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Patterns of Chemosensory Behavior in a Closed Population of Wild African Elephants (*Loxodonta Africana*)

Russell W. Blogg

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PATTERNS OF CHEMOSENSORY BEHAVIOR IN A CLOSED POPULATION
OF WILD AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

by

RUSSELL W. BLOGG

(Under the direction of Bruce A. Schulte)

ABSTRACT

Chemosensory behaviors are used by many mammalian species to assess chemical signals in the environment. These chemical signals may contain important information about reproductive state, identity, status, or location of conspecifics. Elephants are a long-lived species and males reproduce at a much later age than females, which provides a protracted developmental period for males. This study examined chemosensory behaviors in a population of African elephants living in Addo Elephant National Park, South Africa and demonstrated that patterns emerge as elephants develop and approach sexual maturity. Older pubescent males (15-19 year olds) performed more chemosensory behaviors than younger pubescent males (10-14 year olds) and both groups of pubescent females, which supported the hypothesis that there would be an increase of these behaviors as males approach sexual maturity and the age of their first musth. It is clear that developing pubescent male elephants are paying attention to chemical signals in their environment, many of which are directly related to female reproductive condition. Increased chemosensory behavior by pubescent males observed in this study highlights the importance of chemical communication in elephants.

INDEX WORDS: *Loxodonta africana*, Chemical Communication, Chemosensory Behavior, Development, Musth, South Africa

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RUSSELL W. BLOGG

B.S., Illinois Wesleyan University, 2005

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

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DEDICATION

This work is dedicated to my wife Molly. I could not have done it without you.

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Introduction

Pheromones are important components of mammalian communication systems that contain vital information for some conspecifics. Mammalian pheromones can affect physiology, behavior and reproduction (Rekwot et al. 2001). The exchange of chemical signals functions in intrasexual selection (Kappeler 1998) and development of chemosensory behaviors (Loizi et al. 2009); scent marking serves a role in territorial demarcation as well as a potential honest advertisement of physical condition and mate quality in some species (Sillero-Zubiri and Macdonald 1998; Whittle 2000; Schulte et al. 2007); and chemicals in urine signal reproductive state (Rasmussen et al. 1997; Swaisgood et al. 2002b). Correctly detecting and understanding these chemical signals is critical to the reproduction and survival of many long-lived mammalian species, especially as they develop and approach sexual maturity.

Developmental patterns of chemosensory behaviors are important to understand when considering chemical communication in mammals. Studies have been done on several mammals which focus on the ontogeny of these behaviors. A study on rats (*Rattus norvegicus*) found the development toward adult patterns of behavior was associated with the period of sexual maturation, between days 36 and 50 (Meaney and Stewart 1981). The development of sexual behavior in male rats is characterized by increases in circulating levels of testicular androgens and chemosensitivity to conspecific urine, which increases dramatically with age (Alberts and May 1980; Meaney and Stewart 1981). In ring-tailed lemurs (*Lemur catta*), olfactory investigation appears and matures earlier than scent-marking (Palagi et al. 2002). Lemurs use the juvenile and adolescent periods to develop many behaviors typical of adulthood, notably the olfactory behavioral pattern, which is important for effective chemical communication in this species (Palagi et al. 2002). A study by Hong et al. (2007) examined the developmental pattern

of behavioral responses of male giant pandas (*Ailuropoda melanoleuca*) to urine odors of conspecifics; chemosensory cues from conspecific urine were found to induce age specific responses in male pandas. Exposure to adult female urine elicited a significant increase in sniffing and licking behaviors in male pandas; adult males spent more time licking than adolescents and juveniles; another interesting developmental observation was that adolescent and adult males displayed high levels of environmental sniffing/licking, which was absent in juvenile males (Hong et al. 2007). These investigative behaviors to environmental stimuli may precede flehmen, a chemosensory behavior which presents chemical signals to the vomeronasal organ and is referred to as vomerolfaction (Rasmussen and Schulte 1998; Rasmussen 2006)

Flehmen has been investigated in numerous mammals and this behavior, when displayed by adults, is associated with evaluating sexual condition of conspecifics (Estes 1972; Pfeifer 1985; Johnston 2000; Weeks et al. 2002; Bagley et al. 2006). Flehmen is not only performed by adults, but also by younger individuals. Juvenile male horses (colts, less than 4 years old of *Equus caballus*) perform more flehmen behaviors than females, and foals (less than 1 year old) of both sexes perform flehmen more than their mothers (Crowell-Davis and Houpt 1985). Colts also have a higher mean rate of flehmen than females in the same age class (fillies) and they have a threefold higher rate of flehmen compared to adult females. Semi-wild cattle (*Bos indicus*) display flehmen frequently after 4 months of age (onset of puberty) and after this age, male calves display the behavior more frequently than and mostly toward females (Reinhardt 1983). Flehmen rate is highest in adult males with adolescent males ranking second and juvenile males ranking third. For both sexes of sable antelope (*Hippotragus niger*), flehmen rates increase with age, and mean flehmen rates for males exceed those of females throughout development

(Thompson 1995). Sex differences are observed in sable antelope with males exhibiting flehmen earlier in life and at greater frequencies than females.

Olfaction and vomerolfaction are well developed in many mammals and have been studied extensively in species such as ring-tailed lemurs, pandas, and elephants (Palagi et al. 2002; Swaisgood et al. 2002a; Brennan and Keverne 2004; Schulte et al. 2005). In ring-tailed lemurs, olfactory communication is very important and consists of investigative and scent-marking behaviors (Kappeler 1990). In lemurs, chemical signals convey information about identity, sex, emotional state, social status, and reproductive condition (Palagi et al. 2003; Palagi and Dapporto 2006). Sniffing genitals, a common chemosensory behavior that male lemurs perform to female lemurs, occurs at the highest frequency during the breeding season and it is likely that males use olfactory cues to determine the onset of estrous in females (Palagi et al. 2003). Giant pandas rely on pheromones, depositing them in areas used communally by conspecifics, for intraspecific communication (Swaisgood et al. 2000; White et al. 2002; Swaisgood et al. 2002b; Hong et al. 2007; Nie et al. 2012). These chemical signals help pandas locate and choose mates, assess potential competitors, regulate their use of space, and advertise their social status (Swaisgood et al. 1999, 2002a). Male pandas scent mark year-round with higher frequency during the mating season while female pandas scent mark predominantly during the mating season (Yuan et al. 2004). Pandas depend on chemical communication to coordinate their social lives and it is believed that scent marks play a crucial role in bringing mates together during the female's annual 1-3 day fertile period (Schaller et al. 1985). Both Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants detect chemical signals in the environment (e.g., urine, feces, temporal gland secretions, conspecifics) through specific chemosensory behaviors (Rasmussen and Schulte 1998; Langbauer 2000; Schulte et al. 2005).

Elephants use four main chemosensory behaviors (sniff, check, place and flehmen) to detect chemical cues in their environment (Figure 1; Schulte et al. 2005). Adult males rely on chemosensory behaviors to locate other elephants and receptive mates (Langbauer 2000; Loizi et al. 2009). In the case of the flehmen chemosensory behavior, the trunk is the transport facilitator and allows the chemical signal to contact the vomeronasal organ system (Rasmussen and Schulte 1998). Elephants are similar to other animals in that they use the vomeronasal organ to detect chemicals signals of reproductive significance (Rasmussen 1988; Schulte and Rasmussen 1999; Meyer et al. 2008). A well-supported hypothesis suggests that the flehmen behavior allows males to determine the estrous state of a female through the chemosensory functions of the vomeronasal organ (Weeks et al. 2002). Male black-tailed deer (*Odocoileus hemionus columbianus*) typically perform flehmen in response to a urinating female (Altieri and Müller-Schwarze 1980). The rate of flehmen by horse stallions varies with the estrous cycles of mares (Stahlbaum and Houpt 1989). These researchers proposed that the flehmen response may contribute to the chemosensory priming of the stallion for reproduction. Flehmen is not limited to males; females and juveniles (of both sexes) also perform this behavior, potentially to assess reproductive state (Estes 1972). In most mammals, however, females perform flehmen far less commonly than do males. Similarly, in African elephants, males appear to perform chemosensory behaviors more extensively than females (Bagley et al. 2006; Loizi et al. 2009; Merte et al. 2010), and it is often adult males in the rut-like state of musth that perform flehmen to females to assess their reproductive condition (Hall-Martin 1987; Poole and Moss 1989).

Chemical communication plays an integral part in the reproductive and social success of elephants (Rasmussen and Schulte 1998; Langbauer 2000; Loizi 2004). The elephant trunk, an extension of the nose and upper lip, is almost constantly in motion, investigating the surrounding

environment and guiding the animal through its habitat and facilitating interactions with conspecifics (Schulte et al. 2005; Rasmussen 2006). Fluids from the anal/genital areas and the temporal gland are the most likely, physiologically dependent sources for intraspecific chemical signals in elephants (Rasmussen and Schulte 1998). These chemical signals serve as reliable indicators of a sender's physiological condition, providing information about status, health, and reproductive condition such as estrus in females or musth in males (Rasmussen and Schulte 1998; Rasmussen and Greenwood 2003; Rasmussen 2006).

Male and female elephants differ in their age of sexual maturation (Poole 1994). Ovulation may begin in females as young as seven years and pregnancy can occur as early as eleven years (Moss 1996). Male elephants start producing gametes at a similar age during their development, but are not able to compete with older bull elephants for females until their early twenties. Developmental differences are observed between sexes even during the pre-pubescent years. Male calves play more aggressively than female calves, often taking part in mock fights, while female calves spent more time near their mothers and relatives developing strong familial bonds (Lee 1986). These developmental differences in play behaviors and interactions between young male and female elephants are related to later mating strategies and association patterns in adult elephants (Lee 1986; Meyer 2006). In a study on African elephants, Merte et al. (2010) examined the presence of sexual dimorphism in trunk behaviors that are indicative of olfactory investigation; this study found that females exhibited adult-like rates of chemosensory behavior at an earlier age than males because females mature sooner; they also observed that adult males displayed greater investigatory rates to conspecific feces than females and younger males. Another study found that older elephants showed higher rates of chemosensory behaviors than younger elephants; male elephants, especially the older age classes (pubescents and adults) were

more likely to investigate urine and feces with their trunk than female elephants (Loizi et al. 2009). Pubescent elephants are equivalent to adolescent elephants, referring to sub-adult individuals between the ages of 10-19; elephants in this age class have been called either pubescents or adolescents (Evans and Harris 2008; Loizi et al. 2009).

African elephants are a polygynous species that live in matriarchal societies with related females and offspring remaining together in relatively small herds. When young males reach puberty, usually at about 14 years of age (Poole 1994), they become independent and disperse from their natal group (Dublin 1983). Adult males spend a majority of their lives alone or in temporary, loosely organized bachelor herds, joining female groups occasionally for breeding purposes (Dublin 1983; Evans and Harris 2008). Since adult males and females can be widely separated, finding each other for mating requires reliable signals (Schulte et al. 2005; Schulte et al. 2007; Goodwin and Schulte 2009). In Asian elephants, for example, males can detect a chemical signal from female urine to determine if she is reproductively ready (Rasmussen et al. 1982; Rasmussen et al. 1997). In African elephants, the presence of a functionally similar chemical is suggested (Bagley 2004; Bagley et al. 2006). Female elephants have a 16-week estrous cycle and are only receptive for a 5-7 day period during the cycle (Rasmussen and Schulte 1998). During this relatively short receptive period, an acceptable male must locate and form a mating pair with a female.

There have been several different studies prior to this one that examined different aspects of chemosensory behavior on the same population of elephants as the current study. Loizi (2004) initiated the research by studying male and female elephants of all ages at Addo Elephant National Park (AENP), trying to better understand the chemosensory investigatory behaviors and how sexual dimorphism may affect the development of these behaviors. Loizi (2004) found

significantly more males than females responding to urine and feces, potential sources of chemical signals. Pubescent males showed a consistent peak in the rate of their chemosensory behaviors; chemosensory behaviors for male elephants increased from calves, peaked with pubescent males and then showed a slight decline for adult males. In addition, pubescent and adult male elephants demonstrated more flehmen behaviors than younger males and females of all ages. Bagley (2004) continued the research by focusing on male elephants in the Addo population as well as male elephants in captivity. Male calves performed more flehmens when compared to older males. Bagley et al. (2006) found that adult males in captivity showed greater chemosensory responses to periovulatory urine than luteal urine of female elephants, which supports the hypothesis that there is a preovulatory pheromone in African elephants. Merte (2006) took the research a step further by studying female elephants at AENP. Check and place behaviors were performed primarily by sexually mature males and females, indicating that these behaviors may be associated directly with reproductive capability; Merte (2006) also observed that the average rate of sniffs to all substrates increased from the calf to the juvenile stage for females, and for male elephants the increase was from the juvenile to the pubescent stage. Meyer (2006) examined pre-pubescent elephants (calves and juveniles) and studied the impact of play on the development of chemosensory behaviors. Young male elephants showed greater interest to urine and feces than young females, similar to patterns seen in adult elephants (Loizi 2004). The cumulative efforts of these researchers helped set a foundation for a better understanding of how elephants develop and use chemosensory behaviors to respond to chemical signals in their environment. The current study built upon knowledge gained by past work at AENP and sought to broaden our understanding on developmental patterns of chemosensory behavior in elephants by examining these behaviors in pubescent elephants.

The first objective of this study was to examine patterns of chemosensory behavior in adolescent elephants, also termed pubescent elephants. Females at this 10-19 year stage are in their early reproductive years, while males are transitioning to the size of adult females and approaching the age of their first musth (Poole and Moss 1989). During these adolescent years, it is likely that elephants begin to pay more attention to chemical signals in their environment and respond appropriately. For this study, adolescent elephants, henceforth called pubescent elephant, were categorized into two groups: 10-14 year olds and 15-19 year olds. Males in the younger group are still living with their family group or have recently left while males in the older group have been living away from their family group for several years, are going through a growth spurt, and are associating primarily with other males in temporary bachelor groups (Lee 1986; Poole 1994; Moss 1996; Evans and Harris 2008). Females in the younger group are still growing and have recently had their first calf while females in the older group have reached their adult size, are old enough to have had their second calf, and are more experienced mothers (Lee 1987; Poole 1994; Moss 1996). Because of these developmental differences between pubescent males and females and between 10-14 and 15-19 year olds, I hypothesized that there would be some behavioral differences between these four groups. I predicted that 15-19 year old males would perform more chemosensory behaviors than the other three pubescent groups because they are nearing sexual maturity and the age of their first musth. Also, these older pubescent males are independent of their natal groups and are sexually interested in females (even though socially, they are not old or mature enough to mate) so it stands to reason that older pubescent males will have higher rates of chemosensory behaviors than younger pubescent males. Both groups of pubescent females are caring for offspring as mothers or allomothers and as with all female elephants do not need to search for mates, so I predicted that there would be no difference

in their rates of chemosensory behaviors. Because pubescent individuals bridge the juvenile and adult developmental stages, and because extensive data were lacking on this age group, I focused my efforts on pubescent elephants during this study.

The second objective of this study was to examine the occurrence and development of chemosensory behaviors for the entire population. The main purpose of this objective was to compare my results with findings of past researchers that studied the same population of elephants (Loizi 2004; Bagley 2004; Merte 2006; Meyer 2006). For this objective, data were collected on males and females in four age classes (Calves: 0-4 years old, Juveniles: 5-9 years old, Pubescents: 10-19 years old, Adults: 20+ years old). Because elephants are sexually dimorphic, I hypothesized there would be behavioral differences between males and females. I predicted that males would perform more chemosensory behaviors than females, which would support previous observations at this site (Loizi 2004; Loizi et al. 2009). With respect to the development of these behaviors, I hypothesized that the rate of chemosensory behaviors would increase with age. I predicted that the pubescent and adult age classes would perform more chemosensory behaviors than the calf and juvenile age classes, which would match the findings of previous studies conducted in Addo Elephant National Park (Loizi 2004; Merte 2006; Loizi et al. 2009; Merte et al. 2010). By comparing results with past elephant researchers, the hypotheses and predictions made in those studies as well as this study may be strengthened if the results are found to corroborate each other.

Methods

Study Site

This study was conducted in Addo Elephant National Park (AENP), which is located approximately 70 km northeast of Port Elizabeth in the Eastern Cape Province of South Africa (Figures 2 and 3). The entire park covers approximately 700 sq. km, but the elephants are restricted to a fenced area of 103 sq. km (Whitehouse et al. 2001; Whitehouse and Hall-Martin 2000). The park's vegetation is classified as xeric succulent thicket (Low and Rebulo 1996 as cited in Whitehouse and Schoeman 2003). The vegetation is largely dense thorny thicket with a wide range of shrubs, grasses, herbs, succulents, and other plants (Paley and Kerley 1998). Lateral visibility within the thicket is poor (i.e. < 5 m); however, roads, open grasslands, and water holes are scattered throughout the park, providing visual access to the elephants (Whitehouse and Schoemen 2003).

Study Population

In 1931, AENP was created to preserve the eleven remaining elephants in the area, and they became the founder population of the current Addo elephant population (Whitehouse and Hall-Martin 2000). From 1931 to 1954, insufficient fencing failed to keep the elephants within their designated land reserve. An elephant-proof fence was built in 1954, securely containing the elephants within AENP (Whitehouse 2002). The population has since rebounded dramatically.

As of November 2003, there were approximately 340 elephants in six elephant family groups that comprised the AENP elephant population (Bagley 2004). Another sixty elephants were located in a separate fenced area called Nyathi, a newer section of AENP (Loizi 2004). As of 12 December 2006, there were 365 elephants in the AENP Main Section and 75 elephants in

the Nyathi Section (personal observation and personal communication with doctoral candidate K. Gough). All focal observations in this study were performed on elephants in the AENP Main Section. The Addo elephants have been studied extensively and all individual elephants have been carefully identified and catalogued (Whitehouse and Hall-Martin 2000). Since careful records have been kept, most Addo elephants can be distinguished by individual ear notch patterns, ear venation patterns, and individual body characteristics (i.e. tusk presence/absence, tusk placement, tusk size, body scars, physical deformities, tail length). Identification files/binders have been compiled and updated by elephant researchers in the Schulte Lab (Loizi 2004; Bagley 2004; Merte 2006; Meyer 2006). For this study, the age of each elephant was determined by matching each focal individual to their corresponding identity file. In the rare instance when identity could not be firmly established, estimations of size and comparisons to other elephants were used to assess age (Moss 1996). AENP was chosen as a study site because it is an ideal environment to study elephant behavior. The population is well characterized and the ages of nearly all individual elephants are known due to careful recordkeeping. In addition, because it is a closed population with a fenced boundary, it was reasonably easy find and observe elephants on a daily basis.

Behavioral Observation Techniques

Observations took place in 2006 from January to mid-December. Observations were made on males and females in four age classes (Calf: 0-4 years, reliant on mother; Juvenile: 5-9 years, reliant on natal group; Pubescent: 10-19 years, entering or achieving early reproductive readiness; Adult: 20+ years, reproductively active and becoming more socially dominant). Focal-animal sampling, as described by Altmann (1974) was used to collect data on the behavior

of elephants. Twenty-minute observations with continuous recording of all behaviors were conducted on each focal animal. The mean length of all focal observations (excluding musth male focal observations) was 19.7 ± 0.46 minutes (Figure 4). If a focal individual left the field of view, I made an attempt to reposition myself to keep the focal individual in sight. If the focal individual disappeared into a group of elephants or into the thick vegetation, then I would stop the focal observation. Focal observations shorter than 10 minutes were not included in the data analysis. An ethogram defining all relevant behaviors was developed and used with all behavioral data being recorded in a notebook (Table 1). Observations were made from a vehicle, using binoculars as needed. The elephant population in Addo is habituated to vehicles and also tends to ignore passengers within vehicles.

Because my main research objective was to examine the pubescent age class, I attempted to make focal observations on multiple individuals of each year within this age class. I ended up with focal observations on 26 pubescent males and 27 pubescent females (Table 2). Because sufficient sample size is necessary to ensure accurate statistical inferences, my goal for the population comparison was to record focal observations on a minimum of 10 individuals of each sex within each age class (goal of 80 total focal observations). I surpassed this goal and finished the study with a total of 157 focal observations (Figure 6). The discrepancy in number of pubescent males between Figure 6 and Table 2 is due to the fact that 10 pubescent males on which focal observations were made were either XMs or UMs (meaning unknown males). I was able to place them in the pubescent age class (by comparing them to known individuals), but since I did not have an exact age on them, I did not use those 10 males for my analysis requiring specific ages of pubescent elephants.

Focal observations took place in a variety of locations around the park. Upon entering the park, the first task was to locate elephants. During the warmer months, it was easy to locate elephants at one of the waterholes in the park. During the cooler months, elephants spent less time at waterholes so I had to seek them out in other places. Upon entering the park, I drove up to Zuurkop Lookout, Zuurkop Ridge, or Kadouw Lookout and scanned the park for elephants. These locations were elevated and provided good vantage points to spot elephants and note their directional heading. Once I located a group of elephants, I would park the vehicle in a position that was close enough to make observations without affecting their behavior. After finding an adequate position, I would record my location and then identify the elephants present. I also checked my identifications with K. Gough, a doctoral candidate at Nelson Mandela Metropolitan University, who had been observing the population for several years.

Prior to going out in the field, I made a randomized list of focal animals that I needed to observe that week. With males and females in four age classes, there were eight categories from which to randomly select. If an age class/sex combination was not available at a particular location, the next one on the list would be selected for focal observations. With the exception of a few adult males, no individuals were sampled twice in order to avoid pseudoreplication.

Data Analysis

All behaviors were analyzed as mean rates (frequency per minute). There were several adult males for which I had multiple focal observations; these samples were averaged to yield a mean rate of each behavior for each nonmusth adult male. I analyzed the responses of males in musth separately (see Appendix A), so I did not include these data in the calculation of mean rates of behaviors for the adult male group (for the comparison with the other 7 age class/sex

combinations). Data were analyzed with JMP 4.0.4. Data were transformed when they did not meet the assumptions of normality and equal variance. If the assumptions were still not met after the transformation, then non-parametric statistical tests were used. For all statistical tests, the significance level was $\alpha = 0.05$.

To determine the rate of behaviors in pubescents, behavioral data were log-transformed in order to meet the assumptions of normality and equal variance. I grouped the pubescent data into four groups (10-14 year old males, 10-14 year old females, 15-19 year old males, and 15-19 year old females) and used analysis of variance (ANOVA) tests to analyze behavioral differences between sex, age group, and their interaction to see if any patterns were evident. Sex and age group were the independent variables and mean rate of behavior was the dependent variable. Tukey Kramer HSD test was used for *a posteriori* analysis.

When analyzing data for the entire population (males and females in all four age classes; to check for consistency with past studies), ANOVA tests were used to analyze behavioral differences between sex, age class, and their interaction. Sex and age class were the independent variables and mean rate of behavior was the dependent variable. Tukey Kramer HSD test was used for *a posteriori* analysis. When assumptions were not met, Kruskal-Wallis tests were used.

Results

Pubescents

Chemosensory behaviors

Sex had a significant effect on all four chemosensory behaviors (sniffs, checks, places, and flehmens) and age group had a significant effect on two out of four chemosensory behaviors (checks and places), yet no significant interaction was apparent between sex and age group.

Males performed more sniffs than females, but 15-19 year old elephants, with sexes combined, did not differ from 10-14 year old elephants, with sexes combined (2-Way ANOVA – Sex: $F = 8.84$, $df = 1, 49$, $p = 0.0046$; Age group – $F = 2.97$, $df = 1, 49$, $p = 0.091$; Interaction: $F = 3.81$, $df = 1, 49$, $p = 0.057$). Males in the 15-19 year old group performed more sniffs than 10-14 year old males, 10-14 year old females, and 15-19 year old females (Tukey HSD, $\alpha = 0.05$; Figure 7).

Males performed more checks than females and 15-19 year olds displayed more checks than 10-14 year olds (2-Way ANOVA – Sex: $F = 10.77$, $df = 1, 49$, $p = 0.0019$; Age group – $F = 4.37$, $df = 1, 49$, $p = 0.042$; Interaction: $F = 1.35$, $df = 1, 49$, $p = 0.25$). Males in the 15-19 year old age group performed more checks than 10-14 year old males, 10-14 year old females, and 15-19 year old females (Tukey HSD, $\alpha = 0.05$; Figure 8). Mean rate of places differed by sex and age group (2-Way ANOVA – Sex: $F = 13.24$, $df = 1, 49$, $p = 0.0007$; Age group – $F = 11.01$, $df = 1, 49$, $p = 0.0017$; Interaction: $F = 3.83$, $df = 1, 49$, $p = 0.056$). Males in the 15-19 year age group performed more places than 10-14 year old males, 10-14 year old females, and 15-19 year old females (Tukey HSD, $\alpha = 0.05$; Figure 9). Males displayed more flehmen than females, but no differences were observed between age groups (2-Way ANOVA – Sex: $F = 4.39$, $df = 1, 49$, $p = 0.04$; Age group – $F = 0.0003$, $df = 1, 49$, $p = 0.99$; Interaction: $F = 0.20$, $df = 1, 49$, $p = 0.66$). Rate of flehmen did not differ between 10-14 year old males, 10-14 year old females, and 15-19 year old males (Tukey HSD, $\alpha = 0.05$; Figure 10). Flehmen is a relatively uncommon behavior and it was observed much less frequently than the other three chemosensory behaviors.

Auxiliary chemosensory behaviors

Sex had a significant effect on one out of four chemo-related behaviors (horizontal sniffs) and age group had a significant effect on two out of four chemo-related behaviors (horizontal

sniffs and periscope sniffs), yet no significant interaction was apparent between sex and age group. Males had a higher mean rate of horizontal sniffs than females with significant differences between age groups (2-Way ANOVA – Sex: $F = 6.00$, $df = 1$, 49, $p = 0.018$; Age group – $F = 5.53$, $df = 1$, 49, $p = 0.023$; Interaction: $F = 0.70$, $df = 1$, 49, $p = 0.41$). Males in the 15-19 year old age group performed more horizontal sniffs than 10-14 year old males and females, but there was no difference in rate of horizontal sniffs between 15-19 year old males and females (Tukey HSD, $\alpha = 0.05$; Figure 11). For periscope sniffs, no significant differences were apparent between males and females, but the rate did differ by age group (2-Way ANOVA – Sex: $F = 1.28$, $df = 1$, 49, $p = 0.26$; Age group – $F = 6.27$, $df = 1$, 49, $p = 0.016$; Interaction: $F = 2.54$, $df = 1$, 49, $p = 0.12$). Males in the 10-14 year old age group performed more periscope sniffs than 15-19 year old males; however, there was no difference in rate of periscope sniffs between 10-14 year old males and females (Tukey HSD, $\alpha = 0.05$; Figure 12). Rate of genital sniffs and trunk to genital area did not differ by sex or age group (2-Way ANOVA for genital sniffs – Sex: $F = 3.04$, $df = 1$, 49, $p = 0.088$; Age group – $F = 0.53$, $df = 1$, 49, $p = 0.47$; Interaction: $F = 0.99$, $df = 1$, 49, $p = 0.32$; Figure 13); (2-Way ANOVA for trunk to genital area – Sex: $F = 1.81$, $df = 1$, 49, $p = 0.18$; Age group – $F = 0.014$, $df = 1$, 49, $p = 0.91$; Interaction: $F = 1.60$, $df = 1$, 49, $p = 0.21$; Figure 14).

