PHYSIOLOGICAL MEASURES OF VIGOR FOR THE EASTERN SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR) (ISOPTERA: RHINOTERMITIDAE)

by

TIMOTHY JOSEPH ARQUETTE

(under the direction of Brian T. Forschler)

ABSTRACT

Termite populations vary in vigor (Lenz and Williams, 1980). This is a problem in laboratory trials when termites from weak populations decline in vigor or die before a study is completed (Carter and Smythe, 1972a). No method has been established for identifying healthy termites before placement in bioassay arenas. The current study was undertaken to consider predictive methods for describing the vigor of *Reticulitermes flavipes* (Kollar) termites stored in a laboratory under conditions similar to control groups in bioassay. These novel methods were based on measurements for levels of biomolecules (uric acid, soluble proteins, lipid, and glycogen), percent water content, and running speed. Also considered were two established, non-predictive methods for determining vigor, survivorship and consumption rate, as well as body weight, considered an indicator of declining termite populations. Novel measures of vigor were also applied to termites collected from field populations as a survey of levels under natural conditions. Low body water percentage was concluded to be an indicator of weak groups of termites.

INDEX WORDS: vigor, consumption, survivorship, uric acid, soluble protein, glycogen, cellulase, lipid, body water.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Termites are considered the most economically important structural pests (Howick and Creffield, 1975). In the United States, the cost of treatment and prevention of termite infestation is over one billion dollars annually (Suiter and Forschler, 2004). Consequently, termites are included in laboratory trials aimed at measuring efficacy of insecticides and resistance of materials to feeding (Lenz et al, 1984). Conclusions drawn from termite bioassays depend on the health or vigor of the test animals (Becker, 1969; Carter et al, 1972b; Lenz and Williams, 1980; Su and LaFage, 1984a). Termites collected from field populations are typically maintained in the laboratory under standard conditions for weeks or months prior to use in experiments (Becker, 1969; Lenz, 1985). Factors that contribute to differences in vigor between field populations, such as genetic variation and population age, cannot be controlled in laboratory culture; however, environmental conditions including moisture and temperature can be, ensuring the best possible conditions for maintaining healthy test animals (Becker, 1969; Lenz, 1985).

Currently, there is no way to measure the health of termites destined for bioassay, with the result that experiments are often discarded after being conducted, based on low survivorship or decreasing food consumption rate in the controls (Su and LaFage, 1984a and 1984b). This practice results in the establishment of numerous replicates from separate field or laboratory stock populations to ensure data can be obtained for the duration of the experiment. An ability to identify the health of termite populations would assist in such diverse activities as choosing vigorous groups to include in bioassay and to provide the basis for decision criteria for pest management professionals in determining the threat toward infesting structures.

Variation in vigor between termite populations affects conclusions drawn from laboratory studies, and makes comparison of results from different laboratories difficult (Lenz and Williams, 1980; Su and LaFage, 1984a; Lenz, 1985). To ensure that experimental termites

remain healthy throughout a bioassay, food consumption and survival are measured with the assumption that these data correlate to vigor (Lenz et al, 1984; Su and LaFage, 1984a). Consumption rate and survivorship are not predictive measures of vigor, as both techniques require an experiment to be finished or well underway before measurement (Su and LaFage, 1984a and 1984b).

Additional measures could describe healthy or weak groups of laboratory cultured termites. Nutrient stores, water content, running speed, or metabolic wastes accumulated by storage excretion may potentially be predictive measures of termite population vigor. For example, uric acid accumulation may be an indication of starvation, because it is produced when termites digest proteins for energy (Slaytor and Chappell, 1994). Also, uric acid in the fat body of *Reticulitermes flavipes* (Kollar) termites was reported to rise at a linear rate of 2.7% per month in captivity, from 1% to 45% dry weight after 18 months (Potrikus and Breznak, 1980a). Presumably, termite laboratory cultures decrease in vigor over time (Su and LaFage, 1984b), and if uric acid increases predictably, uric acid levels could negatively correlate with vigor. Establishment of a threshold separating healthy and unhealthy groups of termites based on levels of accumulated uric acid could help researchers determine whether a laboratory population of termites is suitable for use in experiments.

The current study considered novel measures of termite population vigor. These measures might provide advantages over traditional determinants of vigor – food consumption rate or survivorship – by being predictive. In this chapter, traditional measures of termite vigor are reviewed, and the choices of potentially predictive measures of vigor are examined.

CONSUMPTION RATE

The consumption rate of wood-eating termites is often measured in laboratory bioassay for determining the resistance of materials to termite attack (Lenz, 1982). Measuring consumption rate is also useful for describing the vigor of termites used as experimental controls (Su and LaFage, 1984a); feeding studies should be regarded as valid only when control termites eat favored foods above a predetermined rate (Lenz, 1982). A table presented by Wood (1978) summarized typical feeding rates for a number of termite species fed palatable foods.

The consumption rate of termites in bioassay is affected by group mortality, requiring a correction factor for accurate measurement (Su and La Fage, 1984b). Consumption rate is also affected by the food source, such as wood species, hardness, moisture content, and degree of decay (Becker, 1969; Smythe et al, 1971; Haverty and Nutting, 1974). The size of wood blocks used in laboratory experiments affects feeding rate. Feeding increases when larger blocks of wood are used, irrespective of the number of termites present (Waller, 1991; Lenz, 1994).

