

DISSERTATION

DUNG BEETLES AND THEIR NEMATODE PARASITES AS  
ECOSYSTEM ENGINEERS AND AGENTS OF DISEASE

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## ABSTRACT

### DUNG BEETLES AND THEIR NEMATODE PARASITES AS ECOSYSTEM ENGINEERS AND AGENTS OF DISEASE

Dung beetles (Order Coleoptera, Subfamily Scarabaeoidea), are a magnificent group of insects noted for both their physical beauty and ecologically significant role in parasite suppression and agricultural management. These insects feed on feces in both their larval and adult forms and are classified into one of three groups based on the way they procure fecal resources to their young. Paracoprid dung beetles collect chunks of feces and bury them in tunnels/nests dug directly below the site of deposition, telocoprid beetles create carefully crafted balls of dung and roll them away from the pat before burying them in underground nests, and endocoprid beetles create nests in the feces without moving it from the original deposition site. Because dung beetles interact with feces on a regular basis, and because many parasites use feces as a medium for distributing their eggs, it is not uncommon for dung beetles to come in contact with parasite propagules at a rate higher than that seen in other animals.

While the majority of parasite propagules cannot survive consumption by a dung beetle, several nematode species have found a way to use these insects as their intermediate hosts. After being consumed by a dung beetle, both *Streptopharagus pigmentatus* (found throughout East Asia) and *Physocephalus sexalatus* (found in the Southeastern United States) migrate from the dung beetle's digestive tract into the body cavity of the beetle where they wait to be consumed by their final host. Trophically transmitted parasites often change the behavior of their host and examples of behavioral modification have been found in most major host taxa. This study asks

whether these two nematodes alter dung beetle behavior in ways that affect transmission dynamics and/or their role as ecosystem engineers.

Indeed, when nematode infections are present, both paracoprid and telocoprid beetles reduce their feeding rate and interaction with feces. Paracoprid beetles (from the genus *Onthophagus* and *Phanaeus*) infected with nematodes consume only half of the feces consumed by uninfected beetles. Further studies on beetles from the genera *Phanaeus* indicate that these beetles also bury less feces and do so at a shallower depth. This is relevant because dung beetles are responsible for removing and processing the majority of fecal material in both agricultural areas and forest ecosystems. Feces serve as breeding grounds for many insect pests and carry nutrients that, when buried by dung beetles, are essential for plant health. I show that both *S. pigmentatus* and *P. sexalatus*, through alterations in their host's behavior, alter the availability of fecal resources to both plant and animal communities and should therefore be classified as an ecosystem engineers.

Telocoprid beetles infected by the nematode parasite *P. sexalatus* also exhibit reduced feeding behavior and provide a unique opportunity to quantify the fecal resources provided to young. It is not uncommon for males and females of many host to respond to infection in different ways and indeed that is what we see in telocoprid beetles from the genus *Canthon*. Both *C. pilularis* and *C. nigricornis* females show reduced feeding whereas this difference in consumption is not present in male beetles. Male beetles of both *C. pilularis* and *C. nigricornis* consistently make smaller brood balls than beetles that are not infected. Because of the direct correlation between adult size and fecal resources available at emergence, this type of behavioral modification in adults is relevant to the outcome of competitive interactions seen in future offspring.

Typical tests for predator avoidance (use of shelter, use of substrate, and activity level) did not indicate a change behavior based on infection for any of the species studied. The fact that activity, shelter use and substrate use are not altered is interesting given that a large number of parasites with complex lifecycles play an active role in increasing the probability of transmission. While this study asks if the most common forms of predator avoidance in dung beetles are changed by parasites, it is not exhaustive and it is possible that the parasite alters its host's predator avoidance behavior in some other way.

Uninfected dung beetles are extremely beneficial and a variety of countries have actually imported exotic dung beetles because of the services they provide. Some of the most common and beneficial dung beetles in the United States (*Onthophagus taurus* and *O. gazelle*) were introduced from Asia and Africa in the early 1980s and continue flourish throughout the country. These insects are so important in agricultural and disease management and are believed to save the American cattle industry \$380 million per year. It is therefore imperative that we understand how the parasites transmitted by dung beetles alter host behavior, especially if the behavioral changes affect their role as ecosystem engineers.

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## CHAPTER 1 – LITERATURE REVIEW

Changes to host behavior as a consequence of infection are a common feature of host-parasite interactions (Moore, 2002). Altered behaviors may include altered responses to light (Cezilly, 2000; Tain *et al.* 2007), altered activity level (Adamo *et al.* 1997), and even altered foraging habits or choice of food (Khan, 1988; Adamo *et al.* 1997). These changes in host behavior are frequently cited in relation to parasite transmission (Moore, 1984; Lafferty, 1992) but can also have ecological effects that extend beyond the host-parasite system. Despite being small, parasites often account for a significant proportion of the biomass in natural ecosystems (Minchella and Scott, 1991; Kuris *et al.*, 2008) and serve as a food source for many animals, giving them great potential for affecting disease transmission in addition to food web properties (Johnson *et al.*, 2010). Thomas *et al.* (1999) suggest that parasites have the potential to serve as ecosystem engineers through phenotypic alterations in their host, and there is growing recognition of the important role manipulative parasites play in the ecology of natural ecosystems (Lefevre *et al.*, 2008). Nonetheless, the broader impact that parasite manipulators have on ecological processes is rarely examined; these impacts can include rates of decomposition, or energy flow along food chains (Lefevre *et al.*, 2008).

This study tests the prediction that nematode parasites of dung-feeding beetles alter aspects of intermediate hosts' behavior that make them more conspicuous to the final hosts (where parasites mature and eventually reproduce). The ecological roles of both dung beetles and parasites as modifiers of dung beetle behavior are also explored. I argue that dung beetles are ecosystem engineers in and of themselves, and that parasites become ecosystem engineers when they modify the dung processing behavior of their host. This study is novel because it addresses the effects of parasite induced behavioral change on transmission and ecosystem

function. Furthermore the parasites of interest use ecologically sensitive hosts including the Japanese Macaque, which is on the Red List of Endangered Species, and the feral hog, a financially and ecologically significant invasive species.

### **Ecosystem Engineering as a Concept**

The concept of organisms as ecosystem engineers was first introduced in 1994. Jones *et al.* (1994, 1997) describe these organisms as those that directly or indirectly modulate the availability of resources to other species by altering physical surroundings or changing the flow of resources, thereby creating and/or modifying habitat. A key characteristic of ecosystem engineers is that they must change the availability (quality, quantity, or distribution) of resources used by other taxa. There are two types of engineers currently defined: autogenic engineers, which are those that change the environment via their own living or dead tissues, and allogenic engineers, which transform living or non-living material from one state to another by mechanical means.

All organisms modify their environments to some extent, and the concept of ecosystem engineering has been criticized as trivial because of this (Reichman and Seabloom, 2002). However, unlike other organisms, ecosystem engineers are thought to change their habitat in relatively large scale ways that have critically important and influential outcomes for community and ecosystem processes. In this regard ecosystem engineers resemble keystone species (Paine 1966, 1969) as both have a disproportionate ecological effect when compared to other organisms. Jones *et al.* (1994) distinguishes ecosystem engineers from keystone species by suggesting that ecosystem engineers have a profound, but not necessarily positive, effect on

biodiversity. Despite organizational schemes that group keystone species and ecosystem engineers into different categories, it is important to note that both ecosystem engineers and keystone species affect processes influencing the distribution and abundance of organisms, in addition to the relationships between organisms and their environment.

Lawton (1994) combines the concepts of keystone species and ecosystem engineering, pointing out that keystone species are often considered to be relevant because they change the impact of engineers. For example, sea otters are considered both keystone species and engineers because they change the density, and therefore the impact, of sea urchins, which in turn directly influence the health of kelp forests and the species dependent on those forests. Seeing each individual as a part of the whole ecosystem and documenting the trophic consequences of each animal's behavior allows for a better understanding of the interactions that are relevant to management or conservation, and that are necessary for a full understanding of the system.

Beavers (*Castor canadensis*) were one of the first animals described as allogenic ecosystem engineers and their ecological role has been thoroughly documented. They cut down trees and build dams, which alters the hydrology of an area by creating ponds. This modifies nutrient cycling and decomposition dynamics, along with influencing the character of water and organic materials transported downstream. Plant communities change as a result of these ponds. So ultimately, beavers indirectly affect the stability and diversity of the plant and animal communities. In this way, beavers are an excellent example of ecosystem engineers (Jones *et al.*, 1994).

Given the physical disturbance caused by elephants (*Loxodonta africana*) as they destroy trees and shrubs, elephants are also ecosystem engineers under the initial definition (Jones *et al.*,

1994). The widespread vegetation changes caused by elephants alter fire regimes, which affect the food supply and population dynamics of other animals. In terrain altered by elephants, fire has more fuel and burns more intensely than it does in other areas. This changes soil formation, riparian zones, and the flow of chemical elements/compounds between organisms, making elephants a critical part of the entire ecosystem.

While environmental engineering may have both positive and negative effects on biodiversity, Crain and Bertness (2006) stress the importance of the overall community effect and how information in this area can be used to aid conservation efforts. They argue that engineers modifying limited resources or constraining variables within an ecosystem have the greatest impact and should therefore be the focus of ecosystem engineering research. Many organisms cannot live in their native communities without the habitat provided by ecosystem engineers (Crain and Bertness, 2006). Thus, understanding the role of ecosystem engineers and the subsequent effects they have on organismal groups is important. While traditional conservation efforts focus on charismatic megafauna, smaller organisms such as insects and parasites are also vital for the maintenance of a community and their roles should also be documented. Ecosystem engineers set the stage for communities and ecosystems to perform their services, be they creation of habitat, sustained biodiversity, nutrient cycling, forest regeneration, etc.

Organisms will and always have played an important role in modifying the environment so this is not a new phenomenon. All organisms engage in ecosystem engineering to some extent and it is difficult to imagine an organism that does not in some way alter its environment (Wright and Jones, 2006). Darwin (1881) documented the relevance of the earthworm's actions in soil formation long before the concept of ecosystem engineering was developed. Despite

these criticisms, Write and Jones (2006) argue that the field of ecosystem engineering is relevant because it encourages people to develop conceptual tools and uncover general patterns associated with behavioral processes. Those encouraging the development of this field hope that the tools created will allow for ecological modeling and the ability to predict the impact of various groups. While understanding the role of individual species is an important first step, a multispecies perspective is absolutely essential for a good understanding of trophic interactions and any animal's role in the ecosystem.

In order to predict when and where ecosystem engineers will have the greatest effect Jones *et al.* (1994) propose six criteria to assess their impact: (1) lifetime *per capita* activity of individual organisms (2) population density (3) local and regional distribution of the population (4) length of time a population has been present at a site (5) durability of constructs in the absence of the original engineer (6) number and types of resource flows that are modulated by the constructs or artifacts, and the number of other species dependent on these flows.

Despite the limited durability of dung beetle burrows, dung beetles are relevant ecosystem engineers because of their lifetime *per capita* activity, population density, and ability to alter the flow of dung and soil nutrients which are essential to a variety of plant and animal species. I will show that both a variety of dung beetle species and also their nematode parasites, which alter the beetle behavior, act as important ecosystem engineers. The extent to which each of these animals acts as an allogenic ecosystem engineer, and the ways in which they alter their community are explored in both the warm temperate forests of Yakushima Japan and the coastal plains regions of Coastal Plains region of Texas.

## **Dung Beetles as Ecosystem Engineers**

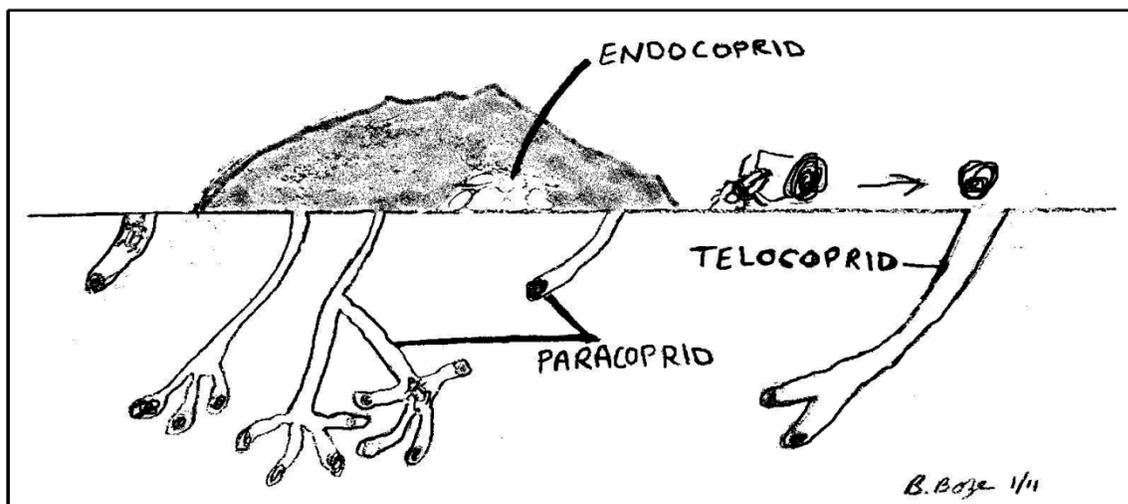
Dung beetles belong to the taxonomic order Coleoptera, which includes the family Scarabaeidae (~5000 species), subfamily Aphodiinae (~1800 species), and the subfamily Geotrupinae (~300 species). The scarcity of dung beetle species, relative to many other insect groups, is often in great contrast with the abundance of individuals (Hanski and Cambefort, 1991). Seeing that dung is such a valuable resource, there are many species from other taxa (Lepitoptera and Diptera) that use dung at some point of their lifecycles. Dung beetles are typically better competitors than these other animals because of their ability to move feces from its original deposition site.

Dung beetles are classified as such because they feed on the microorganism-rich components of vertebrate feces in both their larval and adult forms. They have sucking/lapping mouthparts and typically search for food by direct straight line flight close to the ground in a normal long-distance cruising pattern. When food is encountered they switch to a zig-zag pattern of close range search and land directly on or near the fecal pat of interest (Halffter and Matthews, 1966). Adults feed and then remove feces to create brood balls which are buried underground and used to provide nutrients to young. Both sexes participate in the nidification and brooding process. Females are typically responsible for burrowing while the males gather feces for sustenance and brood ball creation (Gullan and Cranston, 2000).

Dung beetles are divided into three groups based on the way they procure fecal resources for their young (Figure 1.1). Paracoprid dung beetles, also known as tunnelers, bury brood balls in chambers below the original deposition site. Telocoprid beetles, called rollers, remove a chunk of feces from the original pile and roll it away from the source before burying it.

Endocoprid species, called dwellers, brood their eggs in the original fecal mass without moving the dung from its original location (Halffter and Edmonds, 1982). In each case, the eggs are laid in dung and the developing larvae feed off the fecal resources provided by their parents. Adult dung beetles are holometabolous and typically iteroparous. Because the eggs of most dung beetles are quite large, and their production requires great amounts of, beetles spend several hours creating brood balls and tunnels which help prevent predation and desiccation of young.