Entire Population (Calves, Juveniles, Pubescents, and Adults)

Chemosensory behaviors

Sex had a significant effect on all four the chemosensory behaviors and age class had a significant effect on three out of four chemosensory behaviors, yet no significant interaction was evident between sex and age class. Males had a higher mean rate of sniffs than females and there were significant differences between age classes (2-way ANOVA – Sex: $F = 11.66$, $df = 1$,

149, $p = 0.0008$; Age class: $F = 12.21$, $df = 3$, $p < 0.0001$; Interaction: $F = 0.46$, $df = 3$, $p = 0.71$). Pubescents performed significantly more sniffs than calves and juveniles; adults performed significantly more sniffs than calves (Tukey HSD, $\alpha = 0.05$) (Figure 15). Males had a higher mean rate of checks than females with significant differences between age classes (Kruskal-Wallis – Sex: $H = 21.18$, $df = 1$, $p < 0.0001$; Age-Class: $H = 59.09$, $df = 3$, $p < 0.0001$). Pubescents performed significantly more checks than calves and juveniles; adults performed significantly more checks than calves (Tukey HSD, $\alpha = 0.05$) (Figure 16). Males had a higher mean rate of places than females, with significant differences between age classes (Kruskal-Wallis – Sex: $H = 15.40$, $df = 1$, $p < 0.0001$; Age-Class: $H = 38.70$, $df = 3$, $p < 0.0001$). Pubescents performed significantly more places than calves and juveniles (Tukey HSD, $\alpha = 0.05$) (Figure 17). Males had a higher mean rate of flehmen than females, but no differences were observed between age classes (Kruskal-Wallis – Sex: $H = 16.03$, $df = 1$, $p < 0.0001$; Age-Class: $H = 6.33$, $df = 3$, $p = 0.097$) (Figure 18).

Discussion

Distinct patterns of chemosensory behaviors were observed within the pubescent elephants of AENP. Older pubescent males performed significantly more chemosensory behaviors than younger pubescent males. In a study on ring-tailed lemurs, Palagi et al. (2002) noticed that use of olfactory behaviors increased during the juvenile and adolescent age periods. Reinhardt (1983) discovered that in semi-wild cattle, flehmen became a regular part of the behavioral repertoire during adolescence with males primarily displaying the behavior toward females. In a study on giant pandas, Hong et al. (2007) observed that adolescent and adult males exhibited high levels of chemosensory behaviors that were not observed in juvenile males. It

seems consistent across many mammalian taxa that chemosensory and olfactory behaviors are occurring at adult-like rates by the end of adolescence. Appropriate use of these behaviors, which are critical for reproduction, is important as individuals develop and approach sexual maturity. In this study, pubescent male elephants in the 15-19 year old age group had the highest rates of sniffs, checks, and places, which supported my prediction. This older group of pubescent males performed higher rates of chemosensory behaviors than 10-14 year old males as well as 15-19 year old females, indicating that rate of chemosensory behaviors differed by age group and sex. Chemosensory reception helps the signal receiver assess identity and sexual state/status of potential mates (Rasmussen and Schulte 1998; Rasmussen 2006). During the pubescent years, it is clear that developing teenage elephants are paying attention to chemical signals in their environment and in the case of males, responding to them more frequently as they approach adulthood.

There was no difference in rate of chemosensory behaviors between 10-14 and 15-19 year old females, which supported my prediction. Females used chemosensory behaviors, but not nearly as often as pubescent and adult males. Both groups of pubescent females are busy nurturing and raising offspring and do not actively seek out males, but rather when in estrous appear to attract males (Poole 1994). The priority of pubescent females is to comfort, assist, and protect calves, which enhances mother-offspring bonds and family group stability (Lee 1987). Females in the 15-19 year old group did not display any trunk tip to genital area behavior, while females in the younger pubescent group displayed a low rate of this behavior (Figure 14). Sometimes this behavior is combined with chemosensory behaviors (personal observation) and may be involved with determining reproductive state, which is probably why pubescent males displayed higher rates of this behavior. The older group of pubescent males displayed the

highest rate (although not statistically different from the other groups), but there was lots of variability because only two 18 year old males performed high rates of this behavior, while the rest of the males in the 15-19 year old group displayed very low rates. The reason that 15-19 year old females were not observed displaying this behavior may simply be that these females were not concerned or interested in the reproductive status of their female relatives.

An interesting pattern of elevated sniffs (horizontal sniffs and periscope sniffs) was observed within the pubescent age class. Elevated sniff behaviors are used to determine direction from which odors originate as well as location of conspecifics (Rasmussen 2006). Males in the 15-19 year old group had a higher rate of horizontal sniffs than 10-14 year old males, while 10-14 year old males had a higher rate of periscope sniffs than 15-19 year old males. These findings suggest that younger pubescent males tend to use the periscope sniff behavior as an investigatory tool, but as they mature they transition to using horizontal sniffs as a way to investigate similar types of chemical signals and gather information about their surroundings. For the entire population (all four age classes; Figure 19), a similar pattern was evident for these elevated sniff behaviors. Adults and pubescents performed significantly more horizontal sniffs than calves, suggesting that the horizontal sniff behavior may be a more advanced auxiliary chemosensory behavior. Another possible reason for this behavioral transition could be related to the size of the elephants. Younger elephants need to reach higher (i.e., periscope sniff) to catch the same air currents that older and taller elephants are reaching with horizontal sniffs (personal communication with B. Schulte). Further research is warranted to better understand the development and transition of these elevated sniff behaviors.

Clear patterns emerged while examining the occurrence and development of chemosensory behaviors for the entire population of elephants at AENP. Because elephants are

sexually dimorphic, I hypothesized there would be behavioral differences between male and female elephants. Males did perform more chemosensory behaviors than females which supported my prediction and matched the findings of a previous study by Loizi (2004). Maturational changes in the behavior of elephant calves results in increasing independence from mothers as well as development of social relationships with other elephants (Lee 1986). During this developmental period, behaviors and patterns of social interaction often differ between males and females. Young female elephants tend to stay closer to their mothers and family members with frequent friendly and supportive interactions, while young male elephants are increasingly independent and actively explore their environment and play/spar with known and unknown peers. When males reach puberty, they disperse from their family group and set out on their own, relying on their own ability to find food, water, stay out of harm's way (e.g., musth males) and successfully navigate their environment using their developing chemosensory abilities. As pubescent males grow older and approach adulthood, they have been living independently from their family for years and they have a fairly developed set of chemosensory abilities. As sexually mature adults, males rely heavily upon their chemosensory skills to navigate their environment and locate receptive mates. When considering the trajectory of male development, from young calf to mature adult, it makes sense that males at all stages of development are displaying higher rates of chemosensory behaviors than females.

I also hypothesized that the mean rate of chemosensory behaviors would increase with age. My prediction that the two older age classes (pubescents and adults) would perform more chemosensory behaviors than the two younger age classes (calves and juveniles) was partially supported. Pubescents performed more sniffs, checks, and places than calves and juveniles, while adults only performed more sniffs and checks than calves. In other species as well as

elephants, it is often the older age classes that are responding to chemical stimuli in their environment. Reinhardt (1983) found that in semi-wild cattle, rate of flehmen was highest in adult males; adolescent males performed less flehmen than adult males and juvenile males performed less flehmen than adolescents. Similarly, Thompson (1995) found that in sable antelope, flehmen rates increased with age. In the current study, pubescent and adult elephants performed more chemosensory behaviors than calf and juvenile elephants. Since adult males and females live separately, it is very important for elephants to correctly assess chemical signals they come across if they want to successfully reproduce (Langbauer 2000; Schulte et al. 2005). All elephants utilize chemosensory behaviors, but it is the pubescents and adults that display the highest rates of chemosensory behavior because they are frequently assessing important chemical signals, many of which pertain to reproductive state of conspecifics (Loizi et al. 2009; Merte et al. 2010).

The findings of this study are largely consistent with those of past studies on the same population of elephants. In this study, males performed more chemosensory behaviors than females. One previous study described males displaying more chemosensory behaviors than females to urine and feces (Loizi 2004). Another previous study found that adult males took longer to approach waterholes because they performed more chemosensory behaviors to conspecific feces than females (Merte 2010). Meyer (2006) reported that young male elephants showed greater interest to urine and feces than young females, similar to patterns seen in adult elephants. Loizi (2010) observed that male elephants appear more dependent than females on signals in excretions with subsequent inspections of conspecifics through trunk tip contact behaviors. Males reproduce at a much later age than females, which provides a protracted developmental period for males. During adolescence and adulthood, male elephants are

constantly evaluating other males and searching for sexually active females; as a result of these social pressures, males are more likely than females to investigate chemical signal sources associated with conspecifics (Loizi 2010).

In this study, pubescent elephants performed more chemosensory behaviors than juveniles and calves. Merte (2006) found that two chemosensory behaviors, check and place, were exhibited more frequently by sexually mature elephants, which suggests that these behaviors could be associated with reproductive capability. Merte (2006) also found that for males, the rate of sniffs to all substrates increased from the juvenile to the pubescent stage, which is consistent with the findings of this study. Loizi (2004) found that pubescent males displayed more chemosensory behaviors than the younger age classes, which is directly in line with the findings of this study. Chemosensory behaviors in elephants are investigative behaviors that are performed in part for reproductive functions so it seems logical that males and females would show an increase in the rate of chemosensory behaviors just before they begin reproducing (Merte 2010). Reproduction occurs later in males than females so it is not surprising to find the highest rates of chemosensory behavior in the older group of pubescent males (15-19 year olds).

Although this study was conducted on a closed population of African elephants that went through a population bottleneck in the early 1900s (Whitehouse 2002), the findings of this study are still relevant to other populations of elephants and even other species of long-lived mammals. African elephants in Tanzania and both African and Asian elephants in captivity have similar behavior repertoires and development patterns to the elephants in AENP (Rasmussen 1998; Rasmussen and Schulte 1998; Vyas 2006; Napora 2007). Both species of elephants rely on chemosensory behaviors to discern important physiological information about the reproductive status of other elephants. Other mammalian species such as ring-tailed lemurs, sable antelope,

semi-wild cattle, and giant pandas all undergo important development phases with juveniles and adolescents increasingly using chemosensory behaviors to interpret chemical signals as they approach adulthood (Reinhardt 1983; Thompson 1995; Palagi et al. 2002; Hong et al. 2007). There may be subtle differences between species, but general patterns of chemosensory behaviors in this study would certainly have relevance when considering patterns of similar behavior in other long-lived mammals.

The patterns of chemosensory behaviors observed in this study and the development of these behaviors bear some resemblances to patterns in other species. Hong et al. (2007) observed that juvenile giant pandas did not display scent-marking behavior, but adult male pandas had well-developed scent-marking behavior. Hong et al. (2007) also established that the frequency of scent marking increased with age in pandas which parallels the findings of this study in relation to chemosensory behaviors in elephants. Reinhardt (1983) found the flehmen behavior to be much more frequent in adult males than young males, which suggests that adult males are more alert and sensitive to chemical signals from females; the same study found that subadult males performed flehmen more frequently than juvenile males to pregnant females. Since flehmen helps males discern reproductive status of females, males who are approaching sexual maturity have greater interest and higher sensitivity to female chemical signals because those signals provide information necessary for successful reproduction (Reinhardt 1983; Rasmussen et al. 1997; Bagley et al. 2006).

As pubescent African elephants develop, their behavioral repertoires become more refined and their behavioral patterns begin to resemble those of sexually mature and reproductively active adult elephants. This study examined chemosensory behaviors in elephants and demonstrated that patterns emerge as elephants approach sexual maturity. The rates of

chemosensory and auxiliary chemosensory behaviors increased with the age of pubescent male elephants. Similar patterns of increased chemosensory behavior are displayed by adult males in musth, which is discussed in Appendix A. Pubescent males displayed more chemosensory behavior as they approach adulthood and the age of their first musth. This is likely an important step in their development, preparing these teenage males for the challenging mating system in which only the biggest and oldest males have access to estrous females, a scarce yet vital resource to the fitness of these African elephants.

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Table 1. Ethogram used to record event behaviors performed by wild African male and female elephants during continuous focal-animal sampling (Loizi 2004; Bagley 2004; Merte 2006; Meyer 2006)

<u>Event behavior categories</u>	<u>Defined event behaviors</u>	<u>Definition</u>
<i>Chemosensory</i>	Check*	Touch ground with tip of either finger
	Flehmen*	Tip of trunk touches substrate or conspecific then placed in the VNO ducts in the roof of the mouth
	Place*	Entire nasal opening is placed on ground or conspecific and held momentarily
	Sniff*	Nasal openings hover over ground or conspecific (excluding genitals) without contact
<i>Auxiliary Chemosensory</i>	Blow	Expel air out through trunk
	Defecate	Expel feces from body
	Genital Sniff	Nasal openings hover over genital area of conspecific without contact
	Horizontal Sniff	Elevated sniff occurring with trunk extended horizontally (between ground and mouth level)
	Periscope Sniff	Elevated sniff occurring with trunk raised above mouth level
	Pinch	Both tips of trunk close and touch each other
	Trunk Flick	Distal end of trunk curls up slightly and then flicks away from the body, usually toward the ground
	Trunk Shake	Entire trunk wriggles/twists once and then returns to a hanging position; slower than a trunk flick
	Urinate	Expel urine from body

*Chemosensory definitions defined by Schulte & Rasmussen (1999)

Table 2. Number of focal observations for different male and female pubescent elephants at AENP.

Age (years)	Pubescent Males	Pubescent Females
10	2	4
11	4	3
12	2	3
13	4	2
14	2	2
15	3	2
16	2	2
17	3	4
18	2	3
19	2	2
Total	26	27

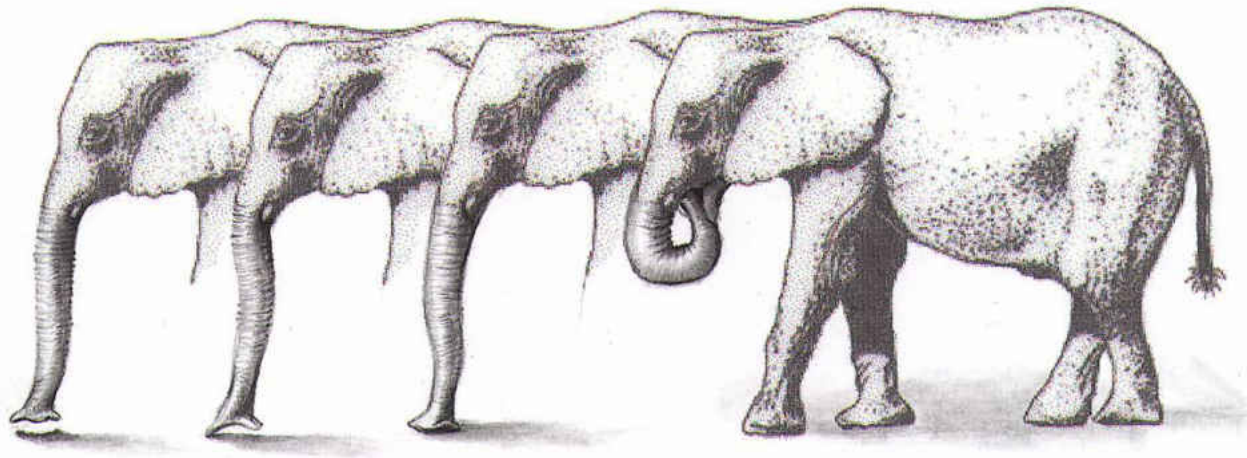


Figure 1. Illustration depicting the four primary chemosensory behaviors. Behaviors from left to right: Sniff (nasal openings hover over ground or conspecific without contact), Check (touch ground or conspecific with tip of either trunk finger), Place (entire nasal opening is placed on ground or conspecific and held momentarily), and Flehmen (tip of trunk touches substrate or conspecific then placed in the vomeronasal organ ducts in the roof of the mouth). Chemosensory behaviors defined by Schulte and Rasmussen (1999). Illustration by Mary Amaral.

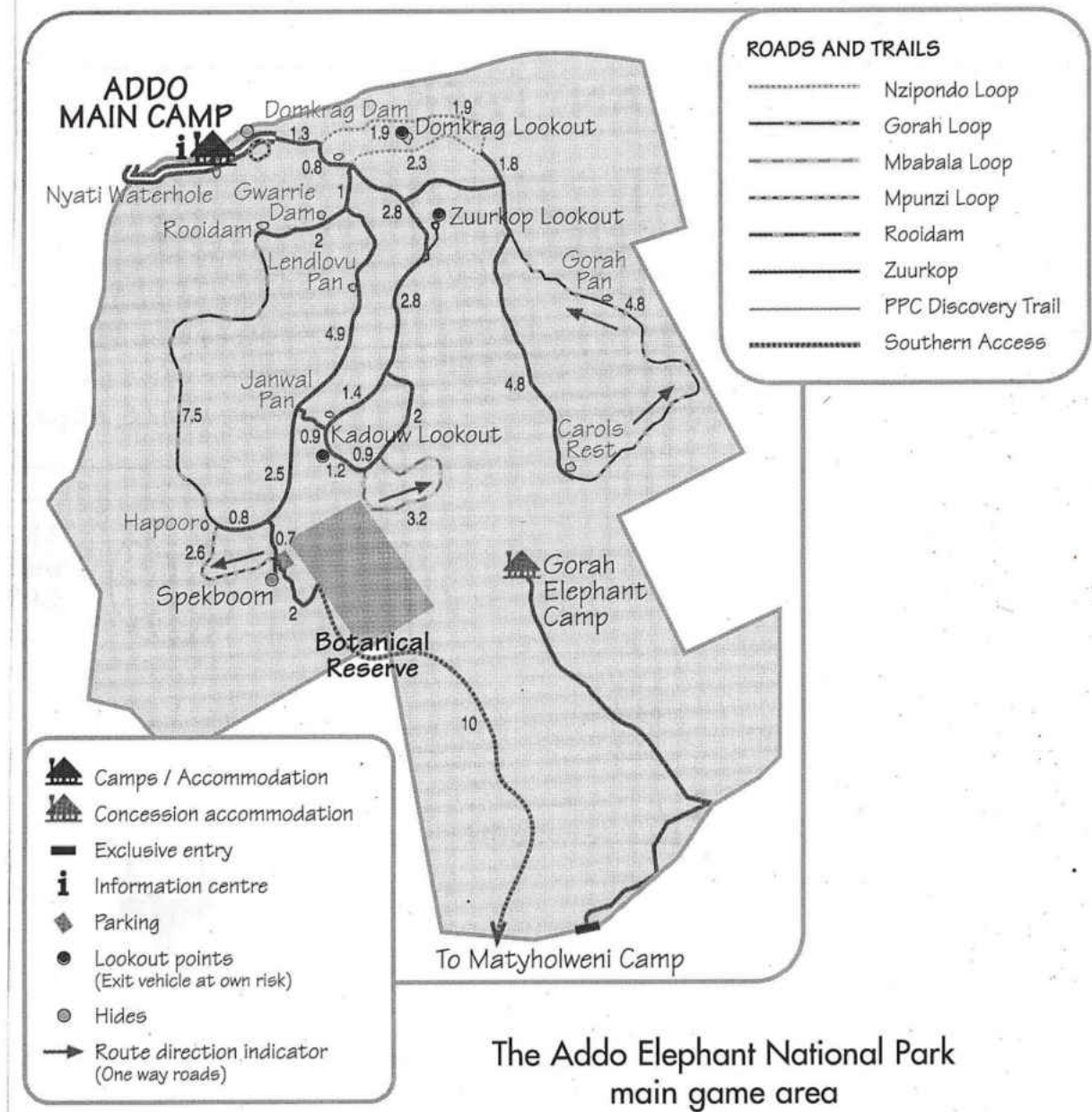


Figure 2. Map of Addo Elephant National Park's Main Camp (study site). Image scanned from Addo Elephant National Park Official Guide (2006).

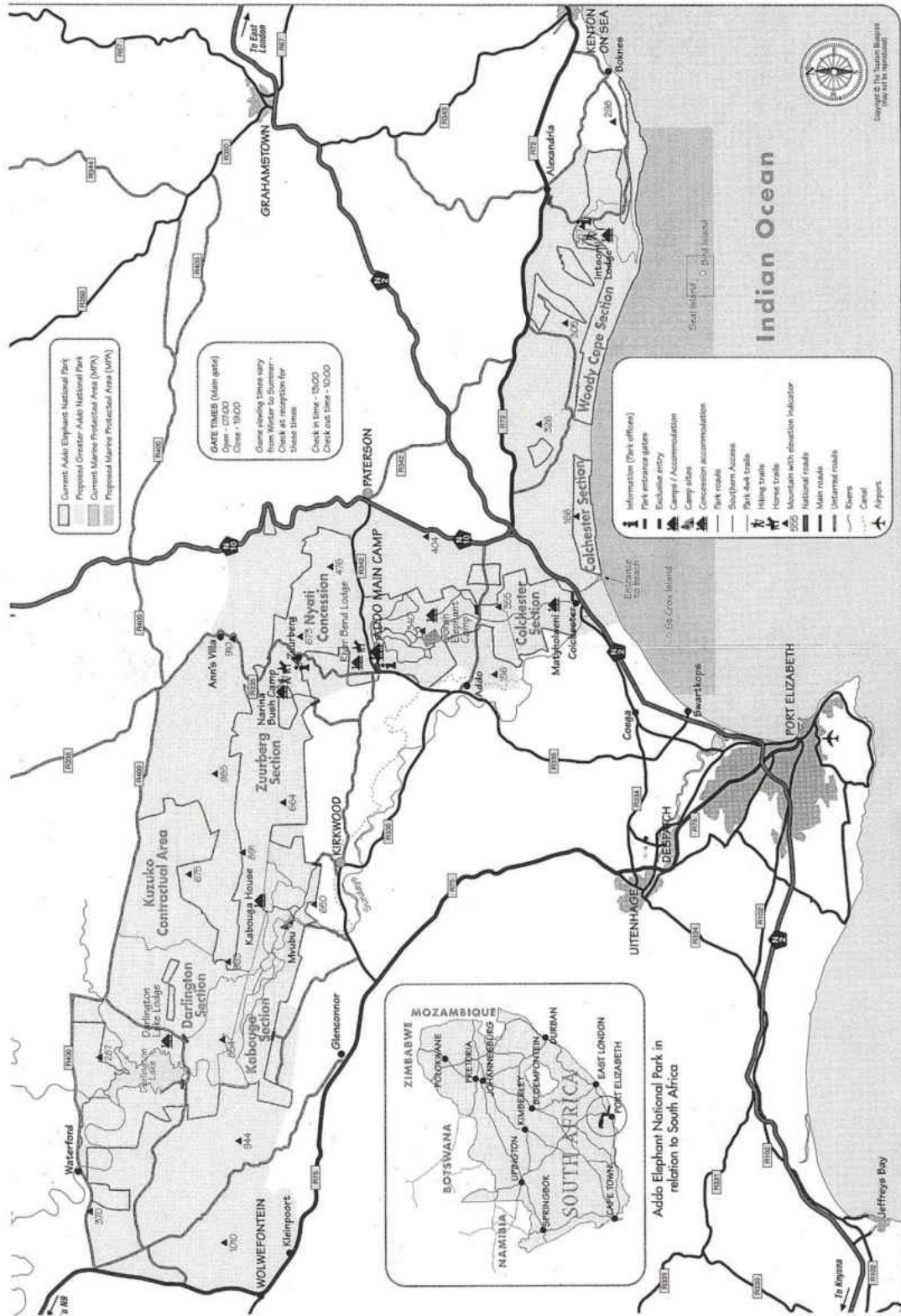


Figure 3. Map of the Greater Addo Elephant National Park. Image scanned from Addo Elephant National Park Official Guide (2006).

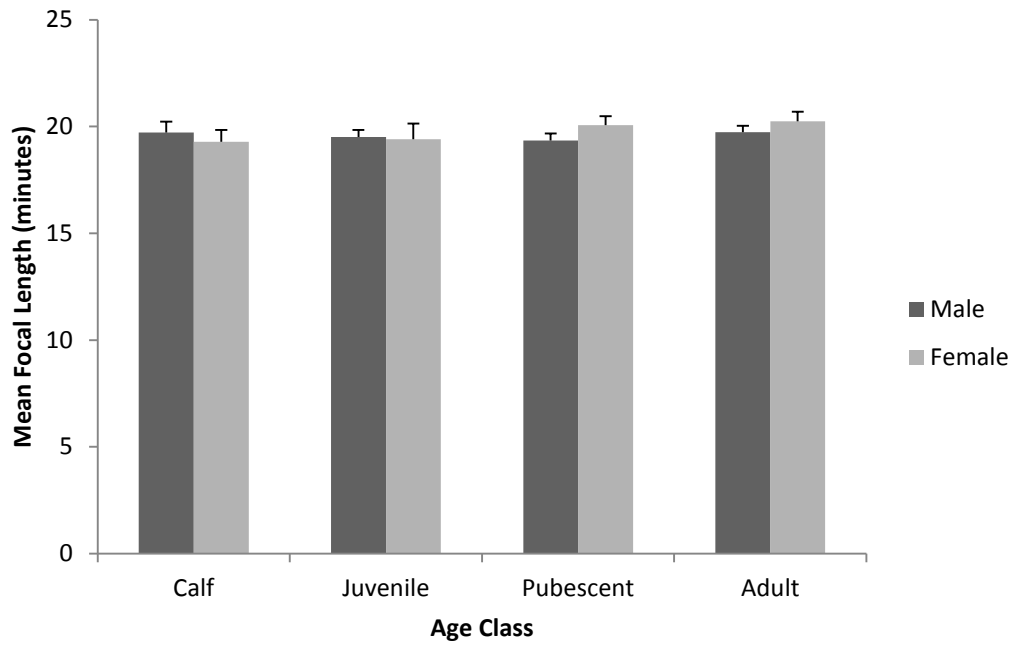


Figure 4. Mean (+SE) focal length per age class for male and female elephants at AENP, excluding males in musth.

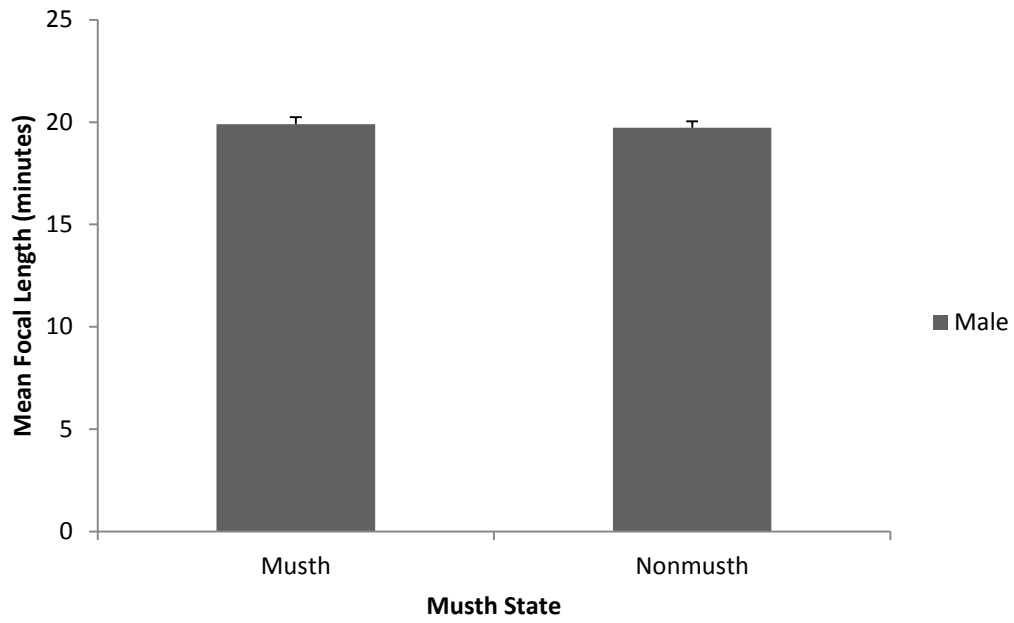


Figure 5. Mean (+SE) focal length for musth and nonmusth adult male elephants at AENP.