Consumption rate can be an indicator of the health of termites in feeding experiments (Lenz et al, 1984; Su and LaFage, 1984b). Su and LaFage (1984b) devised a method to assess the vigor of experimental termites by monitoring the consumption rate of the control group. They measured *Coptotermes formosanus* (Shiraki) workers feeding at a constant rate for 42 days before declining, and concluded that at the point when feeding rates start to decline, termites are no longer healthy. Consumption rate may also be related to live weight. Su and LaFage (1984a) reported mean worker weight from eight *C. formosanus* colonies ranged between 2.0 mg and 3.7 mg. Using linear regression, they demonstrated a relationship between weight and consumption rate of workers, with the larger termites eating less. They cited a publication by Nakajima (1960, in Japanese) that concluded heavier termites result from less vigorous, declining colonies, and

they consume less food. Grace et al (1995) also described termites in older, declining colonies as weighing more, although the study did not report feeding rates.

Termites in laboratory culture as well as in control groups should be offered favored woods (Becker, 1969). Yellow pine is a typical wood fed to control groups in experiments with *R. flavipes* termites (Carter et al, 1972b). Termites have also been shown to prefer certain hardwoods such as beech, poplar, birch, apple, and pear (Becker, 1969). Alpha-cellulose and filter paper are acceptable diets for termites kept in laboratory culture (Becker, 1969). Artificial diets that promote toxic mold growth should not be used (Mauldin and Rich, 1975). Woods toxic to protozoan symbionts must be excluded from the diet of termites in captivity, as the termites need these symbionts to survive (Becker, 1969; Smythe and Carter, 1969; Waller, 1991; Lewis, 2003).

SURVIVORSHIP

Besides measuring consumption rate, another established method for determining the vigor of termites in experiments is to monitor the survivorship of the control group (Su and La Fage, 1984a; Lenz, 1985). When survivorship drops below 80%, experiments should be stopped (Su and LaFage, 1984a). As with consumption rate, an experiment must be underway or completed before an assessment of vigor from survivorship can be performed.

Carter et al (1972b) reported that vigorous termites might withstand the effects of toxic woods if the duration of an experiment is short (i.e., 4 weeks). Survivorship of six wild populations fed toxic woods ranged from 5%-80% at 4 weeks, while nearly all were dead at 8 weeks. However, control groups from each of the six populations maintained 85-90% survivorship through 8 weeks. The study concluded that the variation in survivorship of trial insects at 4 weeks was due to differences in vigor between populations. Vigorous termites

survived at rates similar to control groups through 4 weeks, so if the experiment had been stopped at that point, an erroneous conclusion could have been made that the test woods were nontoxic. Carrying out experiments for 8 weeks was recommended to ensure that lethal effects of toxic woods are apparent for healthy groups.

Laboratory conditions promoting the optimal survival of termites in culture have been described in various reviews (Becker, 1969; Howick and Creffield, 1975; Lenz, 1984). Survivorship of laboratory termites is affected by container size, aeration, humidity, and type of food (reviewed by Becker, 1969). Termites used in laboratory experiments should be species that are known to survive in laboratory culture (Becker, 1969). Each species has its own requirements for optimal survival in captivity. For instance, many termite species survive at 26-28°C, but the optimum temperature range for the Pacific dampwood termite, *Zootermopsis angusticollis* (Hagen) is between 18-22°C (Becker, 1969).

C. formosanus colonies are estimated to persist for about 35 years, with a 15-year declining phase (Grace et al, 1995). Termites in older colonies are heavier and may be less vigorous, with declining survivorship (Grace et al, 1995). Individual termites from a single *C. formosanus* field population sampled over 16 years increased in weight from 3 mg to 6 mg, while estimated population number decreased (Grace et al, 1995). Similar effects on live weight have been reported over time in captivity. *C. formosanus* termites increased in body mass by about 30% after several months in culture (Waller and LaFage, 1987). However, the weight increase may have been due to an increase in the number of older, heavier instars because *C. formosanus* does not develop secondary reproductives in captivity (Becker, 1969, Waller and LaFage, 1987; Grace et al, 1995).

Mold fungi and white-rot fungi are toxic to termites, and other fungi cause disease (Becker, 1969). Certain fungi may also enhance termite health (reviewed by Cornelius, 2002). An example of fungi benefiting termites is degradation of wood by brown-rot fungi, which attracts termites and improves termite nutrition (Becker, 1969; Smythe et al, 1971). Parasites, other invertebrates, and bacteria can also influence termite survivorship (Becker, 1969). Parasitic mites can kill weak termite populations (Becker, 1969). Centipedes prey on termites and can kill off small cultures (Becker, 1969).

The health of bacterial and protozoan symbionts is crucial for the survival of lower termites, as they contribute to the termite's metabolism by fixing nitrogen, digesting uric acid, synthesizing vitamins, converting glucose in cellulose to acetate, and synthesizing proteins and amino acids (Moore, 1969; Smythe, 1972; Mauldin and Rich, 1975; Potrikus and Breznak, 1980a; Odelson and Breznak, 1983; Slaytor, 1992; Nation, 2002).

URIC ACID

Uric acid is the primary metabolic waste product of most insects (Chapman, 1998; Nation, 2002). It is probably produced in the fat body (Candy, 1985). The uricolytic pathway is the main route of uric acid production, starting with the digestion of proteins (Nation, 2002). This same pathway is used in mammals and birds (Downer, 1982). A less common process is the nucleolytic pathway, using as precursors nucleic acids, purines, or pyrimidines rather than proteins to make uric acid (Downer, 1982).

Some insects store uric acid in specialized fat body cells (Chapman, 1998). These cells, called urocytes, contain precipitated spherules of potassium or sodium urates (Mullins, 1979). Some insects that store uric acid are tobacco hornworm larvae, larval *Chrysopa* lacewings, and adult mosquitoes (Chapman, 1998). Termites and cockroaches store uric acid at all life stages

and in captivity are known to store it in large quantities. American cockroaches given food with high protein content accumulated up to 31% of their dry weight as uric acid (Mullins and Cochran, 1975). *R. flavipes* termites accumulated uric acid up to 45% of their dry weight after 18 months in a laboratory (Potrikus and Breznak, 1980a).