The fecal material provided by parents is extremely important as it is the only nutritional resource larvae consume, and it makes up the only environment encountered during the first months of life. Some scarabs are generalist and will consume/use any feces encountered while others specialize on feces according to texture, moisture, age, fiber content and even host species (Gullan and Cranston, 2000). As a general rule, feces from omnivores are more desirable than feces from herbivores and carnivores.



**Figure 1.1:** Dung beetles are divided into three groups based on where they oviposit and how they procure fecal resources for their young. Endocoprid species lay their eggs directly in the fecal pat, paracoprid species lay their eggs in fecal balls buried directly below the fecal pat, and telocoprid species remove chunks of feces from their original location and then lay their eggs in fecal balls below ground.

While not typically referred to as ecosystem engineers, dung beetles provide varieties of ecosystems functions, and affect a multitude of species through their processing of fecal material. Fincher *et al.* (1971) summarize the importance of dung beetles in four major areas of research. They (1) are vectors of parasites and animal diseases in both domestic and wild animals, (2) remove and decompose organic waste material, returning nutrients to the soil that would otherwise be lost by volatilization or tied up in fecal pats, (3) reduce pests such as the horn fly (*Haematobia irritans*) which breeds in cattle feces, and (4) prevent parasitism in livestock by rapid removal of feces containing eggs and larvae of internal parasites, thereby reducing the contamination of surface soil and herbage. This study will focus primarily on the first and second topics by assessing the role of dung beetles as intermediate hosts and as removers of organic waste material. In order to fully understand the ecological role of dung beetles we must first understand the behaviors associated with the act of burying feces and the consequences of these actions. Burying behaviors associated with feces aid ecological processes including but not limited to maintenance of habitat and pest reduction.

From an ecosystem perspective, nutrient cycling is one of the most important acts carried out by dung beetles. Soil health is often assessed through measurements of carbon and nitrogen content, and Vitousek *et al.* (1997) state that nitrogen is one of the critically limiting elements structuring plant communities. Most nitrogen loss occurs through ammonia (NH<sub>3</sub>) volatilization which dung beetles prevent by burying dung below the surface where soil moisture tends to be slightly elevated (Gillard, 1967). Dung beetles also affect the nitrogen cycle by accelerating soil mineralization rates. Both mineralization and volatilization are bacteria-mediated processes, which are increased as beetles create a more aerobic environment via their movement in and around feces (Lussenhop *et al.*, 1980). Several studies suggest that dung beetles elevate nitrogen

and carbon levels in the upper surfaces of soil as well as lower levels where feces are buried. The increased nitrogen and carbon levels stimulate bacterial growth and are responsible for promoting nitrogen-mineralization which increases soil productivity (Yokoyama and Kai, 1991). In some cases the results of nutrient mobilization by dung beetles on plant growth are more effective than chemical fertilizers (Miranda *et al.*, 2000). Nichols *et al.* (2007) believe that dung beetles, in addition to affecting soil fertility and parasite transmission dynamics, also contribute to the timber and non-timber forest production industries and reforestation/restoration projects. Since dung beetles directly affect soil components, and ultimately the whole plant community, they should indeed be classified as ecosystem engineers.

While dung beetles are not the only group of animals that use feces I argue that they are the most important. A variety of explanations have been put forward to explain the relationship between species diversity and ecosystem function. One such idea is supported by the redundant species hypothesis (Walker 1992, Lawton and Brown 1993) which suggests that there is a minimal number of species required for basic ecosystem function but that a variety of species carry out the same role and are therefore redundant. Erik Stockstad (2004) reviews this hypothesis by looking at the role of dung beetles as dispersers of seeds in feces and finds that species diversity is not a safeguard against ecosystem collapse. Nichols *et al.* (2007) also reports that decreased dung beetle diversity leads to a surplus of dung, increased seed predation and a reduction in buried or naturally planted seeds. When larger dung beetle species disappear, smaller species are not capable of reducing the surplus dung that remains on the surface of the soil. This shows that larger dung beetles play a surprisingly important role in ecosystem function. While other insects use feces, and birds or worms can bury seeds, dung beetles are

seen as extremely important for ecosystem integrity as no other animal is as efficient in carrying out these activities.

A large proportion of dung beetle research has focused on dung beetles in agricultural or natural ecosystems in the context of parasite suppression. The vast majority of parasite eggs cannot survive consumption by dung beetles, a fact that benefits those working in the domestic animal and/or food trade industries. Based on estimates published by Fincher (1981) and Anderson *et al.* (1984), Losey and Vaughn (2006) estimate an annual dung beetle value of \$380 million per year in the United States pastured cattle industry. This estimate includes reduced use of fertilizer, minimized parasite transmission, and less damage to livestock from pestiferous flies. Dung beetles are such efficient removers of feces that exotic dung beetles are often introduced to new areas for this purpose (Anderson and Loomis, 1978). While dung beetles do significantly decrease pestiferous flies and enteric parasites in herbivorous animals like cattle (Bryan, 1976) and elk (Bergstrom, 1983), they also serve as intermediate hosts for some nematode parasites and aid in transmission of diseases to avian and mammalian hosts such as monkeys, racoons, hogs and other animals that consume the beetles (Alicata, 1935). Both the stomach worm of apes and monkeys (*Streptopharagus pigmentatus*) and the stomach worm of hogs (*Physocephalus sexalatus*) are transmitted in this manner and are therefore the focal parasites of this research.

### **Parasites as Ecosystem Engineers**

Parasites affect ecosystem function as profoundly as any element in the system and their roles are as abundant, complex, subtle and important as any other (Price *et al.*, 1986). The direct and indirect ways in which parasites alter the abundance of other species are many, and removal

or altered behavior due to parasitism often changes the relative composition of the whole community (Minchella and Scott 1991, and Poulin 1999). There is evidence that parasites can influence a diversity of processes including mate choice and sexual selection (Hamilton and Zuk 1982, Howard and Minchella 1990), population regulation via altered birth and/or death rates (Anderson and Gordon 1982, Thomas *et al.* 1995), spatial distribution (Anderson, 1972), increased energetic demand and altered flow of energy through food webs (Lefevre *et al.*, 2008), and even interspecific and intraspecific competition (Park, 1948). When changes such as these have a genetic basis, parasites can even lead to an evolutionary change in the population (Thomas *et al.*, 2000).

By altering the life history traits of their hosts, parasites also alter population dynamics and community structure. Robert Poulin (1999) summarizes the three basic mechanisms by which parasites alter communities of free-living organisms. Parasites can (1) have different pathological effects on different host species and alter the relative abundance of each, (2) alter the functional importance of their host species in the community, and (3) indirectly increase the functional importance of their host species by inducing alterations in host phenotype (behavior, morphology and/or physiology).

Manipulated hosts exhibit characteristics associated with uninfected individuals but also display characteristics uniquely associated with infected individuals (Lefevre *et al.*, 2008). These altered behaviors are often the cause of new associations, both direct and indirect, between and within species. Some of the most common effects of parasites on invertebrate host behaviors include changes in activity and altered elevation seeking behavior. For example, the trematode *Microphallus papillorobustus* has been shown to split its amphipod host (*Gammarus insensibilis*) into two distinct communities with one residing on the surface of the water and one

near the bottom. These two groups not only differ in spatial distribution but also differ in density, fecundity, physiology, and intermolt duration. This is relevant from an ecological standpoint because it affects *Gammarus* mating habits in addition to avian health. Birds, which serve as the final host for *M. papillorobustus*, are more likely to predate amphipods on the surface of the water than amphipods below the surface, and therefore experience higher exposure to this parasite (Ponton *et al.*, 2005). This study alone shows the contribution of parasites to our understanding of their interconnectedness with larger animals and their ecological significance.

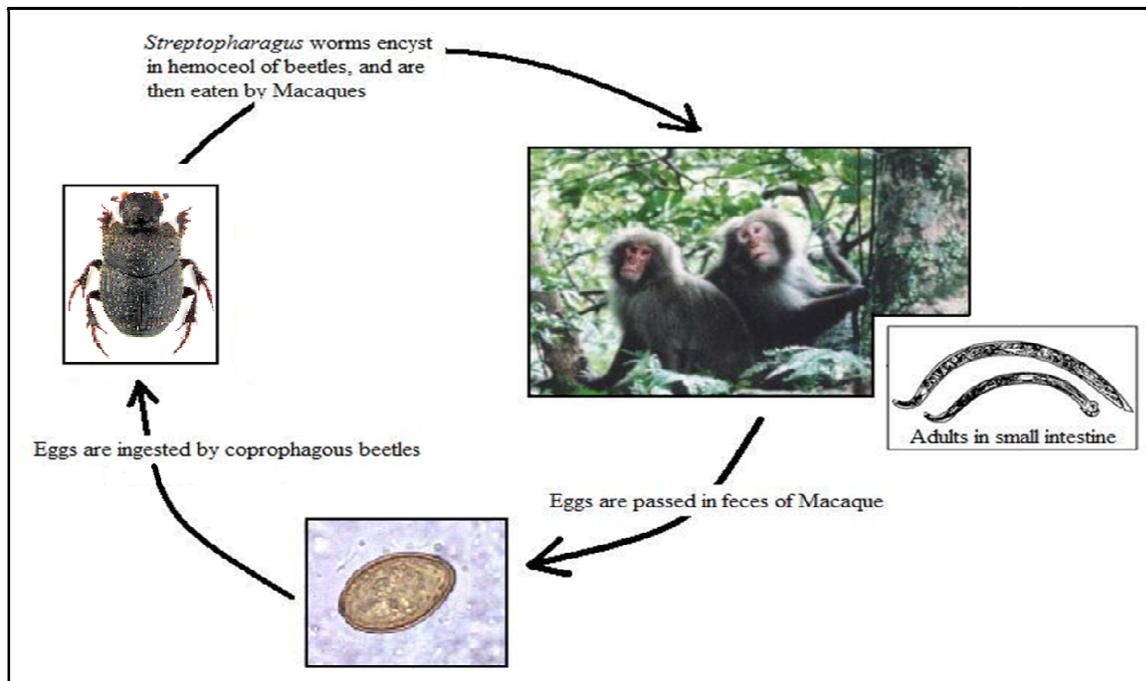
Many parasites act as ecosystem engineers by modifying food webs and energy flow. They can do this by strengthening trophic interactions related to transmission, affecting trophic lengths not directly related to transmission, and creating novel interactions which often provide food and/or habitat. One of the best examples of parasites affecting ecosystem ecology via trophic links that are not involved in transmission involves the isopod *Caecidotea communis* and the acanthocephalan parasite *Acanthocephalus tahlequahensis*. When the isopod, a detritivore, is infected with *A. tahlequahensis*, its consumption rates decrease significantly. The relevance of this behavioral change is most apparent in the fall when leaf litter fails to be broken down and there is a build-up of non usable material in the streams (Hernandez and Sukhdeo, 2008).

Arneberg *et al.* (1996) was one of the first to show that gastrointestinal nematodes could have an important role in ecosystem function because they modify their environment through altering herbivore appetite. Altered feeding behavior resulting from infection are not uncommon and have been documented in a variety of taxa (Moore, 2002). Several nematode parasites use coprophagous beetles as intermediate hosts and could alter their host feeding behavior with consequences that are not unlike those found in the 1996 study by Arneberg *et al.* Until now few studies have tested whether parasites alter the behavior of coprophagous beetle hosts.

The mere existence of manipulative parasites creates at least two distinct groups within a given species, each group having its own ecological traits (Lefavre *et al.*, 2008; Poulin, 2006; Thomas *et al.*, 2005). Measuring the functional importance of any species is virtually impossible because it requires complete removal from the system. However, using laboratory experiments we are able to document the role of parasites in predator avoidance behaviors. This study aims to document and measure the effect of nematode parasites by comparing behavioral changes associated with infection in two nematode parasite-coprotophagous beetle systems which are described below. The first system involves *S. pigmentatus* which resides in the stomach and small intestine of monkeys in Japan, and the second system involves *P. sexalatus* which resides in the stomach of swine throughout Texas and the southeastern United States.

### ***Streptopharagus pigmentatus*: A Trophically Transmitted Parasite of Primates**

*Streptopharagus pigmentatus* (Nematoda, Spirocercidae) are generally found in the stomach or upper part of a monkey's small intestine and are not believed to be a major pathogen, although the mere presence of *S. pigmentatus* can cause intestinal blockage when infections levels are high (Itoh *et al.* 1988). Adult males (30-35 mm) are typically smaller than females (45-55 mm) and both exhibit a hexagonal mouth with four submedian cephalic papillae and two lateral amphids (Machida *et al.* 1978). Thick shelled embryonated eggs (~ 35 x 18 µm) are shed with primate feces and complete their lifecycle after being consumed by a coprophagous beetle and then once again being consumed by a primate (Jessee *et al.* 1970). (See Figure 1.2).



**Figure 1.2:** Lifecycle of *Streptopharagus pigmentatus* (Ph. Nemata). Eggs are passed from host's body with primate feces. Embryonated eggs are then ingested by coprophagous beetles where larval nematodes develop, encyst, and then wait to be ingested by a primate.

Araki *et al.* (1977) showed that dung beetles are indeed the intermediate host for this parasite as his team succeeded at infecting Japanese Monkeys with larvae from *Geotrupus laevistriatus* and several beetles from the genus *Onthophagus*. Gotoh (2002) identified additional beetles from the genus *Onthophagus* and *Aphiodius* suspecting that they, and cockroaches, can also serve as intermediate hosts for *Streptopharagus pigmentatus*. Once inside the body cavity of a coprophagous beetle, *S. pigmentatus* undergoes two molts. These larvae create a cyst which extends viability and allows them to wait for their final host to consume them. Cysts, 0.75-1.25 mm in diameter, are typically spherical and contain one coiled larva although cysts containing two or three have been observed (Machida *et al.* 1978). On occasion, the cysts stick together forming a congregated bunch. Juvenile larvae have transverse striations

on their cuticle and resemble adults with four submedian papillae and two lateral amphids. Larvae have 20-30 irregularly arranged spines on the posterior end and are practically identical to those of *Physocephalus sexalatus* which was described by Alicata in 1935 and is the other parasite of interest in this study (Machida *et al.* 1978).

*Streptopharagus pigmentatus* uses several species of dung beetle as their intermediate host and can infect a variety of Old World Monkeys. The first part of this study focuses on worms and beetles interacting with a subspecies of Japanese Macaque (*Macaca fuscata yakui*) located on Yakushima Island Japan. The study troop, named Umi, consists of 43 individuals, all of which are infected by *S. pigmentatus* to varying degrees. Both nocturnal and diurnal dung beetles from the genus *Onthophagus* are found on the island and approximately half of the beetles carry encysted *S. pigmentatus* larvae. The abundance of beetles, in addition to the approximately equal distribution of infected and uninfected beetles, provides a suitable system for studying behavioral changes associated with parasitism.

