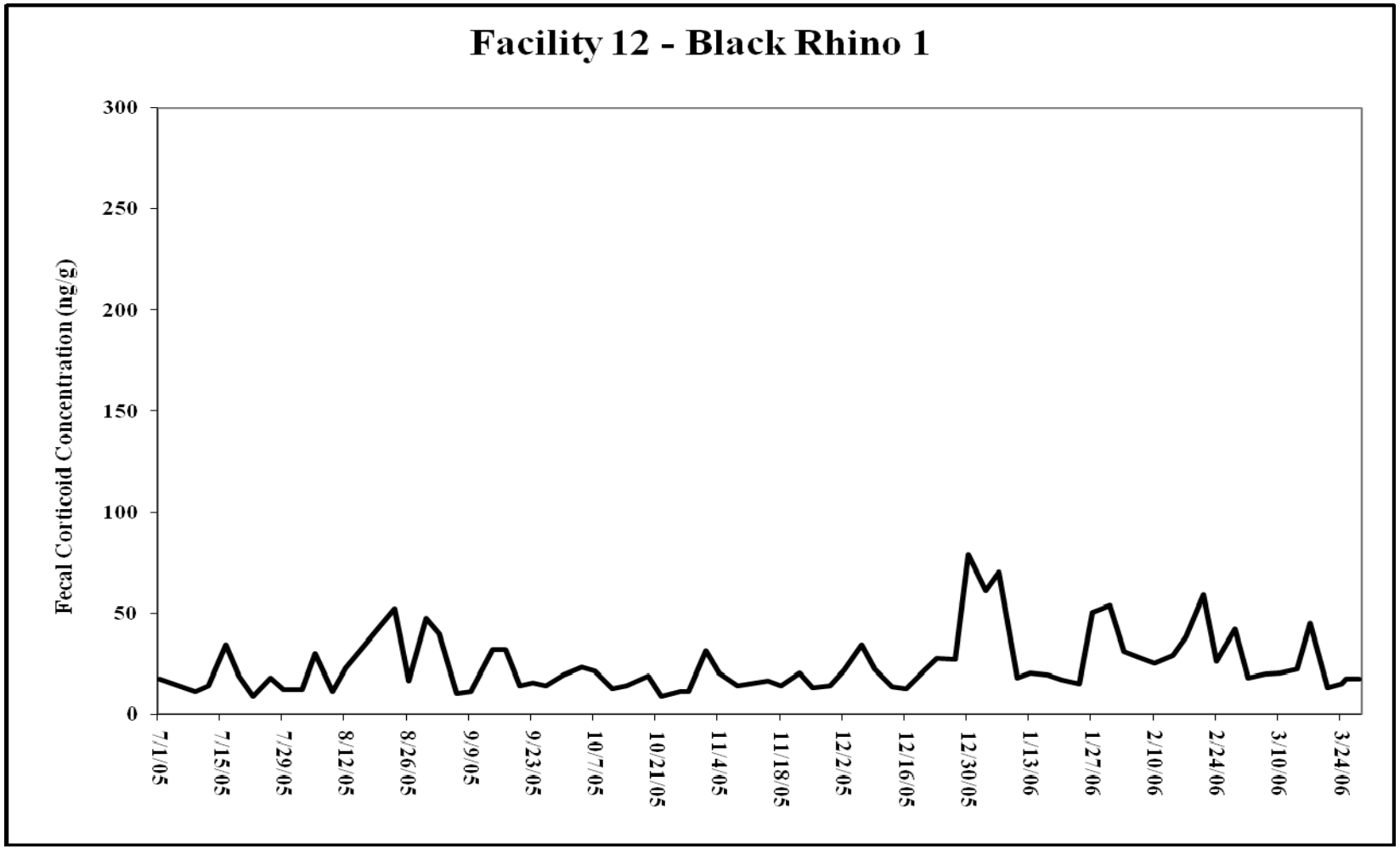

Facility 10 - Black Rhino 2*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
3/10/06
3/17/06
3/24/06
3/31/06
4/7/06
4/14/06
4/21/06
4/28/06
5/5/06
5/12/06
5/19/06
5/26/06
6/2/06
6/9/06
6/16/06
6/23/06
6/30/06
7/7/06
7/14/06
7/21/06
7/28/06
8/4/06
8/11/06
8/18/06

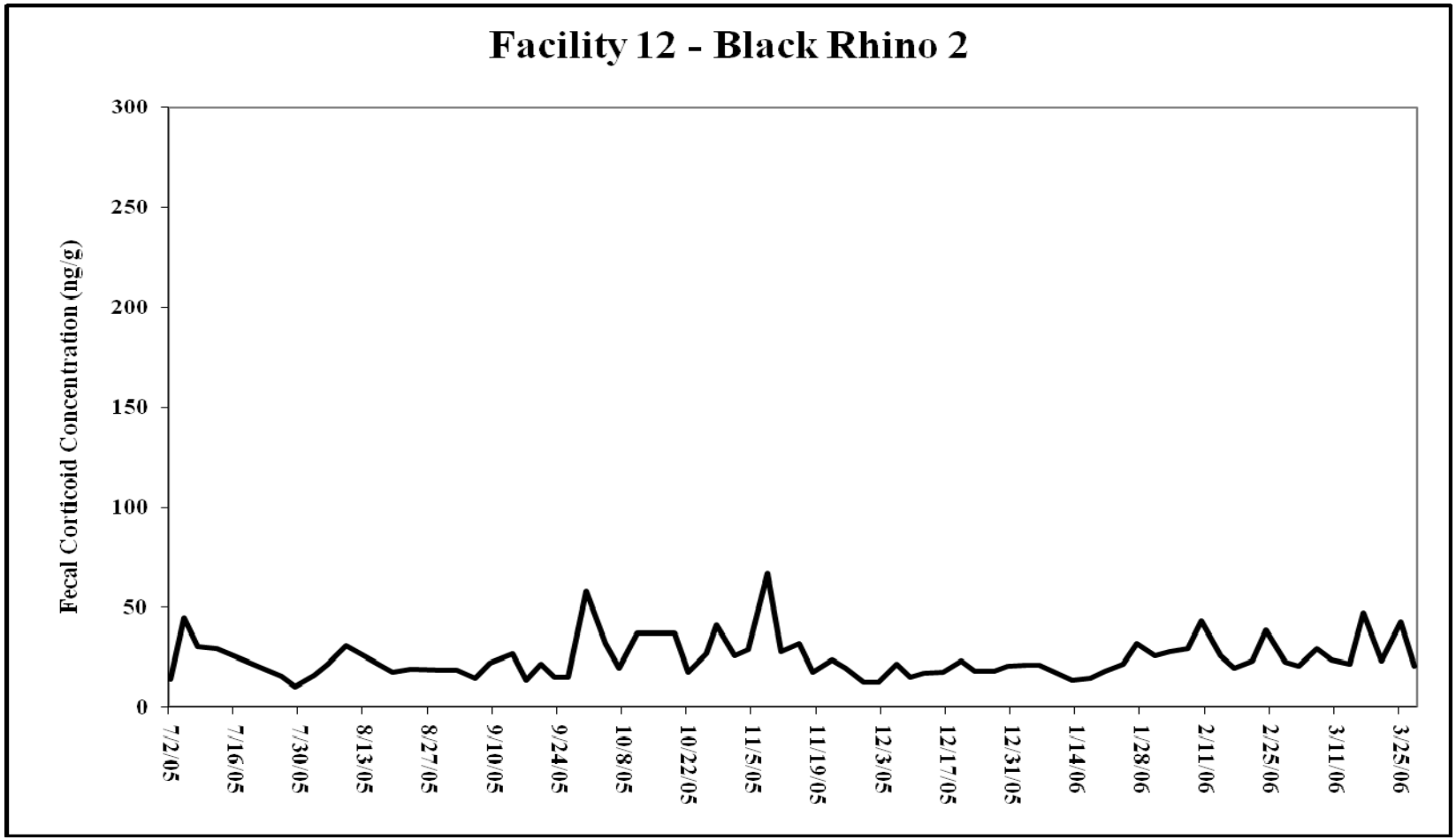

Facility 10 - Black Rhino 3*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
3/24/05
4/24/05
5/24/05
6/24/05
7/24/05
8/24/05
9/24/05
10/24/05
11/24/05
12/24/05
1/24/06
2/24/06
3/24/06
4/24/06
5/24/06
6/24/06
7/24/06