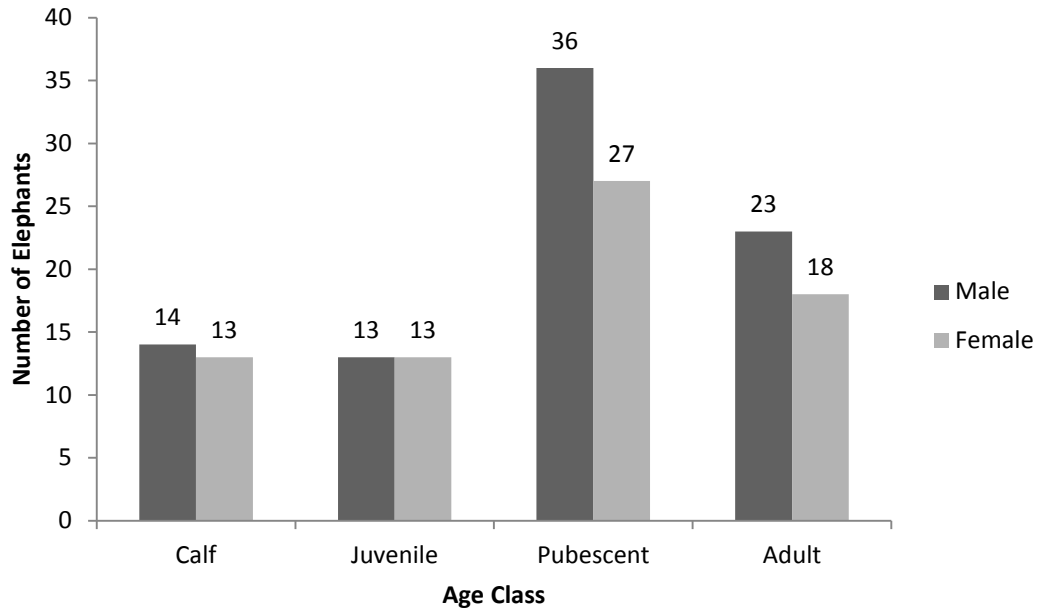


Figure 6. Number of male and female focal individuals in each age class. Sample size is shown above each bar.

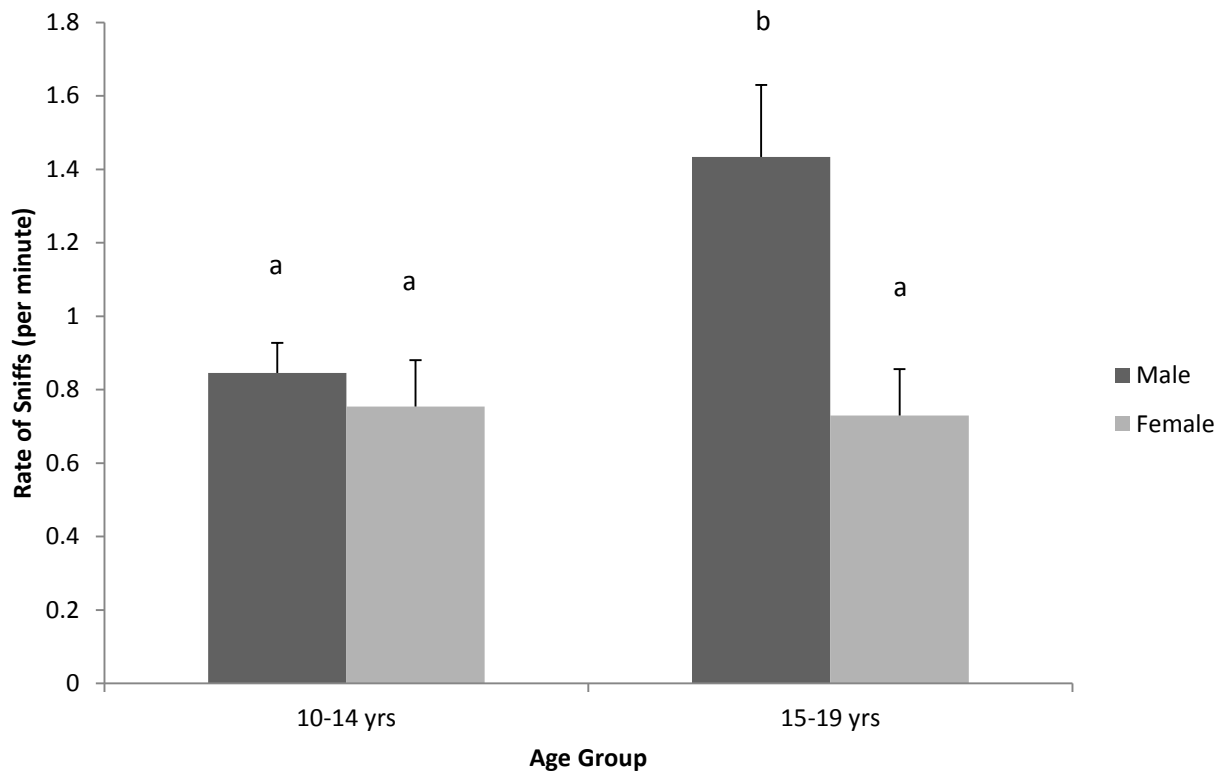


Figure 7. Mean (+SE) rate of sniffs per age group for male and female pubescent elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

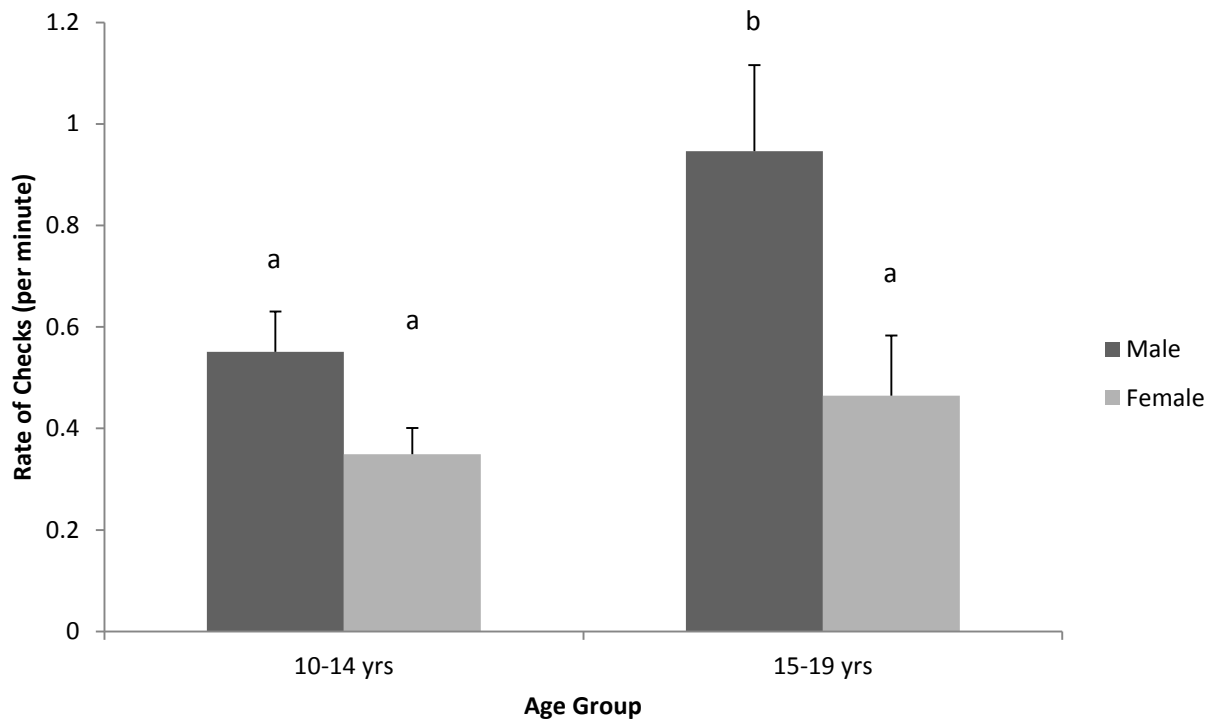


Figure 8. Mean (+SE) rate of checks per age group for male and female pubescent elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

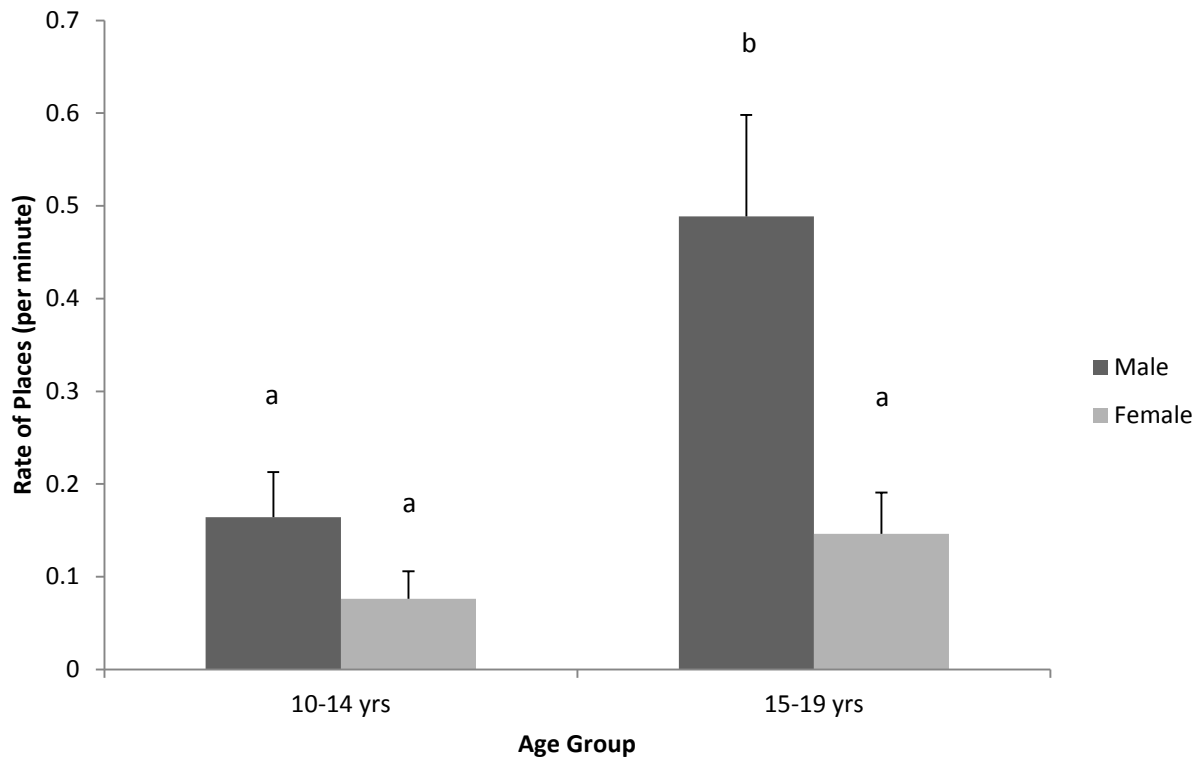


Figure 9. Mean (+SE) rate of places per age group for male and female pubescent elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

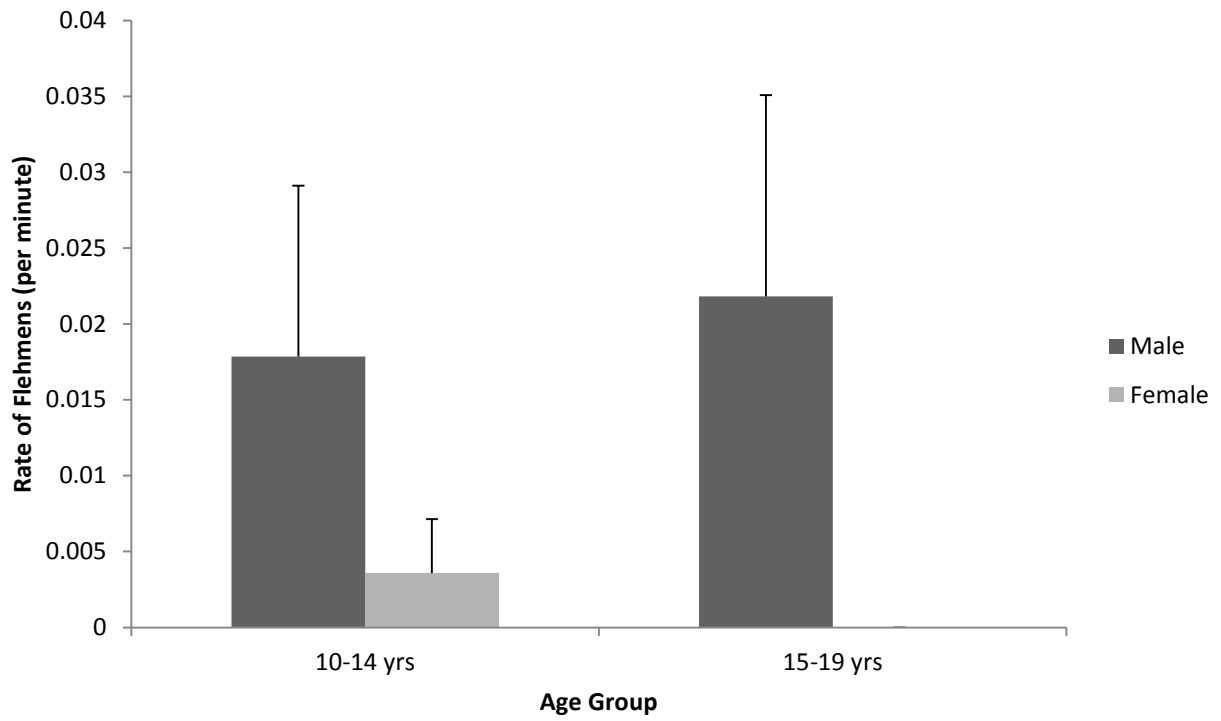


Figure 10. Mean (+SE) rate of flehmens per age group for male and female pubescent elephants at AENP.

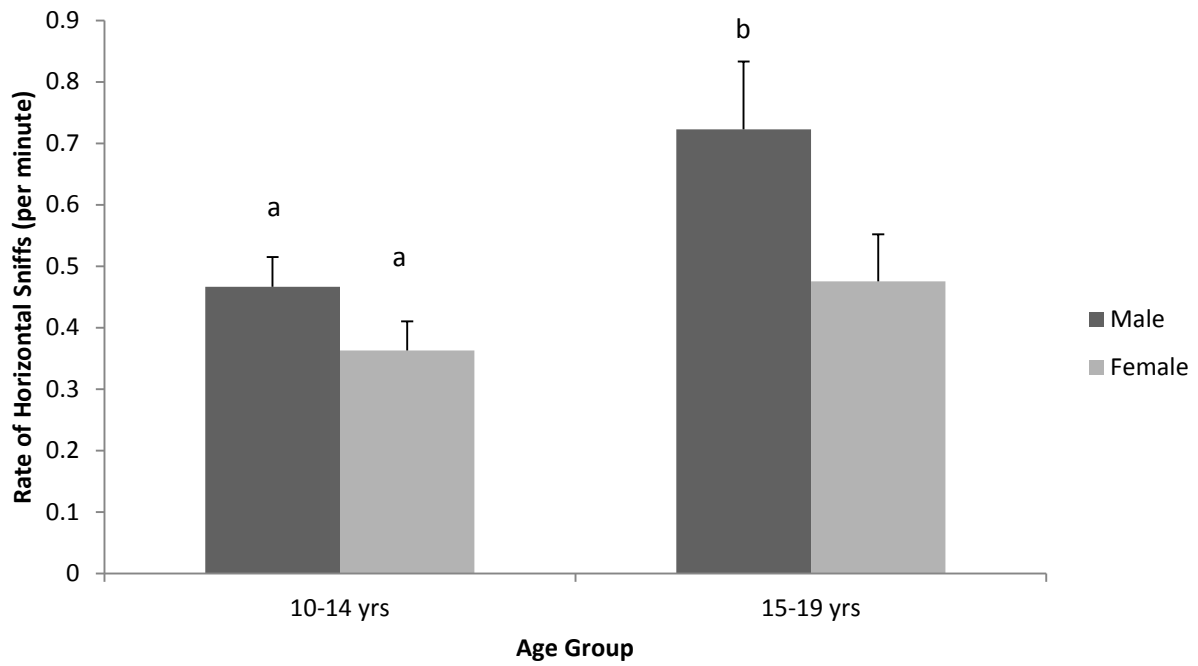


Figure 11. Mean (+SE) rate of horizontal sniffs per age group for male and female pubescent elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

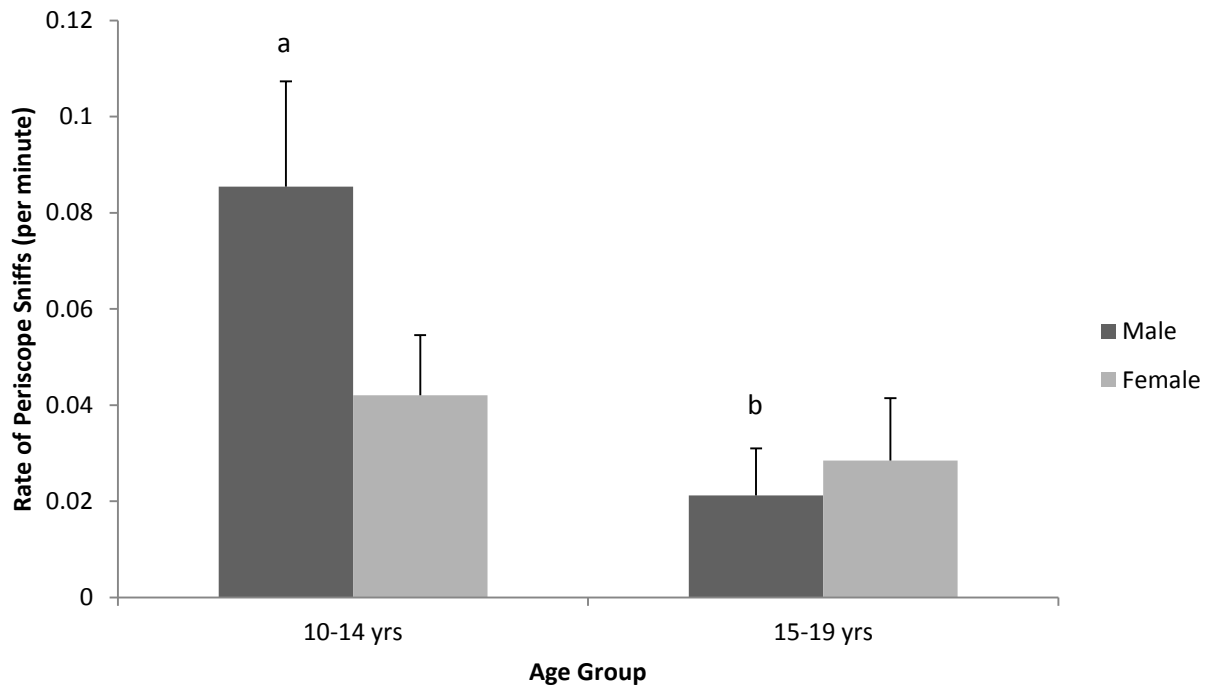


Figure 12. Mean (+SE) rate of periscope sniffs per age group for male and female pubescent elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

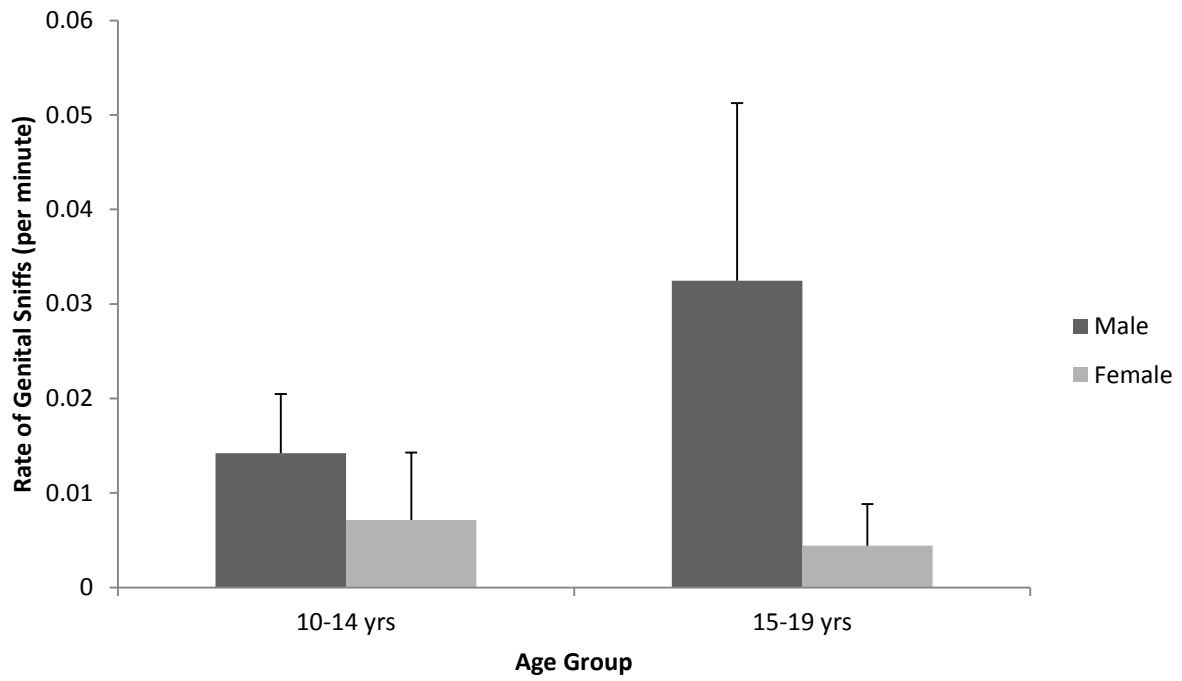


Figure 13. Mean (+SE) rate of genital sniffs per age group for male and female pubescent elephants at AENP.

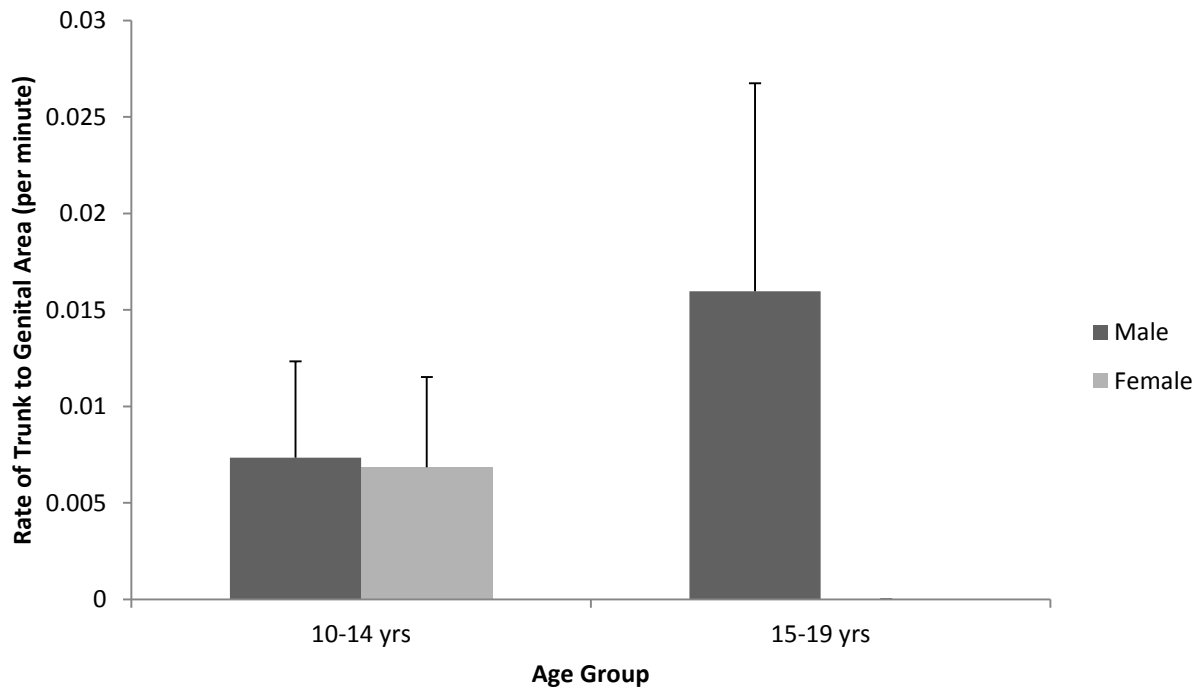


Figure 14. Mean (+SE) rate of trunk to genital area per age group for male and female pubescent elephants at AENP.