In addition to being a good system for studying behavioral change, the final host in this system has provided much about our basic understanding of primate social behavior (Huffman, 1991) and they are widely known for their propensity toward generating cultural behaviors in response to environmental changes: e.g. stone handling (Huffman, 1996; Nahallage and Huffman, 2007) and potato washing (Kawai, 1965). Despite the fact that these monkeys are revered and protected by law, the International Union for Conservation of Natural Resources (IUCN) World Conservation Union's Species Survival Commission has placed them on the "Red List" of threatened species. While there is no current reason to suspect *S. pigmentatus* as a major pathogen, when the infection becomes intense the mere presence of the worms can cause intestinal blockage and may act in concert with other pathogens to make monkeys sick. In

addition to *Streptopharagus pigmentatus*, at least one pathogenic nematode, *Gongylonema pulchrum*, has the potential to use dung beetles as intermediate hosts. Understanding the behavioral changes associated with pathogen transmission in this system may be vital to Japanese macaque health.

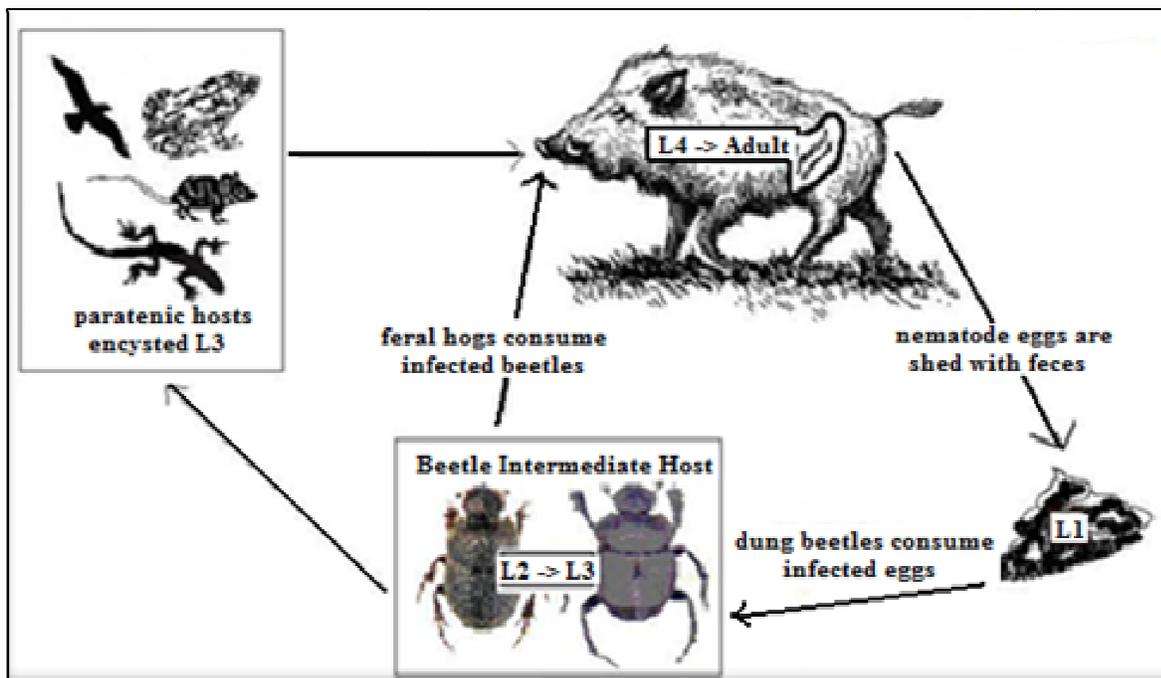
### ***Physocephalus sexalatus*: A Trophically Transmitted Parasite of Swine**

*Physocephalus sexalatus* (Nematoda, Spirocercidae) are commonly called thick stomach worms and reside in the pyloric gland region of feral hog stomachs. *Physocephalus sexalatus* eggs are typically shed with hog feces, and when ingested by a coprophagous beetle, they hatch, grow to third stage larvae and burrow into the beetle's body cavity. Once in the beetle's body cavity, the larvae encyst and wait to be consumed by their final host. In the most direct form of transmission, the beetle is consumed by a hog. Once consumed by a hog, the worm molts twice, completing its life cycle (Figure 1.3) as the worm becomes a sexually mature adult in the hog's stomach.

Eggs of *P. sexalatus* are elliptical in shape (~40 x 20 µm) and contain a fully developed embryo at time of oviposition (Alicata, 1935). About one month after the eggs are consumed by a dung beetle, third stage larvae develop and can be identified by transverse striations on the cuticle and cervical papilla on the right side of the body, opposite the excretory pore. Third stage larvae are small (1.4 to 1.5 mm in length) and can be identified because the tip of the tail ends in a characteristic knob with 20 to 23 digitiform cuticular processes (Alicata, 1935) similar to that seen in *S. pigmentatus*. Cysts develop quickly and are typically found attached to Malpighian tubules or floating freely in the abdominal portion of the body cavity. Cyst and larvae are both

liberated from the beetle by the gastric juices in the stomach of the hog. It is in the lining of the stomach that the adult worms take up residence.

As olfactory and auditory predators, hogs find most of their prey via rooting, which involves using the snout to displace soil. Soft soils suffer the most damage and can contain holes up to three feet deep (Mapston, 2004). These animals are opportunistic omnivores and eat almost anything and everything although their diet composition changes with the seasons and availability of food. Carrion and animal matter from arthropods (especially beetles), amphibians, reptiles, eggs, birds and small mammals are readily consumed when available. While dung beetles are the primary intermediate host for *P. sexalatus*, other animals have been documented as paratenic hosts and can aid in transmission of the parasite to hogs. Paratenic hosts are defined as hosts that sustain the lifecycle of the parasite but are not needed for development to occur.



**Figure 1.3:** Lifecycle of *P. sexalatus* in swine. Adults inhabit the stomach of feral hogs and eggs are shed with fecal material. Parasite larvae develop inside of the eggs and are ingested by coprophagous beetles. Larvae become adults when they are consumed by hogs and make their way back to the stomach of the final host.

Thick stomach worms are common in feral hog populations but rarely seen in domestic swine because they are easily treated with anti-nematode agents such as thiobendazole and sodium fluoride. The reduction of free-ranging hog production has also reduced the prevalence of stomach worms in domestic swine because management techniques reduce the ingestion of dung beetles and transport hosts. When a hog becomes infected, the stomach worms burrow into the stomach mucosa and cause irritation. Similar to *Streptopharagus pigmentatus* infection, clinical illness rarely occurs unless the infection is extreme or the animals are poorly nourished. When clinical signs do occur, they are associated with poor growth, gastritis, anemia, and death.

While several nematodes can inhabit the hog's stomach, this study focuses on the thick stomach worm *Physocephalus sexalatus*, and only three species of dung beetle hosts (*Phanaeus vindex*, *Canthon pilularis* and *C. nigricornis*). The behavior and ecology of *P. vindex*, commonly called the rainbow scarab, has been thoroughly documented because of its large size and bright colors. The behaviors of *C. pilularis* and *C. nigricornis*, which are black and much smaller, are less well documented.

Parasites frequently change the behaviors of their intermediate hosts (Moore, 2001) but dung beetles have been largely ignored. Understanding the role of *P. sexalatus* in dung beetles is important because they reside in the stomach of economically and ecologically significant invasive species. The disease dynamics associated with this parasite are also important as the range of invasive feral hogs continues to grow. The introduction or elimination of a parasite in any ecosystem can affect interactions between a diverse range of species (Thomas *et al.* 2005) making them relevant to conservation and management practices.

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**CHAPTER 2 – DUNG BEETLES AND THEIR NEMATODE PARASITES  
AS ECOSYSTEM ENGINEERS IN A TEMPERATE FOREST ECOSYSTEM OF  
SOUTHERN JAPAN**

**Summary:** Dung beetles play a vital role in the transmission of *Streptopharagus pigmentatus* to its final host, the Japanese Macaque (*Macaca fuscata*). This study compares the behaviors of infected and uninfected beetles with regards to both transmission dynamics and the ecological role of the parasite. The results of this study suggest that parasitism does not alter the beetle's use of shelter or choice of substrate on Yakushima Island, Japan. However, infected and uninfected beetles consume significantly different quantities of feces. This is relevant because dung beetles are responsible for removing the majority of fecal material in this forest ecosystem. Feces serve as breeding grounds for many insect pests and carry nutrients, that when buried by dung beetles, are essential for plant health. I show that the nematode parasite *S. pigmentatus*, through alterations in its host behavior, alters the availability of fecal resources to both plant and animal communities and should therefore be classified as an ecosystem engineer.

## **Introduction:**

Examples of parasite-induced behavioral changes in intermediate hosts range across most major host taxa; these changes can affect a variety of behaviors including phototaxis, geotaxis, activity level or movement, and even choice of food. Such alterations can have a variety of effects, ranging from benefits to the parasite (e.g., enhanced transmission or survival) to benefits to the host (e.g., parasite avoidance or resistance), as well as effects with no currently known beneficiary (Moore, 2002).

Despite many well-documented instances of such alterations (see Moore 2002, and Thomas *et al.* 2005), coprophagous animals have not figured prominently in this literature. This is surprising, given their heightened risk of encountering the propagules of intestinal parasites for which they might serve as intermediate hosts. Because of this, I chose to investigate behavioral changes in dung beetles (*Onthophagus lenzii* and *Geotrupus laevistriatus*), which act as intermediate hosts for the nematode parasite *Streptopharagus pigmentatus*. The definitive host for *S. pigmentatus* is the Japanese Macaque (*Macaca fuscata*).

The Japanese Macaque plays a prominent role in Japanese culture as a messenger of Shinto gods and is also a symbol of success and good fortune. Study of Japanese Macaques has provided much of our basic understanding of primate social behavior (Huffman, 1991). This species is widely known for its propensity toward generating cultural behaviors as it adapts to environmental changes: e.g. stone handling (Huffman, 1996; Nahallage and Huffman, 2007) and potato washing (Kawai, 1965). The primates in this study are a sub-species of Japanese Macaque (*M. fuscata yakui*) that live on Yakushima Island, Japan (a UNESCO World Heritage Site since 1993). Despite the fact that these monkeys are revered and protected by law, the

International Union for Conservation of Natural Resources (IUCNR) World Conservation Union's Species Survival Commission has placed them on the "Red List" of threatened species.

Coprophagous beetles serve as intermediate hosts for a variety of nematode parasites and play an important role in natural and agricultural ecosystems by removing feces from the upper surfaces of soil. For example, telocopride (dung-rolling) species such as those from the genus *Onthophagus* depend on feces for food, and for construction of brood balls that are buried and inoculated with eggs. Natural feeding and breeding behaviors associated with dung-processing aid in dispersal of seeds and help preserve the regenerating capacity of the forest (Estrada *et al.* 1999). Dung beetles also contribute to ecosystem health by increasing the rate of nutrient cycling and fertilizing by aerating soils (Halffter and Mathews, 1966). Because of these roles alone, dung beetles can be considered ecosystem engineers which are defined as organisms, plant or animal, that directly or indirectly modulate the availability of resources to other species by causing physical state changes to biotic or a-biotic materials (Jones *et al.* 1994 and Jones, 1997).

Adult *S. pigmentatus* typically inhabit the primate host intestine and depend on coprophagous beetles such as *Onthophagus lenzii*, *O. atripennis*, *O. ater*, and *Aphodius mizo* for completing their life cycle (Gotoh, 2000). Cockroaches and one other coprophagous beetle (*Geotrupus laevistriatus*) are also believed to be potential intermediate hosts (Gotoh, 2000). The specific pathology of *S. pigmentatus* has not been identified although there is ongoing work to identify the role of this nematode in primate health. While there is no current reason to suspect it as a major pathogen, when the infection becomes intense the mere presence of the worms can cause intestinal blockage and *S. pigmentatus* may act in concert with other pathogens to make monkeys sick. Moreover, at least one pathogenic nematode of primates, *Gongylonema pulchrum*, has the potential to use dung beetles as intermediate hosts and understanding the

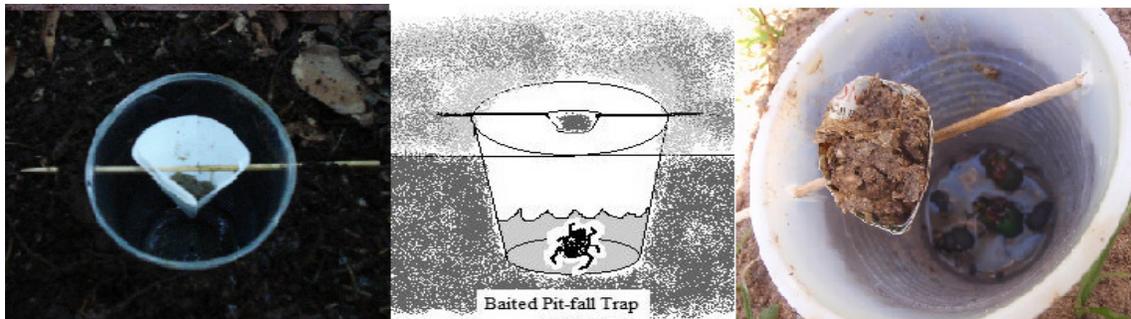
behavioral changes associated with pathogen transmission in this system may be important for our understanding of Japanese Macaque health.

The majority of research on parasite manipulated behavior focuses on transmission effects, but there is growing interest in other outcomes of parasite-induced transmission effects. For instance, Thomas *et al.* (1999) suggest that parasites can serve as ecosystem engineers through phenotypic alterations in their host, and there is increasing recognition of the important role manipulative parasites play in the ecology of natural ecosystems (Lefevre *et al.* 2008). This study therefore has a dual purpose: it explores the effect of *S. pigmentatus* on behaviors associated with parasite transmission (e.g., activity, use of shelter, choice of substrate), and asks if the nematode influences behaviors integral to the beetle host's role as an ecosystem engineer (e.g., rate of fecal consumption).

## **Methods:**

**Collection and Maintenance:** Dung beetles from the genus *Onthophagus* were collected from the subtropical, warm-temperate evergreen forest of Yakushima Island located in southwest Japan (30°N, 131°E). Traps were constructed from twelve ounce plastic cups buried with the rim level to the ground and modified from those used by Kanda *et al.* (2005) with water instead of ethanol added at the base (Figure 2.1). Traps were set within several isolated plots (10 x 10 m) inside the home range of a troop of Japanese Macaques and baited with feces from active troop members known to be shedding nematode eggs. The study troop (named Umi) consisted of 43 individuals; all infected to different degrees with *Streptopharagus pigmentatus* (Hernandez *et al.* 2009). Because some *Onthophagus* species are known to be nocturnal and others diurnal,

traps were set twice a day. Traps were set at 7:00 am (just after sunrise), collected and reset at 7:00 pm (just before sunset) and once again collected at 7:00 am. Because of behavioral differences associated with daily activity patterns beetles remained in two groups with those collected between 7 am and 7 pm henceforth referred to as diurnal, and those collected between 7 pm and 7 am referred to as nocturnal. While these patterns are typically consistent throughout life, nocturnal and diurnal behavioral patterns in some *Onthophagus* species are known to change with seasonal variation (Sasayama *et al.* 1984).