In contrast to numerous studies that concluded cockroaches mobilize urates from urocytes (reviewed by Cochran, 1975), only a limited number of similar studies have been done with termites (reviewed by Breznak, 2000). Subsequently, there is controversy as to whether termites can mobilize stored urates. One view holds that termites do transport urates by hypothesizing that the molecules are mobilized to the hindgut via the Malpighian tubules, and upon digestion by uricolytic bacteria harbored in the hindgut, provide nitrogen molecules that can be used to manufacture new proteins for the termite (Potrikus and Breznak, 1981; Breznak, 2000). Three known species of uricolytic bacteria are known to live in the *R. flavipes* hindgut, each capable of converting uric acid to carbon dioxide, ammonia, and acetate (Breznak, 1982 and 2000). These bacteria are present in sufficient numbers to provide termites with a significant amount of dietary nitrogen (Potrikus and Breznak, 1981). Cannibalism and necrophagy are also cited as sources of uric acid for the uricolytic bacteria (Potrikus and Breznak, 1981; Slaytor and Chappell, 1994; Breznak, 2000). Most uric acid reaching the hindgut is digested by the bacteria, as evidenced by nearly undetectable amounts of the molecule in feces of *R. flavipes* (Potrikus and Breznak, 1980b).

An alternate view from Slaytor and Chappell (1994) considers uric acid to be permanently stored in termite fat body. They consider nitrogen-fixing spirochetes rather than uricolytic bacteria to be the significant providers of nitrogen for amino acid synthesis. Slaytor and Chappell attribute the phenomenon of high uric acid accumulation in laboratory cultured

termites to a drop in nitrogen fixing ability, which has consistently been reported to occur in termites soon after field collection (reviewed by Slaytor, 2000). The reduction or loss of nitrogen fixing capability when termites are collected from the field is easily explained by the fact that CO_2 levels inside termite tunnels and wood are elevated compared to outside air. Nitrogen fixation has been reported to increase with higher carbon dioxide levels in *R. flavipes* and *Reticulitermes virginicus* (Hageni) (Curtis and Waller, 1996).

While only a handful of studies have investigated whether uric acid has a role in termite metabolism (reviewed by Breznak, 2000), the storage, mobilization, and metabolism of uric acid has been well documented in cockroaches (reviewed by Cochran, 1975). Experiments with female *P. americana* cockroaches fed a high protein diet showed uric acid levels increasing to over 30% of the dry weight, with levels dropping in starving insects (Mullins and Cochran, 1975). Cockroaches with blattabacteria removed were shown be smaller-sized and slow in maturing with diminished fecundity (Brooks and Richards, 1956). Fat body of cockroaches without blattabacteria had uric acid levels 20-fold higher than cockroaches with the symbionts (Cochran, 1985). Transplants of normal cockroach fat body into cockroach nymphs lacking blattabacteria caused the insects' growth rate to increase. After four months, the bacteria-free part of the host's fat body was white due to accumulated uric acid, while the transplanted section with mycetocytes was translucent with no apparent accumulation (Brooks and Richards, 1956).

Mycetocytes containing uricolytic bacteria are found in the fat body of cockroaches, as well as a termite species, *Mastotermes darwiniensis* (Bandi and Sacchi, 2000). Mycetocytes contain blattabacterium, a rod-like, uricolytic bacteria (Bandi and Sacchi, 2000). Blattabacteria have uricase and xanthine dehydrogenase activity (Wren and Cochran, 1987; Breznak, 2000) and are capable of breaking down uric acid to pyruvate *in vitro* (Donnellan and Kilby, 1967). The

pathway by which isolated blattabacteria metabolize uric acid begins with the conversion of uric acid to allantoin, followed by allantoic acid, glyoxylate, glycerate, and finally pyruvate (Donnellan and Kilby, 1967). *R. flavipes* do not have mycetocytes, so uric acid would have to be mobilized to the hindgut for digestion by the uricolytic bacteria harbored there. The close association of mycetocytes with urocytes in cockroaches (Downer, 1982) may explain the ability of cockroaches to utilize uric acid for a variety of metabolic uses. The lack of mycetocytes in *R. flavipes,* as well as all other termite species except *M. darwiniensis*, illustrates the difficulty in demonstrating that termites have a similar ability to utilize uric acid metabolically, unless it could be shown that stored urates are mobilized from termite fat body to the uricolytic bacteria in the hindgut.

Irrespective of the fate of uric acid in the termite fat body, its accumulation there may assist in determining whether a group of termites is vigorous. This is because uric acid formation has been attributed to the digestion of body proteins during starvation (Slaytor and Chappell, 1994). Termites with elevated uric acid levels could therefore be regarded as less vigorous.

SOLUBLE PROTEINS

Proteins are involved in almost all cellular functions (Lehninger et al, 2000). Protein types include contractile proteins, allowing for movement; structural proteins; transport proteins, including lipophorins in the hemolymph; regulatory proteins, including hormones of insects; defense proteins; and enzymes (Neville, 1975; Chapman, 1998; Lenhninger et al, 2000). Over one hundred different kinds of proteins are known to occur in different areas of insect cuticle and contribute to function, pigmentation, or degree of hardness (Hopkins and Kramer 1992; Chapman, 1998).

While structural proteins are fixed in particular regions of the insect body, many proteins are mobile. For instance, lipophorin is a conjugated protein that shuttles diacylglycerols through the hemolymph (Chapman, 1998). Urates may also be transported through hemolymph by proteins (Cochran, 1975). Some proteins can be mobilized to sites where they are needed. For instance, some of the cuticular proteins that are secreted from the epidermis during molting originate in the hemolymph (Reviewed in Neville, 1975; Hopkins and Kramer, 1992).