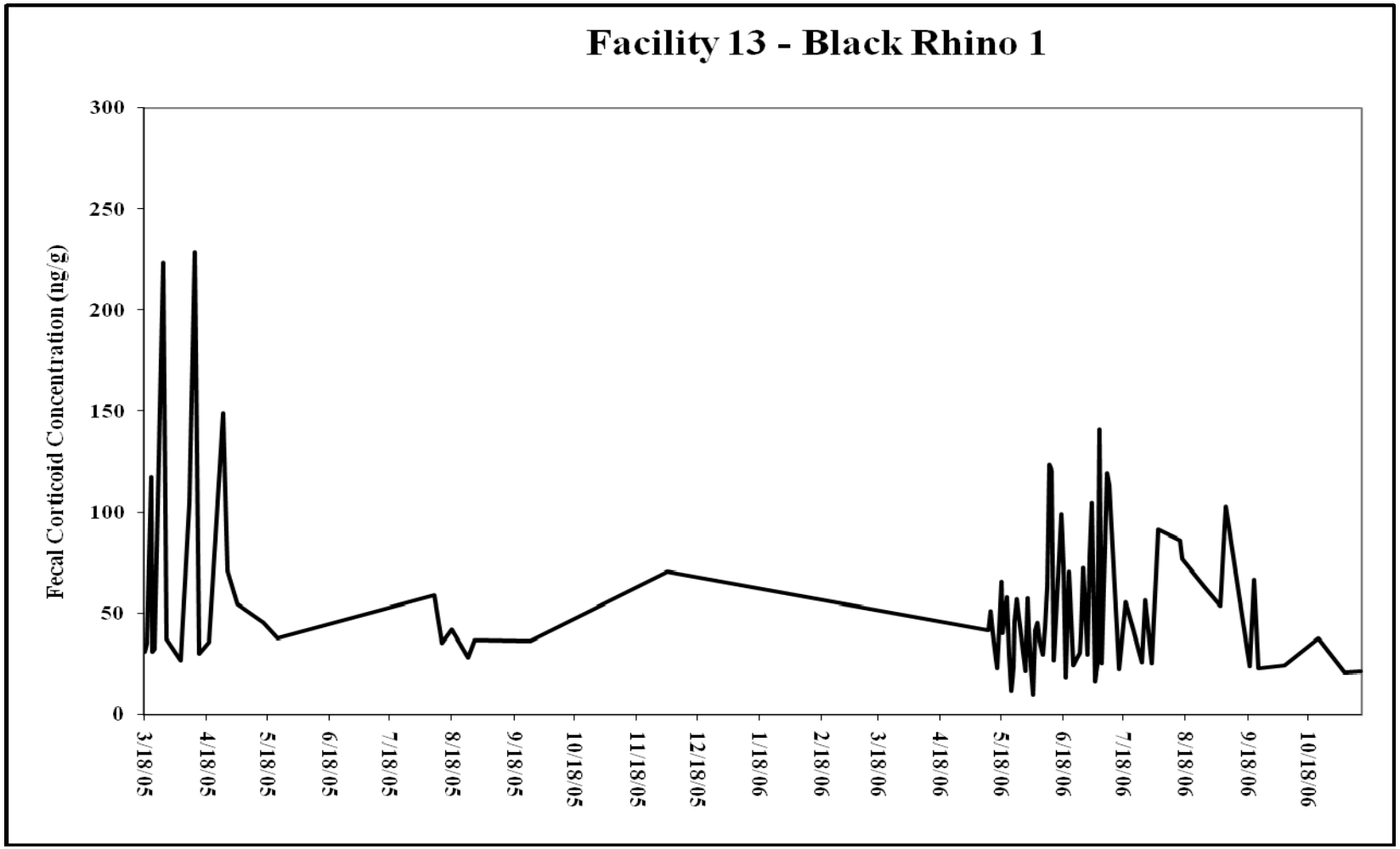

Facility 10 - Black Rhino 4
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
3/24/05
5/24/05
7/24/05
9/24/05
11/24/05
1/24/06
3/24/06
5/24/06
7/24/06
9/24/06
11/24/06
1/24/07
3/24/07