**Figure 2.1:** Pitfall traps baited with dung from wild primates known to be infected with *Streptophagagus pigmentatus*. Water in base prevents beetles from escaping trap.

Once collected, beetles were placed in twenty-eight liter aquariums (40 x 25 x 28 cm) and allowed to habituate to tank life for a minimum of twenty-four hours before the start of any experiments. Beetles collected during daylight hours were kept separate from those collected at night. Beetles belonging to the genus *Onthophagus* are classified as paracoprid beetles, meaning they procure dung in tunnels directly below the fecal source. A minimum of five inches of soil was added to each tank to accommodate this tunneling behavior. Dung beetles can live on feces alone and were fed monkey feces *ad libitum*, which served as a source of both food and water. Because of field conditions and the lack of controlled rearing chambers, tanks were kept outside in natural conditions. Average temperature was  $30.7 \pm 3.86$  °C and average humidity was 67.08

$\pm 5.64$  %. Conditions were monitored throughout experimental trials and did not appear to influence the behaviors addressed here.

After habituation, beetles were tested for several behavior changes: a substrate preference (white vs. black), shelter preference (shelter vs. no-shelter), and the amount of dung consumed in forty-eight hours. Preference tests were done during daylight hours because this is when monkeys are most likely to engage in insect foraging behavior. Thus, any observed changes in insect host behaviors recorded as a result of infection are likely to have potential consequence to the outcome of the interaction between monkeys and beetles.

Preference tests were done with individual beetles. Each individual was placed in either the substrate or shelter test apparatus with the order of tests randomized. Preliminary observations suggested that fifteen minutes were sufficient time for the beetles to habituate to the apparatus before data was collected. Habituation was determined to have occurred after flight escape behaviors ceased, and the beetles began to crawl around the tank instead of sit with legs tucked in close to their body.

When running choice experiments, the location of beetles was recorded every thirty seconds during a fifteen-minute period. Individuals were assigned a score between zero and thirty for each test based on their location at each thirty-second interval. One point was assigned for every observation that found the beetle in either the open area or on white substrate, and no points if they were underneath shelter or on black substrate. Each experimental apparatus was cleaned thoroughly between trials to prevent the potential for behavioral alteration due to pheromones released in previous trials.

After the choice experiments were complete, beetles were deprived of food for twenty-four hours before being placed in an isolated container with a pre-weighed and re-hydrated

amount of feces (dry weight = 0.2 grams). The dry weight of feces remaining after forty-eight hours was then weighed and the amount consumed by the individual beetle determined. At the termination of all preference and dung feeding trials, beetles were fixed in seventy percent ethanol for later dissection. Length, width, weight, and sex were recorded for each individual. Presence and intensity of infection was determined post-hoc after they were dissected and examined for the presence of nematodes encysted in the hemocoel.

### **Experiments:**

Shelter Preference: In order to assess predator avoidance behavior, the amount of time beetles spent out in the open versus underneath shelter was recorded. Black styrofoam with a three cm clearance provided shelter on one half of the tank. The styrofoam provided a compact area void of light while the other half of the tank remained open and had no type of structure for the beetle to hide under or in. As described earlier, beetles were placed in this apparatus for fifteen minutes prior to data collection. The location of each beetle was recorded as open if it could be seen, and hidden if it was under the shelter and out of sight. Both tank and shelter were washed and reset between trials.

Substrate Preference: To test for predator avoidance via crypsis we assessed whether beetles spent more time on the surface of a substrate lighter or darker than the natural color of their body (black). A tank, similar to those used in rearing, was divided into equal halves with one side of the aquarium containing a thin layer of black aquarium gravel and the other white aquarium gravel. The amount of time spent on each half of the tank was recorded. No soil was used

during these experiments to prevent beetles from burrowing out of sight. Gravel was washed, dried and replaced after each test to ensure consistency between trials.

Consumption: Beetles were isolated and deprived of food for twenty-four hours before being placed in a small 18-ounce container with 0.2 grams (dry-weight) of primate feces rehydrated with 1 ml of water. Beetles were left in this apparatus for forty-eight hours at which point the remaining feces was desiccated and the dry weight was again recorded. The amount of dung consumed by each individual was found by subtracting the final weight from the initial weight of the dried feces.

## **Results:**

A total of 146 dung beetles were used in this study (diurnal N = 75, nocturnal N = 71). There was no difference in prevalence of infection based on collection area ( $F_{2, 291} = 2.15$ ;  $p = 0.192$ ) so data from various plot locations were pooled for analysis.

Nocturnal beetles ( $8.296 \pm 0.918$  mm) found on Yakushima Island Japan were slightly larger than those active during daylight hours ( $6.74 \pm 1.58$  mm) ( $F_{1, 144} = 52.11$ ;  $p < 0.0001$ ). Despite the difference in size based on activity patterns, length did not affect prevalence of infection in either the diurnal (Mann-Whitney U = 2850, P = 0.1015) or nocturnal groups (Mann-Whitney U = 2556, P = 0.4119).

Both intensity ( $F_{1, 77} = 12.499$ ;  $p = 0.00069$ ) and abundance ( $F_{1, 144} = 15.58318$ ;  $p = 0.00012$ ) of infection were higher in the smaller diurnal beetles than they were in those collected at night (Figure 2.3). Approximately 62 percent of diurnal beetles were infected and had an

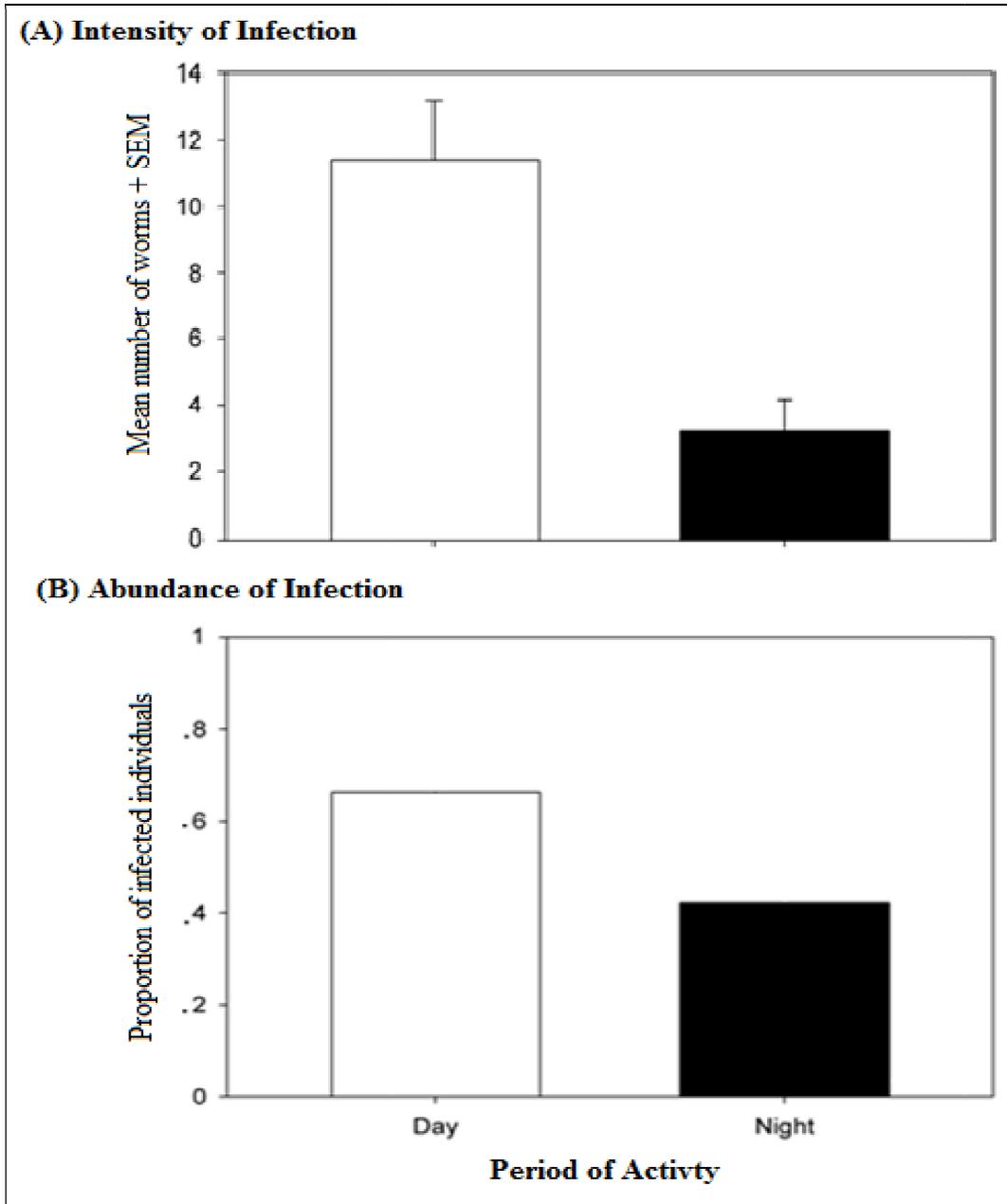
average of  $11.59 \pm 12.04$  larval nematodes per individual. Only 45 percent of nocturnal beetles were infected and the intensity of infection was much lower with only  $3.2 \pm 5.21$  nematodes per individual.

Status of infection did not affect substrate choice or use of shelter in either the nocturnal or diurnal groups (see Table 2 for statistical results). Both infected and uninfected beetles exhibited similar predator avoidance behaviors and spent more time underneath the shelter than out in the open. Both infected and uninfected individuals spent more time on the dark colored substrate (which matches their external markings) than the light colored substrate.

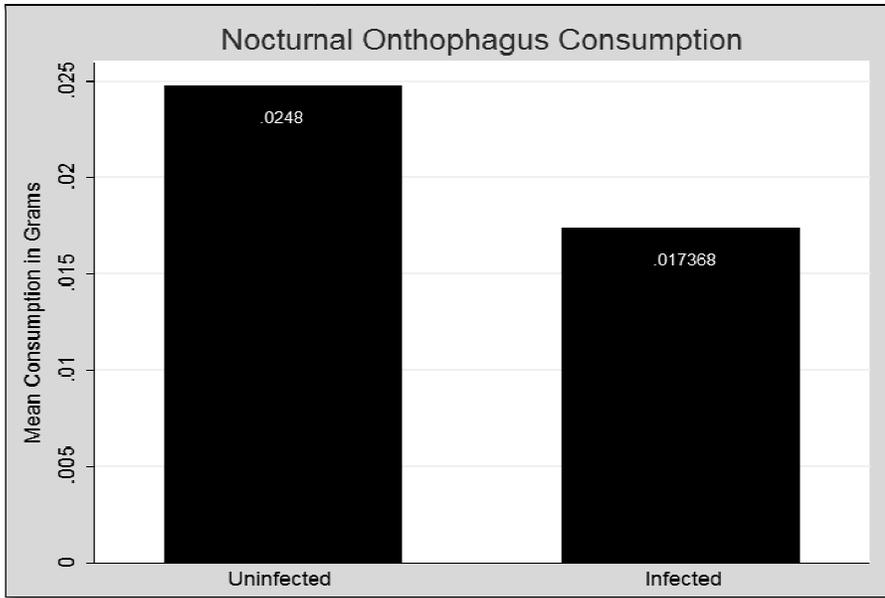
Infected beetles consumed less feces than uninfected beetles in both the nocturnal (Figure 2.4) and diurnal groups (Figure 2.5). Infected nocturnal beetles consumed almost 30 percent less feces than uninfected nocturnal beetles (Mann-Whitney U = 1540, P = 0.0472); and infected diurnal beetles consumed approximately 22 percent less feces than their uninfected counterparts (Mann-Whitney U = 990, P = 0.0093).

**Table 2:** Statistical results showing no difference in use of shelter or choice of substrate color in infected and uninfected beetles.

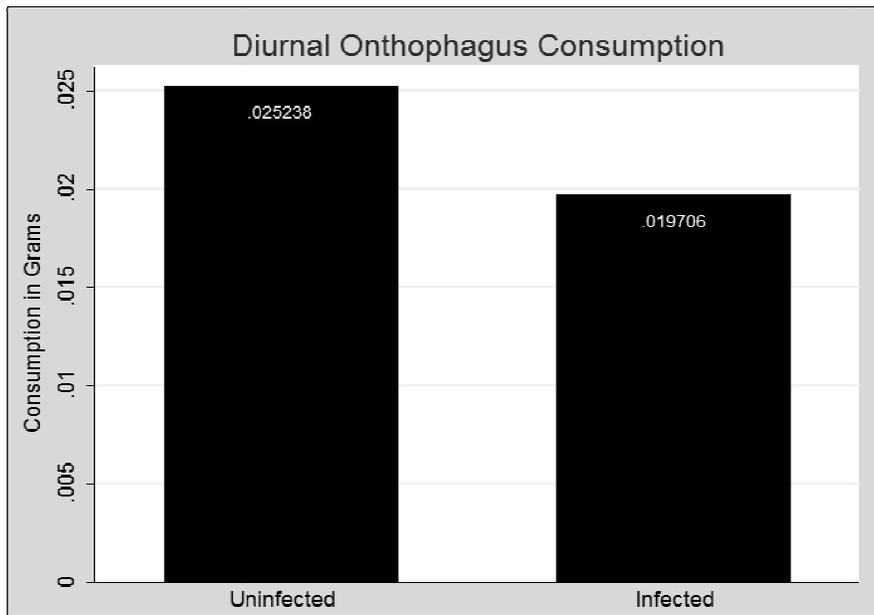
	<b>Nocturnal</b>	<b>Diurnal</b>
<b>Substrate Test</b>	Mann-Whitney U = 2556, P = 0.5433	Mann-Whitney U = 2850, P = 0.4894
<b>Shelter Test</b>	Mann-Whitney U = 2556, P = 0.6100	Mann-Whitney U = 2850, P = 0.6529



**Figure 2.3:** Both intensity and abundance of infection are higher in diurnal dung beetles than nocturnal dung beetles. (A) Intensity of infection shown as mean number of *Streptopharagus* larvae found in hemoceol of infected beetles (B) Abundance of infection shown as proportion of infected individuals within two beetle populations



**Figure 2.4:** Mean consumption of nocturnal *Onthophagus* beetles during a forty-eight hour period (results shown in grams). Mann-Whitney U = 1540, P= 0.0472



**Figure 2.5:** Mean consumption of diurnal *Onthophagus* beetles during a forty-eight hour period (results shown in grams). Mann-Whitney U = 990, P= 0.0093

## **Discussion:**

This study shows that both intensity of infection within individuals, and abundance of infection within the population are greater in diurnal beetles. This can be explained by the differences in lifestyle and foraging habits associated with each group. Many nematode eggs are subject to desiccation and cannot survive for great lengths of time outside their host. The likelihood of finding viable parasite eggs at night is thus decreased as feces remain on the surface of the soil and lose moisture. While more work needs to be done in this area, we believe that dung beetles exhibiting similar activity patterns as their final host are more likely to encounter fresh feces than those that forage on alternate cycles. Arthropods with overlapping sleep cycles are also more likely to serve as intermediate hosts because overlapping foraging times increases the likelihood of interaction and therefore predation.