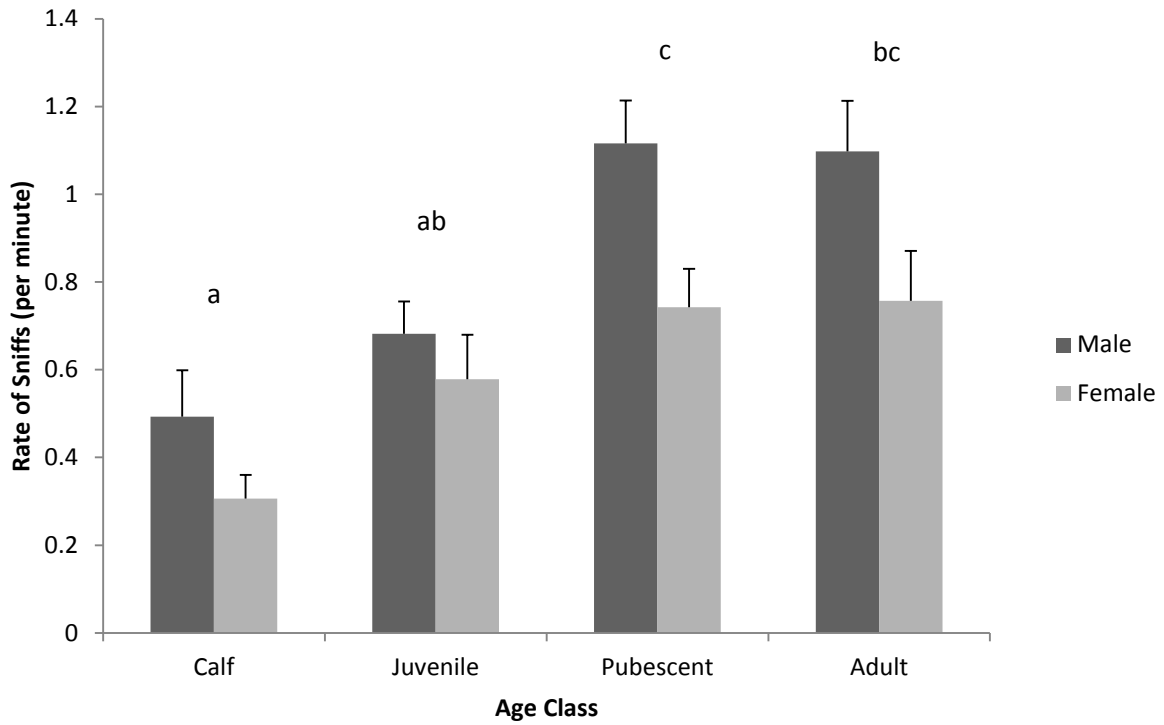


Figure 15. Mean (+SE) rate of sniffs per age class for male and female elephants at AENP. Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

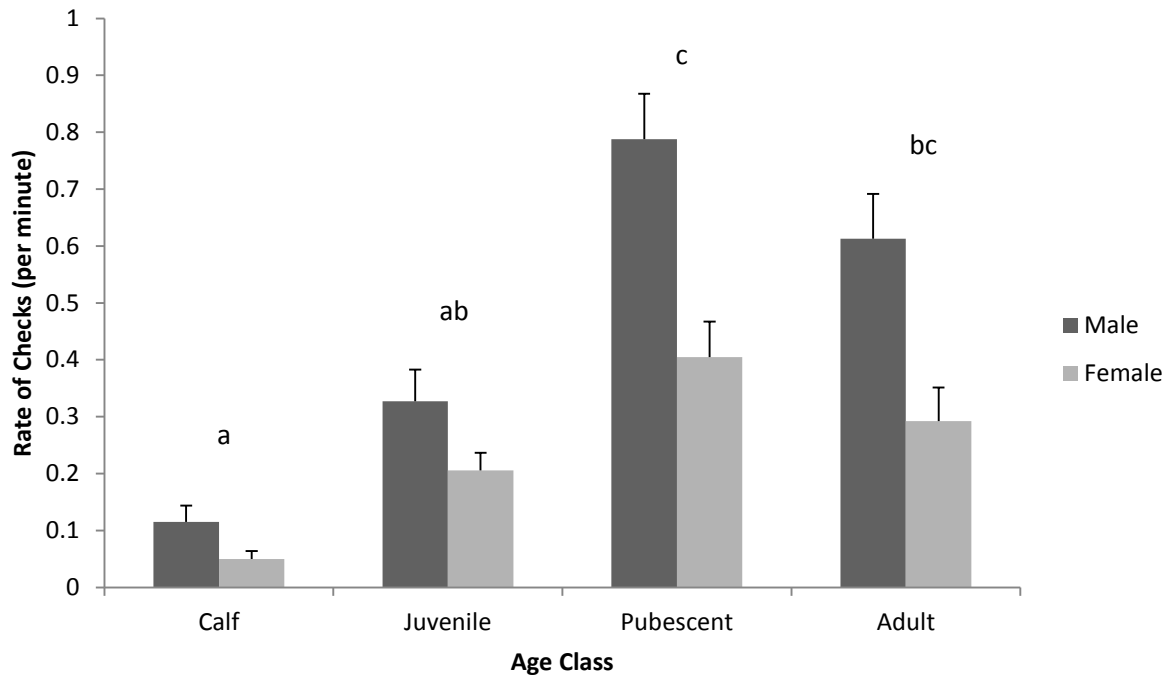


Figure 16. Mean (+SE) rate of checks per age class for male and female elephants at AENP. Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

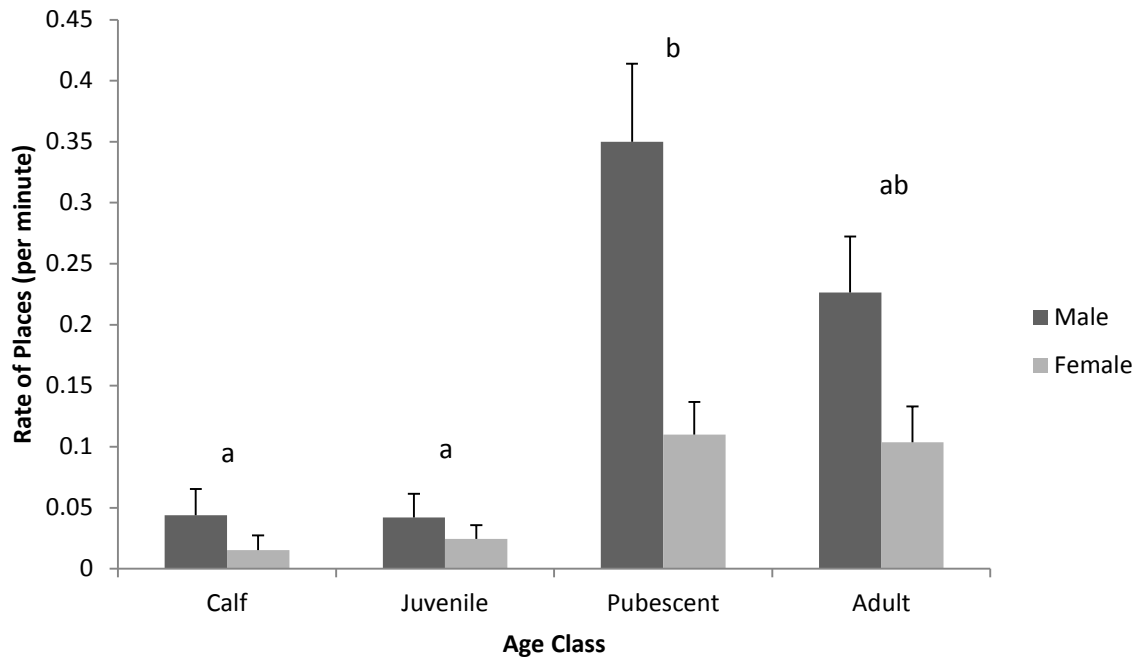


Figure 17. Mean (+SE) rate of places per age class for male and female elephants at AENP. Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

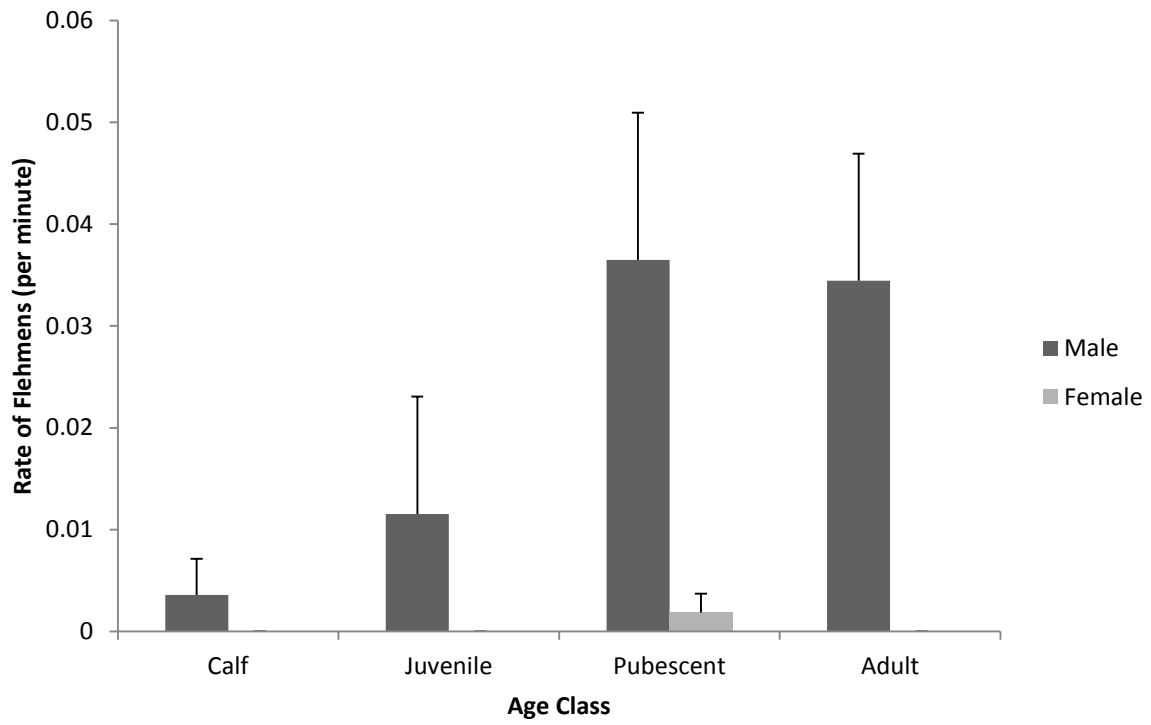


Figure 18. Mean (+SE) rate of flehmens per age class for male and female elephants at AENP.

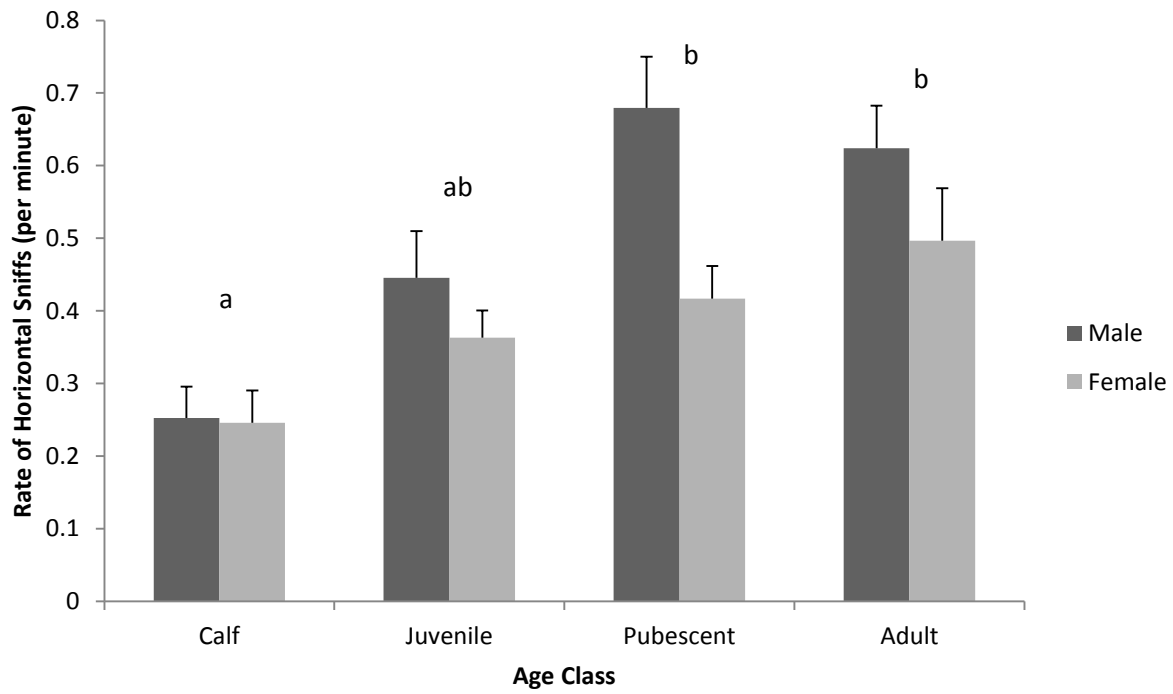


Figure 19. Mean (+SE) rate of horizontal sniffs per age class for male and female elephants at AENP. Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

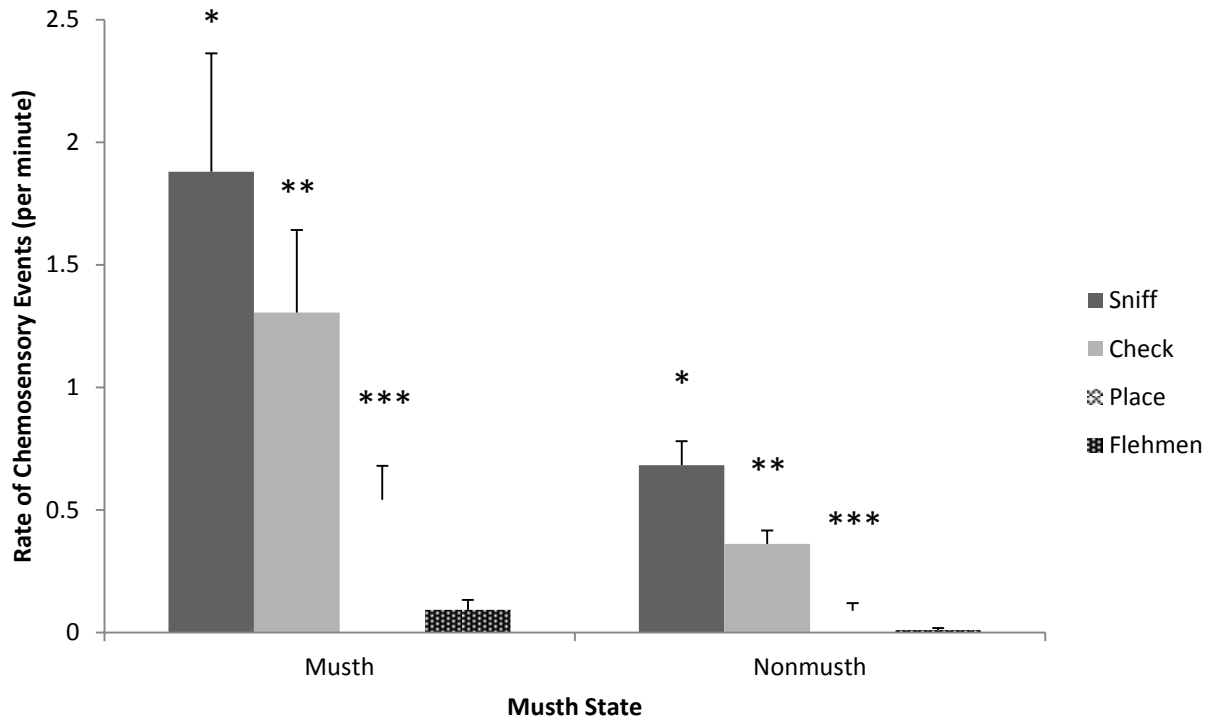


Figure 20. Mean (+SE) rate of chemosensory events for musth and nonmusth male elephants at AENP.

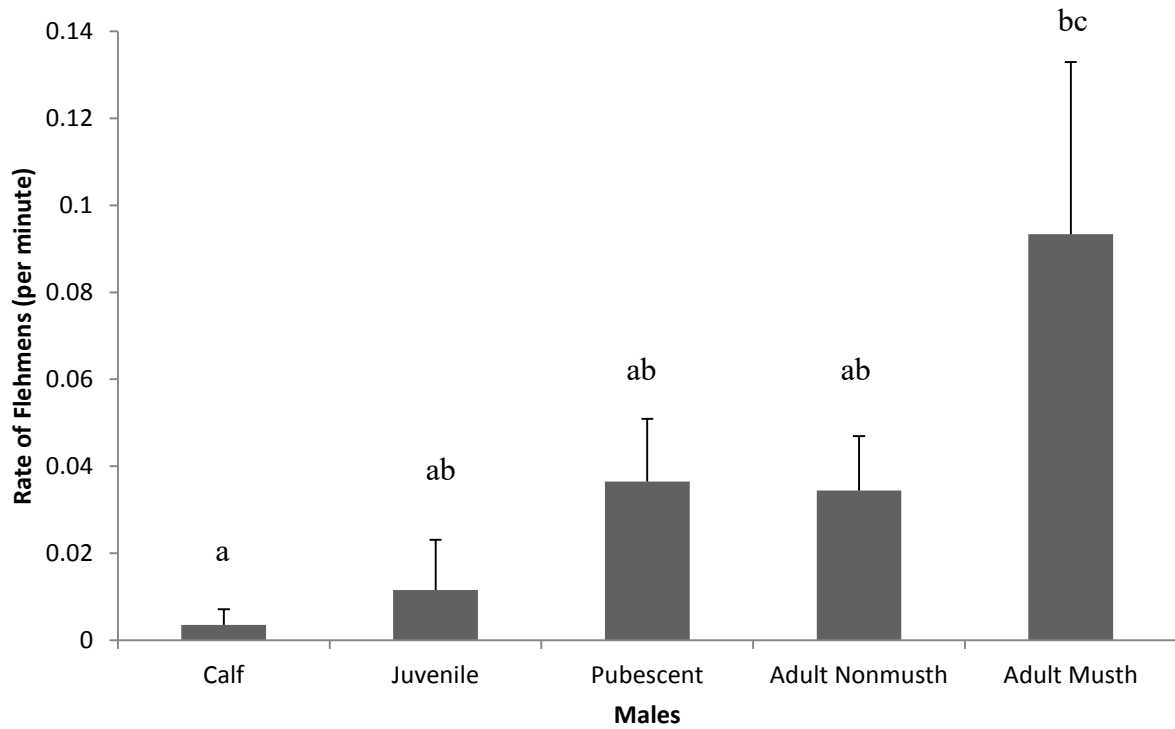


Figure 21. Mean (+SE) rate of flehmen per age class for male elephants at AENP. Different letters indicate significant differences between groups (Tukey HSD, $\alpha = 0.05$).

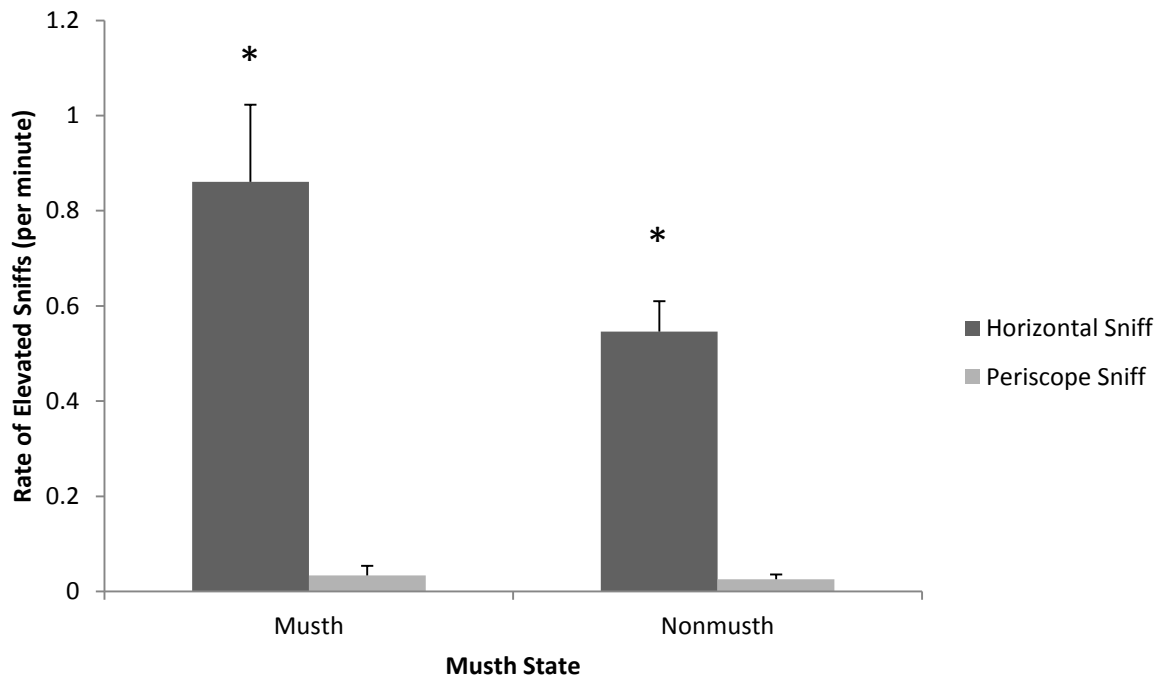


Figure 22. Mean (+SE) rate of elevated sniffs for musth and nonmusth male elephants at AENP.

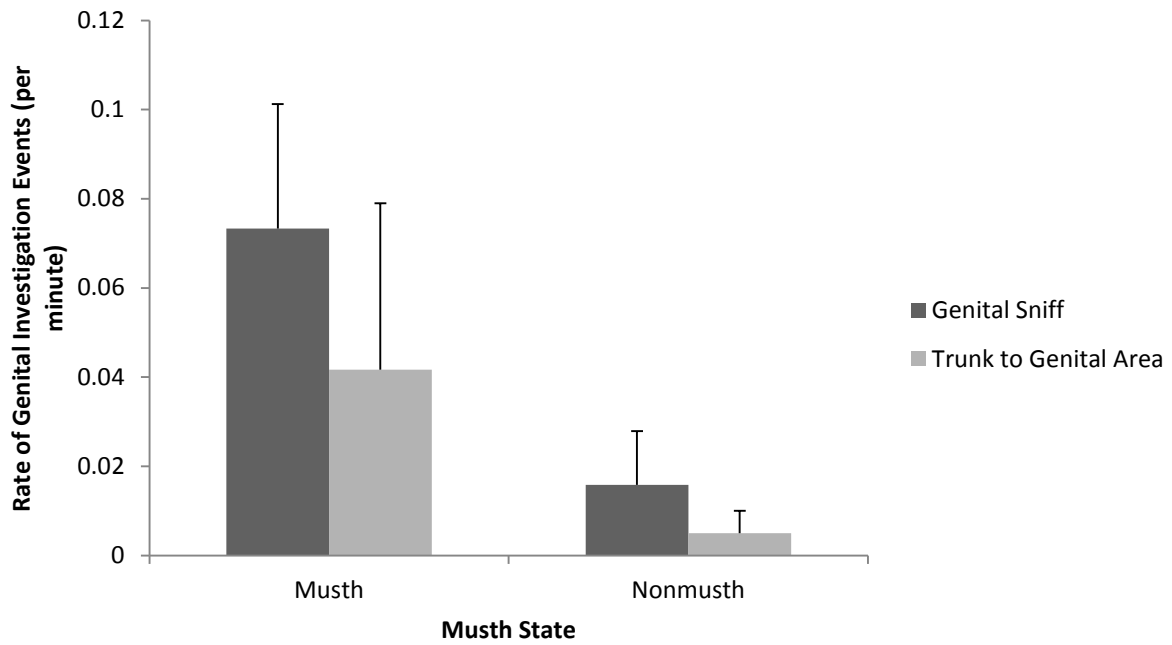


Figure 23. Mean (+SE) rate of genital investigation events for musth and nonmusth male elephants at AENP.

Appendix A

PATTERNS OF CHEMOSENSORY BEHAVIOR IN REPRODUCTIVELY MATURE ADULT MALE ELEPHANTS

Introduction

Musth is an annual occurrence for adult male Asian and African elephants and has been likened to rutting behavior in ungulates (Eisenberg et al. 1971). Musth is a period of highly elevated testosterone levels, accompanied by heightened interest in females and increased aggressiveness (Poole and Moss 1981; Hall-Martin 1987). A male in musth is dominant to all non-musth males, regardless of age and size. Older males experience long and predictable musth cycles while younger males have shorter and more sporadic musth episodes (Poole and Moss 1989). Along with increased testosterone levels, musth males produce low-frequency calls, increase their activity levels, expel a large amount of odorous secretion from their temporal gland, and dribble pungent urine trails (Poole and Moss 1981; Poole and Moss 1989; Rasmussen et al. 1984; Rasmussen et al. 1990; Kahl and Armstrong 2002). Males frequently use chemosensory behaviors to assess female reproductive status (Rasmussen 1988; Langbauer 2000; Loizi et al. 2009). Using these behaviors, males are likely able to detect a chemical in female urine which signals the female is approaching estrous (Bagley et al. 2006). Females actively prefer mating with males in musth; older males in musth tend to mate more often and more frequently than younger males in musth (Poole 1989).

The objective of this study was to examine the effect of musth on rate of chemosensory behavior. Because adult males in musth spend more time searching for receptive females, I predicted that males in musth would have higher rates of chemosensory behavior than nonmusth males. Males in musth actively search for receptive mates (Hall Martin 1987; Ganswindt et al.

2005), so I predicted that musth males would perform more auxiliary chemosensory behaviors (horizontal sniffs, periscope sniffs, genital sniffs, and trunk tip to genitals) than nonmusth males.

Methods

This study took place in Addo Elephant National Park on the same population of elephants as the aforementioned study. Observations occurred in 2006 from January to mid-December. Observations were made on adult males capable of entering the state of musth (approximately 23 years and older in the AENP population). Focal-animal sampling, as described by Altmann (1974) was used to collect data on the behavior of these adult male elephants. Twenty-minute observations with continuous recording of all behaviors were conducted on each focal animal. The mean length of adult male focal observations was 19.8 ± 0.32 minutes (Figure 5). If a focal individual left the field of view, I made an attempt to reposition myself to keep the focal individual in sight. If the focal individual disappeared into a group of elephants or into the thick vegetation, then I would stop the focal observation. Focal observations shorter than 10 minutes were not included in the data analysis. An ethogram defining all relevant behaviors was developed and used, with all behavioral data being recorded in a notebook (Table 1). Observations were made from a vehicle, with binoculars if necessary. The elephant population in Addo is habituated to humans and tends to ignore vehicles as well as the passengers within those vehicles.

Twelve adult males were sampled multiple times in order to obtain behavioral data on a male in musth and then that same male not in musth. State of musth was determined by observing external/physical signs including temporal gland secretion (TGS), continuous secretion and dribbling of urine, and presence of a characteristic odiferous smell (Poole and

Moss 1981; Poole and Moss 1989; Rasmussen et al. 1984; Rasmussen et al. 1990; personal communication with B. Schulte). Because musth males are quite mobile and their state of musth is temporary (generally 1-3 months), finding a particular male in musth was often a challenge. Therefore, if I happened to find a musth male and did not have a focal observation on that individual yet, I generally stopped to record a focal observation on that male. In order to make a comparison between musth and nonmusth males, I sought out the same 12 males in an effort to record focal observations of their behavior while they were not in musth. I compared the 12 males in musth to the same 12 males when they were not in musth using the Wilcoxon Signed-ranks test (non-parametric equivalent of a paired t-test). I also performed focal observations on 13 other adult males (2 were in musth and 11 were not in musth). I did not analyze these data because the nonmusth males were typically younger than the musth males and I would not be able to distinguish an age from a musth effect.

Results

Chemosensory behaviors

As predicted, elephants in musth exhibited higher rates of chemosensory behaviors compared to when they were not in musth (Figure 20). Musth males performed significantly more sniffs ($t = 29.0$, $df = 11$, $p = 0.007$), checks ($t = 39.0$, $df = 11$, $p < 0.00001$) and places ($t = 39.0$, $df = 11$, $p < 0.00001$) than nonmusth males. Although the rate of flehmens did not differ between musth and nonmusth males ($t = 11.0$, $df = 11$, $p = 0.078$), six males performed flehmen while in musth compared to only two of these males displaying flehmen when not in musth. When comparing musth males to all ages of males, musth males displayed significantly more flehmens per minute than calf males ($H = 9.77$, $df = 4$, $p = 0.044$; Figure 21).

