THE BIOLOGY OF THE FLORIDA SCRUB ENDEMIC MILLIPEDE:

FLORIDOBOLUS PENNERI

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Master of Arts

By

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The undersigned, appointed by the Dean of Graduate School, have examine the thesis entitled

THE BIOLOGY OF THE FLORIDA SCRUB ENDEMIC MILLIPEDE:

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Presented by Danielle Antoinette Sattman

A candidate for the degree of Master of Art

And hereby certify that in their opinion it is worthy of acceptance.

Professor James Carrel

Professor Raymond Semlitsch

Professor Bruce Barrett

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

Figure Page

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

ABSTRACT

Even though millipedes provide a number of important ecosystem services, the group overall is understudied and nowadays largely ignored in the USA. Most millipedes that are well-studied occur in temperate, mesic environments, yet millipedes can be found to one degree or another in most habitats around the world. I report on several laboratory and field tests designed to learn about the ecology and life history of the large millipede, *Floridobolus penneri that* is endemic to native scrub only in south central Florida. *F. penneri* was found preferentially in patches of rosemary scrub, a xeric habitat containing extensive gaps of barren sand. Both male and female millipedes were active above-ground during the rainy season (July – October). Immature millipedes were captured fairly steadily during the entire period the pitfall traps were open (June-December), though peak activity for immature millipedes was early November, more than two months after the adult peak. Maximum temperature and weekly precipitation were not significant predictors for capturing mature millipedes. However, maximum temperature, but not weekly precipitation, was a significant predictor for capturing immature millipedes. Time-since-fire in rosemary scrub did not affect the number of millipedes captured at a site. Based on laboratory feeding trials, stable isotope analysis, and laboratory observations of feeding behavior, it seems unlikely that leaf litter and root tissues from woody scrub plants are major dietary inputs. The identity of the main food source of *F. penneri* remains an enigma.

CHAPTER 1: INTRODUCTION TO THE STUDY SYSTEM

Millipedes: Evolution, Phylogeny, and Taxonomy

Millipedes (Class Diplopoda) deserve much more respect and scientific attention than they get. Fully terrestrial millipedes were the first land animals, arising in the Ordovician Period more than 450 million years ago (mya) (Wilson and Anderson 2004). As abundant detritus feeders and occasional herbivores, diplopods served as the primary consumers of early plants and they played a major role in soil formation and nutrient cycling in Paleozoic ecosystems. In addition, millipedes may have been a selective pressure on early vascular plants to evolve defenses against herbivores. Subsequently in the Devonian, millipedes rendered primary productivity available to early terrestrial predators, such as amphibians and arachnids (Shear and Kukalova-Peck 1990). According to the fossil record, diplopods became very diverse and exceedingly common in the forests of the Devonian and Carboniferous (410-290 mya) and again in the early Triassic (248-220 mya) (Shear and Kukalova-Peck 1990, Wilson and Anderson 2004).

Millipedes are a major component in the evolutionary systematics of the phylum Arthropoda. Taxonomists traditionally place the Class Diplopoda within the Superclass Myriapoda (Table 1). Together with insects and their close relatives (the Superclass Panhexapoda), the myriapods comprise the Infraphylum Atelocerata [referring to the rudimentary antennae present only in early embryos]. As indicated in Table 1, the atelocerates and crustaceans are placed currently within the Subphylum Mandibulata.

Of the many models for the evolution of the Subphylum Mandibulata and the rest of the arthropods that have been proposed in the last 150 years, two alternative schemes

have received most support by modern systematists (Telford and Thomas 1995, Grimaldi and Engel 2005). The traditional "atelocerate" model, which is represented in the classification scheme in Table 1, indicates that crustaceans arose apart from atelocerates, and subsequently the lineages of myriapods and hexapods diverged from one another (Figure 1). The alternative "pancrustacean" model (Figure 2) places the myriapod-crustacean split fairly early and much latter the hexapods branched off from within the crustacean linage.

Not only does fossil evidence, but also the results of recent molecular, genetic, and neuroembryological studies, support the pancrustacean model in which millipedes diverged early on from the crustacea-insect clade (Telford and Thomas 1995). For example, hemocyanin proteins (Hc's) in insects and crustaceans are more similar in amino acid sequences to one another than they are to millipede Hc's (Hagner-Haller et al. 2004). Likewise, phylogenetic analysis using the structure of three nuclear protein-coding genes places the insect/hexapod clade deep within the Crustacea, far distant from the myriapods (Regier et al. 2005). Morphological data on neurogensis in euarthropod groups does not support the atelocerate model but it is consistent with the pancrustacean model of arthropod phylogeny (Fanenbruck et al. 2004, Stollewerk and Chipman 2006, Harzsch 2006).

The phylogeny of the Class Diplopoda at the ordinal level appears to be well established (Hoffman 1969). Extant millipedes are organized into fifteen taxonomic orders (Shelley 1999). The orders are combined into two subclasses (Penicillata and Chilognatha) based on the presence or absence of a hard, calcified exoskeleton. The majority of millipedes belong to the Subclass Chilognatha, which has a calcified exoskeleton, so the

orders in this subclass are further grouped into two infraclasses (Pentazonia and Helminthomorpha) Pentazonia are relatively short, broad millipedes, whereas Helminthomorpha are elongated, worm-like millipedes. Using morphological characteristics and cladistic analyses, all the orders of millipedes, with the exception of the enigmatic Siphoniulida, can be organized into a preferred cladogram (Figure 3). A recent phylogenetic analysis using nuclear protein-coding yields a cladogram (Figure 4) that largely agrees with the morphological tree. The discrepancies between the two approaches are considered minor and easily explained by the authors (Regier et al. 2005).

The millipede that is the subject of this thesis is *Floridobolus penneri,* Family Floridobolidae, Order Spirobolida. The Order Spirobolida contains medium to large-bodied species with smooth, cylindrical bodies having 35-60 segments. All spirobolids coil into a spiral when disturbed (Shelley 1999). Spirobolid millipedes occur worldwide. The species are grouped into ten families, most of which are considered to be well-defined and logical taxa (Hoffman 1969). A major, yet unresolved point concerns the question of "whether the genus *Floridobolus* represents a distinct monotypic family, or should better be added to the Spirobolidae as a third, fairly specialized, subfamily" (Hoffman 1969).

General Biology of Millipedes

Millipedes are functionally important in facilitating nutrient cycling and decomposition of dead plant tissues, perhaps much more so than is envisioned (Hättenschwiler and Gasser 2005). In addition, millipedes can be bioindicators for environmental changes in ecosystems. Even though millipedes provide a number of important ecosystem serv-

ices, the group overall is understudied and nowadays largely ignored in the USA. Most millipedes that are well-studied come from a narrow geographic distribution (England, Western Europe, and the Appalachian Mountains in the eastern USA) dominated by temperate, mesic environments, yet millipedes can be found to one degree or another in most habitats around the world. They are especially common in some moist, tropical forests.

Morphology and growth. One of the most notable characteristics of millipedes is that they have two pairs of legs on most body segments. Hence, the name "Diplopoda". They have a relatively inflexible body composed of three units: the head, a variable number of trunk segments, and a pygidial segment that contains the anus. Most millipedes have the ability to burrow below ground. In fact, some millipedes spend the majority of their lives below ground. On the other hand, there are some millipedes that have lost the ability to burrow and they live entirely above-ground in leaf litter, in cracks in the soil substrate, or up in trees (Shear 1999, Shelley 1999).

Millipedes exhibit a wide range of body lengths. The smallest millipede (in the family Polydesmidae) is 3 mm whereas the largest millipede (*Archispirostreptus gigas*) is 275 mm when fully mature. Finally, in comparison to insects and spiders, millipedes as a taxon are long-lived. The pill millipede, *Glomeris marginata,* takes several years to mature sexually and can live up to 11 years. In this case, adults molt annually and continue to increase in body size and mass (Carrel 1990).

Reproduction. Millipedes have separate sexes and are typically sexual. Some species exhibit unique mating rituals, while others do not display any courting behavior. For example, in taxonomically primitive millipedes (Subclass Penicillata, Order Polyxen-

ida), the male deposits a spermatophore in a mesh of threads, which is secreted by penis glands on the eighth and ninth pair of legs. A conspecific female is attracted to the threads and picks up the spermatophore with her mouth and deposits it in her spermatheca (Hopkin and Read 1992). In contrast, mating in taxonomically advanced hard-bodied millipedes (Subclass Chilognath) is accomplished by prolong clasping of the male and female. Spermatic material is extruded onto male gonopods either prior to clasping or after the gentalia is engaged, and then it is transferred to the female seminal receptacle. After mating, female millipedes store the spermatophore in their spermatheca. Eggs are fertilized just before oviposition. In addition to sexual reproduction, some millipedes can reproduce parthenogenetically, especially those species that have low ratios of males to females or no males at all (Hopkin and Read 1992, Shelley 1999).

Millipede eggs come in various forms and shapes. They are usually laid in the soil. Some millipedes (e.g. *Narceus americanus*) coat their eggs with their fecal matter, which disguises and protects the developing eggs (Shear 1999, Shelley 1999). Eggs are typically very yolky. They must be nutritious to nurture the offspring until after emergence following the second molt because many immature millipedes remain in an egg-capsule and do not feed till then (Hopkin and Read 1992). The number of eggs laid by a female millipede ranges widely: as few as 3 or 4 and up to 2,000 eggs are present in one clutch. Hatchlings are usually legless; they develop legs and add segments in each molt post eclosion. Thus, millipedes exhibit anamorphic development.

Enemies and defense. Diplopods are attacked by a variety of organisms, including other invertebrates, mammals, fungi, protozoans and reptiles. Millipedes typically

are slow moving and non-aggressive. Even though they may appear defenseless, the majority of millipedes exhibit three kinds of defensive mechanisms against a number of predators. First, most millipedes have a thick, hard exoskeleton that gives some protection. Second, most millipedes have the ability to curl into a spiral or ball that protects the sensitive head of the animal. For example, Eisner and Davis (1967) explained that an African pill millipede (*Sphareotherium sp.*) is able to escape predation from a number of enemies because of its very hard exoskeleton and the ability to form in a tight ball. However, this millipede is unable to escape predation from the banded mongoose, which hurls the millipede through its hind legs and smashes the millipede against a rock or another hard surface, so it can be eaten. Third, many millipedes employ defensive secretions that can cause stinging, irritation, or sedation to potential predators. In many instances the exudates contain hydrogen cyanide or quinones, but others discharge unusual molecules. Carrel and Eisner (1984) found that predacious wolf spiders (*Lycosa sp.*) that attempted to prey upon quinazolinone-secreting millipedes (*Glomeris marginata*) were quickly deterred by the exuded fluid; those few that persisted and consumed some secretion were sedated for hours if they ingested less than one droplet of secretion. On the other hand, Polyzoniidae millipedes (such as *Polyzonium rosalbum)* secrete polyzonimine and nitropolyzonamine when disturbed. Polyzonimine is a known ant deterrent. It has been shown when ants attack this millipede they will immediately retreat when the millipede secrets its toxins. Thus, the millipede escapes the attack unharmed (Eisner et al. 2005).

Activity patterns. In general, millipede activity above-ground is usually correlated with seasonal climatic variations. Most species of millipedes are active when it is

warm, especially during the rainy season or after a single rain event (Hopkin and Read 1992, Shelley 1999). For example, Barlow (1960) showed that temperate *Cylindroiulus frisius* millipedes are most active during wet, warm conditions. Usually millipedes are found in habitats containing much leaf litter so they can feed, remain hidden, and stay moist, all at the same time. Millipedes are more susceptible to desiccation than other terrestrial arthropods because many apparently lack a waterproofing epicuticular lipid layer (Edney 1977, Apple 1988). Thus, many millipedes stay hydrated by living in the moist leaf litter. Edney (1977) concluded because millipedes lack an epicuticular lipid layer, they are concentrated in humid habitats. Also, within a given species of diplopods, immatures in general are more susceptible to desiccation than adults. Immature millipedes not only are relatively large (macroarthropods), but also they have a large surface area relative to their volume. Thus, these animals are more prone to desiccation relative to adult individuals (Edney 1977).

Millipedes living in extreme environments use climatic conditions as cues to coordinate their behavior, which assists in their survival. Karamaouna (1987) showed that Mediterranean millipedes were only active during the rain season; they remained in burrows in the ground during the dry season. Contrary to Edney's claim, some millipedes may have evolved a waterproofing epicuticular lipid layer. Crawford (1979) confirmed that that the desert millipede (*Othoporus ornatus*) has a thin layer of wax on the surface of the exoskeleton to reduce water loss.

Nutrition. Millipedes require calcareous soils or other sources to provide calcium for their exoskeletons (Cromack et al. 1977, Hopkin and Read 1992). Lyford

(1943) found that the common brown millipede (*Diploiulus londonensis*) prefers to consume leaves that contain higher calcium contents. In calcium poor ecosystems, invertebrates obtain calcium mainly from eating exoskeletons of dead invertebrates (Seastedt and Tate 1981). Some millipedes routinely consume their own exuvia after molting to obtain calcium and other nutrients (Hopkin and Read 1992). The need for diplopods to accumulate calcium means that, for this reason alone, they are an important component in the cycling of calcium in some terrestrial ecosystems.

The vast majority of millipedes are detritivores that feed opportunistically on decaying leaves or wood on the ground (Mundel 1990, Hopkin and Read 1992, Shelley 1999). A few species eat living plant tissue or decaying animal tissue (Mundel 1990, Hopkin and Read 1992). This does not mean that millipedes indiscriminately consume any decaying plant material that they encounter. In fact, most species of millipedes when tested have shown a clear feeding preference for some types of leaf litter (Hopkin and Read 1992). If millipedes are given a choice among several different plant substrates, they usually demonstrate a preference for some and an aversion for other kinds of leaves or wood. Carcamo et al. (2000) showed that the Pacific Northwest millipede *Harpaphe haydeniana* exhibits a preference for rotting Douglas-fir needles (*Pseudotsuga menzieii*) and, to a lesser extent for Sitka spruce litter (*Picea sitchnesis*), rather than other coniferous litter. Among broadleaf species, swordfern (*Polystichum munitum*) was clearly avoided. On the other hand, paper birch (*Betula papyrifera*), bigleaf maple (*Acer macrophyllum*), vine maple (*Acer circinatum*) and red adder were consumed at a much higher rate by *H. haydeniana*.

The above information is an overview of the general biology of millipede species found mostly in mesic habitats. Yet, some millipedes are found in dessert, tundra, alpine, chaparral, savanna, and scrub habitats. Diplopods living in these atypical ecosystems might be expected to exhibit different characteristics than those described above. Thus, the millipede *F. penneri* I studied in xeric Florida scrub was, from the start of my work, expected to have some atypically life history traits.

The Florida Scrub Ecosystem

The Florida scrub ecosystem exhibits a number of paradoxical characteristics. Scrub is like a seasonally wet desert: it is dominated by xeromorphic shrubs in a region known for its for high humidity and rainfall. High temperatures prevail in summers, whereas winters are comparatively cool; occasionally temperatures in December through February briefly drop below freezing. Annual rainfall exceeds 1200 mm per year, but the majority of the rainfall typically occurs from June to September, whereas relatively little rainfall occurs during the rest of the year (the "dry season"). Another anomaly is that native scrub is subjected to frequent fires caused by lighting, typically at the start of the rainy season (May-June). Yet the vegetation regenerates quickly after a burn by resprouting because the dominant shrubs all have subterranean stems, allowing full recovery in only a few years. Finally, the scrub is low in species diversity, yet importantly globally in biodiversity ratings because it has one of the highest levels of endemism in the United States. Because Florida scrub exhibits a wide many life-history oddities in a rapidly developing state, it is an ecosystem of high importance in studying speciation, endemism, evolution, behavioral biology, and conservation biology.

Geomorphology of scrub. The Florida scrub ecosystem occurs naturally on fragmented ridges that were formed from Pleistocene sand dunes running North and South in central and costal Florida. The peninsula of Florida is exceptionally flat except for the series of low sand ridges, ranging in elevation from thirty-seven to sixty-two meters above sea level (Figure 5). In the late Pleistocene, when Florida's climate was much cooler and drier than now, scrub vegetation was more widespread in the peninsula, even off ridges, amounting to twice its current size (Abrahamson et al. 1984; Myers 1990). Throughout this era the scrub vegetation went through a succession of transformations from rosemary scrub to oak savanna and then to sand pine scrub (Myers 1990). The scrub's range contracted to the sandy ridges when climatic conditions became moister and sea levels rose, about 5,000 -7,000 years ago (Abrahamson et al. 1984; Myers 1990).

Scrub vegetation. There are several distinctive vegetative associations in present day Florida scrub. The habitats are distributed as a patchwork across the landscape; their presence is correlated in part with soil types, topography, distance to seasonal ponds, and burn history. The tops of the ridges, at elevations > 50m, are called "sandhill" (Figure 5). They have sand and slash pines growing with oaks in the sloping, excessively well-drained sands. The rest of the ridges, which are quite flat for the most part, are a mosaic of scrub associations that vary in openness and species composition.

The rarest component (ca 5%) of Florida scrub is called "rosemary scrub". It is dominated by Florida rosemary (*Ceratiola ericoides*) (Empetraceae), an evergreen aromatic shrub that grows slowly in an urn-shape and inhibits other shrubs with allelopathic chemicals, causing formation of "balds". Rosemary scrub is confined to barren knolls

that rise gently like pillows in the otherwise flat ecosystem to elevations of 40-50 meters (Figure 5). Thus, rosemary balds are not affected by summer flooding, unlike other scrub habitats. Long fire intervals are required to perpetuate rosemary balds because rosemary plants are obligate seeders (Menges and Kohfeldt 1995).

Scrubby flatwoods (oak scrub) is the most abundant type of vegetation found in the Florida scrub ecosystem. Typically, this vegetation consists of a dense matrix of oaks, palmettos, and lyonia and scattered sand and slash pines. Scrubby flatwoods is found on white or gray sands that are moderately well-drained, thus seasonal summer flooding occurs in this vegetation. Typically, this vegetation burns frequently, about every 5-20 years. The majority of plants resprout and spread clonally to regenerate the same community post-fire (Abrahamson 1984a and 1984b; Johnson and Abrahamson 1990; Menges and Kohfeldt 1995).