Many parasites alter the behavior of their host in ways that are thought to increase predation and thus transmission. However, we do not see this type of behavioral change occurring in the nematode-beetle-primate system of focus in this study. Both the infected and uninfected beetles spend the majority of their time on black substrate where they are less conspicuous to predators. They also spend more time under shelter than out in the open. The lack of behavioral changes associated with transmission may be related to the fact this parasite can use multiple intermediate hosts. A parasite that can use multiple species as its intermediate host, and has the ability to slow its growth rate by encysting in its host's body cavity greatly reduces the urgency with which the final host must be encountered. A trophically transmitted parasite with many options therefore, has less incentive to invest energy in modifying the behavior of its host than a parasite that must be eaten by a specific host or cannot slow its growth rate by creating a cyst. This idea has not been tested but has great potential for future research.

While this study intended to focus on behavioral changes associated with transmission the data show something of much greater interest. The mean consumption of feces by infected individuals is significantly lower than the mean consumption of feces by uninfected individuals. In this case the mere presence of *S. pigmentatus* within *Onthophagus* beetles seems to be creating two distinct groups of beetles with different ecological roles; those that are consuming fecal matter and aiding the breakdown of feces, and those that are not. The two groups of beetles inevitably share the same trophic niche, thus the parasite has potential to alter interspecific competition processes. The infected population of beetles consume less feces than the uninfected population which also has potential to alter food availability and energy flow within the greater population of animals on the island. This would not be the first time we see parasites affecting food web dynamics (Thompson *et al.*, 2005; Lafferty *et al.*, 2008; Hernandez and Sukhdeo, 2008). Because feces serve as the only food source for these beetles, decreased intake means less energy for carrying out functional roles such as feces removal and brood ball creation for young. With few exceptions, parasitic manipulation dramatically reduces host fitness (Lefevre *et al.*, 2008). This could have serious consequences on Yakushima Island as it has potential to alter the diversity and abundance of dung beetles.

While often considered detrimental to the host, anorexia can serve as a mechanism of host defense, essentially starving the parasite or preventing establishment of infection (Wing and Young, 1980). Murray and Murray (1979) showed that anorexic rats infected with *Listeria monocytogenes* live longer than infected rats that are force fed. Decreased feeding in hosts has also been shown to be adaptive for parasitic organisms. For example, when infected with the LaCrosse Virus, mosquitoes (*Aedes triseriatus*) have difficulty engorging and therefore probe their host with greater frequency (Grimstad *et al.* 1980 and Patrican *et al.* 1985). In this case

feeding/foraging increases the opportunity for parasite transmission to occur as more and more hosts become exposed. Whatever the reason, suppressed feeding not only affects the sick or infected animal but has potential to indirectly affect a variety of animals within a food web and/or ecosystem. This is especially true if the organism of interest is trophically linked to a large number of other organisms.

Dung beetles play a vital role in terrestrial ecosystems by driving a series of ecological processes including nutrient recycling, parasite suppression, soil aeration, pest control, and secondary seed dispersal (Nichols *et al.* 2008). These processes are driven by consumption and burying of feces below the surface soil. The physical movement of feces from above to below the soil surface relocates nutrient rich organic material and instigates micro-organismal and chemical changes in the upper soil layers. In addition, the consumption of feces helps reduce the number of viable parasite eggs in the environment and removes larvae of pestiferous insects. Future studies should assess the formation and use of brood balls in addition to measuring the consumption of feces by individual groups. Dung beetles can move many times their weight in feces and are often the predominant competitor when it comes to procuring fecal resources. If behaviors associated with the use of feces are changed by parasitism, then both the parasite and the beetle have great potential to offset natural flow of resources through the environment. As such important modifiers, both the parasite and dung should be classified as ecosystem engineers.

## Notes on *Geotrupes laevistriatus* on Yakushima Island, Japan

We collected a total of twelve *Geotrupes* (out of 53) that were infected with *Streptopharagus pigmentatus*. The low prevalence of infection, relative to that seen in *Onthophagus* species, may be explained by the fact that beetles in this genus do not feed in their adult form. While this has not been tested, I predict that larval beetles are less likely to encounter feces infected with viable eggs and will therefore show lower infection rates than individuals that are actively seeking fresh feces throughout their life.

There was a significant difference between the mean abundance of infection in male and female beetles (Mann-Whitney U = 438, P= 0.03). Approximately 30% of the female population was infected with *Streptopharagus* whereas only 11% of the males contained larval nematodes in their hemocoel. Male *Geotrupes* were significantly smaller than females (Male length:  $13.124 \pm 0.161$  mm; Female length:  $13.825 \pm 0.173$  mm; Mann-Whitney U= 491, P= 0.01).

There was no significant difference between infected and uninfected in their substrate choice score (Mann-Whitney U= 243.5, P=0.957). Similarly, there was no significant difference in their use of shelter (Mann Whitney U = 325.5, P= 0.09). While *Geotrupes* beetles spend the majority of their time on dark substrate we found it curious that they spent slightly more time in the open than underneath shelter.

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**CHAPTER 3 - THE EFFECT OF PHYSOCEPHALUS SEXALATUS,  
A NEMATODE PARASITE ENGINEER, ON THE FEEDING AND DUNG BURYING  
BEHAVIOR OF PHANEAUS VINDEUS,  
ANOTHER IMPORTANT ECOSYSTEM ENGINEER**

**Summary:** Dung beetles feed on feces in both their larval and adults forms, and because of their diet are likely to encounter parasitic propagules at a rate higher than that of other animals. It is not uncommon for parasites to alter the behavior of their hosts and we set out to test the potential of *Physocephalus sexalatus* (a nematode parasite) in altering the behavior of dung beetles in ways that affect predator-prey relationships (transmission of the parasite) and the beetle's role as an ecosystem engineer. Classic tests of anti-predator behavior (e.g. use of conspicuous backgrounds, use of shelter, activity) did not reveal any differences between infected and uninfected beetles. However, this parasite does have a significant effect on beetle behavior that is critical to its role in ecosystem engineering. We found that infected beetles consume only half of the feces consumed by uninfected beetles, which is relevant because many parasite eggs (those of *Ascaris*, *Trichuris* and others) cannot survive consumption by dung beetles. In several instances we have seen dung beetles introduced to agricultural areas because of their phenomenal role in parasite suppression. We also found that infected beetles bury fewer feces than uninfected beetles and do so in tunnels that are shorter than those created by uninfected beetles. Fecal burial is beneficial because it aerates the soil, incorporates nitrogenous compounds, and increases water flow making soil and pastureland more productive. We show that dung beetles are ecosystem engineers and that *P. sexalatus* becomes an ecosystem engineer as it modifies the ecologically significant behaviors of its hosts.

## **Introduction:**

Beetles from the genus *Phanaeus* have been studied for almost 100 years and were first noted by scientists and collectors because of their relatively large size (up to 25 mm in length) and vibrant metallic exoskeleton (see Ohaus 1913, Becton 1930, Lindquist 1935). In the past hundred years our knowledge about these beetles has expanded greatly and the role they play in maintaining ecosystem functions has been well documented. *Phanaeus* beetles have unique mating and reproductive rituals in which beetles form temporary pair-bonds that last from first encounter to completion of nidification (Halffter and Matthews, 1966). The pair typically work together to build a nest for their young even though the female is capable of doing it alone. While most dung beetles create brood chambers in the feces or several yards away from the fecal deposition site, *Phanaeus* beetles nidification process is characterized by tunneling directly below the fecal source. Members of the genus *Phanaeus* fill a unique ecological niche as they are the only dung beetles in the United States to form balls of feces underground and bury them in this manner (Fincher, 1973).

Tunneling, rather than dwelling or rolling behavior is beneficial to soil fertility because it expediently removes feces from the surface of the soil, where nitrogenated compounds are quickly lost or broken down (Bornemissza, 1960). Burrows built for storing food and/or rearing young are ecologically significant as they increase soil porosity, gas exchange and water flow. Incorporation of dung into these chambers and surrounding soils substantially increases crop fertility and soil productivity (Bornemissza 1960, Halffter and Matthews 1966, Fincher *et al.* 1981, Yokoyama *et al.* 1991, Bang *et al.* 2005) in addition to altering disease dynamics by removing breeding ground for insect and helminth pests (Fincher *et al.* 1971). Despite being small, these dung beetles are classified as ecosystem engineers because their burrowing behavior

changes not only soil health and plant community structure but also the growth rate of microbial populations and parasitic diseases contained within the feces and surrounding soil (Anduaga and Huerta, 2007). Given the ecological significance of dung beetle behavior, any agent that modifies that behavior is likely to have widespread consequences. Because parasites frequently change the behavior of their hosts (see below), they have the potential to be one such agent.

*Phanaeus vindex* is the dung beetle most commonly infected with third stage larvae of *Physocephalus sexalatus*, a spirurid nematode of swine. Despite this, the role of *P. vindex* as a vector of disease has been largely ignored (Fincher *et al.* 1969). It is not uncommon for parasites to dramatically change the behavior of their hosts (Hamilton and Zuk 1982, Thomas *et al.* 1995, Poulin 1999, Moore 2002, Lefevre *et al.* 2008) and ecologically significant behavioral changes associated with parasitism have been documented in a variety of taxa. For instance, trematodes can change the behavior of their mollusk hosts in ways that alter competitive interactions between limpets and sea anemones (Thomas *et al.* 1998). Nematodes can alter plant community structure by changing the feeding behavior of their ungulate hosts (Arneberg *et al.* 1996) and acanthocephalans can alter the trophic biology of streams by changing feeding rates of isopods (Hernandez and Sukhdeo, 2008). In each of these cases parasites are acting as ecosystem engineers by modifying the behavior of their host. Because of these examples, we ask whether nematode parasites can alter the behavior of dung beetles in ecologically significant ways.

The focal parasite in this study is the cosmopolitan nematode *Physocephalus sexalatus* (Spiruvida: Spirocercidae, subfamily Ascaropsinae). The adult worm lives in the stomachs of a wide range of ungulate definitive hosts (e.g. swine, wild boar, peccary, tapir, cattle, horses, and dromedary). Embryonated eggs are shed from the ungulate with its feces. Dung beetles serve as intermediate hosts, and become infected by consuming *P. sexalatus* eggs along with their normal

intake of feces. Over 20 species of beetle from 14 genera can serve as intermediate hosts for this parasite. However, beetles from the genus *Phaneaus* and *Canthon* are believed to be the primary intermediate host for *P. sexalatus* because of their abundance and ability to out compete smaller species. Once inside a dung beetle, the parasite hatches, molts and then migrates from the gut to the hemocoel and molts again becoming a third stage larvae which encysts and then waits in a dormant state to be consumed by a hog or other ungulate host, where it can develop and reach sexual maturity. A wide range of animals can act as paratenic hosts to *P. sexalatus* and it is not uncommon to find larvae in a variety of accidental or paratenic hosts including blue jays (*Cyanocitta cristata*), red bats (*Lasiurus borealis*), sagebrush lizards (*Sceloporus graciosus*) and fence lizards (*Sceloporus occidentalis*) (Shimalov *et al.*, 1999).

Dung beetles themselves are ecosystem engineers, and they provide ecosystem services, that is, they provide a subset of ecological functions that are relevant or beneficial to the human condition (Groot *et al.*, 2002). Because the ecosystem engineering and services associated with dung beetles largely depend on their behavior, and because of the major behavioral role that parasites can play, this study asks if healthy dung beetles behave differently than those infected with nematode parasites. Although parasite-induced behavioral changes are common, this study is novel because it documents the behaviors of a host that is itself a significant ecosystem engineer and documents the effect of the parasite on the ecosystem services that host provides.

This study documents the relationship of *P. sexalatus* with its most common intermediate host by comparing feeding, burrowing and predator avoidance behaviors of both infected and uninfected *Phaneaus* dung beetles. In so doing, we ask two types of ecologically significant questions: are predator avoidance behaviors in dung beetles altered in a way that affects

transmission of this parasite, and does this parasite become an ecosystem engineer by altering the burrowing behavior of its already significant host.

## **Methods:**

### **Study Site and Collections:**

Live coprophagous beetles were collected from Oakridge Ranch in Colorado County, Texas, an area abundant with wild hogs, dung beetles, and parasitic stomach worms of swine. Oakridge Ranch is a private residential community comprising approximately 4400 acres of land managed by the Texas Parks and Wildlife Association. Collections took place on a small plot of land located at 29°33'28"N, -96°40'19"W. This area is dominated by sandy soils with mesquite and live oak vegetation.

Beetles were collected in pitfall traps constructed from 12-ounce plastic cups buried with the rim level to the ground and modified from those used by Kanda *et al.* (2005) with water instead of ethanol at the base. Traps were baited with feces of wild, but captive feral hogs (Wild Boars, Russian Boars and Hybrids). Fecal samples for these traps were collected from wild hogs that had been trapped and kept in captivity for anywhere from one day to three months. Captive hogs were fed a diet of commercially distributed deer feed, but were kept in an outdoor pen (approximately 1-acre in size) where vegetation and other prey could still be consumed.

*Phanaeus vindex* is a diurnal beetle species with activity levels that peak during the late morning and early afternoon (Price and May, 2009). Traps were set between 9 am and 4 pm with fecal contents monitored on an hourly basis and replaced or rehydrated with a spray bottle when a dried out crust was present. Beetles found swimming in the base of the trap were quickly

removed by hand and placed in plastic transport aquaria containing 15 to 20 cm of regional sandy soil.

### **Care and Maintenance of Beetles:**

Once in the lab, beetles were transferred to ten-gallon glass aquaria (51 x 26 x 32 cm) containing approximately 24 cm of soil and allowed to acclimate to tank life for a minimum of one week before any experiments began. They were maintained on a 12:12 light:dark cycle with temperatures ranging from 24 to 28°C and relative humidity kept between 50 and 60 percent. In order to prevent additional infection, and therefore estimate natural prevalence, beetles were fed an unlimited amount of feces from a domestic, uninfected pot belly pig. Dung beetles feed exclusively on feces and do not seek water from other sources so this was the only source of sustenance available.

### **Experiments:**

Individual beetles were tested for a variety of behaviors including choice of substrate, use of shelter, level of activity, rate of consumption, and depth/width of tunnels formed. Preference tests took place between the second and fifth hour of the photophase because this is when the beetles are most likely to be active. Preliminary trials suggested that fifteen minutes were sufficient for predator avoidance and flight escape behaviors to cease and thus a fifteen minute acclimation period was provided at the beginning of each trial. Because these were field collected animals, the infection status was known only upon dissection and this study conforms to a double blind design.

Substrate Preference Test – To assess predator avoidance via crypsis, a glass aquarium, similar to that used in rearing, was divided into equal halves with each side of the aquarium containing either black or white aquarium gravel. Gravel size and texture did not differ between colors and was rinsed with water, dried and replaced after each test to ensure consistency between trials. To estimate the amount of time beetles spent on each half of the tank we recorded their location every thirty seconds for a fifteen-minute period. Individuals were assigned a score between zero and thirty points for each test based on the sum of their location scores at each thirty-second interval. One point was assigned for every observation of the beetle on white substrate and no points when they were on black substrate. Despite the bright colored elytra on these beetles, the majority of their body is black and they blend in well with dark substrate.