Auxiliary chemosensory behaviors

Male elephants in musth compared to when not in musth showed significantly higher rates of horizontal sniffs ($t = 26.5$, $df = 11$, $p = 0.015$; Figure 22) and nearly significant higher rates of genital sniffs ($t = 9.5$, $df = 11$, $p = 0.063$; Figure 23). Rates of periscope sniffs ($t = 2.5$, $df = 11$, $p = 0.719$; Figure 22) and genital contacts with the trunk ($t = 1.0$, $df = 11$, $p = 0.750$; Figure 23) did not differ by musth or nonmusth condition.

Discussion

There were clear behavioral differences between musth males and nonmusth males in the Addo elephant population. My prediction that musth males would have higher rates of chemosensory behavior than nonmusth males was supported by the results of this study. Males in musth performed higher rates of sniff, check, and place behaviors than nonmusth males. When looking at the males of all ages, musth males had the highest rate of flehmen behavior. Once males enter the state of musth, they spend a large proportion of their time seeking out estrous females, a scarce and highly mobile resource (Hollister-Smith et al. 2007). Musth is an energetically costly state to maintain. A study by Poole (1989) found that males in musth lose weight and their physical condition visibly declines as time in musth increases; weight loss during musth is largely attributed to decreased feeding, increased metabolism (due to elevated testosterone/androgen levels), and increased activity levels as musth males spend much of their time trying to locate mates (Poole 1989; Ganswindt et al. 2005). Musth males often perform elevated sniffs and other investigatory behaviors to locate receptive females and determine their sexual state. For this study, my prediction that musth males would perform more investigative behaviors was partially supported. Musth males performed more horizontal sniffs than nonmusth males, but the rate of periscope sniffs, genital sniffs, and trunk to genital area did not

differ between musth and nonmusth males. Horizontal sniffs, interestingly enough, were also performed significantly more by 15-19 year old males than 10-14 year old males, indicating that this investigative behavior may become more important and useful as pubescent males approach adulthood and the age of their first musth or it may simply be related to size. Musth influences the association patterns and behavior of both male and female elephants (Hollister-Smith et al. 2007). In adult males, chemosensory behaviors are strongly influenced by musth, as evidenced in this study. As musth males wander around in search of receptive mates, they actively use chemosensory behaviors to assess chemical signals of reproductive importance in an effort to locate estrous females and pass their genes on to the next generation.

Appendix B

The following graphs and associated statistics were created and compiled during the data analysis phase of this project. As the manuscript was developed it became clear that these behaviors, interesting as they may be, were not directly relevant to the objectives of this research project or the hypotheses of this thesis. Therefore, they were included in the appendix just in case this information might be useful to another elephant researcher down the road.

Table A.1. Ethogram used to record event behaviors performed by wild African male and female elephants during continuous focal-animal sampling (Loizi 2004; Bagley 2004; Merte 2006; Meyer 2006)

<u>Event behavior categories</u>	<u>Defined event behaviors</u>	<u>Definition</u>
<i>Chemosensory</i>	Check*	Touch ground with tip of either finger
	Flehmen*	Tip of trunk touches substrate or conspecific then placed in the VNO ducts in the roof of the mouth
	Place*	Entire nasal opening is placed on ground or conspecific and held momentarily
	Sniff*	Nasal openings hover over ground or conspecific (excluding genitals) without contact
<i>Auxiliary Chemosensory</i>	Blow	Expel air out through trunk
	Defecate	Expel feces from body
	Genital Sniff	Nasal openings hover over genital area of conspecific without contact
	Horizontal Sniff	Elevated sniff occurring with trunk extended horizontally (between ground and mouth level)
	Periscope Sniff	Elevated sniff occurring with trunk raised above mouth level
	Pinch	Both tips of trunk close and touch each other
	Trunk Flick	Distal end of trunk curls up slightly and

		then flicks away from the body, usually toward the ground
	Trunk Shake	Entire trunk wriggles/twists once and then returns to a hanging position; slower than a trunk flick
	Urinate	Expel urine from body
<i>Trunk tip to conspecific</i>	Body	Torso or areas not listed
	Foot	Area below ankle
	Genital Area	Penis/vulva region, between rear legs
	Head	Forehead and superior most point of head
	Leg	Area from ankle to shoulder/ankle
	Mouth	Trunk tip inserted into mouth
	Pinnae	External area around ear (outer ear)
	Tusk	Trunk tip to tusk
<i>Reproductive</i>	Present Rear	Turn backside toward conspecific
<i>Threat (no contact)</i>	Ear Expansion	Pinnae extend out completely, wave, and then rapidly brought back against body
	Trunk Toss	Trunk is thrown forward in a threatening way
<i>Comfort (contact)</i>	Head Into	Gently presses head into body of another individual and rests it there
	Lean	Places body weight against the body of another individual
	Rub	Using the torso to brush against another individual's torso
<i>Multiple meanings</i>	Ear Flap	Pinnae are extended away from body and then brought back against body at a moderate speed
	Headshake	Head is dropped and twisted and then rapidly returned to place, with ears and trunk flailing
	Incidental Contact	Accidental contact with conspecific
	Penis Drop	Penis pushes out of sheath and is fully extended
	Trunk Curl	Trunk rolls up toward mouth but does not touch body
	Trunk Dig	Trunk tip digs at ground, substrate, or object
	Trunk Entwine	Trunk twists around the trunk of a conspecific

	Trunk Over Back	Placing trunk across the back of a conspecific and holding position for at least two seconds
	Vocalize	Audible sound emits from mouth or trunk

*Chemosensory definitions defined by Schulte & Rasmussen (1999)

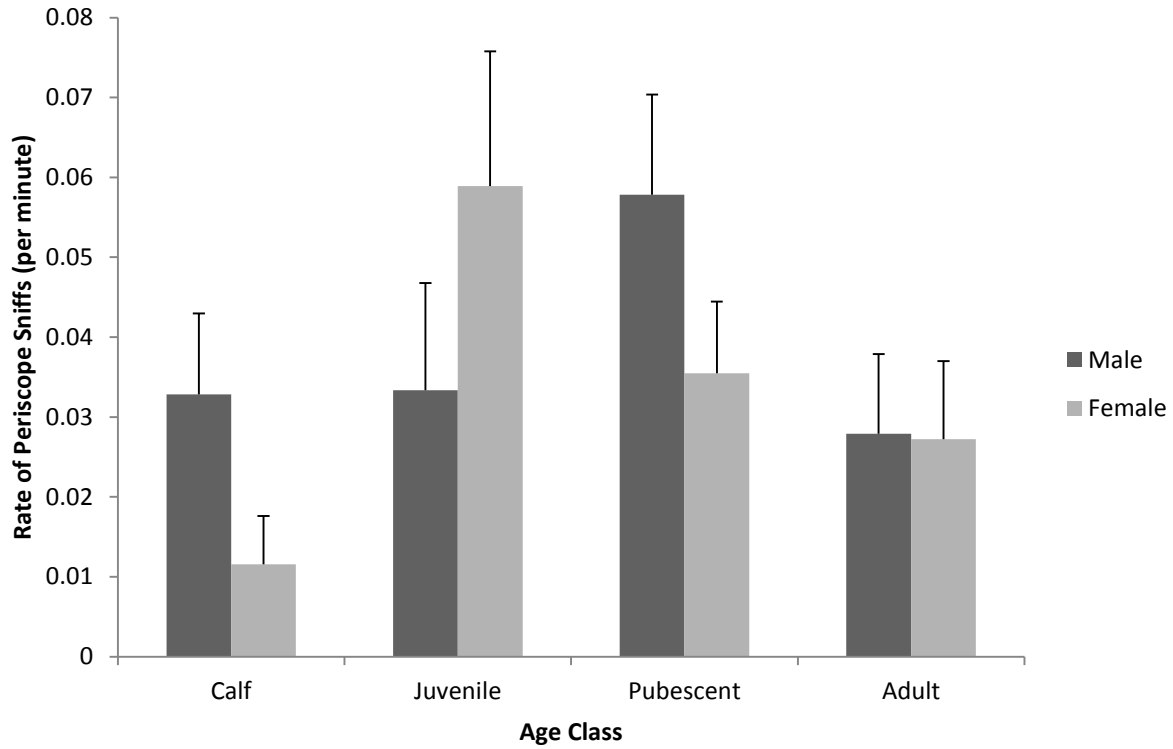


Figure A.1. Mean (+SE) rate of periscope sniffs per age class for male and female elephants at AENP. There was no significant difference in the mean rate of periscope sniffs between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.43$, $df = 1$, $p = 0.515$; Age-Class: $H = 4.98$, $df = 3$, $p = 0.173$).

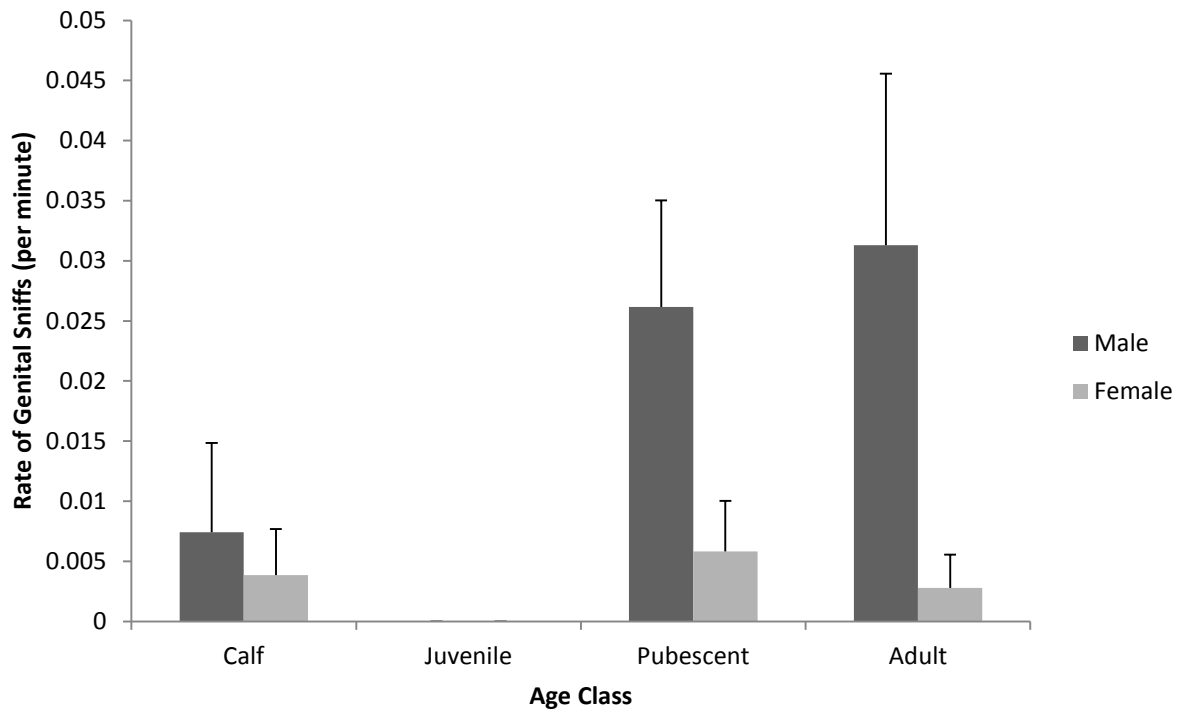


Figure A.2. Mean (+SE) rate of genital sniffs per age class for male and female elephants at AENP. Males had a higher mean rate of genital sniffs than females, but there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 6.14$, $df = 1$, $p = 0.013$; Age-Class: $H = 6.75$, $df = 3$, $p = 0.080$).

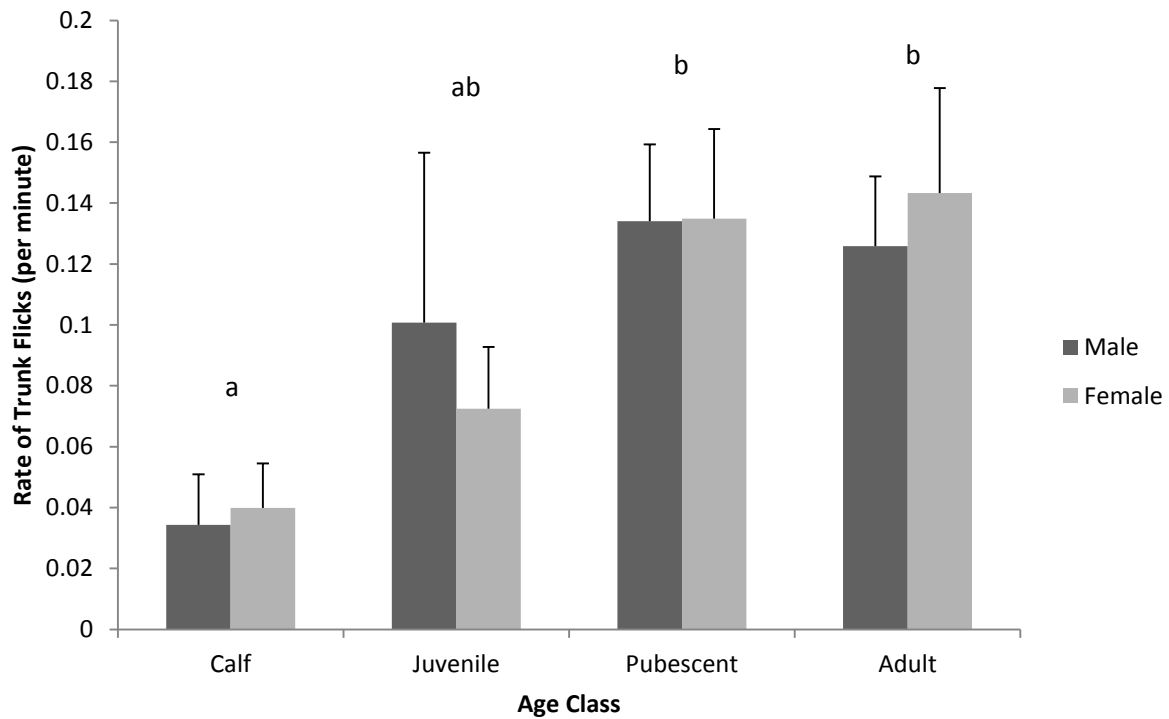


Figure A.3. Mean (+SE) rate of trunk flicks per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk flicks between males and females, but there were significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.033$, $df = 1$, $p = 0.856$; Age-Class: $H = 17.46$, $df = 3$, $p = 0.0006$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

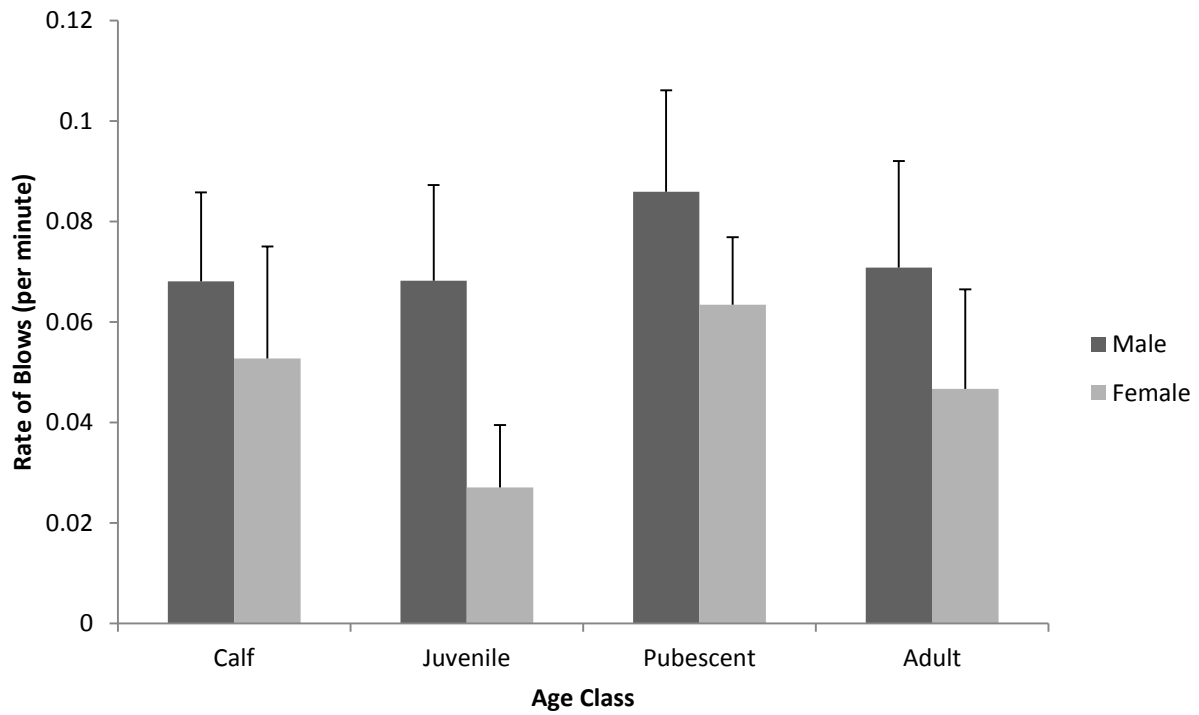


Figure A.4. Mean (+SE) rate of blows per age class for male and female elephants at AENP. There was no significant difference in the mean rate of blows between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 3.52$, $df = 1$, $p = 0.061$; Age-Class: $H = 2.29$, $df = 3$, $p = 0.52$).

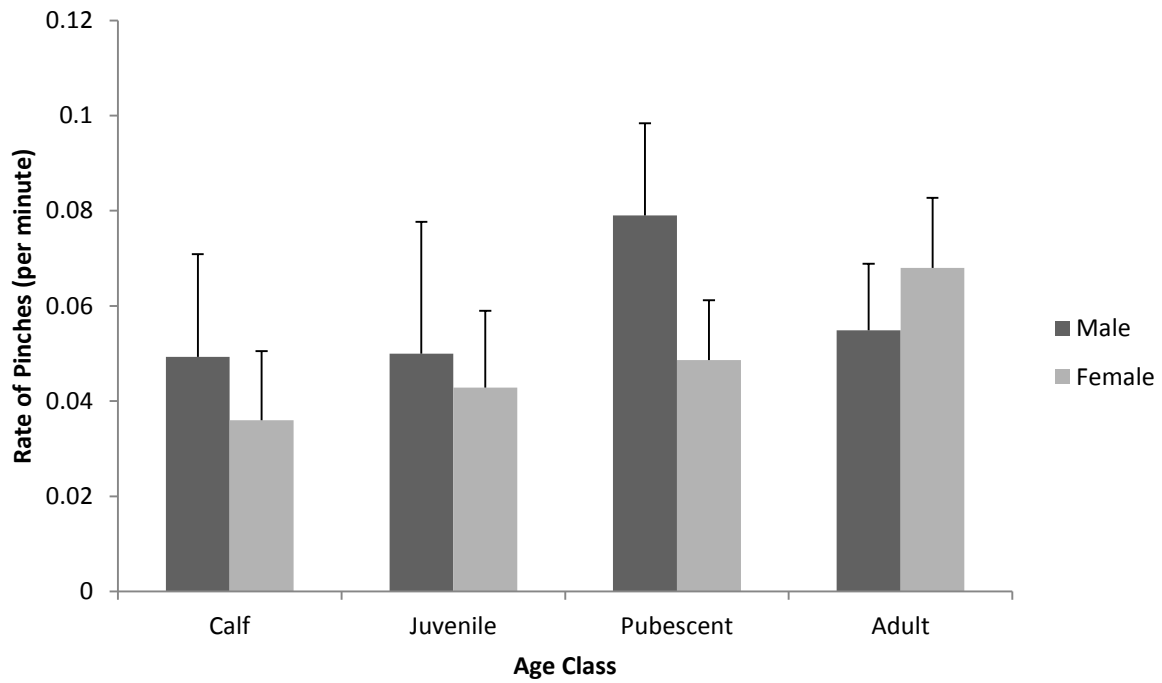


Figure A.5. Mean (+SE) rate of pinches per age class for male and female elephants at AENP. There was no significant difference in the mean rate of pinches between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.006$, $df = 1$, $p = 0.94$; Age-Class: $H = 4.38$, $df = 3$, $p = 0.22$).

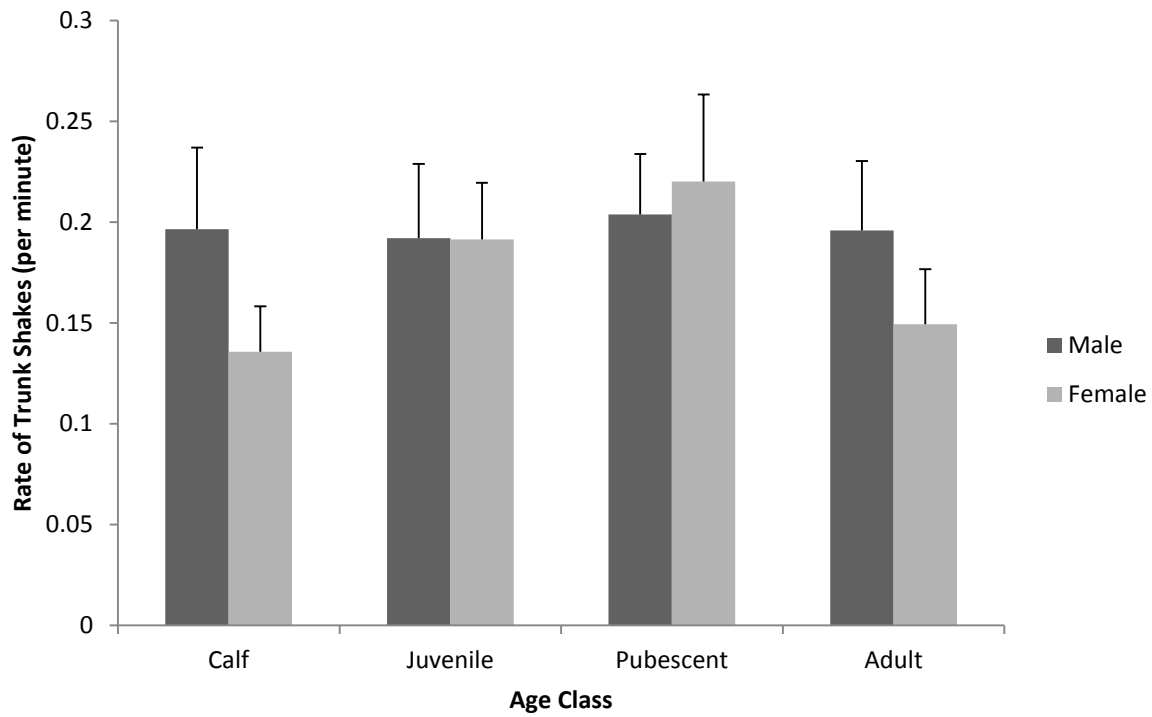


Figure A.6. Mean (+SE) rate of trunk shakes per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk shakes between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.31$, $df = 1$, $p = 0.58$; Age-Class: $H = 0.84$, $df = 3$, $p = 0.84$).

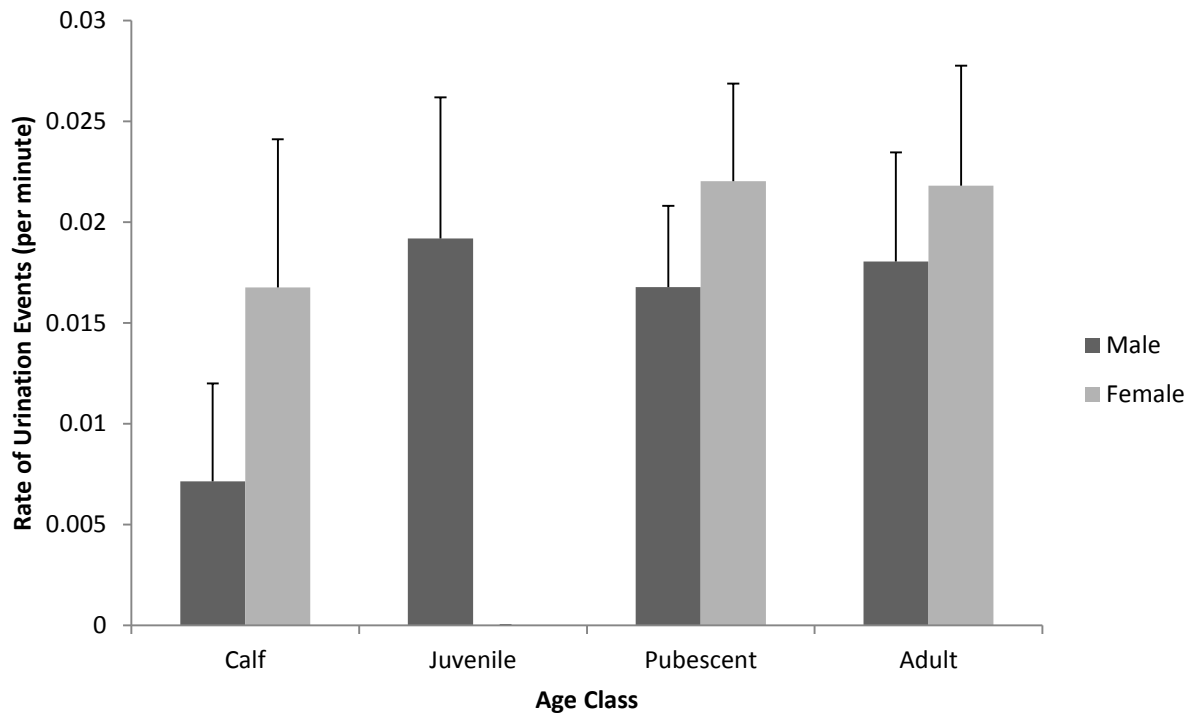


Figure A.7. Mean (+SE) rate of urination events per age class for male and female elephants at AENP. There was no significant difference in the mean rate of urination events between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.028$, $df = 1$, $p = 0.87$; Age-Class: $H = 5.99$, $df = 3$, $p = 0.11$).

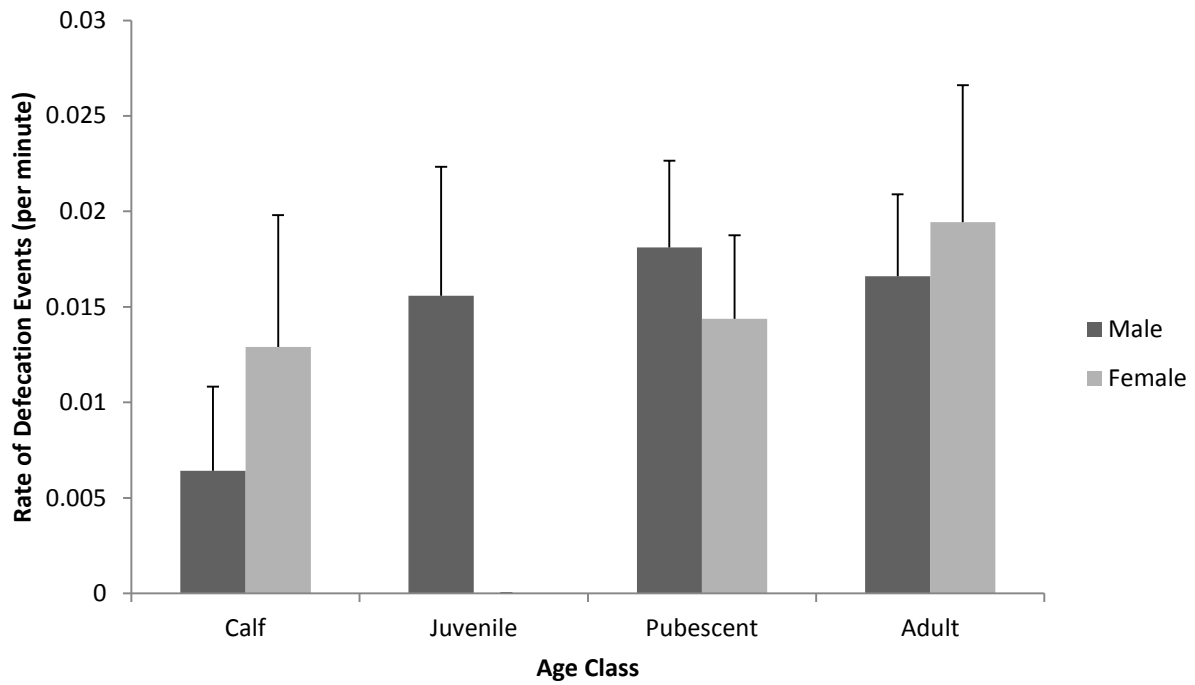


Figure A.8. Mean (+SE) rate of defecation events per age class for male and female elephants at AENP. There was no significant difference in the mean rate of defecation events between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 1.14$, $df = 1$, $p = 0.29$; Age-Class: $H = 5.42$, $df = 3$, $p = 0.14$).