Termites obtain some protein from their diet. Fungal tissue, a primary food source of subterranean termites (Waller and Curtis, 2003), consists of 20%-40% protein (dry weight). Other dietary sources of protein include shed cuticle, dead protozoans from proctodeal feeding, cannibalism, and necrophagy (Noirot and Noirot-Timothee, 1969; Collins, 1983; Hunt and Nalepa, 1994). All termites have proteolytic enzymes in their digestive systems (Collins, 1983).

Insects synthesize amino acids predominantly in fat body cells (Chapman, 1998). Higher organisms synthesize some amino acids from intermediates of glycolysis and the citric acid cycle, while other amino acids are essential in the diet. Bacteria synthesize all amino acids (Lenhninger et al, 2000). *C. formosanus* obtain amino acids synthesized by their bacterial and protozoan symbionts (Mauldin et al, 1978). Intracellular symbionts of the German cockroach are able to synthesize amino acids from urate precursors (McFarlane, 1985). Amino acids are stored in hemolymph. Amino acids in hemolymph are concentrated at 100-300 times the levels found in human blood, in excess of amounts needed for protein synthesis (Chen, 1985).

In addition to comprising the primary structure of proteins, amino acids serve as precursor molecules. For example, glucogenic amino acids are used for synthesis of glucose during starvation (Lenhninger et al, 2000). Some amino acids are used to synthesize acetyl-CoA.

In adult insects, protein is always being replaced (McFarlane, 1985). However, the rate of protein synthesis may decline in aging insects due to an inability to repair or replace mitochondria (Sohal, 1985). Consequently, protein synthesis as well as enzyme activity is diminished (Sohal, 1985; Brunk and Terman, 2002).

The termite diet is too low in nitrogen, amino acids, and proteins for growth and egg development (Chen, 1985). Nitrogen for adequate protein synthesis in termites has been hypothesized to come from various sources. Atmospheric nitrogen, fixed by bacterial symbionts in the paunch, is thought to be incorporated into termite tissues at high enough levels to compensate for the low levels of nitrogen found in wood (Slaytor and Chappell, 1994). Bacteria harbored in the paunch of subterranean termites may provide the insect with significant amounts of dietary nitrogen for protein production by digesting uric acid (reviewed by Breznak, 2000). Dead protozoan symbionts, and ammonia excreted by protozoans, are considered to be nitrogen sources for protein production by termites; however, this has not been demonstrated by experiment (Breznak, 1982; Collins, 1983). Chitin may be a source of dietary nitrogen; it is always associated with proteins in nature and contains 7% nitrogen (Breznak, 1982; Chapman, 1998). When an insect molts, chitin, as well as protein digested in molting fluid, is re-absorbed through epidermal cells or taken in through the mouth or anus (Neville, 1975; Breznak, 1982).

Published reports of protein levels of termites are scant. Mauldin et al (1973, 1978) measured amounts of protein-bound amino acids in *C. formosanus* after extraction of proteins from whole termites into hydrogen chloride solution. Slaytor and Chappell (1994) described a relationship between decreasing protein levels and increasing uric acid levels in *Nasutitermes walkeri*. These reports concluded that protein levels remain constant in healthy termites, while falling levels indicate protein metabolism during starvation.

LIPID

Lipids are integral components of an insect's cell membranes, cuticular wax, and other layers of the cuticle, and are a major source of energy and metabolic water (Fast, 1964; Nation, 2002). Esters of fatty acids, called glycerides, are the main lipid type in insects; these include triacylglycerols stored in the fat body (Fast, 1964). Total lipids in insects are at least 78% triaclyglycerols, with the proportion in fat body about 90% (Fast, 1964; Beenakkers et al, 1985). Diacylglycerols, which make up about 3% of the total lipid content, are the main lipid class found in hemolymph and are an important energy reserve (Bailey, 1975; Beenakkers et al, 1985). Other lipid types are phospholipids and sterols (Lehninger et al, 2000).

Insects obtain lipids from their diet, synthesize them from precursor biomolecules, or acquire them from symbionts (Chapman, 1998). Insects usually synthesize glycerides from carbohydrates in the fat body (Bailey, 1975). Lipid synthesis from glucose begins when it is converted to pyruvate by glycolysis. Pyruvate is converted to acetyl-CoA, used in long-chain fatty acid synthesis (Bailey, 1975). Long fatty acid chains and glycerols are esterified, forming triacylglycerols for storage in the fat body (Bailey, 1975). Lower termites also produce glycerides in the fat body from acetate produced by protozoan symbionts in the paunch (Potrikus and Breznak, 1981). These symbionts ferment cellulose to acetate and other short-chain fatty acids. Acetate makes up about 87% of the short-chain fatty acids produced from cellulose digestion by protozoan symbionts of *R. flavipes* (Potrikus and Breznak, 1981). Acetate in hindgut fluid is absorbed and used for lipid synthesis in the termite fat body (Potrikus and Breznak, 1981). Carter et al (1972a) reported that oleic acid makes up about 60% of the fatty acids in *R. flavipes* termites; lineolic acid (18 carbon atoms) and palmitic acid (16 carbon atoms) each constitute about 10% of the total.

Acetyl-CoA is the substrate for fatty acid synthesis in cell cytoplasm, and is formed in the mitochondrial matrix of cells (Lehninger et al, 2000). However, the molecule is too large to pass through the mitochondrial wall. Citrate produced from acetyl-CoA passes through the mitochondrial wall, and then is regenerated into acetyl-CoA in the cytoplasm (Lehninger et al, 2000).

