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Feeding Behavior of Wild and Captive Oxpeckers (Buphagus spp.): A Case of Conditional Mutualism

Tiffany B. Plantan
University of Miami, tiffany@bio.miami.edu

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UNIVERSITY OF MIAMI

FEEDING BEHAVIOR OF WILD AND CAPTIVE OXPECKERS (BUPHAGUS SPP.): A CASE OF CONDITIONAL MUTUALISM

By

Tiffany B. Plantan

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FEEDING BEHAVIOR OF WILD AND CAPTIVE OXPECKERS (BUPHAGUS SPP.): A CASE OF CONDITIONAL MUTUALISM

Tiffany B. Plantan

Approved:

Michael S. Gaines, Ph.D. Professor of Biology	Terri A. Scandura, Ph.D. Dean of the Graduate School
David Janos, Ph.D. Professor of Biology	William Searcy, Ph.D. Professor of Biology
Judith Bronstein, Ph.D. Professor of Biology University of Arizona	

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Mutualisms are delicately balanced partnerships and are increasingly recognized as being fundamental to patterns and processes within ecological systems. Changes to the ecological setting in which such interactions operate can disrupt this balance. By understanding the context-dependent nature of such associations, researchers can begin to understand how changes in the environment can have cascading effects on the entire community within which they occur. Oxpeckers (*Buphagus* spp.) feed on the ectoparasites of ungulates in sub-Saharan Africa and from the blood of ungulate wounds. Because of this dichotomy in feeding behavior, the role of oxpeckers as consistently beneficial partners of their hosts has been questioned, and it has been suggested that the concept of conditional mutualism be applied to this interaction. I combined observational studies of oxpeckers in Kruger National Park, South Africa, with experiments on oxpeckers in captivity at the Mokopane Biodiversity Conservation Centre, South Africa, to test the hypothesis that the ectoparasite cleaning service provided by oxpeckers to their hosts is context-dependent. The results of my dissertation research demonstrate that the oxpecker-ungulate association has conditional outcomes. Under certain conditions, the oxpecker-ungulate relationship is a nutritional mutualism where ungulates provide food (ticks) for oxpeckers in exchange for a cleaning service. Under other conditions, oxpeckers exploit their hosts to feed from their blood. My findings suggest that a high

abundance of the tick species and tick stages oxpeckers prefer is necessary to maintaining an alignment of interests between oxpeckers and ungulates. When ungulates host few ticks of the species oxpeckers prefer, these birds will wound-feed to meet their nutritional demands. Such wound-feeding threatens the balance of the oxpecker-ungulate mutualism. Although oxpeckers frequently wound-fed in captivity, the frequency of wound-feeding events on wild host species was only 3.1% of feeding events. Whether the relationship between oxpeckers and domesticated hosts differs from that between oxpeckers and wild hosts, or if this observed difference is actually a result of researcher constraints in the wild, needs further exploration.

This thesis is dedicated to my family.
You provided me with the love and support I needed to pursue my dream in Africa.

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And to Kenny…

Thank you for sacrificing the world to make my dream a reality.

McAttee (1944): "The perching of birds upon animals is not altogether an innocent and mutually advantageous arrangement; like so many relations, it has become perverted in some instances and has a sinister side."

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The Potential for Conditionality in the Oxpecker-Ungulate Interaction

The prevalent idea that [oxpeckers] free domesticated stock of ticks appears to be entirely erroneous…These birds feed mainly on the raw tissue of open wounds and it is by their action that the open wounds usually occur.

Game warden quoted in Moreau 1933

BACKGROUND

Historically, interspecific interactions were placed into one of three categories: mutualism, commensalism or parasitism. The current view of these relationships, particularly mutualistic partnerships, recognizes their variability. Rather than being fixed attributes of species interactions, benefits and costs can, and often do, vary with the biotic and abiotic setting in which the interaction occurs (Holland *et al*. 2002). Such situations, in which the outcome of an interspecific interaction is context-dependent, have been termed conditional (Bronstein 1994a). An interspecific relationship may conditionally fall into more than one category, thereby creating a continuum from mutualism to commensalism to parasitism. Over evolutionary time, many interactions that were once antagonistic have shifted towards commensalism and even mutualism (Bronstein 1994a). In other cases, mutualistic and commensal relationships have shifted towards parasitism (Bronstein 2001b).

One interspecific interaction that may exhibit conditional outcomes is the oxpecker-ungulate interaction. The oxpecker (*Buphagus* spp.) is a small passerine bird restricted to sub-Saharan Africa. Oxpeckers engage in a reputedly mutualistic relationship with the perrisodactyl and artiodactyl species of the African savanna. Oxpeckers provide a cleaning service by gleaning ectoparasites from their partners' hides. In the first half of the twentieth century, oxpecker populations became threatened in South Africa due to the treatment of livestock with poisonous acaricides, the over-hunting of wild game, and the rinderpest epidemic of 1896-1897 (Sutterheim and Brooke 1981; Stutterheim 1982b; Mundy 1983; Hall-Martin 1987), each of which contributed to the decline of oxpecker hosts. The loss of large herbivores not only reduced the number of hosts available for the birds to forage, perch, display and mate on, but also decreased the number and species of ticks available for the birds to feed on. Some investigators hypothesized that this decrease in tick abundance drove the birds to create host wounds and feed from the blood and tissue (van Someren 1951; Attwell 1966), behaviors still frequently observed today. It is just as likely, however, that oxpeckers have always wound-fed, but this behavior simply was overlooked. A decrease in tick abundance could have resulted in wound-feeding being a more commonly observed behavior. The role of oxpeckers as consistently beneficial partners to ungulates has been questioned as a result of their vampire tendencies, which likely impose more costs than benefits to their hosts.

My Ph.D. dissertation explores the interspecific relationship between oxpeckers and ungulates in South Africa to determine whether this interaction has conditional outcomes based on the feeding behavior of the oxpecker. The two possible outcomes in an oxpecker-ungulate relationship are: 1) oxpecker tick-feeds and is a mutualist or a commensal, or 2) oxpecker wound-feeds and is a parasite or commensal.

Oxpeckers prey on ticks of the Ixodidae (hard tick) family, ticks that have four developmental stages: egg, larva, nymph, and adult (Moreau 1933; Attwell 1966; Oliver 1989). Whereas there are no obvious costs to an ungulate of a tick-feeding bird, the benefits are many. Blood loss, anemia, appetite suppression, weight loss, and metabolic disturbances are just some of the effects of ticks on wild and domesticated ungulate species (Little 1963; O'Kelly and Seifert 1969; Seebuck *et al.* 1971; Williams *et al.* 1978; Price 1980; Mulilo 1985; Norval *et al.* 1988; Hart *et al.* 1990; Oorebeek and Kleindorfer 2008). Engorging adult ticks, in particular, can drain body reserves through the removal of large amounts of blood (Little 1963; Seebeck et al. 1971; Turner and Short 1972; Williams et al. 1978; Sutherst et al. 1983; Hart and Hart 1988; Norval et al. 1988; Kaiser et al. 1991; Scholtz et al. 1991; Hart et al. 1992). Some studies have documented that in growing calves a single engorging tick produces a growth decrement of 0.6 g-0.6 kg per calf per year (Little 1963; Seebeck et al. 1971; Turner and Short 1972; Williams et al. 1978; Hart and Hart 1988; Hart et al. 1992). However, the effect of *Boophilus* infestations is proportional to the number of ticks of this genera engorging on the host animal (Uilenberg 1992). Results obtained in a study in South Africa indicated that each engorging *B. decoloratus* female can produce a growth decrement of 8.0-9.0 g per calf (Scholtz et al. 1991). Immature hard ticks, however, do not appear to affect weight gain in calves (Norval *et al.* 1988; Norval *et al.* 1989).

Ticks are vectors of more kinds of microorganisms than any other arthropod taxon, including mosquitoes (Hoogstraal 1985 in Oliver 1989). Ixodid ticks can transmit bacterial diseases such as Anaplasma and Erhlichia and protozoan diseases such as Theileria and Babeseia. Large infestations of certain tick species, such as *Rhipicephalus appendiculatus*, can cause tick toxicosis, which results in a general loss of host condition, tissue damage, abscesses, and a decrease in resistance to disease. A tick can become infected when it feeds on an infected host animal during the larval stage and then transmit the pathogen to different hosts during subsequent nymphal and adult stages. Alternatively, a tick can become infected during the nymphal stage and transmit the pathogen during the adult stage. The infected tick transmits the pathogen through its saliva as it engorges with blood. Some, but not all, tick pathogens are transmitted to the next generation of ticks via the eggs. Oxpeckers can prevent disease transmission to their ungulate hosts by removing pathogenic ticks before they engorge.

Oxpecker tick predation prevents tick reproduction. Engorged female ixodid ticks lay their eggs in a single batch on the ground. There is a positive relationship among tick size, amount of blood meal, and number of eggs produced. Some hard tick females, such as species belonging to *Amblyomma* and *Hyalomma*, can oviposit 15, 000-20, 000 eggs (Oliver 1989). Intermediate sized ticks of *Boophilus* and *Rhipicephalu*s usually produce 3, 000-6, 000 eggs, and small tick species such as *Haemaphysalis* may oviposit only 250-1, 000 eggs (Oliver 1989).

In contrast to a tick-feeding oxpecker, the benefits conferred by a wound-feeding oxpecker are questionable. Several authors have proposed that a wound-feeding oxpecker may actually benefit a host by cleaning wounds of maggots and blowfly larvae (Attwell 1966; Breitwisch 1992, 1996). There is no direct evidence for this positive effect, and oxpeckers have been observed feeding from already clean wounds and opening old wounds thereby increasing the chance of secondary infection (van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988; Weeks 1999, 2000; McElligott *et al.* 2004). Most interesting is that oxpeckers have been observed creating wounds on their hosts, for which there is not obvious benefit to the host. Wound-feeding causes blood loss and irritation, attracts flies, allows secondary infection, and weakens the host. Of course, the level of wound-feeding, size of the wound per host animal, and size of the ungulate host will affect the cost of a blood-feeding bird.

STUDY SPECIES

One-hundred and fourteen species comprise the Sturnidae family, including the starlings, mynas and oxpeckers (Feare and Craig 2001). Because oxpeckers, or "tickbirds" as they are so fittingly named, differ from starlings in the shape of their feet, claws, bills and certain behaviors, they are often placed in the sub-family Buphaginae (Attwell 1966; Stutterheim *et al.* 1975). The red-billed oxpecker (*Buphagus erythrorhynchus*, Stanley 1814) and yellow-billed oxpecker (*Buphagus africanus*, Linnaeus 1776) are strictly African species found from 1400 to 2500 m in altitude (Van Someren 1951; Attwell 1966; Hustler 1987). Their range covers much of sub-Saharan Africa, but because oxpeckers are dependent on wild and/or domesticated ungulates, their distribution is often patchy (Stutterheim 1982b). Red-billed oxpeckers are principally an eastern species, ranging from the Central African Republic east to Ethiopia and south to South Africa. The range of yellow-billed oxpeckers extends from Senegal in West Africa east to Sudan and south to South Africa. Both species co-occur in several countries, including Botswana, Namibia, Zimbabwe and South Africa (Pitman 1956; Koenig 1997). In South Africa, yellow-billed oxpeckers are restricted to the northern section of Kruger National Park, but red-billed oxpeckers are found in seven of the nine South African Provinces: Limpopo, Northwest, Mpumalanga, Guateng, KwaZulu Natal, Northern Cape and Eastern Cape (le Roux, personal communication). Red-billed oxpeckers have been observed in 29 different vegetation types in South Africa (Acocks 1975).

The sizes of the two species are comparable: yellow-billed oxpeckers are 23 cm long and weigh approximately 60 g, whereas red-billed oxpeckers are 3 cm shorter and have a mean weight of 50 g (Pitman 1956; Stutterheim 1976; Mundy 1983). Despite many physical similarities, the two species can be distinguished by a few characteristics. Red-billed oxpeckers have a dark rump and red beak with a yellow ring around the eyes; yellow-billed oxpeckers have pale colored rumps, yellow beaks with a red tip, and no ring around the eye (Pitman 1956). Juveniles of both species have dark brown to black beaks, but the adult pigmentation becomes apparent before the birds are 12 months old, usually around 7-8 months (Grobler 1976). The red-billed oxpecker has a narrow, bill, whereas the yellow-billed oxpecker has a broader bill (Attwell 1966; Buskirk 1975; Stutterheim *et al.* 1976; Stutterheim *et al.* 1988).

Oxpeckers have sharp, curved claws for clinging to and crawling across their hosts' bodies in search of food. Their tails are long and stiff to provide balance and support, and their beaks are flattened with a sharp edge for plucking, pecking, scissoring and manipulating ticks (Attwell 1966; Breitwisch 1992; Koenig 1997). Based on four studies in which the stomach contents of oxpeckers were analyzed, the diet of these birds appears to be primarily composed of hard ticks (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980). Bezuidenhout and Stutterheim (1980) found that ticks composed 52.3% by mass of the stomach contents of 53 red-billed oxpeckers. A yellow-billed oxpecker can consume approximately 13,600 larvae or 109 adult engorged female blue ticks (*Boophilus decoloratus*) per day, and the red-billed oxpecker 12,500 larvae or 98 adult female blue ticks per day (Stutterheim *et al.* 1988). But the oxpecker diet is varied, although almost entirely dependent on the host (Pitman

1956). Grass, seeds, and ungulate hair, earwax, dung, urine, scurf cells, epidermal tissue, and nose, mouth and eye secretions are consumed in varying amounts (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980; personal observation). Oxpeckers also consume insects, mostly flies, but also lice, termites, and grasshoppers (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988). Insect hawking, stalking, and snapping are generally observed while oxpeckers are on the host. The amount of time oxpeckers spend consuming insects off the host is unknown.

Oxpeckers are believed to have a narrow home range (Grobler 1979; Irwin 1981 in Hall-Martin 1987). Yellow-billed oxpeckers released into the Matobos National Park, Zimbabwe moved 15 km within three months of their release and red-billed oxpeckers moved 20-30 km (Hall-Martin 1987). One red-billed oxpecker was recovered 45 km from its ringing site in Zambia (Mundy 1983). Oxpeckers near the Skukuza restcamp of Kruger National Park exhibited no local movements (Stutterheim and Stutterheim 1980). The maximum distance oxpeckers were observed away from animal pens was 8.0 km with a mean distance of 3.37 km (Stutterheim 1976).

Generally, oxpeckers occur in areas with an annual rainfall of more than 500 mm (Stutterheim and Brooke 1981; Stutterheim 1982b). Oxpeckers can be seen in small flocks of 4-5 individuals or up to a dozen, and are colonial roosters at night (van Someren 1951; Pitman 1956). They roost in trees, holes in trees, and reed beds (McLachlan and Liversidge 1957; van Someren 1951). Red-billed oxpeckers can often be observed roosting in the ilala palms, *Hyphaene atalensis*, of northern Kruger National Park. Yellow-billed oxpeckers, in particular, often roost on their hosts at night (Dowsett 1969).

Oxpeckers are cooperative breeders. The previous season's brood is believed to remain with the parents to help raise the present year's nestlings (Stutterheim 1976; Breitwisch 1992). Although oxpeckers have been recorded building their own nests in captivity (Kaiser-Benz 1975), they generally utilize natural tree holes (Henderson 1958; Meinertzhagen 1959 in Grobler 1980; Dowsett 1965; Mundy and Cook 1975; Stutterheim 1976). Preferred nesting trees are leadwood, *Combretum imberbe*; knob-thorn, *Acacia nigrescens*; tambotie, *Spirostachys africanus*; mopane, *Colophospermum mopane*; and sausage tree, *Kigelia pinnata* (Stutterheim 1976, 1982). The lifespan of oxpeckers is unknown.

OXPECKER CONTROVERSY: THE BEGINNINGS

Since the early twentieth century, the nature of the oxpecker-ungulate relationship has engendered debate (Moreau 1933). Some argue that the oxpecker benefits ungulates through tick removal, while others believe the oxpecker harms ungulates through wound-feeding (van Someren 1951; Pitman 1956; Attwell 1966). The interaction between oxpecker and ungulate is undoubtedly beneficial for the oxpecker. But whether ungulate hosts receive a net benefit from the interaction is unclear (Breitwisch 1992; Weeks 1999). What follows is a brief review of studies documenting oxpecker feeding behavior and the conclusions drawn with regard to the nature of the oxpecker-ungulate relationship.

Moreau (1933) provided the first written record of oxpeckers wound-feeding, although this behavior was reportedly known for many years prior (van Someren 1951). Moreau was the first to conduct a systematic study of the feeding habits of red-billed oxpeckers and to assess their status as pests. Moreau begins his report by citing several opinions on the bird, several claiming that oxpeckers rarely eat ticks, but instead primarily wound-feed. Moreau found little evidence that the oxpecker actually creates wounds. He believed that when livestock are kept in healthy condition and any wounds are tended to, oxpeckers do more good than harm. He analyzed the stomach contents of 58 red-billed oxpeckers and found 2,291 ticks of all sizes and stages in 55 of the birds examined, with an average of 41 ticks per bird. Ninety-five percent of these ticks were potential vectors of disease, leading Moreau to conclude that oxpeckers are more of a benefactor to livestock than a pest.

Van Someren (1951) and Attwell (1966*)* addressed the economic significance of oxpeckers in relation to livestock in Kenya and Zambia, respectively. In Kenya, dipping practices (i.e. the chemical treatment of cattle against ticks) had spread by 1951, and farmers began to complain that red-billed oxpeckers were inflicting damage because of a shortage of their regular food source, ticks. Van Someren decided to evaluate the behavior of oxpeckers in relation to cattle. In 1947 he sent out questionnaires to local farmers in Kenya. Based on the responses, 51% of the surveyors reported that the birds actually break the skin and create wounds, 27% claimed that the birds only exploit and enlarge existing wounds, and the remaining 22% did not believe the birds wound-fed. Van Someren personally observed oxpeckers inflicting wounds. He made note of an irrefutable incident in which an oxpecker created a wound in an area of unbroken skin, free of ticks, at the base of a bull's tail. He also closely examined 19 sores on a single bull. Each one had been "worked by the birds into a shallow saucer-shaped depression almost perfectly round, and varying from ½ in to 3 in in diameter and 2-3 mm deep." Like Moreau, van Someren also conducted a stomach content analysis, examining the stomach contents of 12 red-billed oxpeckers. His findings were similar to those of

Moreau: the main bulk of food is ticks followed by flies, lice and mites are eaten in small quantities, and hair is found in varying amounts. Van Someren concluded his report with the statement "Many people do not mind [oxpeckers] in small numbers, but consider them a nuisance in quantity." However, he suggested that oxpeckers cause more damage to livestock when tick abundance is depleted through livestock dips and sprays. Attwell concurred, stating that the birds are "benefactors on beasts in good condition, but aggravate existing sores and prevent them from healing; serious damage can be caused to stock in bad condition."

Mengesha (1978) studied the interaction between oxpeckers and their hosts in Ethiopia. That oxpeckers feed on both ticks and animal tissue was corroborated by both direct observation and stomach analyses. Oxpeckers created wounds on cattle and enlarged sores up to 1.5 cm x 3 cm. A single bird was observed pecking at the nostril of a bull that started to bleed after 10 min. Such behavior drains blood, can cause anemia, and allows the entry of septic germs into the wound, resulting in abscesses and inflammation. Mengesha also conducted stomach content analyses on 15 oxpeckers. He found hair, feathers, scurf cells, tissue fibers with blood vessels, dark brown materials, two parasitic lice and a leg of an insect, and numerous male and female tick remnants. Mengesha's overall assessment was that the interaction between oxpecker and domesticated host depends on the type and condition of the host animal and the availability of food on that host. He suggested that in cases where cattle are treated against ectoparasites, oxpeckers appear to be parasitic and may be "more dangerous than the tick." The birds repeatedly utilized cattle with sores more than those with ticks, suggesting a preference for wounds.

Bezuidenhout and Stutterheim (1980) studying oxpeckers and twelve species of ungulates in Kruger National Park, found that red-billed oxpeckers spent 0.4% time wound-feeding out of a total of 17,057 feeding observations. Competition between oxpeckers to feed at wounds indicated a preference to wound-feed, but this feeding behavior was rarely observed. Bezuidenhout and Stutterheim also conducted the first set of controlled experiments on oxpeckers. Captive red-billed oxpeckers reduced female *Boophilus* ticks on cattle by 95.7%, and obtained a mean level of control of 59.8% and 36.9% for nymphae and larvae, respectively. The level of tick control in a laboratory setting depended on the number of birds per host and the species, size, and number of ticks present. Stutterheim *et al.* (1988) then conducted a second series of controlled experiments on five birds of each oxpecker species in captivity. The two species were held in separate aviaries with a single cow and oxpecker feeding-behavior was recorded. Tick-feeding accounted for 72% of red-billed oxpecker feeding activities, whereas yellow-billed oxpeckers spent 65% of their time engaged in this activity. Wound-feeding was a behavior more commonly exhibited by captive yellow-billed oxpeckers (4.7%) than captive red-billed oxpeckers (0.5%). To examine the tick preferences of oxpeckers, six tick species were placed in petri dishes and offered to captive birds. Tick preferences were recorded. Then the two oxpecker species were confined in separate aviaries with engorged ticks attached to cattle. The results of both experiments suggested that both oxpecker species prefer adult blue ticks, *Boophilus decoloratus*. In addition to determining the tick species and stage preferences of oxpeckers, Stutterheim *et al.* were able to show that both species of oxpecker are efficient at removing lice infestation on cattle within 5-6 weeks.

Weeks (1999, 2000) performed the third set of oxpecker experiments, which was also the first study to follow individually marked oxpeckers in the field (Weeks 1999). He conducted focal watches of 41 individually ringed red-billed oxpeckers on cattle in the Limpopo Valley of Zimbabwe between August 1996 and September 1997. The birds fed mainly from wounds, in the ears, and by scissoring for ticks. Observable tick-feeding accounted for a small amount of the total feeding time, with oxpeckers seemingly preferring blood. Cattle attempted to dislodge the birds in response to wound-feeding, but were unable to do so. Weeks (1999) concluded that oxpeckers may not be beneficial, at least not to cattle. Weeks (2000) further tested the hypothesis that the oxpecker-host relationship is mutually beneficial by excluding oxpeckers from one herd of cattle as part of a controlled field experiment in Zimbabwe. Contrary to expectation, adult tick abundance was unaffected by excluding the birds. The absence of oxpeckers had no statistically significant effect on the change in total tick load in any of the three replicate experiments. In addition, control cattle exposed to oxpeckers had significantly more wounds than experimental cattle in all treatments, and oxpeckers significantly prolonged the healing time of wounds (Weeks 2000). These results suggest that the oxpecker-mammal relationship is partially parasitic (Attwell 1966; Weeks 2000).

McElligott *et al.* (2004) observed five red-billed oxpeckers in captivity at the Zurich Zoo, Switzerland creating and feeding from wounds on two captive black rhinoceri (*Diceros bicornis*). Foraging time of oxpeckers was divided as follows: 56.3% wounds, 19.3% ears, 15.3% skin, 6.8% anogenital, and 2.3% other. These birds were fed on an artificial mixture of cereals, vegetables, minced meat and mealworms, but still resorted to wound-feeding. Based on their observations, McElligott *et al.* proposed that oxpeckers may be conditional mutualists.

Despite early evidence and many reports, researchers have tended to ignore the fact that oxpeckers spend a considerable amount of time consuming blood. As Weeks (2000) noted, "tick-feeding has been of much attention, but the oxpecker's habit of wound-feeding has generally been ignored (e.g. Stutterheim *et al.* 1988) or regarded as having a negligible effect (e.g. Dale 1992b) despite strong evidence that blood is the birds' preferred food (e.g. Bezuidenhout and Stutterheim 1980; Weeks 1999) ." For example, Breitwisch (1992) said oxpeckers "may remove dead tissue and maggots from wounds in mammal's hide and *occasionally* help themselves to a sip of blood." In all of Stutterheim's 19 oxpecker studies, wound-feeding is rarely acknowledged. There is considerable field evidence for the consumption of blood-tissue from the host's hide. Blood-feeding has been observed on the majority of ungulate hosts and the ability of the smaller hosts to control their oxpecker attendants may explain why oxpeckers are observed more often on larger hosts (Koenig 1997). Weeks (1999) speculates that wound-feeding on wild game may be underestimated for it was not specifically looked for on many occasions. Also, the wounds oxpecker inflict or exploit are often small, only the size of the tip of the beak, and therefore unnoticeable from a distance. Furthermore, oxpecker stomach content analyses cannot conclude whether or not an oxpecker has wound-fed.

In summary, the oxpecker has been a focus of ecological and behavioral research due to its unique association with African perrisodactyls and artiodactyls. Several of these studies have demonstrated that oxpeckers feed from and create host wounds, and that this activity seems to offer no benefits to the host. Despite this, oxpeckers are still widely regarded as consistently beneficial cleaning symbionts to the large herbivores that support them.

CONDITIONAL CLEANERS

Many interspecific relationships are not always either mutualistic or parasitic (Douglas 2008). Mutualism and parasitism are the outer extremes of a dynamic continuum of interspecific relationships (Johnson *et al*. 1997). Mutualisms of all kinds show evidence of conditionality (e.g. ant-aphid, Offenberg 2001; ant-plant, Cushman and Addicott 1991, Bronstein 1998, Wirth and Leal 2001, Palmer *et al*. 2008; ant-treehopper, Cushman and Whitham 1989, Billick and Tonkel 2003, Del-Claro and Oliveira 2000; plant-fungi, Hoeksema and Kummel 2003; Egger and Hibbett 2004, Johnson *et al.* 1997, Tuomi *et al.* 2001; trichomycete fungus-black fly, McCreadie *et al.* 2005; fig-fig wasp, Jousselin *et al.* 2001; kelp-colonial epifauna , Hepburn and Hurd 2005). Cleaning associations, in particular, in which cleaner organisms remove ectoparasites from the body surfaces of larger host species, exhibit context-dependent outcomes (Feder 1966). In the cleaner-client fish association, for example, cleaners cooperate by gleaning ectoparasites from client fish. But cleaners actually prefer to cheat by feeding on client mucus and tissue (Bshary 2002; Bshary *et al.* 2008). Although such conflict of interest is common in interspecific associations, it is generally controlled (Douglas 2008). To prevent cleaners from foraging against their preference, client fish jolt their bodies or terminate interactions by swimming away in response to exploitation (Bshary *et al.* 2008). In the oxpecker-ungulate cleaning association, it is beneficial for ungulates to associate with oxpeckers when net benefits exceed net costs, but is detrimental when net costs exceed net benefits. A mutually beneficial relationship between oxpecker and host depends on the degree to which partner interests match and on the degree to which hosts are able to end an interaction in response to wound-feeding. Host animals respond to oxpecker presence by exhibiting toleration or rejection behaviors. For example, these large herbivores will run through the bush to scrape the birds off, kick their legs, swing their tails, and roll on the ground. However, many of these host species (e.g. rhinoceros, buffalo, and giraffe) have very large body surfaces, and efforts to control oxpecker feeding behavior can be ineffective. The level of host control over oxpecker behavior may vary across host species (Johnstone and Bshary 2002). Host species with little control over attendant oxpeckers may be more frequently exploited.

Another factor that may influence the outcome between oxpecker and host is density. The benefits and costs to a species can vary as a function of its own density and also by variation in the abundance of its partner species (Holland and DeAngelis 2001). If densities occur at which costs exceed gross benefits, then the outcome of the interaction will be parasitic; at other densities when the benefits exceed the costs, the outcome will be mutualistic (Holland and DeAngelis 2001). Oxpeckers may be mutualistic at low population densities, but commensal or parasitic at high densities. An increase in the number of oxpeckers per host animal may lead to the rapid depletion of ticks on that host. In another situation, a low density of host animals may create a similar tick shortage because tick density is generally proportional to host density (Norval and Lightfoot 1982). As the tick supply is exhausted, hungry oxpeckers competing for food may wound-feed.

In addition to partner interest, the level of host control, and the density of partners, the multispecies nature of the oxpecker-ungulate interaction may also make it vulnerable to conditional outcomes (Bronstein 1994a). This African interaction involves two oxpecker species, over twenty species of large ungulates, and a variety of ixodid tick species. In such a diverse interaction, the species involved often vary in terms of the benefits or services they provide; some may provide strong benefits, others weak benefits (Hoeksema and Kummel 2003). For example, ungulates exhibit intraspecific and interspecific variation in tick species composition and tick load. Certain tick species exhibit host specificity (Hoogstraal and Aeschlimann 1982 in Cumming 1998; Oliver 1989). Certain ungulates are more susceptible to ticks when they are in poor condition and under nutritional stress (Sutherst *et al*. 1979; Norval and Lightfoot 1982; Gallivan *et al.* 1995; Gallivan and Horak 1997), which reduces their resistance to tick infestations (O'Kelly and Seifert 1969; Sutherst *et al.* 1983 in Gallivan and Horak 1997). Ungulates foraging in tick dense habitats, with larger body sizes, and that spend less time engaged in grooming activities also generally support higher tick loads (Horak 1982; Horak *et al.* 1983; Hart *et al.* 1990; Hart *et al.* 1992; Olubayo *et al.* 1993; Gallivan and Horak 1997). Because livestock are usually chemically treated against ticks, they often support lower tick loads than wild ungulate species. Also, oxpeckers exhibit a preference for four tick species out of the more than eighty-three species of ixodid ticks that occur in South Africa (Walker 1991). The presence or absence of these tick species on a host animal may regulate oxpecker feeding behavior.

My research addressed the conditionality of the oxpecker-ungulate association. In agreement with Weeks (1999) and based on preliminary evidence, I hypothesize that the multispecies oxpecker-ungulate relationship has conditional outcomes based on the feeding behavior of oxpeckers. Specifically, I predicted that oxpeckers are conditional cleaners. Oxpeckers may cooperate and remove ectoparasites from their ungulate hosts, or they may cheat by pecking and feeding from the blood and tissue of host wounds. A tick-feeding oxpecker tips the relationship towards mutualism, but a wound-feeding oxpecker shifts the interaction towards parasitism. To reject the hypothesis that oxpeckers are conditional mutualists, I would expect to observe only tick-feeding oxpeckers. Factors that can lead the benefits of mutualism to be greater are a high tick load per host, the presence of the tick species and tick stages that oxpeckers prefer to consume, a low density of oxpeckers per host, a small number of available hosts, and hosts that exert control over oxpecker feeding behavior. Factors that lead the costs of mutualism to be greater are a low tick load per host, the absence of the tick species and tick stages that oxpeckers prefer to consume, a high density of oxpeckers per host, low host abundance, and hosts with little control over oxpecker feeding behavior. In addition, the benefits of the mutualism between oxpecker and host can vary in magnitude. An oxpecker consuming many pathogenic ticks and ticks that take a large blood meal from their hosts is more beneficial than an oxpecker consuming a single non-infected and unengorged tick.

Designing studies that accurately measure the costs and benefits per interaction in the oxpecker-ungulate system, and the biotic and abiotic factors affecting these trade-offs, is challenging (Axén *et al*. 1996; Herre *et al.* 1999; Del-Claro 2004; Bronstein 1994b; 2001a; personal communication). Empirical studies showing bilateral exchange and the persistence of an interaction are not enough to demonstrate that an interaction is mutualistic (Boucher *et al.* 1982; Egger and Hibbet 2004). Ideally, studies of conditional mutualism should 1) document variation in aspects of the relationship, 2) identify the mechanisms generating the variation, and 3) link that variation to the fitness of at least one, if not both, of the partners (Billick and Tonkel 2003). Unfortunately, this research will not meet all the requirements necessary to measure the net outcome of the oxpecker/host interaction, but it will address the first two. In reference to the third requirement, the fitness effect of a single tick has been examined. However, each tick species and tick stage likely induces a different effect on its host, an effect that also varies depending on the host species parasitized, the susceptibility of that host, and the number of ticks per host animal. A cost-benefit analysis that derives the exact conditions favoring tick-feeding is necessary. Furthermore, the effects of a wound-feeding oxpecker on ungulate survival and/or reproduction need to be quantified.

In order to provide a clearer understanding of the spatio-temporal variation in oxpecker feeding behavior, I complemented field studies of oxpeckers in Kruger National Park with experimental studies on captive oxpeckers. I tested the hypothesis that this interaction is not a simple bilateral exchange between partners, but rather a complex interspecific relationship with conditional outcomes. By viewing the oxpecker-ungulate interaction on a continuum, the question switches from “is the oxpecker-ungulate relationship mutualistic?” to “under what conditions is the oxpecker-ungulate interaction mutualistic?”

CHAPTER TWO

CHAPTER TWO

The Tick and Wound-Feeding Behavior of Oxpeckers (*Buphagus* spp.) in Kruger National Park, South Africa

Summary

Oxpeckers prey on the ectoparasites of ungulates in sub-Saharan Africa. The diet of oxpeckers includes not only ectoparasites, but also the blood of host wounds. Because of this dichotomy in feeding behavior, the role of oxpeckers as consistently beneficial partners of their hosts has been questioned, and it has been suggested that the concept of conditional mutualism be applied to this interaction. Fluctuations in the biotic and abiotic environment may regulate oxpecker feeding behavior and thus the net outcome of this interspecific interaction. This was the first study to quantitatively examine the frequency of the wound-feeding behavior of both oxpecker species in a wild area where they coexist. Red-billed oxpeckers (*Buphagus erythrorhynchus*), yellow-billed oxpeckers (*B. africanus*), and their wild ungulate hosts were observed daily from January to July 2007 in northern Kruger National Park, South Africa. Fifty-eight percent of all oxpecker observations (n=992) were feeding events. Although oxpeckers have been reported to feed frequently on the wounds of domesticated host species, the frequency of wound-feeding events on wild host species in this study was only 3.1% of 578 of feeding events. The majority of these wound-feeding events were by yellow-billed oxpeckers, which is the slightly larger and behaviorally dominant oxpecker species. Wound-feeding was observed only on buffalo, giraffe, and hippopotami. Oxpecker feeding behavior was related to season with wound-feeding significantly increasing in the dry season. This suggests that tick abundance decreased, or the frequency of wounded ungulates

increased, although it is also possible that I observed more wound-feeding events in the dry season by chance. Host animals were highly tolerant of oxpeckers, regardless of oxpecker behavior, but were significantly more tolerant of non-wound feeding oxpeckers and more rejecting of wound-feeding oxpeckers. I conclude that wound-feeding appears to be a behavior rarely used by oxpeckers on wild ungulates. Whether the nature of the relationship between oxpeckers and domesticated hosts differs from that between oxpeckers and wild hosts, or if this observed difference is actually a result of researcher constraints in the wild, needs exploring. The conditions regulating wound-feeding are still unclear.