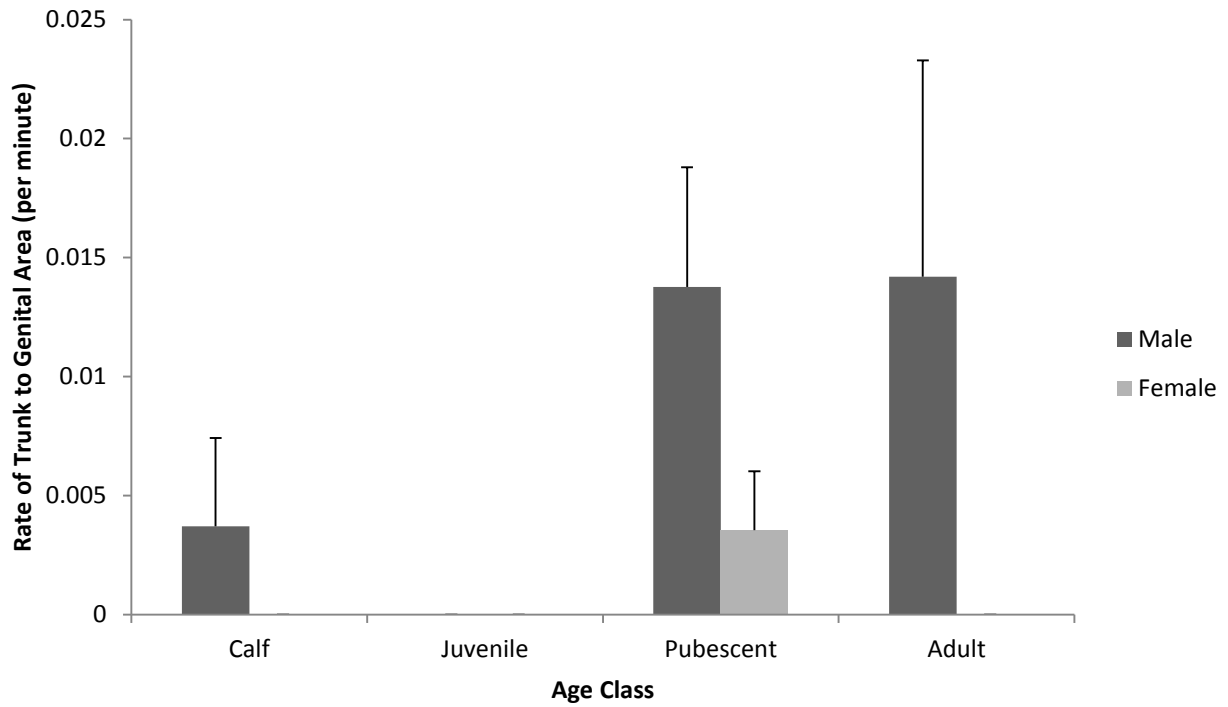


Figure A.9. Mean (+SE) rate of trunk touches to genital area per age class for male and female elephants at AENP. Males had a higher mean rate of trunk tip to genital area than females, but there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 6.18$, $df = 1$, $p = 0.013$; Age-Class: $H = 5.66$, $df = 3$, $p = 0.130$).

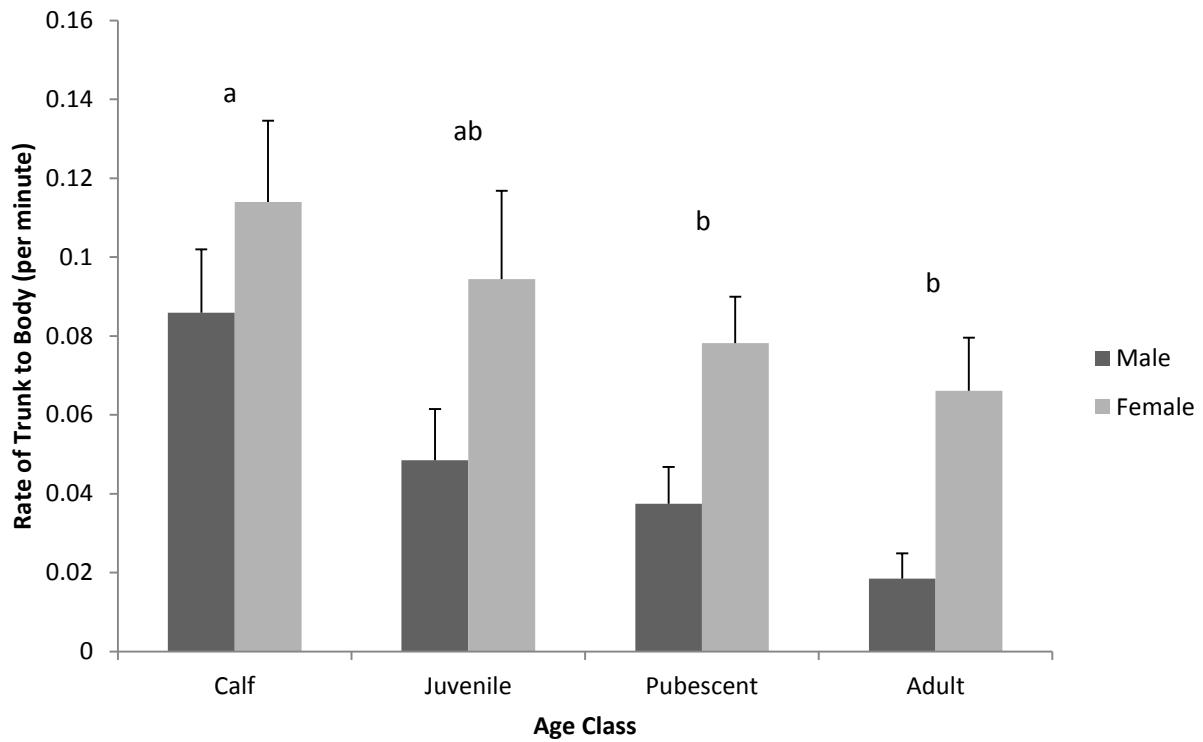


Figure A.10. Mean (+SE) rate of trunk touches to body per age class for male and female elephants at AENP. Females had a higher mean rate of trunk touches to body than males and there were significant differences between age classes (Kruskal-Wallis – Sex: $H = 19.58$, $df = 1$, $p < 0.0001$; Age-Class: $H = 15.41$, $df = 3$, $p = 0.0015$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

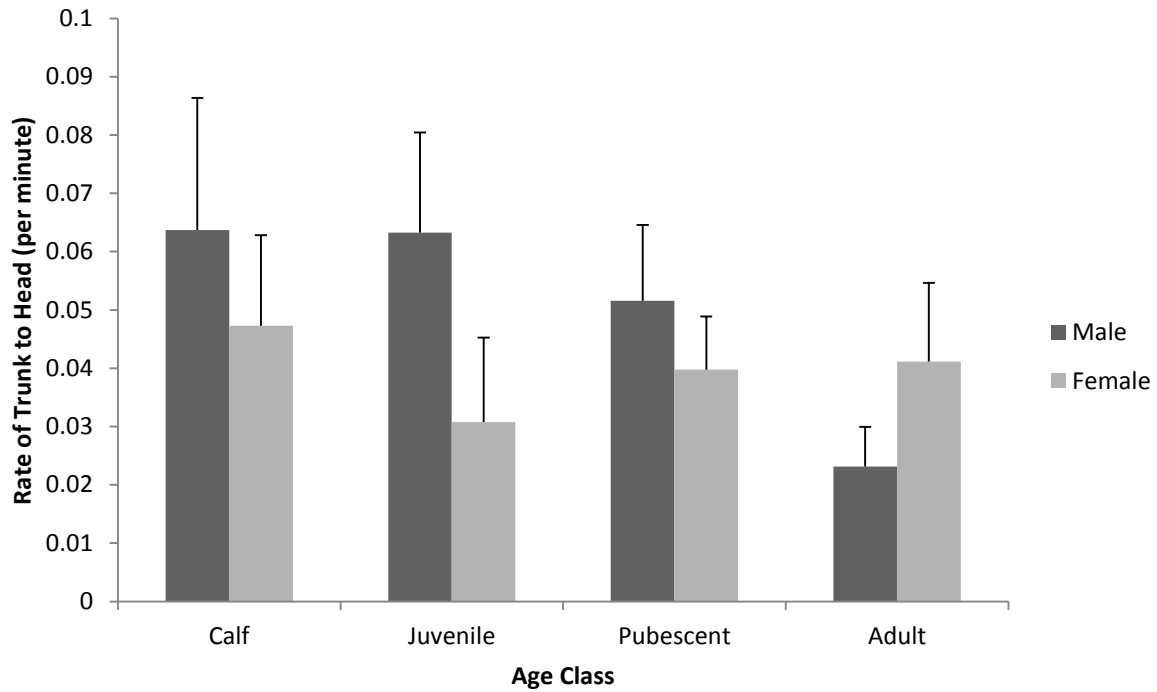


Figure A.11. Mean (+SE) rate of trunk touches to head per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk touches to head between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.42$, $df = 1$, $p = 0.52$; Age-Class: $H = 2.34$, $df = 3$, $p = 0.50$).

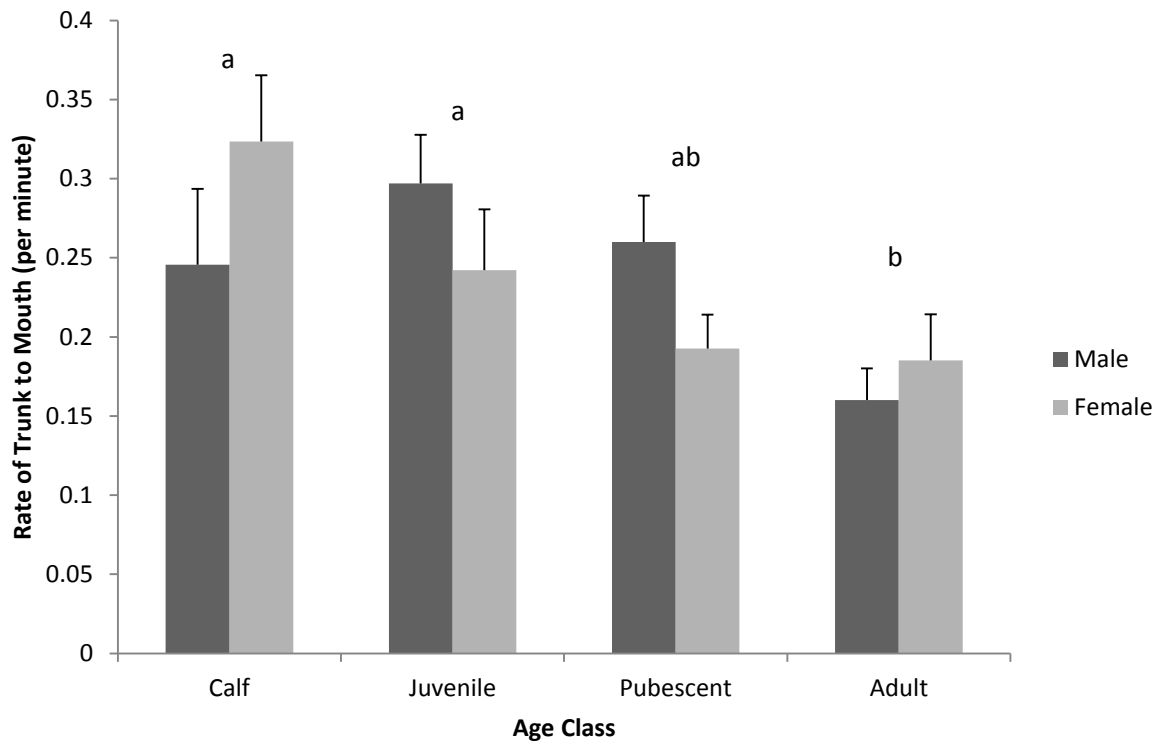


Figure A.12. Mean (+SE) rate of trunk touches to mouth per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk touches to mouth between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 0.034$, $df = 1$, 149 , $p = 0.86$; Age class: $F = 4.48$, $df = 3$, 149 , $p = 0.0048$; Interaction: $F = 2.17$, $df = 3$, 149 , $p = 0.094$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

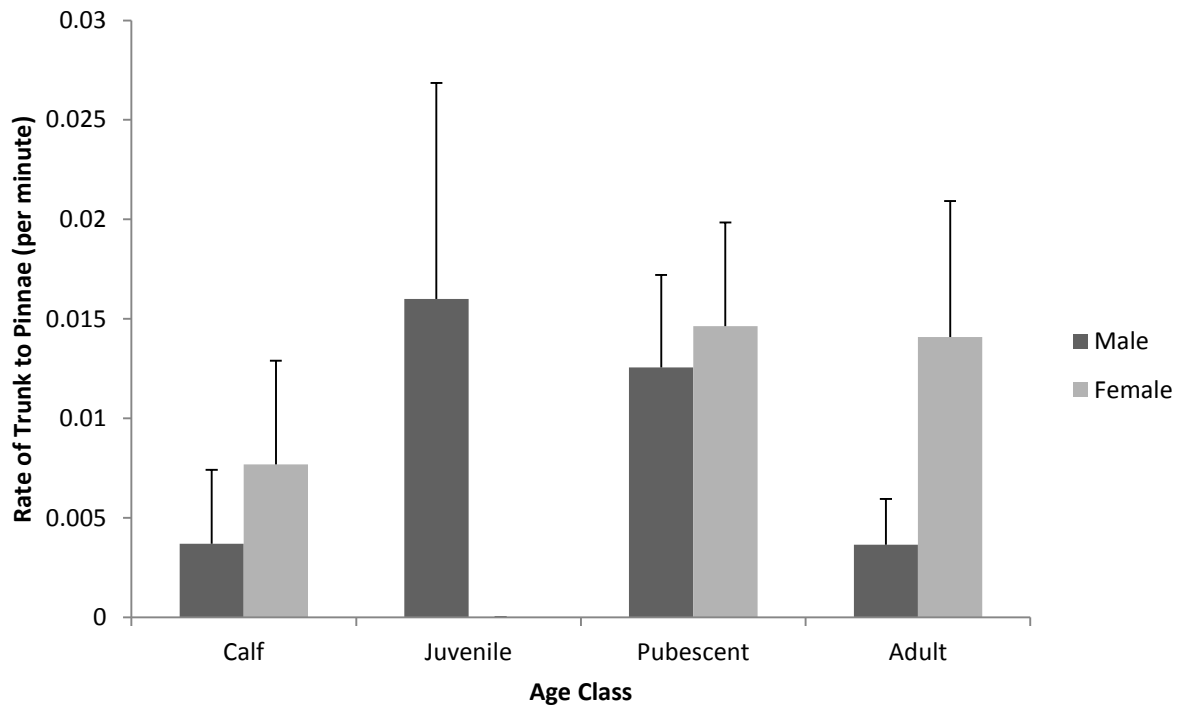


Figure A.13. Mean (+SE) rate of trunk touches to pinnae per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk touches to pinnae between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.28$, $df = 1$, $p = 0.60$; Age-Class: $H = 3.21$, $df = 3$, $p = 0.36$).

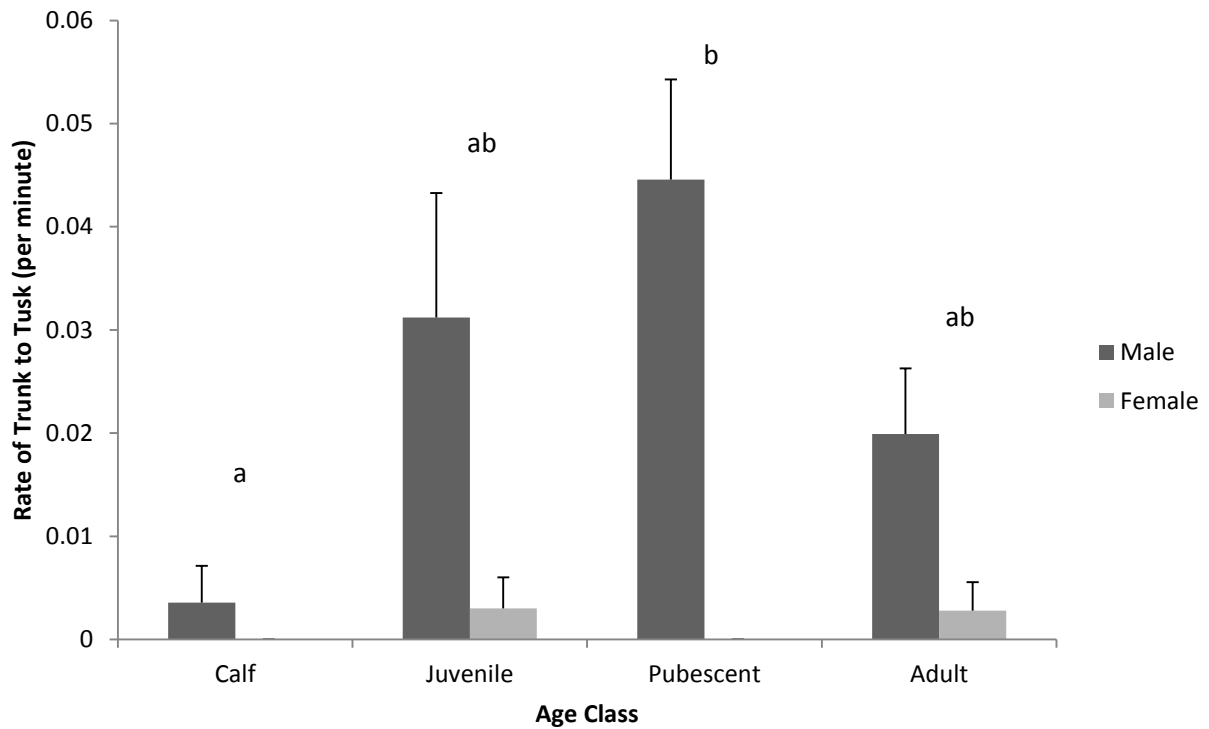


Figure A.14. Mean (+SE) rate of trunk touches to tusk per age class for male and female elephants at AENP. Males had a higher mean rate of trunk touches to tusk than females and there were significant differences between age classes (2-way ANOVA – Sex: $F = 17.37$, $df = 1$, 149 , $p < 0.0001$; Age class: $F = 2.59$, $df = 3$, 149 , $p = 0.05$; Interaction: $F = 2.83$, $df = 3$, 149 , $p = 0.040$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

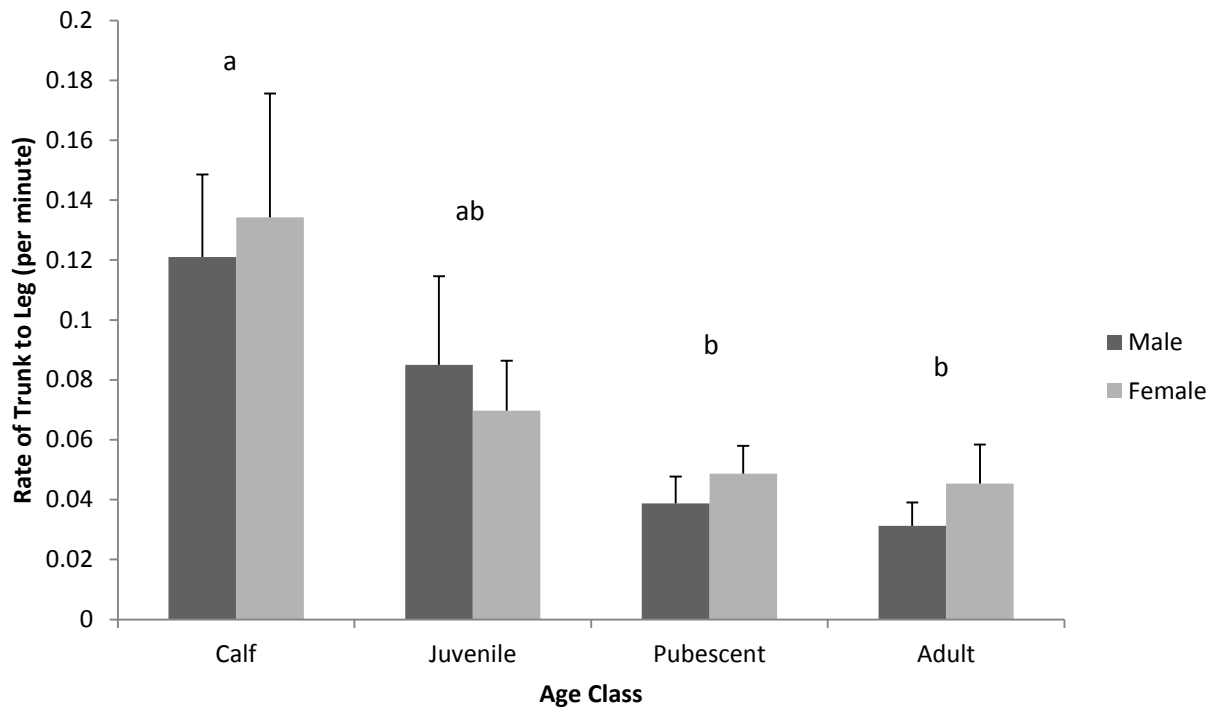


Figure A.15. Mean (+SE) rate of trunk touches to leg per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk touches to leg between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 0.19$, $df = 1$, 149 , $p = 0.67$; Age class: $F = 9.99$, $df = 3$, 149 , $p < 0.0001$; Interaction: $F = 0.22$, $df = 3$, 149 , $p = 0.88$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

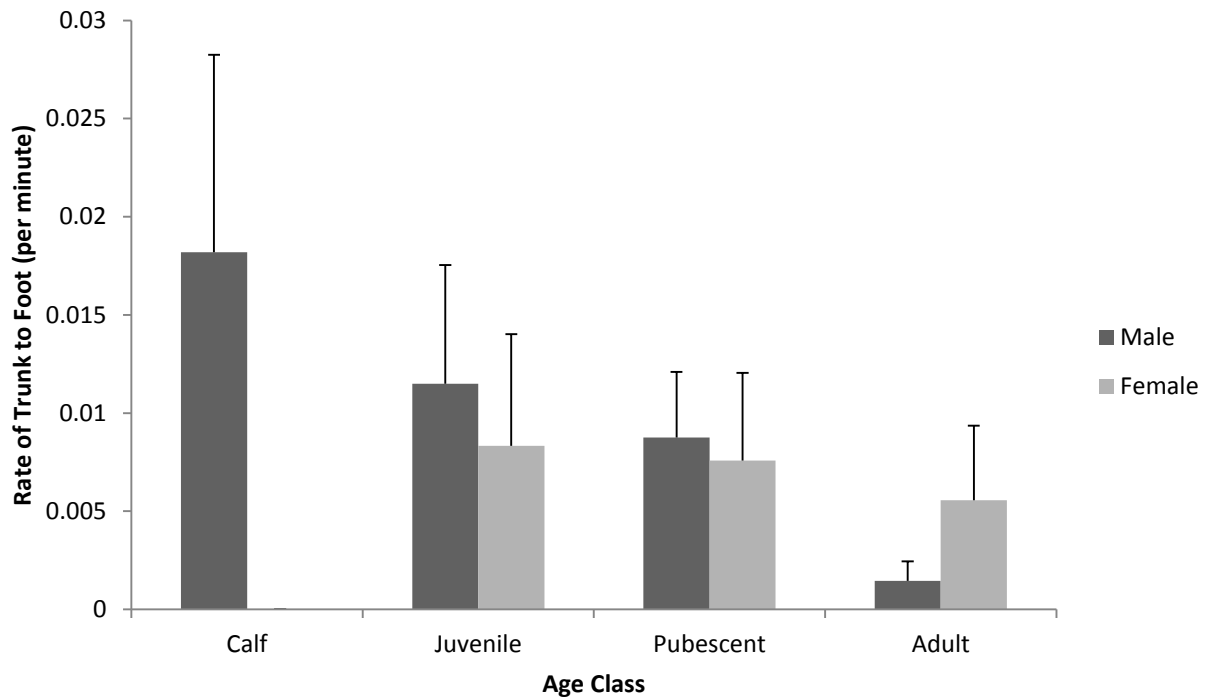


Figure A.16. Mean (+SE) rate of trunk touches to foot per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk touches to foot between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 1.22$, $df = 1$, $p = 0.27$; Age-Class: $H = 1.37$, $df = 3$, $p = 0.71$).

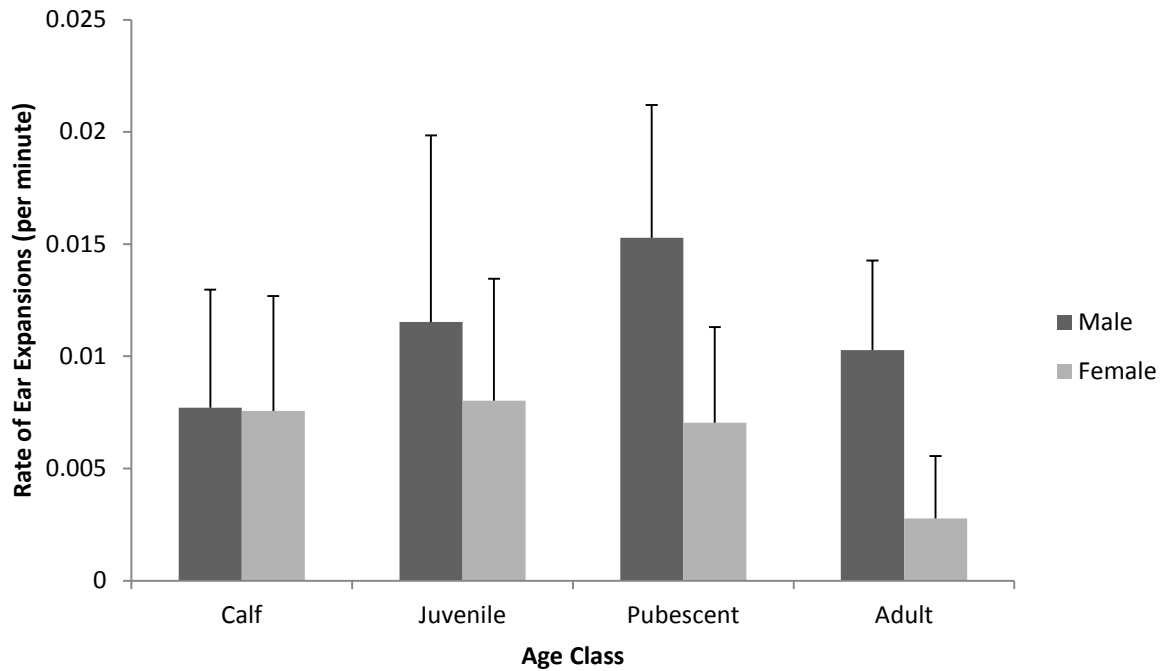


Figure A.17. Mean (+SE) rate of ear expansions per age class for male and female elephants at AENP. There was no significant difference in the mean rate of ear expansions between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 2.21$, $df = 1$, $p = 0.14$; Age-Class: $H = 0.046$, $df = 3$, $p = 0.10$).