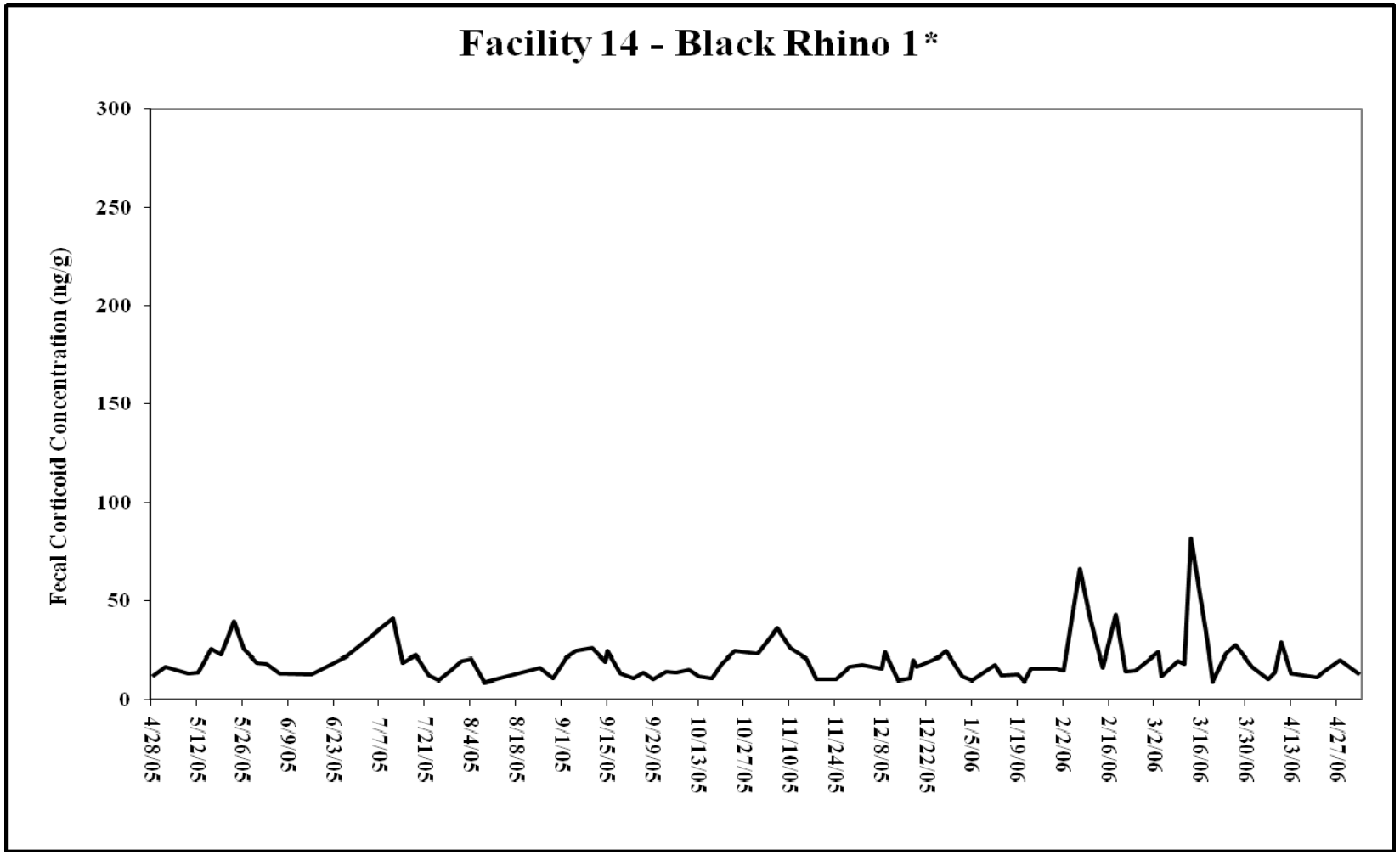

Facility 10 - Black Rhino 5
Fecal Cortiocid Concentration (ng/g)
300
250
200
150
100
50
0
8/24/06
7/24/06
6/24/06
5/24/06
4/24/06
3/24/06
2/24/06
1/24/06
12/24/05
11/24/05
10/24/05
9/24/05
8/24/05
7/24/05
6/24/05
5/24/05
4/24/05
3/24/05

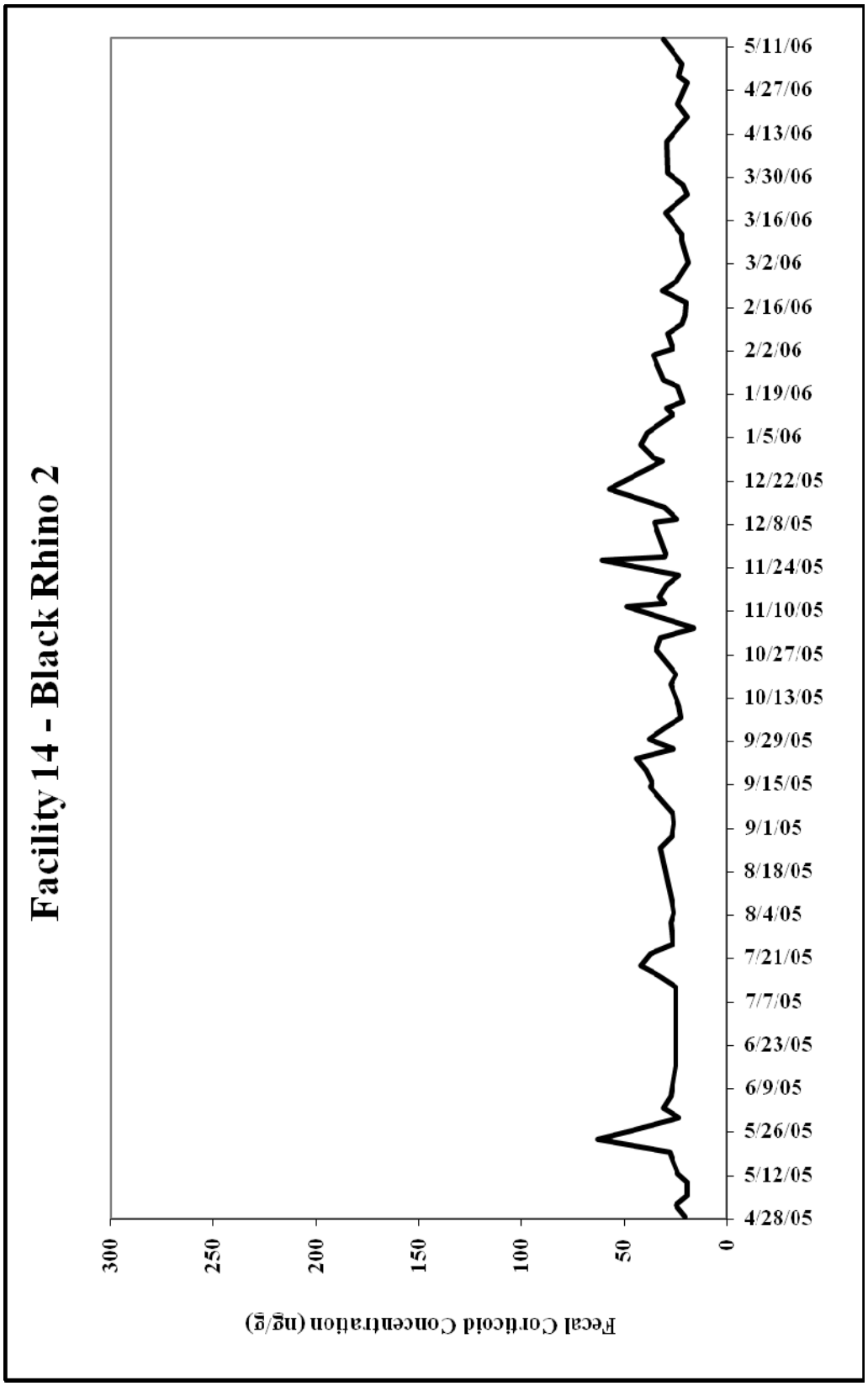

Facility 11 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
6/3/06
5/20/06
5/6/06
4/22/06
4/8/06
3/25/06
3/11/06
2/25/06
2/11/06
1/28/06
1/14/06
12/31/05
12/17/05
12/3/05
11/19/05
11/5/05
10/22/05
10/8/05
9/24/05
9/10/05
8/27/05
8/13/05
7/30/05
7/16/05
7/2/05
6/18/05
6/4/05
5/21/05
5/7/05
4/23/05
4/9/05


Facility 12 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
7/1/05
7/15/05
7/29/05
8/12/05
8/26/05
9/9/05
9/23/05
10/7/05
10/21/05
11/4/05
11/18/05
12/2/05
12/16/05
12/30/05
1/13/06
1/27/06
2/10/06
2/24/06
3/10/06
3/24/06


Facility 12 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
7/2/05
7/16/05
7/30/05
8/13/05
8/27/05
9/10/05
9/24/05
10/8/05
10/22/05
11/5/05
11/19/05
12/3/05
12/17/05
12/31/05
1/14/06
1/28/06
2/11/06
2/25/06
3/11/06
3/25/06


Facility 13 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
3/18/05
4/18/05
5/18/05
6/18/05
7/18/05
8/18/05
9/18/05
10/18/05
11/18/05
12/18/05
1/18/06
2/18/06
3/18/06
4/18/06
5/18/06
6/18/06
7/18/06
8/18/06
9/18/06
10/18/06


Facility 14 - Black Rhino 1*
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
4/28/05
5/12/05
5/26/05
6/9/05
6/23/05
7/7/05
7/21/05
8/4/05
8/18/05
9/1/05
9/15/05
9/29/05
10/13/05
10/27/05
11/10/05
11/24/05
12/8/05
12/22/05
1/5/06
1/19/06
2/2/06
2/16/06
3/2/06
3/16/06
3/30/06
4/13/06
4/27/06