Shelter Preference Test – Half of an aquarium, similar to that used in rearing, contained a black plexiglass shelter with one inch clearance. Opaque black tape covered the glass area below the shelter creating a compact area devoid of light. The other half of the tank remained open and had no structure for the beetle to hide under or in. The location of each beetle was recorded every thirty seconds for a fifteen-minute period, once again giving a total of thirty observations per beetle. One point being assigned for every observation that found the beetle in the open and zero points assigned when it was found underneath the shelter. Both tank and plexiglass shelter were rinsed, dried and replaced between trials.

Activity Test – In order to compare activity levels of healthy versus infected beetles we divided a glass Petri dish (fifteen cm diameter) into four quadrants and assigned individual beetles an activity score based on the number times it moved from one quadrant to another. A single beetle

was observed for a ten minute period and received a score equal to the number of times it moved from one quadrant to another.

Consumption Test – Individual beetles were isolated and deprived of food for 24 hours before being placed in a small 2-ounce container containing 1 gram (dry weight) of homogenized pig feces that was rehydrated with 1.5 ml of tap water. After 48 hours we determined the dry weight of remaining feces and calculated consumption based on the difference in initial and final values of the feces.

Tunneling Behavior Test – While *P. vindex* typically pair up with the opposite sex to create burrows, both sexes are capable of creating a burrow on their own and do so on a regular basis. Because of this, individual beetles were isolated and placed in a 30 x30 x2.5 cm plexiglass terraria containing 20 cm of wet sandy soil and 50-60 mL of moist feces from a domesticated and uninfected pot belly pig. Because these terraria are only 2.5 cm wide we can see the tunnels created by beetles, much as one views an ant farm. The depth, width and number of branches/galleries in tunnels were recorded for each individual.

Beetles began experiments in either the shelter, substrate or activity apparatus with the order of these tests randomized and completed in one day. Beetles were then isolated into separate containers, deprived of food and prepared for consumption and burrowing trials. Upon termination of experiments individual beetles were preserved in AFA (50 95% EtOH: 10 Formalin: 5 Glacial Acetic Acid: 45 Distilled Water) and stored until they could be dissected and examined for the presence of nematode larvae in their hemoceol. For each beetle we recorded

the intensity of infection in addition to length from pronotum to pygidium, width of thorax, weight, sex and horn length.

### **Statistical Analysis:**

Because the majority of experiments tested for a difference in infected and uninfected individuals, Wilcoxon Rank Sum Tests (Mann-Whitney U) were applied to non-normal data and significance was established at 0.05. All analyses were carried out using Stata 11 software.

### **Results:**

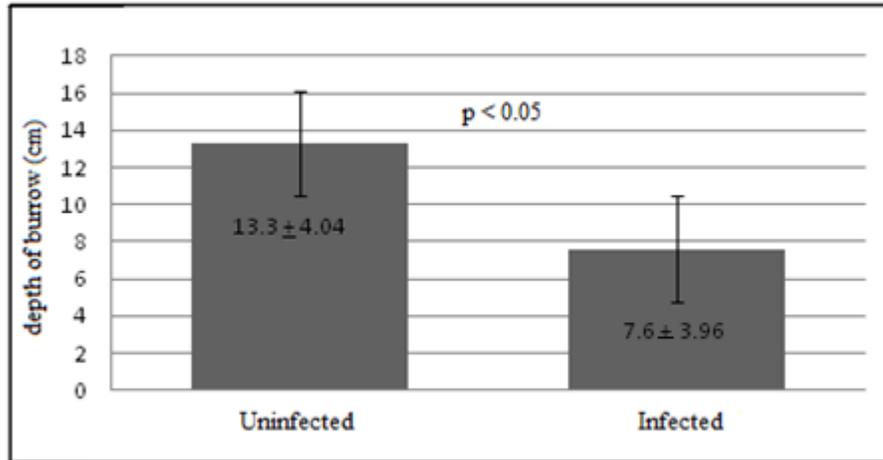
A total of 47 adult dung beetles (*Phanaeus vindex* Macleay) were used in this study. While not significantly different (Mann-Whitney U = 703, n = 47, p = 0.336) prevalence of infection was slightly higher in female beetles with 92.8% of females and only 82.1% of male beetles containing third stage larvae of *P. sexalatus*. Sex of host did not affect the intensity of infection (p = 0.8081) with the average adult containing  $52.2 \pm 56.4$  nematodes per individual.

Price and May (2009) indicate that tunnels created by *P. vindex* are approximately 12.3 cm deep when they are created by individuals working alone. Our data show that parasites dramatically alter the depth of tunnels created by these individual beetles (Mann-Whitney U = 594, n = 40, p < 0.05). Infected beetles dig tunnels that are an average of  $7.6 \pm 3.9$  cm deep and uninfected beetles digging tunnels that average  $13.3 \pm 4.04$  cm in depth (See Figure 3.1). The number of galleries (or rooms) within these tunnel systems did not vary based on status of infection (Mann-Whitney U = 597, n=40, p = 0.1937) but we did see slightly more females with branched burrows than males (Mann-Whitney U = 859, p < 0.05).

We observed 100% of uninfected beetles removing feces from the surface of the soil to a gallery located within their tunnel system. Uninfected beetles used the entirety of feces provided to them leaving no feces unused. However, only 19 of 34 (54.2%) beetles infected with *P. sexalatus* engaged in feces burying behavior so a large amount of feces remained on the soil surface. Dry weight measurements of the remaining feces were unattainable due the presence of sand in the sample.

In addition to not burying feces we found that infected beetles consume only 46% of the feces that uninfected beetles consume (Mann-Whitney U = 1118, n = 47, p <0.05). Uninfected beetles consumed an average of  $0.157 \pm 0.116$  grams of feces in a 48 hour period where as infected beetles consumed an average of  $0.073 \pm 0.066$  grams.

Status of infection did not affect the proportion of time beetles spent under shelter (Mann-Whitney U = 1074, n = 47, p = 0.1311), their substrate choice (Mann-Whitney U = 1116, n = 47, p = 0.8108), or level of activity (Mann-Whitney U = 1118, n = 47, p = 0.4369). Both groups exhibited normal predator avoidance behavior and spent the majority of their time on black substrate or underneath the shelter provided.



**Figure 3.1:** Depth of burrows created by dung beetles infected with third stage larvae of *Physocephalus sexalatus* are almost half as deep as those created by uninfected beetles.

### Discussion:

We set out to test the potential of *Physocephalus sexalatus* to alter the behavior of dung beetles in ways that affect predator-prey relationships (transmission of the parasite) and the beetle's role as an ecosystem engineer. Our classic tests of anti predator behavior (e.g. use of conspicuous backgrounds, use of shelter, activity) did not reveal any differences between infected and uninfected beetles. However, this parasite does have a significant effect on beetle behavior that is critical to its role in ecosystem engineering. The altered behaviors most relevant to ecosystem processes are those of feces consumption and feces burial. Moreover, some of those effects are likely to expose the beetle to increased hog predation.

*Phanaeus* dung beetles infected with the nematode parasite *P. sexalatus* consume only 46% of the feces that uninfected beetles consume. This altered feeding rate is ecologically relevant because it alters disease dynamics. A large number of helminth eggs and protozoan cysts cannot survive ingestion by a dung beetle and dung beetles are often introduced to

agricultural areas because of their ability to reduce these organisms. The exact mechanism by which parasitic propagules are destroyed by dung beetles is not well understood but the molar surfaces may be used to crush eggs and other small particles which allows excess liquid component to be consumed by the dung beetle (Holter, 2002). Miller *et al.* (1961) report that dung beetles from the *Phanaeus* and *Canthon* genera can reduce the passage of round worm eggs by nearly 100%. Laboratory studies show that fecal consumption by a dung beetle dramatically reduces the survival of harmful and costly parasites including *Ascaris lumbricoides*, *Necator americanus*, *Trichuris trichiura*, *Entamoeba coli*, *Endolimax nana*, *Giardia lamblia* (Miller *et al.* 1961) and *Cryptosporidium parvum* (Mathison and Ditrich, 1999). Feeding and the act of burying feces below the surface of the soil are beneficial to humans as they prevent both livestock and humans from coming into contact with feces and the harmful agents or propagules contained within. *Physocephalus sexalatus* reduces the feeding and burying behavior of these dung beetles thus increasing the prevalence of other parasites.

In addition to altering the amount of feces consumed by dung beetles, *Physocephalus sexalatus* also alters the depth at which dung beetles bury fecal material. The tunnels created by these beetles have important ecological significance as they help maintain both soil fertility and porosity in the upper layers (Bang *et al.*, 2005). A significant proportion of nutrients are expelled with vertebrate feces (Steinfeld *et al.*, 2006) and whether or not these nutrients are incorporated back into the soil can dramatically affect plant productivity. *Phanaeus* beetles that are not infected with *P. sexalatus* readily bury feces and incorporate nitrogen and other limiting agents back into the soil. Nitrogen is a critically limiting agent when it comes to plant growth (Vitousek *et al.*, 1997) and dung beetles prevent the loss of nitrogen by moving feces underground where loss through ammonia (NH<sub>3</sub>) volatilization is less likely to occur (Gillard,

1967). *Physocephalus sexalatus* appears to limit the beetle's role in incorporating nutrients back into the soil as only half of the infected beetles were seen engaging in dung burying behavior. In areas like Texas where the natural prevalence of this parasite in dung beetles is extremely high (eighty to ninety percent), the amount of feces that remains unprocessed due to the presence of this parasite could be highly significant.

In addition, given the foraging habits of hogs and some other ungulates, a beetle that is closer to the surface of the ground may very well incur a larger risk of intentional or accidental predation. Hogs root anywhere from several centimeters to one meter below the soil surface although most forage resources are found at or near the ground level (Mayer and Brisbin, 2009). Insects make up only about five percent of the hogs diet with their increased presence being explained by availability and accessibility rather than prey selection (Klaa, 1992). Because *P. sexalatus* is causing its dung beetle host to move to the upper layers of the soil it is reasonable to assume that the encounter rate between hogs and beetles is increased. The fact this host does not actively seek out its dung beetle prey can explain the lack of behavioral change associated with normal predator avoidance activity. While many parasites modify anti-predator behavior in their intermediate hosts, anti-predator behavior can remain intact and predation risk can nonetheless increase if encounter rates between final and intermediate host increase (Holmes and Bethel, 1972).

We have shown that *Physocephalus sexalatus* can have significant effects on the behavior and ecology of the ecosystem engineer, *Phanaeus vindex*. While it is beyond the scope of this study to quantify these effects, a brief review of the dung beetle literature can put them into some perspective. Uninfected dung beetles are extremely beneficial and a variety of countries have imported exotic dung beetles because of the services they provide (Anderson and Loomis, 1987).

Between 1964 and 1985 Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) launched The Dung Beetle Project introducing approximately fifty species of dung beetle from Africa and Europe. Twenty-three species of dung beetles still flourish in Australia pasturelands, greatly improving the quality and fertility of their cattle industry (Bornemissza, 1976). Some of the most common and beneficial dung beetles in the United States (*Onthophagus taurus* and *O. gazelle*) were introduced from Asia and Africa in the early 1980s (Fincher *et al.* 1983). Because these insects are so important in agriculture management, and are believed to save the American cattle industry \$380 million per year (Losey and Vaughn, 2006), understanding the ways that common parasites alter their participation in agriculture becomes increasingly relevant.

As feral hog populations continue to grow and spread across the United States, an understanding the diseases they carry becomes increasingly important. The nematode parasite *Physocephalus sexalatus* has been largely ignored because of its minor pathology in hogs. Because *P. sexalatus* can infect a variety of dung beetle species, and can potentially remove the beneficial services they provide, this parasite should be considered an ecosystem engineer and its affect on additional hosts should also be explored.

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## **CHAPTER 4 - THE NEMATODE PARASITE PHYSOCEPHALUS SEXALATUS AND ITS EFFECT ON FECAL USE AND REPRODUCTIVE STRATEGIES IN CANTHON DUNG BEETLES**

### **Introduction:**

Dung beetles are extraordinary creatures that play a significant role in both natural and agricultural ecosystems by decreasing parasitism and increasing nutrient cycling, soil structure and forage growth. Adult dung beetles feed exclusively on the liquid components of feces and use the remaining material to feed and house their offspring. All dung beetles (family Scarabaeidae) provision their young with food and are classified into three groups based on their nesting strategy (Halffter and Edmonds, 1982). Paracoprid dung beetles bury feces directly below the fecal pat, endocoprid beetles feed and nest within the dung pat, and telocoprid beetles remove a portion of feces before rolling it from the original deposition site to bury it. Most dung beetles lay a single egg in the brood ball, coat the brood ball in sand to prevent desiccation and then abandon the nest (Halffter and Edmonds, 1982). Hatched beetle larvae remain underground for several months feeding exclusively on the feces provided by their parents, making this resource essential to their survival and future reproductive success. Adult dung beetle size is directly related to the quantity of initial provisions in the brood ball and often determines their success in competitive interactions (Lee and Peng, 1981). In addition, insect body size is often correlated with reproductive success and fitness (Lee and Peng 1981, Thornhill and Alcock 1983) although the heritability of size is low (Gordon, 1984). Because larger dung beetles are often better competitors and capable of creating brood balls larger than themselves, why is such a

large variation found between and within species in the size of dung beetles and their brood balls?

Parasites often change the behavior of their host and we see that several nematode species that alter the way dung beetles interact with feces. Both *Streptopharagus pigmentatus* and *Physocephalus sexalatus* (Phylum Nematata, Class Secernentea, Subclass Spiruria) reduce the feeding behavior of their paracoprid dung beetle hosts (Boze *et al.*, 2011). Because paracoprid beetles bury the feces directly below the fecal pat, and feces is directly in contact with soil, it is often hard to measure the amount of feces provided to offspring. However, telocoprid species create brood balls on the surface of the soil and create distinct brood balls which are easy to measure and weigh. *Canthon pilularis*, a telocoprid species, serves as one of the most common intermediate host to the nematode *Physocephalus sexalatus* and provides a unique opportunity to ask if parasitism could account for the differential size in brood balls created by this species.

*Physocephalus sexalatus* is often referred to as the common stomach worm of swine. Adult worms are found throughout the world and reside in the stomach of their mammalian definitive host and body cavity of arthropod intermediate hosts. Eggs are similar in appearance to those of *Ascarops strongylina*, and contain a fully developed embryo at the time of ovoposition (Alicata, 1935). First and second stage larvae are similar in appearance and often difficult to differentiate from those of *Ascarpos strongylina*. However, third stage larvae of *P. sexalatus*, which are found in the body cavity of dung beetles, possess a knobby protrusion on their tail that contains 20-30 finger-like processes not found in *A. strongylina*. While *P. sexalatus* can use a variety of dung beetle intermediate hosts, it is most commonly found in those from the genus *Phanaeus* and *Canthon* (Fincher *et al.*, 1969). Little is known about the behavior of either *Canthon pilularis* or *Canthon nigricornis*, but Fincher (1969) identifies these beetles as excellent

intermediate hosts for stomach worms of swine because they are abundant in swine infested areas, available during the day, and present in all seasons except winter (Fincher, 1969).