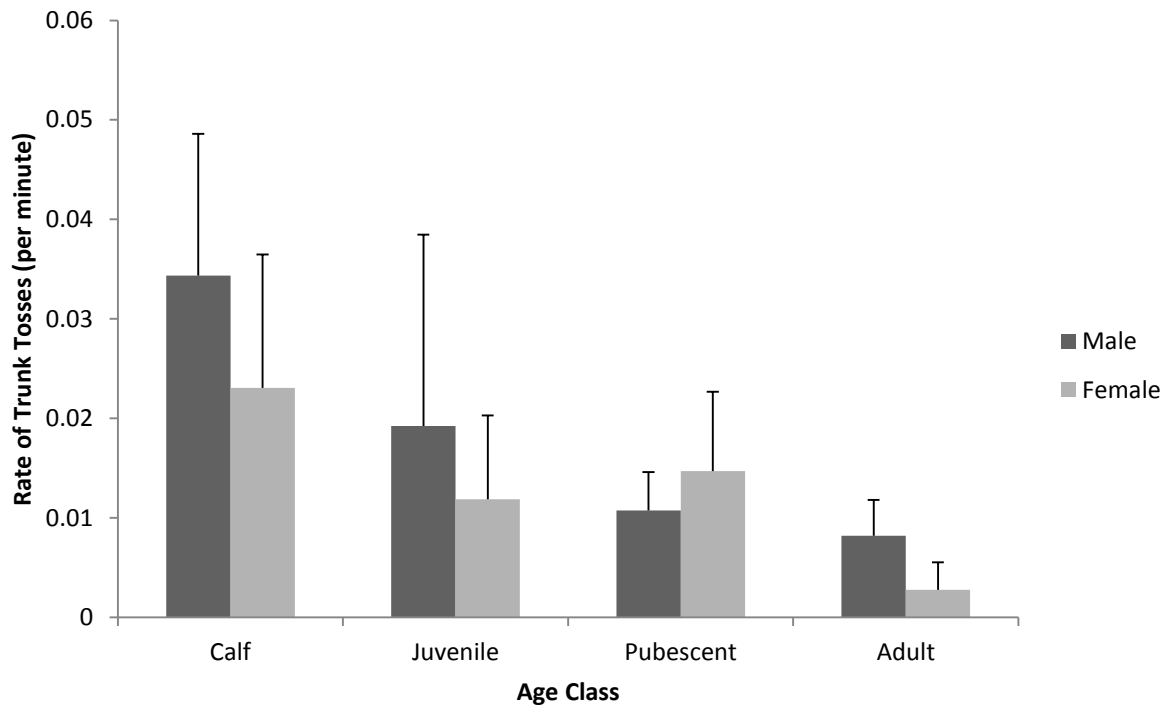


Figure A.18. Mean (+SE) rate of trunk tosses per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk tosses between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.89$, $df = 1$, $p = 0.35$; Age-Class: $H = 4.45$, $df = 3$, $p = 0.22$).

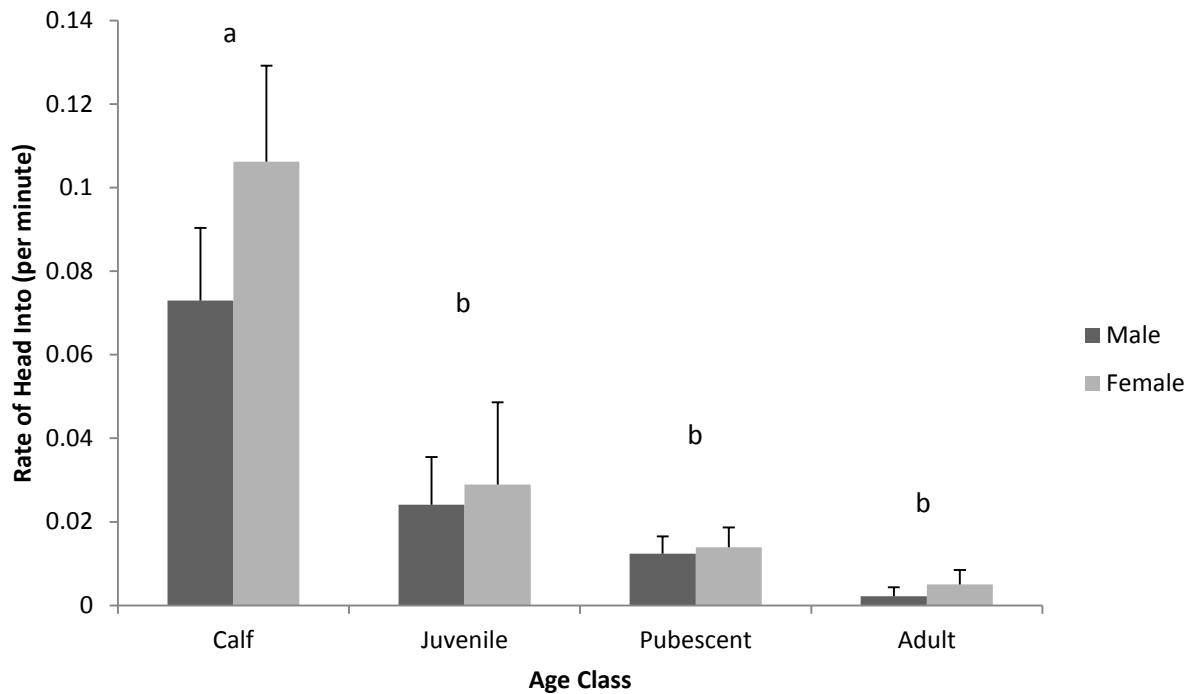


Figure A.19. Mean (+SE) rate of head into conspecific per age class for male and female elephants at AENP. There was no significant difference in the mean rate of head into conspecific between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 2.09$, $df = 1, 149$, $p = 0.15$; Age class: $F = 27.77$, $df = 3, 149$, $p < 0.0001$; Interaction: $F = 0.97$, $df = 3, 149$, $p = 0.41$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

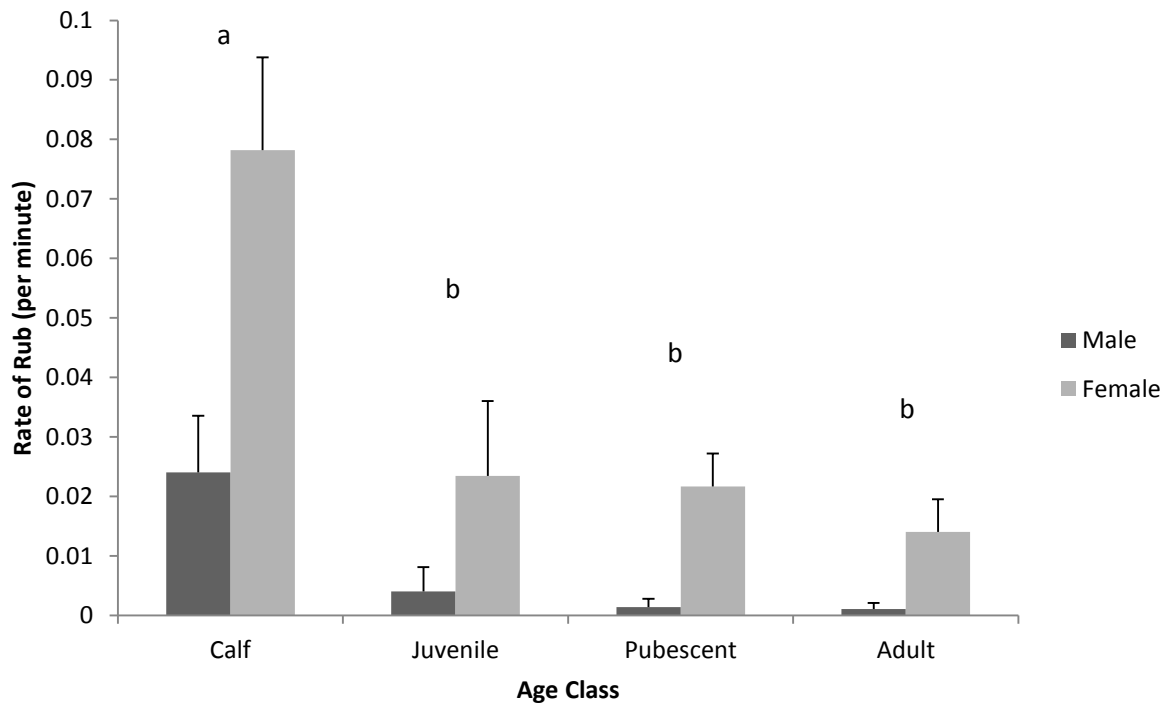


Figure A.20. Mean (+SE) rate of rub against conspecific per age class for male and female elephants at AENP. Females had a higher mean rate of rub against conspecific than males and there were significant differences between age classes (2-way ANOVA – Sex: $F = 32.27$, $df = 1$, 149 , $p < 0.0001$; Age class: $F = 16.12$, $df = 3$, 149 , $p < 0.0001$; Interaction: $F = 3.21$, $df = 3$, 149 , $p = 0.023$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

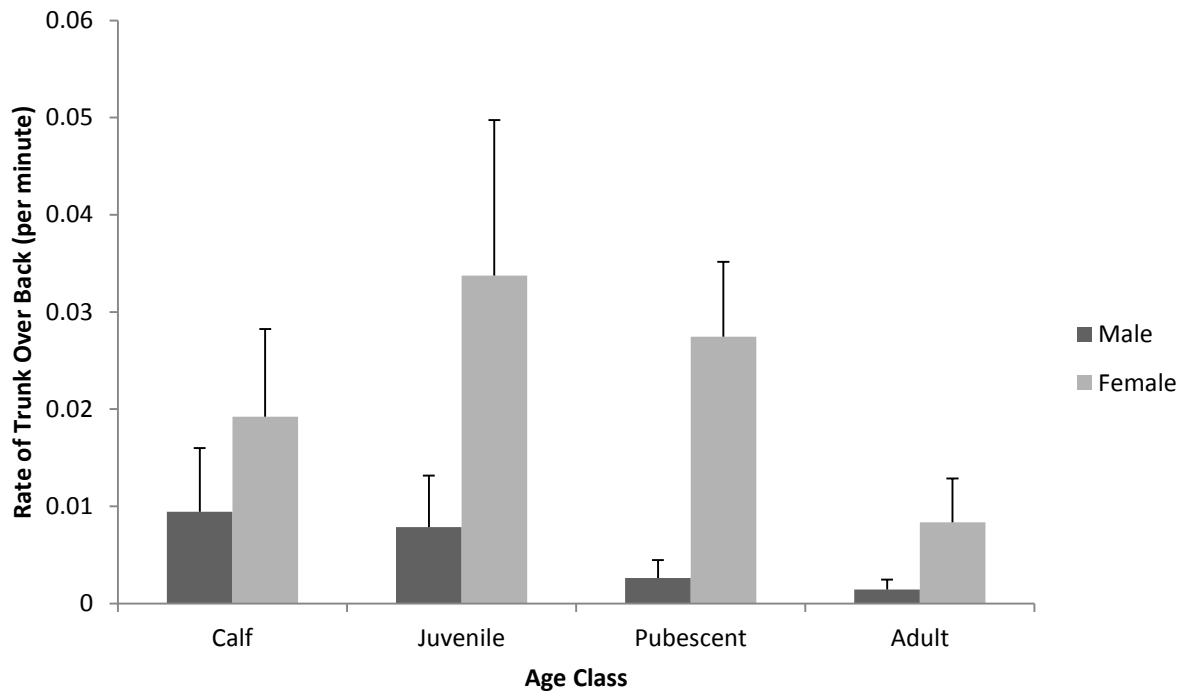


Figure A.21. Mean (+SE) rate of trunk over back per age class for male and female elephants at AENP. Females had a higher mean rate of trunk over back than males, but there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 15.39$, $df = 1$, $p < 0.0001$; Age-Class: $H = 3.37$, $df = 3$, $p = 0.34$).

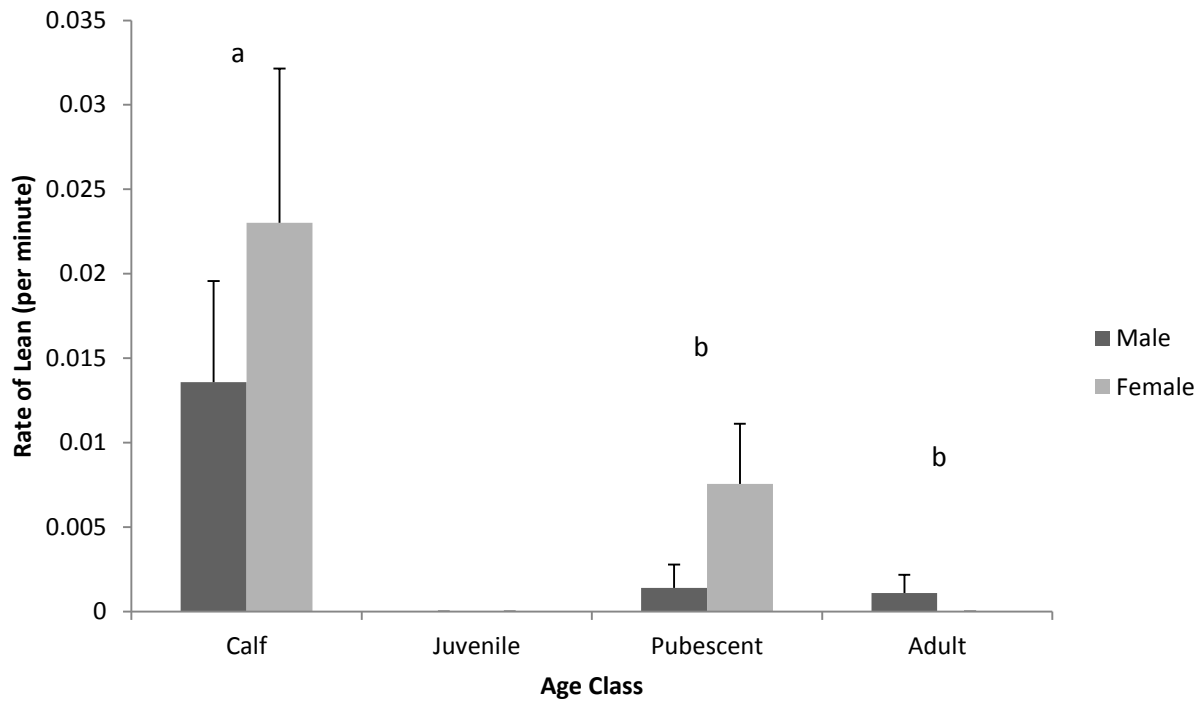


Figure A.22. Mean (+SE) rate of lean against conspecific per age class for male and female elephants at AENP. There was no significant difference in the mean rate of lean against conspecific between males and females, but there were significant differences between age classes (Kruskal-Wallis – Sex: $H = 1.64$, $df = 1$, $p = 0.20$; Age-Class: $H = 22.85$, $df = 3$, $p < 0.0001$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

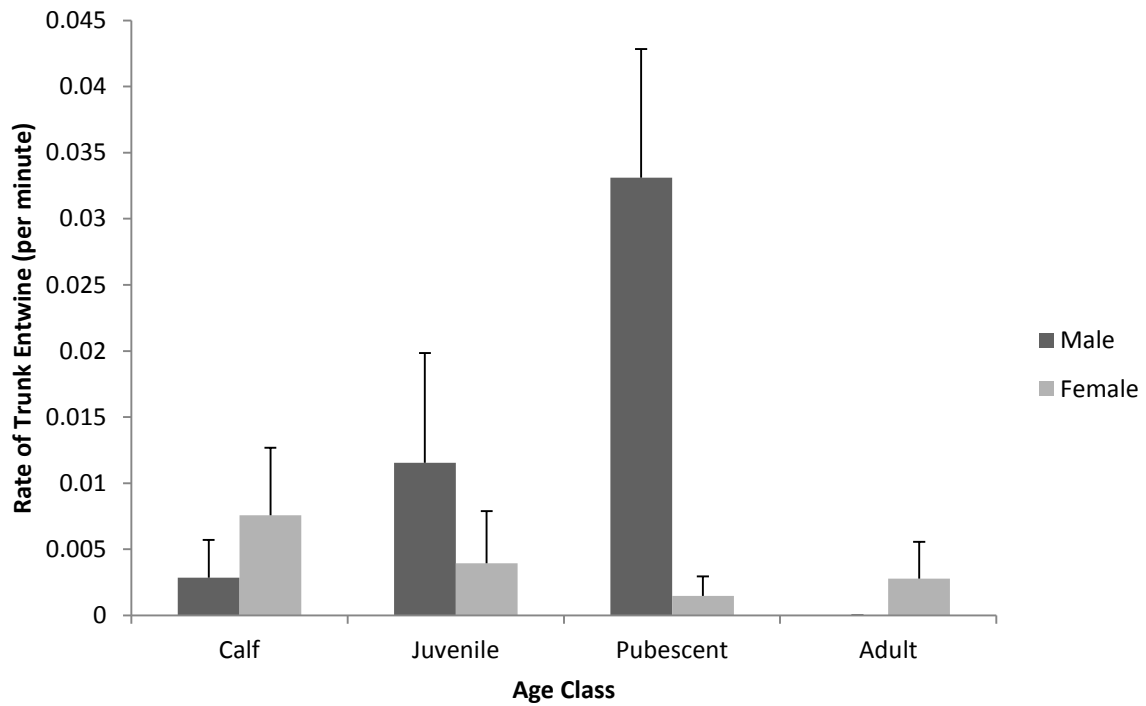


Figure A.23. Mean (+SE) rate of trunk entwine with conspecific per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk entwine between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 3.57$, $df = 1$, $p = 0.059$; Age-Class: $H = 6.90$, $df = 3$, $p = 0.075$).

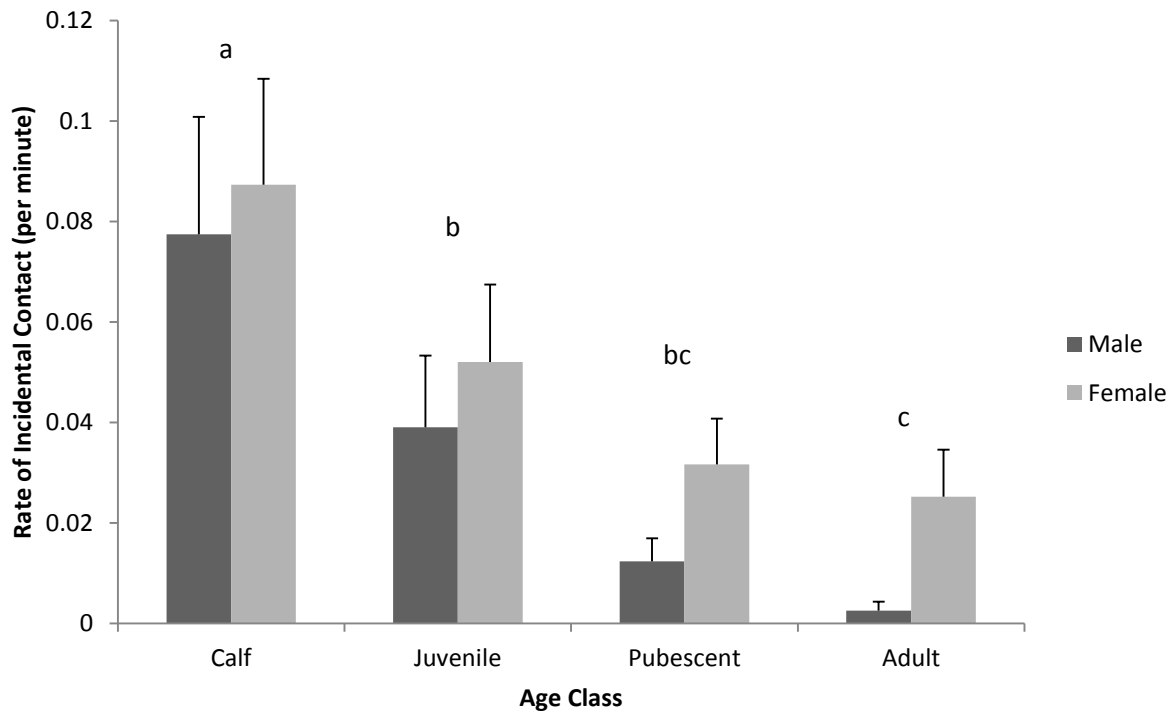


Figure A.24. Mean (+SE) rate of incidental contact with conspecific per age class for male and female elephants at AENP. Females had a higher mean rate of incidental contact with conspecific than males and there were significant differences between age classes (2-way ANOVA – Sex: $F = 4.12$, $df = 1, 149$, $p = 0.044$; Age class: $F = 13.05$, $df = 3, 149$, $p < 0.0001$; Interaction: $F = 0.12$, $df = 3, 149$, $p = 0.95$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

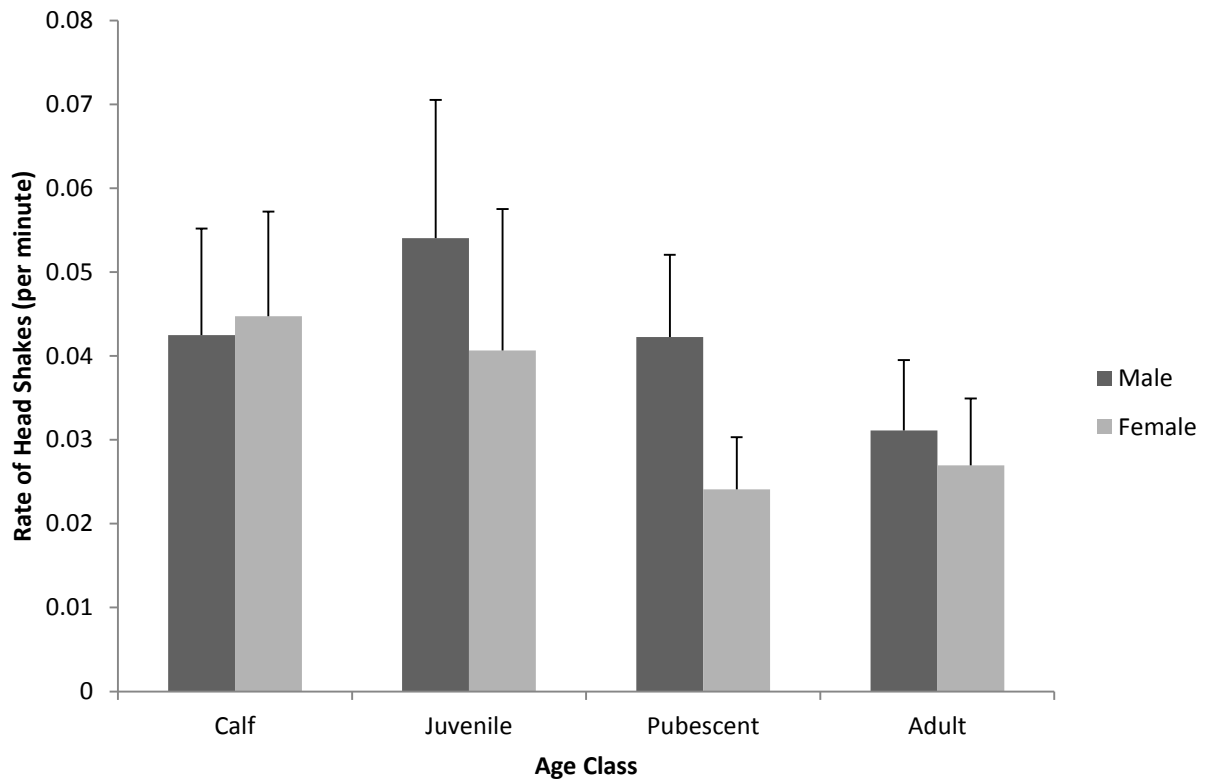


Figure A.25. Mean (+SE) rate of head shakes per age class for male and female elephants at AENP. There was no significant difference in the mean rate of head shakes between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 1.22$, $df = 1$, $p = 0.27$; Age-Class: $H = 2.01$, $df = 3$, $p = 0.57$).