Background

Interspecific relationships, in which two species live in close association to one another, are commonly observed in nature. The current view of these relationships recognizes the context-dependent nature of such associations. The fitness effects of an interaction can, and often do, vary temporally and spatially (Cushman and Whitham 1989; Bronstein 1994a,b; Holland *et al.* 2002; Billick and Tonkel 2003). Such situations, in which the outcome of an interspecific interaction is context-dependent, have been termed conditional (Bronstein 1994a). An interspecific relationship may conditionally fall into more than one category, thereby creating a continuum from mutualism to commensalism to parasitism. Over evolutionary time, many interactions that were once antagonistic have shifted towards commensalism and even mutualism (Bronstein 1994a). In other cases, mutualistic and commensal relationships have evolved towards parasitism (Bronstein 1994a). Moreover, the costs and benefits exchanged in an interaction may change from one interaction to the next, or even oscillate during a single interaction creating dynamic outcomes (Axén *et al.* 1996). The relationship between birds called oxpeckers (*Buphagus* spp.) and the ungulate species that typify the African savanna is one interaction that may exhibit conditional outcomes.

Red-billed oxpeckers (*B. erythrorhynchus*) and yellow-billed oxpeckers (*B. africanus*) are sympatric throughout much of southern Africa (Mackworth-Praed and Grant 1960 in Koenig 1997), frequently being observed together at the same time on the same host animal. Oxpeckers have been labeled dietary opportunists because of their behaviors of hawking flies and feeding on host dung, ear wax, and nose, mouth and eye secretions (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980). But they are believed to be primarily tick predators, consuming ectoparasites attached to the hides of Africa's large mammal species (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988). Through tick removal, oxpeckers provide a cleaning service in exchange for a food reward. Because of this seemingly beneficial exchange between partners, the oxpecker-ungulate association traditionally has been viewed as a mutualistic cleaning interaction (Moreau 1933; van Someren 1951; Pitman 1956; Attwell 1966). Circumstantial evidence, however, suggests that the nature of this relationship may vary from strong to weak mutualism, to commensalism, and even into parasitism.

The fitness effects of the oxpecker-ungulate association have been a subject of uncertainty. Some oxpeckers appear to be conditional exploiters. They cheat their partners by feeding from the blood and tissue of host wounds. The cleaning service provided by one mutualist species to another can vary with changes in the biotic and abiotic environment (Cushman and Whitham 1989). Furthermore, multispecies interactions lend themselves to context-dependent outcomes (Bronstein 1994a; Knowlton and Rohwer 2003; Stanton 2003). When the benefit of a mutualism scales with individuals of a third species, in this case ectoparasites, variation in the abundance of that third species is likely to lead to variable outcomes (Bronstein and Barbosa 2002). The conditions regulating tick-feeding and wound-feeding are unknown, but because of the dichotomy in feeding behavior, some researchers have proposed that the concept of conditional mutualism be applied to this cleaning association (Weeks 1999; McElligott *et al.* 2004). I investigated this possibility by observing oxpecker behavior in Kruger National Park, South Africa.

In any analysis of the nature of an interspecific association, the benefits to each partner need to be weighed against the costs. The fitness effects of a tick-feeding and wound-feeding bird to an ungulate host need to be quantified. Tick removal is imperative to ungulate health and survival (Mooring *et al.* 2004). A tick-feeding bird decreases host tick load, which in turn prevents blood loss, host anemia and appetite suppression, the spread of pathogens, and tick reproduction (Little 1963; Williams *et al.* 1978; Mulilo 1985; Norval *et al*. 1988). The body size principle states that smaller ungulates, with a greater surface area-to-mass ratio, incur higher costs for a given density of tick infestation relative to larger ones (Hart *et al.* 1992; Olubayo *et al.* 1993). Some studies have documented that in growing calves the equivalent of one engorging tick produces a growth decrement of at least 4.4 g due to blood loss and tick-induced anorexia (Little 1963; Turner and Short 1972; Williams *et al.* 1978; Hart and Hart 1988; Norval *et al.* 1988; Kaiser *et al.* 1991; Hart *et al.* 1992). Results obtained in a study in South Africa indicated that one engorging female blue tick, *Boophilus decoloratus*, produces a growth decrement of 8.0-9.0 g per calf (Scholtz *et al.* 1991). A fully engorged female bont tick, *Amblyomma hebraeum*, can cause a loss in liveweight gain of approximately 10 g per calf (Norval *et al*. 1989) or a loss in milk production of approximately 7g (Norval 1990 in Norval *et al.* 1994). Heavy infestations of bont ticks can lead to teat damage (Asselbergs and Lopes Pereira 1989 in Norval *et al*. 1994; Norval *et al.* 1989). Wound-feeding, by contrast, is usually assumed to have a detrimental effect on hosts. Blood loss and susceptibility to secondary infection are negative effects of wounds. A few authors have proposed that a wound-feeding oxpecker may actually benefit a host by cleaning wounds of maggots (Attwell 1966; Breitwisch 1992, 1996). There is no direct evidence for this positive effect, and oxpeckers have been observed feeding from already clean wounds, opening old wounds, and creating new wounds, thereby increasing the chance of secondary infection (van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988; Weeks 1999, 2000; McElligott *et al.* 2004). Most of these wound-feeding observations have been of oxpeckers associating with domesticated species (Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Stutterheim *et al.* 1988; Weeks 1999, 2000). Wound-feeding behavior on wild host species, in contrast, has been minimally investigated (Bezuidenhout and Stutterheim 1980; McElligott *et al.* 2004).

The majority of studies examining the interaction between oxpeckers and their ungulate hosts have focused on host preference and oxpecker host usage patterns (Table 2.1). The focus of this study was oxpecker feeding behavior. I specifically investigated the occurrence of tick-feeding and wound-feeding behavior of wild oxpeckers on large ungulates in South Africa to elucidate the nature of this interspecific relationship. Potential factors regulating oxpecker feeding behavior and creating conditional outcomes in this multispecies African interaction are: 1) the species of oxpecker and species of host, 2) the relative abundance of ticks and wounds per host, 3) the species and developmental stages of ticks available, and 4) the density of partners. Although it is impossible to quantify the number of ticks and wounds per host animal, the proportion of total feeding events that are tick-feeding and the proportion of total feeding events that are wound-feeding can be determined, and these values can be compared among oxpecker species, wild host species, and seasons.

This research addressed four questions: 1) Do oxpeckers spend more time tick-feeding or wound-feeding, and do these feeding activities differ between red-billed and yellow-billed oxpeckers? The majority of feeding observations are predicted to be tick-feeding if oxpeckers are mutualists. But because host density, vegetation, temperature, relative humidity and rainfall are factors influencing the distribution and abundance of ticks (Londt and Whitehead 1972; Semter and Hair 1973; Norval 1977; Howell *et al.* 1978; Grobler 1980; Norval and Lightfoot 1982; Barnard 1986; Hart *et al.* 1990; Garris *et al.* 1990; Needham and Teel 1991; Carroll and Schmidtmann 1996; Oorebeek and Kleindorfer 2008), wound-feeding may be conditional on periods of tick scarcity. The two oxpeckers species are similar in body size, tick preferences, and host species utilized. The only other study to document wound-feeding by yellow-billed oxpeckers concluded that this species spent 4% more time feeding on cattle wounds than did red-billed oxpeckers (Stutterheim *et al.* 1988). Yellow-billed oxpeckers were observed competing with each other and with red-billed oxpeckers for access to wounds (Stutterheim *et al.* 1988). Based on these observations, I predicted that the majority of wound-feeding observations would be of yellow-billed oxpeckers.

2) Is wound feeding related to host species and/or the number of oxpeckers per host animal? Ungulate species differ in characteristics such as body size, hair thickness, skin thickness, habitat preference, and wounds (Attwell 1966; Menegsha 1987; Grobler 1980; Hart *et al.* 1990; Dale 1992a,b; Mooring and Mundy 1996a). These factors influence how susceptible an ungulate is to tick infestation. Large ungulate species generally have greater tick abundance, as well as a higher proportion of adult ixodid ticks than small ungulate species (Horak 1982; Horak *et al*. 1983). Nymphal and larval

infestations, however, tend to be proportional to the host animal's surface area (Horak 1982; Horak *et al*. 1983; Hart *et al.* 1990; Gallivan and Horak 1997; Mooring and Mundy 1996a). Impala is the smallest antelope, and warthog, the smallest ungulate, with which oxpeckers associate (Hart *et al.* 1990). Impala can support a high abundance of *Boophilus* ticks, and warthogs have very fine hair which facilitates easy tick removal (Hart *et al.* 1990; Mooring and Mundy 1996a). Woodland habitats generally have a higher tick density than grassland areas (Londt and Whitehead 1972; Semter and Hair 1973; Norval 1977; Grobler 1980; Barnard 1986; Garris *et al.* 1990; Hart *et al.* 1990; Carroll and Schmidtmann 1996). Thus, ungulates inhabiting woodland habitats are predicted to have higher tick loads. Some ungulates, such as impala, can control tick abundance by grooming (Olubayo *et al.* 1993). Additionally, due to intra and interspecific fighting, ungulate species can differ in wounds. Hippos, for example, often have numerous wounds due to a high level of intraspecific aggression (Attwell 1966). Oxpecker and ungulate abundance also are factors that may cause this association to have conditional outcomes. High numbers of oxpeckers per host animal are predicted to quickly reduce the host's tick load, causing wound-feeding to be more commonly observed on hosts with many oxpeckers. Wound-feeding could, however, be a function of the occurrence of wounded ungulates.

3) Are there seasonal effects on oxpecker feeding behavior? The abiotic environment in which the oxpecker-ungulate interaction occurs may affect if and how this interaction moves along the continuum. Tick species and abundance varies seasonally, dependent on temperature, rainfall and humidity (Hart *et al.* 1990; Needham and Teel 1991; Cumming 1999). Many tick species increase in the wet season and

decrease in the dry season (Mulilo 1985; Mooring 1995; Mooring and Mundy 1996a; Speybroek *et al.* 2004). Wound-feeding is predicted to be conditional on tick scarcity, and hence increase in the dry season.

4) Does the response behavior of the host to a tick-feeding, wound-feeding, or non-feeding oxpecker differ? Mutualisms are subject to exploitation, leading to conflicts of interests between partner species that can disrupt the relationship (Bronstein 2001b). Therefore, host animals are expected to terminate antagonistic interactions to reinforce the mutualistic behavior of oxpeckers. In the cleaner-client fish association, the client exhibits considerable control over whether a cleaner fish gleans ectoparasites or "cheats" by feeding on client mucus and tissue (Bshary 2002; Johnstone and Bshary 2002). Client fish make short jolts in response to cleaner bites, chase the cleaner, or swim away and visit another cleaning station (Bshary 2002; Bshary and Grutter 2002). To deter oxpeckers, ungulates kick and stomp their legs, shake their heads, roll on the ground, run through the bush to scrape the birds off, and push the birds with their noses. These behaviors all are classified as rejection behaviors (Attwell 1966; Hart *et al.* 1990; Mooring and Mundy 1996b). I predict wound-feeding to elicit a rejection behavior from the host animal, whereas hosts are predicted to tolerate non-feeding and non-wound feeding birds, especially tick-feeding birds. However, a host animal with a large surface area can have a difficult time removing a bird. In addition, oxpeckers are very persistent and will continue to utilize a host despite repeated attempts by the host to remove them (personal observation). Therefore, the relationship between oxpeckers and ungulates, in terms of costs and benefits, may vary along a continuum from mutualism to parasitism

based on the feeding behavior of oxpeckers and the level of host control over oxpecker feeding behavior.

Mutualisms are increasingly recognized as fundamental to patterns and processes within ecological systems (Holland and Bronstein 2007; Bronstein 2001c). Studies of conditionality help us understand how changes to the environment, including anthropogenic disruptions, can influence the entire community within which these interspecific interactions occur.

Methods

In South Africa, red-billed and yellow-billed oxpeckers coexist only in northern Kruger National Park, a two million ha reserve. This area was chosen as the study site. I observed oxpeckers interacting with ungulates daily from January to July 2007. This time period included three wet months (January, February, March) and three dry months (April, May, June). The average rainfall in the wet and the dry season of Kruger National Park is 470 mm and 87 mm, respectively. Field observations were conducted from a 4-wheel drive vehicle using 7-15 x 25 binoculars (FOV 238ft @ 7x and FOV 156ft @ 15x) and/or a spotting scope (25x, 37x, 50x). Oxpeckers were observed up to a distance of 200 m from the vehicle. Shingwedzi research camp (23°06’30.48’’S, 31°23’57.97’’E) was the base camp for this six-month study (Fig. 2.1). The area between the Punda Maria (22°41’34.66’’S, 31°00’58.02’’E) and Mopani (23°31’21.48’’S, 31°23’57.97’’E) rest camps of Kruger National Park, a distance stretching 130 km, was routinely searched in the morning (08h00-11h00) and late afternoon (15h00-18h00) hours when oxpeckers are most active (Fig. 2.1). Oxpeckers have morning and late afternoon feeding peaks within these time periods (Stutterheim 1976; Mengesha 1978). Each observation session lasted two to three hours depending on the number of oxpeckers observed and the route taken. The route searched in the morning hours was always different from that searched in the afternoon. During an observation session, precaution was taken to avoid double counting a host animal.

An observational event was the observational period after an oxpecker was spotted on a host animal. An oxpecker was observed until it flew away, was no longer visible, or for a maximum of two minutes. For each event, oxpecker species, host species, location of the oxpecker on the host (back, neck, head, side, leg, anogenital, or tail region), oxpecker behavior, number of oxpeckers per host animal, and host response behavior were observed and recorded. These location categories were not mutually exclusive, for oxpeckers are very mobile when on their hosts and can move to several different locations during a single observational event. Bird behavior was categorized as wound-feeding, non-wound feeding (tick-feeding, ear-feeding, eye-feeding, mouth-feeding, nose-feeding, and/or anogenital-feeding), or non-feeding (i.e. preening, perching). Host response behaviors, which are summarized in Table 2.2, were classified as objectively as possible as either tolerate or reject (Attwell 1966; Hart *et al.* 1990; Mooring and Mundy 1996b). Behaviors were not mutually exclusive. During a single observational event a bird could exhibit more than one behavior and a host animal could exhibit more than one response behavior. After an observational event, the next bird on the same host animal was observed for two minutes, and so on, until all the oxpeckers on a particular host had been observed and their behaviors recorded. I then began observations of the next visible host carrying an oxpecker, using the same scanning technique. There were several instances in which an oxpecker was located, but then flew away before all data could be recorded. In addition, on a few occasions the host animal moved into the bush and its attendant oxpeckers were out of view. Such incomplete data were used in the analyses.

I considered a wound to be any visible break in the skin whether or not blood was present (see Weeks 2000). This is because even if blood was not initially present, an oxpecker can simply peck at the exposed area and draw blood in seconds (personal observation). Notes were made when potential hosts had an exploitable wound, whether or not oxpeckers were in attendance. All of the ungulates observed during an observation session were recorded, regardless of oxpecker presence, in order to calculate the frequency of utilization for all potential host species. Table 2.3 lists the ungulate species that occur in Kruger National Park and are potential hosts of oxpeckers. These species are "potential" hosts because they have been observed being utilized by oxpeckers in the past in various areas of sub-Saharan Africa. There are difficulties with counting large numbers of ungulates, particularly impala and buffalo. For these groups, probable underestimates were used when ungulate numbers were too large for an accurate count.

Statistical Analyses

I first calculated the total number of observational events of each oxpecker species. These values were then used to test whether observations of both oxpecker species on the same host animal were more or less frequent than would be expected based on their relative abundances using a Chi-square test. To determine the host species most commonly observed with oxpeckers, I calculated the proportion of observational events per host species per season. I then tested whether the host species oxpeckers were most frequently observed on in the wet season differed from those in the dry season using a

Two-proportion z-test. Oxpeckers may differentiate between ungulates they use primarily as perching substrates vs. those they use primarily for feeding purposes. For each host species, the proportion of observational events that were feeding and non-feeding events were calculated and compared using a Chi-square test. The location of oxpeckers on their hosts was recorded to determine whether oxpeckers frequent body areas where hosts cannot oral-groom. To examine this, I calculated the proportion of observational events per season that were of oxpeckers on the back, neck, head, side, leg, anogenital, or tail areas.

I categorized observational events as either feeding or non-feeding events and compared these proportions using a Chi-square test. I also compared the proportion of events that were feeding and non-feeding for each oxpecker species using Chi-square tests. To detect differences in feeding behavior within and between red-billed and yellow-billed oxpeckers, feeding events were separated into categories (tick-feeding, ear-feeding, eye-feeding, mouth-feeding, nose-feeding, and anogenital-feeding) and then compared within oxpecker species using Chi-square tests and between oxpecker species using Two-proportion z-tests. I then tested the hypothesis that there are seasonal effects on oxpecker feeding behavior and wound-feeding increases in the dry season. The proportion of wound-feeding events and non-wound feeding events per season were compared with a Chi-Square test. The proportion of wound-feeding events per species was tested for seasonality using Two-proportion z-tests.

To examine whether wound-feeding was related to host species, the proportion of wound-feeding events per host species was calculated and compared. Then, for each ungulate species oxpeckers utilized for wound-feeding, I conducted a Two-proportion z-test to test whether wound-feeding events increased from the wet to the dry season.

To examine whether oxpecker abundance per host affects oxpecker behavior, observational events were divided into two categories: 1) events with more than one oxpecker present per event per host and 2) events with only one oxpecker per event per host. To test the hypothesis that wound-feeding would be more commonly observed on ungulates with more than one oxpecker, the proportion of non-feeding events, feeding events, wound-feeding events, and non-wound-feeding events with more than one oxpecker were compared using Two-proportion z-tests. Furthermore, I used Two-proportion z-tests to test whether the proportion of observational events, feeding events, non-feeding events, wound-feeding events, and non-wound feeding events with more than one oxpecker per host increased in the dry season.

Finally, I tested the hypothesis that wound-feeding elicits rejection behaviors from the host animal, but hosts tolerate non-feeding and non-wound feeding birds. The proportion of response behaviors that were tolerant responses and rejection responses were calculated per host species and tested for significance using Chi-square tests and Two-proportion z-tests.

Tests were two-tailed with a significance level of $p<0.05$ (SPSS Statistics Gradpack 17.0).

Results

Oxpecker Observations and Host Utilization Patterns

A total of 992 independent observational events (386 wet season; 606 dry season) of oxpeckers (696 red-billed; 267 yellow-billed; 29 unidentifiable) were recorded (Table 2.4). Observational drives were made on 111 days, with oxpeckers being observed on

105 of those days (Table 2.4). The average number of oxpeckers per host animal per observational event was 2.75, per feeding event was 2.98, and per non-feeding event was 2.42 (Table 2.4). Both species were observed simultaneously on the same host animal during 17 observational events (7 wet season; 10 dry season), which is less than expected based on their relative abundances (χ^2=715.195, df=2, p<0.001).

Oxpeckers utilized eight of 17 potential host species observed (Table 2.3; Fig. 2.2). The total number of oxpecker sightings was greatest on impala, giraffe and buffalo (totaling 90% of all observations; Fig.2.2a), and this did not change from the wet (94%; Fig. 2.2b) to the dry (87%; Fig. 2.2c) season (Two-proportion z-test, z=3.58, n_1=386; n_2=606, p<0.001). Observations of red-billed oxpeckers were primarily on impala, giraffe and buffalo (Fig. 2.3a) and this was consistent for both seasons. Yellow-billed oxpeckers were most frequently observed on giraffe and buffalo (Fig. 2.3b), and no seasonal difference was observed. Impala and zebra were used almost exclusively by red-billed oxpeckers, and only one observation was made of oxpeckers on bushbuck.

Although host utilization patterns were consistent from the wet to the dry season, oxpeckers appeared to differentiate between those hosts they used primarily as perches versus those used primarily for feeding purposes (Table 2.5). Buffalo-oxpecker observations were predominantly of oxpeckers perching (χ^2=83.31, df=1, n=281, p<0.001); whereas giraffe (χ^2=163.51, df=1, n=297, p<0.001) and impala (χ^2=36.01, df=1, n=312, p<0.001) mostly served as feeding substrates. There was no statistically significant difference in perching versus feeding behavior for the other five host species (Table 2.5).

The large percentage of oxpecker observations on certain host species might simply reflect the abundance of these species in the study area. I made records of all ungulates observed during the observation sessions, whether or not oxpeckers were present. For each of the eight host species used by oxpeckers in Kruger National Park, I then calculated a ratio of the number of hosts utilized to the total number of hosts observed (Table 2.6). Giraffe, kudu, buffalo and hippo were the species with the highest ratio values. Bushbuck had the lowest ratio. Interestingly, impala also had a low ratio, despite being the species with the highest percentage of oxpecker observational events. The ratio values for only two of the eight species, buffalo and impala, differed significantly between the wet and the dry seasons (Table 2.6).

Many ungulate species engage in grooming behaviors to control their tick loads (Hart and Hart 1988; Olubayo *et al*. 1993; Mooring *et al.* 1996; Mooring *et al.* 2002). Oxpeckers were predicted to most frequently be observed on the areas of ungulates that they cannot self-groom, and the frequency of these locations was not predicted to change seasonally. The greatest percentage of oxpecker observations were of oxpeckers on the back of the host animal (40% wet, 46% dry). The neck (19% wet, 18% dry) and head (17% wet, 12% dry) were less utilized. The lowest percentage of observations were of oxpeckers on the side (10% wet; 13% dry), leg (6% wet; 4% dry), anogenital (4% wet, 3% dry) and tail (4% wet, 4% dry) regions. In summary, the majority of observations were of oxpeckers on areas where ungulates cannot self groom.

Oxpecker Feeding Behavior

The majority of oxpecker observations were predicted to be feeding observations because observation sessions were intentionally conducted during the peak feeding hours of oxpeckers. Fifty-eight percent of all observational events were feeding, which was statistically higher than non-feeding observational events (χ^2=27.11, df=1, n=992, p<0.001). Although the proportion of red-billed oxpecker observational events that were feeding were significantly higher than non-feeding events (χ^2=88.37, df=1, n=696, p<0.001), the reverse was observed for yellow-billed oxpeckers, with non-feeding events predominating (χ^2=29.67, df=1, n=267, p<0.001; Fig. 2.4). The feeding activities for each oxpecker species are presented in Fig. 2.5. For both red-billed and yellow-billed oxpeckers, the majority of feeding events were tick-feeding. The proportion of feeding events that were tick-feeding, wound-feeding, and other-feeding (ear, eye, mouth, nose and anogenital) differed significantly for both red-billed oxpeckers (χ^2=687.41, df=2, n=472, p<0.001) and yellow-billed oxpeckers (χ^2=36.43, df=2, n=89, p<0.001). Out of the total oxpecker observations, there were significantly more tick-feeding than wound-feeding events (χ^2=434.57, df=1, n=504, p<0.001). Red-billed oxpeckers were observed tick-feeding significantly more than yellow-billed oxpeckers (Two-proportion z=6.71, n_1=472; n_2=89, p<0.001). Yellow-billed oxpeckers were observed other-feeding significantly more than red-billed oxpeckers (Two-proportion z=4.03, n_1=472; n_2=89, p<0.001). The occurrence of wound-feeding behavior was only 3.1% of 558 total feeding observations. As predicted, wound-feeding was a behavior more commonly exhibited by yellow-billed oxpeckers than by red-billed oxpeckers, and this difference was statistically significant (Two-proportion z-test, z=6.27, n_1=472; n_2=89, p<0.001).

An oxpecker could wound-feed and non-wound feed (tick, nose, mouth, ear, eye, anogenital) during a single observational event. Events in which an oxpecker only non-wound fed (i.e. did not wound-feed, but fed in other locations on the host) were grouped and compared to events where an oxpecker only wound-fed, and these frequencies differed significantly (χ^2=525.01, df=1, n=572, p<0.001; Fig. 2.6). The proportion of feeding events that were only non-wound feeding and only wound-feeding also differed significantly for both red-billed oxpeckers (χ^2=464.03, df=1, n=472, p<0.001; Fig. 2.6) and yellow-billed oxpeckers (χ^2=56.640, df=1, n=89, p<0.001; Fig. 2.6), with a higher proportion of non-wound feeding events.

Oxpecker feeding behavior was related to season. Wound-feeding events significantly increased in the dry season (χ^2=5.29, df=1, n=578, p=0.022; Fig. 2.7). However, the proportion of wound-feeding events of each species did not differ seasonally (red-billed: Two-proportion z-test, z=1.17, n_1=217; n_2=255, p=0.241; yellow-billed: Two-proportion z-test, z=1.56, n_1=24; n_2=65, p=0.118).

Only eighteen observational events were made of a wound-feeding oxpecker of either species (Table 2.7). Several of these observations were repeated sightings of the same host animal (Table 2.7). Giraffe 1 suffered from a skin disease resulting in wart-like growths over the entire surface of its body. The oxpeckers plucked off the warts, exposing the blood and tissue from which they then feed. This giraffe was observed on three different days and was the host during five observational wound-feeding events. Giraffes 2, 3 and 4 were observed only once with wound-feeding oxpeckers. Giraffe 5 had a missing horn with tissue and blood exposed. Oxpeckers wound-fed on this giraffe during five observational events. Giraffe 6 had a wound at the upper part of its tail that was utilized once by oxpeckers. Hippopotamus and buffalo were the only other two species on which wound-feeding observations were made. A wound-feeding bird was observed on a hippo during only one observational event (Hippo 1) and two buffalo were observed with wound-feeding birds. Buffalo 1 had two red-billed oxpeckers simultaneously feeding from a wound behind the horns, and Buffalo 2 had one red-billed oxpecker feeding from a tail wound. During the study period, an oxpecker was never observed inflicting a wound on a host, only exploiting existing wounds.

Although eighteen wound-feeding events were recorded, seven of these events involved oxpeckers in the presence of other wound-feeding oxpeckers (Table 2.7). On Apr 23 two yellow-billed oxpeckers wound-fed from Giraffe 5, on May 28 two red-billed oxpeckers wound-fed from Buffalo 1, and on May 30 three yellow-billed oxpeckers wound-fed from Giraffe 1. These events are highlighted in Table 2.7. Of the nine wounded hosts, only Giraffe 1 supported both oxpecker species during an observational event (May 30 and May 31). In each instance, only the yellow-billed oxpeckers wound-fed (Table 2.7). During three of the eighteen events, only the wound-feeding oxpecker was present on the host animal (Jan 19, Mar 7, June 14).

Counts were made of wounded-host animals observed during the morning and afternoon observation sessions whether or not oxpeckers were in attendance. A wounded host animal was observed on 50 different occasions, 22 of which were believed to be the same host animals observed more than once. Fourteen wounded hosts had wound-feeding oxpeckers; nine hosts had non-wound feeding oxpeckers; and 27 did not have oxpeckers in attendance.

Wound-Feeding Related to Host Species and Oxpecker Abundance

Wound-feeding was observed on only three host species: buffalo, giraffe and hippopotamus. The total wound-feeding and non-wound feeding observations on these three host species in the wet and the dry season are listed in Table 2.8. Three wound-feeding and 151 non-wound feeding events were observed in the wet season, whereas 15 wound-feeding and 167 non-wound feeding events were observed in the dry season. The frequency of wound-feeding events on giraffes increased from the wet to the dry season and this difference was statistically significant (Two-proportion z-test, z=2.18, n_1=3; n_2=11, p=0.029; Table 2.8). The frequency of wound-feeding events on buffalo and hippo also increased from the wet to the dry season, but this was not statistically significant (Table 2.8).

Table 2.9 presents the percentage of events with more than one oxpecker present per host. Sixty-four percent of the total observational events had more than one oxpecker per host (58% wet; 68% dry). The percentage of feeding events (67%) with more than one oxpecker was statistically greater than that of non-feeding (61%) events (Two-proportion z-test, z=2.05, n_1=578; n_2=414, p=0.04). There was no significant difference in the percentage of wound-feeding (78%) and non-wound feeding (67%) events with more than one oxpecker present (Two-proportion z-test, z=0.99, n_1=18; n_2=560, p=0.32). Nor was there a statistical difference in the proportion of wound-feeding and non-feeding events with more than one oxpecker present (Two-proportion z-test, z=1.46, n_1=18; n_2=414, p=0.14). When these events were compared seasonally, a statistically significant difference was found for observational events (Two-proportion z-test, z=3.30, n_1=386; n_2=606, p=0.001), total feeding events (Two-proportion z-test, z=3.16, n_1=249; n_2=329, p=0.002), wound-feeding events (Two-proportion z-test, z=2.03, n_1=3; n_2=15, p=0.043) and non-wound feeding events (Two-proportion z-test, z=2.86, n_1=246; n_2=314, p=0.004). Events with more than one oxpecker increased from the wet to the dry season. Only non-feeding events showed no seasonal difference in the percentage of events with more than one oxpecker present per host.

Host Response Behavior

There was a statistically significant difference between the response behaviors of host animals to oxpeckers (Fig. 2.8). Hosts exhibited more tolerant behaviors than rejection behaviors (χ^2=797.69, df=1, n=962, p<0.001; Fig. 2.8; Table 2.10) and this was consistent between the wet season and the dry season. The host response behavior to a feeding or non-feeding oxpecker did not differ significantly (χ^2=0.06, df=1, n=950, p=0.802). Hosts were more tolerant than rejecting of wound-feeding (χ^2=7.12, df=1, n=17, p=0.008), non-wound feeding (χ^2=466.51, df=1, n=551, p<0.001), and non-feeding (χ^2=302.15, df=1, n=367, p<0.001; Table 2.10) oxpeckers. However, host response behavior to wound-feeding and non-wound feeding oxpeckers was statistically different. Hosts were significantly more tolerant of non-wound feeding oxpeckers and more rejecting of wound-feeding oxpeckers (Two-proportion z-test, z=2.70, n_1=17; n_2=551, p=0.007; Fig. 2.9). Two of these rejection events were of red-billed oxpeckers foraging on a wound behind buffalo horns, and the third rejection response was to a yellow-billed oxpecker pecking at a wound between the eyes of a giraffe.

Discussion

Interspecific associations in a complex environment are subject to variation (Boucher *et al.* 1982; Cushman and Whitham 1989; Bronstein 1994a,b; Herre *et al.* 1999; Billick and Tonkel 2003; McCreadie *et al.* 2005). They involve inherent costs and benefits for each interacting species, and the net effect of each partner can vary as a function of the physical and biological setting. Mutualistic interactions are often skewed, with one species benefiting more than the other (Hoeksema and Bruna 2000). The association between ticks, oxpeckers, and ungulates was hypothesized to exhibit conditional outcomes because of the inherent complexity of this interaction. Two oxpecker species forage on a variety of ungulate species, which vary in tick burden and tick species. Oxpeckers unquestionably benefit from their association with the large ungulates of South Africa. Whether using them as a perch for preening and displaying, as a source of hair for their nests, or as a feeding substrate, oxpeckers require their hosts for survival. With the exception of some off-host insect-feeding, the diet of oxpeckers is entirely dependent on that of their ungulate hosts (Moreau 1933; van Someren 1951; Pitman 1956; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988). The net effect of the interaction to the ungulate host, however, depends on whether oxpeckers provide a cleaning service to their hosts. Oxpeckers may benefit their hosts through tick removal, but the relationship can turn antagonistic when oxpeckers feed from the tissue and blood of ungulate wounds.

Although oxpeckers have been observed regularly wound-feeding on livestock (Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Weeks 1999,2000; personal observation), I rarely observed this behavior in relation to wild ungulate species in Kruger National Park. In the few wound-feeding events observed, yellow-billed oxpeckers were the primary wound-feeders. However, the low wound-feeding occurrence of only 1.8% of observational events and 3.1% of feeding events, of which nine events involved repeated sightings of same host, is insufficient to draw a strong conclusion on wound-feeding differences between oxpecker species. Oxpecker abundance per host did not appear to influence oxpecker behavior. Several wound-feeding events occurred on hosts with a single oxpecker. During wound-feeding events, oxpeckers made intense vocalizations that were not made during non-wound feeding events. Oxpeckers also squabbled with each other for access to wounds. Stutterheim (1981) suggested that observations of red-billed oxpeckers competing with each other for wounds indicates a preference to feed at wounds, but the observed low occurrence of this behavior indicates that it is an infrequent feeding behavior on wild ungulates. It is possible that oxpecker rarely wound-feed on wild species. Domesticated species often are treated with chemicals to eradicate ticks, whereas wild host species are not. As a result, wild ungulates presumably support higher tick loads than livestock. It is also possible, however, that wound-feeding on wild ungulates is more common than it is observed. The inability to observe oxpeckers and their hosts in close proximity is a major constraint of wild observations. Observations of oxpeckers in captivity demonstrated that oxpeckers can create tiny wounds on their hosts, the size of the tip of their bill (personal observation). In a wild setting, where the observer can be 100-200 m from the oxpecker-ungulate interaction, wound-feeding of this sort may be misidentified as tick-feeding.

The low occurrence of wound-feeding observations also might be attributable to the absence of pre-existing host wounds. Although oxpeckers can inflict wounds on livestock (van Someren 1951; Mengesha 1978; personal observation), the frequency at which oxpeckers inflict wounds on wild host species is unknown. In this study, oxpeckers were never observed creating wounds. However, I made 50 observations of a wounded host animal: 54% did not have oxpeckers, 18% had non-wound feeding oxpeckers and 28% had wound-feeding oxpeckers. Despite their presence in the study area, wounded animals were observed more frequently than they were exploited by oxpeckers. This suggests that these ungulates had high tick loads.

In general, certain ungulates appear to be highly utilized while others are rarely selected. All researchers agree that it is uncommon to find oxpeckers utilizing elephants. There are only two records of elephants with oxpeckers in attendance: one record in the Luangwe Valley, Zambia in 1953 (Benson and White 1957 in Attwell 1966) and one in Hwange National Park, Zimbabwe in 1990 (Dale and Hustler 1991). That oxpeckers do not associate with elephants has been attributed to elephants' ability to dislodge the birds with their trunks (Dale and Hustler 1991). Although I observed many elephants, I never observed oxpeckers associating with them. Waterbuck and bushbuck also are uncommonly used hosts, an observation attributed to their thick woolly coats and frequent occurrence in dense bush. I observed only two red-billed oxpeckers on a single bushbuck. But because this bushbuck was foraging along the bank of a river near bathing hippos that also had oxpeckers, it is likely that the birds flew from the hippos to the bushbuck.

The species utilized by oxpeckers are dependent on the number of potential hosts available, the specific ungulate species composition in a particular region, and the density and species of ticks parasitizing the ungulates. For example, Stutterheim (1979) observed red-billed oxpeckers in southern Kruger National Park and concluded that red-billed oxpeckers only regularly associate with 15 species, showing a preference for giraffe, black rhino, roan antelope, and sable antelope. Grobler (1980) also studied red-billed oxpeckers in southern Kruger National Park. Eleven ungulate species of nineteen observed were utilized as hosts, with giraffe most commonly used. In the Hluhluwe-

Umolozi Game Reserve complex of KwaZulu-Natal, South Africa, the red-billed oxpecker associated with nine species, utilizing black rhino, white rhino, buffalo and giraffe most commonly (Stutterheim 1980). But in the Mkuzi Game Reserve (Kwazulu-Natal, South Africa) and Nxwala State Land (KwaZulu-Natal, South Africa) red-billed oxpeckers associated with six species, including white rhino, giraffe, kudu, and zebra (Stutterheim and Stutterheim 1981). In northern Kruger National Park, where the present study was conducted, there are potentially 17 host species available to the birds (Table 2.3). I never observed oxpeckers on black rhino, white rhino, sable and roan antelope, species that were rare in the study area. During non-observational periods in other sections of Kruger National Park, however, red-billed oxpeckers were observed associating with some of these species. Yellow-billed oxpeckers were observed on impala on only one occasion in this study, but in Zimbabwe they regularly associated with impala (Mooring and Mundy 1996b).