This study uses both *C. pilularis* and *C. nigricornis* to assess whether the presence of *Physocephalus sexalatus* infection alters adult dung beetle feeding rate and provisioning of young with resources. The amount of feces consumed by individual beetles in addition to activity rate and the size/weight of brood balls is recorded for each individual. Dung beetles are extremely important insects from an environmental perspective because of their ability to move and quickly disseminate feces. They are the insect group primarily responsible for disturbing dung, and thereby limiting nuisance-level populations of flies (Gullen and Cranston, 2000). Young (1978) found that ball-rolling species consistently out-compete species that butt food over land or bury it at the food source making them extremely important for agricultural management. Dung beetles are already classified as important ecosystem engineers and there are books written on their ecological relevance (Hanski and Cambefort, 1991). If *P. sexalatus* does indeed alter the reproductive strategy of its host and therefore alters the amount of dung processed by its host, then it too should be classified as an important and ecologically relevant ecosystem engineer.

## **Methods:**

### **Study Site and Collections:**

Live coprophagous beetles were collected from Oakridge Ranch in Colorado County, Texas. This is an area rich with wild hogs, dung beetles, and parasitic stomach worms of swine

(*P. sexalatus*). Oakridge Ranch is comprised of approximately 4400 acres of land managed by the Texas Parks and Wildlife Association and located at 29°33'28"N, -96°40'19"W.

Beetles were collected in pitfall traps constructed from 12-ounce plastic cups buried with the rim level to the ground and modified from those used by Kanda *et al.* (2005) with water instead of ethanol at the base. Traps were baited with feces of wild, captive Suids (European wild hogs, feral hogs, and European-feral crossbreeds). Fecal samples for these traps were collected from wild hogs that had been trapped and kept in captivity for up to three months. Captive hogs were fed a diet of commercially distributed deer feed, and kept in a 1-acre outdoor pen with natural vegetation and prey available.

Traps were set between 9 am and 4 pm with fecal contents monitored hourly and replaced or rehydrated if a dry crust developed. Beetles found swimming in the base of the trap were quickly removed and placed in plastic transport aquaria containing 15 to 20 cm of regional sandy soil. Both *C. pilularis* and *nigricornis* are recognized because of their shared characteristics including a black exoskeleton, broadly shaped body, flattened fore-tibiae with teeth on outer edges and highly visible flattened head used for digging (Matthews, 1963). Despite their shared traits, the species are easily distinguished because *C. pilularis* is much larger (10-19 in length) than *C. nigricornis* (5-9 mm in length), making the species easy to differentiate.

### **Care and Maintenance of Beetles:**

In the lab, beetles were transferred to ten-gallon glass aquaria (51 x 26 x 32 cm) containing approximately 24 cm of soil and allowed to acclimate to tank life for one week before experiments began. Beetles were separated by species and maintained on a 12:12 light:dark cycle with temperatures ranging from 24 to 28°C and relative humidity kept between 50 and 60

percent. To prevent additional infection, and estimate natural prevalence, beetles were fed an unlimited amount of feces from a domestic, uninfected pot belly pig. As dung beetles feed exclusively on feces and do not seek water from other sources this was the only source of sustenance available.

### **Experiments:**

Individual beetles were tested for a variety of behaviors including choice of substrate, use of shelter, level of activity, rate of consumption, and weight/diameter of brood balls formed. Preference tests took place between the 2<sup>nd</sup> and 5<sup>th</sup> hour of the photophase because this is when the beetles are naturally active. Preliminary trials suggested that 15 minutes were sufficient for predator avoidance and flight escape behaviors to cease. Thus a 15 minute acclimation period was provided at the beginning of each trial. Because these were field collected animals, the infection status was known only upon dissection and this study conforms to a double blind design. There is no sexual dimorphism in these species meaning that sex determination was based on internal anatomy after experiments were concluded.

Ball Formation Experiments – Dung beetles create two types of balls; food balls which are used for adult consumption, and brood balls which are used to house and feed offspring. Food balls take 12-20 minutes to make and are often crude and asymmetrical, whereas brood balls take 35-50 minutes to make and are made with great care and precision (Guertin, 1993). When provided with a pat of feces, beetles quickly moved to the resource and began eating or making balls of feces. When balls were complete they were rolled away from the fecal deposition site and coated in sand. The individual(s) rolling the ball and the ball itself were then removed from the

feeding/rearing tank. Fecal balls were identified as either food or brood balls and then measured with electronic calipers and weighed. The beetles associated with ball formation were isolated and then participated in the shelter, substrate, activity or feeding experiments.

**Substrate Preference Test** – A glass aquarium, similar to that used in rearing, was divided in half with each side containing either black or white aquarium gravel. Gravel size and texture did not differ between colors and was rinsed with water, dried and replaced after each test to ensure consistency between trials. To estimate the amount of time beetles spent on each substrate type, beetle location was recorded every thirty seconds for fifteen minutes. One point was assigned for every observation on white substrate and no points for black substrate. Individuals scored between zero and thirty points for each test based on their location scores at each thirty-second interval.

**Shelter Preference Test** – Half an aquarium, similar to that used in rearing, was covered by a sheet of Plexiglass with one inch clearance. Opaque black tape covered the walls below this shelter creating a compact area devoid of light. The other half of the tank remained open and had no structure for the beetle to hide under or in. Beetle location was recorded every thirty seconds for fifteen minutes, for a total of 30 observations per beetle. One point was assigned for observations where the beetle was in the open and zero points were assigned if the beetle was out of sight. Both tank and Plexiglass shelter were rinsed, dried and replaced between trials.

**Activity Test** – To compare activity levels of healthy versus infected beetles a glass Petri dish (15 cm diameter) was divided into four quadrants with a cross drawn on the bottom. An

individual beetle was observed for ten minutes and received a score equal to the number of times it moved from one quadrant to another.

Consumption Test – Individual beetles were isolated and deprived of food for 24 hours before being placed in a 2-ounce container containing 1 gram (dry weight) of homogenized pig feces rehydrated with 1.5 ml of tap water. After 48 hours, the dry weight of remaining feces was subtracted from the initial weight and total consumption was calculated.

Beetles were selected to participate in this study because of their fecal ball formation although not all individuals were found making balls at the time of their selection. Beetles began experiments in either the shelter, substrate or activity apparatus with the order of these tests randomized and completed in one day. Beetles were then isolated into separate containers, deprived of food and prepared for consumption experiments. Upon termination of experiments individual beetles were killed and preserved in AFA (50 95% EtOH: 10 Formalin: 5 Glacial Acetic Acid: 45 Distilled Water) and stored until they could be dissected and examined for the presence of nematode larvae in their hemoceol. Despite Baermannization being a more efficient method of extracting larval nematodes, each individual was dissected and thoroughly examined. This allowed for all larvae, including those who have entered arrested development and become encysted, to be identified. The intensity of infection, length from pronotum to pygidium, width of thorax, weight, sex and species of each beetle was recorded.

## Statistical Analysis:

Because the majority of experiments tested for a difference in infected and uninfected individuals, Wilcoxon Rank Sum Tests (Mann-Whitney U) were applied to non-normal data and significance was established at 0.05. Logistic regression was also used when the control of extraneous variables was necessary. All analyses were carried out using Stata Corporation Software, Version 11.

## Results:

A total 120 beetles were used in this study (65 *C. pilularis* and 55 *C. nigricornis*). While the prevalence of infection was extremely high in both species, we found that it was slightly higher in *C. nigricornis* with approximately 84 percent of individuals containing at least one *S. pigmentatus* larva. Despite being the larger species, only 66 percent of *C. pilularis* were found to be infected. Neither prevalence ( $p=0.6691$ ,  $n=65$ ) nor intensity ( $p=0.9043$ ,  $n=65$ ) of infection were different in *C. pilularis* males and females. *Canthon pilularis* males carry an average of  $23.2 \pm 42.6$  worms per individual and approximately 68% of males are infected. *Canthon pilularis* females carry an average of  $25.1 \pm 57$  worms per individual and approximately 63% of individuals are infected. Similar results were found between male and female *C. nigricornis* with neither prevalence ( $p=0.3100$ ,  $n=55$ ) nor intensity ( $p=0.3751$ ,  $n=55$ ) differing significantly. *Canthon nigricornis* males carry an average of  $28.5 \pm 31.6$  worms per individual and approximately 78 percent of individuals are infected. *Canthon nigricornis* females carry an average of  $20.79 \pm 27.5$  worms per individual with approximately 88 percent of individuals infected. It is often the case that prevalence and intensity differ between males and females and

while we do not see this here, we do find extraordinarily different behavioral responses to infection.

### **Feeding Behavior:**

We saw a definite trend in altered feeding behavior for both male and female *C. pilularis* (Figure 1a). When looking at the entire collection of *C. pilularis*, the difference in fecal consumption was highly significant with infected individuals consuming an average of  $0.0448 \pm 0.0387$  grams of feces whereas uninfected individuals consumed  $0.0887 \pm 0.0585$  grams of feces ( $p=.0006$ ,  $n=65$ ). For females the difference was even more significant ( $p=.0042$ ,  $n=33$ ) with healthy females consuming  $0.0975 \pm 0.062$  grams of feces and infected females consuming  $0.0431 \pm 0.039$  grams of feces. Infected males consume less feces than uninfected males but the difference is no longer statistically significant ( $p=0.07128$ ,  $n=32$ ). Infected males consume an average of  $0.0465 \pm 0.0385$  grams of feces and uninfected males consume an average of  $0.0781 \pm 0.0553$  grams of feces.

The total sample of *Canthon nigricornis* showed a non-significant trend toward altered fecal consumption based on infection ( $p=.0908$ ,  $n=55$ ) (Figure 4.1 b). When separating *C. nigricornis* by sex we saw absolutely no change in consumption for males ( $p=.9363$ ,  $n=28$ ) but an overwhelmingly significant change in females ( $p=.0010$ ,  $n=27$ ). Uninfected females consume an average of  $0.0846 \pm 0.026$  grams of feces and infected females consume only  $0.0180 \pm 0.029$  grams.

### **Ball-Rolling Behavior:**

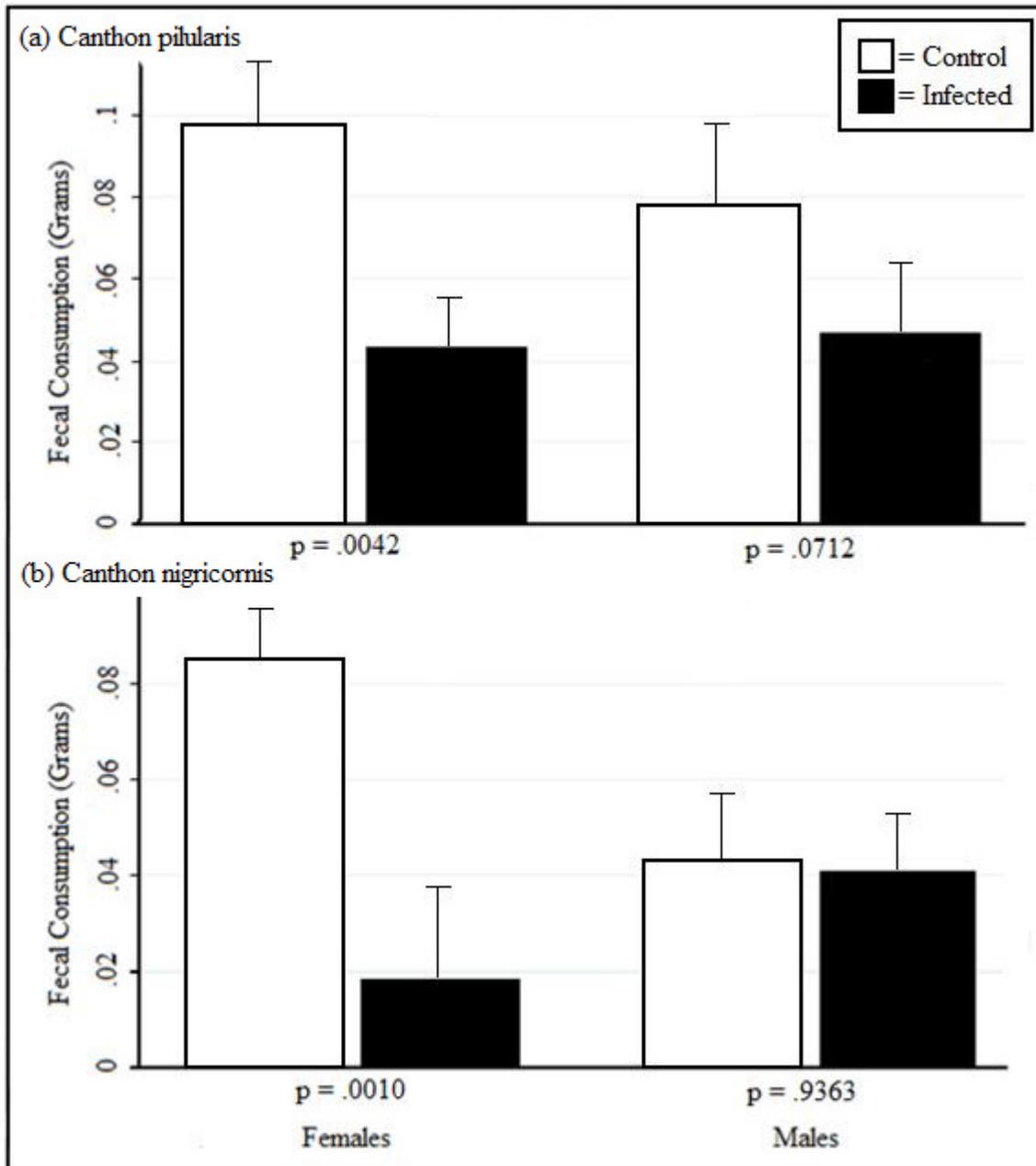
Males of both species are primarily responsible for making brood balls and were seen creating fecal masses more frequently than females. Brood balls created by infected *C. pilularis* males were smaller in both diameter ( $p=0.0286$ ,  $n=20$ ,  $r=0.1781$ ) and weight ( $p=0.0374$ ,  $n=20$ ,  $r=0.1608$ ) than those created by uninfected males (Figure 4.2 a). The average brood ball created by uninfected males working alone weighed an average of  $3.212 \pm 2.002$  grams were as those created by infected males working alone were  $2.421 \pm 0.786$  grams. Brood balls created by control males were  $17.018 \pm 2.822$  mm in diameter and those created by infected individuals averaged  $14.448 \pm 2.268$  mm in diameter. When controlling for size of individuals (based on length from pronotum to pygidium) the difference in diameter of brood balls based on infection was still significant ( $p=0.0485$ ,  $n=20$ ,  $r=0.2248$ ) although the weight difference was not ( $p=0.0611$ ,  $n=20$ ,  $r=0.207$ ).