Fire in scrub. The scrub is a pyrogenic ecosystem maintain by frequent-infrequent, patchy, and short-lived but intense burns. Historically, lighting ignited these stand-replacing burns. In fact, south central Florida has one of the highest frequencies of lighting strikes in the United States (Abrahamson 1984a; Myers 1990). Ninety-seven percent of all lighting-caused wildfires occur in the transition from dry-to-wet season and less so later during the summer rainy season (Abrahamson 1984a and 1984b; Myers 1990). Scrub is neither particularly flammable nor easy ready to ignite. Often scrub is ignited by adjacent grassy vegetation that is on fire. Several conditions must be present for the fire to intensify and spread. High temperatures, low humidity, and low surface soil moisture are typical requirements, which happen almost daily in the afternoon in cen-

tral Florida. Actual burns in scrub so short-lived that heat from the flames does not penetrate more than a few centimeters into soil.

After fire, the vegetative community regenerates by two main mechanisms. First, the dominant woody shrubs quickly resprout and grow both vertically and horizontally. Within 2-4 years the shrub matrix returns or surpasses pre-fire levels and species composition is completely restored (Abrahamson 1984; Johnson and Abrahamson 1990). On the other hand, herbaceous plants recover by seed germination in gaps between the shrubs. For many "obligate seeders" fire is a necessity for population persistence or recovery since it is the only mechanism for gap generation. It takes obligate seeders 10 to 12 years to return to pre-fire population densities in rosemary balds, whereas in other habitats where fire is excluded for decades the seed-bank may be depleted and fire/ burning cannot allow the community to recover (Johnson and Abrahamson 1990). Thus, obligate seeders are more sensitive to frequent fire intervals. Most importantly, without fire-induced perturbations in the Florida scrub, there is a decline in the presence of endemic herb species, a decline in plant biodiversity, and colonization of invasive species (Menges 1999). Ultimately, fire is an imperative force for the plants and vertebrates in this ecosystem.

***F. penneri* Millipedes at Archbold Biological Station .** A little-known invertebrate endemic in the Florida scrub is *F. penneri,* a giant burrowing millipede. *F. penneri* is the only member of its genus and its family, Floridobolidae. In all probability, *F. penneri* is the only scrub endemic millipede. Despite its large size and easy identification, virtually nothing is known about *F. penneri*'s life history. The first taxonomic description

of *F. penneri* was based on two males collected approximately 16 km west of Archbold Biological Station, in Highlands County, Florida. A more detailed description of the species was based on a few males and one female. *F. penneri* has an average body length between 74 to 92 millimeters and width between 10.8 to 11.6 millimeters. A habitat preference for *F. penneri* in Florida scrub is not reported, but the species has been collected only in scrubby areas of both Highlands and Polk Counties. It is believed that *F. penneri* is restricted the Lake Wales Ridge, like its vertebrate analog the federally listed sand skink, *Neoseps reynoldsi* (Deyrup and Franz 1994).

Archbold Biological Station (ABS) owns and manages 2,077 hectares (5,193 acres) of scrub habitat, which makes it the largest, private preserve of the Florida scrub ecosystem. Richard Archbold, an internationally known aviator and explorer, founded ABS in 1941 and lived on the station until his death in 1975. While Mr. Archbold was alive and for twenty years thereafter, the lawns bordering the driveways were maintained in tip-top condition by regular mowing, raking, and fertilizing using a 6-6-6, N-P-K blend. During the dry season, lawns were irrigated weekly (J. Layne, personal communication). In 1995-1996 both fertilizing and watering of lawns was halted permanently at ABS. In addition, fire was suppressed or excluded on main grounds at ABS continuously until recently (1929-1990).

In June of 1958, Dr. Thomas Eisner happened upon the ABS by chance while on a field trip after the end of the spring semester at Cornell. In Dr. Eisner's book, For the Love of Insects, he mentions that on his first night at ABS he encountered at least 100 *F. penneri* and *Narceus gordanus* millipedes on the station grounds just east of the main

building. In addition, Dr. Jim Carrel (Eisner's former graduate student) recalls being able to catch a surplus of *F. penneri* millipedes on warm, wet summer nights (June-August) on the station's driveways and adjoining lawns in the late 1960's. The reason for mentioning this information is that when I was at ABS in the summer of 2003, I never found *F. penneri* around the buildings or on the driveway and lawns at night nor did I ever see more than 5 millipedes together at once in the scrub despite repeated attempts to find them. Dr. James Carrel has had the same experience since 2000 during the rainy season. The millipedes seem to have disappeared from the main grounds and the numbers of these animals may have sharply declined at the station over the past 10 years or more. Could Mr. Archbold's lawn maintenance have inadvertently promoted high *F. penneri* and *N. gordanus* densities around the station's buildings, giving investigators an erroneous view of millipede abundance and distribution in Florida scrub? The purpose of this thesis project was to gain more insight into the natural history of *F. penneri* in native Florida scrub.

Figure Legends

Figure 1. Atelocerate hypothesis indicates that crustaceans arose apart from atelocerates, and subsequently the lineages of myriapods and hexapods diverged from one another.

Figure 2. Pancrustacean hypothesis places the myriapod-crustacean split fairly early and much latter the hexapods branched off from within the crustacean linage.

Figure 3. Phylogeny of all millipede orders based on morphological characteristics, with exception of the Siphoniulida, can be organized into a preferred cladogram (Regier et al. 2005). The arrangement of orders is taken from the molecular phylogeny in Figure 4. Two semicircular lines indicate alternate arrangements from morphological data.

Figure 4. Phylogeny of all millipede orders based on nuclear protein-coding (Regier et al. 2005).

Figure 5. Elevation transect across the peninsula of Florida, passing through the highest point at the Archbold Biological Station.

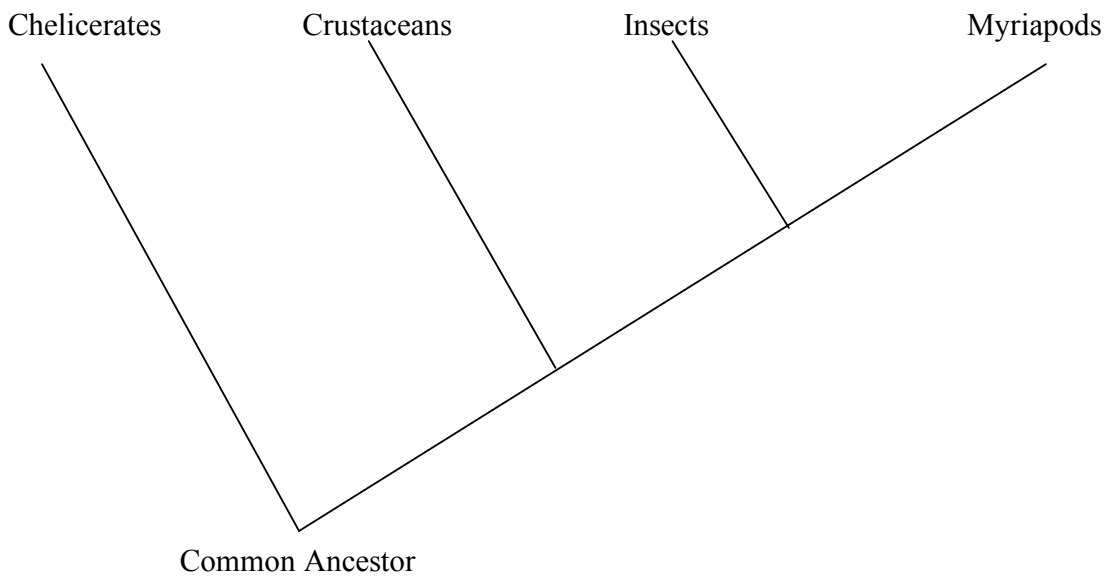
Chelicerates
Crustaceans
Insects
Myriapods
Common Ancestor

Figure 1

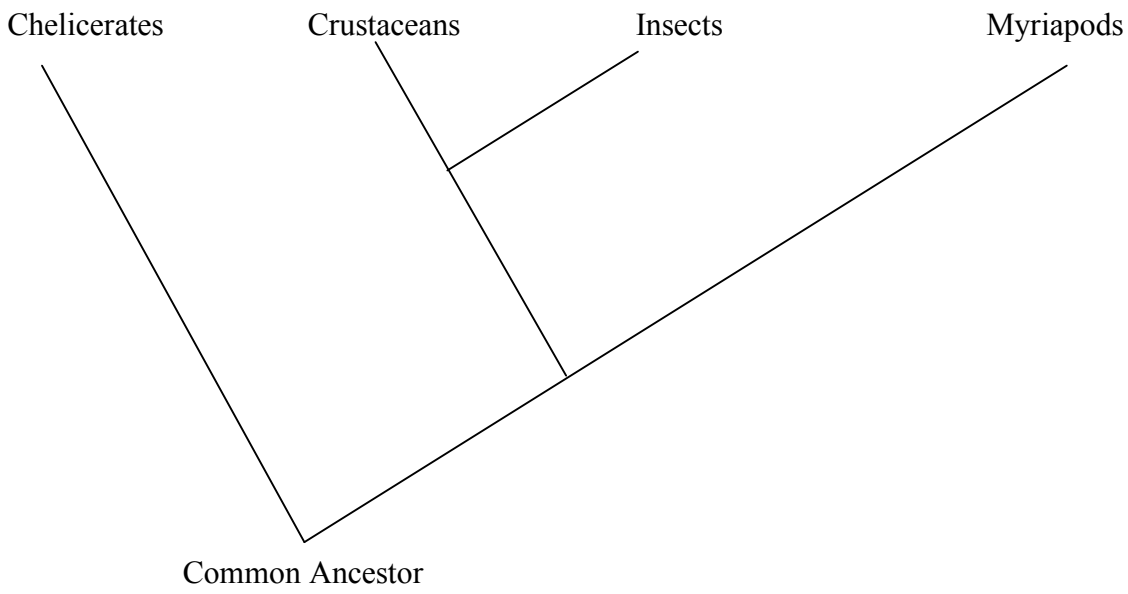
Chelicerates
Crustaceans
Insects
Myriapods
Common Ancestor

Figure 2

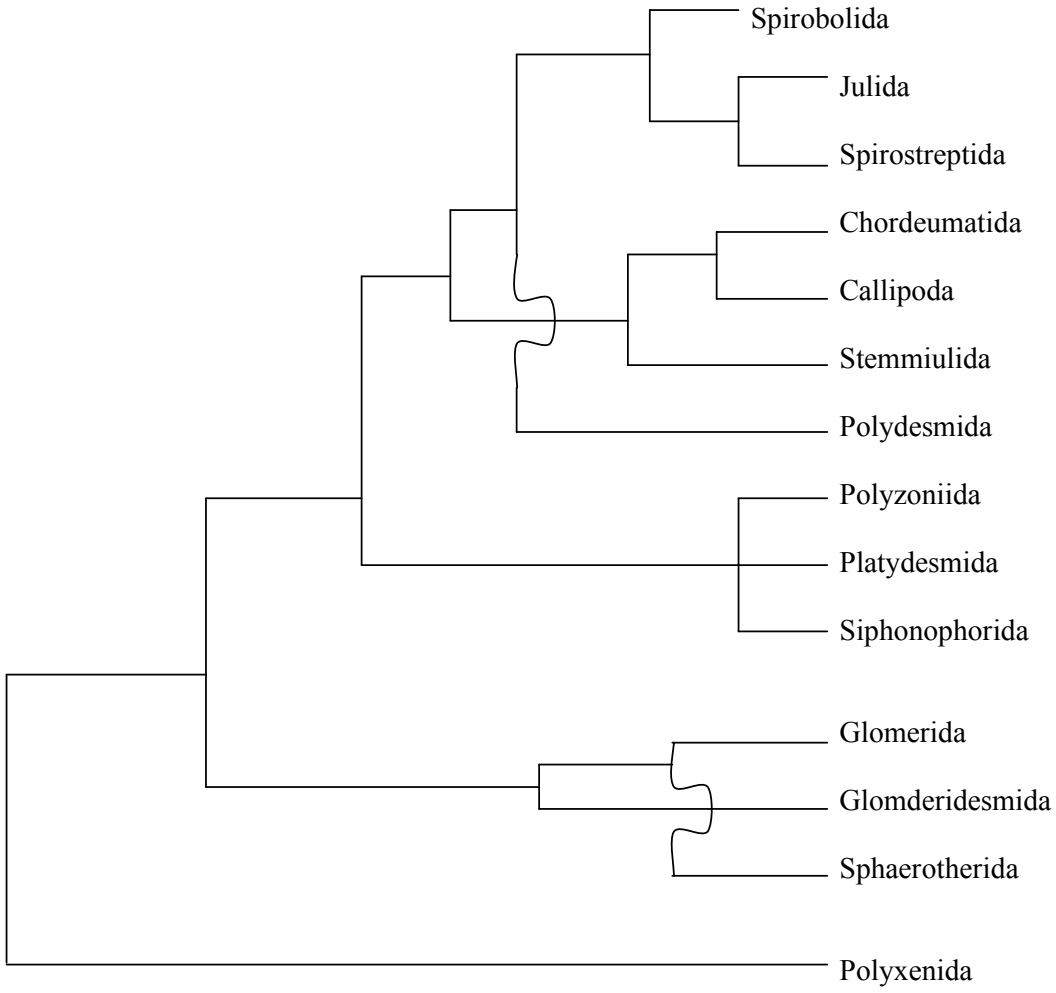
Spirobolida
Julida
Spirostreptida
Chordeumatida
Callipoda
Stemmiulida
Polydesmida
Polyzoniida
Platydesmida
Siphonophorida
Glomerida
Glomderidesmida
Sphaerotherida
Polyxenida

Figure 3

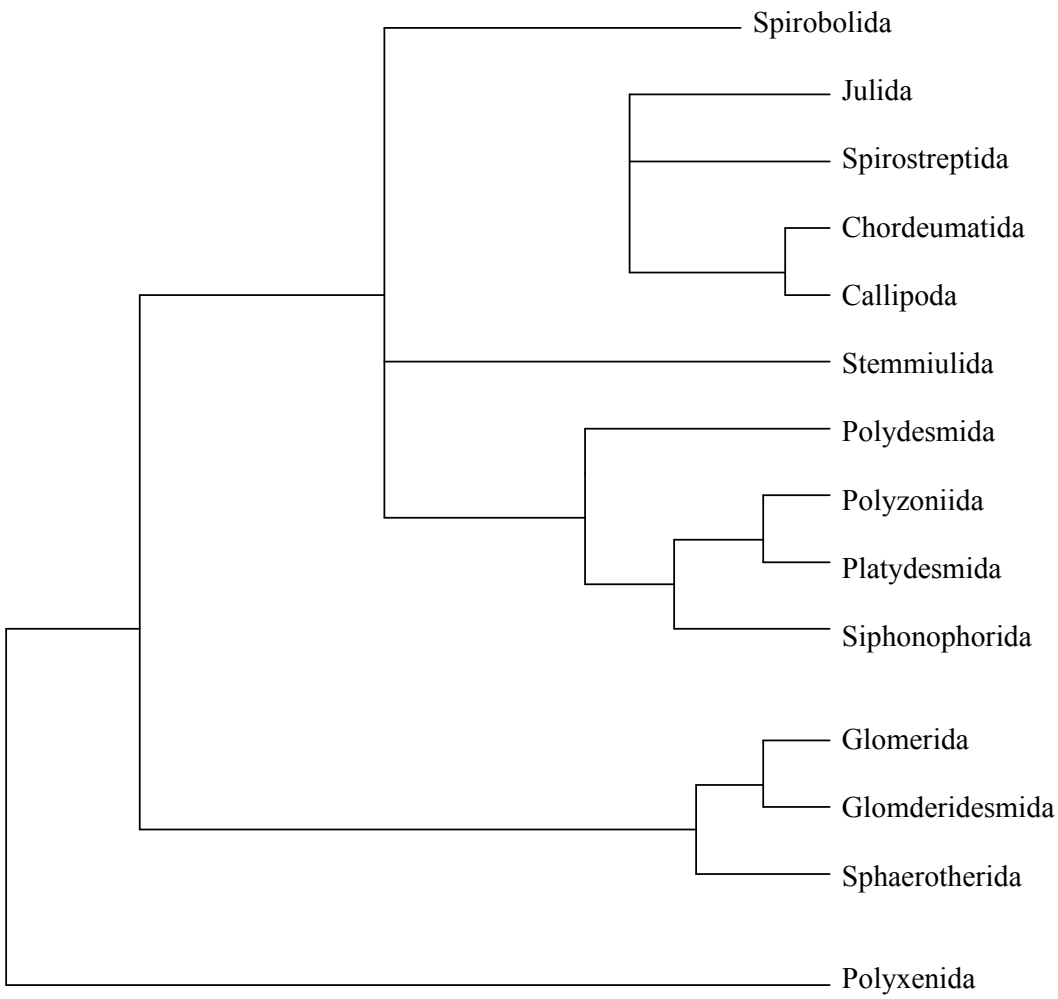
Spirobolida
Julida
Spirostreptida
Chordeumatida
Callipoda
Stemmiulida
Polydesmida
Polyzoniida
Platydesmida
Siphonophorida
Glomerida
Glomderidesmida
Sphaerotherida
Polyxenida

Figure 4

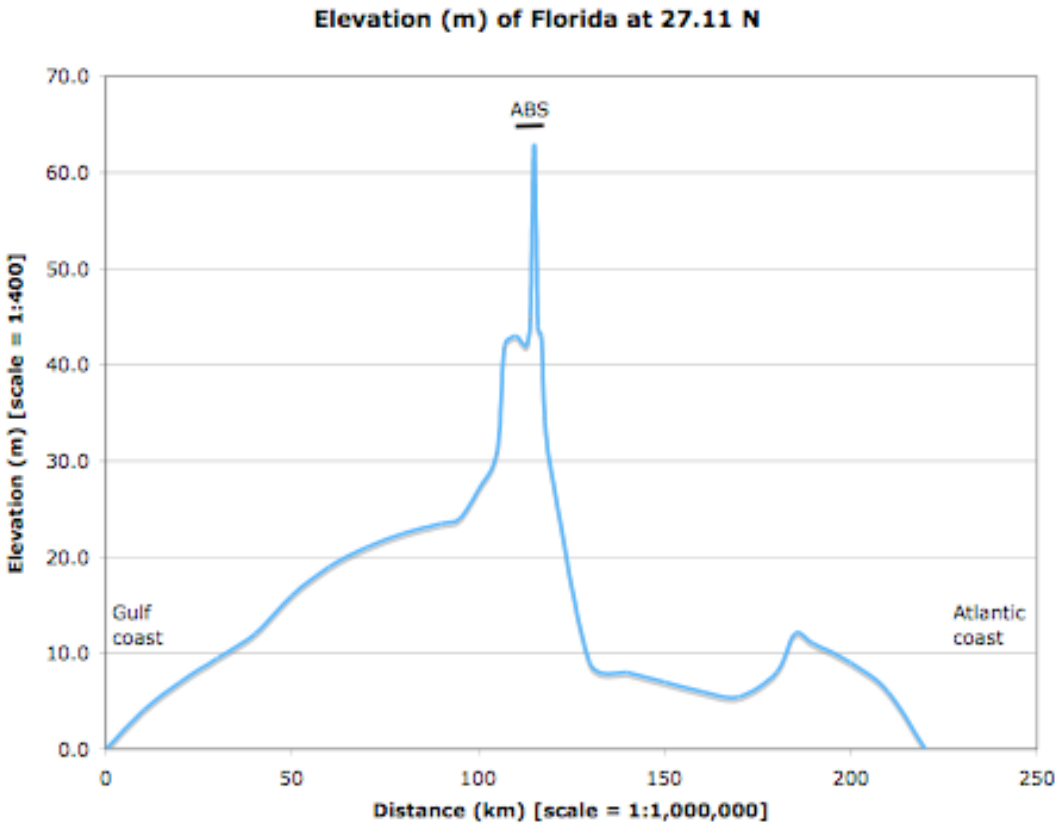
Elevation (m) of Florida at 27.11 N
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Elevation (m) [scale = 1:400]
ABS
Gulf coast
Atlantic coast
0
50
100
150
200
250
Distance (km) [scale = 1:1,000,000]

Figure 5

Table 1. Classification of Extant Arthropods, with Emphasis on Millipedes.