Ticks may select certain hosts in a given environment or select certain environments and feed on the host within these environments (Cumming 1998). Certain tick species parasitize a variety of host species, others are more selective, and some are species-specific (Oliver 1989). The host animals that oxpeckers are commonly seen foraging on are expected to be infested with the tick species and tick stages that oxpeckers prefer. In this study, feeding observations were greatest on impala and giraffe. Buffalo were frequently observed with oxpeckers, but the majority of these observations were non-feeding events. Oxpeckers exhibit a preference for four species of tick: blue tick, *Boophilus decoloratus*; brown ear tick, *Rhipicephalus appendiculatus*; bont-legged tick, *Hyalomma truncatum*; and bont tick, *Amblyomma hebraeum* (Bezuidenhout and

Stutterheim 1980; Stutterheim *et al.* 1988). Buffalo carry large burdens of most of the stages of these tick species (Horak *et al.* 1983). Impala and giraffe also support these tick species in varying amounts. The most preferred tick species of oxpeckers, *B. decoloratus*, attaches to antelope species such as impala and kudu, with impala supporting high infestations (Horak *et al.* 2003). Stutterheim and Brooke (1981) concluded that when searching for *Boophilus*, oxpeckers should associate mainly with antelope species, but when foraging for *Rhipicephalus*, they should select buffalo and rhino. Adult *A. hebraeum* also prefer large herbivore hosts such as giraffes (Horak *et al.* 2003), and adult *H. truncatum* frequent large thick-skinned ungulates (Walker 1991). In this study, oxpeckers were observed wound-feeding on three species: buffalo, giraffe and hippo. Hippos generally have low tick loads but many wounds due to a high level of intraspecific fighting (Attwell 1966). Buffalo and giraffe, however, are expected to host many ticks as a result of the body-size principle. This principle states that generally larger ungulates host more ticks than smaller ungulates (Gallivan and Horak 1997). The frequency of wound-feeding events on buffalo and giraffe may have been the result of a higher incidence of wounded individuals of these species relative to the other ungulate species.

In the current study, I observed oxpeckers primarily on impala, buffalo and giraffe. This does not necessarily indicate that these are the host species most heavily used or preferred by oxpeckers. Instead, these were the species most commonly encountered and observed with oxpeckers by the researcher. Giraffe, kudu, and buffalo were the three species with the highest ratio values of oxpecker utilization. However, these numbers reflect the abundance of these species in the park. Certain ungulate species are abundant and commonly observed in Kruger National Park (e.g. impala, buffalo), whereas others are rare and more elusive.

Tick populations generally increase in the wet season and decrease in the dry season (Mooring and Mundy 1996a,b; Stutterheim and Stutterheim 1980), and these seasonal dynamics may have an indirect effect on oxpecker foraging behavior. Wound-feeding may be a behavior more frequently observed when tick abundance is low. In the present study, wound-feeding increased and non-wound feeding decreased in the dry season, as predicted. However, wound-feeding could have been an artifact of an increased frequency of wounded ungulates. It should be noted that this study was conducted over a period of six months and among-year variation in oxpecker feeding behavior due to climate variation was not examined.

Whether oxpeckers are tolerated or rejected by a host animal will influence their presence on that particular host (Hart *et al.* 1990). As Mooring and Mundy (1996b) suggest, hosts that suffer from high tick loads are expected to tolerate oxpecker presence in order to benefit from tick removal. But if oxpecker feeding-behavior is antagonistic, hosts should exhibit a response strategy to deter exploitation and reinforce the mutualistic actions of oxpeckers (Bronstein 2001b). As predicted, host animals were more rejecting of wound-feeding oxpeckers than non-wound feeding oxpeckers. However, in general, hosts were very tolerant to wound-feeding oxpeckers. Why a host animal would tolerate a wound-feeding bird is unclear. Ungulates with high tick loads may tolerate oxpeckers even if the birds occasionally wound-feed. Or, it is possible that the longer an oxpecker feeds from a wound, the less tolerant the host becomes over time. I may have observed oxpeckers at the beginning of their wound-feeding events, and hence, the host animal was not yet irritated by oxpecker presence. Another possibility is that, generally, wounds are small and wound-feeding may not be recognized by the host animal.

Conclusion

Despite the uncertainty surrounding the possibility that oxpeckers create small wounds on wild ungulates from which to blood-feed, I conclude from my observations that oxpeckers rarely wound-feed from their wild hosts. Based on previous reports in the literature, perhaps wound-feeding is more common on domesticated ungulate species. Whether the relationship between oxpeckers and domesticated hosts differs from that between oxpeckers and wild hosts, or if this observed difference is actually a result of researcher constraints in the wild, needs exploration. Wound-feeding was more commonly observed in the dry season, indicating the possibility that oxpeckers employ this behavior under times of nutritional stress (e.g. tick shortages). Experimental manipulation in which tick species and tick abundance per host animal can be controlled would help isolate the conditions under which oxpeckers wound-feed. Also, a study on the dynamics of an interspecific relationship is not complete unless the fitness costs to each partner are examined. The costs and benefits to the host animal of a wound-feeding bird compared to a tick-feeding bird need to be critically assessed to generate a clear picture of the nature of this unique African interaction.

Table 2.1. Studies examining the ungulate host species preferred by oxpeckers.

Author	Study Location	Species	Study Period
Robertson & Jarvis 2000	Caprivi, Namibia	RBO YBO	16-26 Nov 1997, 13-24 Feb 1998, 14-19 May 1998
Koenig 1997	Lake Nakuru National Park & Masai Mara Reserve, Kenya	RBO YBO	26 July -17 August 1990
Mooring & Mundy 1996	Matobo National Park, Zimbabwe	YBO	20 Oct -26 Nov 1992 & 3 March - 10 April 1993
Dale 1992	Matobo National Park, Zimbabwe	YBO	15-19 May 1990
Hart, Hart, & Mooring 1990	Mara region & Lake Naivasha area, Kenya	RBO YBO	N/A
Brown & Brown 1987	Eastern Caprivi, Namibia	RBO YBO	17-23 Sept 1983
Hustler 1987	Hwange National Park, Zimbabwe	RBO YBO	Sept 17-Oct 12 1983
Stutterheim & Panagis 1985	Kavango & Caprivi, Namibia	RBO YBO	April 1984
Stutterheim & Panagis 1985	Moremi Game Reserve, Botswana & Caprivi, Namibia	RBO YBO	April -June 1984
Stutterheim 1981	KNP, South Africa; southern Botswana; Hluhluwe-Umfoloxi Game Reserve	RBO	March 1973-Jan 1975 in KNP; January 1977 in Botswana; June 1977 in Hluhluwe-Umfoloxi Game Reserve Complex;
Stutterheim & Stutterheim 1981	Mkuzi Game Reserve and Nxwala State land, Mozambique	RBO	7 days, Jan 1978
Stutterheim 1980	Hluhluwe-Umfolozi Game Reserve Complex , South Africa	RBO	33 days, May-June 1977
Stutterheim & Stutterheim 1980	Shingwedzi, KNP, South Africa	RBO	Nov 1974; Dec 1979
Grobler 1980	Southern KNP, South Africa	RBO	Jan-Feb 1979
Grobler 1979	Rhodes Matopos National Park, Rhodesia	RBO YBO	1975-1977
Grobler & Charsley 1978	Rhodes Matopos National Park, Rhodesia	YBO	May 1977-April 1978
Mengesha 1978	Ethopia	RBO	N/A
Buskirk 1975	Botswana	RBO YBO	June-July 1973
Dowsett 1969	Luangwa Valley National Park, Zambia	RBO YBO	Aug 1966; Dec 1967
Attwell 1966	Luangwa Valley & Kafue National Park, Zambia	RBO YBO	1960-1964

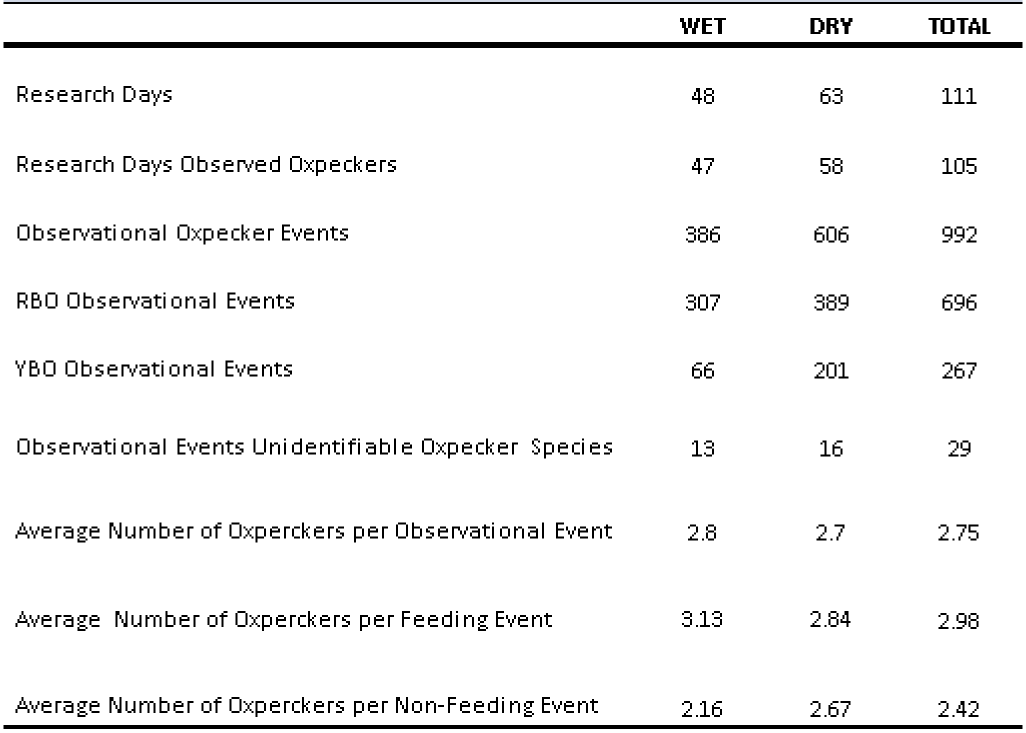
Table 2.2. Oxpecker behaviors and host response behaviors to oxpeckers, as tabulated in this study.

	Oxpecker Behavior	Description of Behavior	Host Behavior	Description of Behavior
Wound-Feeding	wound-feeding	oxpecker creates, opens, pecks at host wound, removes scab material, feeds on blood and/or tissue	tolerate	host lowers ear, inclines head, stands still, lifts tail, or exhibits no visible response to bird
Non Wound-Feeding	tick-feeding	oxpecker uses scissoring motion to comb through hair of the host animal for ectoparasites; oxpecker using plucking motion to pull off ticks	reject	host attempts to dislodge oxpecker by head-shaking, nose-pushing, stomping, kicking, rolling, running, etc.
	ear-feeding	oxpecker feeds on earwax or ticks within ear cavity		
	eye-feeding	oxpecker cleans inner and outer corners of eye		
	mouth-feeding	oxpecker pokes around the mouth region of the host animal		
	nose-feeding	oxpecker feeds on nose secretions		
	anogenital-feeding	oxpecker feeds around anogenital region, particularly when host animal lifts tail to defecate or urinate		
Non-Feeding	preening	oxpecker preens while on the host animal		
	perching	oxpecker rests, displays sun-bathes and/or volcalizes on host animal		

Table 2.3. Ungulate species observed and utilized by oxpeckers in Kruger National Park (●species utilized; x species on which wound-feeding was observed).

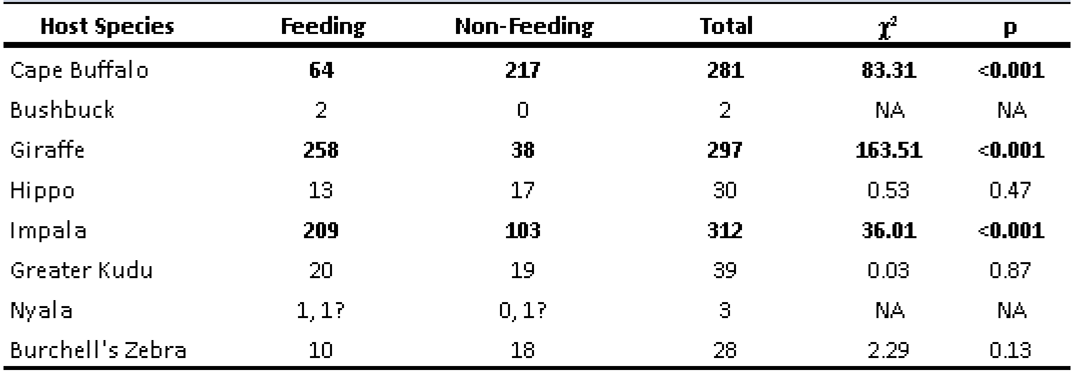
Scientific Name	Common Name
Syncerus caffer	● x Cape Buffalo
Tragelaphus scriptus	● Bushbuck
Giraffa camelopardalis	● x Giraffe
Hippopotamus amphibius	● x Hippo
Aepyceros melampus	● Impala
Tragelaphus strepsiceros	● Greater Kudu
Tragelaphus angasii	● Nyala
Equus burchelli	● Burchell's Zebra
Sylvicapra grimmia	Duiker
Raphicerus sharpei	Grysbok
Oreotragus oreotragus	Klipspringer
Hippotragus equinus	Roan Antelope
Raphicerus campestris	Steenbok
Damaliscus lunatus	Tsessebe
Phacochoerus aethiopicus	Warthog
Kobus ellipsiprymnus	Waterbuck
Connochaetes taurinus	Blue Wildebeest

Table 2.4. Number of research days, number of research days oxpeckers were observed, number of oxpecker observational events, and the average number of oxpeckers observed per event per the wet and dry season (RBO = red-billed oxpecker; YBO = yellow-billed oxpecker).



	WET	DRY	TOTAL
Research Days	48	63	111
Research Days Observed Oxpeckers	47	58	105
Observational Oxpecker Events	386	606	992
RBO Observational Events	307	389	696
YBO Observational Events	66	201	267
Observational Events Unidentifiable Oxpecker Species	13	16	29
Average Number of Oxperckers per Observational Event	2.8	2.7	2.75
Average Number of Oxperckers per Feeding Event	3.13	2.84	2.98
Average Number of Oxperckers per Non-Feeding Event	2.16	2.67	2.42

Table 2.5. Species and number of hosts utilized for feeding and non-feeding purposes. Chi-square, $p<0.05$.



Host Species	Feeding	Non-Feeding	Total	χ^2	p
Cape Buffalo	**64**	**217**	**281**	**83.31**	**<0.001**
Bushbuck	2	0	2	NA	NA
Giraffe	**258**	**38**	**297**	**163.51**	**<0.001**
Hippo	13	17	30	0.53	0.47
Impala	**209**	**103**	**312**	**36.01**	**<0.001**
Greater Kudu	20	19	39	0.03	0.87
Nyala	1, 1?	0, 1?	3	NA	NA
Burchell's Zebra	10	18	28	2.29	0.13

Table 2.6. Ratio of the number of ungulates utilized to the number of ungulates observed for each ungulate species per season. Two proportion z-test of significance between seasons, $p<0.05$.

	Wet	Dry	z	p
Cape Buffalo	0.06	0.04	2.12	0.03
Bushbuck	0.00	0.02	0.81	0.42
Giraffe	0.39	0.36	0.45	0.65
Hippo	0.04	0.06	0.65	0.52
Impala	0.02	0.01	2.34	0.02
Greater Kudu	0.16	0.12	0.53	0.59
Nyala	0.02	0.01	0.88	0.38
Burchell's Zebra	0.02	0.03	0.89	0.38

Table 2.7. Wound-feeding observations (n=18) of oxpeckers in Kruger National Park. Highlighted observations represent events when more than one oxpecker wound-fed per host animal (RBO = red-billed oxpecker; YBO = yellow-billed oxpecker).

	Date	Host Spp.	Oxpecker Spp.	Presence of Other Oxpeckers	Wound Location
WET	18-Jan	Giraffe 1	unidentifiable spp.	yes; unidentifiable spp.	skin disease
	19-Jan	Giraffe 2	YBO	alone	side
	7-Mar	Giraffe 3	RBO	alone	neck
DRY	4-Apr	Giraffe 4	YBO	YBO	between eyes
	4-Apr	Giraffe 5	YBO	YBO	missing horn
	23-Apr	Giraffe 5	YBO	YBO	missing horn
	23-Apr	Giraffe 5	YBO	YBO	missing horn
	24-Apr	Giraffe 5	YBO	YBO	missing horn
	19-May	Giraffe 5	YBO	YBO	missing horn
	24-May	Hippo 1	RBO	RBO	side
	28-May	Buffalo 1	RBO	RBO	behind horns
	28-May	Buffalo 1	RBO	RBO	behind horns
	30-May	Buffalo 2	YBO	RBO	tail
	30-May	Giraffe 1	YBO	YBO; RBO	skin disease
	30-May	Giraffe 1	YBO	YBO; RBO	skin disease
	30-May	Giraffe 1	YBO	YBO; RBO	skin disease
	31-May	Giraffe 1	YBO	YBO; RBO	skin disease
	14-Jun	Giraffe 6	RBO	alone	tail

Table 2.8. Number of wound-feeding events (WF) and non-wound feeding events (NWF) per host species per season. Two proportion z-test, $p<0.05$.

	Wet Season		**Dry Season**		**Seasonal Variation in WF Behavior**	
	WF	**NWF**	**WF**	**NWF**	**Z**	**p**
Buffalo	0	23	3	38	1.33	0.184
Giraffe	3	126	11	119	2.18	0.029
Hippo	0	2	1	10	0.444	0.657

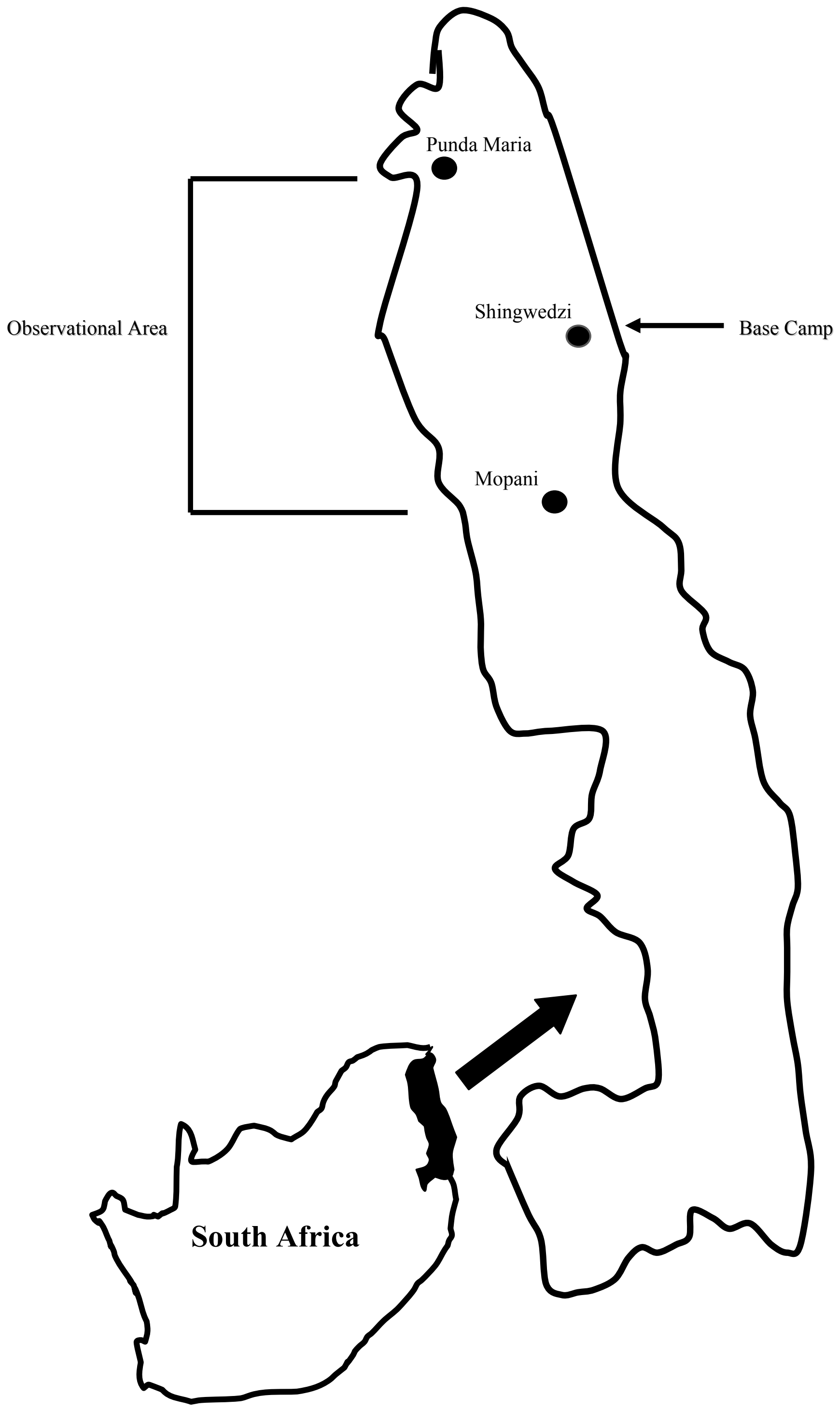
Table 2.9. Percentage of events with >1oxpecker.

Event	**Total**	**Wet**	**Dry**
Observational	64	58	68
Non-Feeding	61	55	64
Total Feeding	67	60	72
Wound-Feeding	78	33	87
Non-Wound Feeding	67	60	72

Table 2.10. Response behaviors of host animals to oxpecker wound-feeding, non-wound feeding and non-feeding events, Chi-square, $p<0.05$.

	Wound-Feeding	**Non Wound-Feeding**	**Non-Feeding**
% Tolerate	82	96	95
% Reject	18	4	5
χ^2	7.118	466.514	302.15
p	0.008	<0.001	<0.001

Fig. 2.1. Study area in Kruger National Park, South Africa.



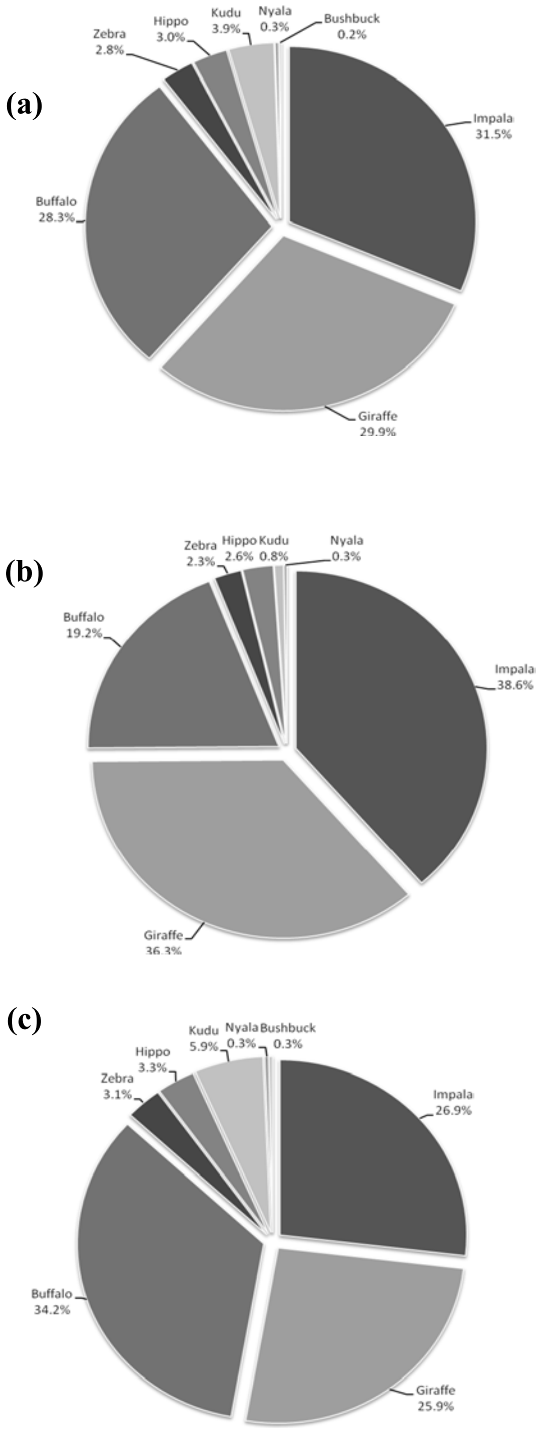


Fig. 2.2. Percentage of total oxpecker utilization (a) of eight host species in Kruger National Park and percentage of total oxpecker utilization of these hosts in the wet (b) and the dry (c) seasons.

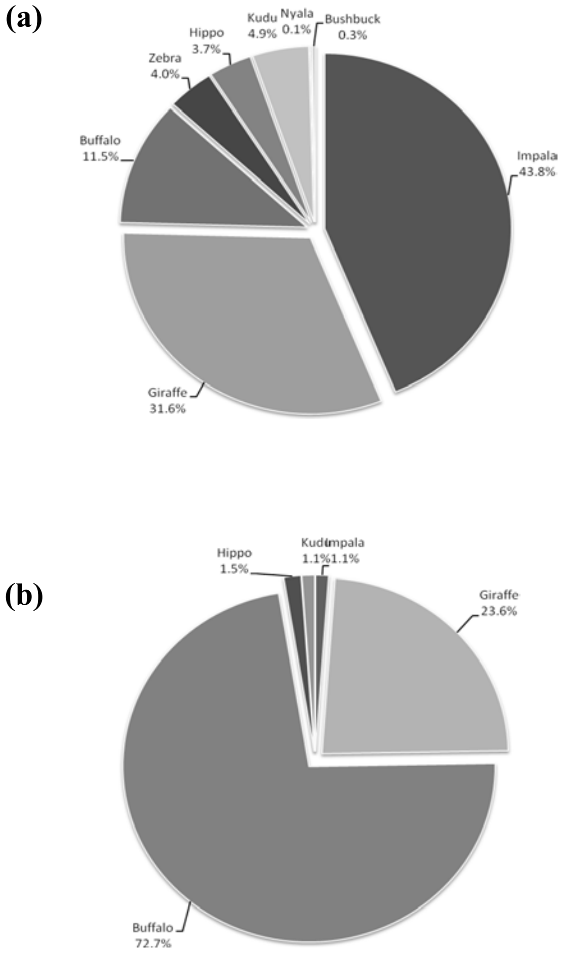


Fig. 2.3. Percentage of red-billed oxpecker observational events per host species (a) and percentage of yellow-billed oxpecker observational events per host species (b).

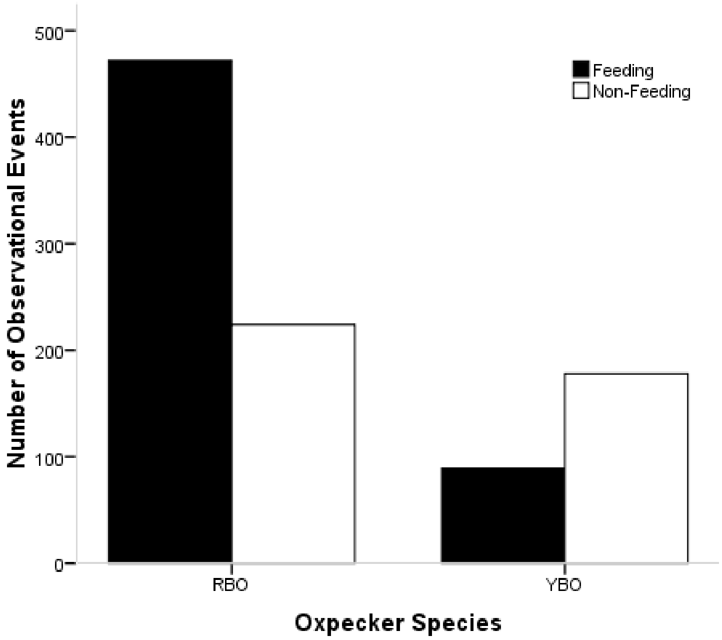


Fig. 2.4. Feeding versus non-feeding events for each oxpecker species (RBO = red-billed oxpecker; YBO = yellow-billed oxpecker).

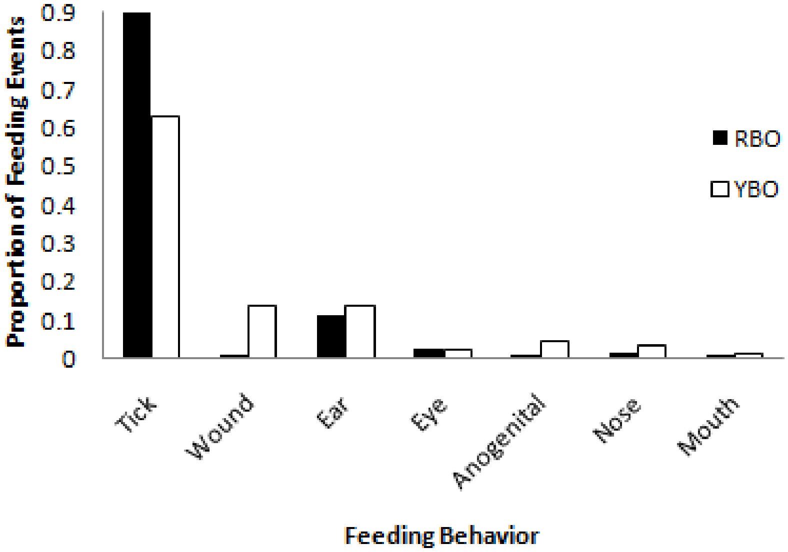


Fig. 2.5. Proportions of the observed feeding behaviors of red-billed oxpeckers (RBO; n=472) and yellow-billed oxpeckers (YBO; n=89).

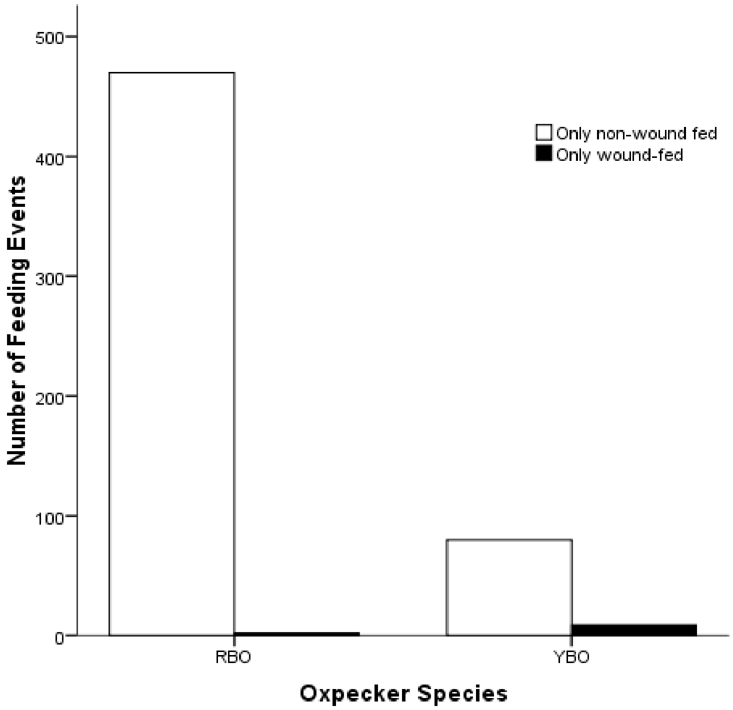


Fig. 2.6. Number of red-billed oxpecker (RBO) and yellow-billed oxpecker (YBO) feeding events during which an oxpecker only non-wound fed (tick, ear, eye, anogenital, nose, mouth) or only wound-fed.

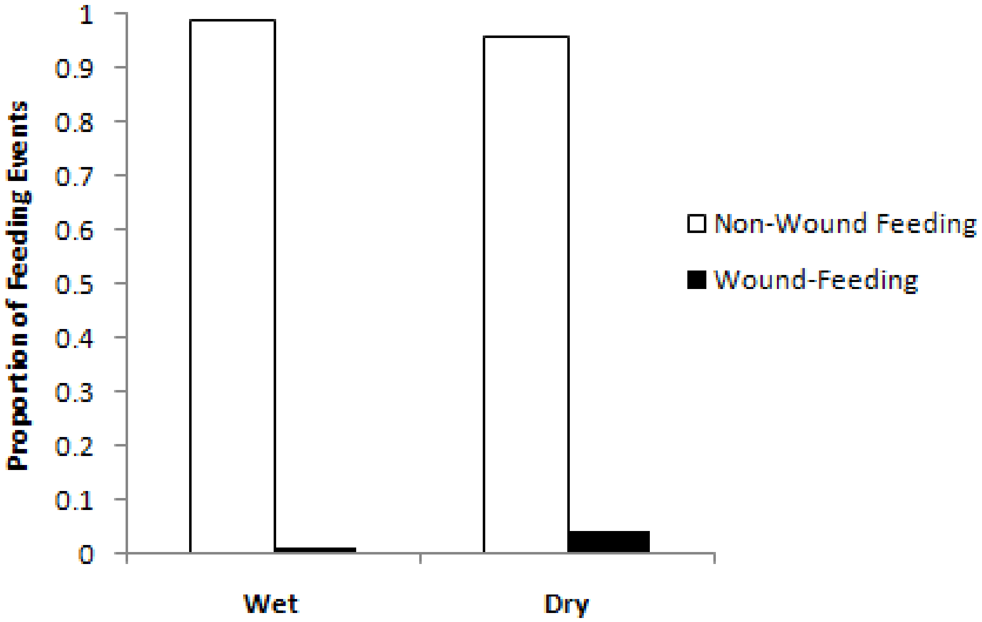


Fig. 2.7. Total oxpecker feeding behavior related to season.

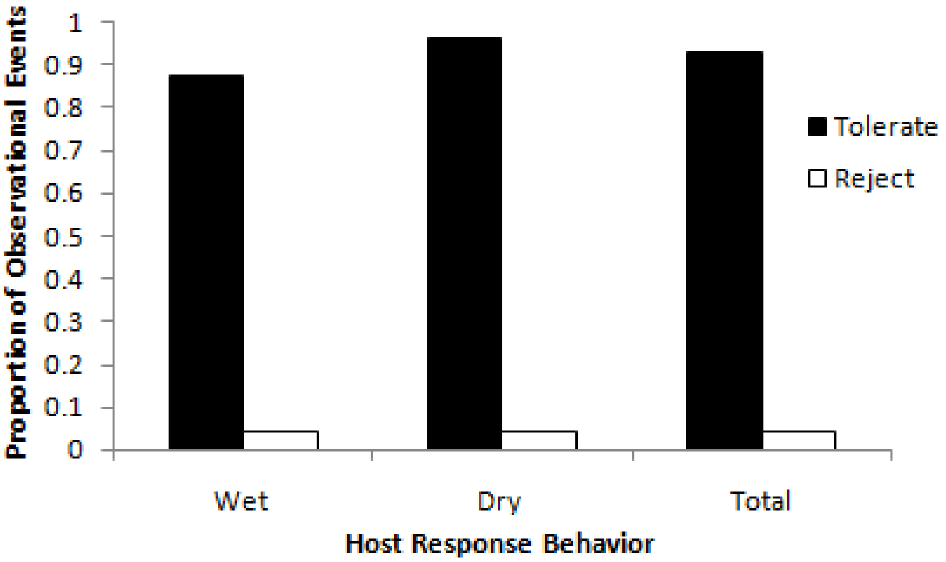


Fig. 2.8. Proportion of host response behaviors to oxpeckers in the wet and the dry season.