Brood balls created by *C. nigricornis* males working alone were also smaller in infected individuals (Figure 4.2 b). When controlling for length of adult males we found the average brood ball created by infected individuals to be  $4.327 \pm 1.715$  mm in diameter and those created by uninfected individuals to be  $6.761 \pm 1.561$  mm in diameter ( $p=0.0451$ ,  $n=11$ ,  $r=0.4299$ ). The average weight of brood balls was  $0.395 \pm 0.183$  grams for infected individuals and  $0.742 \pm 0.215$  grams for uninfected individuals ( $p=0.0189$ ,  $n=11$ ,  $r=0.5506$ ).

### **Predator Avoidance Behavior:**

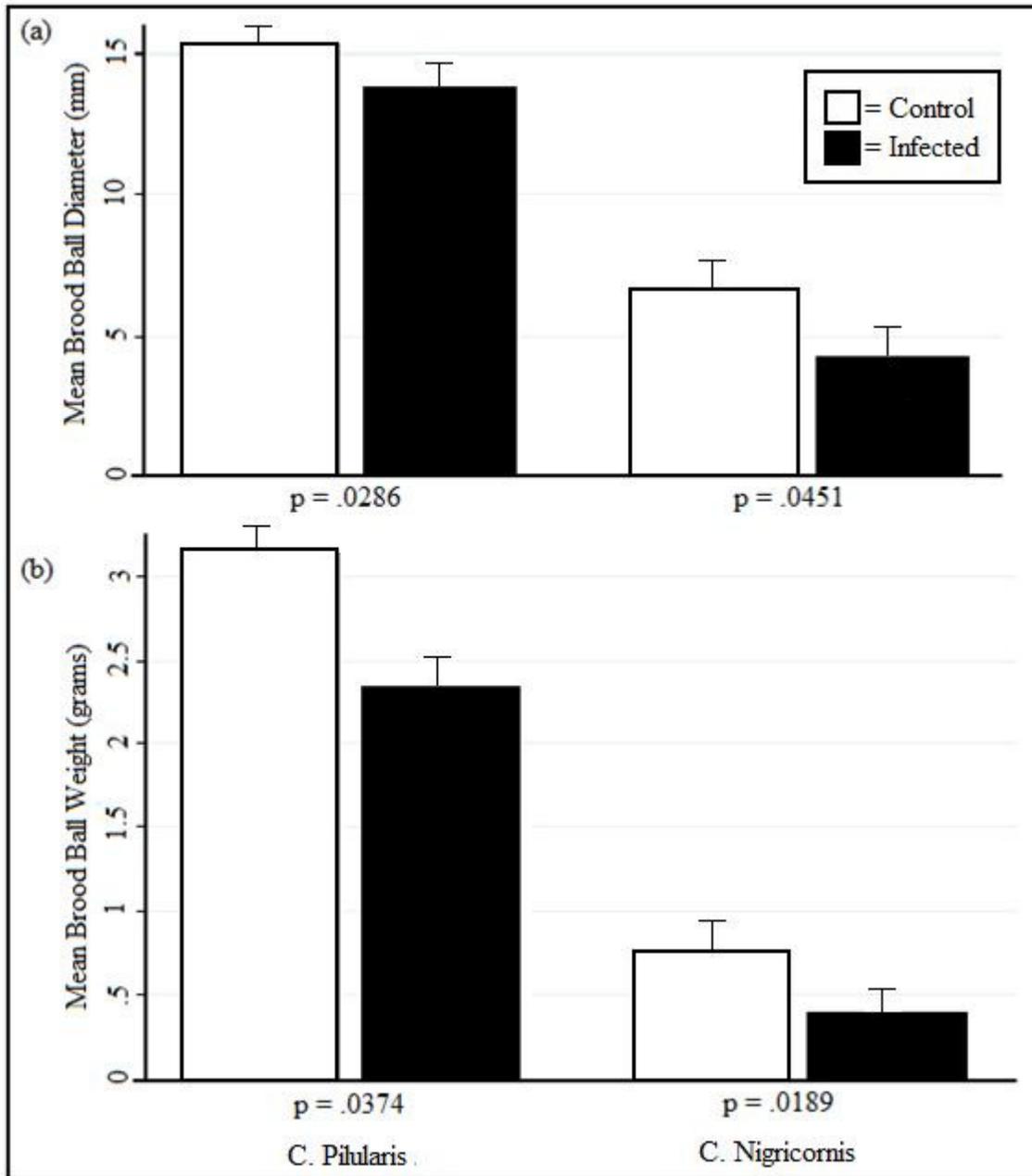
Predator avoidance did not differ based on presence of infection. Neither uninfected nor infected *C. pilularis* differed in terms of general activity ( $p=0.6975$ ,  $n=65$ ), the amount of time spent on black/white substrate ( $p=0.1771$ ,  $n=65$ ), or use of shelter ( $p=0.1221$ ,  $n=65$ ). The same tests of

predator avoidance behavior were non-significant for *C. nigricornis* with uninfected and infected individuals displaying similar activity levels ( $p=0.1721$ ,  $n=55$ ), spending the same proportion of time on black/white substrate ( $p= 0.8556$ ,  $n=55$ ), and underneath shelter ( $p=0.1408$ ,  $n=55$ ).



**Figure 4.1:** Effect of *Physocephalus sexalatus* infection on feeding behavior of both *Canthon pilularis* and *Canthon nigricornis*. Infected individuals consistently consume less feces than

uninfected individuals and females show a more dramatic difference in consumption than males do.



**Figure 4.2:** Effect of *Physocephalus sexalatus* infection on size of brood balls created by male dung beetles working alone.

## Discussion:

Dung beetles typically feed off the same food they provide for their larvae, making feeding and reproduction hard to separate (Price and May, 2009). While, paracoprid dung beetles make fecal balls for both feeding and reproduction it was possible to identify the intended use of their fecal masses based on shape. By isolating the beetles' by activity, in containers containing a specific amount of feces, it was possible to quantify consumption based on the feces remaining after a predetermined period. When infected by the nematode parasite *P. sexalatus*, both *C. pilularis* and *C. nigricornis* showed reduced interaction with feces. Females of both species significantly reduced their consumption and males of both species created brood balls that were much smaller than those created by uninfected individuals.

Reduced feeding as a result of infection is not uncommon, and examples of illness-induced anorexia are seen across the animal kingdom (Adamo, 2006). Upon initial examination, reduced feeding appears to be maladaptive because excess energy is often needed to initiate the immune response. However, reduced feeding alters a variety of physiological processes, and several studies report a heightened immune response and/or reduced susceptibility associated with anorexia (Ayres and Schneider, 2009). Murray and Murray (1979) showed that mice who were unable to reduce their food intake, and were infected with *Listeria monocytogenes*, show increased mortality relative to those who could reduce food intake. The majority of studies related to illness-induced anorexia have focused on vertebrates although several benefits of illness-induced anorexia are seen amongst the invertebrates as well. Reduced feeding in crickets adjusts energy needs away from digestion and ultimately increases the immune response and survival of crickets by altering the amount and distribution of lipoproteins (Adamo *et al.*, 2010).

Tenebrionid beetles with reduced lipid intake also show increased resistance to the parasitic nematode *Heterorhabditis thaica* (Shapiro-Ilan *et al.*, 2008).

While dung beetles feed exclusively on feces, the nutrient contents within fecal types varies and it is possible that the reduced feeding we observed is an attempt to minimize lipid intake. In most cases parasite-induced anorexia amongst the insects does not result in complete cessation of feeding (Adamo *et al.*, 2010) because nutrient intake is still necessary for initiating an immune response (Humphrey and Klasing, 2004). The reduced intake of food seen in previous studies is consistent with the altered feeding seen in both *C. pilularis* and *C. nigricornis*. While the physiological responses to altered feeding can be different (Woodring, 1984), reduced feeding as a way of increasing the beetle's immune response provides one possible explanation for the altered feeding associated with *P. sexalatus* infection. Despite the fact that male feeding behavior is not statistically different for infected and uninfected individuals, a slight trend toward reduced feeding is nevertheless observed.

Differences in infection rate and susceptibility, based on behavioral differences amongst the sexes, are fairly common (Herd *et al.* 1992). Because males and females differ from each other in many aspects of biology and behavior, several authors have encouraged further examination of sex differences associated with parasitism (Grossman 1989, Williams 1975, Eloi-Santos *et al.* 1992). It is often the case that prevalence differs between male and female hosts of parasites. While not observed here, extraordinarily different responses to infection on the part of animals that appear to be at equal risk of infection were noted. Both male and female dung beetles feed exclusively on feces and should have approximately equal exposure to the propagules of *P. sexalatus*.

Despite similar feeding behavior by both sexes, the amount and type of parental care provided by each sex is different. While dung beetle males typically provide more parental care than most insects, their investment is still much less than that of females. Parental care is defined as any parent-offspring interaction that promotes the survival, growth, or development of offspring; which, in the most primitive sense is limited to protection of eggs (Tallamy, 1984). In most insect groups only the females protect their eggs from predators, and in most cases parental care ceases after the first larval stage (Bequaert 1935, Wood 1976). In dung beetles, both parents abandon their eggs at a fairly early stage. However, the resources they provided continue to nourish larvae until they can emerge from the soil as full grown adults.

Both male and female dung beetles are said to have significant parental investment. Males initiate the making of the brood balls and are exclusively responsible for rolling, defending and burying them (Matthews, 1963). Females play a passive role while the ball is being formed and it is not uncommon to see a male completing the formation of the brood ball before a female joins him (Matthews, 1963). When the ball is complete it is rolled to another location for burial. Females do not aid the rolling process, although they have been seen walking on the surface of the ball, keeping time with its rotation (Matthews, 1963). After the nest is created and ball is buried, the pair copulate and males stay in the nest no longer than 22 hours (Matthews, 1963) making their time investment approximately 24 hours. After the male abandons its nest the female begins to take an active role. She will spend 4 to 10 days laying an egg on the surface of the brood ball and then modify the ball into a pear shaped structure as she covers the egg in moist dirt (Lindquist, 1935).

Infected hosts frequently make tradeoffs between survival and reproduction (Stearns 1992, Richner *et al.* 1995). The reduced feeding we see in females of both dung species may

well be explained by the potential for increased immune response. However, the reduced brood ball size in males of both species is a little more complicated. We know that the quantity of dung in the brood masses provided to young determine adult body size, with additional dung increasing the size of offspring produced (Emlen 1994, Hunt and Simmons 1997). Upon initial examination, it appears infected males are putting less effort into reproduction because the size of their brood balls are smaller than the ones made by uninfected males. However, not all brood balls made by males are actually used for reproduction. The encounter of the sexes is often by chance and there are many instances when, having completed a ball, males are never joined by females (Matthews, 1963). If the infected males in this study had been successful at finding females, it is reasonable to assume their offspring would be smaller in size and less competitive than those from uninfected parents.

Despite the male and female responses to *P. sexalatus* being dramatically different, both the altered feeding in females, and reduced brood ball size in males have broad implications for ecosystem engineering. Ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species by causing physical state changes to biotic or abiotic materials (Jones *et al.* 1994 and Jones, 1997). As previously mentioned, dung beetles play an active role in reducing the presence of biologically and economically relevant pests by altering their availability to fecal resources. Pesticiferous flies such as the horn fly (*Haematobia irritans*) and the face fly (*Musca autumnalis*) use feces as a breeding ground for their young but are not capable of competing with dung beetles. Bornemissza (1970) shows that the presence of a healthy dung beetle population can reduce the presence of flies by ninety percent. Other harmful and costly diseases reduced by dung beetles fecal use include *Ascaris lumbricoides*, *Necator americanus*, *Trichuris trichiura*, *Entamoeba coli*, *Endolimax nana*, *Giardia lamblia*

(Miller *et al.* 1961) and *Cryptosporidium parvum* (Mathison and Ditrich, 1999). Based on estimates published by Fincher (1981) and Anderson *et al.* (1984), Losey and Vaugn (2006) estimate a \$380 million per year value of dung beetles in the United States pastured cattle industry. Because the benefits provided to humans by dung beetles depend on their consumption and processing of feces any change in these behaviors is likely to have significant consequences. The nematode parasite *P. sexalatus* significantly decreases both the feeding and dung burying behavior of dung beetles. In so doing the parasite very likely removes or diminishing the benefits dung beetles would otherwise provide.

In addition to reducing the presence of flies and parasitic worms dung beetles also play a vital role in nutrient recycling, soil aeration and secondary seed dispersal (Nichols *et al.*, 2008). From an ecosystem perspective, nutrient cycling is one of the most important acts carried out by dung beetles. As feces are removed from the soil and nutrient rich organic materials are incorporated into tunnel systems below ground, dung beetles instigate micro-organismal growth and chemical changes in the upper layers of soil. Soil health is often assessed through measurements of critically limiting agents including carbon and nitrogen (Vitousek *et al.*, 1997). Most nitrogen loss occurs through ammonia volatilization which dung beetles prevent by burying dung below the soil surface where moisture content is elevated (Gillard, 1967). In some cases the introduction of dung beetles has been shown to increased soil productivity and plant growth more effectively than chemical fertilizers (Miranda *et al.*, 2000).

While dung beetles are not the only group of animals that process feces they are the most important. The redundant species hypothesis suggests that a minimum number of species are required for basic ecosystem function but a variety of species carry out the same role and are therefore redundant (Walker 1992, Lawton and Brown 1993). Stockstad (2004) reviews this

hypothesis and shows that species diversity amongst the dung beetles is not a safeguard against ecosystem collapse. Nichols *et al.* (2007) also report that decreased dung beetle populations result in surplus dung and increased seed predation. When larger dung beetle species are removed from the population the smaller species are not capable of dealing with the additional fecal matter that remains (Stockstad, 2004).

While it is challenging to quantify the economic and agricultural importance of *Physocephalus sexalatus* and the role it plays in modifying the behavior of its dung beetle host we can assume a direct relationship between fecal processing and the benefits they provide. We have shown that dung beetles play a vital role in seed dispersal, soil aeration, nutrient cycling, and prevention of harmful flies and parasitic diseases. If fecal processing (including consumption and burial) is reduced by half due to the presence of this parasite, than it is not unreasonable to assume that the benefits provided by dung beetles are reduced by half as well. Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO) was one of the first groups to recognize the importance of dung beetles and actively manage populations for their benefit. Over 50 species of beetle were introduced to the country 23 continuing to thrive and improve the quality and fertility of their cattle industry and human health (Bornemissza, 1976).

Parasites frequently change the behavior of their host and the affects they have on behavior are often ignored. When parasites modify the behavior of their hosts, especially those that are relevant and important ecosystem engineers, they become ecosystem engineers themselves and should not be ignored. Nowhere on the planet are dung beetles considered pests. In some places across the world they are revered and protected (Figure 4.3) while in others they

are simply ignored. Because the benefits they provide are diverse and vast, I urge further exploration of their behavior and how their behavior is altered by parasitic disease.



**Figure 4.3:** Dung beetles are extremely important and even protected in various parts of the world. Sign on left was taken at Addo Elephant National Park near Port Elizabeth, South Africa. Sign on right taken at Ndumu Game Reserve in Kwazulu Natal, South Africa. (Photos taken by Linda Garrison, 2002)

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