(based on Table 3.2 in Grimaldi and Engel 2005)

Phylum Arthropoda

Subphylum Arachnomorpha

Superclass Chelicerata

Epiclass Pycnogonida (sea spiders)

Epiclass Euchelicerata

Class Xiphosura (horseshoe crabs)

Class Arachnida (mites, spiders, etc.)

Subphylum Mandibulata

Infraphylum Crustaceomorpha

Superclass Crustacea

Epiclass Eucrustacea

Classes of crustaceans (n = 6)

Infraphylum Atelocerata (= Tracheata)

Superclass Myriapoda

Class Chilopoda (centipedes)

Class Symphyla

Class Pauropoda

Class Diplopoda (millipedes)

Superclass Panhexapoda

Epiclass Hexapoda

Class Entognatha (springtails)

Class Insecta (insects)

CHAPTER 2: ENERGETICS AND DIETARY STUDIES WITH *F. PENNERI*

INTRODUCTION

Metabolism is the sum total of chemical processes that occur in living organisms, including the breakdown of nutritional molecules, extraction of energy from this breakdown, and the synthesis of new molecules for growth and development, maintenance, and reproduction. In animals, such as millipedes, cells require oxygen to break down sugars and other organic compounds. The metabolic rate measures the rate at which energy is utilized by organisms. Energetic or metabolic transformations underlie all biological activities, from the molecular and biochemical level to the ecological and evolutionary (Brown et al. 2004, Allen et al. 2006).

Most species of millipedes are are considered to be generalist detritivores that feed fairly indiscriminately on decaying leaves and rotting logs. Yet, some species of millipedes are known to select certain plants in the field and others show clear choices in laboratory tests (Kheirallah 1979, Dudgeon et al. 1990, Dangerfield 1993, Carcamo et al. 2000). Some millipedes have a more specialized diet. Those millipedes consume fresh plant material, soil, algae, and even dead invertebrates under natural conditions (Hopkin and Read 1992, Shelley 1999).

Many millipedes only emerge from retreats in soil, beneath rocks, or in leaves and logs for a short period of time to feed. Typically, during this brief period of activity millipedes will spend much of their time feeding. Van der Drift (1975) found that *Glomeris marginata* consumes about ten times its own weight in leaf litter each year. Blower

(1974) determined when *Ophyiulus pilosus* reaches maturity the millipedes have eaten about five times their weight in leaf litter. Similar figures have been found for other temperate species of millipedes (Hopkin and Read 1992). Millipedes that spend the majority of their lives below-ground are able to temporarily decrease metabolism to sustain their lives while they are subterranean.

Feeding trials have traditionally been used for evaluating rates of food consumption and dietary preferences in animals, such as millipedes. For example, Kheirallah (1979) found that *Julus scandinavius* had a clear preference of leaf litter; he found that the species order of preference was ash, sycamore, birch, and oak. Oak leaves were the least preferred leaf substrate, perhaps because they were highest in tannin concentration.

Many feeding trials with millipedes sometimes fail due to the animals' reluctance to feed in the laboratory. Blower (1974) noted that previous feeding trials on the millipede *Ophyiulus pilosus* had failed. He determined that conventional preparation methods, in which leaf litter was dried for 48 hours at 60° C and then crushed, prevented millipedes from consuming the leaf litter. Blower inferred that millipedes did not eat processed leaf litter due to a lack of living bacterial and fungal colonists. He fixed this problem by soaking the leaves in water for a period of time and then successfully conducted the feeding trials on the millipedes. Similarly, O'Neill (1968) noted that the millipede *Narceus americanus* would reject leaf litter and only feed on rotting logs in the laboratory.

Since feeding trials may not accurately reflect the actual diet of an animal in the wild, another complimentary approach for dietary research is stable isotope analysis.

Stable isotope analysis looks at the diet over a longer period of time and, therefore, reflects the long-term feeding behavior of animals. Moreover, Tayasu (1998) determine that stable isotope signatures are useful parameters for investigating detritivores diets.

The isotopic composition of animal tissues serves as a natural tracer of different dietary inputs with distinct signatures. Animal tissues are enriched in ^{15}N (= heavy nitrogen) relative to food resources. On average, the $^{15}N/^{14}N$ ratio of consumers is increased by 3 to 4 percent compared to their food substrate (Peterson and Fry 1987, Cabana and Rasmussen 1994).

The focus of this chapter is on the energetics of the endemic Florida scrub millipede, *F. penneri*. *F. penneri* is found only on the southern tip of the Lake Wales Ridge, an area about 600 km^2. Relatively nothing is known about the life history and ecology of this animal.

It has been speculated that *F. penneri* may feed primarily on decaying leaf litter from scrubby flatwoods vegetation, the most abundant habitat found in the scrub (~30% of total hectarage). Scrubby flatwoods is dominated by species of shrubby oaks (primarily *Quercus inopina* intermixed with *Q. chapmanii, Q. myrtifolia,* and *Q. minima*), two palmettos (*Serenoa repens* and *Sabal etonia*), and three lyonias (*Lyonia ferruginea, L. fruticosa,* and *L. lucida*). These evergreen, woody shrubs form a dense matrix that typically is 1-2 m high, producing a layer of leaf litter 2-15 cm deep except in the occasional, small (≤ 0.1 m^2) gaps In addition, it has also been speculated that the *F. penneri* may also feed on roots of common shrubs, such as *Q. inopina*, since they spend the majority of their lives below ground.

METHODS

Study Site

Archbold Biological Station (ABS) is located near the southern tip of the Lake Wales Ridge in Highlands County, Florida (27° 11′ N lat., 81°21′ W long.), 12 km south of the town Lake Placid. The elevation ranges from approximately 36 to 67 meters above sea level. ABS manages one of the largest remaining contiguous tracts of undeveloped land in peninsular Florida, comprising 2300 ha of scrub and other native communities.

All *F. penneri* were taken from rosemary scrub, the only habitat were they seemed abundant during the study. Rosemary scrub is the least extensive kind of habitat at ABS (~2% of the total hectarage), but it contains the most endemic herbs growing in the extensive sandy gaps. Florida rosemary (*Ceratiola ericoides*) (Empetraceae) is the dominant shrub. This evergreen bush is interspersed with large gaps of open sand where herbaceous plants are found. Occasionally, an over-story of sand pines will be found in rosemary scrub. Rosemary "balds" are found on pillow-like ridges and knolls, 40 to 50 meters in elevation, that rises from the flat scrub wherever well-drained white sands are found (Myers 1990). This vegetation has a low fire frequency, ranging ten to one hundred years (Myers 1990).

Standard Metabolic Rates

Twenty-two immature, nine female, and twelve male *F. penneri* were collected from June to October 2004 and in February 2006. All millipedes (n = 43) were food-

deprived for 48 hours prior to laboratory testing to permit emptying of the gut and achievement of a post-absorptive condition.

Oxygen consumption by single millipedes was measured in a simple manometer involving a single millipede in a 50 mL plastic vial. The manometer contained approximately 4 grams of soda lime wrapped in cotton biopsy bags to absorb carbon dioxide. Millipedes were sealed inside the plastic vials plugging up the open end with a rubber stopper fitted with a calibrated 1mL pipette. After waiting five minutes for equilibration a drop of manometric fluid was added to the pipette to permit detection of oxygen consumption. All experiments, lasting for one hour, were conducted at temperatures ranging from 25°-26°C.

Feeding Trials

F. penneri used for leaf litter consumption trials were collected from rosemary balds from August to November 2003. Millipedes used for root consumption trials were collected from rosemary balds from July to September of 2004. Only mature millipedes were used for both feeding trials. Animals were weighed individually and placed in Sysco 32-ounce deli containers with an 11 cm diameter. In order to maintain hydration, each container was filled approximately two-thirds with moist sand taken from rosemary scrub and covered with a lid having 8-10 holes (5 mm diameter). Millipedes were food-deprived for 48 hours prior to each feeding trial. Millipedes not used for tests were maintained on a diet of fresh, peeled cucumbers.

Leaf litter was collected from the ground from scrubby flatwoods habitat located directly east of the main buildings of ABS, which had not been burned in at least ten

years. Decaying leaves were identified to species level based on leaf shape and the identity of the overarching shrub. The type of leaf litter used was *Quercus myrtifolia, Q. geminata, Q. chapmanii, Q. inopina, Lyonia ferruginea, L. lucida, L. fruticosa, Persea humilis,* and *Serenoa repens.* Leaves were pooled by species, brushed to remove sand, and then soaked in rainwater 5 days prior to a feeding trial.

Roots from dominate shrubs for feeding trials were collected in three, long- unburned rosemary scrub sites. To collect living roots, I found isolated plants and dug up roots from directly underneath the stems. The types of roots used for the root feeding trials were *Q. inopina, L. ferruginea, S. repens,* and *Ceratiola ericoides.* Similar to the leaf litter consumption trials, roots were rinsed with deionized water to wash away sand and soaked in rainwater for 5 days before a feeding trial.

Millipedes were food deprived for two days prior to the feeding trial. If an animal produced any frass, it was removed from the container before a test. Each millipede was given a standard amount of leaf litter (1.5 grams) or roots (1.0 gram) of one plant species for 48 hours. A total of fifty millipedes each were given all nine leaf litter types. Every week each millipede was randomly chosen a new leaf litter type to use for the consumption test. On the other hand, because of the abundance of millipedes at the start of the root feeding trials, millipedes were randomly assigned one four root types. Thus, millipedes were only used once during the entire root consumption experiment. At the end a two-day feeding trial, shoots or roots were removed from containers and weighed.

To determine the actual amount of dry shoot or root tissue consumed first the wet weight of plant tissue at the end of a feeding trial was subtracted from the initial wet

weight at the start of the trial. Subsequently, this value for wet tissue consumed was converted to dry matter using water content values. The dry tissue proportion and water content of soaked plant tissue was calculated from control measurements made using a drying oven at 60°C for 48 hours for each plant substrate.

Stable Isotope Analysis

Millipedes and vegetation substrates were collected from October to November of 2004 from four rosemary bald sites. Four millipedes, each collected at a different site, were brought back to the laboratory and allowed 48 hours to clear their guts before they were killed by freezing at -20º C for a day. Millipedes were thawed, gently washed with distilled water to remove any dirt or detritus from the exoskeleton, and dried at 60°C for 48 hours, then ground individually using a Wiley mill.

In addition, five kinds of vegetative substrates were collected from four rosemary scrub sites and the samples were combined to form one composite sample. For example, when I collected *Q. inopina* leaves I collected four leaves from all four sites. I grounded a total of 16 leaves to make one composite sample. All plant substrates were gently rinsed with distilled water and dried at 60°C for forty-eight hours and ground individually using a Wiley mill.

Samples were analyzed for ^{13}C:^{12}C and ^{15}N:^{14}N ratios using a continuous flow stable isotope ratio mass spectrometer (Delta-Plus CFIRMS) at the W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory at the University of California, Irvine. The CFIRMS was interfaced with a Gasbench II (for CO_2 or H_2O stable isotope analysis) and a Fisons NA-1500 for ^{13}C and ^{15}N analysis. The CFIRMS provided very precise

measurements of stable isotopes (far higher than the precision provided by the AMS system). All isotope ratios were expressed as either $\delta^{13}C$ or $\delta^{15}N$ values (units of $^{0}/_{00}$) according to the following equation: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1]*1000$ where R_{sample} is the $^{13}C:^{12}C$ and $^{15}N:^{14}N$ ratio of the sample and $R_{standard}$ is the $^{13}C:^{12}C$ and $^{15}N:^{14}N$ ratio of the standard.

Statistical analysis

Resting metabolic rates

Standard metabolic rates of millipedes were analyzed using a univariate analysis of variance to compare oxygen consumption and standard metabolic rates of male, female, and immature *F. penneri* millipedes (SPSS, GLM Univariate). Post-hoc comparisons were made using Tukey's HSD test with *P* set at 0.05 to determine which sample means difference exist.

The standard metabolic rate was used to determine the minimal amount of calories consumed to sustain an animal's life. The standard formula (1mL O_2 consumed = 5 calories of energy from glucose metabolism) was used to calculate the minimal energetics of the millipedes' basic existence using Golley's (1961) energetics for leaf litter tissues and root tissues 4.2 Kcalories/grams and 4.7 Kcalories/gram, respectively.

Feeding Trials

Consumption of leaf litter. In 2003, my preliminary pitfall traps only captured about 100 *F. penneri* millipedes. Since I only captured a small number of millipedes I had to reuse the millipedes for the consumption of leaf litter feeding trials. Since I reused millipedes, leaf litter consumption was analyzed using a repeated measures analysis of

variance (SPSS, GLM Repeated Measures). The sphericity assumption was not met so the Huynh-Feldt correction was applied = F (5.6, 34.79). Post-hoc comparisons were preformed using the Bonferroni adjustment for multiple comparisons to determine which sample means difference exists.

Consumption of roots. In 2004, I captured over 500 *F. penneri* millipedes. Since I was able to capture a large number of millipedes I was able to only use each millipede once for the consumption of root feeding trials. Since I only used the millipedes once, root consumption was analyzed using a univariate analysis of variance (SPSS, GLM Univariate). Post-hoc comparisons were made using Tukey's HSD test with P set at 0.05 to determine which sample means difference exist.

RESULTS

Standard Metabolic Rates in Millipedes

There were significant differences in resting metabolism for the different stages of *F. penneri* (F (2, 43) = 10.913, $P < .0005$; Figure 1 and Table 1). Standard metabolic rates for male and female millipedes were similar, 0.06 ± 0.01 mL O_2 h^{-1} g^{-1} and 0.04 ± 0.01 mL O_2 h^{-1} g^{-1} , respectively (Figure 1 and Table 1). On the other hand, standard metabolic rates for immature millipedes (0.24 ± 0.04 mL O_2 h^{-1} g^{-1}) were significantly higher than in adults. Overall, the results show that standard metabolic rates for *F. penneri* vary significantly by stage, but differences are driven by the adult-immature disparity (Figure 1).

Based on the rate of oxygen usage by the millipedes and published equations for converting respiration rate to ingestion rate, we calculated the minimum amount of leaf litter or root tissue that *F. penneri* would need to consume to sustain its life. Male millipedes would need to consume at least 0.0043grams/day of leaf litter or 0.0038 grams/day of root tissue to sustain their lives under minimal activities (Table 2). Likewise, female millipedes would consume at least 0.0049 grams/day of leaf litter or 0.0043 grams/day of roots, respectively to sustain their lives at rest (Table 2). Finally, immature millipedes need to consume a minimal of 0.0094 grams/day of leaf litter and a minimal of 0.0084 grams/day of root tissue to sustain their lives under minimal energetics (Table 2). Given that plant tissues are biochemically complex and much of the material is probably ingestible, the actual intake might be ten or more times greater than we calculated: this would elevate intake to 0.04-0.05 g/day for adults and 0.08-0.10 g/day for immatures.

Feeding Trials

Consumption of leaf litter. Millipedes ate some of every kind of leaf litter offered to them (Figure 2 and Table 3). Analysis of variance showed that the main effect of litter was significant (F (5.6, 34.79) = 2.47, $P < 0.0005$). Post-hoc multiple comparison revealed that there were no significant differences in consumption for *Q. inopina, Q. myrtifolia, Q. chapmanii, Q. geminata, L. ferruginea, L. lucida, L. fruticosa,* and *P. humillis* (Figure 2 and Table 3). However, S. *repens* was consumed significantly less than all other kinds of leaf litter (Figure 2 and Table 3). Overall, although plant species was a significant variable, the species–specific differences in out come were mainly driven by *S. repens* (Figure 2).