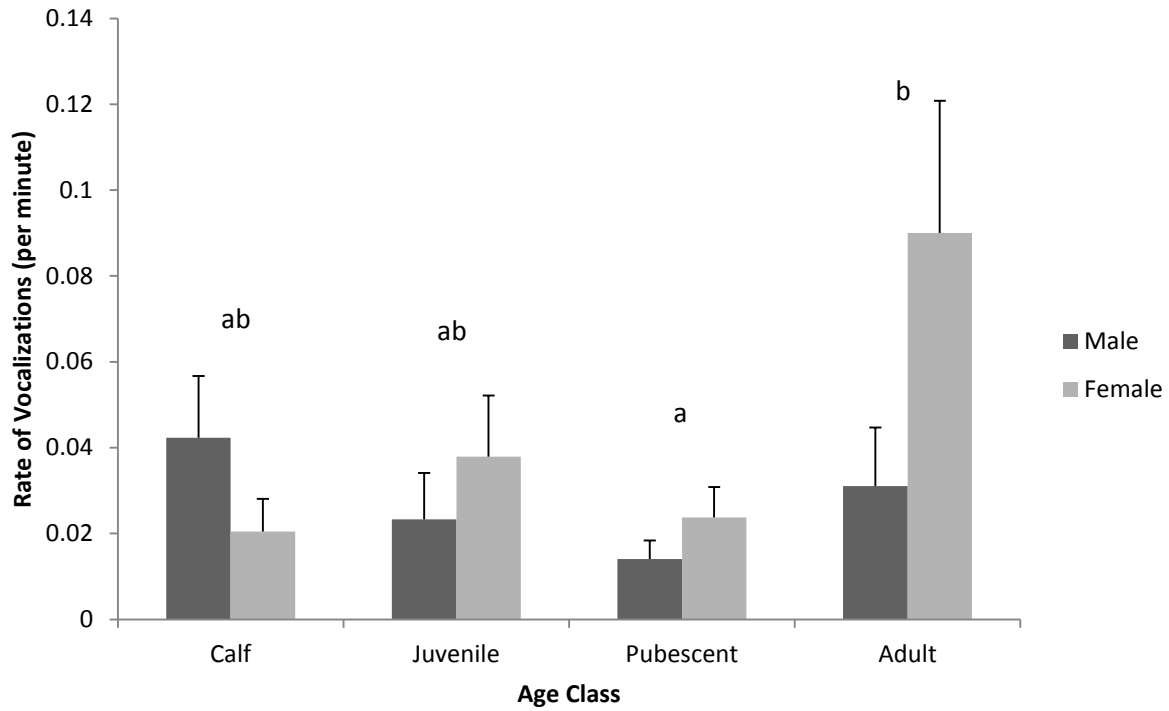


Figure A.26. Mean (+SE) rate of vocalizations per age class for male and female elephants at AENP. There was no significant difference in the mean rate of vocalizations between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 2.20$, $df = 1, 149$, $p = 0.14$; Age class: $F = 3.78$, $df = 3, 149$, $p = 0.012$; Interaction: $F = 2.60$, $df = 3, 149$, $p = 0.054$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

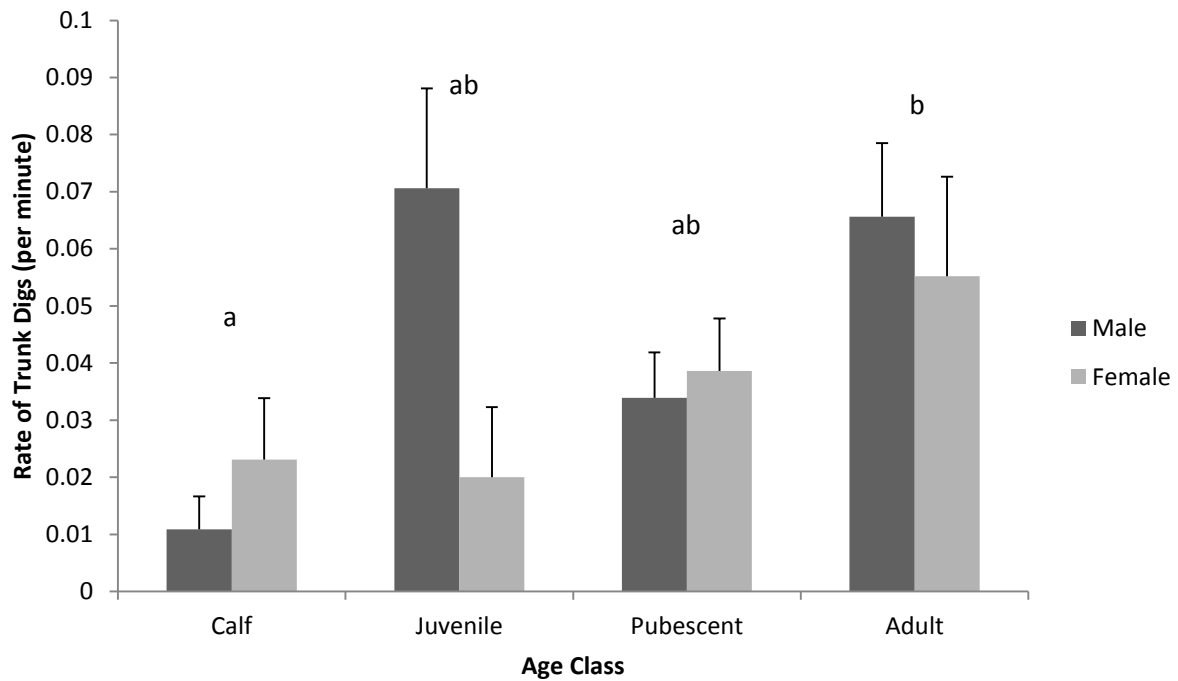


Figure A.27. Mean (+SE) rate of trunk digs per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk digs between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 1.59$, $df = 1$, 149 , $p = 0.21$; Age class: $F = 3.91$, $df = 3$, 149 , $p = 0.01$; Interaction: $F = 2.13$, $df = 3$, 149 , $p = 0.099$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

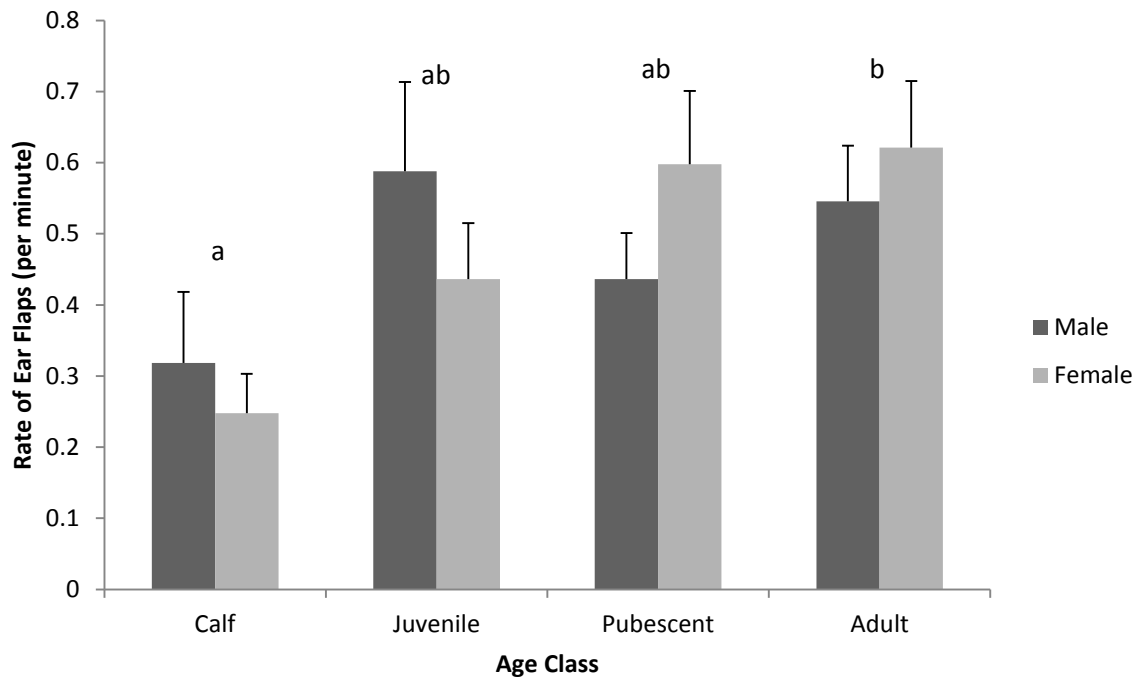


Figure A.28. Mean (+SE) rate of ear flaps per age class for male and female elephants at AENP. There was no significant difference in the mean rate of ear flaps between males and females, but there were significant differences between age classes (2-way ANOVA – Sex: $F = 0.017$, $df = 1$, 149 , $p = 0.90$; Age class: $F = 3.85$, $df = 3$, 149 , $p = 0.011$; Interaction: $F = 0.944$, $df = 3$, 149 , $p = 0.42$). Different letters indicate significant differences between age classes (Tukey HSD, $\alpha = 0.05$).

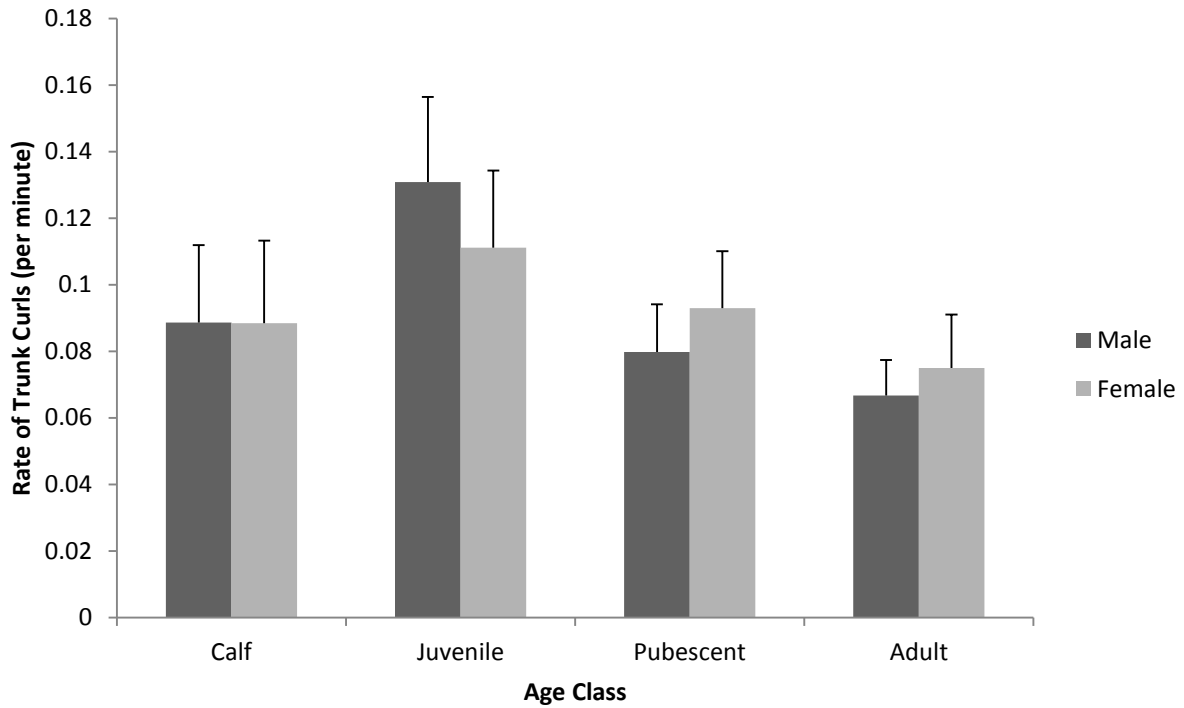


Figure A.29. Mean (+SE) rate of trunk curls per age class for male and female elephants at AENP. There was no significant difference in the mean rate of trunk curls between males and females and there were no significant differences between age classes (Kruskal-Wallis – Sex: $H = 0.17$, $df = 1$, $p = 0.68$; Age-Class: $H = 5.72$, $df = 3$, $p = 0.13$).

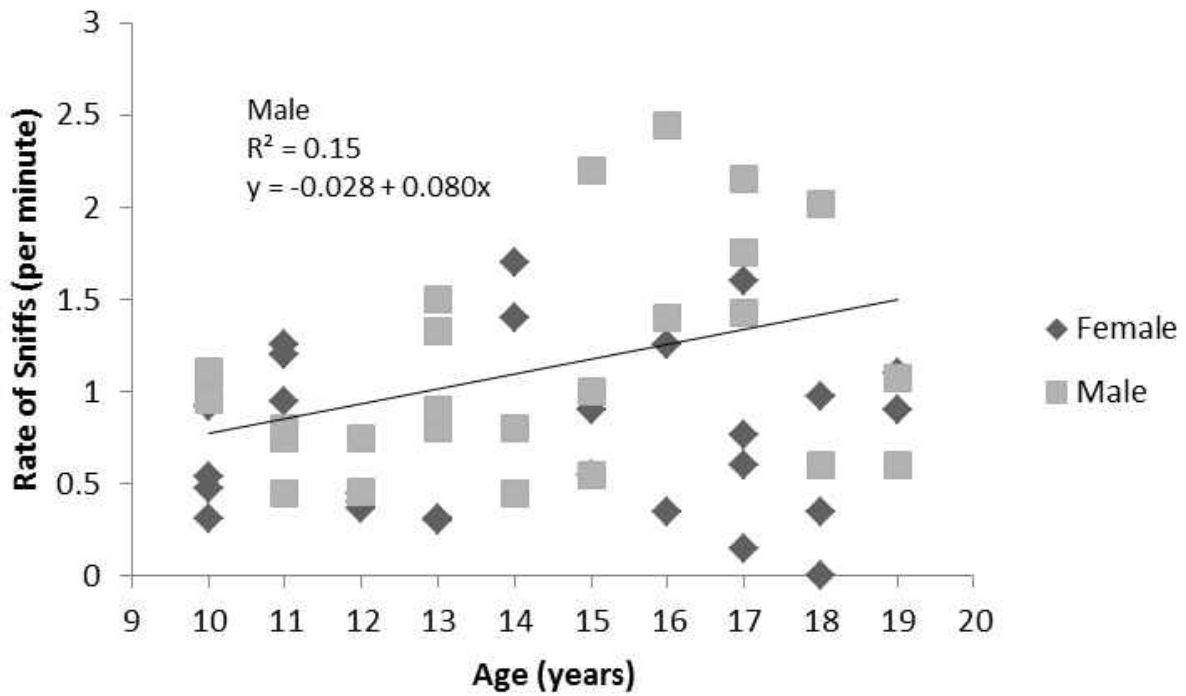


Figure A.30. Mean rate of sniffs by year for pubescent male and female elephants at AENP. The rate of sniffs increased with age for males ($R^2 = 0.15$, $N = 26$, $p = 0.05$) but not for females ($R^2 = 0.003$, $N = 27$, $p = 0.79$).

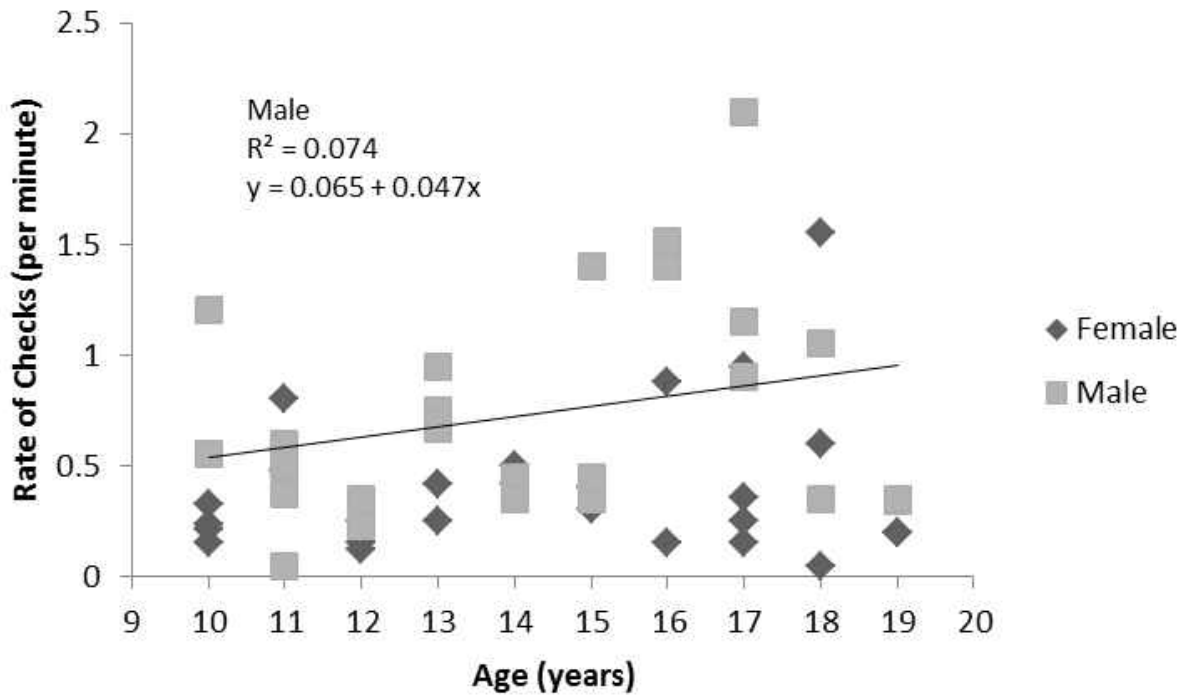


Figure A.31. Mean rate of checks by year for pubescent male and female elephants at AENP. The rate of checks did not increase significantly with age for males ($R^2 = 0.074$, $N = 26$, $p = 0.18$) or females ($R^2 = 0.034$, $N = 27$, $p = 0.35$).

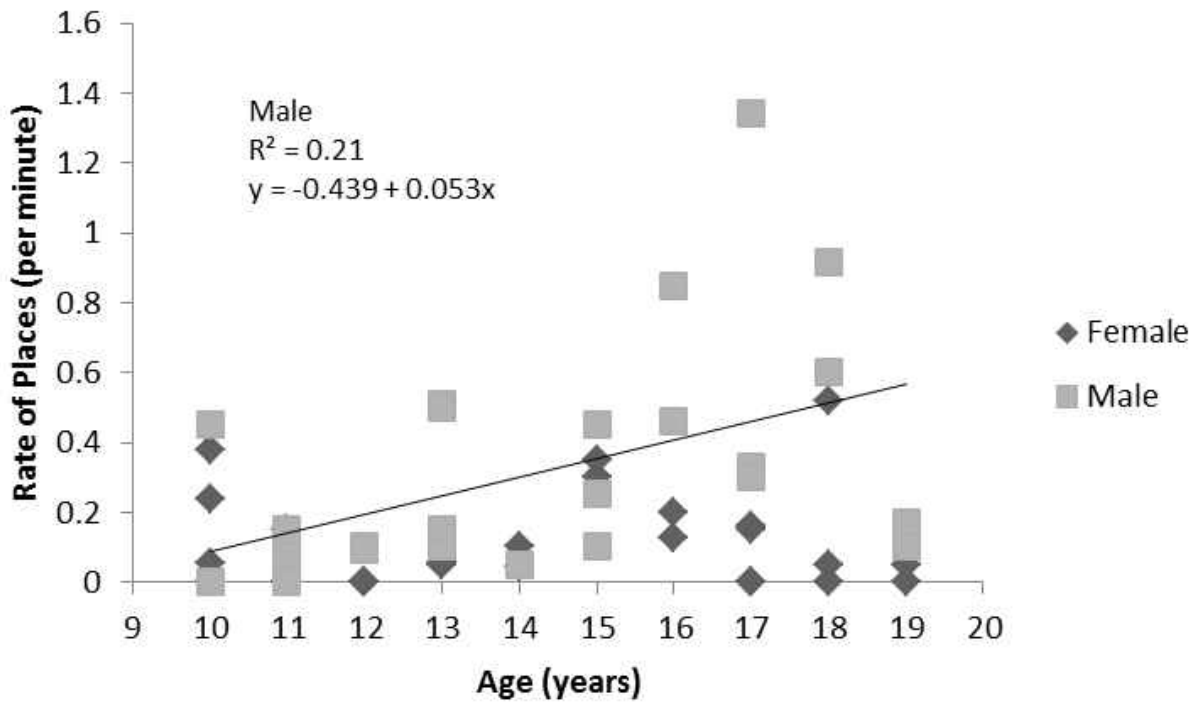


Figure A.32. Mean rate of places by year for pubescent male and female elephants at AENP. The rate of places increased with age for males ($R^2 = 0.21$, $N = 26$, $p = 0.019$) but not for females ($R^2 = 0.005$, $N = 27$, $p = 0.74$).

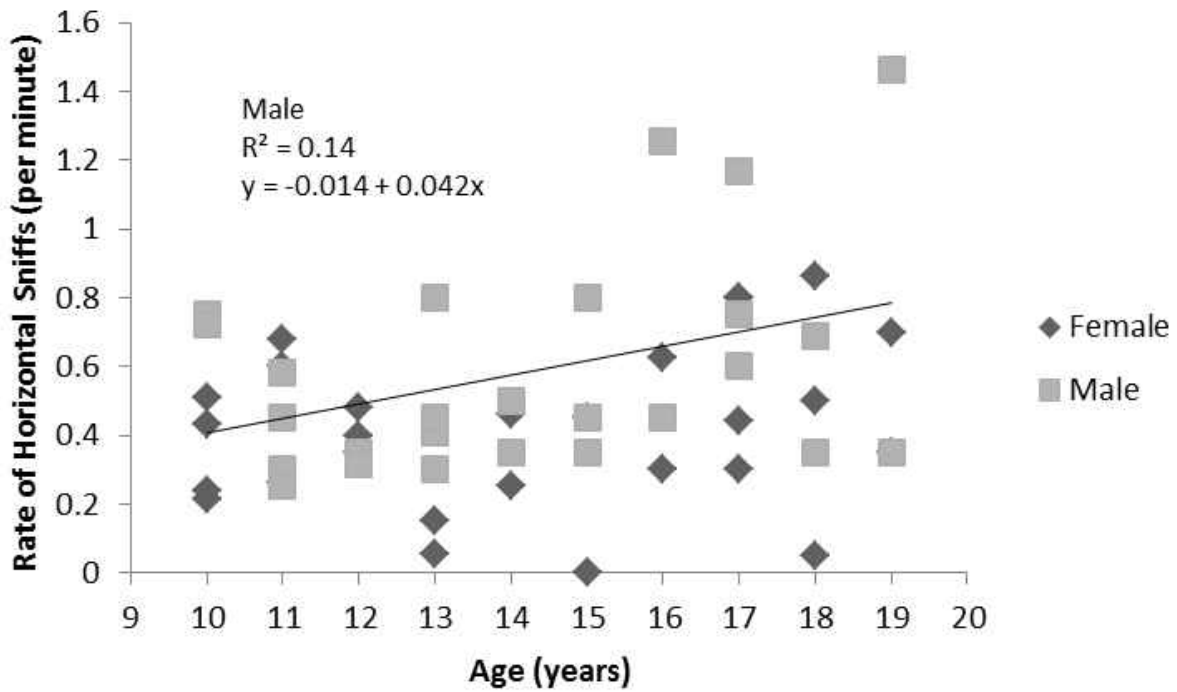


Figure A.33. Mean rate of horizontal sniffs by year for pubescent male and female elephants at AENP. The rate of horizontal sniffs increased with age for males and approached significance ($R^2 = 0.14$, $N = 26$, $p = 0.057$) but not for females ($R^2 = 0.06$, $N = 27$, $p = 0.23$).

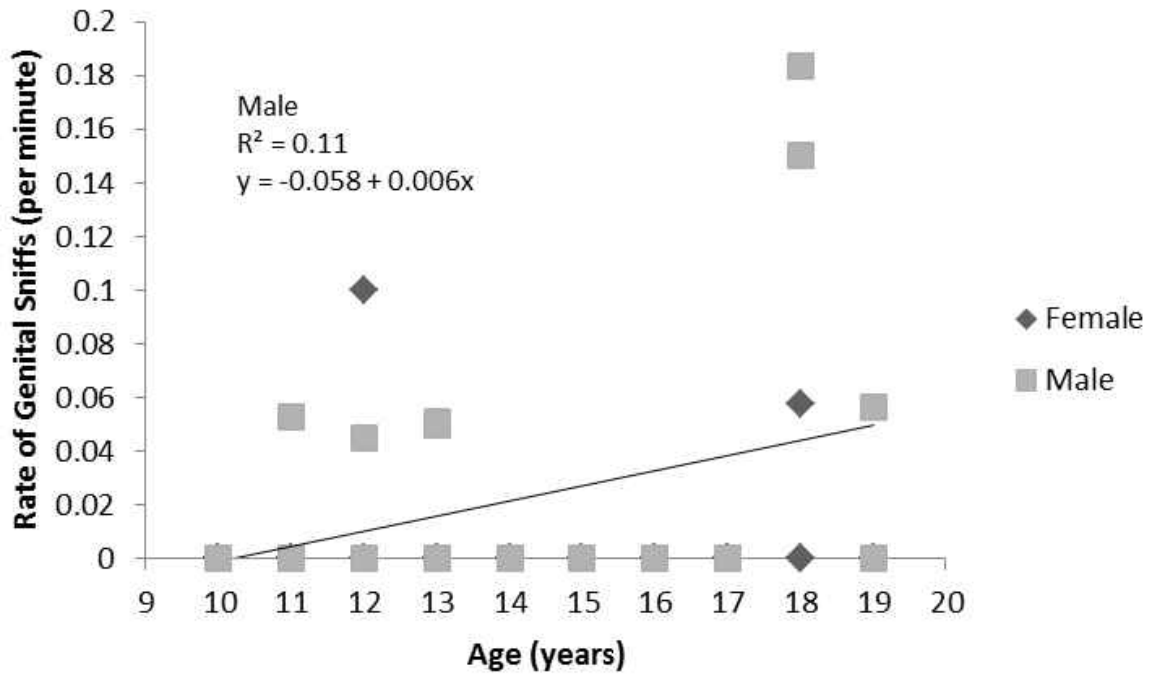


Figure A.34. Mean rate of genital sniffs by year for pubescent male and female elephants at AENP. The rate of genital sniffs did not increase with age for males ($R^2 = 0.11$, $N = 26$, $p = 0.09$) or females ($R^2 = 0.00004$, $N = 27$, $p = 0.97$).

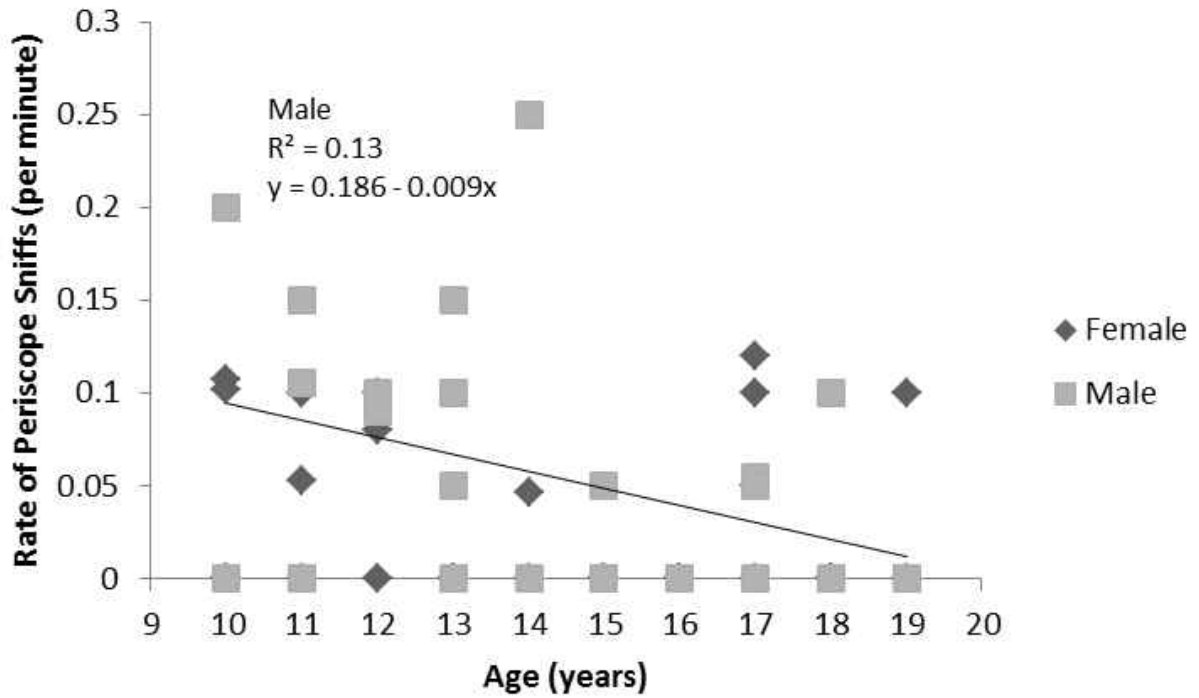


Figure A.35. Mean rate of periscope sniffs by year for pubescent male and female elephants at AENP. The rate of periscope sniffs decreased with age for males and approached significance ($R^2 = 0.13$, $N = 26$, $p = 0.068$) but not for females ($R^2 = 0.03$, $N = 27$, $p = 0.43$).