Facility 14 - Black Rhino 2
Fecal Cortiocid Concentration (ng/g)
300
250
200
150
100
50
0
5/11/06
4/27/06
4/13/06
3/30/06
3/16/06
3/2/06
2/16/06
2/2/06
1/19/06
1/5/06
12/22/05
12/8/05
11/24/05
11/10/05
10/27/05
10/13/05
9/29/05
9/15/05
9/1/05
8/18/05
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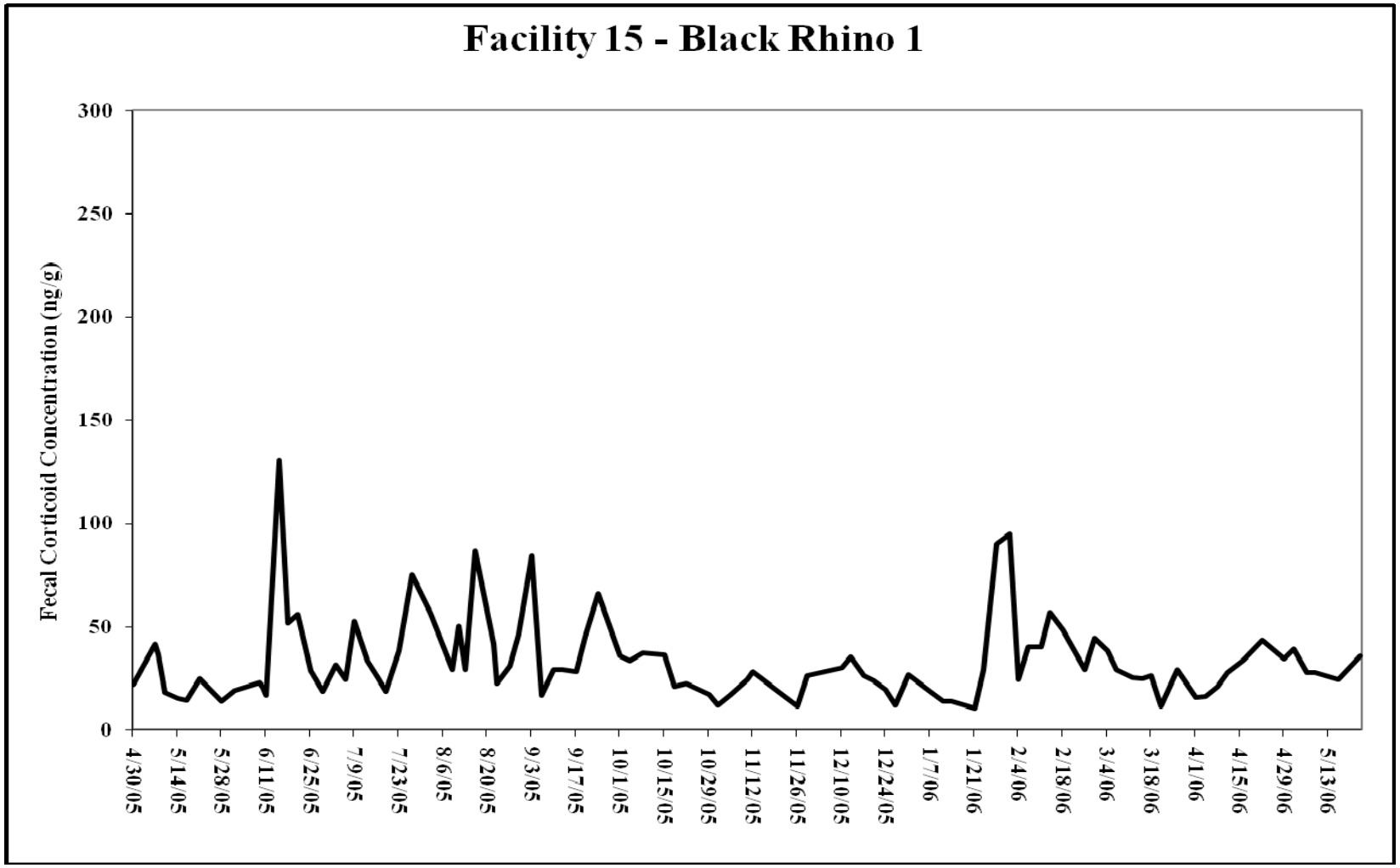
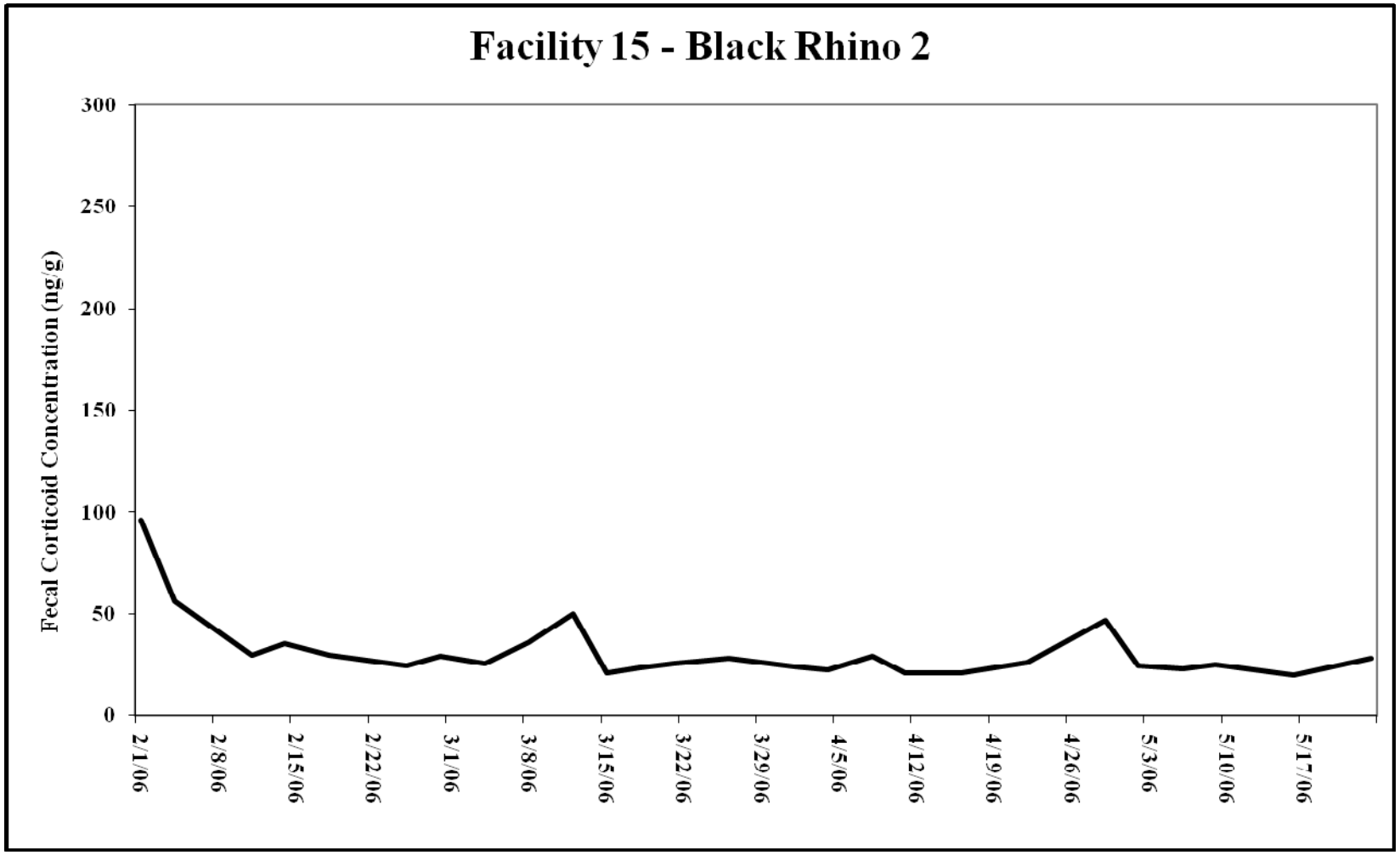
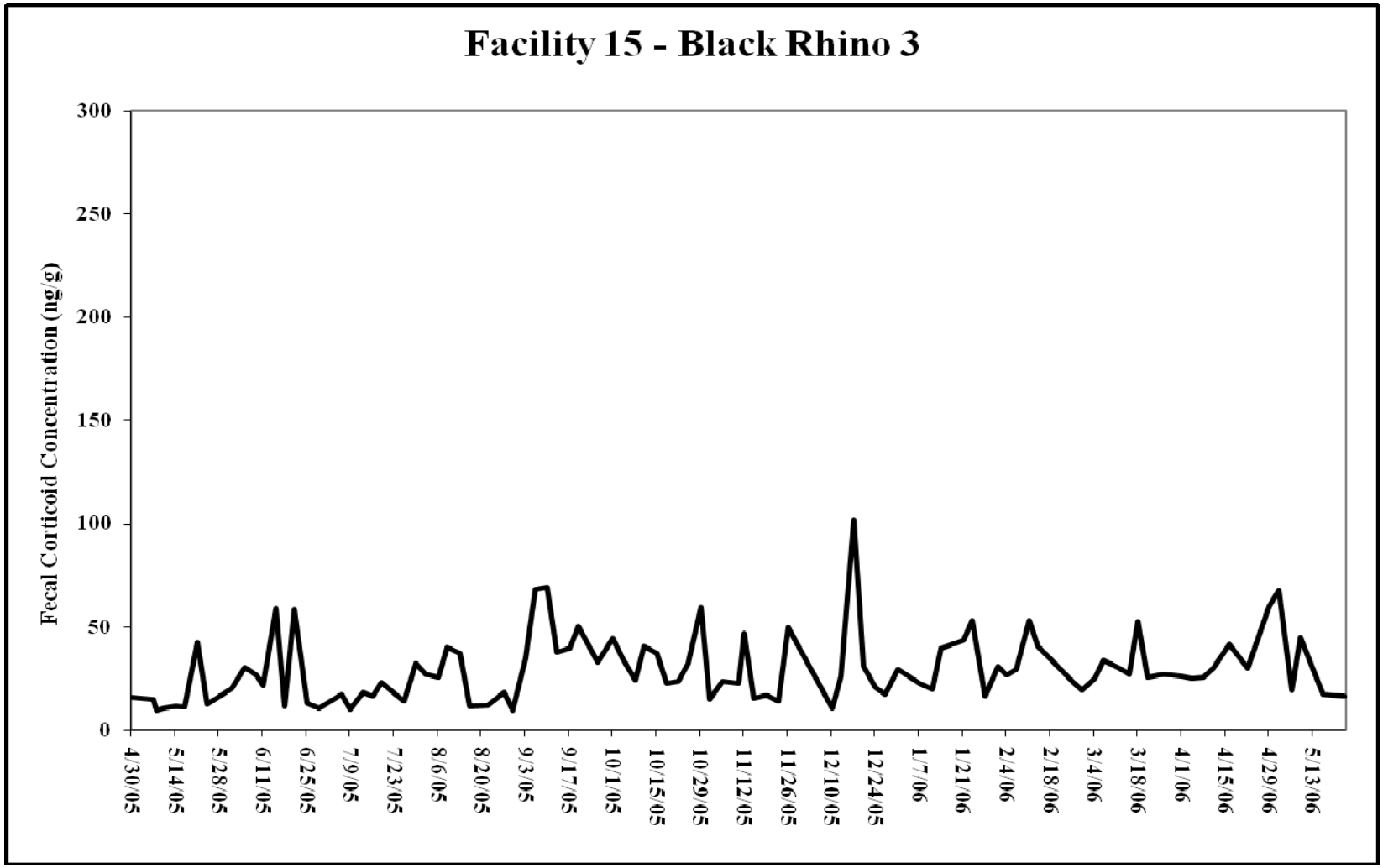
6/9/05
5/26/05
5/12/05
4/28/05