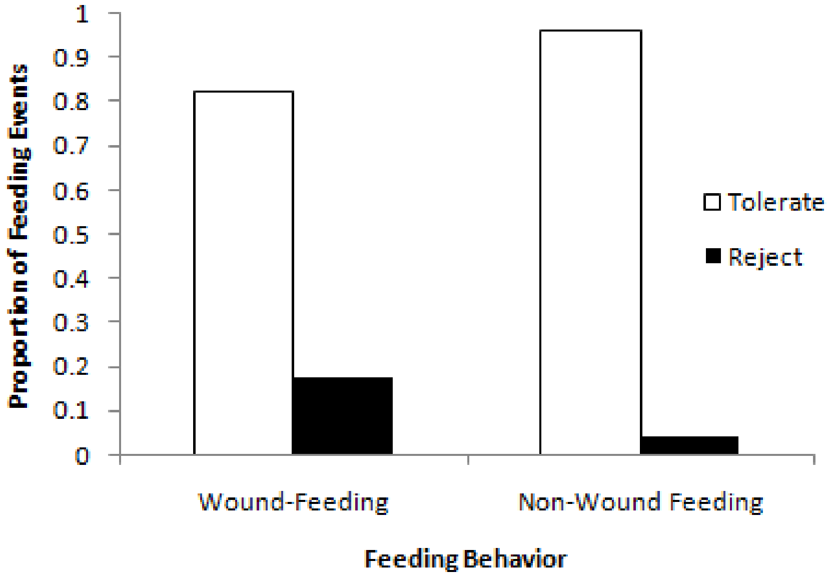


Fig. 2.9. Host response behavior to wound-feeding (n=17) and non-wound feeding (n=578) oxpeckers.

CHAPTER THREE

CHAPTER THREE

Population Dynamics of Free-Living Ixodid Ticks Relative to Oxpecker-Ungulate Presence

Summary

The environment within which an interspecific association occurs can influence the dynamics of that association. I examined the relative importance of spatial and seasonal variability in the oxpecker-ungulate cleaning interaction. In Kruger National Park, South Africa, over 30 species of ixodid ticks parasitize approximately twenty ungulate species. Several of these tick species are fed upon by oxpeckers (*Buphagus spp.*), birds that glean ectoparasites and thereby have been inferred to improve ungulate health. I collected data on the dynamics of free-living ixodid ticks to extrapolate how tick abundance, diversity, and pathogenicity could potentially influence the net outcome of an oxpecker-ungulate interaction in areas where these species coexist. Two sites in Kruger National Park were dragged monthly for ticks from April 2007 through March 2008. Each site (Makhohlola and Shingwedzi) consisted of two plots, an unfenced high mammal use plot (HMU) and a fenced low mammal use plot (LMU). Because the oxpecker diet is dependent on ungulates, oxpeckers were assumed to be present in the HMU areas and absent from the LMU areas. Collected ticks were counted and identified, and adult ticks were then analyzed for pathogens using a Reverse Line Blot hybridization assay. Because South Africa has a bimodal climate based around a wet and a dry season, seasonal differences in tick abundance, tick species composition, and the proportion of pathogenic ticks were also examined by season. Tick abundance and tick species composition varied spatially in Kruger National Park both dependent and independent of

ungulate presence. As predicted, tick abundance and the proportion of ticks belonging to species that oxpeckers consume were higher in ungulate areas compared to non-ungulate areas. Makhohlola in southern Kruger National Park had a higher abundance of the tick species oxpeckers consume compared to Shingwedzi in the north. Approximately 25% of the ticks analyzed were positive for tick-borne diseases. The frequency of pathogenic ticks did not vary spatially or seasonally. Seasonal variation was not found to be a major factor influencing overall tick dynamics. I conclude that areas occupied by ungulates contain large numbers of ticks of the species that oxpeckers consume and carry diseases that can be detrimental to ungulate health. These results suggest that ungulates could benefit from the cleaning services of oxpeckers in areas where ticks, ungulates, and oxpeckers coexist.

Background

Interspecific relationships are commonly observed in nature and their outcomes often exhibit temporal and spatial variability (Bronstein 1994a,b; Axén *et al.* 1996; Bronstein 2001b; Egger and Hibbett 2004; McCreadie *et al.* 2005). Such variation in the outcome of interspecific interactions is referred to as conditionality (Bronstein 1998). In the last two decades, studies on conditionality, particularly conditional mutualism, have come to the forefront of ecological research. Such studies provide evidence that the net outcome of a species interaction in a complex environment can range from positive to negative (Bronstein 2001c). Although mutualisms are not as fully understood as other species interactions, such as competition and predation, they are fundamental to patterns and processes within ecological systems (Bronstein 2001c). By determining how external factors regulate the outcome of interspecific relationships, we can develop a better understanding of community structure and maintenance, and how variation in the biotic and abiotic environment can disrupt the system.

One interaction that may exhibit conditional outcomes is the oxpecker-ungulate interaction. The red-billed oxpecker (*Buphagus erythrorhynchus*) and the yellow-billed oxpecker (*B. africanus*) are two of more than 96 African bird species that glean ectoparasites from mammals (Dean and MacDonald 1981; Appendix A). Through tick consumption, an oxpecker directly reduces host tick load and indirectly prevents tick reproduction and pathogen transmission to the ungulate host animal. As tick abundance and tick species per ungulate vary, so should the costs and benefits of oxpecker presence on ungulates. Oxpeckers can also inflict fitness costs on their ungulate hosts by feeding from the tissue and blood of host wounds.

Oxpecker-ungulate associations may be conditional, dependent on factors such as tick abundance, tick species composition, and tick pathogenicity. Tick abundance and tick pathogenicity may, in turn, be positively associated with ungulate presence, so that the oxpecker-ungulate association shifts towards mutualism where ungulate abundance is high. In addition, if a substantial proportion of ticks carry diseases, this may tip the association towards mutualism, even if oxpeckers occasionally feed in a way that harms the host. I examined the plausibility of such conditionality by investigating the following questions: 1) is tick abundance, tick species composition, and tick pathogenicity associated with ungulate presence? 2) do these factors also vary spatially independent of ungulate presence? and 3) do these factors vary seasonally? I used data collected on the dynamics of free-living ixodid ticks in oxpecker-ungulate areas and non-oxpecker-ungulate areas to extrapolate how tick abundance, diversity, and pathogenicity could potentially influence the net outcome of an oxpecker-ungulate interaction in areas where these species coexist.

Study System

Oxpeckers specialize in gleaning hard ticks of the family Ixodidae from the ungulates of sub-Saharan Africa (see Appendix B). Although remnants of Argasidae (soft) ticks have been identified in oxpecker stomach content analyses (Bezuidenhout and Stutterheim 1980), oxpeckers do not actively consume ticks of this family (see Appendix B). Ixodidae includes 692 species belong to twelve genera. Eighty-three species of ixodid ticks belonging to 10 genera occur in South Africa (Walker 1991; Appendix C) and approximately 30 species of ixodid ticks occur in Kruger National Park (Horak, personal communication). Oxpeckers prefer to feed on the adult ticks of four tick genera

(*Amblyomma*, *Boophilus*, *Hyalomma*, and *Rhipicephalus*), but can significantly reduce larval and nymphal tick loads of these species, as well (Bezuidenhout and Stutterheim 1980). These birds often feed indiscriminately by scissoring their bill through the hair of their hosts (Bezuidenhout and Stutterheim 1980). This method is suitable for consuming immature ticks. However, vision plays a role when oxpeckers search for and pluck adult ticks from the host's hide (Bezuidenhout and Stutterheim 1980). Although ticks are believed to constitute the majority of the oxpecker diet (based on observations and stomach content analyses), oxpeckers are opportunists. They consume the insects, hair, earwax, dung, urine, and nose, mouth and eye secretions of the large herbivores they depend upon (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988). Moreover, oxpeckers exploit the wounds of their hosts, on occasion (van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988; Weeks 1999, 2000; McElligott *et al.* 2004).

The Effects of Tick Removal

The ectoparasite gleaning behavior of oxpeckers is an apparently mutually beneficial service. Tick toxicosis, anemia, appetite suppression, and metabolic disturbances are some of the effects of ticks on wildlife (O'Kelly and Seifert 1969; Seebeck *et al.* 1971; Price 1980; Clayton and Moore 1997 in Oorebeek and Kleindorfer 2008). Secondary bacterial infection of tick-bite marks can also occur (Howell *et al.* 1978). Perhaps the greatest cost of tick parasitization is blood loss. There is much variation in the effect of a single tick, depending on the developmental stage and species of the tick. Engorging adult ticks, in particular, can remove large amounts of blood and drain body reserves (Little 1963; Seebeck *et al.* 1971; Williams *et al.* 1978; Mulilo 1985; Norval *et al.* 1988; Norval *et al.*1989; Hart *et al.* 1990; Hart 1992; Norval *et al.* 1994). Experiments on growing calves have demonstrated that a single engorging adult tick produces a growth decrement between 0.6g and 0.6 kg per calf per year (Little 1963; Seebeck *et al.* 1971; Turner and Short 1972; Williams *et al.* 1978; Sutherst *et al.* 1983; Hart and Hart 1988; Norval *et al.* 1988; Kaiser *et al*. 1991; Scholtz *et al.* 1991; Hart *et al.* 1992). A calf with a moderate tick load can suffer a 10-44 kg reduction in weight gain per year (Norval *et al.* 1988). A similar effect is expected in wild ungulates, but this remains to be tested. Immature hard ticks do not appear to affect weight gain in calves (Norval *et al.* 1988; Norval *et al.* 1989). A single oxpecker can consume up to 13,000 immature ticks or 100 adult female blue ticks, *Boophilus decoloratus*, per day (Samish 2000) and in the process prevent the negative consequences of tick parasitization.

Ticks are vectors of more kinds of microorganisms than any other arthropod taxon, including mosquitoes (Hoogstraal 1985 in Oliver 1989). Ixodid ticks are vectors of bacterial diseases, such as Anaplasma and Ehrlichia, and protozoan diseases such as Theileria and Babeseia. A tick can become infected when it feeds on an infected host animal during the larval stage and then transmit the pathogen to a different host during subsequent nymphal and adult stages. Or, a tick can become infected during the nymphal stage and transmit the pathogen during the adult stage. Some tick pathogens are transmitted to the next generation of ticks via the eggs. Five ixodid tick species that are of concern in South Africa are *A. hebraeum*, *B. decoloratus*, *B. microplus*, *R. appendiculatus*, and *R. evertsi* (Walker 1991). Each of these tick species can transmit pathogens to their hosts and belongs to the oxpecker diet (Stutterheim *et al.* 1988; Table

3.1). *R. appendiculatus,* for example, can transmit east coast fever, *Theileria parva,* which causes 100% mortality in susceptible hosts (Mulio 1985). *Amblyomma hebraeum* transmits heartwater, *Cowdria ruminantium,* which is also fatal (Mulio 1985). Generally, blood parasites do not affect endemic wild animals, but can seriously compromise the health of domesticated species (Olubayo *et al.* 1993). Wild ungulates and/or ticks translocated into non-endemic areas, however, can create problems, and severe losses among susceptible ungulates can occur (Lightfoot and Norval 1981; Fyumagwa *et al.* 2007). Oxpeckers can prevent pathogen transmission to ungulates by consuming ticks before they engorge on the host.

Oxpeckers also play a role in limiting tick reproduction. Engorged female ixodid ticks lay their eggs in a single batch on the ground. There is a positive relationship among tick size, degree of engorgement, and number of eggs produced. Large ticks, such as *Amblyomma* and *Hyalomma* females, can oviposit 15, 000-20, 000 eggs (Oliver 1989). Intermediate sized ticks of *Boophilus* and *Rhipicephalu*s usually produce 3000-6000 eggs, and small tick species such as *Haemaphysalis* may oviposit only 250-1000 eggs (Oliver 1989). Oxpecker tick predation prevents tick reproduction, which indirectly affects ungulate fitness.

The fitness-comprising consequences of ticks can vary with the tick species and tick stage parasitizing individual hosts. For example, male ixodids take small blood meals, feeding intermittently, but do not gorge with blood as female and immature ticks do (Oliver 1989); larger tick species and adult tick stages take larger blood meals relative to smaller tick species and tick stages; and tick species and tick stages vary in their pathogenicity. Oxpeckers consuming pathogenic ticks before they engorge would seem to confer the greatest benefit to the host (Weeks 2000). The effect of an oxpecker consuming only a few larvae may be negligible.

Wound-Feeding Behavior

Oxpeckers have frequently been observed wound-feeding on livestock (e.g. Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Stutterheim *et al.* 1988; Weeks 1999, 2000), while few observations have been made of oxpeckers wound-feeding on wild ungulates (Bezuidenhout and Stutterheim 1980; Weeks 1999, 2000; McElligott *et al.* 2004). The situations surrounding these wound-feeding events have been controversial. Are oxpeckers vampire birds that peck into their hosts to feed on the blood that emerges, or are they opportunists that exploit pre-existing wounds? Do oxpeckers wound-feed only when they are subjected to conditions of tick scarcity? Oxpeckers may increase the frequency of wound-feeding behavior as a result of a lack of tick resources. In this situation, oxpeckers will be more costly to ungulate hosts than oxpeckers gleaning ticks in a tick abundant area. The cost of this behavior to the host is likely to depend on the size of the wound, the degree of blood loss, the size of the host animal and the state of its health, and the occurrence of secondary infection. Wound-feeding likely has a commensal or parasitic effect on host fitness for it results in blood loss and subjects the ungulate to secondary infection. However, the costs of a wound-feeding oxpecker could be balanced by the benefits of tick removal.

Tick Population Dynamics

Tick abundance often varies spatially and seasonally and may be a major factor affecting oxpecker foraging behavior (Mulilo 1985; Kaiser *et al.* 1991). I examined seasonal and spatial variation in tick abundance, tick species composition, and tick pathogenicity at two geographical areas of Kruger National Park, South Africa. South Africa has a bimodal climate based around a wet season and a dry season. Favorable climatic conditions of high rainfall and relative humidity can increase tick numbers (Hart *et al.* 1990; Needham and Teel 1991; Cumming 1999). Several studies on tick population dynamics in South Africa have concluded that tick abundance generally increases in the wet season when temperature, rainfall and relative humidity are most favorable (Mulilo 1985; Mooring 1995; Mooring and Mundy 1996a; Speybroek *et al.* 2004). Therefore, I predicted that overall tick abundance would be higher in the wet season than in the dry season. Because excessive rainfall can actually decrease the abundances of some tick species (Mulilo 1985), tick species composition was hypothesized to vary seasonally. Because of the predicted population dynamics of tick species, tick pathogenicity was also predicted to vary seasonally.

In addition to the seasonal dynamics of tick populations, I examined spatial variation in tick abundance, tick species composition, and the proportion of pathogenic ticks. Each geographical area consisted of a high mammal use plot (HMU) and a low mammal use plot (LMU). The ungulate species utilized by oxpeckers could only access the HMU plots. With the exception of some occasional off-host insect-feeding, the oxpecker diet is almost entirely dependent on ungulates (Moreau 1933; van Someren 1951; Pitman 1956; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim et al. 1988). Therefore, the HMU plots were assumed to be high oxpecker use areas, in contrast to the LMU plots. Because most ixodid tick species require a large herbivore host at some stage in their life cycle (Horak *et al.* 2002; Fyumagwa *et al*. 2007), I predicted that the HMU plots would support higher tick abundances, a higher percentage of pathogenic ticks, as well as a different composition of tick species than the LMU plots. Ungulate biomass has to be high enough for tick populations to become established and maintained (Norval and Lightfoot 1982). But in cases where host density is low, tick populations may still be high if the ungulates present are susceptible hosts (Sutherst *et al.* 1979; Norval and Lightfoot 1982). Tick abundance and tick species composition were hypothesized to vary spatially independent of ungulate presence, as well, due to climatic variation between areas. The degree to which oxpeckers benefit their ungulate partners is dependent on tick removal, which, in turn, is dependent on tick population dynamics. The service provided by oxpeckers to their ungulate partners may be dependent on seasonal (Del-Claro 2004) and spatial variation that can alter tick abundance, tick species composition, and the frequency of pathogenic ticks.

Methods

Study Sites

The first geographical location chosen for tick collection was Makhohlola (25°15’50’’S, 31°54’50’’E) in southern Kruger National Park (Fig. 3.1). Flat plains with basalt (clay) soil characterize the landscape. This area is an open grassy savanna with sparse trees. Typical tree species in the area include marula, *Sclerocarya caffra*, knob-thorn, *Acacia nigrescens*, leadwood, *Combretum imberbe* and sickle-bush, *Dichrostachys cinerea*. Annual rainfall is approximately 620 mm. The second location chosen for tick collection was near the Shingwedzi Research Camp (23°06’30’’S, 31°23’58’’E), approximately 300 km north of Makhohlola (Fig. 3.1). The Shingwedzi landscape is also typified by basalt and is classified as an open to moderately dense tree savanna (Frandsen 2007). Mopane shrubveld characterizes the landscape, with mopane, *Colophospermum mopane*, umbrella-thorn, *Acacia tortilis* and the transvaal mustard tree, *Salvadora angustifolia* the most common tree species. Annual rainfall is approximately 500 mm (Frandsen 2007). Each location included an HMU unfenced plot and an LMU fenced plot, which were sampled monthly for ticks (Fig. 3.2). The ungulate species that oxpeckers associate with could not access the LMU plots, but could access the HMU plots. The dimensions of the Makhohlola HMU and LMU plots were 100 m x 100 m and 200 m x 100 m, respectively. These plots were separated by 35 m. The 100 m x 100 m HMU Shingwedzi plot lies 1 km southeast of Shingwedzi research camp. The LMU plot, located 0.5 km northeast of Shingwedzi research camp, measured 75 m x 50 m.

Sampling Technique

Ixodid ticks have a one-host, two-host or three-host life-cycle, depending on the tick species (Howell *et al.* 1978). Upon hatching, larvae crawl to the top of the vegetation with their legs extended, a behavior called questing. Here they wait for a suitable host to pass to which they then attach. This behavior allows unengorged free-living ticks to be collected by means of the drag method, in which the researcher drags a 1200 mm horizontal wood pole with ten 1000 mm x 100 mm weighted flannel strips attached to it along a vegetation transect (Spickett *et al.* 1991; Horak *et al.* 2006; Fig. 3.3). The drag apparatus is pulled by a rope harness attached to the pole's ends. At the end of each drag, ticks are individually removed with forceps and placed into vials containing 70% ethanol. One constraint of this method is that tick drags are most suitable for collecting immature ticks questing on the vegetation. Unengorged adults and nymphs are only occasionally caught on the drags (Zimmerman and Garris 1985; Spickett *et al.* 1991). As a result, adult ticks present in the tick plots were underrepresented in tick collections. A second constraint is that although the bont-legged tick, *H. truncatum* is one of the three tick species preferred by oxpeckers (Stutterheim *et al.* 1988) and occurs in Kruger National Park, this species, for unknown reasons, is not collected on tick drags (Horak, personal communication). Five transects, each 100 m long, were dragged for ticks in each Makhohlola plot and in the Shingwedzi HMU plot. Because of the smaller plot dimensions of the Shingwedzi LMU plot, ten transects, each 50 m long, were dragged to equalize sampling effort in the two sites. Monthly surveys were treated as independent sample points.

Sampling was conducted between 0730 and 1300 hours, when temperature and relative humidity favor tick collection (Mooring 1995; Mooring *et al.* 1994). Drags were made once per month in the dry season (April to September) and once per month in the wet season (October to March) at each location. With few exceptions, drag sampling was conducted during the second week of each month. No drags were made in November. Temperature (°C) and rainfall (mm) at each collection site were also recorded monthly. Collected ticks were transferred to the Onderstepoort Veterinary Institute where they were counted and identified using a stereoscopic microscope.

Pathogenic Analyses

Following identification, adult ticks were analyzed for pathogens using a Reverse Line Blot hybridization assay (RLB) (Taoufik *et al*. 2004). Ticks transmit a greater variety of blood parasites than any other arthropod vector group (Taoufik *et al*. 2004). These include both tick-borne protozoa and tick-borne bacteria (Taoufik *et al*. 2004). Two common African tick-borne protozoan diseases are theileriosis and babesiosis, and two tick-borne bacterial diseases are anaplasmosis and ehrlichiosis. Because both ticks and mammalian hosts can harbor more than one species of tick-borne pathogens at a time, RLB allows multiple samples to be analyzed against multiple probes to enable simultaneous detection and differentiation of *Theileria*, *Babesia*, *Anaplasma* and *Ehrlichia* species. RLB analysis cannot be conducted on larvae.

Statistical Analyses

Between site and between plot comparisons of number of ticks collected were conducted using Mann-Whitney *U* tests. To examine within-site and within-plot seasonal variation in number of ticks collected. Wilcoxon Signed-Rank tests were performed. Between plot comparisons of seasonal variation in number of ticks collected were conducted using Mann-Whitney *U* tests. Five tick species in Kruger National Park are known to be consumed by oxpeckers. These are the bont tick, *Amblyomma hebraeum*, blue tick, *Boophilus decoloratus*, brown ear-tick, *Rhipicephalus appendiculatus*, red-legged tick, *R. evertsi*, and glossy brown ear-tick *R. simus* (Moreau 1933; van Someren 1951; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1980). These tick species were used in calculations of the proportion of ticks collected in each plot that are consumed by oxpeckers (Oxpeckers may also consume *R. turanicus* and *R. zambeziensis*, but this has not been confirmed. Therefore, these two tick species were not included when calculating proportions of ticks collected that oxpeckers consume). Two-proportion z-tests were then used to compare these proportions between plots and between sites. The proportion of ticks that were pathogenic at each site also were compared using Two-proportion z-tests. Statistical tests were two-tailed, $p<0.05$ (SPSS Statistics Gradpack 17.0).

Results

Tick Species, Abundance and Spatial Dynamics

As predicted, both tick abundance and tick species varied independently (Makhohlola vs. Shingwedzi) and dependently (HMU vs. LMU) of ungulate presence. A total of 2304 ticks from ten species and 129 ticks from six species were collected in the Makhohlola plots and Shingwedzi plots, respectively (Table 3.2; Table 3.3). This difference in the total number of ticks collected at the two Kruger National Park sites was statistically significant (Mann-Whitney U=6, $n_1=n_2=11$, $p<0.001$). Ninety-eight percent of the ticks collected at Makhohlola were larvae, compared to 72% of ticks collected at Shingwedzi (Fig. 3.4). One percent of the ticks collected at each site were nymphae (Fig. 3.4). One percent of the ticks collected at Makhohlola and 27% of ticks collected at Shingwedzi were adults (Fig. 3.4).

As predicted, significantly more ticks were collected in the Makhohlola HMU plot (n=2145) than the Makhohlola LMU plot (n=159) (Mann-Whitney U=11.5, $n_1=n_2=11$, $p<0.001$). At Shingwedzi, tick abundance was also higher in the HMU plot (n=84) compared to the LMU plot (n=45), but this difference was not statistically significant (Mann-Whitney U=38.5, $n_1=n_2=11$, $p=0.13$). The number of ticks collected in the HMU plots of each site also differed significantly (Mann-Whitney U=5.5, $n_1=n_2=11$, $p<0.001$), as did the LMU plots of each site (Mann-Whitney U=29, $n_1=n_2=11$, $p=0.03$), with more ticks collected in the Makhohlola plots.

Makhohlola and Shingwedzi differed in tick composition. The Makhohlola plots had a statistically higher percentage of ticks belonging to the oxpecker diet compared to the Shingwedzi plots (99% vs. 47%; Two-proportion z-test, z=33.08, $p<0.001$). Although *A. hebraeum*, *B. decoloratus*, and *R. appendiculatus* totaled 95% of the ticks collected in the Makhohlola plots (Fig. 3.5a), *B. decoloratus* (43%), *A. marmoreum* (26%), and *R. turanicus* (24%) were the most abundant tick species collected at Shingwedzi (Fig. 3.5b).

The percentage of ticks belonging to the oxpecker diet that were collected in the HMU plots of each study site was statistically greater than the percentage collected in the LMU plots (Makhohlola: Two-proportion z-test, z=5.39, p<0.001; Shingwedzi: Two-proportion z-test, z=6.27, p<0.001; Fig. 3.6). In the Makhohlola HMU plot, 99% of collected ticks were species that oxpeckers are known to consume (45% *A. hebraeum*, 35% *B. decoloratus*, 15% *R. appendiculatus*, 4% *R. evertsi*, and 0.1% *R. simus*). In the Shingwedzi HMU plot, 66% of collected ticks were species that belong to the oxpecker diet (63% *B. decoloratus*, 1% *R. appendiculatus*, 2% *R. simus*). *R. turanicus*, a tick species that may or may not belong to the oxpecker diet, comprised 32% of Shingwedzi HMU ticks. Only 9% of Shingwedzi LMU ticks belonged to the oxpecker diet (4.5% *B. decoloratus* and 4.5% *R. simus*), whereas 97% of Makhohlola LMU ticks were species known to be consumed by oxpeckers (72% *A. hebraeum*, 3% *B. decoloratus*, 15% *R. appendiculatu*s, 1% *R. evertsi*, and 6% *R. simus*). Table 3.4 summarizes the species and numbers of ticks, as well as the tick stages, collected in the HMU and LMU plots of Makhohlola and Shingwedzi.

Pathogenic Ticks

Fifty-eight adult ticks were collected in this study, but only 48 were subjected to RLB analysis due to contamination of the other ten samples. Table 3.5 lists the ticks analyzed from each plot in the wet and the dry season and whether or not they were positive for pathogens. All of the adult ticks analyzed were *Rhipicephalus* spp. (Table

3.5). Oxpeckers are known to consume *R. appendiculatus* and *R. simus,* but only four of the pathogenic ticks were of these two species (Table 3.5). Oxpeckers may also consume the other two *Rhipicephalus* species analyzed, but the proportion of their diet composed of these species is unknown.

The frequency of pathogenic ticks did not vary spatially. Twenty-seven percent of the analyzed ticks were pathogenic: 20% of the Makhohlola ticks ($n = 15$) and 30% of the Shingwedzi ticks ($n=33$). There was no statistical difference between the percentage of pathogenic ticks collected at each of these two locations (Two-proportion z-test, $z=0.74$, $p=0.46$). Furthermore, there was no significant difference in the proportion of pathogenic ticks collected in the HMU vs. LMU plot of each study site. At Makhohlola, 1 out of 4 ticks collected in the HMU was pathogenic, and 2 out of 11 ticks collected in the LMU plot were pathogenic (Two-proportion z-test, $z=0.29$, $p=0.77$). At Shingwedzi, 8 out of 28 ticks collected in the HMU plot were pathogenic, and 2 out of 5 ticks collected in the LMU plot were pathogenic (Two-proportion z-test, $z=0.51$, $p=0.61$).

Seasonal Dynamics of Ticks

Tick Abundance

Makhohlola and Shingwedzi did not show within-site or within-plot seasonal variation in tick abundance (Fig. 3.7). The total number of ticks collected at Makhohlola in the wet season did not differ significantly from the number collected in the dry season (Wilcoxon Signed-Rank, $z=-0.37$, $n_1=5$; $n_2=6$, $p=0.72$). The number of ticks collected within each Makhohlola plot (Makhohlola HMU Wet vs. Makhohlola HMU Dry; Makhohlola LMU Wet vs. LMU Dry) also did not differ statistically between the wet and the dry season (Wilcoxon Signed-Rank, $p<0.05$; Table 3.6). The same pattern was observed in the Shingwedzi plots. The total number of ticks collected at Shingwedzi in the wet season did not differ significantly from the number collected in the dry season (Wilcoxon Signed-Ranked, z=-1.21, n_1=5; n_2=6, p=0.23). The number of ticks collected within each Shingwedzi plot (Shingwedzi HMU Wet vs. Shingwedzi HMU Dry; Shingwedzi LMU Wet vs. LMU Dry) also did not differ statistically between the wet and the dry season (Wilcoxon Signed-Rank, $p<0.05$; Table 3.6). Although the total number of ticks collected at each site did not differ statistically between the wet and the dry season, the number of ticks collected monthly varied greatly (Fig. 3.8).

In comparison with the Shingwedzi HMU plot, significantly more ticks were collected in the Makhohlola HMU plot irrespective of season (WET Mann-Whitney *U*=2, n_1=n_2=5, p=0.03; DRY Mann-Whitney *U*=0.5, n_1=n_2=6, p=0.01). But the Makhohlola LMU plot and Shingwedzi LMU plot did not differ significantly in tick abundance in the wet (Mann-Whitney *U*=6, n_1=n_2=5, p=0.15) or the dry (Mann-Whitney *U*=7.5, n_1=n_2=6, p=0.09) season. The number of ticks collected in the Makhohlola HMU plot was statistically greater than the number collected in the Makhohlola LMU plot in both the wet (Mann- Whitney *U*=3, n_1=n_2=5, p=0.05) and the dry season (Mann-Whitney *U*=3.5, n_1=n_2=6, p=0.02). The number of ticks collected in the Shingwedzi HMU and LMU plot did not differ statistically in the wet (Mann-Whitney *U*=6, n_1=n_2=5, p=0.15) or dry season (Mann-Whitney U=15, n_1=n_2=6, p=0.62), although more ticks were collected in the HMU plot.

Tick Species

There were some differences in the tick species collected at each geographical location in the wet and the dry season (Fig. 3.9a,b). At Makhohlola, 99% of ticks collected in the wet season and in the dry season were species that oxpeckers consume.

In the wet season, the most abundant tick species were: 86% *A. hebraeum*, 7% *B. decolorautus*, and 4% *R. evertsi*. In the dry season, the most abundant tick species were: 54% *B. decoloratus*, 26% *R. appendiculatus*, and 15% *A. hebraeum*. At Shingwedzi, 53% of ticks collected in the wet season, and 17% of ticks collected in the dry season, were species that belong to the oxpecker diet. This difference was statistically significant (Two-proportion z-test, $z=3.25$, $n_1=105$; $n_2=24$, $p=0.001$). The most commonly collected tick species at Shingwedzi in the wet season were: 50% *B. decoloratus*, 31% *A. marmoreum*, and 15% *R. turanicus*. In the dry season, *R. turanicus* (63%), *D. rhinocerinus* (21%), and *B. decoloratus* (13%) were most abundant. Table 3.7 provides the number of ticks collected by species April 2007 through March 2008.

Tick Pathogenicity

There was no seasonal variation in tick pathogenicity. The percentage of Shingwedzi ticks that were pathogenic in the wet season was greater than in the dry season, but this difference was not statistically significant (Two-proportion z-test, $z=0.19$, wet=19; dry=14, $p=0.85$). The same pattern was observed at Makhohlola, where twenty-percent of ticks collected in the wet season, and none of the ticks collected in the dry season, were pathogenic (Two-proportion z-test, $z=0.76$, wet=13, dry=2, $p=0.45$).

Climate Data

The annual temperature (Fig. 3.10) and rainfall (Fig. 3.11) patterns of Makhohlola and Shingwedzi differed over the study period. Shingwedzi had a lower annual average temperature, but a higher annual average rainfall, than Makhohlola (Table 3.8; There were no rainfall data for Makhohlola in May 2007). At Makhohlola, the mean annual rainfall for April 2007-March 2008 (435 mm) was lower than the cumulative mean annual rainfall (620 mm). The mean annual rainfall of Shingwedzi (561 mm) was higher than the cumulative mean annual rainfall (500 mm). For both Makhohlola and Shingwedzi, temperature was higher in the wet season than the dry season, but this was not statistically significant (Wilcoxon Signed-Rank, $z=-0.67$, $n_1=5$; $n_2=6$, $p=0.50$).

Discussion

These results suggest that the oxpecker-ungulate association has the potential to shift towards mutualism in areas where ungulates, oxpeckers, and ticks co-occur. Tick abundance and tick species varied spatially in Kruger National Park. Makhohlola and Shingwedzi differed significantly in tick abundance and diversity, indicating that tick dynamics also vary independently of ungulate presence. Furthermore, the HMU plot at each site had higher tick abundance and than the LMU plots, demonstrating that tick dynamics are also positively associated with ungulate presence. The tick species collected also differed between plots, with a higher percentage of ticks belonging to the oxpecker diet collected in areas where oxpeckers and ungulates interact. Because tick abundance was high, and the tick species collected were those oxpeckers consume, oxpeckers could potentially play a significant role in tick reduction in areas where they coexist with ungulates.

There are a few explanations for why tick populations varied independently of the occurrence of ungulates. Both Makhohlola and Shingwedzi are characterized as open to moderately dense tree savannas. The ungulate species composition in these areas also overlaps, with a few differences (e.g., rhinoceri and wildebeest are more abundant in Makhohlola). However, ungulate density and small mammal density could have varied between sites, thus influencing tick abundance. Tick overdispersion and climatic differences between Makhohlola and Shingwedzi also could be factors responsible for the differences in tick collections at these two sites. First, inherent in tick collections using the drag method is the problem of tick overdispersion (Petney *et al.* 1990). Female ixodid ticks lay up to several thousand eggs in a single clump. When the larvae hatch, there is little horizontal movement, and an aggregation of larvae may or may not be encountered during tick drags (Rechav 1979). For example, Rechav (1979) measured the horizontal movement *A. hebraeum* and *R. appendiculatus* larvae to be only 80 cm from the point of hatching, while *R. evertsi evertsi* moved 120 cm. The vertical movements of these three species were regulated by relative humidity. As humidity increased, the proportion of larvae questing increased (Rechav 1979). Tick overdispersion is implicated when a few drags harbor a high proportion of the ticks sampled. In the present study, larvae aggregations were occasionally encountered during tick drags. To deal with this problem of tick overdispersion, Petney *et al*. (1990) suggest replicating data for seasonal abundance between years. Seasonal replication was not possible for this particular study.