Consumption of root tissue. Millipedes ate some, but not much, of every kind of root type offered to them (Figure 3 and Table 4). There were significant differences in the root species consumed (F (3, 108) = 9.69, $P < .0005$). Mean root consumption of *L. lucida, S. repens,* and *C. ericoides* were similar for all three roots, (0.08 - 0.11 grams) and did not differ significantly (Figure 3 and Table 4). On the other hand, millipedes consumed significantly less *Q. inopina* root tissue than roots from other species (Figure 3). Overall, the results show plant species is significant, but differences are driven by *Q. inopina* (Figure 3).

Stable Isotope Analysis

The natural-abundance $\delta^{15}N(^{0}/_{00})$ analysis based on an expected trophic $\delta^{15}N(^{0}/_{00})$ enrichment (i.e., increase in $\delta^{15}N(^{0}/_{00})$ values of an organism compared with its food) of +2 to $+4^{0}/_{00}$ (Peterson and Fry 1987) provided little information (Figure 4 and Table 5). In addition, natural abundance $\delta^{13}C(^{0}/_{00})$ analysis based on an expected trophic $\delta^{13}C(^{0}/_{00})$ enrichment of $+1^{0}/_{00}$ (Peterson and Fry 1987) provided little information, too (Figure 5, Table 6). Based on expected trophic enrichment of stable isotopes, it was expected that the values for the millipedes as presumptive herbivores, would be in the range of -1 to $6^{0}/_{00}$ for $\delta^{15}N$ and -28 to $-25\ ^{0}/_{00}$ for $\delta^{13}C$ (Figure 6). However, the observed values for *F. penneri* clustered at much more negative $\delta^{15}N$ and less negative $\delta^{13}C$ (Figure 6). There were no millipedes and food sources that had similar comparisons even without nitrogen and carbon enrichment. Hence, the stable isotope analysis did not indicate potential dietary preferences in *F. penneri*.

DISCUSSION

Resting metabolism in *F. penneri* was in the range expected for a poikilothermic animal, but the slope of the intraspecific body size-metabolic rate curve was much steeper than expected. This is not surprising since Hemmingsen's curve depicts an interspecific relationship and detailed studies with single species have shown that the slope of the regression of SMR on body mass may differ considerably from the classic -0.25 value (Hemmingsen 1950, Prosser 1973). The data indicated that small millipedes have a disproportionately high metabolic rate compared to adult *F. penneri.* This implies that immatures not only would need to consume more food, but also they would need disproportionately more water than adults.

The standard metabolic rate was also used to estimate the minimal amount of energy (calories) required to sustain a millipede (Golley 1961). One must keep in mind that the standard metabolic rate is a minimal level of metabolism and likely accounts for only 20 – 30% of total energetic expenditure of a free ranging millipede.

Keeping in mind that the feeding trials lasted for two days and that only adult millipedes were used, it can be concluded that all the millipedes in the feeding trials consumed more than enough plant material (leaf litter and root tissues) to sustain total energetic expenditure for the millipedes.

From the leaf litter feeding trials it was found that the millipedes consumed *Q. inopina* more than any other type of scrub leaf litter including other oak species (Figure 2 and Table 3). This result was expected because *Q. inopina* is a dominant shrub in the scrub and its leaves are not very leathery or hairy. In addition, it was not surprising that

the millipedes consumed *Q. geminata* leaves the least compared to the other oak species (Figure 2 and Table 3). *Q. geminata* leaves are recurved, thick, leathery, and hairy on the underside compared to the other oak species or any other leaf litter given in the feeding trial. *F. penneri* probably finds *Q. geminata* leaves are difficult to efficiently feed because of their weak mandibles.

Another unexpected result was millipedes consumed *Q. myrtifolia* more than any other leaf litter type than *Q. inopina* (Figure 2 and Table 3). It was surprising since *Q. myrtifolia* plants are not always found in rosemary scrub like the three other types of oaks used in the feeding trial. One explanation could be that both *Q. inopina* and *Q. myrtifolia* are red oaks where *Q. geminata* and *Q. chapmanii* are white oaks. A previous study has shown that the two red oaks are more nutritious than the other two white oaks (Abrahamson and Abrahamson 1989). In addition, both *Q. inopina* and *Q. myrtifolia* are less thick, leathery, and hairy on the underside compared to *Q. geminata* and *Q. chapmanii*.

There were similar results with the lyonia leaf litter (Figure 2 and Table 3). The both *L. ferruginea* and *L. lucida* are less thick and leathery compared to *L. fruticosa* and they were consumed more by the millipedes. Similar to the oaks, *L. lucida* is thinner than *L. fruticosa* but is not found throughout the Florida scrub like *L. fruticosa* and was not consumed as much.

One of the seemingly odd outcomes was that the millipedes consumed leaves of saw palmettos (*S. repens*) significantly less than any other leaf litter given for the feeding trials (Figure 2 and Table 3). This is surprising because *S. repens* is commonly found throughout the scrub and is not as thick as some as the other leaf litter consumed by the

millipedes. Though herbivory in the scrub is rarely understood it has been noted that *S. repens* seldom exhibits signs of herbivory compared to other scrub plants (M. Deyrup personal communication). Perhaps *S. repens* chemical composition makes the leaves unpalatable to the millipedes. For example, leaves higher in nitrogen and water content are usually preferred over those with a lower content in herbivores and detritivores (Gurevitch et al. 2002). *S. repens* leaves contain half as much nitrogen as *Q. inopina* (Table 5*)*.

For root consumption trials millipedes consumed similar amounts of *C. ericoides, S. repens,* and *L. ferruginea* (0.0950 g, 0.0844 g, and 0.1073 g respectively) and consumed *Q. inopina* roots the least (0.0341 g). This is especially interesting because the leaf litter consumption trials revealed the opposite trend. Thus, for leaf material millipedes consumed *Q. inopina* shoots ten more times than *S. repens* shoots ((0.2909 g versus 0.0285 g, respectively). However, for root tissues millipedes consumed *S. repens* (0.0844 g) more than *Q. inopina* (0.0341 g) root tissues. One explanation for this oddity is a possible difference in the chemical composition between roots and shoots of the plants, which makes them unpalatable to the millipedes.

Natural-abundance stable isotope analysis relies on substantial separation between potential food resources (generally at least 3-4$^{0}/_{00}$ for $\delta^{15}N$; 1% for $\delta^{13}C$). Based on expected trophic enrichment of stable isotopes, it was expected that the values for the millipedes, as presumptive herbivores, would be in the range of -1 to 6 $^{0}/_{00}$ for $\delta^{15}N$ and -28 to -25 $^{0}/_{00}$ for $\delta^{13}C$. However, the observed values for *F. penneri* clustered at much more negative $\delta^{15}N$ and less negative $\delta^{13}C$. Even if one assumes that the $\delta^{15}N$ values for milli-

pedes tissues are systematically biased by stored nitrogenous wastes to a large degree, say -6 to -8 $^{0}/_{00,}$ the adjusted data set still is largely non-overlapping with expected trophic enrichment data set (Figure 6). Hence the stable isotope analysis results do not indicate potential dietary preferences. In addition, the stable isotope analysis results did not confirm any plant substrates used in the consumption trials are a major dietary source of *F. penneri*.

There are three possible explanations for the non-conclusive results of natural-abundance stable isotope analysis and consumption trials. First, wild millipedes are eating a food source that was not sampled. Second, an organism may assimilate a portion of the food resource that has a different stable isotope ratio than the bulk material sampled (Peterson and Fry 1987, Lajtha and Michener 1994). For example, millipede cells that have different $\delta^{15}N$ or $\delta^{13}C$ values than the material as a whole. The strengths of the natural-abundance stable isotope approach are that it integrates over time and that organisms are likely to be at isotopic equilibrium with respect to their food resources, assuming there have been no recent shifts that alter the relative abundance of food resources (Peterson and Fry 1987, Lajtha and Michener 1994). Third, the stable isotope values for the millipedes' maybe decreased in natural-abundance of $\delta^{15}N$ because of the process millipedes dispose of nitrogenous waste (Checkley and Miller 1989, McCutchan et al. 2003).

In summary, both leaf litter and root consumption trials revealed that *F. penneri* could survive feeding on scrub leaf litter and root tissues. However, stable isotope analysis revealed that the millipedes' diet does not consist of leaf litter or roots tested. In the laboratory millipedes did not appear to consume leaf litter or roots in great quantities.

Yet, the millipedes did appear to consume peeled cukes in great quantities. Thus, based on the feeding trials, stable isotope analysis, and laboratory observations it seems unlikely that leaf litter and root tissues are major dietary inputs. One potential food source that was not tested in the feeding trials or stable isotope analysis is seeds, especially *C. ericoides* seeds. These seeds are eaten by the harvester ant *Pogonomyrmex badius* and birds, especially the resident rufus-side towhee *Pipilo erthyropthalamus* and the Florida scrub jay *Aphelocoma coerulescens* (Johnson 1982). In addition, Dangerfield et al. (1992) found three species of juliform millipedes in the savanna habitat in southeast Botswana were observed eating seeds, which is a high quality food. During this study, *F. penneri* was mainly found in rosemary scrub, which contains more seed bearing plant species than in other scrub habitats. Having a lot of seed bearing plants like Florida rosemary (*C. ericoides*) can provide reliable, widely available, and unlimited food resources for *F. penneri*. If seeds are the major component of the millipedes' diet *F. penneri* may be important for seed dispersal, which may have an imperative effects in the Florida scrub ecosystem, especially in rosemary scrub.

Figured Legends

Figure 1. Standard metabolic rate for 12 male, 9 female, and 22 immature *F. penneri* millipedes. Bars indicate mean values and brackets show standard errors. Bars having the same letter are not significantly different (Tukey's HSD test, $p > 0.05$).

Figure 2. Consumption of dry weight leaf litter by 50 adult *F. penneri* millipedes. Bars indicate mean values and brackets show standard errors. Bars having the same letter are not significantly different (Bonferroni adjustment, $p > 0.05$).

Figure 3. Consumption of dry weight root tissues by 116 adult *F. penneri* millipedes. Bars indicate mean values and brackets show standard errors. Bars having the same letter are not significantly different (Tukey's HSD test, $p > 0.05$).

Figure 4. The natural-abundance $\delta^{15}N(^{0}/_{00})$ of *F. penneri* and potential food sources. For each potential food item the closed triangles represent a composite sample. The composite sample was formed from a food source take at four different sites. Each millipede represents only one millipede.

Figure 5. The natural-abundance $\delta^{13}C(^{0}/_{00})$ of *F. penneri* and potential food sources. For each potential food item the closed triangles represent a composite sample. The composite sample was formed from a food source take at four different sites. Each millipede represents only one millipede.

Figure 6. Stable nitrogen isotope ratios ($\delta^{15}N$) vs. stable carbon isotope ratios ($\delta^{13}C$) of *F. penneri* millipedes and potential food sources. Triangles represent the actual nitrogen and carbon isotope. Extended line from plant substrates and mushroom represent the expected trophic isotropic enrichment: $^{15}N \sim 3^{0}/_{00,}$ $^{13}C \sim 1^{0}/_{00.}$ The shaded oval area

represents where potential foods sources of the millipedes might be placed on the graph if N-values were systematically biased by stored wastes in millipede samples.

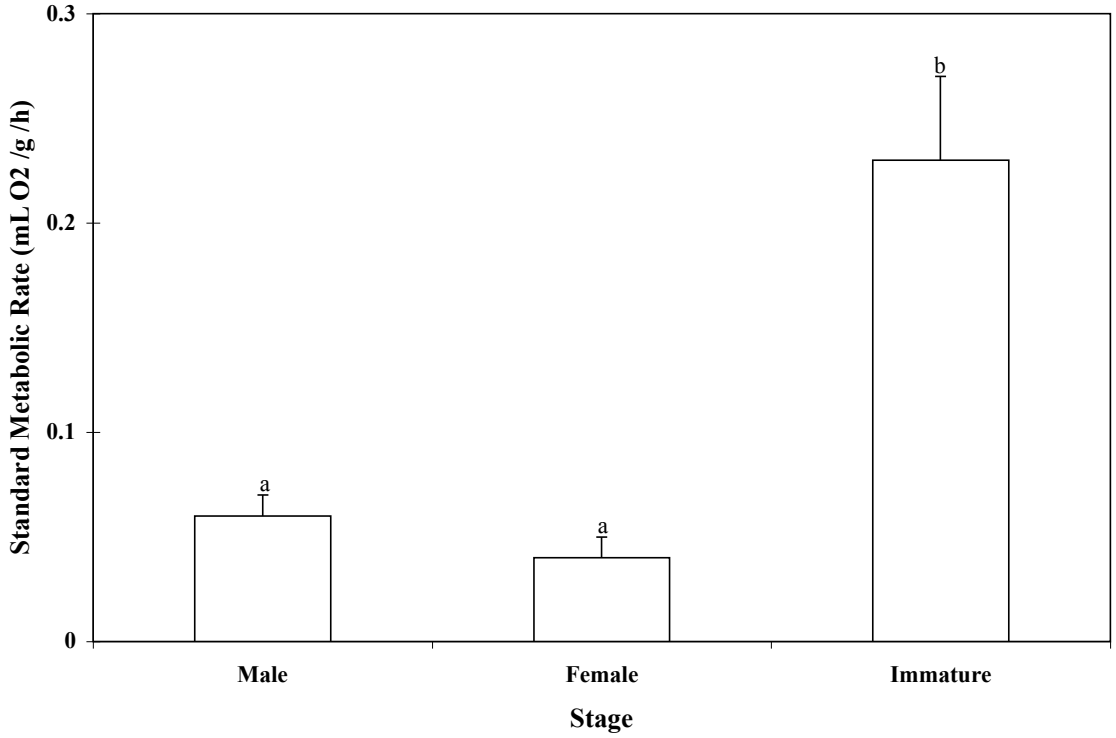
0.3
0.2
0.1
0
Standard Metabolic Rate (mL O2 /g /h)
a
a
b
Male
Female
Immature
Stage

Figure 1

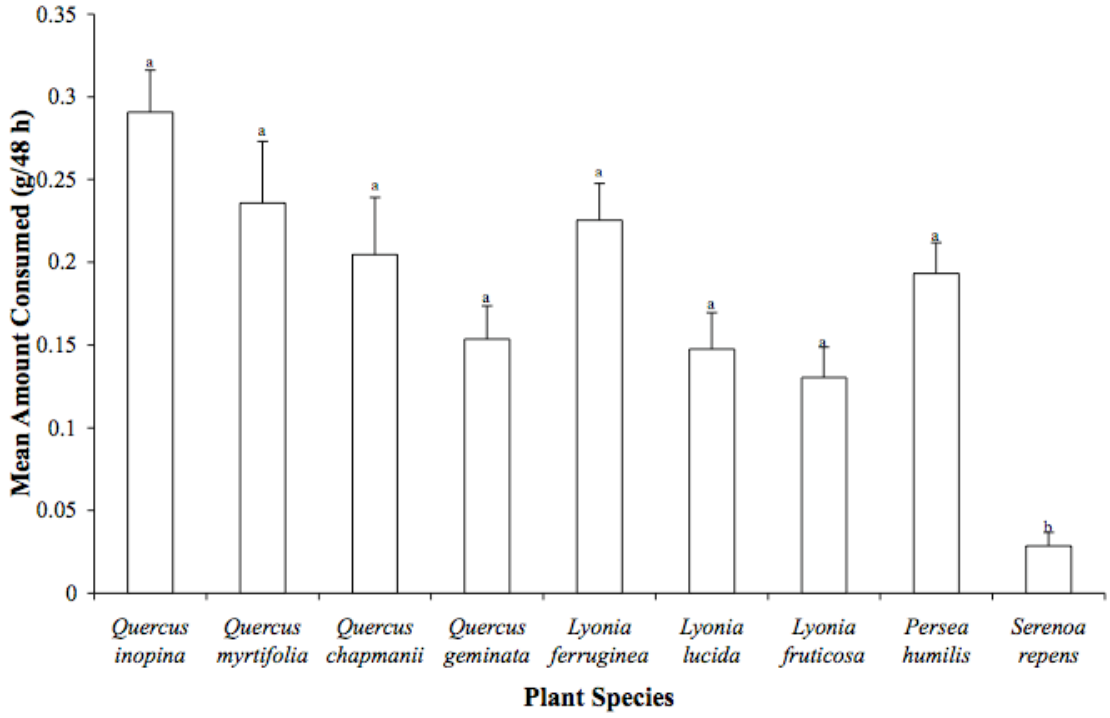
Mean Amount Consumed (g/48 h)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
a
a
a
a
a
a
a
a
b
Quercus inopina
Quercus myrtifolia
Quercus chapmanii
Quercus geminata
Lyonia ferruginea
Lyonia lucida
Lyonia fruticosa
Persea humilis
Serenoa repens
Plant Species

Figure 2

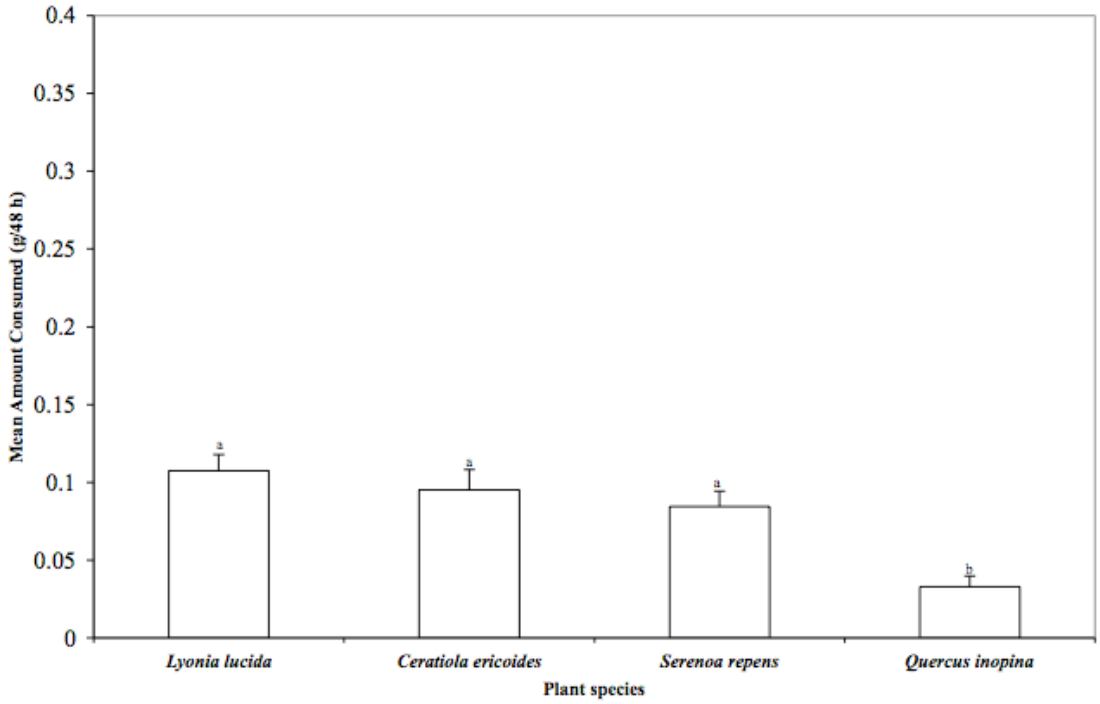
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Mean Amount Consumed (g/48 h)
a
a
a
b
Lyonia lucida
Ceratiola ericoides
Serenoa repens
Quercus inopina
Plant species