Facility 15 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
4/30/05
5/14/05
5/28/05
6/11/05
6/25/05
7/9/05
7/23/05
8/6/05
8/20/05
9/3/05
9/17/05
10/1/05
10/15/05
10/29/05
11/12/05
11/26/05
12/10/05
12/24/05
1/7/06
1/21/06
2/4/06
2/18/06
3/4/06
3/18/06
4/1/06
4/15/06
4/29/06
5/13/06


Facility 15 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
300
250
200
150
100
50
0
2/1/06
2/8/06
2/15/06
2/22/06
3/1/06
3/8/06
3/15/06
3/22/06
3/29/06
4/5/06
4/12/06
4/19/06
4/26/06
5/3/06
5/10/06
5/17/06


Facility 15 - Black Rhino 3
Fecal Corticoid Concentration (ng/g)
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250
200
150
100
50
0
4/30/05
5/14/05
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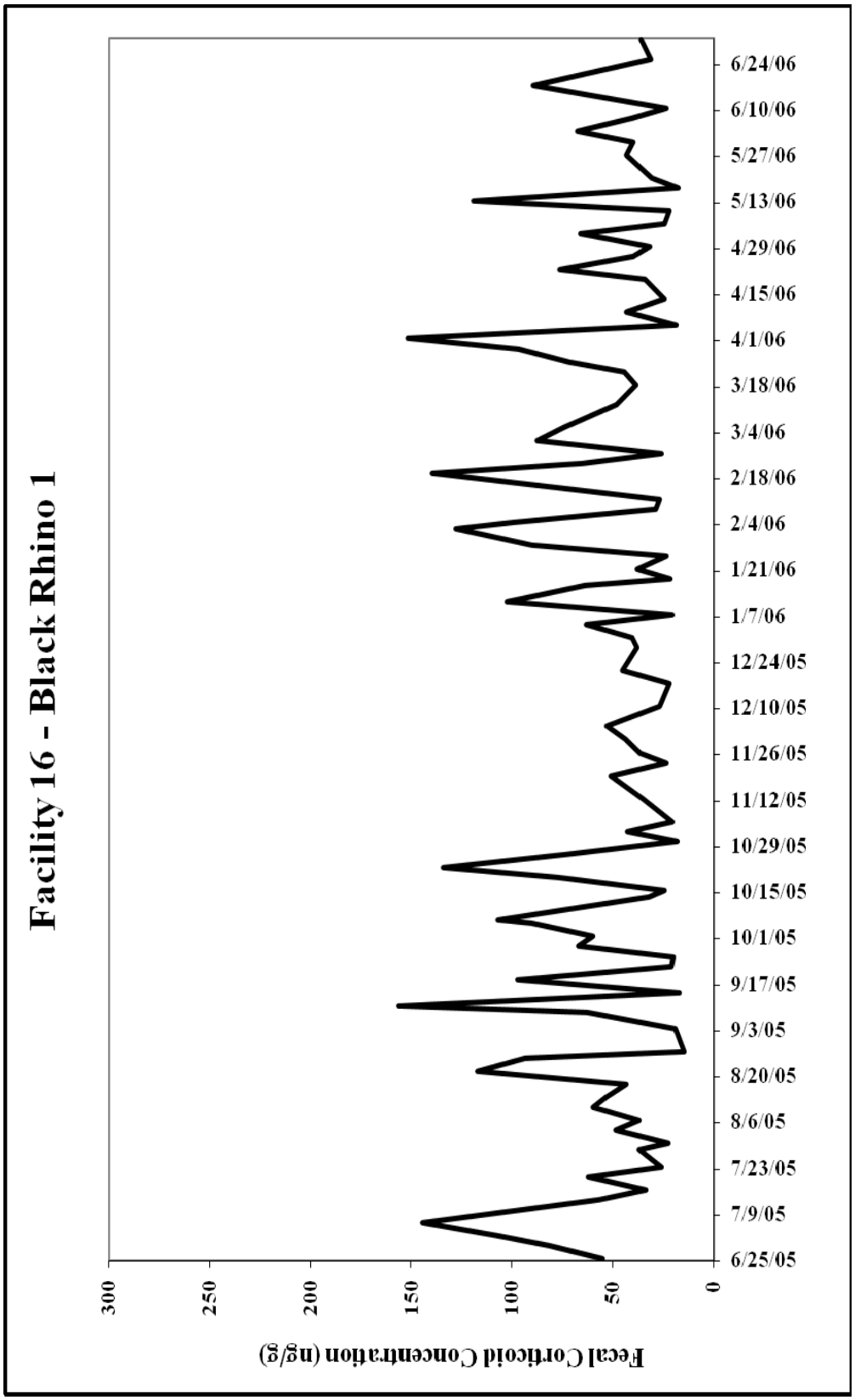
6/11/05
6/25/05
7/9/05
7/23/05
8/6/05
8/20/05
9/3/05
9/17/05
10/1/05
10/15/05
10/29/05
11/12/05
11/26/05
12/10/05
12/24/05
1/7/06
1/21/06
2/4/06
2/18/06
3/4/06
3/18/06
4/1/06
4/15/06
4/29/06
5/13/06