In addition to tick overdispersion, climatic factors could be responsible for the discrepancy in tick collections between Makhohlola and Shingwedzi. Tick population dynamics are often dependent on local temperature, rainfall and relative humidity. The observed differences in temperature and rainfall at Makhohlola and Shingwedzi could be factors influencing the differences in tick species and tick abundance between these two sites. In general, tick populations increase in the wet season and decrease in the dry season (Mulilo 1985; Mooring 1995; Mooring and Mundy 1996a; Speybroek *et al.* 2004). I did not find evidence of seasonal variation in tick abundance at either site. However, at Shingwedzi, a greater proportion of the ticks collected in the wet season belonged to species that oxpeckers consume than in the dry season. In a study of tick diversity, abundance and seasonal dynamics in Zambia, the majority of tick species had peaks of abundance during the wet season (Mulilo 1985). In another study, *B. decoloratus* larvae and *A. hebraeum* showed no evidence of seasonality, but *R. appendiculatus* exhibited pronounced seasonal periodicity (Horak *et al.* 2006). Mooring *et al.* (1994) examined the effect of rainfall on ticks in Zimbabwe. Larvae of *B. decoloratus* were more abundant during drier periods, the abundance of *R. appendiculatus* adults and nymphs was positively correlated with rainfall, and *R. evertsi* was not influenced by climatic conditions. Although I did not find seasonal variation in tick abundance, it still may be relevant in other areas where the oxpecker-ungulate association occurs. Furthermore, relative humidity could be a factor for the observed differences between sites, but this was not specifically examined.

Shingwedzi served as the research base of a six-month observational study of oxpeckers and ungulates in northern Kruger National Park (Chapter Two). The Shingwedzi HMU tick plot was part of the observational study area. In the observational study, tick-feeding accounted for 84% of oxpecker feeding observations. Wound-feeding behavior was only 3% of feeding observations, but significantly increase in the dry season. Increased wound-feeding was hypothesized to be the result of a seasonal decrease in tick abundance, particularly the preferred tick species of oxpeckers. In the present study, however, I did not observe seasonal fluctuations in tick abundance at Shingwedzi. However, during the wet season I did collect a greater percentage of ticks belonging to the oxpecker diet. Therefore, fluctuations in tick species composition, rather than overall tick abundance, could explain the increase in wound-feeding behavior of oxpeckers previously observed at Shingwedzi.

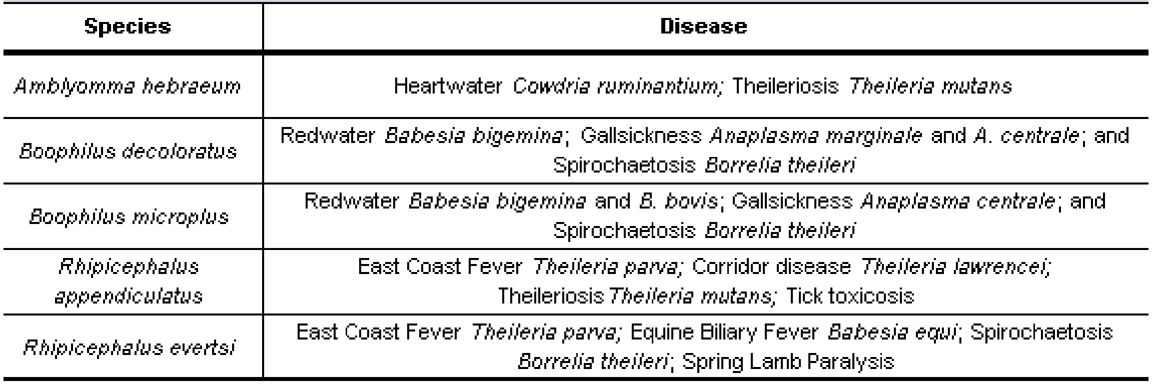
One of the major assumptions of the oxpecker-ungulate interaction is that oxpeckers benefit their hosts by preventing disease transmission. However, this had never been specifically examined. Tick species and tick stages vary in pathogenicity. Adult ticks, in particular, are common vectors of disease, but pathogenicity is also related to tick species. Whether oxpeckers consume all *Rhipicephalus* species remains to be tested. However, there is no reason that oxpeckers would not consume ticks of this species, for *Rhipicephalus* spp. are morphologically similar. Therefore, oxpeckers in the study sites sampled could potentially consume all the pathogenic ticks and prevent them from transmitting disease to their ungulate hosts. A major constraint of this project was that collected larvae could not be subjected to RLB analysis, despite the fact that this tick stage composed 96% of collected ticks. Also, a sample size of 48 adult ticks, all of which were *Rhipicephalus spp.*, was not enough to draw strong conclusions on the frequency of pathogenic ticks in Kruger National Park. Although endemic wild animals can be affected by blood parasites (e.g., *Theileria spp., Babesia spp., Anaplasma spp.,* and *Cowdria ruminantium*), pathogenic ticks are most troublesome to livestock (Olubayo *et al.* 1993). Because oxpeckers also glean ticks from domesticated ungulate species, further investigation into the potential role of oxpeckers in preventing pathogen transmission to ungulates would require the removal of unengorged adult ticks from both wild and domesticated ungulate species foraging in high oxpecker use areas, and their subsequent analysis for pathogens. If a high percentage of the adult ticks that oxpeckers consume were positive for tick-borne diseases, then this would provide strong support for the hypothesis that oxpeckers prevent pathogen transmission.

The results of this study demonstrate that ticks oxpeckers consume are abundant in areas where oxpeckers and the ungulates they depend on coexist. However, the point must be made that tick infestations of individual hosts are highly variable despite a large abundance of ticks in an ungulate-occupied area. Various factors such as grooming rate, ungulate health, body size, habitat preference, and tick questing height influence ungulate tick load (Olubayo *et al.* 1993; Gallivan and Horak 1997). Hosts in poor condition and under nutritional stress are more susceptible to tick infestations (Norval and Lightfoot 1982; Horak *et al.* 1983; Gallivan *et al.* 1995; Horak, *et al.* 1988). However, tick density can be high on seemingly healthy animals (Horak *et al.* 1983). The body size principle states that the intensity of tick infestation on ungulates is proportional to the surface area of the host (Gallivan and Horak 1997). But not all species appear to follow this principle. Two notable exceptions are warthogs, *Phacochoerus aethiopicus*, which can carry heavy loads of adult ticks, and the blue wildebeest, *Connochaetes taurinus*, which is known to host very few ticks, if any (Horak *et al.* 1987). Moreover, larger ungulate species generally are more important hosts of adult ticks while smaller species are more important hosts for the immature stages (Gallivan and Horak 1997). Ixodid ticks quest on grasses and wait for a suitable host to pass onto which they then attach. Short, open grasslands are unsuitable habitat for the free-living stages of many ticks because short grasses limit the questing height of ticks (Londt and Whitehead 1972; Short *et al.* 1989a, b). The height at which ticks quest will influence what species they parasitize. For example, *R. appendiculatus* larvae, nymphs, and adults quest at approximately 26 cm, 31 cm, and 70 cm, respectively (Short *et al.* 1989a). Larvae would encounter the body of small ungulates, but only the legs of large ungulates. Gallivan and Horak (1997) found grazers to support fewer ticks than browsers and intermediate feeders. Moreover, there may be an upper limit to ungulate tick load. Repeated feeding of ticks on hosts can induce an immune response which limits the feeding success and survival of ticks (Tatchell 1987).

Conclusion

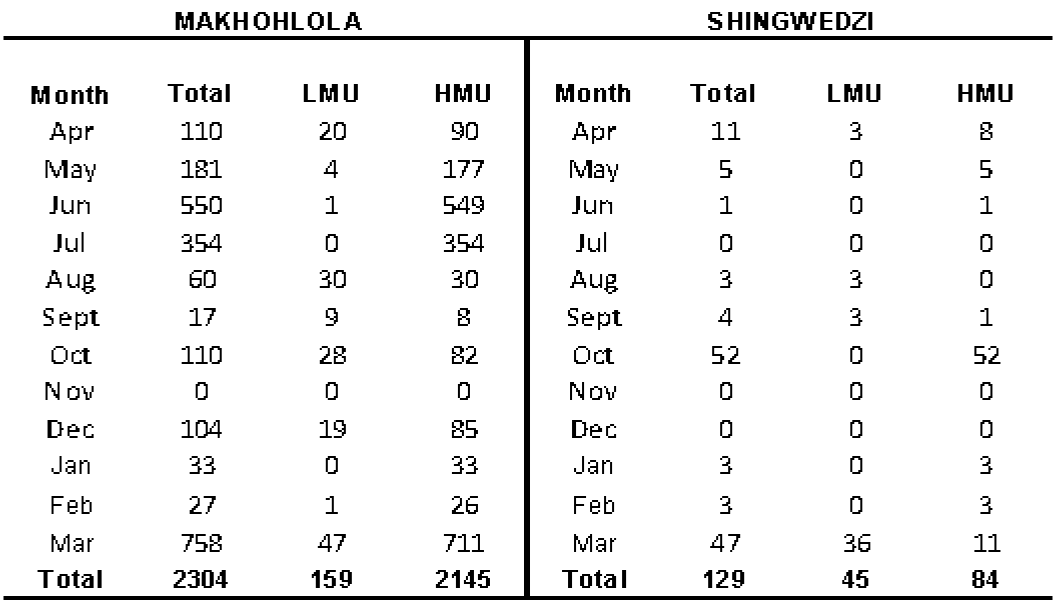
Areas occupied by ungulates contain large numbers of ticks of the species that oxpeckers consume, and a substantial proportion of these ticks carry diseases that can potentially harm ungulates. These results suggest that ungulates could benefit from the tick removal services of oxpeckers in areas where they co-occur. Oxpecker presence is dependent on ungulate density, which in turn influences tick abundance. However, how tick abundance and tick species per host influences oxpecker feeding behavior cannot be measured in the field. However, these factors can be experimentally manipulated and their effects on oxpecker feeding behavior monitored (Chapter 4).

Table 3.1. Ixodid tick species that oxpeckers consume and the wildlife/livestock diseases they transmit.



Species	**Disease**
Amblyomma hebraeum	Heartwater *Cowdria ruminantium;* Theileriosis *Theileria mutans*
Boophilus decoloratus	Redwater *Babesia bigemina*; Gallsickness *Anaplasma marginale* and *A. centrale*; and Spirochaetosis *Borrelia theileri*
Boophilus microplus	Redwater *Babesia bigemina* and *B. bovis*; Gallsickness *Anaplasma centrale*; and Spirochaetosis *Borrelia theileri*
Rhipicephalus appendiculatus	East Coast Fever *Theileria parva;* Corridor disease *Theileria lawrencei;* Theileriosis *Theileria mutans;* Tick toxicosis
Rhipicephalus evertsi	East Coast Fever *Theileria parva;* Equine Biliary Fever *Babesia equi*; Spirochaetosis *Borrelia theileri*; Spring Lamb Paralysis

Table 3.2. Number of ticks collected monthly from April 2007-March 2008 in the low mammal use (LMU) and high mammal use (HMU) plots of Makhohlola and Shingwedzi, Kruger National Park.



MAKHOHLOLA				**SHINGWEDZI**			
Month	**Total**	**LMU**	**HMU**	**Month**	**Total**	**LMU**	**HMU**
Apr	110	20	90	Apr	11	3	8
May	181	4	177	May	5	0	5
Jun	550	1	549	Jun	1	0	1
Jul	354	0	354	Jul	0	0	0
Aug	60	30	30	Aug	3	3	0
Sept	17	9	8	Sept	4	3	1
Oct	110	28	82	Oct	52	0	52
Nov	0	0	0	Nov	0	0	0
Dec	104	19	85	Dec	0	0	0
Jan	33	0	33	Jan	3	0	3
Feb	27	1	26	Feb	3	0	3
Mar	758	47	711	Mar	47	36	11
Total	**2304**	**159**	**2145**	**Total**	**129**	**45**	**84**

Table 3.3. Species of tick and tick stages collected in the Makhohlola and Shingwedzi plots, Kruger National Park. (L=larvae; N=nymphae)

Makhohlola	**Shingwedzi**
A. hebraeum L, N	*A. hebraeum* 0
R. appendiculatus L, N, ♂, ♀	*R. appendiculatus* L
R. simus ♂, ♀	*R. simus* ♂, ♀
R. evertsi L, N	*R. evertsi* 0
R. turanicus ♂, ♀	*R. turanicus* ♂, ♀
R. zambeziensis ♀	*R. zambeziensis* 0
B. decoloratus L	*B. decoloratus* L, N
H. unknown sp. L	*H. unknown sp.* L
D. rhinocerinus L	*D. rhinocerinus* 0
A. marmoreum L	*A. marmoreum* L

Table 3.4. Number, species, and life stage of ticks collected in the low mammal use (LMU) and high mammal use (HMU) plots of Makhohlola and Shingwedzi, Kruger National Park. (L=larvae; N=nymphae)

Tick Species	**Makhohlola**				**Shingwedzi**				
LMU	L	N	♂	♀	L	N	♂	♀	Total
Amlyomma hebraeum	114								114
Rhipicephalus appendiculatus		21	1	2					24
Rhipicephalus simus			3	7				2	12
Rhipicephalus evertsi	2								2
Rhipicephalus turanicus			1				2	2	5
Rhipicephalus zambeziensis				1					1
Boophilus decoloratus	4				2				6
Haemaphysalis unk. sp.	2				4				6
Dermacentor rhinocerinus	1								1
Amblyomma marmoreum					33				33
LMU Total	123	21	5	10	39	0	2	4	204
HMU	L	N	♂	♀	L	N	♂	♀	Total
Amlyomma hebraeum	955	4							959
Rhipicephalus appendiculatus	313	8	1	2	1				325
Rhipicephalus simus				2			1	1	4
Rhipicephalus evertsi	98	1							99
Rhipicephalus turanicus				3			16	11	30
Rhipicephalus zambeziensis									0
Boophilus decoloratus	756				52	1			809
Haemaphysalis unk. sp.					1				1
Dermacentor rhinocerinus	1								1
Amblyomma marmoreum	1								1
HMU Total	2124	13	1	7	54	1	17	12	2229
Total Ticks Collected	2247	34	6	17	93	1	19	16	2433

Table 3.5. The number, species and sex of adult ticks subjected to the RLB analysis. The number is parentheses designates how many ticks of that species and sex were positive (***) for pathogens.

	Makhohlola LMU	**Makhohlola HMU**	**Shingwedzi LMU**	**Shingwedzi HMU**
Wet	1 ♂ *R. appendiculatus* 2 ♀ *R. appendiculatus* (1)*** 2 ♂ *R. simus* 4♀ *R. simus* (1)***	1 ♂ *R. appendiculatus* (1) *** 2 ♀ *R. simus* 1 ♀ *R. turanicus*	2 ♀ *R. simus* 1 ♀ *R. turanicus* (1)***	1 ♂ *R. simus* (1)*** 1 ♀ *R. simus* 5 ♂ *R. turanicus* (2)*** 9 ♀ *R. turanicus* (2)***
	Makhohlola LMU	**Makhohlola HMU**	**Shingwedzi LMU**	**Shingwedzi HMU**
DRY	1 ♀ *R. zambeziensis* 1 ♀ *R. simus*	None	1 ♂ *R. turanicus* (1) *** 1♀ *R. turanicus*	8 ♂ *R. turanicus* (2)*** 4 ♀ *R. turanicus* (1)***

Table 3.6. Comparison of the number of ticks collected seasonally within the Makhohlola plots and Shingwedzi plots, Kruger National Park (Wilcoxon Signed-Rank test, $p<0.05$).

		z	p
Makhohlola	HMU Wet vs. HMU Dry	0.675	0.500
	LMU Wet vs. LMU Dry	1.625	0.104
Shingwedzi	HMU Wet vs. HMU Dry	1.214	0.225
	LMU Wet vs. LMU Dry	0.447	0.655

Table 3.7. Number of ticks collected by species per month April 2007-March 2008, Kruger National Park.

	TickSpecies										
Month	*A. heb.*	*B. dec.*	*R. append.*	*R. evertsi*	*R. simus*	*R. turan.*	*R. zamb.*	*H. unk sp.*	*D. rhin.*	*A.marm.*	Total
Apr	93	10	1	0	2	14	0	1	0	0	121
May	52	3	125	0	1	4	1	0	0	0	186
Jun	11	359	158	22	1	0	0	0	0	0	551
July	1	286	29	37	0	0	0	1	0	0	354
Aug	29	28	3	1	0	0	0	0	2	0	63
Sept	3	1	13	1	0	0	0	0	3	0	21
Oct	65	87	6	2	0	0	0	0	2	0	162
Dec	91	5	7	1	0	0	0	0	0	0	104
Jan	0	29	0	4	1	2	0	0	0	0	36
Feb	1	7	1	18	0	3	0	0	0	0	30
Mar	727	0	6	15	11	12	0	0	0	34	805

Table 3.8. Temperature (°C) and rainfall (mm) data of Shingwedzi and Makhohlola, Kruger National Park April 2007-March 2008.

	Temperature (°C)		**Rainfall (mm)**		
	Range	Mean	Range	Mean	Annual
Makhohlola	21-37	30	0-89	40	435
Shingwedzi	17-32	26	0-193	47	561

Fig. 3.1. Map of study sites in Kruger National Park, South Africa.

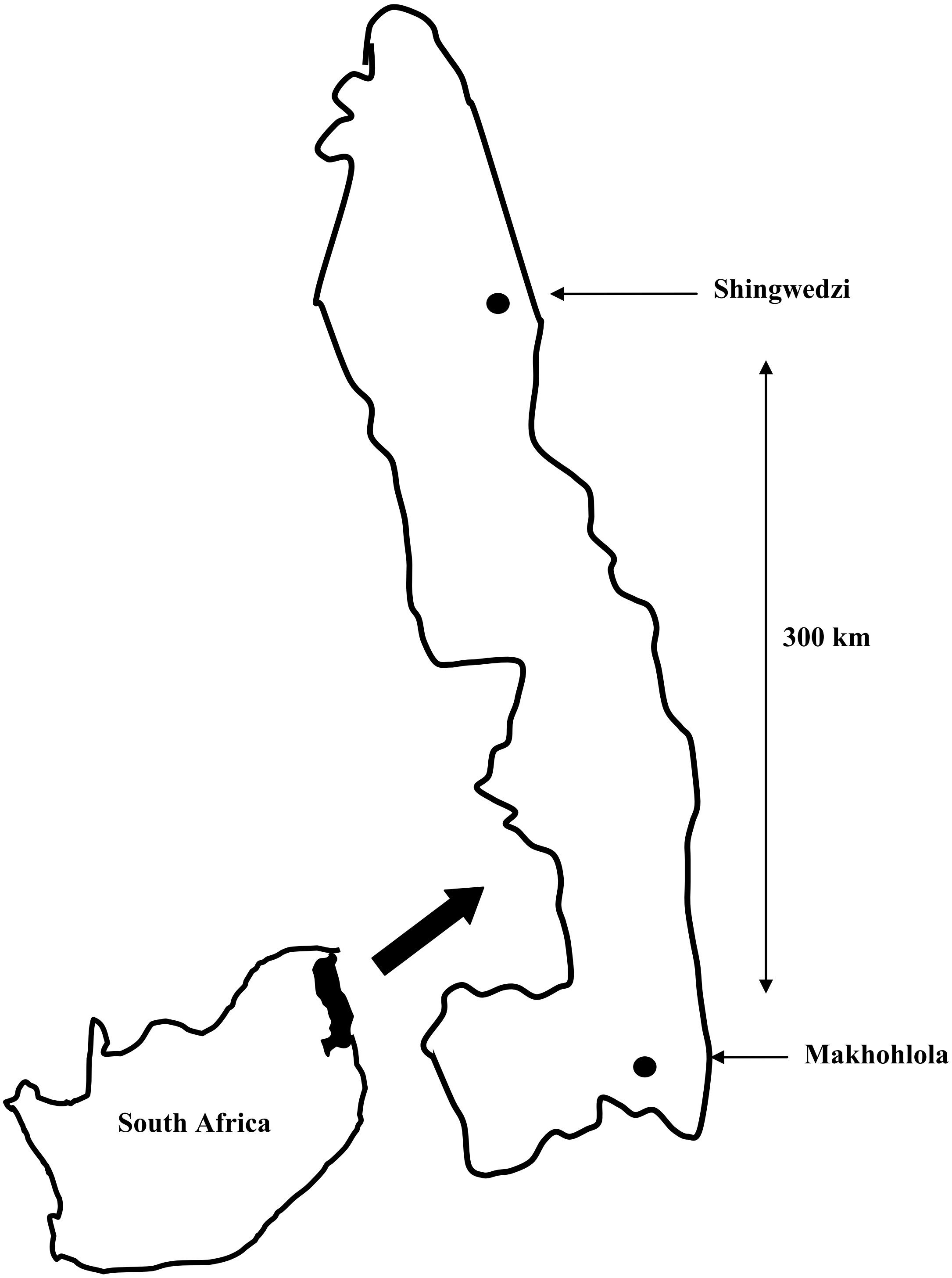


Fig. 3.2. Makhohlola HMU plot (a) Makhohlola LMU plot (b) Shingwedzi HMU plot (c) and Shingwedzi LMU plot (d).

Fig. 3.3. Tick drag apparatus.

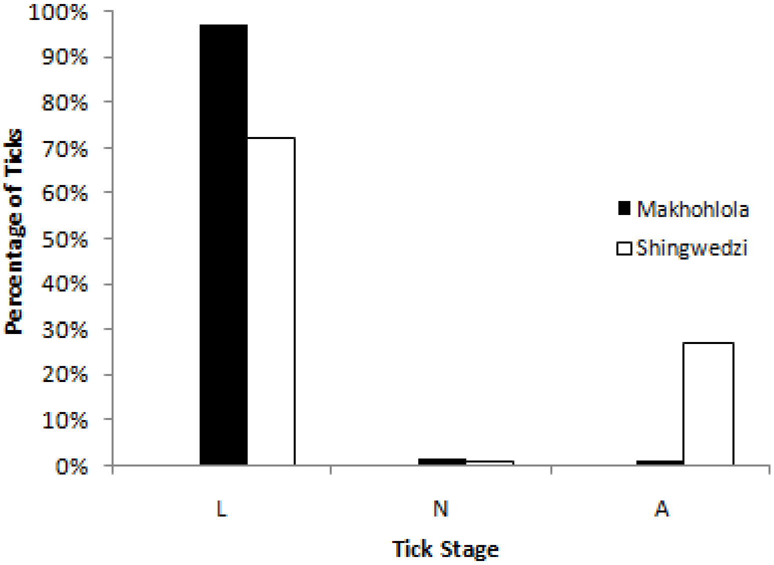


Fig. 3.4. Percentage of larval (L), nymphal (N) and adult (A) ticks collected in Makhohlola and Shingwedzi.

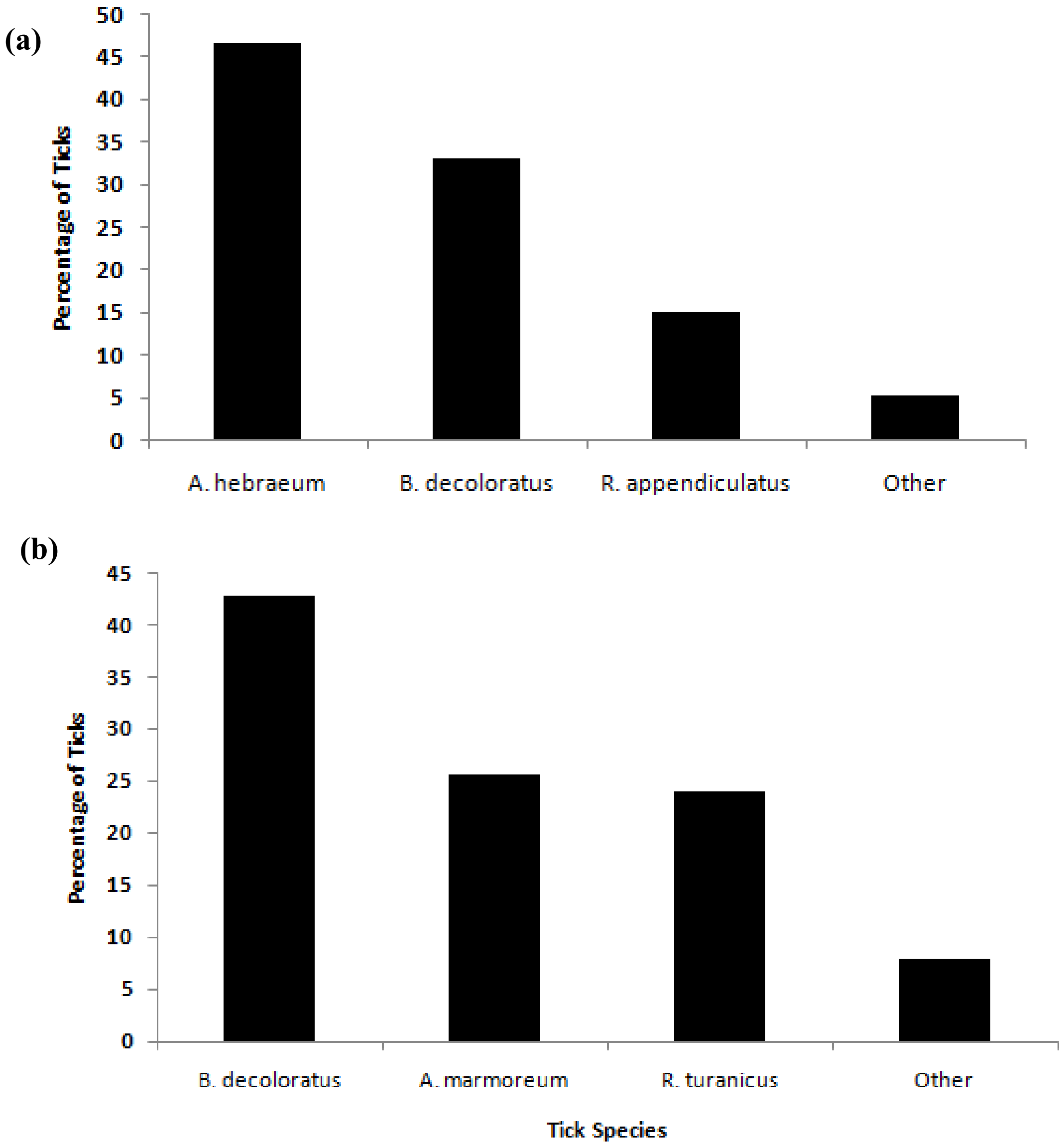


Fig. 3.5. Percentage of ticks of each species collected in Makhohlola (a) and Shingwedzi (b), KNP.

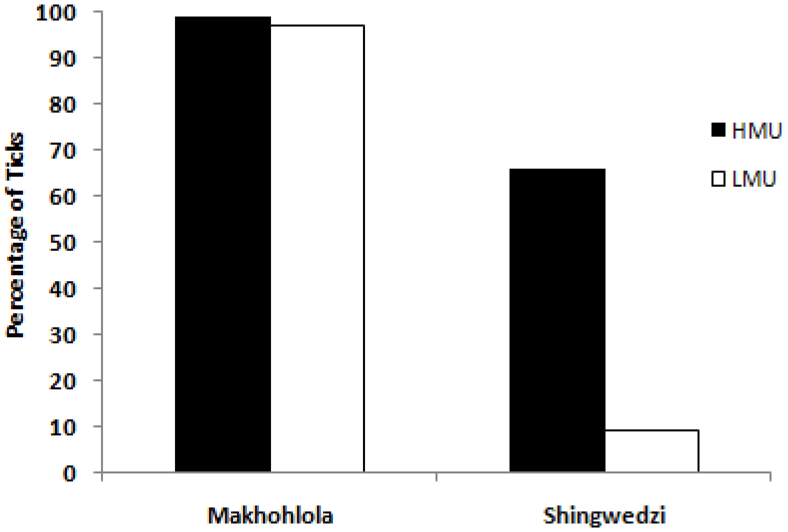


Fig. 3.6. Percentage of ticks belonging to the oxpecker diet that were collected in the high mammal use plot (HMU) and low mammal use plot (LMU) of Makhohlola and Shingwedzi.

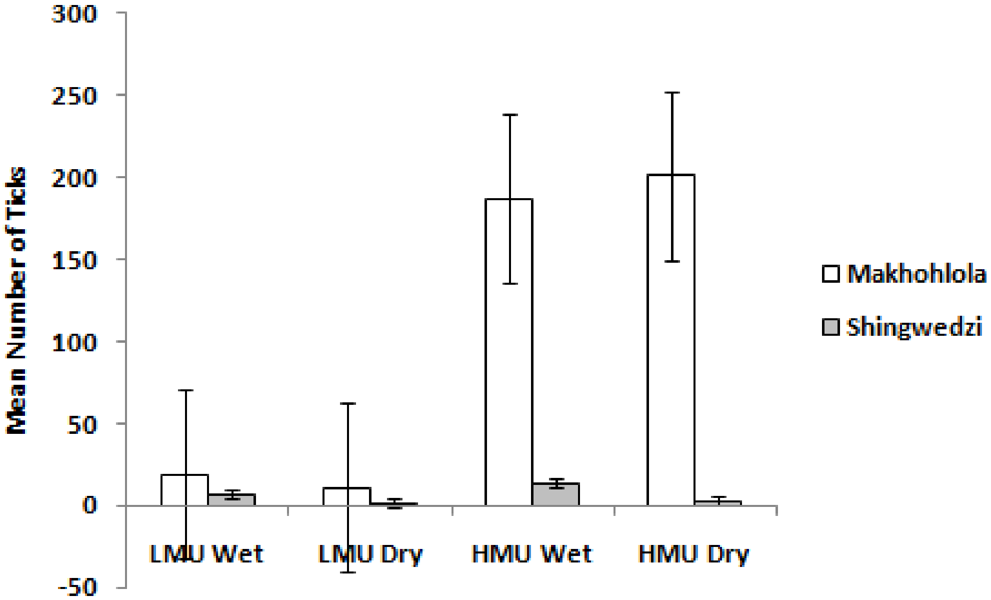


Fig. 3.7. Mean number of ticks (±SE) collected in low mammal use (LMU) and high mammal use (HMU) plots of each site (Makhohlola and Shingwedzi) in the wet and the dry season.

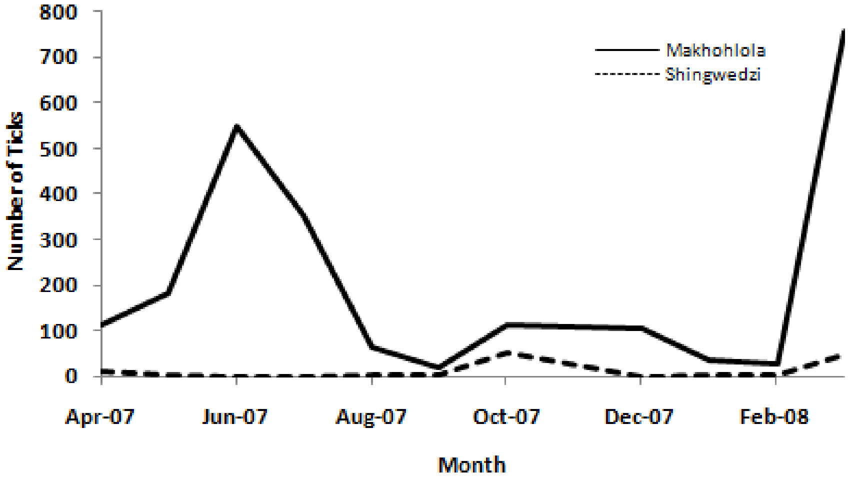


Fig. 3.8. Number of ticks collected monthly in Makhohlola and Shingwedzi April 2007-March 2008, KNP. Dry season April-September; Wet season October-March.

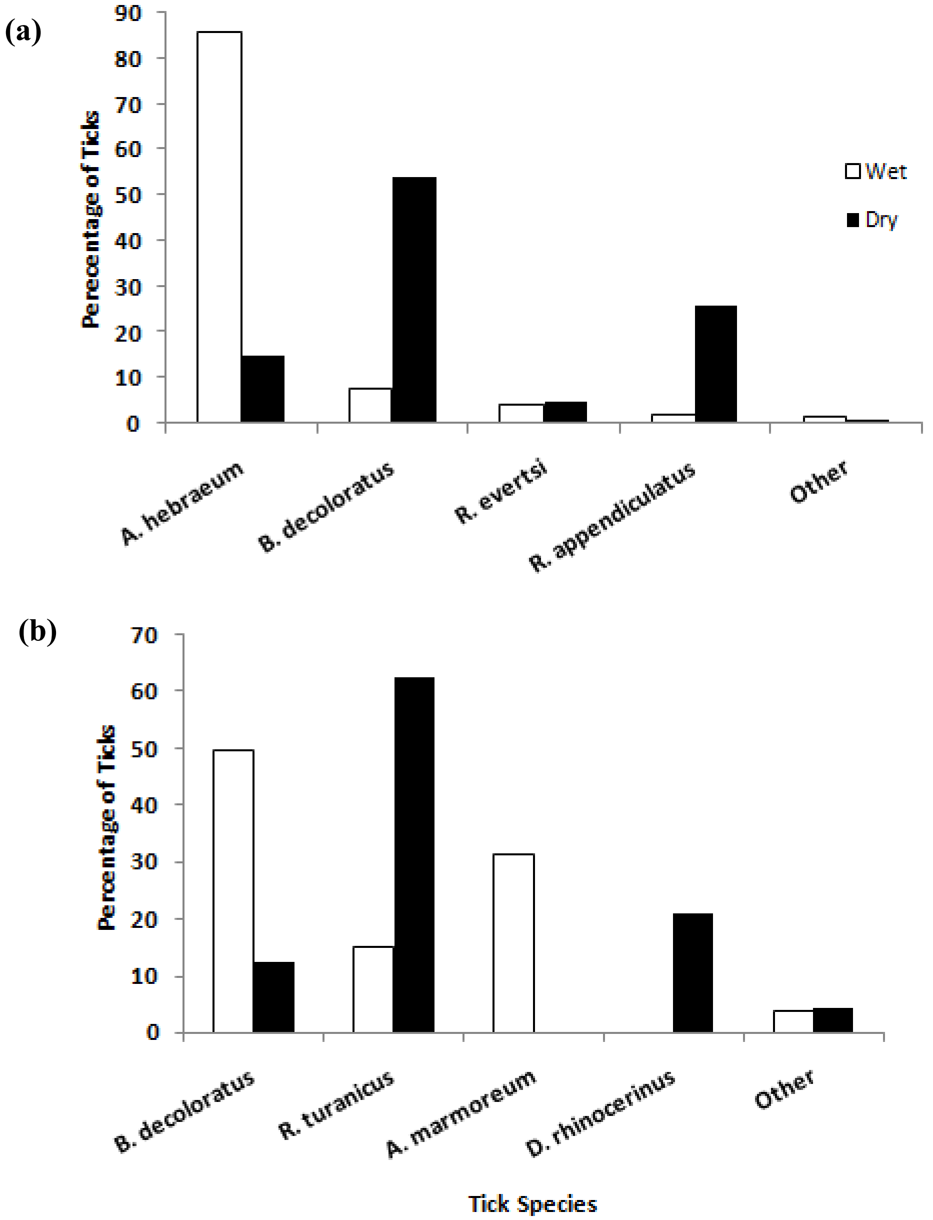


Fig. 3.9. Percentage of ticks of each species collected at Makhohlola in the wet and the dry season (a) and at Shingwedzi in the wet and the dry season (b).