Insects cannot synthesize sterols (McFarlane, 1985). Sterols are either obtained directly from food or are synthesized by microbial symbionts (Chapman, 1998). Clayton (1960) described the production of 22-dehydrocholesterol from radiolabeled acetate fed to the German cockroach. He concluded that bacterial symbionts in the cockroach gut probably converted acetate to ergosterol, which was then converted to 22-dehydrocholesterol.

Most glycerides stored in the fat body must be transported to other sites for oxidation (Candy, 1985). Triacylglycerols are converted to diacylglycerols in trophocytes, which are attached to lipophorins before entering the hemolymph for transport (Bailey, 1975). Lipophorins, reusable shuttles for diacylglycerols, transport diacylglycerol through the hemolymph to muscle for oxidation (Beenakkers et al, 1985). When the fatty acid chains of diacyglycerols are hydrolyzed, most of the remaining glycerol is transferred to the hemolymph (Candy, 1985).

The lipid content of *R. flavipes* has been reported in various studies. Total lipid of workers from 11 field *R. flavipes* populations ranged between 2.8% and 6.7% wet weight (Carter et al, 1972a). Mauldin et al (1977) reported that *R. flavipes* termites had 5.3% lipid (wet weight) shortly after field collection, 1.3% after 18 days after defaunation, and 1.4% 11 days after partial defaunation. The lower lipid levels are probably due to lipid being the main energy reserve used by insects during starvation (Fast, 1964). In the American cockroach, 66% of the energy used

during starvation is from lipid, 22% from glycogen, and 11% from protein (Fast, 1964). Low or decreasing lipid levels in termites may therefore indicate a weakened state from starvation.

GLYCOGEN

Glycogen, the major form of stored glucose in insects (Friedman, 1985), is a branched polysaccharide stored in fat body cells and epithelial gut cells (Nation, 2002). Glycogen synthesis proceeds by the addition of glucose molecules to a glucose primer and is catalyzed by glycogen synthetase (Nation, 2002). Because glycogen is stored intracellularly, freed glucose does not need to be transported and therefore can be used as a quick fuel source (Candy, 1985). Trehalose is also released from glycogen (Candy, 1985).

Carbohydrates are the foundation of termite metabolism (Itakura et al, 2003). However, glucose levels are low or absent in termite hindguts (Slaytor, 1992); the paunch lacks substrates for gluconeogenesis (Itakura et al, 2003); and symbiotic bacteria in the hindgut of both higher and lower termites use acetate and pyruvate for energy, not glucose (Itakura et al, 2003). These observations lead to questions about the source of glucose molecules for termite metabolism. Biology textbooks routinely state that termites are dependent on protozoan symbionts for cellulose digestion, even though the majority of termite species do not harbor protists (Slaytor, 2000). Many studies have established the presence of endogenous termite cellulases (reviewed by Slaytor, 2000), and thus strengthened hypotheses that these enzymes release glucose molecules for midgut absorption.

Glucose synthesis from non-carbohydrate precursors, gluconeogenesis, is important during starvation (Candy, 1985; Lehninger et al, 2000). Amino acids are the main precursors for gluconeogenesis; other precursors are pyruvate, lactate, and glycerol (Candy, 1985). Wigglesworth (1942) first demonstrated the conversion of amino acids to carbohydrates in the

mosquito, *Aedes aegypti*; glycogen levels increased when the mosquitoes were fed alanine or glutamine. Gluconeogenesis is essentially the reverse of glycolysis, but three steps of glycolysis are irreversible and are bypassed by alternate pathways (Candy, 1985; Lenhninger et al, 2000).

When glucose is required for energy, it is cleaved from glycogen at the terminal of each branch by the activated form of glycogen phosphorylase. Glycogen phosphorylase *b* is the stored, inactive form of the enzyme and is activated to glycogen phosphorylase *a* to release glucose units from glycogen (Friedman, 1985). The release of glucose from glycogen results in either free or phosphorylated glucose molecules (Friedman, 1985).

Glycolysis is the main energy pathway for termites and many other insects (Candy, 1985; Slaytor, 1992). Itakura (2003) confirmed that mitochondria isolated from tissues of *N. walkeri* and *C. formosanus* workers oxidize pyruvate to acetyl-CoA. Glucose is also used for lipid synthesis, amino acid synthesis, and chitin production (Candy, 1985; Friedman, 1985; Nation, 2002).

Chitin obtained in the termite diet from fungi or cannibalism (Waller and LaFage, 1987) is chemically similar to cellulose and glycogen, and it may be used by insects as a nutrient (Friedman, 1985). Chitinases have been detected in extracts of three species of mature termites (reviewed by Breznak, 1982). There may be an inverse correlation between glycogen levels and chitin production in arthropods (reviewed by Neville, 1975).

Because insects have been shown to use glycogen during starvation (Wigglesworth, 1942; Satake et al, 2000), glycogen depletion in termites may be a sign of decreased vigor. No studies of glycogen levels in termites have been published.

CELLULASES

Cellulases produced by termites and their microbial symbionts allow the insects to survive on a diet of wood. Cellulose molecules in wood are about 10,000 glucose units long, arranged in microfibrils. Cellulose chains in the core of microfibrils run parallel to each other in a densely packed, crystalline arrangement. Cellulose at the periphery of a microfibril is randomly arranged; this is referred to as amorphous (noncrystalline) cellulose. Cellulose in wood microfibrils is thought to be about 33% amorphous. Crystalline cellulose chains are anhydrous, held together by hydrogen bonds between the hydroxyl groups of adjacent molecules. The hydroxyl groups of amorphous cellulose are not hydrogen bonded to other cellulose molecules, and react with water (reviewed by O'Sullivan, 1997 and Muller et al, 2000).