Facility 16 - Black Rhino 1
Fecal Corticoid Concentration (ng/g)
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200
150
100
50
0
6/24/06
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4/15/06
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3/18/06
3/4/06
2/18/06
2/4/06
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1/7/06
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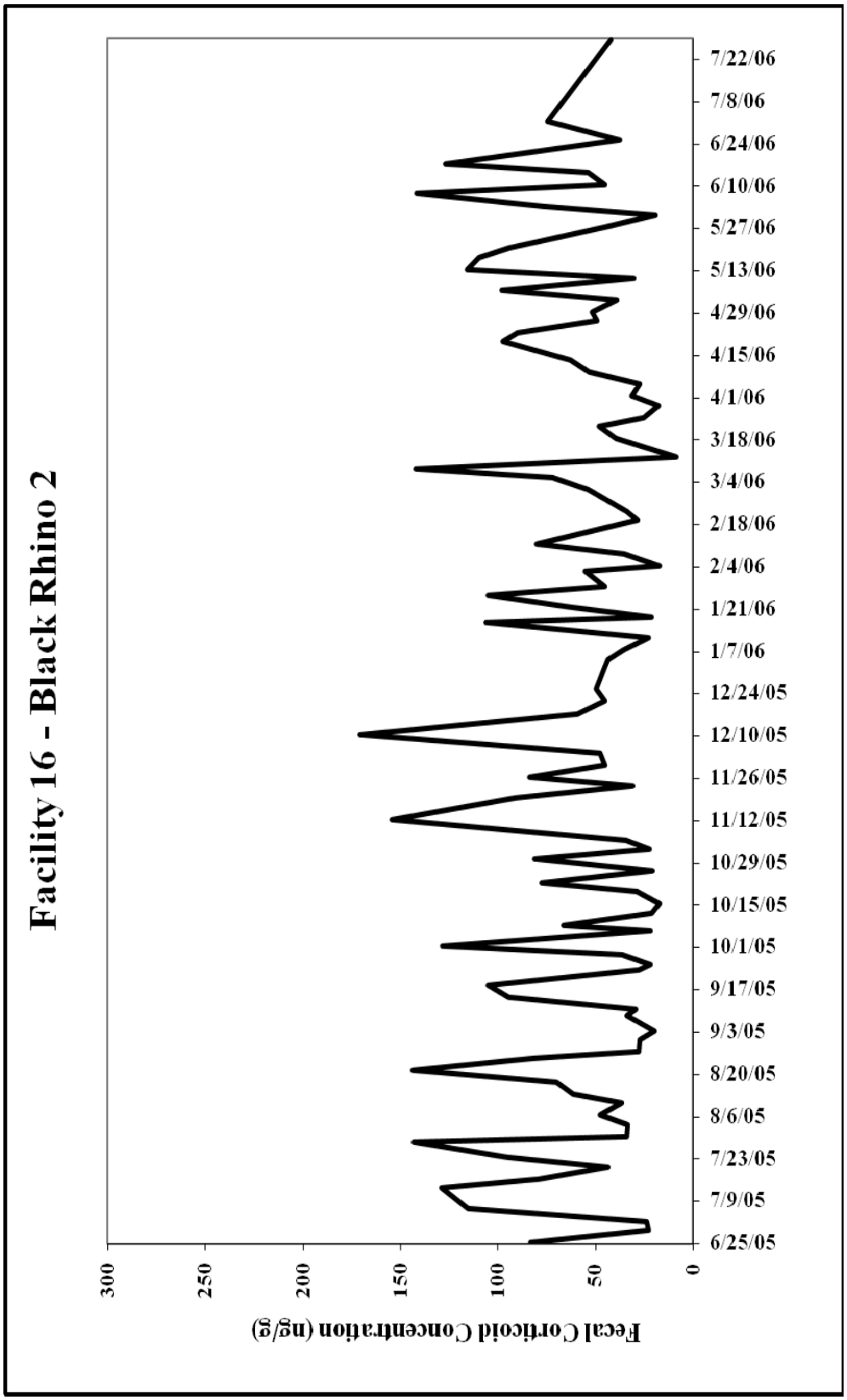
Facility 16 - Black Rhino 2
Fecal Cortiocid Concentration (ng/g)
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150
100
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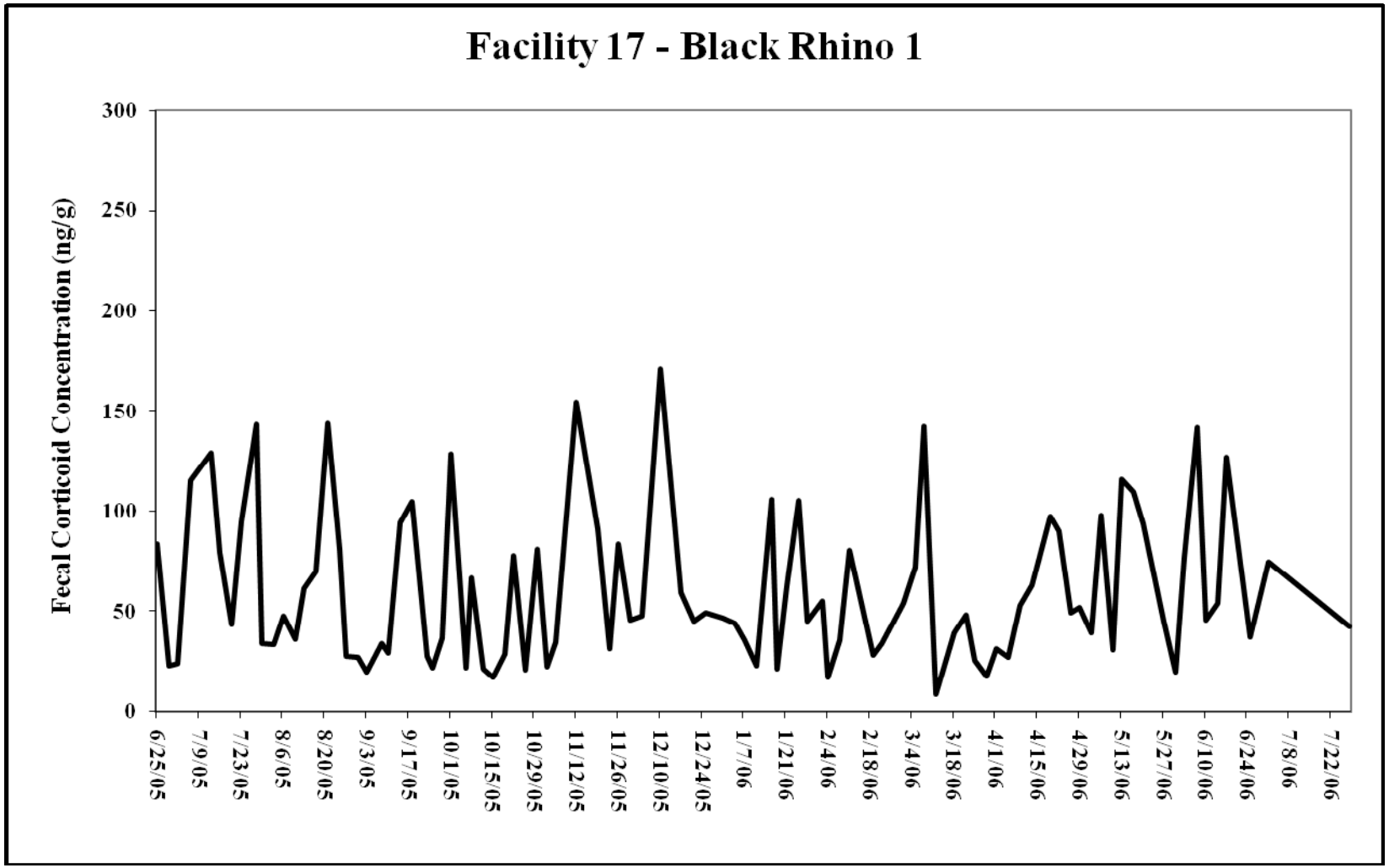
7/9/05
6/25/05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Fecal Corticoid Concentration (ng/g)
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200
150
100
50
0
6/25/05
7/9/05
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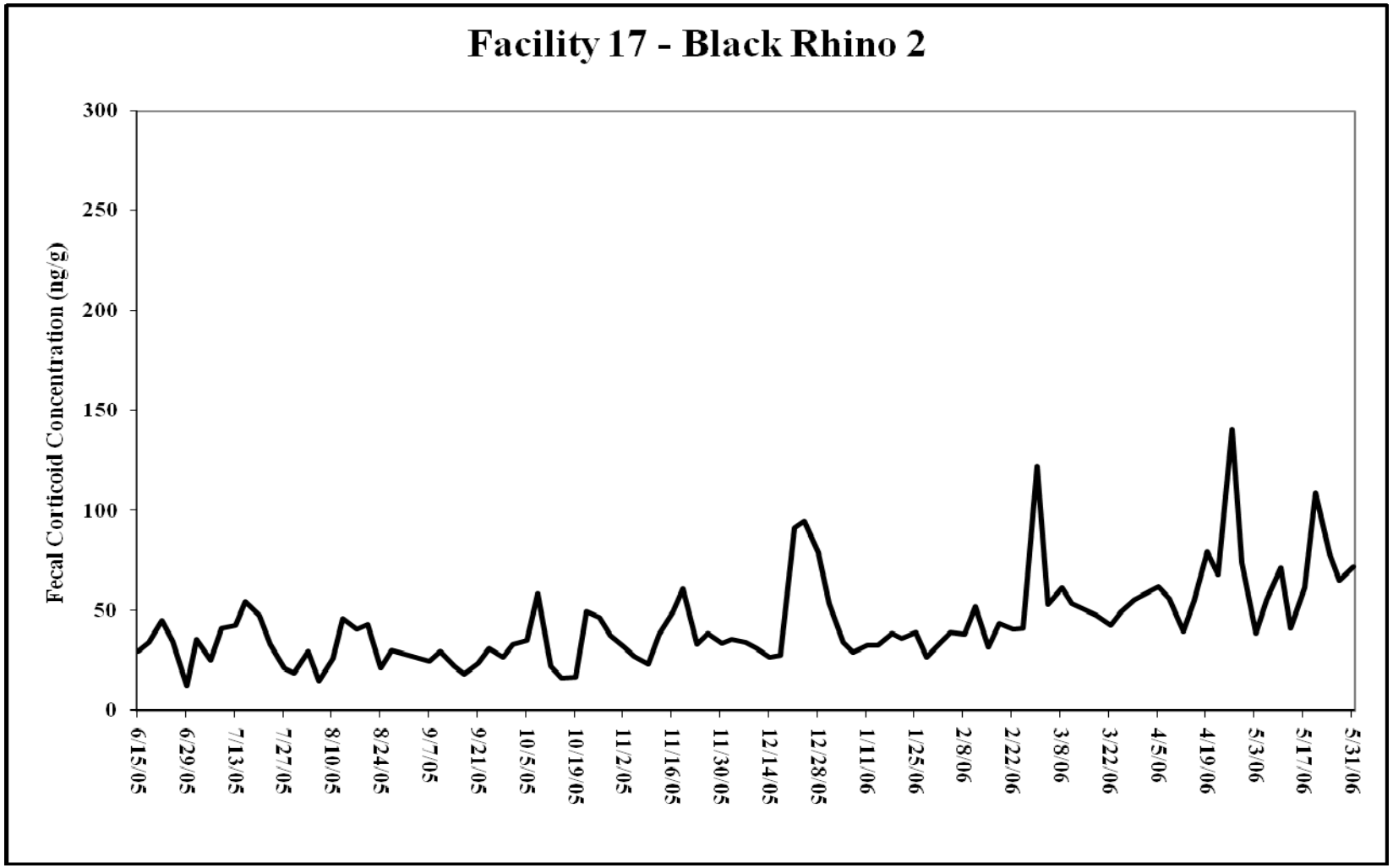
7/22/06