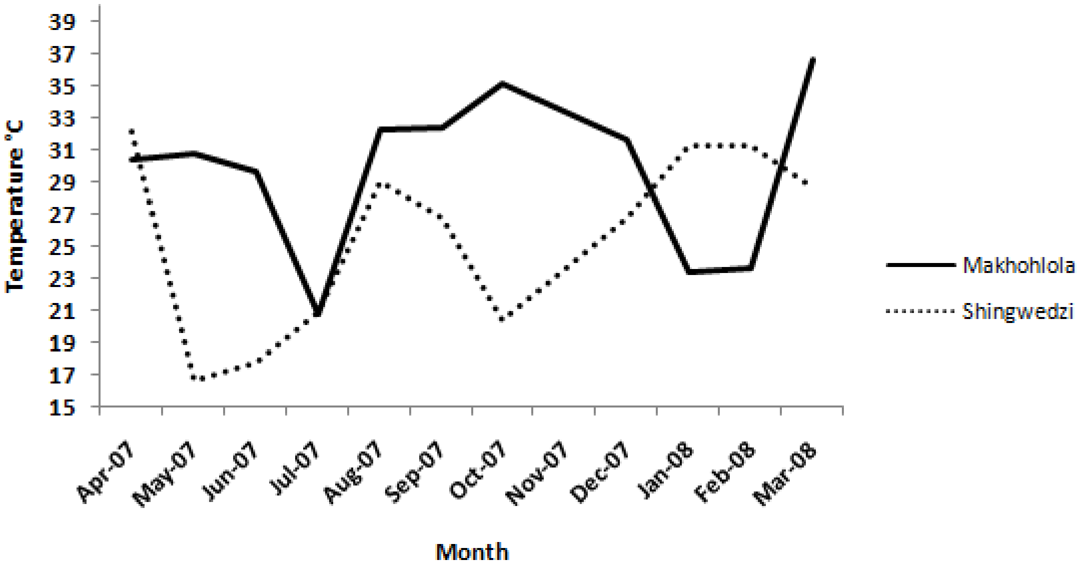


Fig. 3.10. Temperature of Makhohlola and Shingwedzi, Kruger National Park, April 2007 through March 2008.

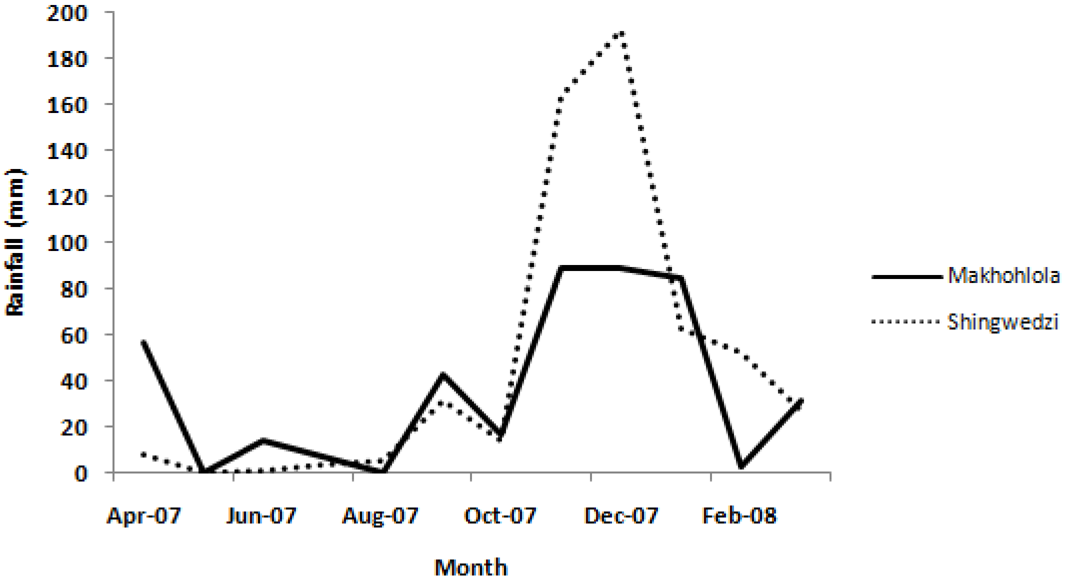


Fig. 3.11. Rainfall of Makhohlola and Shingwedzi, Kruger National Park, April 2007 through March 2008.

CHAPTER FOUR

CHAPTER FOUR

Feeding Preferences of the Red-Billed Oxpecker (*Buphagus erythrorhynchus*): A Bird That Pecked Its Way to Parasitism

Summary

Interspecific relationships are sometimes simple with a single predictable outcome, but also can be complicated associations with multiple dynamic outcomes. The oxpecker-ungulate association of sub-Saharan Africa is an example of a complicated interspecific association subject to variation in outcome. Oxpeckers (*Buphagus* spp.) are unusual birds because they not only glean ticks from an array of African ungulates, but are one of the few avian species known to wound-feed from their living hosts. The conditions under which oxpeckers wound-feed, and hence the mechanisms generating the variation in oxpecker-ungulate association, are unclear. To assess whether the relationship of oxpeckers to their hosts fluctuates from mutualism to antagonism dependent upon tick species and/or tick abundance, a series of feeding preference experiments were conducted on captive red-billed oxpeckers (*B. erythrorhynchus*). In multi-choice cafeteria-style experiments, oxpeckers fed on both ticks and liquid blood. In experiments with donkeys, oxpeckers spent more time wound-feeding when a less preferred tick species was available and when tick abundance was low compared to when a preferred tick species was available and when tick abundance was high. However, oxpeckers still wound-fed even when they were given a large number of the ticks they prefer. The results of these experiments indicate that although the amount of time oxpeckers wound-feed is conditional on tick species and tick abundance, the occurrence

of wound-feeding is not conditional. Oxpecker-feeding behavior is more complex than previously reported.

Background

Mutualisms can be defined as mutually beneficial interspecific interactions in which each species receives a net benefit from the interaction (Bronstein 1994a,b, 1998, 2001; Holland and DeAngelis 2001). Despite a net increase in the reproduction and/or survival of each partner, these reciprocally beneficial interactions also involve costs (Bronstein 1998). The outcome of an interspecific relationship can be strongly conditioned on the ecological setting in which it occurs (Cushman and Whitham 1989; Bronstein 1994a; Del-Claro and Oliveira 2000; Egger and Hibbet 2004) and thus can range from strong to weak mutualism, to commensalism and even to antagonism (Bronstein 1998). Spatial or temporal variation in the magnitude of either the costs or benefits of the interaction will influence the net effect for each partner (Bronstein 1998). To understand how interspecific interactions vary in space and time and move along the continuum, it is necessary to identify and quantify the costs and benefits to each partner species, and to identify the mechanisms generating variation in those costs and benefits (Billick and Tonkel 2003; Herre *et al.* 1999).

The oxpecker-ungulate association of sub-Saharan Africa is one example of a complicated interspecific association subject to temporal and spatial variation. Red-billed oxpeckers (*Buphagus erythrorhynchus*) and yellow-billed oxpeckers (*B. africanus*) are passerines whose diet is almost entirely dependent on African ungulates. Oxpeckers consume the earwax, dung, urine, lice, mites, insects, scurf cells, hair, and nose, eye and mouth secretions of their hosts (Moreau 1933; Attwell 1966; Weeks 1999; personal observation). But ticks are believed to constitute the majority of the oxpecker diet, based on feeding observations and stomach content analyses of oxpeckers (Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980;

Mundy 1983; Stutterheim *et al.* 1988). In addition to obtaining nutritional benefits from them, oxpeckers use their hosts as mobile perches and sources of nesting material. Ungulates reap the benefits of ectoparasite removal, which includes the prevention of blood loss, appetite suppression, tick toxicosis, and skin, bacterial and protozoan diseases (Little 1963; O'Kelly and Seifert 1969; Seebuck *et al.* 1971; Williams *et al.* 1978; Price 1980; Mulilo 1985; Norval *et al.* 1988; Hart *et al*. 1990; Oorebeek and Kleindorfer 2008). The cleaning behavior of oxpeckers is why the oxpecker-ungulate interaction is generally categorized as mutualistic. But, like so many mutualisms, the oxpecker-ungulate interaction appears to have dynamic outcomes.

In addition to being beneficial cleaners, oxpeckers sometimes are parasitic blood-feeders. Oxpeckers have been observed wound-feeding from both domesticated and wild ungulate species. Oxpeckers may inflict small wounds, open scars, or use open wounds to consume tissue and blood (Samish 2000). The percentage of their diet composed of wound blood, tissue and scab material is unknown because this behavior has rarely been specifically examined (see Weeks 1999, 2000; McElligott *et al.* 2004 for exceptions), and stomach content analyses cannot distinguish between the blood of engorged ticks and the blood of wounds. A wound-feeding oxpecker primarily, if not exclusively, inflicts a negative effect on its host. Wound-feeding causes blood loss, an open wound is subject to secondary infection (Samish 2000), and the wound attracts flies that irritate the host.

Only a few bird species are known to wound-feed (e.g. kea parrot, Jackson 1962; magpies, Schorger 1921; sharp-beaked ground finches and Galapagos mockingbirds, Curry and Anderson 1987; Appendix D), and oxpeckers are the only birds in Africa that exhibit this behavior. Oxpeckers are often called "vampire birds" in an attempt to compare them to the vampire bats of the New World (Weeks 2000). Moreau (1933) claimed that it is the blood of engorged ticks, rather than the ticks' tissues, that form the oxpecker's main food (Moreau 1933; Attwell 1966). Other researchers agree, arguing that oxpeckers feed on ticks, particularly engorged ticks, because their main diet is blood (Cole 1992). Anecdotal evidence suggests that the birds strongly prefer blood to ticks when it is available (Bezuidenhout and Stutterheim 1980; Weeks 1999), and studies of both domesticated and wild ungulates have reported oxpeckers ignoring clearly visible ticks (Bezuidenhout and Stutterheim 1980; Weeks 1999). Red-billed oxpeckers were found to have no impact on the number of adult ticks living on domesticated cattle, and they prolonged the healing time of wounds (Weeks 1999). In addition, a group of captive oxpeckers proceeded to create and feed on rhinoceros wounds even though they were being fed a supplementary diet by zoo keepers (McElligott *et al.* 2004). Although there is direct evidence that oxpeckers tick-feed and wound-feed (Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Weeks 1999, 2000; McElligott *et al.* 2004), the conditions surrounding these behaviors have not been specifically examined.

Mutualisms that are facultative, involve a third species, and in which costs and benefits are a function of the partner abundance have been hypothesized to be prone to conditional outcomes (Bronstein 1994a). The oxpecker-ungulate association meets these criteria. The oxpecker-ungulate interaction involves two oxpecker species that consume an assortment of tick species that attach to more than 15 ungulate species. Aside from intrinsic host characteristics (e.g. body size, hair cover), tick abundance and the composition of tick species carried by hosts should be a major factor influencing oxpecker foraging and host selection (Mooring and Mundy 1996a). In a mutualistic relationship, if the service provided by a partner is food, as is the case in the oxpecker-ungulate interaction, then the availability and quality of alternative food sources will influence the value of that service (Axén *et al.* 1996). The availability (tick abundance) and quality (tick species and tick stage preferred by oxpeckers) of ticks may influence oxpecker feeding behavior, and hence the fitness costs and benefits exchanged in the oxpecker-ungulate interaction.

I investigated whether oxpeckers wound-feed because they prefer this food source, or whether wounds are not preferred and wound-feeding occurs exclusively during periods of tick-scarcity. Tick-scarcity can have several meanings: a host animal supports few ticks of the species oxpeckers consume (i.e. small numbers of several tick species), few ticks of a single tick species that oxpeckers consume (e.g. few engorged female blue ticks), or few ticks of the tick stages oxpeckers prefer. Optimally foraging oxpeckers are expected to maximize food intake and minimize search time (Mooring and Mundy 1996a,b). Perhaps it becomes more cost-efficient for an oxpecker to wound-feed under conditions of tick scarcity rather than to spend more time searching for ticks or for an alternative host. There have been no experimental tests of whether oxpeckers resort to wound-feeding on the host animal only when ticks are scarce, or if they actually exhibit a general preference for wounds.

To address this question, I conducted a series of controlled multi-choice feeding-preference experiments on twelve captive red-billed oxpeckers. The first series were cafeteria-style experiments designed to test red-billed oxpeckers for feeding preferences when simultaneously offered blue ticks, ungulate blood, and minced ungulate meat

(Table 4.1). Oxpeckers were hypothesized to prefer a single food type, and to have the greatest preference for blue ticks, when offered this food type along with blood or minced meat. The second series of experiments involved manipulating tick load and tick species on donkeys, then measuring the feeding behavior of oxpeckers in response to these manipulations (Table 4.2). First, I tested the hypothesis that fluctuations in tick abundance per host animal influence oxpecker feeding-behavior. I predicted that wound-feeding would increase as tick load decreased. Second, I examined the effect of tick species on oxpecker feeding-behavior. I predicted that tick-feeding decreases and wound-feeding increases when a less preferred tick species is available. Third, I tested the hypothesis that hunger level influences oxpecker feeding behavior. Specifically, I tested whether a food-deprived oxpecker exposed to a host animal with no ticks would spend more time wound-feeding than a satiated oxpecker. Finally, I tested the hypothesis that oxpeckers do not wound-feed when they are given a supplemental source of liquid blood.

Methods

Study Site and Oxpecker Capture

Feeding preference experiments on captive red-billed oxpeckers were conducted at the Mokopane Game Biodiversity Conservation Centre (MBCC, 24°10’S, 29°1’E) in Mokopane, South Africa. The MBCC is one of three satellite facilities of the National Zoological Gardens of South Africa. In July 2007, red-billed oxpeckers were live-trapped using mist nets erected around cattle kraals in the Platjan area of the Limpopo Province, South Africa. Oxpeckers were transferred to the MBCC and ringed with a unique combination of celluloid colored rings on the left leg so that each bird could be individually recognized. The details of the capture procedure can be found in Plantan *et al.* 2009.

MBCC Oxpecker Enclosure

A specially designed enclosure for the permanent holding of red-billed oxpeckers was built at the MBCC. This outdoor aviary measured 10 m x 7.5 m x 3.1m (Fig. 4.1a). It was divided into five parallel sections, each section measuring 6.5 m x 2.0 m x 3.1 m. Each of the five sections was further divided into a permanent "bird area" (Fig. 4.1b) measuring 4.0 m x 2.0 m x 3.1m and an "experimental area" (Fig. 4.1c) measuring 2.5 m x 2.0 m x 3.1 m. The bird and experimental areas had sand and concrete floors, respectively. Shade cloth lined the walls of the experimental areas to prevent distractions to non-experimental birds. Three sections held two birds, and two sections held three birds. Oxpeckers were permanently held in the bird areas. A trap measuring 60 cm x 60 cm x 40 cm was built into the dividing wall of each section to allow birds to be passively moved from a bird area into an experimental area (Fig. 4.2a,b). A 10 m x 1 m concrete passageway ran parallel to the entrances of the five experimental areas to allow donkeys to be moved into and out of the enclosure at ease.

Diet Plan

The diet plan used for the twelve captive oxpeckers consisted of a mixture (hereafter referred to as the maintenance food mixture) of two raw eggs, 250 g of lean minced meat, 130 g original ProNutro, and 40 g softbill mynah food mix. A small amount of water was added for easy mixing. This diet was supplemented with mealworms when supplies were available. Pieces of fresh donkey dung were occasionally placed into the enclosure to enrich the oxpecker diet. Because oxpeckers are continuous eaters with peak feeding events in the early morning and early evening (Stutterheim *et al.* 1988), moist food was available at all times to oxpeckers in the bird areas.

Capture Schedule

During the afternoon before the following day's experimental trials, five oxpeckers (one bird from each bird area) were captured using the passive capture system and moved from the bird areas to the experimental areas. The trap consisted of two screen doors attached to a pulley system (Fig. 4.2a). The doors were opened and the trap was baited with the maintenance food mixture and mealworms (Fig. 4.2b). As soon as a single bird entered the trap, the back screen door was shut, the front screen door opened, and the bird was released into the experimental area. After an oxpecker finished an experimental trial, it was released back into the bird area of the enclosure. Then the next bird from each section was captured that afternoon for the next day's experiments. This capture method and schedule allowed the easy rotation of birds from the bird areas to the experimental areas ensuring that each bird received all experimental treatments.

Experimental Procedure

Eight months elapsed between the time of capture and the beginning of the experiments to allow the oxpeckers time to adjust to captive conditions. In non-experimental periods and between trials, oxpeckers were fed the maintenance food mixture. The maintenance food mixture was available for oxpeckers in the experimental areas until dusk, at which time it was removed. This ensured that the first food available on the morning of the experiment was the experimental food and that oxpeckers were hungry. Water was always available, during both experimental and non-experimental periods. Feeding trials occurred between 0700 and 1100. This time period was selected because it incorporated the peak morning feeding activity times for red-billed oxpeckers (Stutterheim *et al.* 1988). In the oxpecker feeding preference experiments, the stopping rule (Lockwood III 1998) was a fixed-time design where an oxpecker was allowed to feed for 30 minutes, after which the trial was terminated. The experimental trial began immediately following the placement of the experimental food within the enclosure. Observations were timed with a stopwatch. Feeding behavior was recorded every minute per 30 minute (min) feeding trial. Each of the twelve oxpeckers went through three trials of each experiment independent of conspecifics to prevent feeding biases that may be caused by interactions between consumers (Peterson and Renaud 1989). Experiments were spread out over a period of four months, and no bird was subjected to more than one trial per day. Ticks used in the experiments were collected in two ways. Frozen stocks of blue ticks were provided by Intervet Malelane Research Unit in Malelane, South Africa and Clinvet International in Bloemfontein, South Africa. Bont ticks were collected from cattle and dead wild ungulates and frozen. Ticks were thawed in cold water immediately before the experiments. Cattle blood and meat were collected from the local abattoir. Blood was refrigerated between experiments. Minced meat was frozen and thawed before use.

Cafeteria-Style Experiments

Whether oxpeckers spend most of their time feeding on the blood or tissue of ungulate wounds is unknown. It can be assumed that they primarily feed on blood because they also consume ticks engorged with blood. However, oxpeckers have been observed feeding on carrion (Moreau 1933; van Someren 1951; Attwell 1966;

Bezuidenhout and Stutterheim 1980). To examine whether oxpeckers prefer the blood or tissue of host wounds, minced meat was used to represent the tissue of ungulates. The blood of wounds is primarily in the liquid form. However, during preliminary observations of oxpeckers in captivity, oxpeckers were observed selectively feeding from pieces of coagulated blood when this food item was offered to them. Coagulated blood cut into small pieces 0.5 cm x 0.5 cm resembles blue ticks. I wanted to test whether oxpecker feeding preference would differ when oxpeckers were offered a choice between ticks and blood in both a liquid and solid form. Eight cafeteria experiments were conducted: ticks vs. liquid blood (performed twice), ticks vs. coagulated blood, ticks vs. minced meat, liquid blood vs. minced meat, ticks vs. maintenance food mixture, liquid blood vs. maintenance food, and minced meat vs. maintenance food mixture (Table 4.1). The ticks used in these experiments were engorged female blue ticks, *Boophilus decoloratus*, which are the tick species and tick stage preferred by oxpeckers (Moreau 1933; Stutterheim *et al.* 1988).

The ticks vs. liquid blood experiment was the only experiment to be repeated for another 36 trials. This was the first experiment performed, and I wanted to assess whether oxpecker preferences would change when this experiment was repeated. The maintenance food mixture was tested against ticks, blood and minced meat to determine whether the birds had become conditioned to this food type, or if the preference for other food types would override any conditioning.

In the cafeteria experiments, a single consumer was given the choice between equal amounts of two different food types per trial. It was necessary to provide food items in equal amounts and in quantities that exceeded the possible intake of a single oxpecker in a 30-min trial. Approximately 100 engorged female blue ticks, weighing approximately 20 g, is the maximum number of adult engorged blue ticks that a single oxpecker can consume in a 24 hour period (Stutterheim *et al.* 1988; Bezuidenhout and Stutterheim 1980). Therefore, in a single experimental trial, a bird received a total of 20 g of each of the two food items offered. Because the different configuration of food items can affect oxpecker choice, two food types were offered simultaneously to individual birds in a single black plastic tray (diameter 43.5 cm). This tray was divided into five sections with each of the two food items divided equally among four sections of the tray (Fig. 4.3). Each section contained one food item, and food items were alternated from one section to the next. The fifth section remained empty. Such presentation of food types allowed a bird to express a dietary choice. The food tray was placed in the center of the experimental area on a wood box measuring 86 cm x 27.5 cm x 58.5 cm (Fig. 4.1c). Food items were not replenished as they were consumed. Oxpecker preference was measured as the time (min) spent consuming each food item. The order in which food items were consumed was also recorded. The first item consumed was predicted to correspond with the preferred food choice.

During preliminary work, food items were weighed before and after a 30 min period. Evaporative losses were insignificant because the duration of this period was short relative to the amount of time needed for autogenic change to occur in each of the food items offered. In an attempt to equalize the handling time of the different food items, the minced meat, coagulated blood, and the maintenance food mixture were broken up into small pieces in a size comparable to the blue ticks. Nothing could be done to standardize the handling time of the liquid blood.

Donkey Experiments

Donkeys are domesticated hosts of oxpeckers (Stutterheim and Panagis 1987). Prior to experimentation, five male African donkeys (*Equus asinus*) were purchased from a farm in Pretoria, South Africa and transferred to the MBCC. Eight oxpecker feeding preference experiments involving two of these donkeys were conducted (Table 4.2). Again, each of the12 birds was subjected to each experiment three times, for a total of 36 trials. The experiments were spread out over a period of four months, and no bird was subjected to more than one trial per day. The same donkey was used for all trials of a particular experiment. One cafeteria experiment (second series of Ticks-Liquid blood) had 34 trials and one donkey experiment (Donkey-fed bird) had 33 trials due to a death of one bird during the course of the experiments. Also, only 24 trials of the Donkey-30 bont tick females experiment were completed.

During each experiment, a square plastic dish measuring 10 cm x 10 cm x 4.5 cm was secured onto the back of the donkey with a belt (Fig. 4.4a,b,c). Oxpeckers were conditioned to feed from the dish. Preliminary work with the captive oxpeckers and donkeys demonstrated that the birds could quickly (30-60 sec) and easily create wounds on their hosts (Fig. 4.5). With the described experimental design, a bird had the opportunity to feed from the dish attached to the donkey’s back, create new wounds to feed from the blood and/or tissue, utilize existing or scabbed wounds on the donkey, or “other-feed” (feed on earwax, scurf cells, and nose, mouth, and eye secretions). A donkey could tolerate the bird’s presence, or exhibit an intolerant response to the oxpecker; this involves attempts to dislodge the oxpecker by shaking its head, stomping or kicking its legs, or nudging the bird. Food items, dependent on the experiment in progress, were placed into the dish. An oxpecker was released into the experimental area, one at a time, with a donkey. Before the donkey was placed into the enclosure, it was searched for naturally-attaching ticks. Any ticks found were removed. Also, the presence and location of wounds on the donkey before and after a trial were recorded on a diagram. Each 30-min trial began as soon as the donkey was placed into the experimental area with a single oxpecker. The oxpecker's and donkey's behaviors were recorded every minute per 30-min trial.

The hypothesis that the oxpecker-host relationship is conditioned upon the number of ticks available was experimentally tested by manipulating the number of ticks offered to oxpeckers. Engorged female blue ticks were used for these experiments. The maximum number of ticks consumed by an oxpecker in a single 30 min cafeteria experiment was 30. Therefore, to document the effect of tick load on oxpecker feeding preference, three donkey experiments were conducted: high tick load (30 blue ticks), medium tick load (20 blue ticks), and low tick load (10 blue ticks). The selected tick loads allowed documentation of any change in oxpecker feeding behavior as tick abundance fluctuated.

The hypothesis that the oxpecker-host relationship is conditioned upon tick species was experimentally tested using engorged female blue ticks and unengorged adult bont ticks, *Amblyomma hebraeum*. Engorged female blue ticks are the most-preferred tick oxpeckers consume, whereas adult bont ticks are the least-preferred tick. Each experiment involved 30 ticks of either engorged female blue ticks, unengorged male bont ticks, or unengorged female bont ticks placed in the dish secured to the donkey's back.

The hypothesis that the oxpecker-host relationship is conditioned upon oxpecker hunger level was experimentally tested by exposing hungry and fed birds to a donkey without ticks. Experimental hungry birds were food-deprived until a tick-free donkey was placed into the enclosure. For the fed experiments, the maintenance food mixture was not removed at dusk the evening before the experiment. Oxpeckers were allowed to feed on the maintenance food mixture up until the experimental trial. Also, ten minutes before an experimental trial, the experimental bird was given 15 mealworms or blue ticks, depending on availability. The oxpecker was assumed to be satiated. The maintenance food mixture was removed and a tick-free donkey was placed into the experimental area.

The hypothesis that oxpeckers would not wound-feed when an additional source of blood was provided to oxpeckers was tested by securing 50 ml of fresh cattle blood (<5 days old) to the back of the donkey. This amount was chosen because it was more blood than a single oxpecker could consume in 30 min, and because it was an amount that would not dry out during an experimental trial, but also would not spill out of the dish as the donkey moved.

Statistical Analyses

For each cafeteria experiment, the number of minutes per 30 min trial each oxpecker spent feeding on each of the two food items was calculated. Because every oxpecker went through three trials of the same experiment, the results of the three trials were averaged per bird ($n=12$). The time spent feeding on each food item per experiment was then statistically compared using Wilcoxon-Signed Rank Tests, $p<0.05$. The mean number of minutes oxpeckers spent feeding on each food item are reported ± the standard deviation.

For each donkey tick load and tick species experiment, the number of minutes per 30 min trial each oxpecker spent wound-feeding and tick-feeding was calculated. Again, the results of the three trials were averaged per bird (n=12). The amount of time oxpeckers spent wound-feeding and tick-feeding per experiment was statistically compared using Wilcoxon-Signed Rank Tests, $p<0.05$. The average number of minutes oxpeckers spent tick-feeding, wound-feeding, and other-feeding (feed on earwax, scurf cells, and nose, mouth, and eye secretions) are reported ± the standard deviation. I conducted a Friedman test ($p<0.05$) to test for conditionality dependent upon tick load. Post-hoc pairwise comparisons using Wilcoxon tests were then conducted to test the hypothesis that oxpeckers increase wound-feeding as tick load decreases. I conducted pairwise comparisons using Wilcoxon tests to test the hypothesis that oxpeckers spend more time wound-feeding when a less preferred tick is available (male bont ticks) compared to when a preferred tick is available (blue ticks).

To test for effects of oxpecker hunger level on feeding behavior, the number of minutes per 30 min trial each oxpecker spent wound-feeding and other-feeding was calculated. Trial results were averaged per bird (n=12). The amount of time oxpeckers spent wound-feeding and other-feeding per experiment was statistically compared using a Wilcoxon-Signed Rank Test, $p<0.05$. The average number of minutes oxpeckers spent wound-feeding and other-feeding are reported ± the standard deviation. Then, a Wilcoxon Signed-Rank test ($p<0.05$) was conducted to test the hypothesis that hungry birds would spend more time wound-feeding than oxpeckers fed prior to the experiment.

To test the hypothesis that the presence of an additional blood source would curb wound-feeding behavior, I first calculated the number of minutes per 30 min trial each oxpecker spent wound-feeding and feeding from the blood dish. As in the other experiments, the results of the trials were averaged per bird (n=12). The amount of time oxpeckers spent wound-feeding and feeding from the dish per experiment was statistically compared using a Wilcoxon-Signed Rank Test, $p<0.05$. The average number of minutes oxpeckers spent engaged in both feeding activities is reported ± the standard deviation.

Statistical tests were conducted using SPSS Statistics Gradpack 17.0.

Results

Cafeteria-style Experiments

In the first ticks vs. liquid blood experiment, oxpeckers preferred ticks (Wilcoxon Signed-Rank, $z=-3.06$, $n_1=n_2=12$, $p=0.002$; Table 4.3). The average number of minutes oxpeckers spent tick-feeding per trial was 8 min (±3.07) and the average number minutes spent blood-feeding was 2 min (±2.11). Ticks were the first item consumed by oxpeckers during 30 trials, whereas blood was the first item consumed during only six trials. When this experiment was repeated, and an additional 36 trials were conducted, oxpeckers exhibited no preference for ticks or liquid blood (Wilcoxon Signed-Rank, $z=-1.47$, $n_1=n_2=12$, $p=0.142$; Table 4.3). Oxpeckers spent an average of 6 min (±2.80) tick-feeding per trial and 5 min (±3.80) blood-feeding per trial. The first item oxpeckers consumed was ticks during 25 trials and blood during nine trials. Oxpeckers exhibited no preference for ticks or coagulated blood (Wilcoxon Signed-Rank, $z=-0.55$, $n_1=n_2=12$, $p=0.582$; Table 4.3). On average, oxpeckers spent 6 min (±3.17) tick-feeding and 6 min

(±3.64) feeding on coagulated blood per trial. Oxpeckers consumed ticks first during 24 trials and blood first during 12 trials.

Oxpeckers preferred both ticks and blood to minced meat. Oxpeckers spent significantly more time tick-feeding than feeding on minced meat (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Table 4.3). Oxpeckers spent an average of 8 min (±2.46) tick-feeding and 2 min (±1.55) feeding on minced meat per trial. The first item oxpeckers consumed was ticks during 31 trials and minced meat during five trials. The birds also spent significantly more time feeding on liquid blood than minced meat (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Table 4.3). Oxpeckers spent, on average, 11min (±6.16) blood-feeding and 5 min (±2.04) feeding on minced meat per trial. Even though oxpeckers preferred blood, the first item they consumed during trials did not always reflect this preference. Blood was the first food item consumed during 17 trials and minced meat during 19 trials.

The maintenance food mixture was the least preferred food item offered to oxpeckers. Oxpeckers preferred ticks, blood, and minced meat to the maintenance food. Oxpeckers spent an average of 9 min (±4.42) tick-feeding and 3 min (±3.05) feeding on the maintenance food mixture per trial (Wilcoxon Signed-Rank, z=-3.07, $n_1=n_2=12$, p=0.002; Table 4.3). The first item consumed was ticks during 33 trials and the maintenance food mixture during three trials. In the blood vs. maintenance food experiment, oxpeckers spent an average of 13 min (±5.84) blood-feeding and 3 min (±1.38) feeding on the maintenance food mixture per trial (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Table 4.3). The first item consumed was blood during 25 trials and the maintenance food mixture during eleven trials. In the minced meat vs.

maintenance food mixture experiment, the average number of minutes per trial that oxpeckers fed on the minced meat and the maintenance food mixture was 7 min (±3.01) and 2 min (±1.57), respectively (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Table 4.3). Oxpeckers consumed minced meat first in every trial.

In summary, oxpeckers exhibited a preference for ticks over liquid blood in the first experiment, but exhibited no preference when this experiment was repeated. Oxpeckers also exhibited no preference when given a choice between ticks and coagulated blood. Oxpeckers preferred both ticks and blood to the minced meat. Oxpeckers preferred ticks, blood, and minced meat to the maintenance food mixture, which was the least preferred food type.

Donkey Experiments

Wound-feeding Conditional on Tick Load

I examined whether oxpeckers spent more time tick-feeding or wound-feeding when offered a high tick load (30 blue ticks) of the tick species they prefer. Oxpeckers still wound-fed even when offered a high tick load of the tick species they prefer. There was no significant difference in the time oxpeckers spent tick-feeding and wound-feeding (Wilcoxon Signed-Rank, z=-1.02, $n_1=n_2=12$, p=0.306; Table 4.4). The average number of minutes oxpeckers spent tick-feeding per trial was 7 min (±3.10), the average number of minutes spent wound-feeding per trial was 10 min (±6.22), and the average number minutes spent other-feeding per trial was 10 min (±6.65). In only one trial were all 30 ticks consumed. In all trials, wound-feeding began before all ticks were consumed. In only one trial were all ticks consumed before other-feeding began (feed on earwax, scurf cells, and nose, mouth, and eye secretions).

I examined how much time oxpeckers tick-fed and wound-fed when offered a medium tick load (20 blue ticks). Again, there was no significant difference in time oxpeckers spent tick-feeding and wound-feeding (Wilcoxon Signed-Rank, $z=-0.86$, $n_1=n_2=12$, $p=0.388$; Table 4.4). The average number of minutes oxpeckers tick-fed per trial was 8 min (±2.74), the average number of minutes oxpeckers wound-fed was 10 min (±6.90), and the average number of minutes oxpeckers other-fed was 9 min (±6.02). All 20 ticks were consumed in 18 of the trials. In only four trials were all ticks consumed before wound-feeding began. In only five trials were all ticks consumed before other-feeding began.

I examined whether oxpeckers spent more time wound-feeding or tick-feeding when offered a low tick load (10 blue ticks). Oxpeckers spent significantly more time wound-feeding than tick-feeding (Wilcoxon Signed-Rank, $z=-3.06$, $n_1=n_2=12$, $p=0.002$; Table 4.4). The average number of minutes per trial that an oxpecker tick-fed was 3 min (±1.06), compared to 17 min wound-feeding (±5.61), and 6 min other-feeding (±5.1). There was only one trial during which an oxpecker did not wound-feed. Although wound-feeding was high, oxpeckers consumed the majority of ticks during each trial. The average number of ticks consumed out of all trials was nine (Table 4.5), and 100% of the ticks were consumed in 28 of the trials. As in the high tick and medium tick load experiments, oxpeckers began tick-feeding, on average, at minute one.

A Friedman test was conducted to test for significant differences tick-feeding across the high, medium, and low tick load treatments. The test was statistically signficant (Friedman, $\chi^2=16.13$, $df=2$, $p<0.001$). Post-hoc pairwise comparisons using Wilcoxon tests were then conducted to test whether the amount of time oxpeckers spent tick-feeding increased as tick load increased. Oxpeckers spent significantly less time tick-feeding when offered a low tick load than when offered a high tick load (Wilcoxon Signed-Rank, z=-2.98, $n_1=n_2=12$, p=0.003; Fig. 4.6) and a medium tick load (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Fig. 4.6). The amount of time oxpeckers spent tick-feeding when offered a high tick load did not differ significantly from when they were offered a medium tick load (Wilcoxon Signed-Rank, z=-0.49, $n_1=n_2=12$, p=0.624; Fig. 4.6).

When wound-feeding was compared across treatments, the test was also statistically significant (Friedman, $\chi^2=13.15$, df=2, p=0.001). Post-hoc pairwise comparisons using Wilcoxon tests were then conducted to test whether wound-feeding increased as tick load decreased. Oxpeckers spent significantly more time wound-feeding when offered a low tick load than when offered a high tick load (Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Fig. 4.6) and a medium tick load (Wilcoxon Signed-Rank, z=-2.83, $n_1=n_2=12$, p=0.005; Fig. 4.6). The amount of time oxpeckers spent wound-feeding when offered a high tick load did not differ significantly from when they were offered a medium tick load (Wilcoxon Signed-Rank, z=-0.09, $n_1=n_2=12$, p=0.929; Fig. 4.6). In the high tick load and medium tick load experiments, oxpeckers began tick-feeding, on average, at minute one. They did not begin wound-feeding until 5 min after the start of the experiments, on average. The average number of ticks consumed in both the high tick and medium tick load experiments was 18 ticks (Table 4.5). There were only nine trials (25%) during which an oxpecker did not wound-feed in the high tick load experiments, compared to eight trials (22%) during the medium tick load experiments.