There are three different classes of cellulases, each of which work together to yield glucose molecules from cellulose (Nation, 2002). Endoglucanases randomly digest beta-linkages between glucose molecules within a cellulose chain; exoglucanases attack cellulose chains from the nonreducing ends; and beta-glucosidases break down cellobioses or other oligosaccharide fragments of cellulose chains (Nation, 2002). Both higher and lower termites produce endoglucanases and beta-glucosidases (reviewed by Slaytor, 2000). Exoglucanases are generally regarded as only being produced by fungi, protozoans, and some bacteria (Nation, 2002). However, an exoglucanase was reported in one study to be a minor component of the cellulases produced by *Coptotermes lacteus* (Froggatt) termites (Hogan et al, 1988). Protozoan symbionts in hindguts of lower termites produce each of the three cellulase classes (Nation, 2002). Digestion of cellulose by bacterial symbionts of termites has not been demonstrated, with one possible exception (Reviewed by Bignell, 2000 and Slaytor, 2000).

Although it is widely held that all three cellulase classes work together to convert native cellulose to glucose, endoglucanases and beta-glucosidases might be sufficient to free small amounts of glucose (Martin, 1991; Slaytor, 1992 and 2000). Slaytor (1992) maintained that the "textbook" theory of digestion by termites—that either symbiotic microbes, or cellulases acquired by eating fungi, are ultimately responsible for the breakdown of cellulose—is an ingrained concept that is not supported by current evidence. He suggests that because many cellulolytic bacteria do not produce exoglucanases, termites may not need them either. Martin (1991) hypothesized that the mechanical action of termites chewing wood could disrupt native cellulose crystalline structure prior to enzymatic attack, allowing for limited cellulose digestion without exoglucanase. In either case, the efficiency of the digestion of native cellulose is regarded as much less than if an exoglucanase was present as a component of termite cellulases.

Endoglucanases are active against amorphous cellulose, converting it to cellobiose, which in turn is broken down to glucose by beta-glucosidases. Cellulose molecules at the periphery of microfibrils, being amorphous, are therefore broken down by the termite's own cellulases. Endoglucosidases also have a small degree of exoglucosidase function (Slaytor, 2000). However, the release of glucose from crystalline cellulose by termite cellulases is an inefficient process (Slaytor, 2000).

Endoglucanases and beta-glucosidases have been purified from tissue of the termites *Reticulitermes speratus* (Colbe)*, Nasutitermes takasagoensis* (Shiraki)*, C. formosanus,* and *Neotermes koshunensis* (Shiraki) (Watanabe et al, 1997; Tokuda et al, 1997; Tokuda et al, 2002; Nakashima et al, 2002). Endoglucanases, one of the cellulase classes produced by termites, are active against carboxymethyl cellulose (Watanabe et al, 1997). Carboxymethyl cellulose is a

water-soluble, amorphous derivative of cellulose. Native *N. walkeri* glucosidases are 2,600-fold more active against carboxymethyl cellulose than against crystalline cellulose (Slaytor, 2000).

BODY WATER

Water is essential to the structure and proper functioning of all organisms (Hadley, 1994). It provides insects with a hydrostatic skeleton, and regulates internal temperature. It is the medium for transport of metabolites, nutrients, hormones, respiratory gases, and excretory products. It is a general solvent that dissociates salts into ions, and interacts with most biological molecules (Reviewed by Hadley, 1994). Insects gain water from drinking, food, water vapor absorption, and metabolic water (Hadley, 1994), and lose water through the cuticle, spiracles, mouth, and anus (Edney, 1977; Hadley, 1994). Factors influencing water loss in termites and other insects include temperature and warmer seasons, relative humidity level, air currents, molting, egg production, nutritional state, abrasion of the waterproofing wax layer of the cuticle, and unknown effects of old age (Becker, 1969; Edney, 1977; Hadley, 1994). A termite population with members measuring low in body water could be regarded as unhealthy from dehydration or some other factor affecting metabolism from reduced water content.

Water homeostasis requires the maintenance of proper osmolarity within a lower and higher limit (Hadley, 1994; Nation, 2002). To do this, an insect must take in enough water to offset the amount lost through respiration, transpiration, or excretion. Osmotic balance of body water is essential. If cells could not regulate water uptake, they would swell and rupture. If insects lose too much water, soluble metabolites precipitate, causing irreversible harm (Hadley, 1994).

Water vapor can be taken up passively or actively (Hadley, 1994). Terrestrial arthropods absorb water vapor through the rectum, and sometimes orally (Reviewed by Edney, 1977 and

Hadley, 1994). The relative humidity at which absorption of vapor and dessication are balanced is called the critical equilibrium humidity; below this level, body mass decreases from water loss (Hadley, 1994). This process is not universal in land arthropods, as some species still lose water at saturated humidity levels (Hadley, 1994). The critical equilibrium humidity differs between insect species, ranging from about 43% to near saturated humidity (Reviewed by Edney, 1977 and Hadley, 1994).

Metabolic water is an important source of water for many insects, including termites (Lee and Wood, 1971; Nation, 2002). Metabolic water is produced by insects from oxidation of carbohydrates, lipids, and proteins from food or storage forms (Hadley, 1994; Nation, 2002). Fats are a major source of metabolic water (Nation, 2002). One molecule of palmatic acid, with 16 carbons, produces 108 water molecules when oxidized (Nation, 2002). In contrast, the metabolism of one glucose molecule nets four water molecules. About 1.07 ml of water is produced per gram of metabolized fat (Hadley, 1994). This is about twice the amount of metabolic water produced by weight from the metabolism of carbohydrates, and about two and one-half times the rate produced from the oxidation of protein to urea (Hadley, 1994). However, fats require about two and one-half times more oxygen for metabolism than glucose (Hadley, 1994). Also, water is required for the synthesis of fats (Hadley, 1994).