Facility 17 - Black Rhino 2
Fecal Corticoid Concentration (ng/g)
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250
200
150
100
50
0
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6/29/05
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12/14/05
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1/25/06
2/8/06
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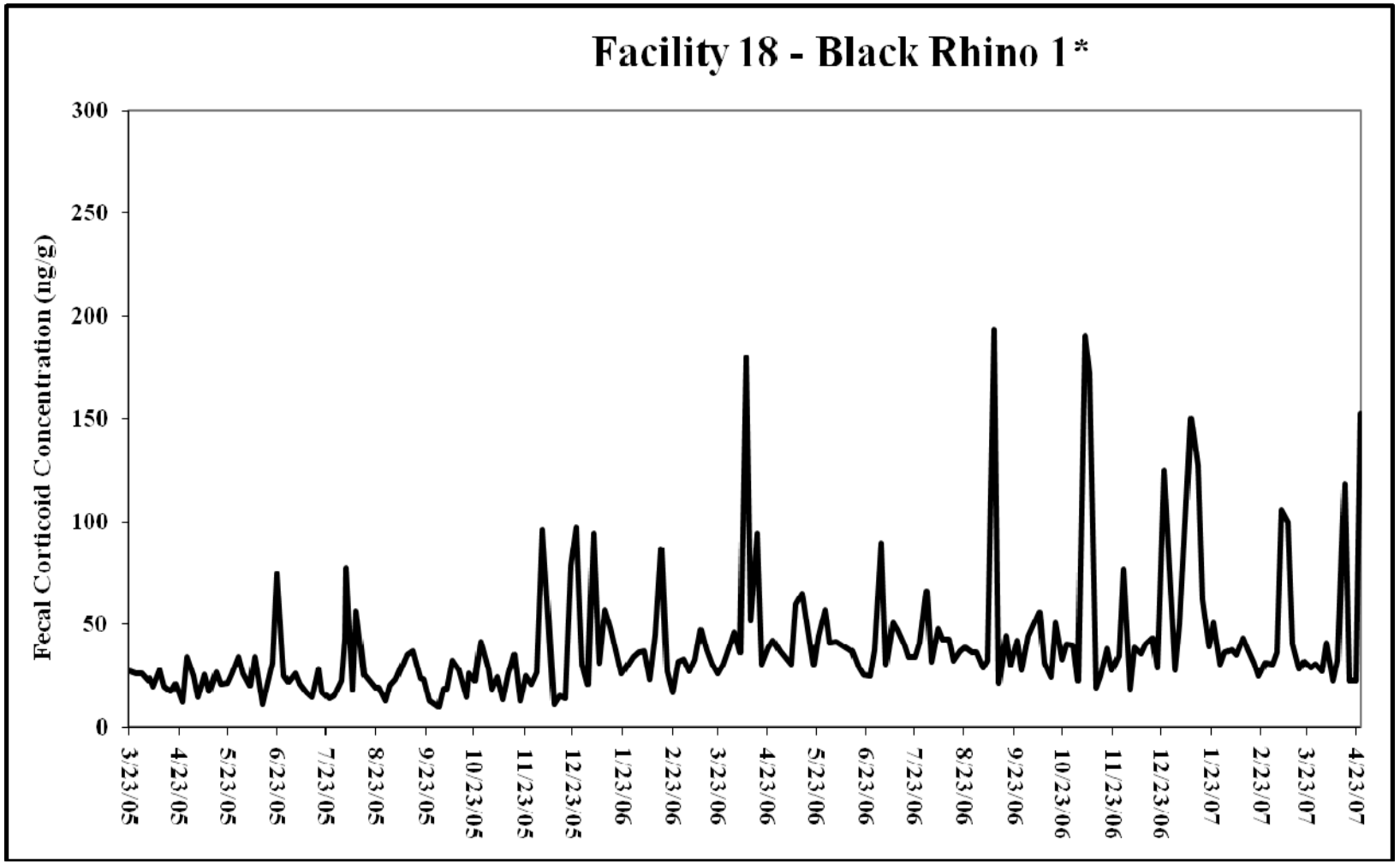
4/5/06
4/19/06
5/3/06
5/17/06
5/31/06