Wound-feeding Conditional on Tick Species

I examined how a less preferred tick species influences oxpecker feeding behavior. In both bont tick experiments, oxpeckers spent significantly more time wound-feeding than tick-feeding (Male Bont Tick: Wilcoxon Signed-Rank, z=-3.06, $n_1=n_2=12$, p=0.002; Female Bont Tick: Wilcoxon Signed-Rank, z=-2.80, $n_1=n_2=10$, p=0.005; Table 4.4). The sex of bont tick did not influence oxpecker feeding behavior. The amount of time oxpeckers spent feeding on bont tick males and bont tick females did not differ statistically (Wilcoxon Signed-Rank, z=-0.34, $n_1=12$, $n_2=10$, p=0.735; Fig. 4.7), nor did the amount of time oxpeckers spent wound-feeding when offered bont tick males and bont tick females (Wilcoxon Signed-Rank, z=-0.87, $n_1=12$, $n_2=10$, p=0.386; Fig. 4.7). In the bont tick male experiments, the average number of minutes oxpeckers tick-fed, wound-fed, and other-fed per trial was 2 min (±3.00), 19 min (±4.46), and 7 min (±4.89), respectively. These values were very similar to those in the bont tick female experiments, in which the average number of minutes oxpeckers tick-fed, wound-fed, and other-fed per trial was 3 min (±4.23), 19 min (±4.80), and 5 min (±4.28), respectively. In both experiments, every oxpecker wound-fed during every trial and wound-feeding began within two minutes after the start of the experiment. The average number of ticks consumed per trial in the bont tick male experiments was seven, compared to two in the bont tick female experiment (Table 4.5). All 30 bont ticks were consumed in only two of the bont tick male trials, and in one of the bont tick female trials. There was only one trial in which all male bont ticks were consumed before wound-feeding began, and no trials in which all bont tick females were consumed before wound-feeding began. It should be noted that in the bont tick experiments, the same four birds consistently fed on many of bont ticks during each experimental trial. The majority of oxpeckers ate only 0-6 ticks during each trial.

I conducted pairwise comparisons using Wilcoxon tests to examine how tick-feeding and wound-feeding differed when oxpeckers were offered the preferred blue ticks and non-preferred male bont ticks. When tick-feeding was examined, oxpeckers spent significantly more time tick-feeding when offered 30 blue ticks than when offered 30 male bont ticks (Wilcoxon Signed-Rank, $z=-2.43$, $n_1=n_2=12$, $p=0.015$; Fig. 4.7). Furthermore, oxpeckers spent significantly more time wound-feeding when offered male bont ticks than when offered blue ticks (Wilcoxon Signed-Rank, $z=-2.75$, $n_1=n_2=12$, $p=0.006$; Fig. 4.7). As predicted, tick-feeding decreased and wound-feeding increased when a less preferred tick species was available.

Wound-feeding Conditional on Hunger Level

Wilcoxon Signed-Rank tests ($p<0.05$) were conducted to examine whether the amount of time oxpeckers spent wound-feeding increased when oxpeckers were food-deprived prior to the experiment compared to when they were fed. The amount of time oxpeckers spent wound-feeding did not differ statistically (Wilcoxon Signed-Rank, $z=-0.89$, $n_1=12, n_2=11$, $p=0.374$). In both the food-deprived and satiated experiments, there were only six trials during which an oxpecker did not wound-feed.

I then examined each experiment separately. The amount of time oxpeckers spent wound-feeding and other-feeding when they were hungry was not statistically different (Wilcoxon Signed-Rank, $z=-0.09$, $n_1=n_2=12$, $p=0.929$; Table 4.4). Oxpeckers spent an average of 15 min (±7.48) wound-feeding and 15 min (±4.21) other-feeding per trial. On average, oxpeckers began wound-feeding 10 minutes after the start of the experiment.

Oxpeckers still wound-fed even when they were fed before the start of the experiment. The amount of time oxpeckers spent wound-feeding and other-feeding when they were satiated was not statistically different (Wilcoxon Signed-Rank, z=-0.49, $n_1=n_2=11$, p=0.625; Table 4.4). The average number of minutes oxpeckers wound-fed per trial was 12 min (±9.26). The average number of minutes oxpeckers spent other-feeding per trial was 11 min (±5.51). On average, oxpeckers began wound-feeding seven minutes after the start of the experiment.

The Effect of an Additional Blood Source on Wound-feeding Behavior

I examined the hypothesis that oxpeckers would not wound-feed when they were offered an additional source of blood. Oxpeckers spent significantly more time feeding from the blood dish than wound-feeding (Wilcoxon Signed-Rank, z=-2.12, $n_1=n_2=12$, p=0.034; Table 4.4). Contrary to prediction, some oxpeckers still wound-fed under these conditions. The average number of minutes oxpeckers fed from the blood dish per trial was 13 min (±4.38). The average number of minutes oxpeckers wound-fed per trial was 6 min (±6.57) and the average number minutes oxpeckers spent other-feeding per trial was 9 min (±6.33). There were 15 trials (42%) during which an oxpecker did not wound-feed. On average, oxpeckers began feeding from the blood dish within two minutes of the start of the experiment and wound-feeding nine minutes after the start of the experiment. Oxpeckers never consumed all the blood from the dish in any given trial.

Host Response Behavior

Donkeys were predicted to be intolerant of wound-feeding oxpeckers. However, in every experiment donkeys exhibited a higher percentage of intolerant responses to other-feeding oxpeckers than they did to wound-feeding oxpeckers (Table 4.6). For each experiment, the percentage of total donkey responses to wound-feeding oxpeckers, other-feeding oxpeckers, and non-feeding oxpeckers (i.e. oxpecker present on head, leg, or side of donkey, but not feeding) is listed in Table 4.7. The percentage of intolerant responses to non-feeding oxpeckers accounted for less than 12% of total responses in each experiment. In each of the bont tick experiments, the percentage of responses to wound-feeding oxpeckers was greater than the percentage of responses to other-feeding and non-feeding oxpeckers. In the other six experiments, the greatest percentage of donkey responses was to other-feeding oxpeckers. In every experiment, ear-feeding accounted for the highest percentage of other-feeding behaviors. I examined donkey ears and only found earwax.

Discussion

Whether an interspecific relationship has a mutualistic, commensal, or antagonistic outcome can be strongly conditioned upon the ecological setting in which that relationship occurs (Cushman and Whitham 1989; Bronstein 1994b; Del-Claro and Oliveira 2000; Egger and Hibbet 2004). Spatial or temporal variation in the magnitude of either the costs or benefits of the interaction will influence the net effect for each partner (Bronstein 1998). To understand how interspecific interactions vary in space and time and move along the continuum, it is necessary to identify the mechanisms generating variation in the outcome of such interactions (Billick and Tonkel 2003; Herre *et al.* 1999).

Because of its inherent complexity, I hypothesized that the relationship between ticks, oxpeckers, and ungulates has conditional outcomes. In a mutualistic relationship, if the service provided by a partner is food, as is the case in the oxpecker-ungulate interaction, then the availability and quality of alternative food sources will influence the value of that service (Axén *et al.* 1996). The results of my research demonstrate that the availability (tick abundance) and quality (tick species and tick stage preferred by oxpeckers) of ticks influence oxpecker feeding behavior and generate conditional outcomes.

Although oxpeckers consumed ticks, they also wound-fed even when donkeys had high tick loads of the tick species oxpeckers prefer. In both the cafeteria experiments and donkey experiments, it is evident that oxpeckers prefer liquid blood over host tissue (minced meat). Whether oxpeckers have a preference for wound-blood or ticks, however, is still unclear. Perhaps there is no preference. Both the cafeteria and donkey experiments revealed that oxpeckers spend time engaged in both tick and wound-feeding behaviors. During both series of experimental feeding trials, oxpeckers repeatedly switched between the two food items. In the first series of ticks vs. liquid blood trials, oxpeckers did exhibit a preference for ticks. When this experiment was repeated, however, oxpeckers spent approximately the same amount of time feeding on ticks and blood. Furthermore, oxpeckers exhibited no preference between ticks and coagulated blood.

An unexpected result was that oxpeckers wound-fed on donkeys even when they were given an additional blood source. This again suggests that oxpecker prefer to wound-feed. To feed from the blood dish, the birds simply had to land on the donkey and lap up the free blood source from the dish. An oxpecker did drink from the dish during every trial, but in 15 out of 36 trials these same birds also wound-fed. Even though fresh blood was purchased from the abattoir every 4-5 days and refrigerated between experiments, the temperature or freshness of the blood cannot be eliminated as factors influencing wound-feeding. It is clear from these experiments that oxpeckers spend much more time feeding on blood than previously reported and will create wounds on their hosts to access free-flowing blood.

During preliminary work with multiple oxpeckers on a single donkey, oxpeckers were very aggressive and frequently squabbled with each other for access to host wounds. Dominant birds would chase others away from wounds and knock them from the donkey. A few oxpeckers were observed pecking wound-feeding birds in the back and pulling them by the leg to remove them from wound-feeding sites. These aggressive behaviors again suggest a preference to feed at wounds, for no such behavior was observed when multiple oxpeckers were given ticks on the donkey.

There were only three donkey experiments in which an oxpecker did not wound-feed during any of its three experimental trials: blood dish, high tick load, and medium tick load. In every other experiment, each oxpecker wound-fed during at least one of its experimental trials. However, variation in tick-load and tick species did influence wound-feeding behavior. Oxpeckers spent more time wound-feeding when donkeys had a low tick load compared to when they had medium and high tick loads. Also as predicted, wound-feeding was higher and tick-feeding lower in the bont tick experiments compared to the blue tick experiments. In each blue tick experiment, an oxpecker tick-fed during every experimental trial. But during the bont tick male and female experiments, a bird did not tick-feed during 19 trials and 15 trials, respectively. Generally, oxpeckers consumed more blue ticks than bont ticks (a few birds were outliers and consumed bont ticks in every trial). Although oxpeckers spent more time wound-feeding when a less preferred tick species was offered, the presence of a preferred tick species did not prevent wound-feeding. Because engorged female blue ticks are the preferred tick of oxpeckers, it can be assumed that wound-feeding would be even greater in the presence of other tick species and tick stages.

One of the experimental birds hatched in captivity at the MBCC and was raised to fledging before the start of the experiments. The feeding choices of this immature bird provide additional insight into the feeding behavior of oxpeckers. This bird wound-fed during every experiment except during the donkey-blood dish experiment. Also, this bird did not consume any female bont ticks or male bont ticks. These experimental trials were the first time this oxpecker was exposed to bont ticks. This bird even created wounds during the bont tick male experiment. Further studies on the feeding preferences of immature naïve birds in the absence of adult birds could shed additional light on development of the feeding behavior of oxpeckers.

When oxpeckers wound-fed from the donkeys, they would rapidly peck the skin of the donkey until the blood would begin to emerge. Once the wound was open, they would place their bill sideways against the wound and lap up the blood. An oxpecker would continue this pecking, blood-lapping cycle for minutes at a time. I suggest a few reasons for why oxpeckers divide their foraging time between tick feeding and wound-feeding. The dietary physiology of oxpeckers, which has never been examined, may prevent them from having a blood-only diet. When oxpeckers were observed feeding on blood for the majority of an experimental trial they exhibited unusual behaviors. The feathers on their head would fluff up, they regurgitated blood on a few occasions (but continued to wound-feed), and they defecated frequently. Blood has a high water concentration that oxpeckers may not be able to process as efficiently as can vampire bats. Vampire bats feed exclusively on the blood of live vertebrates, particularly cattle, in many areas of Latin America (Thompson *et al.* 1977). Because of their blood-restricted diet, vampire bats excrete copious amounts of urine (Wimsatt and Guerriere 1961; Greenhall, Schmidt, and Lopez-Forment 1971; Breidenstein 1982). They have been reported to excrete 30-50% of the blood they ingest as urine (Wimsatt and Guerriere 1962; Breidenstein 1982). Oxpeckers, however, may not have the ability to readily excrete such large amounts. Vampire bats that engorge large quantities of blood also have difficulty flying (Ditmars and Greenhall 1935; Goodwin and Greenhall 1961; all in Greenhall, Schmidt, and Lopez-Forment 1971). A similar effect in oxpeckers could increase oxpecker vulnerability to predation. The nutritional content of ticks and blood also may differ (Chapter Five). Oxpeckers may not obtain all of their nutritional requirements from blood in a non-tick form. Furthermore, the response behavior of the host may prevent an oxpecker from consistently wound-feeding. In this study, donkeys were generally tolerant of wound-feeding oxpeckers even though the wound-feeding locations were usually in areas where the donkeys could easily have removed the birds with a nudge or leg-kick. This same result was also found in an observational study of wild oxpeckers in Kruger National Park (Chapter Two), in which wild ungulate species were generally tolerant of wound-feeding oxpeckers. But these birds divided their time between tick-feeding, wound-feeding, other-feeding, and non-feeding behaviors. Hosts may be less receptive to oxpeckers that exclusively wound-feed.

The relevance of these results to oxpeckers foraging on other ungulate species may be questionable. The amount of time oxpeckers spend wound-feeding may vary between ungulate species. In addition to donkeys, oxpeckers have been observed wound-feeding on cattle, camels, rhinoceri, giraffes, hippopotami and buffalo (Moreau 1933; van Someren 1951; Attwell 1966; Mengesha 1978; Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988; Weeks 1999, 2000; McElligott *et al.* 2004; personal observation). The ability of oxpeckers to easily penetrate the skin of their hosts in order to make wounds may be a factor influencing wound-feeding, but this is highly unlikely. In this study oxpeckers were observed creating wounds on donkeys, a species that has rather thin skin. McElligott *et al.* (2004) observed oxpeckers inflicting wounds on captive rhinoceri, a species that has thick tough skin. Although some ungulate species have thicker skin than others, all species have areas of thin skin. The distribution of the thinnest areas of skin is similar across species, but the location of the thicker areas of skin differs between species (Jarman 1989). The axillary, inguinal and perineal skin is almost always thinnest. However, there are considerable inter-and intraspecific differences in distribution of skin thickness.

There are more than 80 species of ixodid ticks in South Africa (Walker 1991), but oxpeckers are only known to consume eight species of four African ixodid tick genera: *Boophilu*s, *Rhipicephalus*, *Hyalomma,* and *Amblyomma*. Furthermore, oxpeckers exhibit a preference for different developmental stages of these ticks depending on the tick species. For example, while oxpeckers prefer engorged females blue ticks, *B. decoloratus*, they prefer the engorged nymphal stage of bont ticks, *A. hebraeum* (Stutterheim *et al.* 1988). This is because engorged female bont ticks are difficult to remove from the host and are too large for the oxpeckers to manipulate in their bill and swallow whole (Bezuidenhout and Stutterheim 1980; Stutterheim *et al.* 1988). Bont tick nymphs are more manageable. Thus, experimental manipulations on donkeys (and other host species) using the other ixodid ticks oxpeckers do and do not feed upon should also be conducted to fully understand the conditional nature of the oxpecker-ungulate interaction.

In addition to documenting variation in an interspecific relationship and identifying the mechanisms generating that variation, studies of conditional mutualism should link that variation to the fitness of at least one, if not both, of the partners (Billick and Tonkel 2003). The costs and benefits to both participants need to be critically examined. The exact costs of wound-feeding to the host animal are unclear. Some researchers have suggested that wound-feeding benefits mammals because the oxpecker cleans the wound of dead tissue and maggots and prevents bacterial infection (van Someren 1951; Pitman 1956; Breitwisch 1992; Weeks 1999). However, there is no evidence that oxpeckers clean wounds and improve healing time. In a study by Weeks (1999), blowfly maggots did not develop in wounds when oxpeckers were excluded. In addition, I have observed oxpeckers re-opening almost healed wounds and subsequently prolonging healing time. I also have observed oxpeckers creating wounds on multiple occasions, a behavior that is of no plausible benefit to the host. Maggot consumption was never observed. But there are several negative effects of wound-feeding. The host animal suffers blood loss, and the wound attract flies and is open to secondary infection. Such costs likely vary with the size of the wound, the amount of time a bird wound-feeds from a single wound, and the length of time that the wound is open and prevented from healing. The size, age and health of the host animal may also influence how much the host is negatively affected by a wound-feeding oxpecker. In the wild, oxpeckers exist in flocks of up to two dozen birds, and could potentially cause considerable damage if they were to utilize the same host animal every day.

Conclusion

The results of this study confirm that oxpeckers feed on the ticks and wounds of their ungulate hosts. Surprisingly, oxpeckers spent a substantial amount of time wound-feeding from the donkeys in this study. Although wound-feeding decreased when the birds were offered a large number of the tick species they prefer (blue ticks), oxpeckers still wound-fed. These results indicate that the amount of time oxpeckers wound-feed is conditional on tick species and tick abundance, but the occurrence of wound-feeding is not. Future studies on the nature of the relationship between oxpeckers and ungulates should investigate how the other tick species and tick stages that oxpeckers consume affect oxpecker feeding behavior. Adult female blue ticks are the most preferred tick species and tick stage of red-billed oxpeckers, and adult female bont ticks are the least preferred. The amount of time oxpeckers wound-feed when offered the other tick species and tick stages they consume should be intermediate of the values in this study. The fitness effects of a wound-feeding oxpecker and a tick-feeding oxpecker to an ungulate host need to be critically analyzed, and oxpecker response to additional tick manipulations on other host species should be studied. Further experimentation with naïve oxpeckers bred in captivity also could provide insight into the factors that influence dietary choices. Similar experimentation on the feeding preferences of yellow-billed oxpeckers in captivity would also be useful to the investigation of the oxpecker-ungulate association.

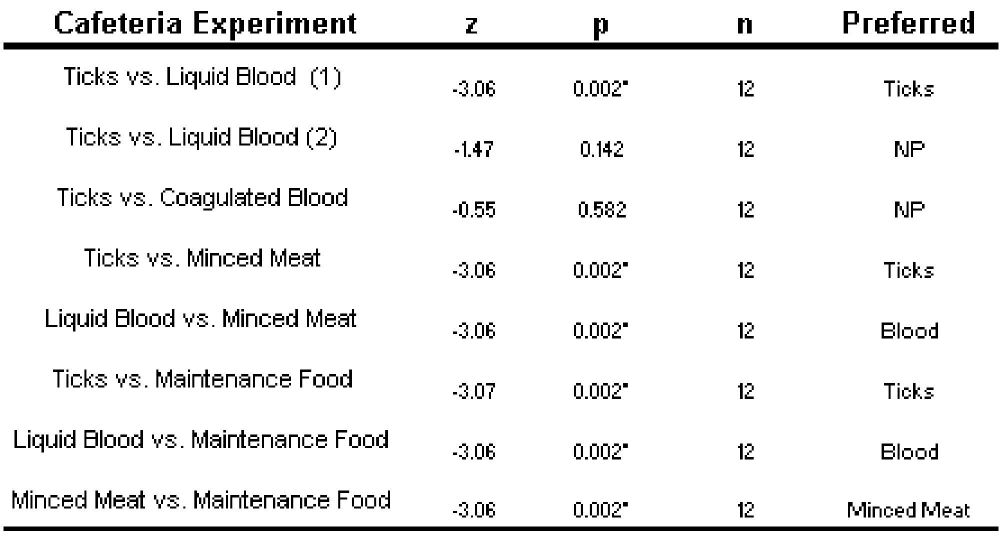
Table 4.1. Cafeteria-style feeding preference experiments and the food item oxpeckers were predicted to prefer.

Cafeteria Experiment	Prediction
Ticks vs. Liquid Blood (1)	Ticks
Ticks vs. Liquid Blood (2)	Ticks
Ticks vs. Coagulated Blood	Ticks
Ticks vs. Minced Meat	Ticks
Liquid Blood vs. Minced Meat	Blood
Ticks vs. Maintenance Food	Ticks
Liquid Blood vs. Maintenance Food	Blood
Minced Meat vs. Maintenance Food	Meat

Table 4.2. Description of the oxpecker-donkey experiments.

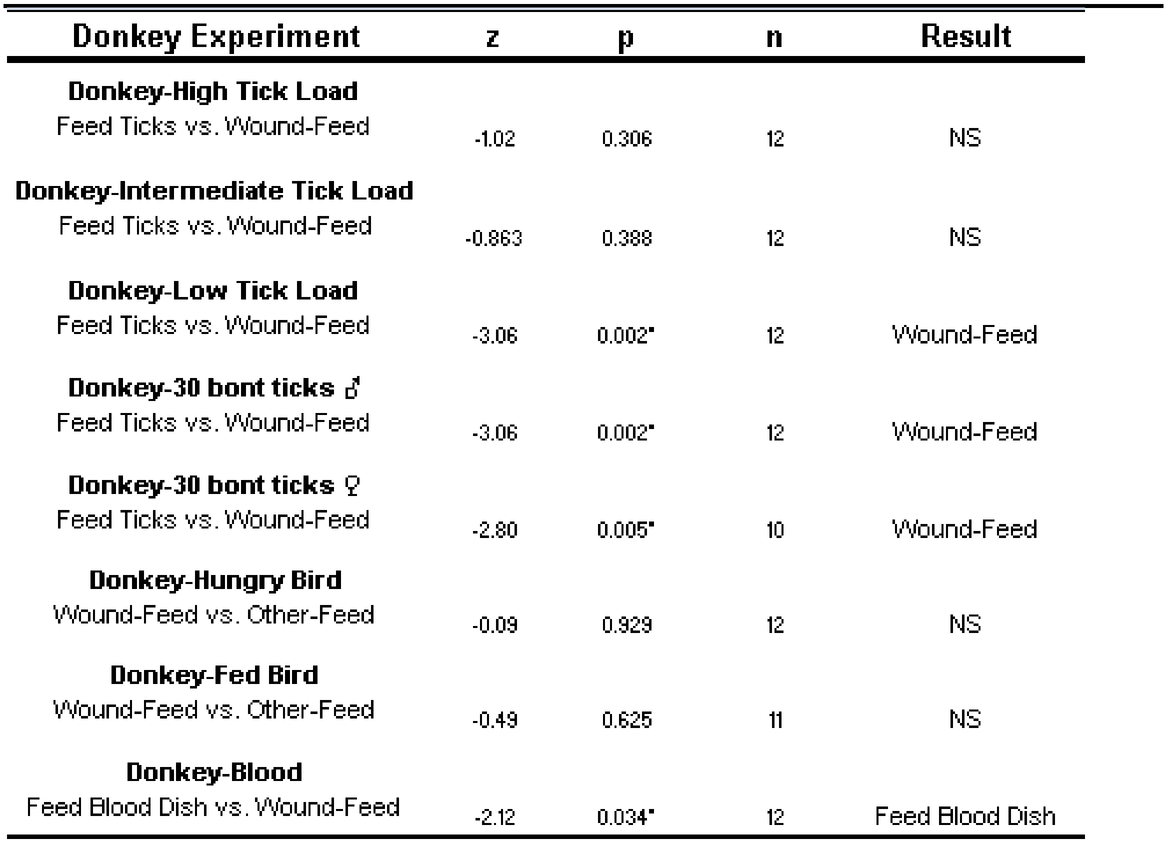
Donkey Experiment	Description
Donkey-Hungry Bird	Food-deprived bird put into experimental area with tick-free donkey. Oxpecker could wound-feed or other feed.
Donkey-Fed Bird	Oxpecker allowed to feed on the maintenance food before the experiment. Oxpecker also given 15 blue ticks or 15 mealworms ten minutes before the experiment to ensure the bird was satiated. A tick-free donkey was then placed into the experimental area with the bird. Oxpecker could wound-feed or other-feed.
Donkey-Blood	50 ml of blood put into dish on donkey's back. Oxpecker could feed from blood dish, wound-feed, or other-feed.
Donkey-High Tick Load	30 engorged female blue ticks put into dish on donkey's back. Oxpecker could feed from tick dish, wound-feed, or other-feed.
Donkey-Intermediate Tick Load	20 engorged female blue ticks put into dish on donkey's back. Oxpecker could feed from tick dish, wound-feed, or other-feed.
Donkey-Low Tick Load	10 engorged female blue ticks put into dish on donkey's back. Oxpecker could feed from tick dish, wound-feed, or other-feed.
Donkey-30 bont ticks ♂	30 unengorged male bont ticks put into dish on donkey's back. Oxpecker could feed from tick dish, wound-feed, or other-feed.
Donkey-30 bont ticks ♀	30 unengorged female bont ticks put into dish on donkey's back. Oxpecker could feed from tick dish, wound-feed, or other-feed.

Table 4.3. Results of the cafeteria-style feeding preference experiments based on time spent feeding on a particular food type (Wilcoxon Signed-Rank, $p<0.05$). NP= no preference.



Cafeteria Experiment	z	p	n	Preferred
Ticks vs. Liquid Blood (1)	-3.06	0.002*	12	Ticks
Ticks vs. Liquid Blood (2)	-1.47	0.142	12	NP
Ticks vs. Coagulated Blood	-0.55	0.582	12	NP
Ticks vs. Minced Meat	-3.06	0.002*	12	Ticks
Liquid Blood vs. Minced Meat	-3.06	0.002*	12	Blood
Ticks vs. Maintenance Food	-3.07	0.002*	12	Ticks
Liquid Blood vs. Maintenance Food	-3.06	0.002*	12	Blood
Minced Meat vs. Maintenance Food	-3.06	0.002*	12	Minced Meat

Table 4.4. Results of the oxpecker-donkey experiments (Wilcoxon Signed-Rank, $p<0.05$).



Donkey Experiment	z	p	n	Result
Donkey-High Tick Load Feed Ticks vs. Wound-Feed	-1.02	0.306	12	NS
Donkey-Intermediate Tick Load Feed Ticks vs. Wound-Feed	-0.863	0.388	12	NS
Donkey-Low Tick Load Feed Ticks vs. Wound-Feed	-3.06	0.002*	12	Wound-Feed
Donkey-30 bont ticks ♂ Feed Ticks vs. Wound-Feed	-3.06	0.002*	12	Wound-Feed
Donkey-30 bont ticks ♀ Feed Ticks vs. Wound-Feed	-2.80	0.005*	10	Wound-Feed
Donkey-Hungry Bird Wound-Feed vs. Other-Feed	-0.09	0.929	12	NS
Donkey-Fed Bird Wound-Feed vs. Other-Feed	-0.49	0.625	11	NS
Donkey-Blood Feed Blood Dish vs. Wound-Feed	-2.12	0.034*	12	Feed Blood Dish

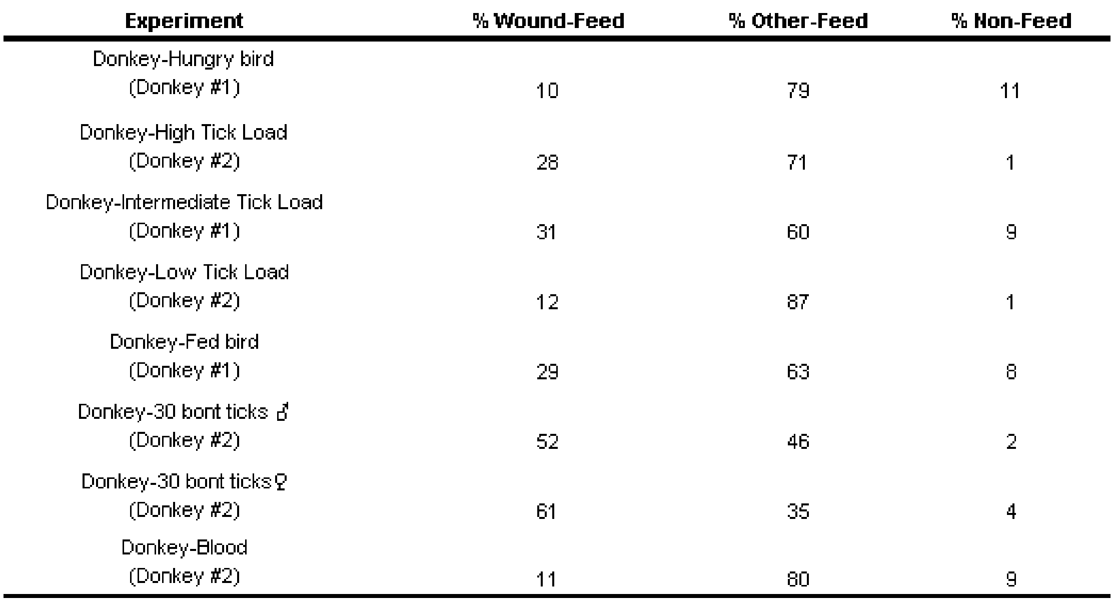
Table 4.5. Average number of ticks consumed (and ranges) per oxpecker-donkey experiment.

Donkey Experiment	Average # Ticks Consumed
Donkey-30 bont ticks ♀	2 (7%)
Range	*0–30*
Donkey-30 bont ticks ♂	7 (23%)
Range	*0–30*
Donkey-High Tick Load	18 (60%)
Range	*5–29*
Donkey-Intermediate Tick Load	18 (90%)
Range	*5–20*
Donkey-Low Tick Load	9 (90%)
Range	*4–10*

Table 4.6. Percentage of wound-feeding events and other-feeding events per experiment that elicited an intolerant response from the donkey.

Experiment	% Wound-Feeding Events	% Other-Feeding Events
Donkey-Hungry bird (Donkey #1)	3	27
Donkey-Low Tick Load (Donkey #2)	7	47
Donkey-Intermediate Tick Load (Donkey #1)	25	41
Donkey-High Tick Load (Donkey #2)	4	30
Donkey-Fed bird (Donkey #1)	4	39
Donkey-30 bont ticks ♂ (Donkey #2)	20	47
Donkey-30 bont ticks ♀ (Donkey #2)	31	65
Donkey-Blood (Donkey #2)	4	17

Table 4.7. Percentage of total donkey responses that were to a wound-feeding, other-feeding, and non-feeding oxpecker per experiment.



Experiment	**% Wound-Feed**	**% Other-Feed**	**% Non-Feed**
Donkey-Hungry bird (Donkey #1)	10	79	11
Donkey-High Tick Load (Donkey #2)	28	71	1
Donkey-Intermediate Tick Load (Donkey #1)	31	60	9
Donkey-Low Tick Load (Donkey #2)	12	87	1
Donkey-Fed bird (Donkey #1)	29	63	8
Donkey-30 bont ticks ♂ (Donkey #2)	52	46	2
Donkey-30 bont ticks ♀ (Donkey #2)	61	35	4
Donkey-Blood (Donkey #2)	11	80	9

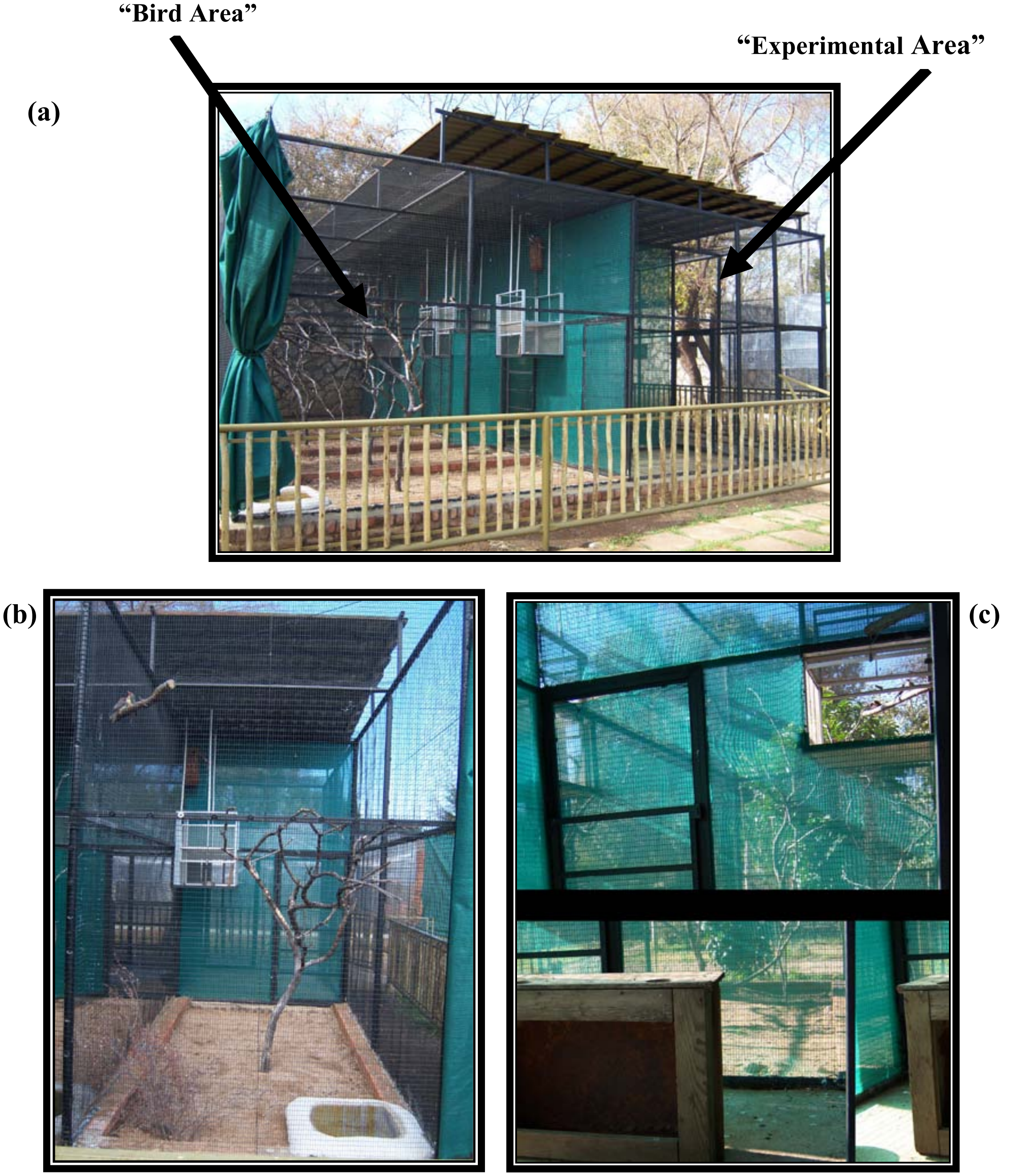


Fig. 4.1. Side-view of the oxpecker enclosure at the Mokopane Biodiversity Conservation Centre (MBCC), South Africa (a); One of the five "bird areas" where oxpeckers were held during non-experimental periods (b); One of the "experimental areas" where feeding experiments occurred. The food tray was placed on the wood box (c).

Fig. 4.2. Trap used for the passive capture of red-billed oxpeckers from the bird areas to move them into the experimental areas. Food dish set inside trap to lure oxpeckers into the trap (a); Oxpecker entering trap to feed from the maintenance food mixture (b).