Figure 3

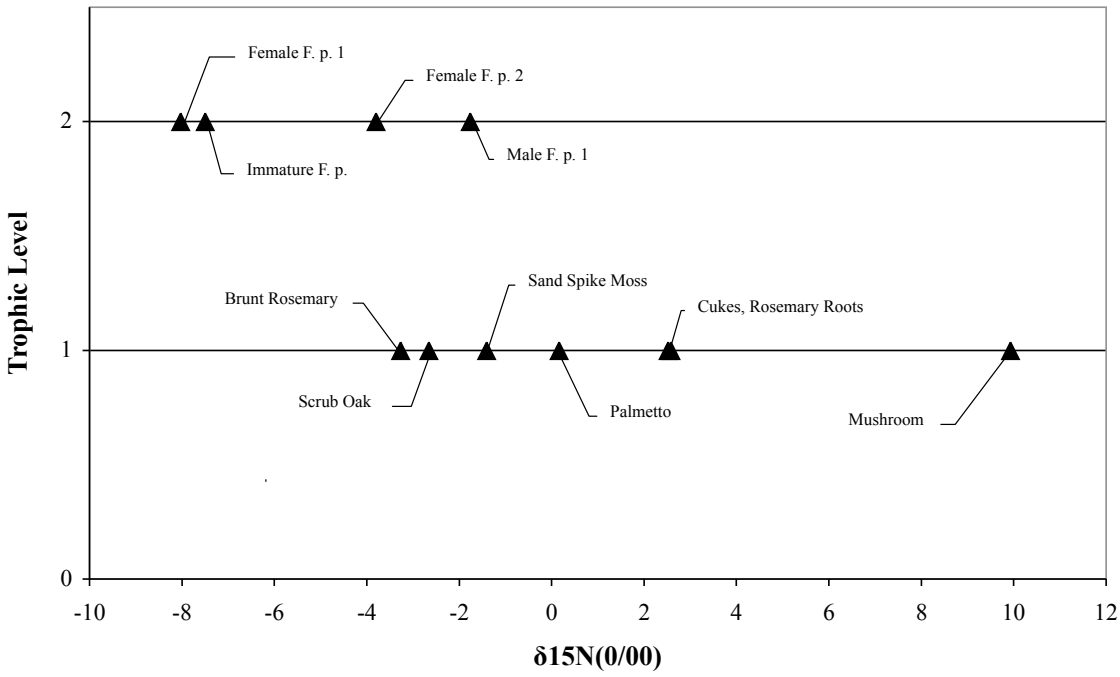
Female F. p. 1
Female F. p. 2
Immature F. p.
Male F. p. 1
Brunt Rosemary
Sand Spike Moss
Cukes, Rosemary Roots
Scrub Oak
Palmetto
Mushroom
Trophic Level
2
1
0
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
δ15N(0/00)

Figure 4

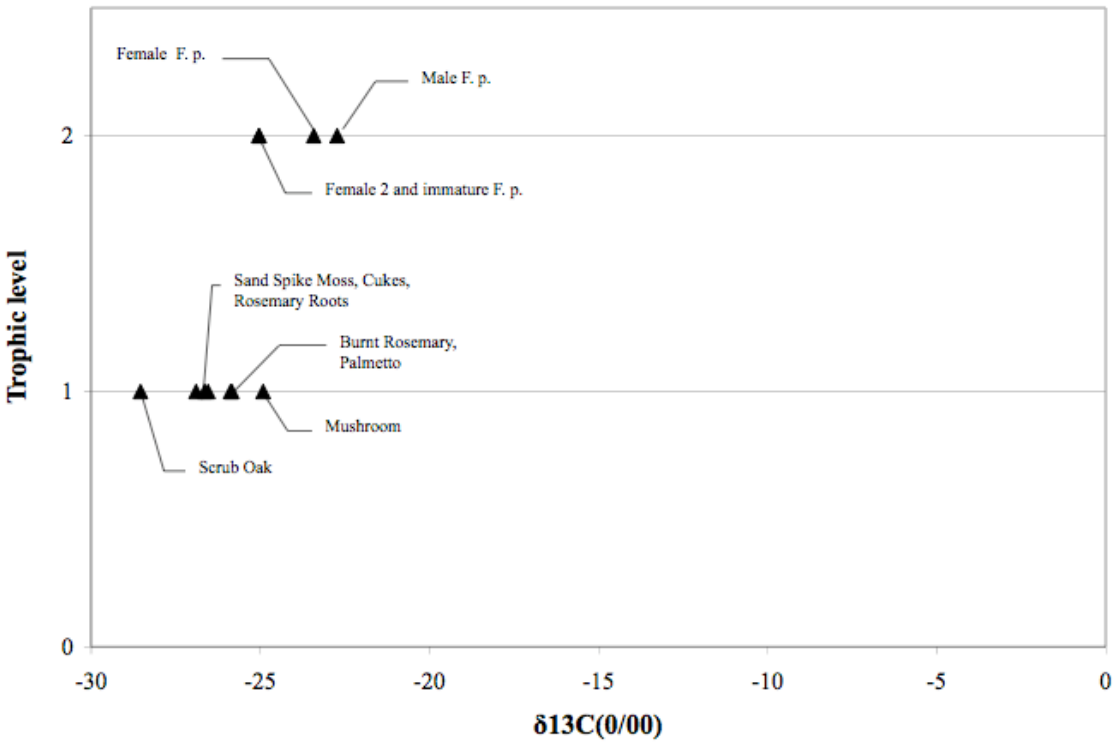
Female F. p.
Male F. p.
Female 2 and immature F. p.
Sand Spike Moss, Cukes,
Rosemary Roots
Burnt Rosemary,
Palmetto
Mushroom
Scrub Oak
Trophic level
2
1
0
-30
-25
-20
-15
-10
-5
0
δ13C(0/00)

Figure 5

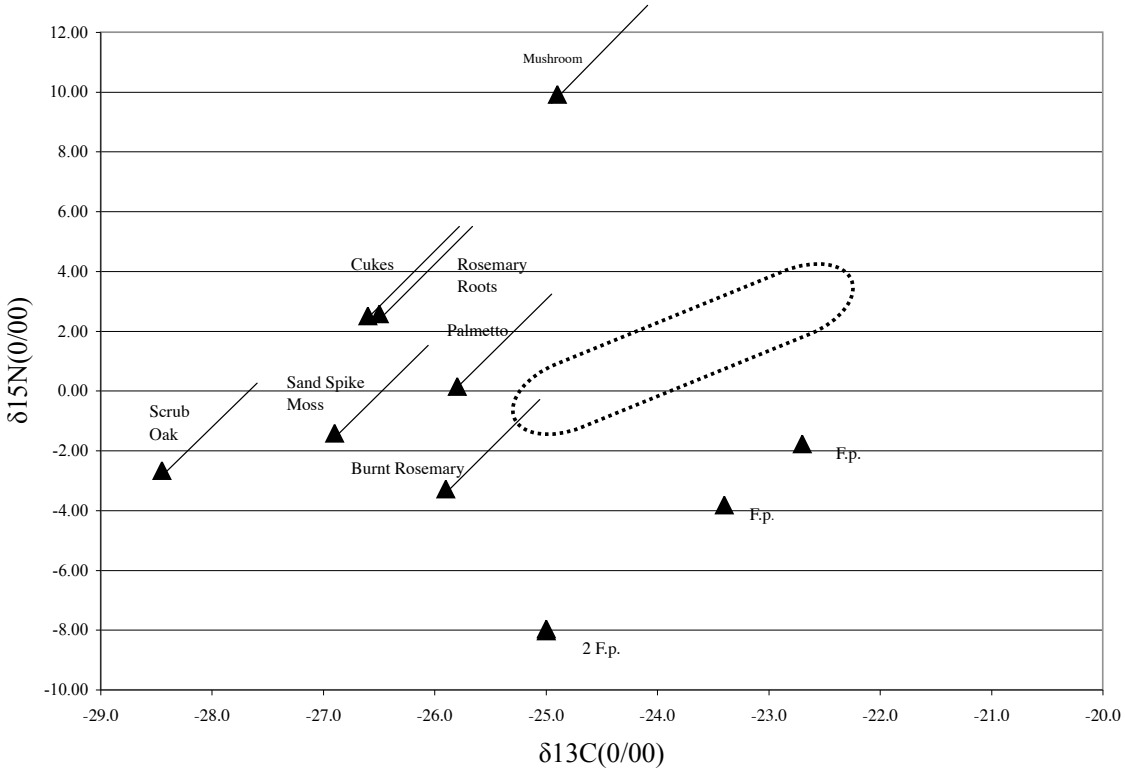
12.00
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
δ15N(0/00)
-29.0
-28.0
-27.0
-26.0
-25.0
-24.0
-23.0
-22.0
-21.0
-20.0
δ13C(0/00)
Mushroom
Cukes
Rosemary
Roots
Palmetto
Sand Spike
Moss
Scrub
Oak
Burnt Rosemary
F.p.
F.p.
2 F.p.

Figure 6

Table 1: Resting metabolism of *F. penneri.*

Stage	N	Live Weight (g) (X ± SEM)	Gross metabolic rate (mL O_2 /h) (X ± SEM)	Standard metabolic rate (mL O_2 /g /h) (X ± SEM)
Immature	22	1.62 ± 0.13	0.33 ± 0.04	0.24 ± 0.04
Female	9	4.78 ± 0.27	0.19 ± 0.03	0.04 ± 0.01
Male	12	5.28 ± 0.42	0.29 ± 0.05	0.06 ± 0.01

Table 2. The minimal amount of dry leaf and root intake for *F. penneri* per day based on resting metabolic rates.

Stage	Average individual metabolic rate		Intake (g/day)	
	mL O_2/h	mL O_2/day	Leaf	Root
Male	0.29	6.96	0.0083	0.0074
Female	0.19	4.56	0.0054	0.0050
Immature	0.33	7.92	0.0094	0.0084

Table 3: Amount of dry leaf litter tissue consumed by *F. penneri* in a 48-hour period.

Plant Species	N	Amount consumed (g) (X ± SEM)
Q. myrtifolia	50	.2360 ± .0372
Q. geminata	50	.1536 ± .0200
Q. chapmanii	50	.2046 ± .0347
Q. inopina	50	.2909 ± .0253
L. ferruginea	50	.2254 ± .0220
L. lucida	50	.1474 ± .0220
L. fruticosa	50	.1302 ± .0185
P. humilis	50	.1932 ± .0187
S. repens	50	.0285 ± .0082

Table 4: Amount of dry root tissue consumed by *F. penneri* in a 48-hour period.

Plant Species	N	Amount consumed (g) (X ± SEM)
C. ericoides	26	.0950 ± .0130
Q. inopina	28	.0341 ± .0070
S. repens	29	.0844 ± .0093
L. lucida	29	.1073 ± .0104

Table 5. The natural-abundance ^{15}N analysis for *F. penneri* and potential food sources.

Sample	% N	$\delta^{15}N(^{0}/_{00})$	C:N
Quercus inopina leaves	1.045	-2.664	47.48612
Serenoa repens leaves	0.524	0.158	89.10305
Wild mushroom	2.294	9.930	11.32868
Ceratiola ericoides burned wood	0.36	-3.275	126.7528
Ceratiola ericoides roots	3.844	2.578	11.09599
Selaginella arenicola roots	0.669	-1.413	38.84006
Cucumber (control)	3.965	2.512	10.89912
Female *F. penneri* 1	5.125	-3.807	5.916488
Female *F. penneri* 2	4.96	-8.031	6.341734
Male *F. penneri*	5.285	-7.952	6.610028
Immature *F. penneri*	5.168	-1.766	6.111262

Table 6. The natural-abundance ^{13}C analysis for *F. penneri* and potential food sources.

Sample	% C	$\delta^{13}C(^{0}/_{00})$	C:N
Quercus inopina leaves	49.623	-28.535	47.48612
Serenoa repens leaves	46.690	-25.844	89.10305
Wild mushroom	25.988	-24.922	11.32868
Ceratiola ericoides burned wood	45.631	-25.872	126.7528
Ceratiola ericoides roots	42.653	-26.542	11.09599
Selaginella arenicola roots	25.984	-26.894	38.84006
Cucumber (control)	43.215	-26.647	10.89912
Female *F. penneri* 1	30.322	-23.427	5.916488
Female *F. penneri* 2	31.455	-25.042	6.341734
Male *F. penneri*	34.934	-25.040	6.610028
Immature *F. penneri*	31.583	-22.725	6.111262

CHAPTER 3: ABOVE-GROUND ACTIVITY OF *F. PENNERI*

INTRODUCTION

Millipede activity is often regulated by a number of climatic influences. Water availability and temperature the foremost abiotic factors. In temperate regions, rapid drops in temperature may be so severe as to interrupt millipede activity, whereas temperature increases may cue feeding and breeding activities (Hopkin and Read 1992). In the tropics where variation in annual temperature can be limited, rain may provide the stimulus for seasonal activities, such as breeding and migration (Hopkin and Read 1992). It has been shown that life histories for some species of millipedes are highly dependent on rainfall and temperature (Dangerfield et al. 1992, Bailey and Kovaliski 1993, Dangerfield et al. 1998).

In addition to precipitation and temperature, the habitat and its stage of ecological succession of vegetation can influence millipede activity (Hopkin and Read 1992). Changes in vegetation in a habitat over a period of time can affect the composition of resident species of millipedes. For example, Dunger and Steinmetzger (1981) recorded the alterations in the millipede community that occurred with transitions of a site from grassland through scrub to woodland over a twenty-five year period. Ultimately, animals are adapted to environments that are consonant with their life history.

Patterns of precipitation, temperature, fire, and their interactions affect the Florida scrub ecosystem and resident animal species. Florida scrub occurs naturally on fragmented ridges that were formed from prehistoric sand dunes. The ecosystem is dominated by an xeromorphic shrub community comprised of oaks, palmettos, and ericads.

Very xeric sites have Florida rosemary (*Ceratiola ericoides*) (Empetraceae) growing on "balds" while moist sites contain an over-story of slash pine and/or sandpine (Myers 1990, Menges 1999). The soil consists of siliceous sand that for the most part is well-drained and exceedingly low in nutrients. Summers are hot, humid, and wet. Winters are characterized as dry and cool. Infrequent, but highly intense fires maintain the scrub ecosystem. Subterranean stems and roots of woody shrubs survive these intense fires and quickly resprout. Thus, most shrub communities are quickly restored to their original species composition (Menges 1999, Carrel 2003). Organisms in the scrub ecosystem have had to adapt to seasonal drought, infertile soils, intense fires, hurricanes, and multidecadal flooding. Endemism in the Florida scrub is among the highest in North America (Menges 1999). Most endemic species studied are plants and vertebrates, but very little is known about endemic invertebrates.

The focus of this chapter is the seasonal activity of the endemic Florida scrub millipede, *F. penneri*. *F. penneri* is found only on the southern tip of the Lake Wales Ridge, an area about 600 km^2. Relatively nothing is known about the life history and ecology of this animal. It is assumed that the millipedes emerge above-ground on humid, summer nights, particularly after rainfall, to feed on moistened leaf litter. This is based on general millipede biology and nocturnal sightings of *F. penneri* in the scrub. In addition, Deyrup and Franz (1994) assumed that only mature F. *penneri* millipedes emerge above-ground during the reproductive season because immature millipedes were rarely seen any time of year, suggesting immatures are largely subterranean.

Based on the following anecdotal information, it was also thought at the start of this study that *F. penneri* would be most abundant in scrubby flatwoods. *F. penneri* was formerly seen in large numbers on lawns bordering the main buildings of Archbold Biological Station (ABS), all of which are embedded in native scrubby flatwoods. Likewise, more than other types of scrub, scrubby flatwoods should also provide a suitable microhabitat for the millipedes because it has a relatively closed multilayered canopy, which provides a humid environment containing much leaf litter.

In addition, it was suspected that *F. penneri* adults would be most active after the rainy season had set in, ranging from mid-July to August. This would correlate with the seasonal pulse of microbial growth and decomposition in leaf litter stimulated by increased temperatures and abundant precipitation. Many species of millipedes are known to prefer leaf litter that has undergone extensive microbial processing (Hopkin and Read 1992, Dangerfield and Telford 1996). Until now there has only been speculation on the biology of *F. penneri*.

METHODS

Study Site

Archbold Biological Station (ABS) is located near the southern tip of the Lake Wales Ridge in Highlands County, Florida (27° 11′ N lat., 81°21′ W long.), 12 km south of the town Lake Placid. The elevation ranges from 36 to 67 meters above sea level. ABS manages one of the largest remaining contiguous tracts of undeveloped land in peninsular Florida, comprising 2300 ha of scrub and other native communities.