Arthropods lose water by diffusion through the cuticle, even if the integument is heavily sclerotized (Hadley, 1994). The exact sites where water is lost through the cuticle is unknown; it may be general, or at fixed points of the cuticle (Hadley, 1994). The surface of the cuticle is very large in comparison with the water reserves of insects: the smaller the insect, the larger the cuticle surface area in relation to water volume (Cloudsley-Thompson, 1988; Nation, 2002). Measurement of water loss through cuticle must take into account the size of an arthropod, in

addition to the thickness of the integument (Edney, 1977). Cuticular lipids of the epicuticle provide insects with a barrier against water loss (Chapman, 1998). Cuticular transpiration increases when lipids in the epicuticle are disrupted from temperature, chemicals, adsorption by dusts, and mechanical disruption (Hadley, 1994). Age is a factor in water loss through the cuticle (Hadley, 1994). It is unknown why this happens, but increased abrasion of the wax layer of the epicuticle over time is a possible reason (Hadley, 1994).

The spiracles are a major site of water loss in terrestrial arthropods (Hadley, 1994). Most insects have spiracular valves that open and close, reducing transpiration (Hadley, 1994). The valves open in the presence of elevated CO_2 in the trachea, as well as by the action of motoneurons from the central nervous system (Reviewed by Hadley, 1994). Metabolism increases with higher temperatures and activity levels, thus requiring spiraclular valves to stay open longer to exchange respiratory gases. Subsequently, more water is transpired through the spiracles (Chapman, 1998). Specific amounts of water lost through spiracles are unknown for most insect species, due to the difficulty in measuring it (Hadley, 1994).

Some water from insect urine is conserved by reabsorption through the ileum and rectum before excretion (Collins, 1969; Nation, 2002). In termites, reabsorption of water from feces occurs only in species that live in dry wood (Lee and Wood, 1971).

Land arthropods are vulnerable to dessication, and usually stay in moist environments (Cloudsley-Thompson, 1988). Arthropods can avoid the dessicating environment on the surface by burrowing just a few centimeters into the soil (Hadley, 1994). Humidity in burrows comes from the surrounding soil or the burrow's inhabitants (Hadley, 1994). Subterranean termites can only survive in high humidity due to their soft, thin cuticle, which requires them to build and live in shelter tubes (Lee and Wood, 1971; Traniello and Leuthold, 2000).

Hadley (1994) described the simplest method of measuring total body water of an insect as weighing the insect live, and then re-weighing it after drying at around 60°C. The difference between live and dry weight is divided by the live weight to give the percent water content of the insect. Vacuum drying is another method for determining water content of arthropods (Hadley, 1994). Most studies of the dessication tolerance of live insects measure water loss at 5% or less relative humidity (Hadley, 1994). Sponsler and Appel (1990) reported lower survivable limits of dehydration in different *C. formosanus* and *R. flavipes* castes. *R. flavipes* workers placed in 0% relative humidity died when they lost 50% of their body water after about 5 hours. *R. flavipes* lose water faster than other *Reticulitermes* species (Collins, 1969).

An emphasis on body water conservation overlooks the fact that an excess of water in an insect's environment is also a possibility. Insects faced with this problem must get rid of excess water from their bodies (Edney, 1977). For example, high humidity has been described as harmful to some termites in captivity (Collins, 1969). A drywood termite, *Cryptotermes brevis* (Walker), when placed in 86% relative humidity swells up and dies due to its inability to transpire metabolically produced water to humid air (Collins, 1969). This particular termite species lives in wood, where it is insulated from the humidity of the atmosphere. Three North American termite species that best survive in arid environments cannot tolerate high humidity (Collins, 1969).

TRAIL-FOLLOWING CHEMICALS

Termites produce several chemicals in their sternal gland that trigger trail-following behavior in other termites (reviewed by Trainello and Leuthold, 2000). Dodecatrienol purified from *Reticulitermes flavipes* has been identified as a trail-following chemical (Matsumura et al, 1968), as has n-hexanoic acid from *Zootermopsis nevadensis* (Hagen) (Karlson et al, 1968).

Some termite species show a trail-following response to lines drawn by ballpoint pens with ink containing 2-phenoxyethanol (Moore, 1969; Chen et al, 1998). Dodecatrienol and 2-phenoxyethanol are both primary alcohols, but are otherwise dissimilar (Chen et al, 1998). Some fungal extracts are known to stimulate trail-following behavior in termites. Extracts of a species of brown-rot fungi, *Gloeophyllum trabeum* (Persoon), contain a molecule very similar to dodecatrienol (Rust et al, 1996). *Serpula lacrymans* (Wulf) is a brown-rot fungi that has been shown to evoke trail-following behavior in *C. formosanus* termites, although the attractant from this fungi is unknown (Cornelius et al, 2002).

Knowledge of chemicals that are attractive to termites could be useful in pest control programs, either by directing termites toward bait or increasing the attractiveness of bait for consumption (Chen et al, 1998). Trail-following chemicals secreted by the termite, or chemicals that elicit a trail-following response such as 2-phenoxyethanol, degrade quickly (Chen et al, 1998). Stable analogs of trail pheromones or 2-phenoxyethanol, or the development of chemicals that would protect trail-following substances from decomposition, may improve the performance of termite baits (Chen et al, 1998).

CONCLUSION

There are no published methods for predicting the vigor of wild or laboratory-cultured termite populations. If predictors of vigor could be established, it would allow for the selection of healthy groups of insects for bioassay. Currently, termites tested in bioassay typically are selected from laboratory populations kept under standard conditions, but no methods are known to demonstrate that such termites are healthy before placement into experimental arenas.