Fig. 4.3. Oxpecker feeding from the tray used for cafeteria-style experiments. This tray has two sections with engorged female blue ticks and two sections with minced cattle meat.

Fig. 4.4. Dish on the donkey's back into which experimental food items were placed (a,b) and oxpecker feeding from blood dish (c).

Fig. 4.5. Red-billed oxpecker wound-feeding on donkey leg.

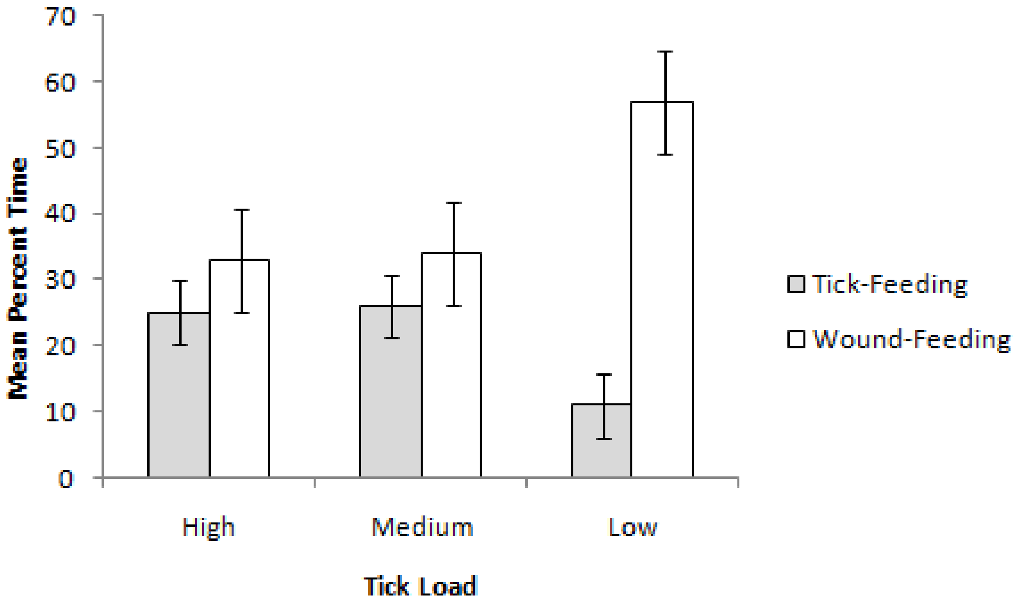


Fig. 4.6. Mean percentage of time (±SE) oxpeckers spent tick-feeding and wound-feeding under conditions of a high tick load (30 blue ticks), medium tick load (20 blue ticks), and low tick load (10 blue ticks).

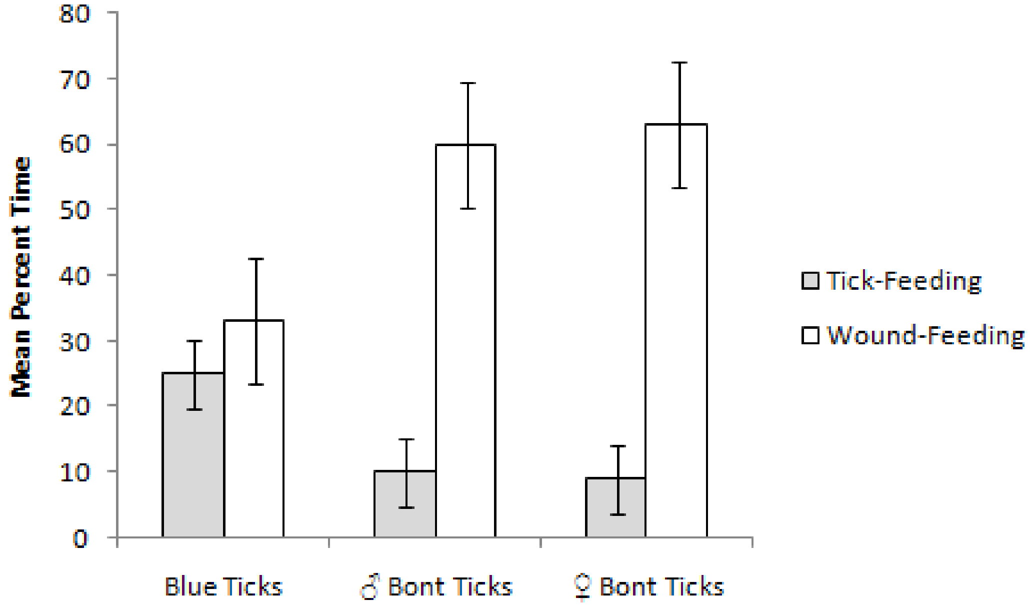


Fig. 4.7. Mean percentage of time (±SE) oxpeckers spent tick-feeding and wound-feeding when offered 30 ticks of a preferred tick species (blue ticks) and 30 ticks of a less preferred tick species (bont ticks).

CHAPTER FIVE

CHAPTER FIVE

The Oxpecker (*Buphagus* spp.) Diet: A Nutritional Analysis of Ticks and Blood

Summary

Oxpeckers (*Buphagus* spp.) have unique dietary habits. These birds coexist with the ungulates of sub-Saharan Africa, and their diet is almost entirely dependent on these large herbivores. Oxpeckers consume the earwax, lachrymal secretions, urine and dung of their mobile hosts. But the ticks and the blood of ungulate wounds compose the majority of the oxpecker diet. The aim of my study was to examine the nutritional composition of blue ticks, *Boophilus decoloratus*, and cattle blood and relate it to oxpecker feeding behavior. To determine their nutritional value, food types were analyzed for energy nutrients (protein, fat, fiber and gross energy), mineral content, and amino and fatty acid content. If diet selection is dominated by the need to meet nutritional criteria, then oxpeckers should prefer the most nutritional food source. The nutritional analyses revealed a few notable differences. The mineral values for ticks were higher than those for blood, with the exception of iron and chlorine. The selenium value for ticks was ten times that of blood, and blood had double the saturated fatty acids of ticks. The energy nutrient content of food types was relatively similar.

Background

Many animal species consume more than one food type. Competition for resources, seasonal and spatial variation in food availability, and differences in the nutritional content of food items can influence food consumption. In addition to observations of feeding behavior, nutritional analyses of food types can provide insight into the feeding activities of a particular species.

Oxpeckers (*Buphagus* spp.) are passerines in sub-Saharan Africa. They are unique birds, whose diet is almost entirely dependent on wild and domesticated ungulates. Oxpeckers are opportunists that feed on the flies, scurf cells, earwax, dung, urine, and nose, mouth and eye secretions of the ungulates with which they associate (Moreau 1933; van Someren 1951; Mengesha 1978; Bezuidenhout and Stutterheim 1980; personal observation). However, oxpeckers are believed to primarily feed on ixodid ticks parasitizing ungulates of the African landscape. In addition, oxpeckers sometimes feed on the blood of ungulate wounds. Oxpeckers are of interest because of these blood-feeding tendencies. The frequency of blood-feeding is unknown, as are the conditions favoring this behavior.

Birds have high basal metabolic rates (BMRs) and passerines notably have the highest rates of any vertebrate group (Tully *et al.* 2000). In comparison to a non-passerine of the same body size, the average BMR of a passerine is 50-60% higher (Tully *et al.* 2000). Stutterheim *et al.* (1988) determined the daily activity budget of oxpeckers in captivity (Table 5.1). Oxpeckers spend almost half of the diurnal period engaged in feeding activities. Oxpeckers have peak feeding periods in the morning and late afternoon (Stutterheim *et al.* 1988; Mengesha 1978), but are continuous diurnal feeders (personal observation).

The food items that compose the oxpecker diet have been identified, but the nutritional values of these food items are necessary to fully understand oxpecker feeding-behavior. Behavioral responses to fluctuating resource availability are critical to maintaining an organism's energy balance (Goldstein 1988). The nutritional content of ticks and blood may differ and influence oxpecker-feeding behavior. The energy stored in biomass is not the same across all taxa. Even when biomass is dried out to remove water, the dry matter differs in its energy content, depending on the type of organism. In an attempt to measure any discernible differences that may influence the food choices of oxpeckers, a series of nutritional analyses were performed on two components of the oxpecker diet: engorged blue ticks, *Boophilus decoloratus* (the preferred tick of oxpeckers; Stutterheim *et al.* 1988), and bovine blood. Because of a paucity of nutritional data, I designed a study to examine the nutritional composition of these two food items.

Methods

One hundred grams of frozen engorged female blue ticks (n=2) and 1.05 L of fresh cattle blood (n=1) were analyzed for nutritional content at the University of Pretoria's NutriLab, South Africa. Blood samples were obtained fresh from the abattoir and frozen ticks were provided by Intervet Malelane Research Unit in Malelane, South Africa and Clinvet International in Bloemfontein, South Africa. Food items were analyzed for dry matter (DM), moisture, crude protein (CP), crude fat (CFat), crude fiber (CF), gross energy (GE), mineral, amino acid and fatty acid contents. Values are reported on a dry matter basis.

Results

Energy Nutrients and Mineral Content

As expected, blood had a slightly higher moisture content than ticks (Table 5.2). The CP, CF, CFat and GE values were similar for ticks and blood (Table 5.3).

Ticks had higher amounts of calcium (Ca), phosphorous (P), magnesium (Mg), copper (Cu), manganese (Mn), potassium (K), and selenium (Se) (Table 5.4). The Se value for ticks was ten times that of blood. The zinc (Zn) value for ticks was higher than that for blood. However, the value may be inaccurate for the Zn because the two tick samples had very different levels (n_1=213.14, n_2=25.71). One of the tick samples may have been contaminated. Blood was only higher in iron (Fe) and chlorine (Cl) compared to ticks.

Amino Acids and Fatty Acids

Amino acid values were relatively similar across food types (Table 5.5). Glutamic acid is an exception, which was much higher for ticks. However, the two tick glutamic acid values were very different (n_1=9.06, n_2=90.01), possibly due to contamination of one of the samples. The percentage of fatty acids that were saturated, monounsaturated, and polyunsaturated are listed for each of the food types (Table 5.6). Blood had double the saturated fatty acids of ticks.

Discussion

A bird's environment plays a critical role in determining its thermoregulatory costs and energy expenditures (Beuchat *et al*. 1979). Food is a limiting resource in that environment, and changing availability of different food types can impact what is consumed. Many birds will opportunistically switch to a new food source that suddenly becomes abundant. Oxpeckers are dependent upon an unpredictable food resource, ticks, in terms of quantity, quality, space, and time. Although oxpeckers are dietary opportunists, they primarily switch between tick-feeding and wound-feeding behaviors. The nutritional composition of ticks and blood was examined in an attempt to discern any differences between these food types that may influence oxpecker feeding-behavior.

The results of these analyses indicate that the energy nutrient values (proteins, carbohydrates, and fats) per 100g were relatively similar for ticks and blood. Moreau stated it is “clear from stomach contents that the oxpecker is capable of digesting ticks completely so that their fats and proteins would become directly available, but the amount of nutriment available from that source would appear to be small.” The nutritional analyses, however, demonstrated that although engorged blue ticks are low in fat, they are highly proteinaceous.

According to Bezuidenhout and Stutterheim (1980), a captive oxpecker has a mean daily food intake of 14.7 g. Wild oxpeckers can consume up to 100 blue ticks per day, which is equivalent to approximately 20 g. A red-billed oxpecker (50 g) consumes up to forty percent of its body weight daily. Based on the results of the nutritional analyses, an oxpecker consuming 100 blue ticks would ingest 19.4 g protein, 0.4 g fiber, and 0.2 g fat per day. Female ticks of the family Ixodidae normally engorge 100 times their unfed body weight, and then drop from the host, produce and lay eggs, and die (Kaufman 2006). An unengorged and engorged female blue tick weighs 0.002 g and 0.2 g, respectively. Thus, a single female blue tick gorges 0.198 g of blood. An oxpecker consuming 100 blue ticks per day would ingest 19.8 g of blood. An oxpecker strictly blood-feeding from wounds would have to drink approximately 20 g of blood per day, an amount that may be greater than can be assimilated. In other words, an oxpecker may need the ticks' tissues in order to efficiently process blood. The impact of a liquid diet on oxpecker physiology is unknown and should be examined. It is possible that the physical composition of ticks and blood may influence oxpecker discrimination among these food types.

In addition to the physiological constraints of a blood-only diet, the energy an oxpecker expends searching for, removing, manipulating and consuming ticks may be greater than that expended when wound-feeding. A bird may have to ingest a larger quantity of ticks to balance this energy expenditure. A balance between energy acquisition and expenditure is crucial to animal survival, growth and reproduction (Goldstein 1988). Furthermore, parental oxpeckers would not be able to have a strictly blood diet. Male and female birds both participate in feeding their offspring, which involves passing food, ticks and occasionally insects, from their bill to that of the chick (Stutterheim 1982a; Plantan *et al.,* unpublished).

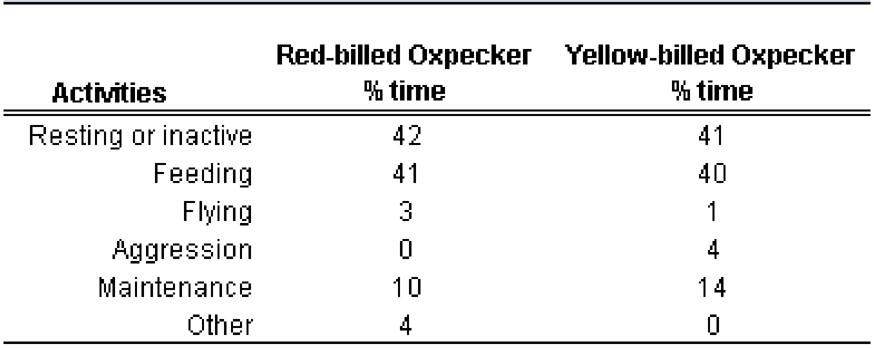
The mineral values of ticks and blood exhibited some differences. Ticks had higher values for all minerals except for Fe and Cl. The Se value for ticks was more than 10 times that for blood. Birds deposit Se into their eggs because it is necessary for embryonic development (Pappas *et al*. 2006). However, very high levels of Se deposited into eggs can lead to embryonic mortality (Pappas *et al*. 2006). The optimal Se concentration for oxpeckers is unknown, but perhaps blood does not provide a sufficient amount. Conversely, ticks may provide too much Se. Ca, which is needed in large quantities for egg production, is another mineral critical for reproductively active female birds. The Ca value for ticks was double that of blood. During breeding, female oxpeckers may especially need to ensure a high level of tick intake to maintain Ca levels required for egg-laying. The implications of a high Fe content in blood and a high P content in ticks are unclear. However, birds such as mynahs and starlings, which are grouped with oxpeckers in the Sturnidae family, can be susceptible to iron storage disease, a disease characterized by excessive amounts of iron in the liver. Excessive dietary intake of Fe has been postulated as a potential cause for this condition (Dierenfield *et al.* 1994; Crissey *et al.* 2000).

The amino acid values of food types were relatively similar with the exception of glutamic acid, which was higher for ticks. However, the glutamic acid values of the two tick samples were highly disparate, indicating the possible contamination of one of the samples. Blood had double the saturated fatty acids compared to ticks, which suggests that ticks would be more nutritional than blood, in this respect. However, very little is known about the fatty acid requirements of non-migratory birds, so it is difficult to draw conclusions.

Conclusion

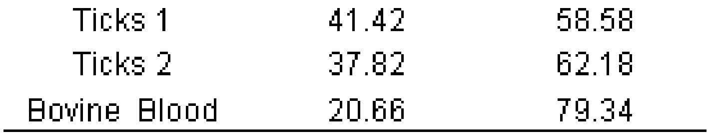
My findings suggest that ticks and blood have relatively similar energy nutrients and amino acid values, but the mineral and fatty acid compositions of the two food items differ. Ticks appear to have a higher overall nutritional value. Although oxpeckers prefer blue ticks, they also feed on at least eight species of ixodid ticks of four genera (*Amblyomma*, *Hyalomma*, *Boophilus*, and *Rhipicephalus*) which vary in size. Furthermore, the amount of blood gorged by these species is variable, dependent on tick species, sex, and developmental stage. Perhaps a mixture of tick species and tick stages can provide a complete set of nutrients for oxpeckers. Oxpeckers also consume many other food items, particularly earwax and flies, which may supplement their diet. It would be useful to analyze the other components of the oxpecker diet and compare the results to those of this study.

Table 5.1. Mean diurnal time spent by captive oxpeckers on various activities (n=1031); Table reproduced from Stutterheim *et al.* 1988.



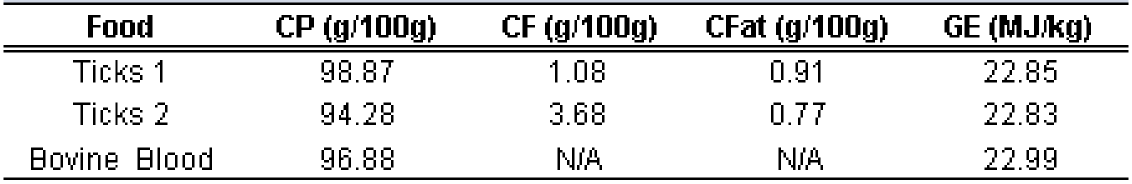
Activities	Red-billed Oxpecker % time	Yellow-billed Oxpecker % time
Resting or inactive	42	41
Feeding	41	40
Flying	3	1
Aggression	0	4
Maintenance	10	14
Other	4	0

Table 5.2. Dry matter and moisture content on an "as is" basis.



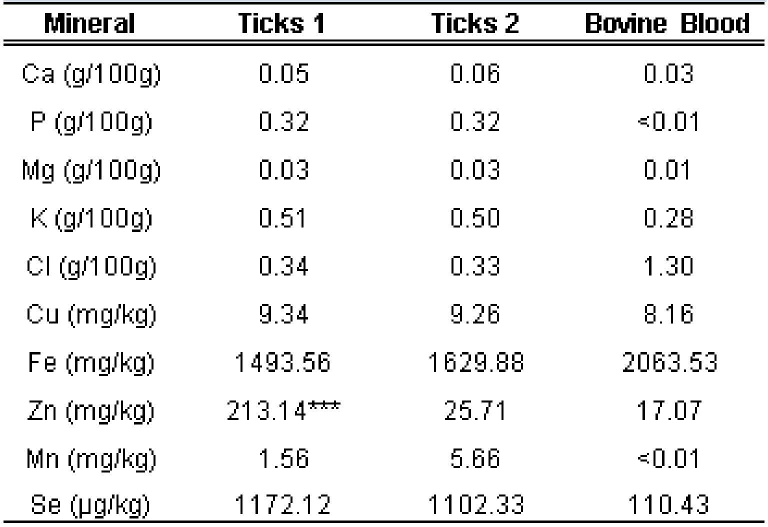
Food	DM (g/100g)	Moist (g/100g)
Ticks 1	41.42	58.58
Ticks 2	37.82	62.18
Bovine Blood	20.66	79.34

Table 5.3. Energy nutrients on a dry matter basis. CP = crude protein; CF = crude fiber; CFat = crude fat; GE = gross energy.



Food	CP (g/100g)	CF (g/100g)	CFat (g/100g)	GE (MJ/kg)
Ticks 1	98.87	1.08	0.91	22.85
Ticks 2	94.28	3.68	0.77	22.83
Bovine Blood	96.88	N/A	N/A	22.99

Table 5.4. Mineral content on a dry matter basis.



Mineral	Ticks 1	Ticks 2	Bovine Blood
Ca (g/100g)	0.05	0.06	0.03
P (g/100g)	0.32	0.32	<0.01
Mg (g/100g)	0.03	0.03	0.01
K (g/100g)	0.51	0.50	0.28
Cl (g/100g)	0.34	0.33	1.30
Cu (mg/kg)	9.34	9.26	8.16
Fe (mg/kg)	1493.56	1629.88	2063.53
Zn (mg/kg)	213.14***	25.71	17.07
Mn (mg/kg)	1.56	5.66	<0.01
Se (µg/kg)	1172.12	1102.33	110.43

*** possible contamination

Table 5.5. Amino acids presented on a % dry matter basis.

Type Amino Acid	Ticks 1	Ticks 2	Bovine Blood
Aspartic acid	8.43	8.65	9.76
Glutamic acid	9.06	90.01***	8.84
Serine	5.19	5.14	5.38
Glycine	3.87	3.82	3.71
Histidine	3.87	3.89	4.59
Arginine	4.01	3.97	3.93
Threonine	4.61	5.02	4.97
Alanine	6.86	6.78	6.55
Proline	3.93	3.89	3.53
Tyrosine	3.69	3.67	2.90
Valine	6.66	6.80	7.00
Isoleucine	1.49	1.44	0.88
Leucine	8.80	9.00	10.43
Phenylalanine	5.67	5.81	3.59
Lysine	7.22	7.70	8.52

*** possible contamination

Table 5.6. % Fatty acid content.

	Ticks 1	Ticks 2	Bovine Blood
Saturated Fatty acids	33.51	30.17	63.72
Monounsaturated Fatty acids	47.70	49.08	26.63
Polyunsaturated Fatty acids	18.79	20.75	9.65

CONCLUSION

Conclusion

One reason mutualisms are intriguing to study is that they often involve interactions between two highly disparate species. The interaction between oxpeckers and ungulates featured in my research is unusual in that these small passerine birds depend on such large African beasts for survival.

Researchers now recognize that the majority of mutualisms are subject to variable outcomes dependent upon the abiotic and biotic environment within which the interaction occurs. These context-dependent situations have been termed "conditional." Mutualisms can be conditional in two ways (Cushman and Whitham 1989): 1) Different mutualist species can vary in the quality of the services they provide to another species, or 2) The services provided by one mutualist species to another can vary with changes in the physical and biological setting.

The oxpecker-ungulate interaction involves inherent complexity, and my research was designed to test the hypothesis that the ectoparasite cleaning service provided by oxpeckers to their hosts is context-dependent. The results of my dissertation research demonstrate that yet another interspecific interaction, the oxpecker-ungulate association in sub-Saharan African, has conditional outcomes. Under certain conditions, the oxpecker-ungulate relationship is a nutritional mutualism where ungulates provide food (ticks) for oxpeckers in exchange for a cleaning service. Under other conditions, oxpeckers exploit their hosts to feed from their blood. The main conclusions of my research are:

1) Oxpeckers tick-feed and wound-feed in relation to both domesticated and wild ungulates in South Africa. In the wild, wound-feeding was not a common feeding behavior and was observed in relation to only buffalo, giraffe, and hippopotami.

2) In the wild, oxpecker feeding behavior was related to season with wound-feeding increasing in the dry season. In the dry season, populations of certain tick species decrease, indicating the possibility that oxpeckers increase wound-feeding behavior under times of nutritional stress (e.g. tick shortages).

3) The oxpecker-ungulate association has variable outcomes. Oxpeckers spent more time wound-feeding on donkeys with few ticks and with the tick species that oxpeckers do not prefer.

4) The presence of an abundant tick supply and/or the preferred tick species of oxpeckers does not completely suppress wound-feeding behavior. However, the length of time oxpeckers wound-fed was conditional on tick species and tick abundance per host.

5) Oxpeckers exploit pre-existing wounds, as well as create wounds. In Kruger National Park, South Africa, I never observed oxpeckers inflicting wounds on their hosts. I only observed them exploiting wounds. In captivity, oxpeckers inflicted wounds on donkeys with minimal effort.

6) Ungulates in both a wild and captive setting were generally tolerant of wound-feeding oxpeckers. However, in the wild, hosts were significantly more tolerant of non-wound feeding oxpeckers than wound-feeding oxpeckers.

7) In addition to tick-feeding and wound-feeding, oxpeckers spent a large amount of time feeding in the ears of their hosts in both the wild and in captivity. The nutritional benefits of earwax to oxpeckers, and the effect of earwax removal on the host, need exploring.

8) Tick abundance and tick species composition varied spatially in Kruger National Park, both dependent and independent of ungulate presence. Oxpecker-ungulate areas contained large numbers of ticks of the species that oxpeckers consume, indicating the potential for oxpeckers to benefit their hosts in areas where ticks, oxpeckers, and ungulates coexist.

9) Blue ticks, *Boophilus decoloratus*, and bovine blood have relatively similar energy nutrients and amino acid values, but the mineral and fatty acid compositions of the two food items differ.

Mutualisms are delicately balanced partnerships and are increasingly recognized as being fundamental to patterns and processes within ecological systems (Bronstein, personal communication). Changes to the ecological setting in which such interactions operate can disrupt this balance. By understanding the context-dependent nature of such associations, researchers can begin to understand how changes in the environment can have cascading effects on the entire community in which they occur (Bronstein, personal communication).

My findings suggest that a high abundance of the tick species and stages oxpeckers consume is necessary to maintaining an alignment of interests between oxpeckers and ungulates. The anthropogenic eradication of ticks, ungulates, and/or oxpeckers is predicted to have drastic cascading effects. The oxpecker-ungulate association in South Africa has been threatened before; populations of red-billed oxpeckers decreased and the yellow-billed oxpecker became locally extinct before being recolonized by populations from Zimbabwe (Sutterheim and Brooke 1981; Stutterheim 1982b; Mundy 1983; Hall-Martin 1987). Over-hunting coupled with chemical tick sprays and dips developed to treat ungulates against ectoparasites threatened the oxpecker. The results of my research show that when ungulates host few ticks of the species oxpeckers prefer, these birds will wound-feed to meet their nutritional demands. Such wound-feeding threatens the balance of the oxpecker-ungulate mutualism. Therefore, in addition to a basic understanding of the dynamics of interspecific interactions, biologists need to be aware of the relevance of conserving these natural associations and ensuring their maintenance. Once a critical component of the system is removed, the mutualism is subject to collapsing with often unpredictable consequences (Palmer *et al.* 2008).

Indeed, the relationship between oxpeckers and their hosts is not a consistently "mutually advantageous arrangement and in some instances has a sinister side (McAttee 1944)."

APPENDICES

APPENDIX A

Bird-Mammal Feeding Associations

Dean and MacDonald (1981) define a bird-mammal feeding association as *a bird intentionally approaching a mammal and thereby gaining some foraging advantage.* Using data accumulated over 15 years in southern Africa, Dean and MacDonald recognized more than 96 species in 37 families of birds exhibiting some form of feeding associations with mammals. They divide such associations into two categories: 1) bird and mammal eat the same general food or 2) bird uses the mammal to help it obtain food that only the bird requires. The various effects of these bird-mammal associations on the mammals involved include an increased chance of finding food, predator alarm calls, and tick removal (Dean and MacDonald 1981).

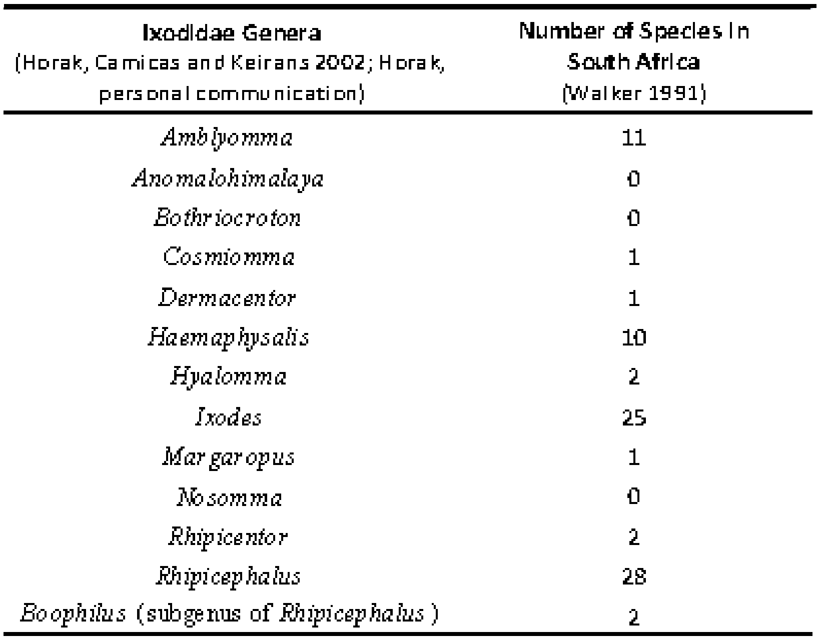
In South Africa, 23.6% of 318 passerines and 17.7% of 657 South African bird species consume arachnids (Rowan 1970). Six of the 43 African Sturnidae species are either obligately or facultatively dependent on mammals for food (Costello 2006/2007). The long-tailed starling *Lamprotornis mevesii* and fork-tailed drongos *Dicrurus adsimilis* follow elephants and forage on the ground behind them (Dean and MacDonald 1981). Cape glossy (*Lamprotornis* nitens), pied (*Spreo bicolor*), red-winged (*Onychognathus morio*) and pale-winged (*O. nabouroup*) starlings, Indian mynahs (*Acridotheres tristis*) and oxpeckers regularly glean ectoparasites from cattle in South Africa (Dean and MacDonald 1981; Costello 2006/2007). Dean and MacDonald suggest that the interspecific associations between Sturnidae and mammals likely began with the birds flushing insect prey, later perching on their hosts' backs, and eventually gleaning ectoparasites (Costello 2006/2007). Avian gleaners are restricted to open habitats with a high biomass of large mammals (Coe *et al.* 1976). Because starlings are of forest origin, their movement to open habitats would have been associated with a shift from a fruit diet to a diet of invertebrates (Costello 2006/2007).

Petney and Kok (1993) conducted an extensive study in which they analyzed the stomach contents from 239 species of South African birds, excluding oxpeckers. Only 36 ticks were found: 24 in 21 cattle egrets (*Bubulcus ibis*), 4 in helmeted guineafowl (*Numida meleagris*), 1 in crowned lapwing (*Vanellus coronatus*) and 7 in a single lilac-breasted roller (*Coracias audate*). With the exception of oxpeckers, birds as predators of ticks in South Africa appeared to be uncommon (Costello 2006/2007).

APPENDIX B

Characteristic	**Argasid**	**Ixodid**
Species	186 species belonging to four genera	692 species belonging to twelve genera
Common name	soft ticks (lack a scutum and are covered by a smooth and leathery integument)	hard ticks (scutum is present in all stages)
Size	smaller	larger
Eyes	eyes are usually absent	mostly one pair of eyes (no eyes on *Ixodes or Haemaphysalis*)
Capitulum	capitulum either subterminal or protruding from anterior margin of body in larval stages	capitulum anterior and always visible from dorsal view
Spiracles	spiracles between third coxae	spiracles behind fourth coxae
Life cycle	mostly one host ticks	one, two or three host ticks
Stages	egg, larval, several nymphal, male and female stages	egg, larval, one nymphal, male and female stages
Habitat	nest dwellers; inhabit dry and hot regions; have lower equilibrium humidity and higher critical temperature limits	inhabit open grasses and quest; have higher equilibrium humidity and lower critical temperature limits
Feeding	feed for short periods; adults feed several times with each feeding followed by egg production in females and sperm production in males	take several days to feed and require larger blood meals; females feed only once and oviposit once before dying; males do not gorge, but feed intermittently
Cement	do not form cement	most form cement to help secure tick in place of attachment
Engorgement	engorged females increase their weight 5-12 times	engorged females increase their weight 80-120 times
Parthenogenesis	occurrence of sporadic parthenogenesis in some species	occurrence of sporadic parthenogenesis in some species
Mating	fed or unfed males only mate off the host; males fully insert their hypostome, chelicerae, and palps into the genital opening of the female; several copulation acts	males must feed prior to mating, which only occurs on the host; most males only insert the tips of their chelicerae; usually do not become fully engorged unless mated
Reproductive strategy	females have a multigonotrophic cycle	females have a single gonotrophic cycle
Oviposition	can oviposit without a blood meal and lay several hundred eggs; females die after laying eggs on soil	cannot oviposit without a blood meal; lay thousands of eggs; females die after laying eggs on soil

APPENDIX C



Ixodidae Genera (Horak, Camicas and Keirans 2002; Horak, personal communication)	**Number of Species in South Africa** (Walker 1991)
Amblyomma	11
Anomalohimalaya	0
Bothriocroton	0
Cosmiomma	1
Dermacentor	1
Haemaphysalis	10
Hyalomma	2
Ixodes	25
Margaropus	1
Nosomma	0
Rhipicentor	2
Rhipicephalus	28
Boophilus (subgenus of *Rhipicephalus*)	2

APPENDIX D

Blood-Feeding Birds

Bird-host relationships are not uncommon. Perhaps among the most recognized are cattle egrets (*Bubulcus ibis*) and cowbirds (*Molothrus* spp.) perching on the backs of large herbivores and feeding on insects flushed by the herbivores, ground finches (*Geospiza fuliginosa*) gleaning ticks from marine iguanas, and Egyptian plovers (*Pluvianus aegyptius*) picking leeches and small bits of food from crocodile teeth. Fork-tailed drongos *Dicrurus adsimilis* and piapiacs *Ptilostomus afer* accompany elephant herds hawking insects dislodged by the large beasts (Rice 1963). Scrub jays (*Aphelocoma coerulescens)* have been observed cleaning ectoparasites from Columbian black-tailed deer, and black-billed magpies (*Pica pica*) consume the ticks hosted by elk and mule deer (Isenhart and DeSante 1985). Roughly 50 species of birds prey on ticks (Samish 2000). However, the oxpecker is rare in that it not only gleans ticks from an array of African ungulates, but it is one of the few avian species known to blood-feed on live animals. Oxpeckers are often called "vampire birds" in an attempt to compare them to the vampire bats of the New World. As is the case with the oxpecker, cattle, sheep, pigs, goats, and horses are all "attacked" by vampire bats (Greenhall, Schmidt, and Lopez-Forment 1971). However, unlike vampire bats, whose sole diet is blood, oxpeckers consume a variety of ectoparasites of which blood is only a component.

The kea parrots (*Nestor notabilis*) of New Zealand are renowned for their attacks on live sheep. But like the oxpecker, the validity of the blood-feeding tendencies of the kea has been questioned. Just as the creation of wounds by oxpeckers has been a source of controversy, the activity of keas preying on their hosts has been debated. In the late

1860s, the first documentation of keas attacking sheep was made (Jackson 1962). A notable difference between the kea and the oxpecker is that the normal diet of the kea is nectar, berries, grubs and beetles, roots and buds (Jackson 1962), whereas the oxpecker's traditional diet includes blood in the form of engorged ticks. The kea only secondarily learned to feed on carrion hanging at slaughterhouses and then live prey, whereas the oxpecker's historic diet is engorged and unengorged ticks (Hanson and Morrison 1991).

In addition to the keas, a handful of bird species are known to drink blood from live hosts. Among them are magpies (Schorger 1921) and the sharp-beaked ground finches (*Geospiza difficilis*) and Galapagos mockingbirds of the Galapagos Islands (Curry and Anderson 1987). Magpies exploit wounds and pluck at swellings and tumors on cattle hides to feed on the tissue and blood beneath. Mockingbirds drink blood from living sea lions, marine iguanas, and masked boobies (Curry and Anderson 1987).

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