Facility 18 - Black Rhino 1*
Fecal Corticoid Concentration (ng/g)
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250
200
150
100
50
0
3/23/05
4/23/05
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7/23/05
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3/23/06
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6/23/06
7/23/06
8/23/06
9/23/06
10/23/06
11/23/06
12/23/06
1/23/07
2/23/07
3/23/07
4/23/07

APPENDIX VI

Fecal corticoid profiles for each individual. Black rhinos indicated by * had ulcerative lesions during the study.

Zoo	Rhino	Overall Mean ± SD	Baseline Mean ± SD	Elevated Mean ± SD	CV	Max	Min
1	**1**	31.4 ± 14.6	27.8 ± 8.4	69.1 ± 9.9	46.3	78.7	16.0
1	**2**	42.7 ± 27.3	35.6 ± 16.2	108.0 ± 20.1	63.8	145.9	14.8
1	**3**	40.2 ± 21.1	35.5 ± 15.3	87.3 ± 12.0	52.5	104.8	12.5
2	**1**	33.6 ± 26.8	27.9 ± 11.0	129.5 ± 33.6	79.8	183.7	8.9
2	**2**	28.4 ± 10.3	27.7 ± 7.7	55.9 ± 16.7	36.3	75.3	9.4
2	**3**	35.6 ± 29.6	28.4 ± 15.9	113.5 ± 32.3	83.1	177.9	10.7
3	**1**	46.9 ± 9.2	46.8 ± 6.4	66.1 ± 4.9	19.7	73.5	22.9
3	**2**	33.7 ± 9.4	32.5 ± 7.5	52.0 ± 4.5	28.0	64.4	15.1
4	**1**	38.4 ± 26.3	32.4 ± 14.5	107.9 ± 32.2	68.4	167.1	15.2
4	**2**	39.0 ± 17.9	35.2 ± 10.8	93.3 ± 13.5	46.0	109.2	20.3
4	**3**	34.5 ± 16.6	30.4 ± 9.5	79.3 ± 12.6	48.3	104.1	17.3
5	**1**	26.0 ± 15.2	23.8 ± 11.7	64.6 ± 20.2	58.6	107.0	4.0
5	**2**	25.1 ± 16.6	22.3 ± 10.7	76.5 ± 42.2	66.2	101.8	8.4
5	**3**	70.5 ± 40.5	62.3 ± 26.6	166.7 ± 43.1	57.5	282.3	9.3
5	**4**	56.3 ± 28.9	50.1 ± 21.0	120.5 ± 18.0	52.0	152.7	16.3
6	**1***	24.7 ± 5.1	23.3 ± 3.8	34.6 ± 1.6	20.9	37.6	17.1
6	**2**	69.0 ± 44.2	57.3 ± 20.2	188.6 ± 44.6	64.0	245.1	25.5

6	**3***	50.8 ± 29.1	43.9 ± 19.2	120.9 ± 15.8	57.3	150.7	19.6
7	**1**	51.6 ± 36.7	44.6 ± 26.7	141.5 ± 29.5	71.2	179.7	9.7
7	**2**	32.3 ± 12.0	33.2 ± 10.0	107.4 ± 58.5	50.2	148.8	2.7
8	**1**	67.8 ± 29.2	64.8 ± 22.9	134.2 ± 11.8	43.1	142.5	20.7
8	**2**	47.7 ± 17.7	43.9 ± 11.0	95.8 ± 16.6	37.2	123.2	22.5
8	**3**	84.9 ± 51.4	71.6 ± 37.7	189.1 ± 39.2	60.6	216.8	28.2
8	**4**	69.7 ± 32.8	62.5 ± 21.1	149.0 ± 18.1	47.0	175.3	14.0
8	**5**	44.8 ± 24.5	41.6 ± 12.2	171.1 ± 59.1	54.6	212.9	17.9
9	**1***	41.0 ± 21.0	35.4 ± 13.8	86.5 ± 10.6	51.2	104.9	13.4
10	**1**	51.4 ± 19.2	46.7 ± 12.9	93.6 ± 14.9	37.4	111.3	24.7
10	**2***	36.4 ± 20.5	30.2 ± 11.0	85.7 ± 5.3	56.4	91.5	16.6
10	**3***	30.2 ± 23.7	27.5 ± 10.6	143.8 ± 107.0	78.7	219.5	9.4
10	**4**	41.8 ± 19.8	37.7 ± 10.8	103.1 ± 23.2	47.3	148.7	15.9
10	**4**	26.7 ± 13.7	23.6 ± 8.4	61.2 ± 14.3	51.2	89.7	10.1
11	**1**	73.3 ± 35.4	65.9 ± 22.3	171.9 ± 29.2	48.3	215.1	40.1
12	**1**	24.3 ± 14.9	20.1 ± 8.5	59.0 ± 10.8	61.3	78.8	8.6
12	**2**	24.9 ± 10.7	22.3 ± 6.7	49.3 ± 9.6	43.0	67.0	10.3
13	**1**	54.9 ± 40.5	46.2 ± 25.0	152.8 ± 46.4	73.8	228.5	9.6
14	**1***	19.3 ± 11.3	17.0 ± 5.9	52.5 ± 17.6	58.2	82.2	8.6
14	**2**	29.2 ± 8.9	27.4 ± 5.8	53.4 ± 7.9	30.5	62.8	16.0
15	**1**	33.2 ± 20.1	28.7 ± 11.4	89.6 ± 20.4	60.7	130.3	10.4
15	**2**	31.4 ± 16.1	27.7 ± 7.6	76.0 ± 28.4	51.1	96.1	19.7
15	**3**	29.9 ± 16.5	26.5 ± 11.7	68.1 ± 14.3	55.1	101.7	9.6

16	**1**	55.5 ± 35.2	47.8 ± 25.5	136.3 ± 14.4	63.4	156.2	14.4
16	**2**	60.1 ± 37.9	51.6 ± 27.9	142.2 ± 13.9	63.0	170.7	8.5
17	**1**	81.4 ± 58.8	72.0 ± 49.0	203.0 ± 37.0	72.3	265.5	2.7
17	**2**	43.9 ± 22.2	39.0 ± 13.9	99.3 ± 22.8	50.5	140.5	12.3
18	**1***	39.9 ± 30.8	32.2 ± 12.9	128.0 ± 37.7	77.0	193.8	10.3

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