Of the seven scrub communities at ABS characterized vegetatively by Abrahamson et al. (1984), for this research I focused on the most and the least abundant kinds of scrub: scrubby flatwoods and rosemary scrub, respectively. Scrubby flatwoods habitat, which accounts for ~30% of total hectarage, is dominated by four species of shrubby oaks (primarily *Quercus inopina* intermixed with *Q. chapmanii, Q. myrtifolia,* and *Q. minima*), two palmettos (*Serenoa repens* and *Sabal etonia*), and three lyonias (*Lyonia ferruginea, L. fruticosa,* and *L. lucida*). Rosemary scrub is not only the rarest vegetative community in Florida scrub (~2% of total hectarage), but it contains the most endemic herbs that grow in the extensive sandy gaps. Florida rosemary (*Ceratiola ericoides*) (Empetraceae) is the dominant, evergreen shrub. Unlike the shrubs in scrubby flatwoods, Florida rosemary is not clonal; this endemic grows slowly only from seeds and consequently, it does not form a dense matrix.

Habitat Selection by Millipedes

As a means to assess habitat selection in *F. penneri*, I monitored millipede activity by counting their tracks in the sand in scrubby flatwoods and in rosemary scrub. I randomly chose fifteen rosemary and fifteen scrubby flatwoods sites among those indicated on the ABS vegetative map prepared by Abrahamson et al. (1984). At each site a ten by two meter plot, delineated with stake flags, was inspected daily for five weeks (July 22, 2004 to September 1, 2004). Every scrubby flatwoods plot was placed in the sandy verge of a primitive road surrounded by scrubby flatwoods. All rosemary scrub sites were placed in the middle of a rosemary bald. The track survey ceased after September 1, 2004 because many sites were completely submerged with water from Hurricane Charley.

I daily inspected each plot and counted and recorded the number of millipede tracks (distinct, parallel, continuous tracks). All millipede tracks were wiped away by brushing the sand after each survey. Thus, any tracks present on subsequent inspections were caused by new above-ground activity of millipedes. Given that rainfall also erased tracks, there were some days it was impossible to count millipede tracks for the day.

Pitfall Trapping of Millipedes

To collect *F. penneri*, fifty-six pitfall traps were placed in each of five rosemary balds. Traps were arranged in a 7 x 8 grid, with traps spaced three meters apart (total trapping area = 21m x 24m). Each trap consisted of a 32 oz plastic deli container 12 cm diameter, and 20 cm height, having four 0.5 cm diameter holes for drainage. Traps were sunk in the sand so that the upper rim was just below the surface of the surrounding soil. A handful of sand was placed in the bottom of each container and moisten periodically with tap water to keep trapped animals hydrated. Traps were checked every one to four days. The traps were open from July 1, 2004 to December 15, 2004, except during hurricane events in September. All traps were closed September 7th. Two sites out of five were opened September 16th and two additional sites were opened September 20th. One site was permanently closed with lids after September 7th due to excessive rain from Hurricanes Charley, Francis, and Jeanne, which completely submerged the site. After a millipede was removed from a pitfall trap, it was brought back the laboratory to identify its sex, to determine its length and weight, and then it was kept in a terrarium on a diet of fresh cucumbers for use in other experiments.

The five rosemary balds were chosen as study sites in part because their burn history was well documented. A record of the date, area, intensity, and location of fires in each study site was available from ABS documents. Two intervals were established for this project. Two rosemary balds were recently burned (0.1 –3 years post fire) and three balds were long unburned (9-36 years post fire).

Statistical Analysis

Habitat Selection

An independent t-test was used to determine if there was a difference in the mean frequency of millipede tracks between rosemary scrub and scrubby flatwoods per day (SPSS, Independent-Sample T Test). Because the data violated the Levene test for homogeneity, I used a t-test based on separate variance estimates.

Mass and Length Measurements

Body length and mass of millipedes were analyzed using a univariate analysis of variance to compare lengths and masses of male, female, and immature *F. penneri* millipedes (SPSS, GLM Univariate). Post-hoc comparisons were made using Tukey's HSD test with *P* set at 0.05 to determine which sample means difference exist.

Temporal Patterns of Millipede Activity

An independent t-test was used to determine if there was a difference in the mean frequency of peak above-ground activity between male and female millipedes (SPSS, Independent-Sample T Test). In addition, another t-test was used to determine if there was a difference in the mean frequency of peak above-ground activity between adult millipedes and immature millipedes (SPSS, Independent-Sample T Test).

Influence of Temperature and Precipitation on *F. penneri* Activity

Staff at the ABS weather station recorded maximum air temperature and precipitation daily. To evaluate the relationship between millipede surface activity (as indicated by number of animals caught) and weather, I used multiple regression to test if weekly catches of *F. penneri* were related to mean maximum air temperature and total precipitation experienced during the week (SPSS, Regression). Prior to analysis, data that violated the nonlinearity of regression function were transformed using log (x + 1) transformation.

Time-since-fire in Rosemary Scrub and Millipede Abundance

To evaluate the relationship between millipede abundance and time-since-burned, I used a univariate analysis of variance to test if there was a difference in mean number of *F. penneri* caught at recently burned sites and long unburned sites (SPSS, GLM Univariate). Prior to analysis, data that violated the nonlinearity of regression function were transformed using log (x + 1) transformation.

RESULTS

Habitat Selection by Millipedes

Millipede tracks were much more abundant (~2.5 times) in rosemary scrub than in scrubby flatwoods. A t-test based on separate variance estimates was used ($F = 9.414$, $P = 0.05$). Thus, significantly 1.18 ± 0.62 more millipede tracks per day were found in rosemary scrub than in scrubby flatwoods scrub ($t = 1.925$, $df = 195$, $P = 0.05$; Figure 1).

Millipede Body Mass and Length

A total of 566 *F. penneri* were trapped alive over the course of 5.5 months (July 1-December 15, 2004). Of those, 467 (83%) were adults and 99 (17%) were immatures.

There were significant differences in overall body length and body mass for *F. penneri* (F_{length} (2, 433) = 253, $P_{length} < 0.0005$ and F_{mass} (2, 436) = 442, $P_{mass} < 0.0005$; Tables 1 and 2). The mean lengths for male and female millipedes were similar (70.5 mm and 69.7 mm, respectively), and mean mass for male and female millipedes were also similar (4.55g and 4.39g, respectively) (Tables 1 and 2). Not surprisingly, post-hoc multiple comparison revealed that immature millipedes were significantly smaller in mass and length than mature millipedes. (Figures 2 and 3). Mean body mass and length of immature *F. penneri* was 1.165g and 41.4 mm (Tables 1 and 2). Thus, the significant differences in body mass and length for all millipedes trapped was driven by immature-mature millipedes developmental gap.

Seasonal Patterns of Millipede Activity

There was no difference between male and female *F. penneri* in the time of year they were active above-ground activity based on trap data (t = 1.495, $P > 0.05$; Figure 4 and Table 3). Both male and female millipedes were captured from mid-July to mid-October. Peak activity for males was July 28 (DOY = 210) and for females it was August 2, 2004 (DOY = 215) (Figure 4). On the other hand, small numbers of immature millipedes were captured fairly steadily during the entire period the traps were open. In fact, on average, immature millipedes were captured later in the year than mature millipedes (t

= -9.397, $P < 0.0005$; Table 3). Peak activity for immature millipedes was early November (DOY = 313), more than two months after the adult peak (Figure 4).

Influence of Temperature and Precipitation on *F. penneri* Activity

Contrary to expectations, the number of mature millipedes captured per week was not significantly related to weekly mean maximum temperature or to total weekly precipitation (linear multiple regression = 0.25, F (2,19) = 2.826, $P > 0.05$; Figures 5 and 6). However the number of immature millipedes captured per week was significantly related to weekly mean maximum temperature and total weekly precipitation (linear multiple regression = 0.33, F (2, 19) = 4.172, $P < 0.05$), with a negative relationship between weekly immature captures and temperature (Pearson's r = -0.279) and a negative relationship between weekly captures and precipitation (Pearson's r = -0.528) (Figures 7 and 8). However, backward stepwise multiple regression analysis revealed that mean weekly maximum temperature was the only significant predictor of weekly immature millipede captures (multiple regression = 0.538, F (1, 19) = 7.338, $P < 0.01$). Thus, weekly precipitation turned out not to be significant as a predictor for trapping immature as well as mature *F. penneri*.

Affect of Time-Since-Fire in Rosemary Scrub on *F. penneri* Activity/Abundance

Slightly more millipedes were captured in recently burned rosemary scrub sites (6.94 ± 8.0) than in long unburned rosemary scrub sites (5.34 ± 9.0; Figure 9). However, this difference was not significant (ANOVA, F (1, 78) = 1.047, $P > 0.05$).

DISCUSSION

This project produced a number of results that were different than what was expected at the outstart based on general millipede biology. This is not surprising, since the majority of biological information about diplopods worldwide is based on species found in temperate, mesic environments, which are dramatically different than Florida scrub where *F. penneri* is located. *F. penneri* millipedes have had to adapt to seasonal drought, infertile soils, intense fires, and multidecadal flooding that have persisted for eons in the Florida scrub. The life history and ecology of present day *F. penneri* reflects their long existence and narrowly endemic condition.

Before the project began it was assumed that *F. penneri* would be found preferentially in scrubby flatwoods vegetation, since it is widespread and it seemingly is most suitable for a millipede. It accumulates more leaf litter and woody debris than other scrub types, implying there is more food for a diplopod. Scrubby flatwoods also has a relatively closed, multilayer canopy; hence, surficial temperatures and relatively humidity are more "mesic" than in other scrub types. Furthermore, anecdotal reports of *F. penneri* abundance near the main buildings of ABS, which extended back a half-century, placed them adjacent to scrubby flatwoods. Thus, it seemed logical to think these millipedes spent the majority of their lives in scrubby flatwoods.

However, the results of this study indicate *F. penneri* prefers xeric rosemary balds. For example, based on my 5-week long track survey, approximately 2.5 times more millipedes were active in rosemary scrub than in scrubby flatwoods (Figure 1).

Most likely *F. penneri* millipedes made the majority of tracks. However, two other large millipedes, which belong to the same order as *F. penneri,* are also found in the scrub. Both *Narceus gordanus* and *Chicobolus spinigerus* have been found in the Florida scrub. These species, not endemic to Florida scrub, can be found throughout peninsular Florida. In the entire period that pitfall traps were open, only 12 *Chicobolus spinigerus* and zero *Narceus gordanus* were captured. In addition, after the track survey was completed pitfall traps were set up (at sites that were not underwater) and only *F. penneri* millipedes were captured. These results suggest that *Chicobolus spinigerus* may at most have made 2-3% of the tracks, so at least 97-98% of the tracks on sand came from *F. penneri*.

Three explanations as to why *F. penneri* is more active in rosemary balds than in scrubby flatwoods come to mind. First, rosemary vegetation occurs on somewhat higher elevations than scrubby flatwoods (Figure 10). Rosemary scrub is found at elevation from 40 to 50 meters, whereas scrubby flatwoods scrub is found at elevations from 37 to 44 meters. Since rosemary balds are found higher, seasonal and multidecadal flooding does not occur in them as much as it does in scrubby flatwoods. In addition, because flooding has persisted between 2002-2005 at Archbold for the first time in nearly a half century, perhaps these animals migrated to rosemary balds from scrubby flatwoods and elsewhere in order to escape seasonal drowning.

Second, a dietary item required by *F. penneri* maybe more abundant in rosemary balds than in scrubby flatwoods. Based on unpublished feeding trials, it does appear that roots and wood of Florida rosemary (*Ceratiola ericoides*) is consumed by *F. penneri.*

However, other leaf litter and roots found in rosemary balds and in scrubby flatwoods were also consumed equally well by *F. penneri* (unpublished feeding consumption trials). Moreover, there is virtually no leaf litter present in rosemary balds and the diversity and abundance of perennial woody species is low. Thus, if rosemary scrub contains a unique dietary source, it remains unclear what that food source is that the millipedes may be eating. Might it be seeds of Florida rosemary and herbs that dominate this habitat?

Third, there could have been a design flaw or a sampling bias in these tests, which can explain why millipede activity was greater in rosemary scrub than in scrubby flatwoods. Rosemary scrub has less leaf litter and rotting woody debris than in scrubby flatwoods and potentially, millipedes in rosemary scrub are more active because they a searching more activity for food resources, which would increase there chances of crossing the track survey sites in the rosemary scrub. On the other hand, millipedes in the scrubby flatwoods sites may have been less active because there were more food sources and decreasing their chance of crossing the track survey sites. Extensive field observations made (both at night and in day time) in the past 10 years at the station agree with the outcome of this study (James E. Carrel unpublished).

Both male and female millipedes had similar body masses and body lengths (Tables 1 and 2). Thus, *F. penneri* did not exhibit sexual size dimorphism. Typically, large millipedes (Julida and Spriobolida) do not exhibit sexual dimorphism. Such big millipedes have been known to exhibit sexual differences in behavior and other functions, rather than in their form (Dangerfield 1993). There was a small difference of range in body mass and length for male and female millipedes captured. Male millipedes were

15% greater for length range and 38% greater for mass range than female millipedes. This seems to be a reflection of sample difference in sample size (1.8 times more males were captured than female millipedes) and not a biological difference. If more female millipedes would have been captured, the sex-based difference in range of body mass and length would likely disappear.

As one would expect, immature millipedes active on the surface were significantly smaller in body length and mass than adult millipedes (Tables 1 and 2). It is difficult to determine the lifecycle of most millipedes. Only rarely have authors been able to rear a species from egg to adult to follow the anamorphosis. In addition, the rate of growth and development in most diplopods is slow, requiring several years for many species; so few scientists have invested in this long term process. First developed by Vachon (1947), the method of counting eyerows can be used along with counting body segments to determine developmental increase form and function in millipedes. The eyerow method may be a more reliable index of growth because, according to Keeton (1959), immature *F. penneri* millipedes prematurely attain their full number of body segments before they reach sexual maturity, which is different from the condition seen known for all other species of Spirobolida. Immature millipedes captured in this study ranged in length from 19 to 58 mm. Based on eyerow counts using preserved millipedes from the 2004 field season, the stadia of immature millipedes caught in pitfall traps ranged from V to VI. The majority of immature millipedes captured were 35 mm, which is approximately stadium VI. Since all adult millipedes have nine eyerows and based on the fact

that the largest immature millipedes have eight eyerows, it is probable to infer that *F. penneri* becomes sexually mature at stadium VIII.

Both male and female millipedes were active above-ground during the same time of year, though female millipedes were captured fourteen days after the last male millipede was captured (Figure 4). Since activity in male and female millipedes was synchronous, it suggests mature millipedes could be emerging in mid-August to copulate near the height of the rainy season (late August). Many other studies have shown that large burrowing millipedes often emerge to copulate and to feed during the rainy season (Dangerfield et al. 1998). Female millipedes may stay above-ground longer than males to feed more in order to produce energetically costly eggs and/or for egg laying.

In addition, 1.8 times as many male millipedes as female millipedes were captured (Table 3). Most likely, male millipedes locomote more than female millipedes, resulting in higher rates of capture for males. Male millipedes may move frequently because they are searching for female millipedes with whom to copulate. In contrast, female millipedes may emerge and spend the majority of their time above-ground feeding due to the high cost of oocyte development. This would explain why fewer *F. penneri* females were captured less than male millipedes. Similarly, Dangerfield et al. (1998) showed in the African millipede (*Alloporus uncinatus*) female millipedes tend to spend proportionately more time feeding, at the same time as males, which are proportionately more active and spend more time walking.

Maximum temperature and precipitation were not significant predictors for capturing mature millipedes. Rainfall events may not influence *F. penneri* adult activity be-

cause their large size renders them somewhat immune to evapotranspirational losses. Spriobolid millipedes, like *F. penneri*, are the only millipedes found in xeric environments because there comparatively large size may retard heat gain and loss and water loss (Hopkin and Read 1992). Even though I did not find a significant relationship between weekly patterns of mature millipede activity, temperature, and precipitation, the data suggest temperature and precipitation most likely influence seasonal or annual activity of *F. penneri.* Adults were not captured in the first half of the cool, dry season (Figures 5 and 6). Other efforts to trap millipedes in winter and spring (January – May) in rosemary balds have produced few captures of *F. penneri* (James E. Carrel, Unpublished), which is consistent with patterns in this study.

Even though weekly precipitation was not a significant factor for finding immature millipedes, the majority of immature millipedes was captured at the onset of the dry season in early October (DOY = 281) (Figure 7 and 8). It is surprising immature millipedes would be most active during the dry season because their small body size renders them more susceptible to desiccation (Edney 1977, Apple 1988). But the fact that immature millipedes are nocturnal may afford them more humid, nearly saturated or foggy conditions near the ground level in fall as days become shorter and evapotranspirational potentials decline seasonally (Cloudsley-Thompson 1988, Handley 1994). Menges and Gallo (1991) found frequent fog during the winter and spring may relieve drought stress in scrub plants. Perhaps small *F. penneri* also get condensate to imbibe like desert arthropods. Studies of water relations at ground level would be needed to address this topic.

On the other hand, daily activity for immature millipedes is dependent on temperature, suggesting immature millipedes use decreasing temperature as a cue for dispersal (Figure 8). Even though immature millipedes are emerging when temperatures are decreasing this could be beneficial to the millipedes because cooler temperatures help reduce water loss. In addition, potential predators of immature *F. penneri* may be less active in the cool, dry season. Predators may be a greater threat than desiccation for immature *F. penneri* millipedes, such as the larva of the phegodid beetle, *Phengodes laticollis*, and sarcophagid flies, *Spirobolomyia singularis*, *S. flaviplapis*, and *Helicoba* sp. I only encountered carcasses of *F. penneri* that had been attacked by phegodid beetle larva and sarcophagid flies during the rainy season and found no others after the onset of the dry season.

Many scrub animals depend on fire and some are most abundant few years after a burn. Endemic reptiles are more abundant following fire than in unburned scrub (Menges 1999). The Florida scrub jay is also dependent on fire. Optimal habitat for the scrub jay includes oaks less than two meters tall, some tall pine trees, and gaps within the shrub matrix for acorn caching (Fitzpatrick et al. 1991). As shrubs grow taller and gaps decrease in size the performance of scrub jays decreases, and they will eventually abandon the area (Fitzpatrick et al. 1991). Periodical fires help to maintain the optimal scrub matrix for the Florida scrub jay. Based on the ecology of some endemic animals of the Florida scrub it is probable that fire might be a positive force for above-ground activity for *F. penneri*.

For *F. penneri,* time-since-fire in rosemary scrub did not affect the number of millipedes captured at a site (Figure 9). First, there may not be an extreme difference in the scrub matrix between the intervals I set for recently burned and long unburned rosemary scrub. Rosemary scrub is the most open vegetation found in the Florida scrub. It retains large barren patches of sand for many years because of the allelopathic chemicals produced by Florida rosemary plants (Myers 1990, Menges 1999). Thus, the shrub matrix for both time-since-fire categories may have been very similar. This is not to suggest that rosemary scrub is insensitive to fire exclusion. It is well documented that if fire is long excluded from rosemary scrub, say for 70 – 100 years, Florida rosemary will eventually die, other shrubby plants will invade gaps, and many organisms that are gap specialists will also disappear from the site (Abrahamson 1984b, Johnson and Abrahamson 1990, Menges and Kohfeldt 1995, Menges and Hawkes 1998, Carrel 2003). On the other hand, if fire is used too frequently, say every decade or so, it will also kill Florida rosemary and other shrubs will invade and close the open gaps in the rosemary scrub (Abrahamson 1984b, Johnson and Abrahamson 1990, Menges and Kohfeldt 1995, Menges and Hawkes 1998). Thus, *F. penneri* may not be very sensitive to fire intervals because the open, patchy matrix remains almost the same unless fire has been severely suppress or over-used.

In summary, mature millipedes were active above-ground during the rainy season and peak activity occurred in mid-August, whereas immature millipedes were active throughout the rainy into the dry season (June-December). Decreasing temperature and precipitation in the fall primarily influenced activity of immature millipedes. Typically,

species of immature millipedes found in mesic condition are more active during the rainy season, but immature *F. penneri* millipedes were not. Activity for mature *F. penneri* millipedes was most likely influenced by seasonal changes in temperature. Peak activity of immature millipedes occurred in early November, nearly two months latter than mature millipedes. Unlike many other animals endemic to Florida scrub, time-since-fire did not have an affect on above–ground activity. It is possible that longer unburned intervals of rosemary scrub could affect millipede activity. Finally, the most surprising outcome of this study is that *F. penneri* millipedes were more prevalent in rosemary scrub than in scrubby flatwoods scrub. Rosemary scrub could provide a safe haven from rising seasonal flooding and it could a provide a unique, necessary dietary substance, the identity of which remains an enigma.

Figure Legends

Figure 1. Number of *F. penneri* millipede tracks in rosemary and scrubby flatwoods scrub. Bars indicate mean values with brackets showing standard errors per day.

Figure 2. Body length of male, female, and immature *F. penneri* millipedes. Bars indicate mean values with brackets showing standard errors. Bars having the same letter are not significantly different (Tukey's HSD test, $p > 0.05$).

Figure 3. Body mass of male, female, and immature *F. penneri* millipedes. Bars indicate mean values with brackets showing standard errors. Bars having the same letter are not significantly different (Tukey's HSD test, $p > 0.05$).

Figure 4. Number of *F. penneri* millipedes trapped as a function of the day in 2004.

Figure 5. Total number of mature *F. penneri* millipedes caught in traps and total precipitation (mm) per week in 2004.

Figure 6. Total number of mature *F. penneri* millipedes caught in traps and mean maximum temperature F° per week in 2004.

Figure 7. Total number of immature *F. penneri* millipedes caught in traps and total precipitation (mm) per week in 2004.

Figure 8. Total number of immature *F. penneri* millipedes caught in traps and mean maximum temperature F° per week in 2004.

Figure 9. Number of *F. penneri* millipedes captured in traps in two time-since-fire categories. Recently burned (0.01-3 years post fire) and long unburned (9-36 years post fire). Bars indicate mean values with brackets showing standard errors.

Figure 10. Elevation transects of the peninsula of Florida. Showing the difference in

elevation for scrubby flatwoods scrub and rosemary scrub.

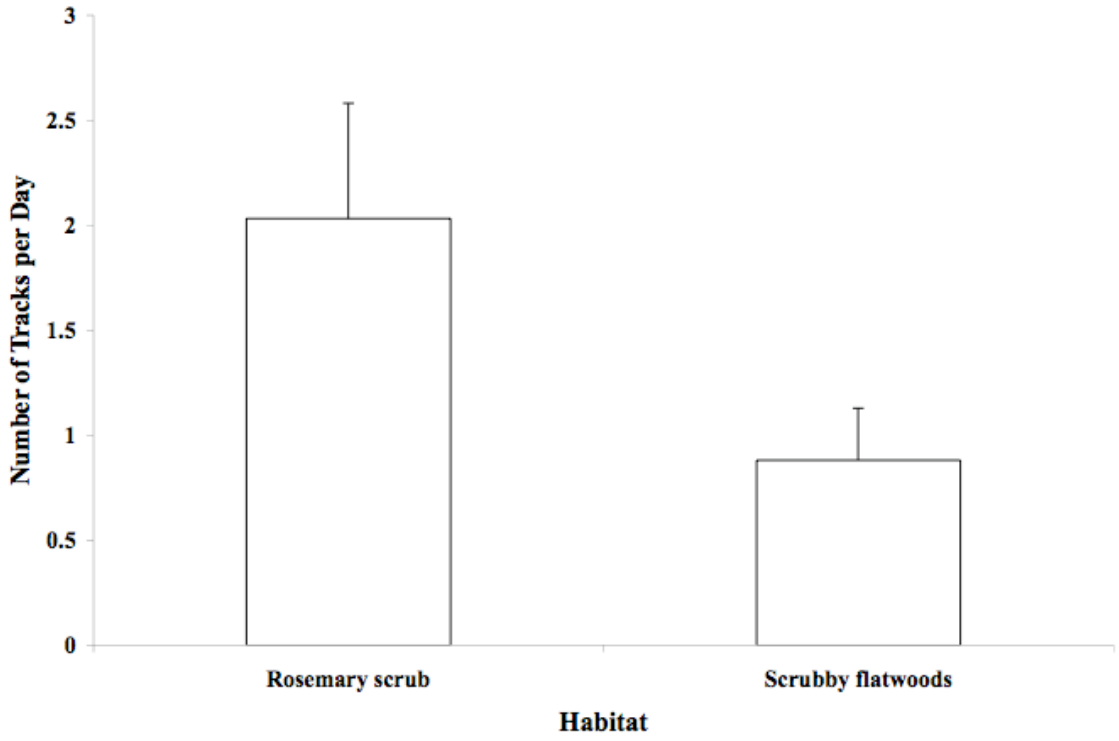
3
2.5
2
1.5
1
0.5
0
Number of Tracks per Day
Rosemary scrub
Scrubby flatwoods
Habitat

Figure 1

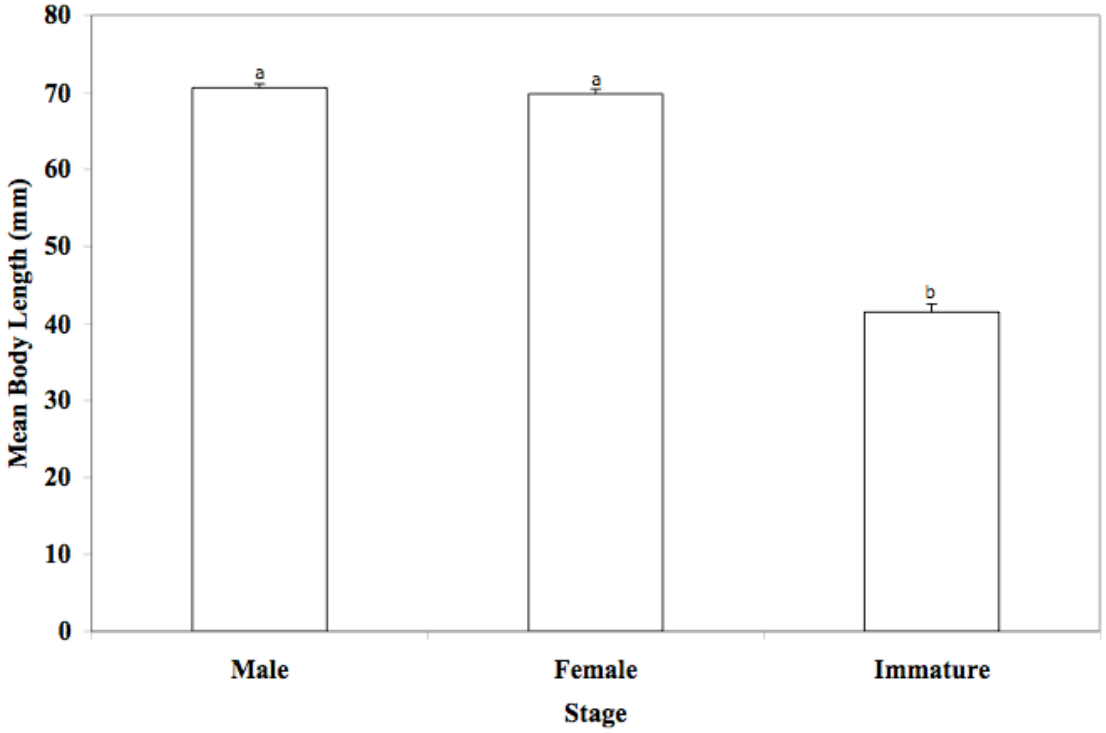
80
70
60
50
40
30
20
10
0
Mean Body Length (mm)
a
a
b
Male
Female
Immature
Stage

Figure 2

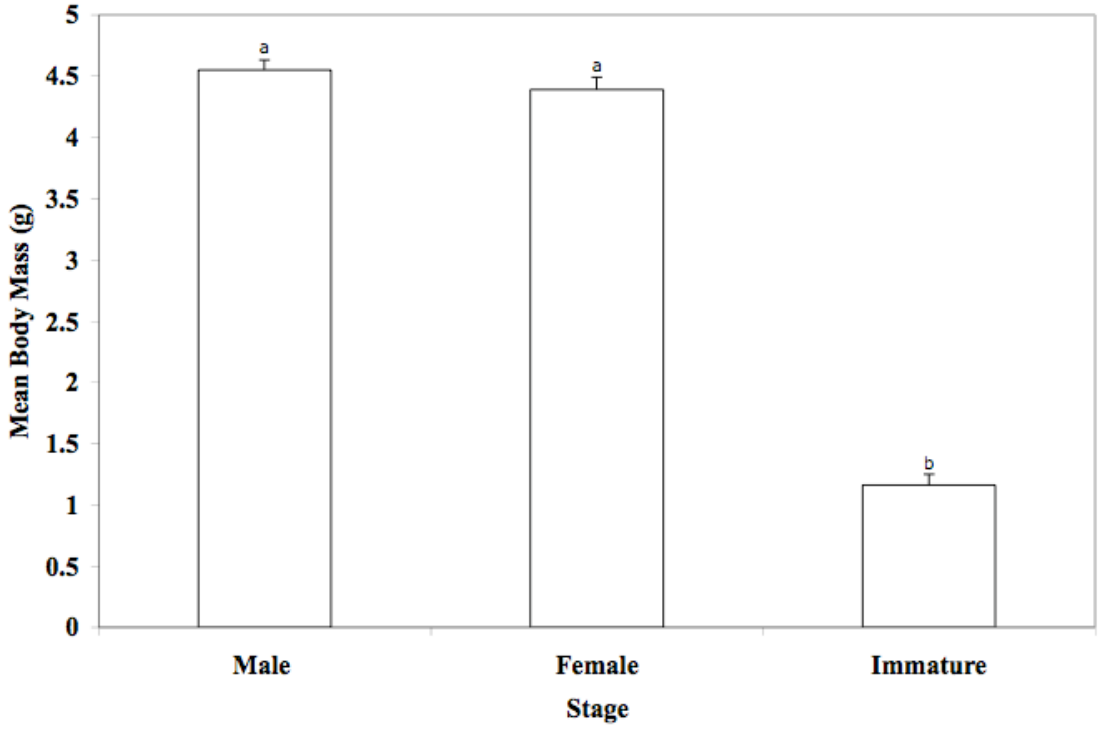
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Mean Body Mass (g)
a
a
b
Male
Female
Immature
Stage

Figure 3

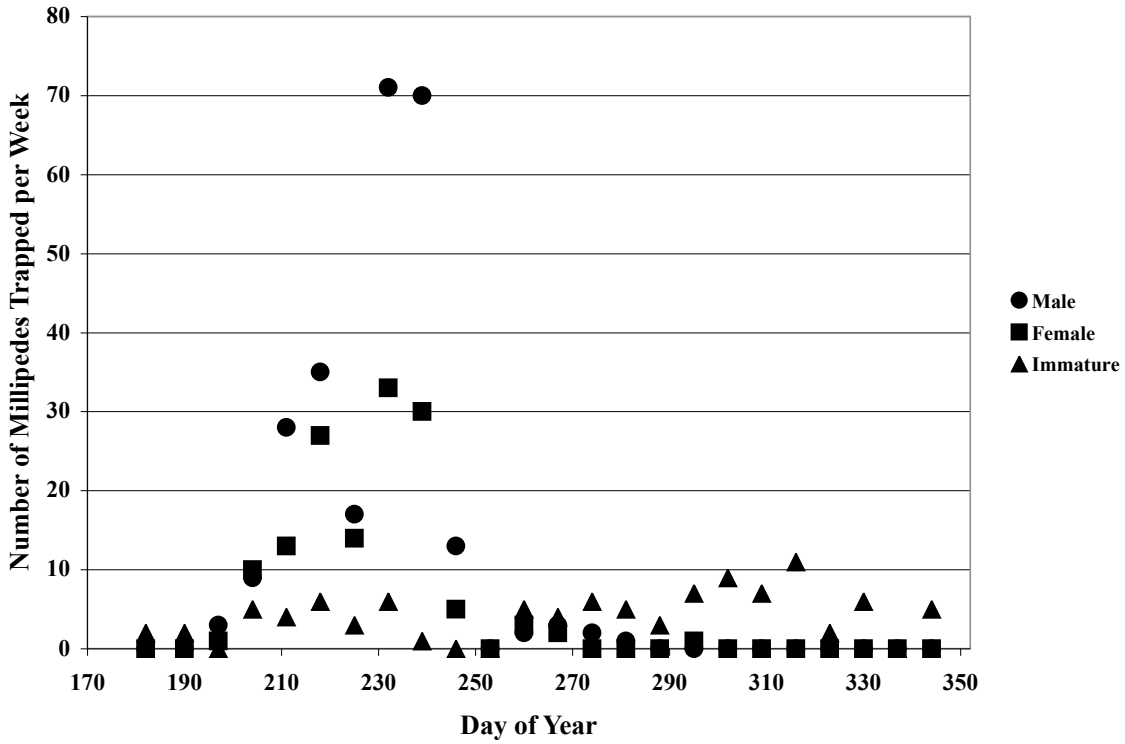
Number of Millipedes Trapped per Week
80
70
60
50
40
30
20
10
0
170
190
210
230
250
270
290
310
330
350
Day of Year
Male
Female
Immature

Figure 4

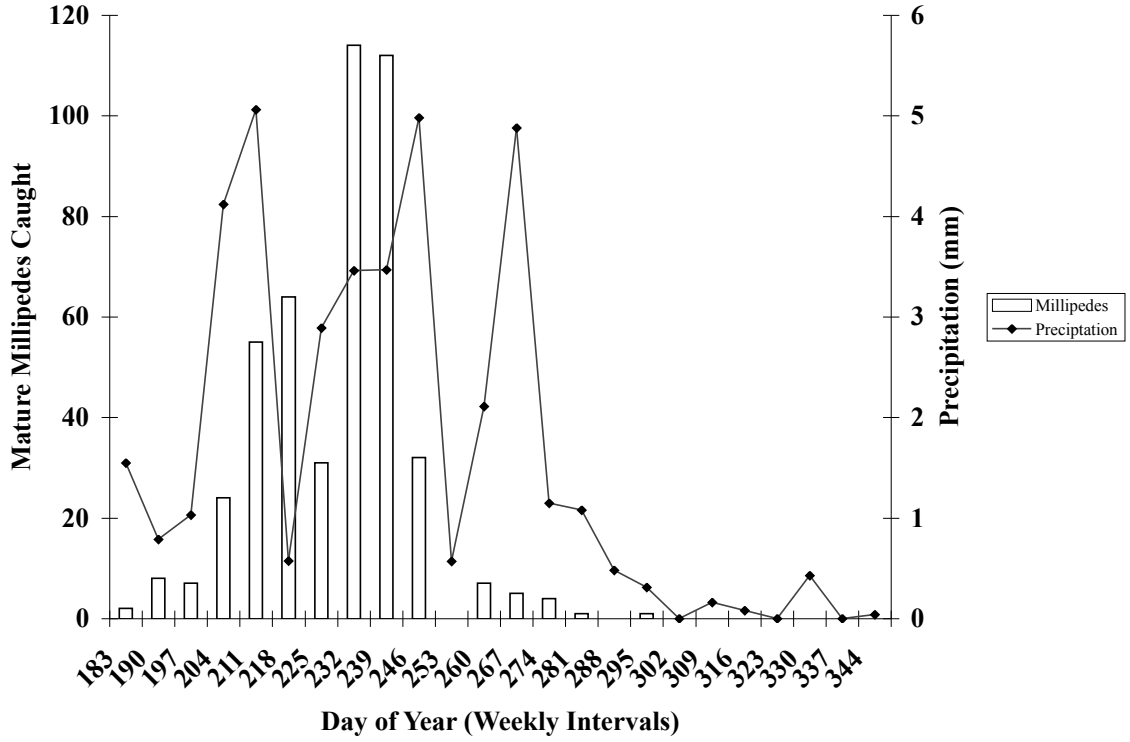
Mature Millipedes Caught
Precipitation (mm)
Day of Year (Weekly Intervals)
Millipedes
Preciptation
120
100
80
60
40
20
0
6
5
4
3
2
1
0
183
190
197
204
211
218
225
232
239
246
253
260
267
274
281
288
295
302
309
316
323
330
337
344

Figure 5

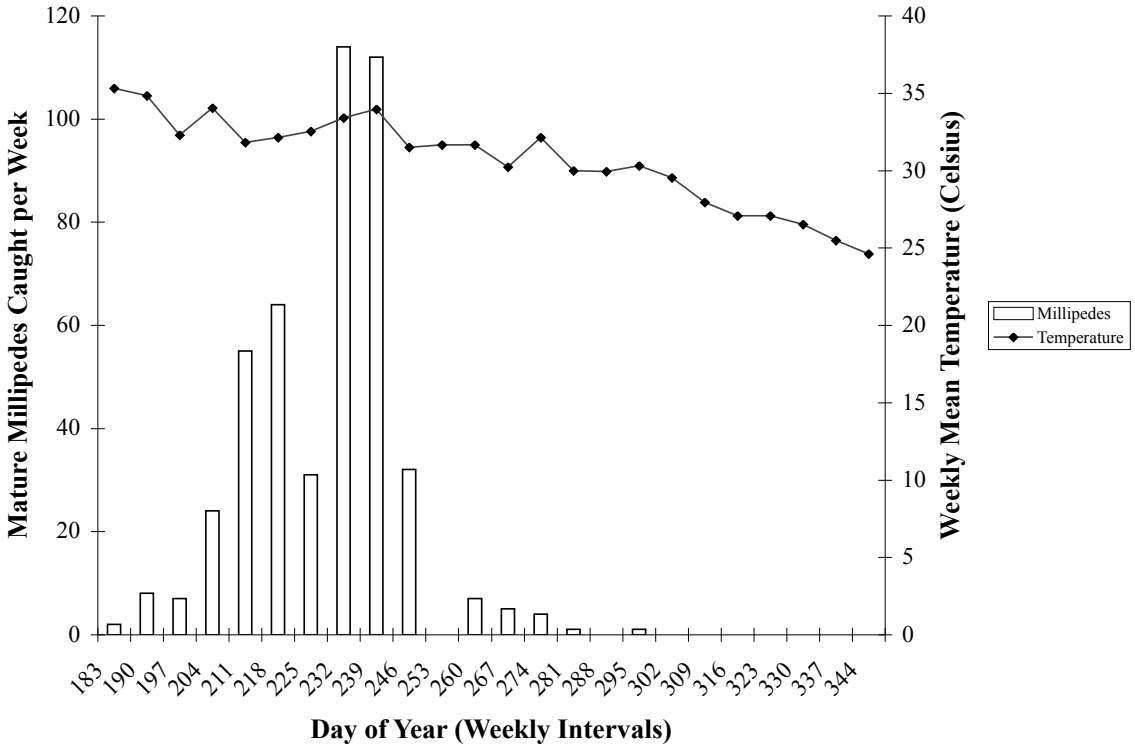
Mature Millipedes Caught per Week
Weekly Mean Temperature (Celsius)
Day of Year (Weekly Intervals)
Millipedes
Temperature
120
100
80
60
40
20
0
40
35
30
25
20
15
10
5
0
183
190
197
204
211
218
225
232
239
246
253
260
267
274
281
288
295
302
309
316
323
330
337
344

Figure 6

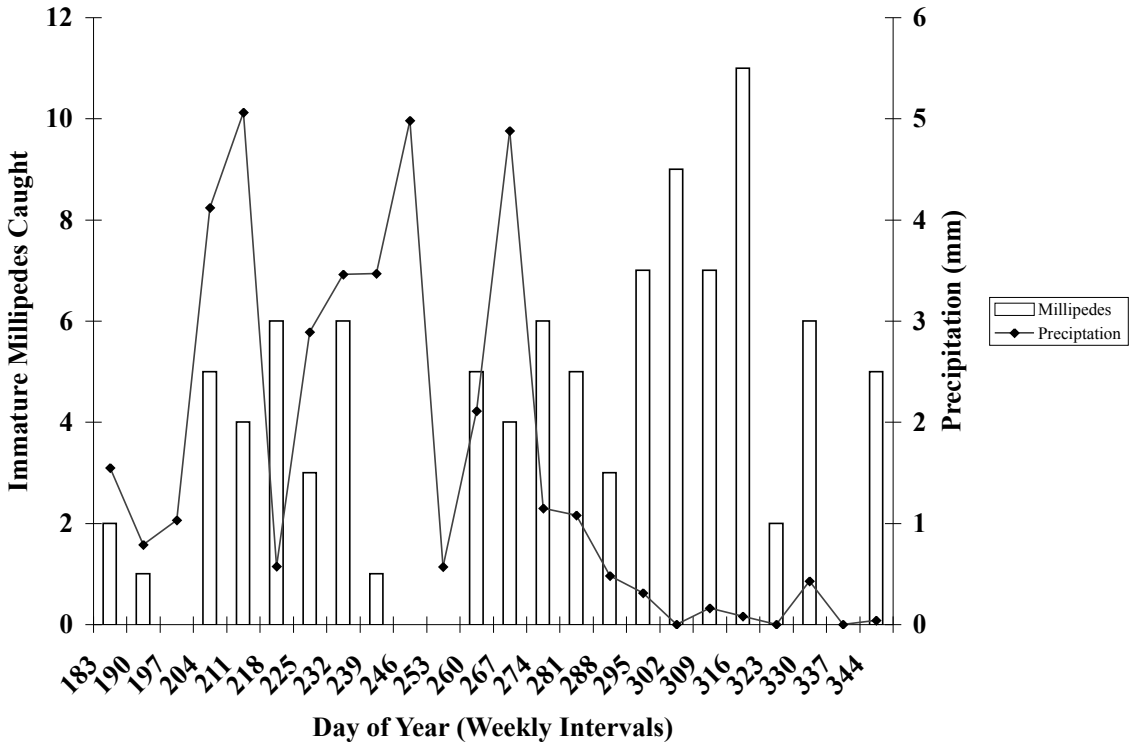
Immature Millipedes Caught
Precipitation (mm)
Day of Year (Weekly Intervals)
Millipedes
Preciptation
0
2
4
6
8
10
12
0
1
2
3
4
5
6
183
190
197
204
211
218
225
232
239
246
253
260
267
274
281
288
295
302
309
316
323
330
337
344

Figure 7

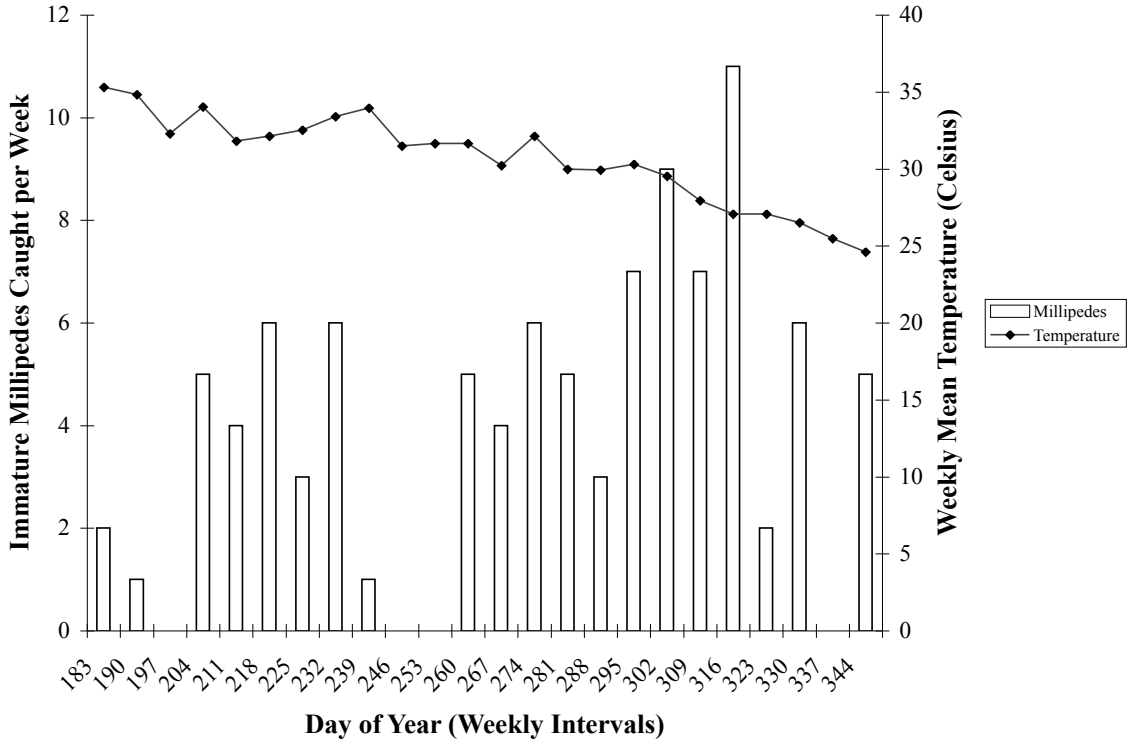
Immature Millipedes Caught per Week
Weekly Mean Temperature (Celsius)
Day of Year (Weekly Intervals)
Millipedes
Temperature
0
2
4
6
8
10
12
0
5
10
15
20
25
30
35
40
183
190
197
204
211
218
225
232
239
246
253
260
267
274
281
288
295
302
309
316
323
330
337
344

Figure 8

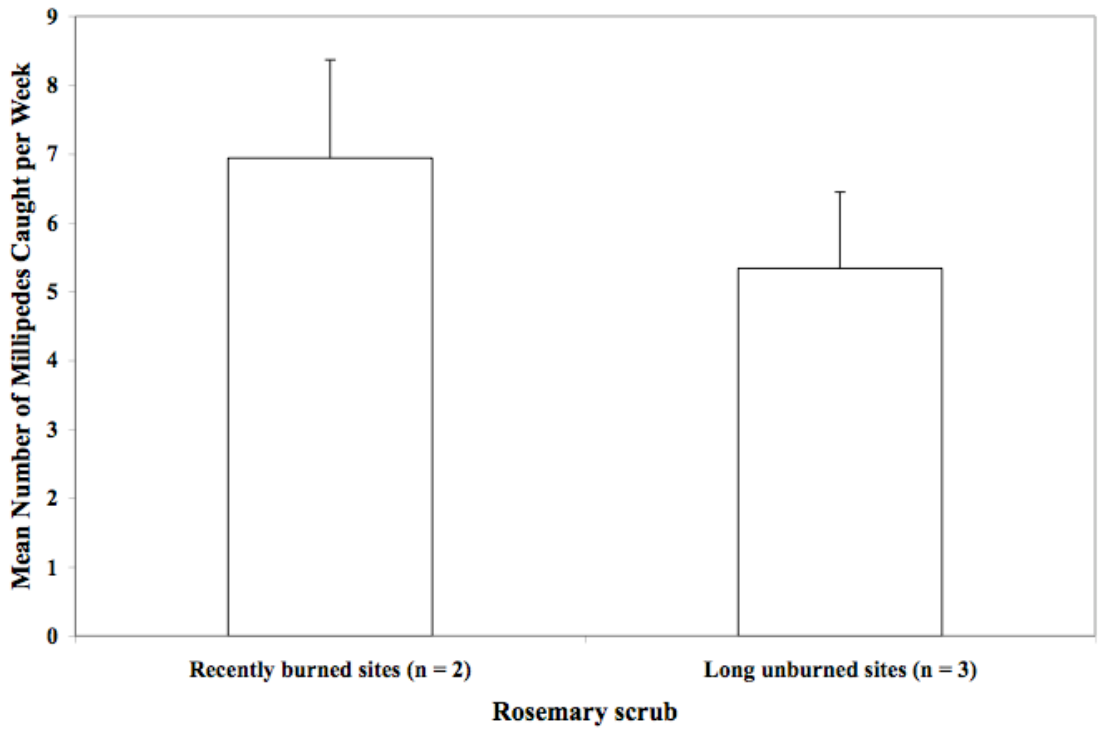
Mean Number of Millipedes Caught per Week
9
8
7
6
5
4
3
2
1
0
Recently burned sites (n = 2)
Long unburned sites (n = 3)
Rosemary scrub

Figure 9

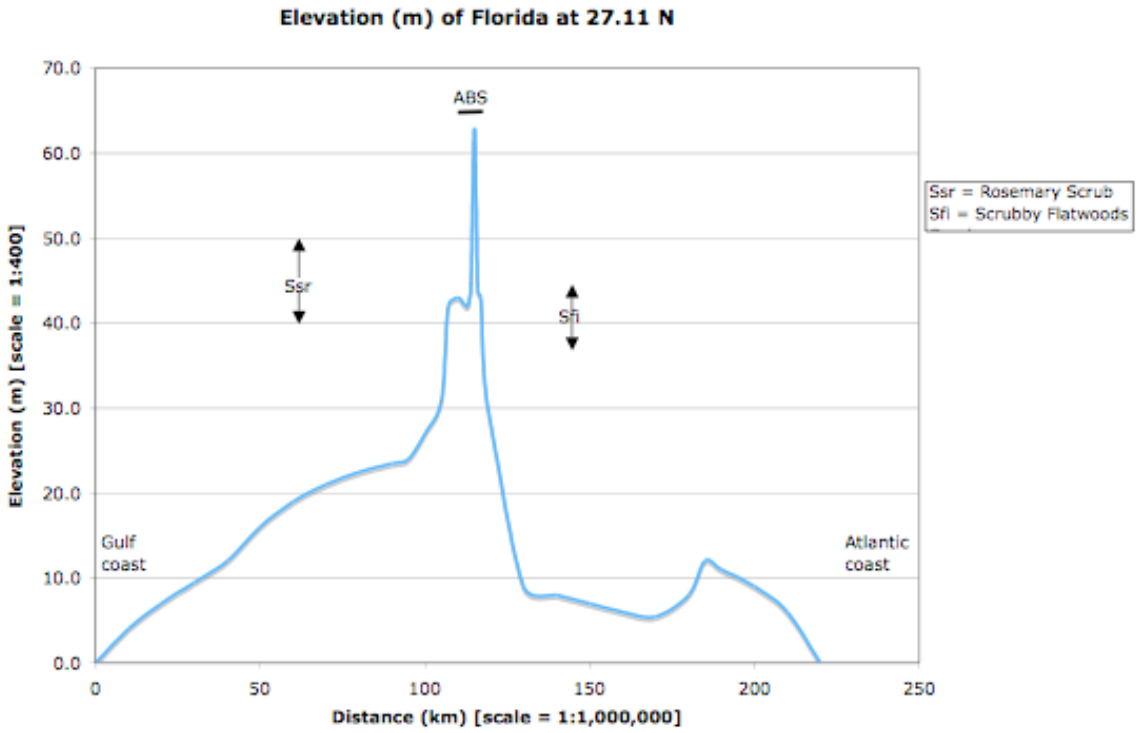
Elevation (m) of Florida at 27.11 N
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Elevation (m) [scale = 1:400]
ABS
Ssr
Sfi
Gulf coast
Atlantic coast
Ssr = Rosemary Scrub
Sfi = Scrubby Flatwoods
0
50
100
150
200
250
Distance (km) [scale = 1:1,000,000]

Figure 10

Table 1: *F. penneri* body length

	N	Maximum (mm)	Minimum (mm)	X ± SE (mm)	Coefficient of variance
Females	130	87.0	55.0	70.6 ± 0.5	0.7
Male	229	93.0	50.0	69.9 ± 0.6	0.8
Immature	77	58.0	19.0	41.5 ± 1.1	2.2

Table 2: *F. penneri* body mass

	N	Maximum (g)	Minimum (g)	Mean (g) X ± SE	Coefficient of variance
Female	130	8.0	3.5	4.4 ± 0.1	0.3
Male	229	8.9	3.4	4.6 ± 0.1	0.3
Immature	74	3.3	0.1	1.2 ± 0.1	.5

Table 3: *F. penneri* day of year millipedes captured

	Maximum		Minimum		Mean	
	DOY	Date	DOY	Date	DOY	Date
Female	294	Oct. 20	196	July 14	226.8 ± 1.2	Aug 14
Male	280	Oct. 6	196	July 14	229.0 ± 0.8	Aug 16
Immature	343	Dec. 8	182	June 10	270.6 ± 4.5	Sept 27

CHAPTER 4: CONCLUSIONS

In this thesis, I report on several laboratory and field tests designed to learn about the ecology and life history of the endemic scrub millipede, *F. penneri.* I found that:

- *F. penneri* apparently prefers xeric barren rosemary balds over more mesic, closed canopied oak scrub. For example, based on my 5-week long track survey, approximately 2.5 times more millipedes were active in rosemary scrub than in scrubby flatwoods. These results are consistent with pitfall trap captures.
- Both male and female millipedes were active above-ground during the same time of year (July – October), though female millipedes were captured in pitfall traps two weeks after the last male millipede was captured. Peak activity for males was July 28 (DOY = 210) and for females it was August 2, 2004 (DOY = 215).
- Immature millipedes were captured fairly steadily during the entire period the traps were open (June-December). In fact, on average, immature millipedes were captured later in the year than mature millipedes. Peak activity for immature millipedes was early November (DOY = 313), more than two months after the adult peak.
- Maximum temperature and weekly precipitation were not significant predictors for capturing mature millipedes. However, maximum temperature, but not weekly precipitation was a significant predictor for capturing immature millipedes.
- Time-since-fire in rosemary scrub did not affect the number of millipedes captured at a site.

- Based on laboratory feeding trials, stable isotope analysis, and laboratory observations of feeding behavior, it seems unlikely that leaf litter and root tissues from woody shrubs are major dietary inputs. The identity of the main food source of *F. penneri* remains an enigma.
- Finally, the most surprising outcome of this study is *F. penneri* millipedes were concentrated in rosemary scrub, a rare native habitat that has barren soil devoid of leaf litter and lacking the dominate shrubs characteristic of Florida. Very little is known about animals that reside in rosemary balds. Rosemary scrub could provide a safe haven from rising seasonal flooding and it could provide a unique, necessary dietary substance, the identity of which remains an enigma. Rosemary scrub is the rarest, most threatened component of Florida scrub and it has the highest concentration of endemic herbs. This study indicates *F. penneri* should be added to the list of species whose very existence is tied to preservation of rosemary scrub.

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