The main objective of this study was to test novel methods for describing the vigor of *R. flavipes* workers predictably. Chapter 2 discusses whether levels of biological molecules,

running speed, consumption rate, survivorship percentage, and live weight are useful in describing and predicting the vigor of termites in captivity. Chapter 3 reports levels of stored biological molecules for *R. flavipes* workers just after field collection, and considers whether field populations are vigorous or weak by applying novel measures of vigor from Chapter 2. Chapter 4 summarizes findings for how the vigor of termite populations could be described and predicted by novel means.

CHAPTER 2

NOVEL AND TRADITIONAL MEASURES OF VIGOR APPLIED TO LABORATORY TERMITES, *RETICULITERMES FLAVIPES* (KOLLAR)[1]

[1]Arquette, T.A. and B.T. Forschler. 2005. To be submitted to *Journal of Insect Physiology*.

ABSTRACT

The current study was undertaken to consider predictive methods for describing the vigor of *Reticulitermes flavipes* (Kollar) termites stored in a laboratory under conditions similar to control groups in bioassay. These novel methods were based on measurements for levels of biomolecules (uric acid, soluble proteins, lipid, and glycogen), percent water content, live weight, and running speed. Also considered were two established, non-predictive methods for determining vigor, survivorship and consumption rate. Low body water percentage was concluded to be an indicator of weak groups of termites.

INDEX WORDS: vigor, consumption rate, survivorship, uric acid, soluble proteins, lipid, glycogen, body water.

INTRODUCTION

Field populations of termites vary in vigor (Lenz, 1985). Although keeping termites in artificial culture allows for comparisons of experimental results between different laboratories (Lenz and Williams, 1980), acclimation under standard conditions eliminates only some of the differences in vigor among field populations (Lenz, 1985). Termites used in laboratory bioassay must be healthy (Su and LaFage, 1984b), but no methods exist for identifying healthy groups before placement in bioassay. Non-predictive methods monitoring food consumption rate and percent survivorship have been used for determining whether termites in bioassay have become too weak for the experiment to continue (Su and LaFage, 1984a and 1984b). An ability to predict termite health might be possible from identification of abnormal levels of metabolic molecules stored by termites, such as lipid or glycogen; metabolic wastes such as uric acid; structural components such as proteins; and metabolic water reserves. Simply timing the movement of termites across a pre-determined distance might be a means of describing vigor. If the health of termite populations could be predicted, not only would researchers have an ability to select groups with a better potential for remaining viable in bioassay, but pest control operators would also benefit by having a tool for identifying the most vigorous populations around structures to target for pesticide treatment.

No prior research has considered predictive indicators of termite vigor. Basic studies quantifying metabolic molecules, water content, and speed of movement did not discuss an association with the vigor of the termites in testing. Studies determined the survivable limit of dehydration (Sponsler and Appel, 1990); changes in protein, lipid, and uric acid levels in starved, defaunated, and normally faunated workers (Carter et al, 1972a; Mauldin, 1977; Potrikus and Breznak, 1980a; Lovelock et al, 1985); and faster speed of movement along pheromone trails

leading to food (Reinhard and Kaib, 2001). Additionally, protein levels have been reported to fall in starved termites with an accompanying increase in stored uric acid (Slaytor and Chappell, 1994), and an inverse correlation between body water and lipid levels has been reported (Shelton and Appel, 2001). A discussion of vigor is relevant to each of these studies. For instance, before reaching a critical point for survival from water loss, termites could be less vigorous from dehydration. Low levels of stored food reserves or a high accumulation of uric acid could show that a population has weakened from starvation. The only distinguishing attribute of termites that has been described in terms of population vigor is live weight, with heavier termites thought to be characteristic of older, declining populations (Su and LaFage, 1984a; Grace et al, 1995). If stored biomolecules, water content, weight, and running speed of termites could be separated between "healthy" or "unhealthy" levels, each could potentially provide a predictive identifier of termite population health. The current study tested novel, predictive measures of termite population vigor.

MATERIALS AND METHODS

Logs infested with *Reticulitermes flavipes* (Kollar) workers were collected in July 2003 from two sites at least 1 km apart at Whitehall Forest, Clarke County, Georgia, designated as Population 1 and Population 2 (P1 and P2). Termites were also collected from an inspection port (P3) located next to the south wall of the University of Georgia Chapel in Athens, Clarke County, Georgia. The inspection port consisted of a buried 17 cm length of 10 cm-diameter polyvinyl chloride (PVC) pipe covered with a plastic lid, with tightly rolled corrugated cardboard placed inside. Logs and cardboard rolls were returned to the laboratory at the University of Georgia, Athens, where termites were removed and processed as described in Forschler and Townsend (1996).

Experimental units consisted of Quikrete® sand (10 g) added to a plastic petri dish (20 mm by 100 mm diameter) and moistened with 2 ml of distilled water. A section of Tygon® tubing (19 mm by 10 mm diameter) was packed with 1 g of finely powdered α-cellulose (Whitmire Micro-Gen, San Antonio TX) and placed in the petri dishes as a food source. Groups of 250 termites were separated into weighing boats and transferred into each experimental unit. For P1, 12 petri dishes were prepared; for P2 and P3, 15 dishes were set up due to a surplus of termites. Experimental units for the P2 population contained first and second instar larvae comprising approximately 5% of the total. Nymphs numbered 6% of the total for P1, and 2% of the P3 group. Experimental units of each population contained 1% or fewer soldiers. Only workers above third instar were used for testing.

Experimental units were kept, by population, inside three plastic boxes (27 cm x 19 cm x 9.5 cm) containing wet paper towels. Boxes were covered with a lid and sealed with Parafilm®, followed by placement in a dark incubator maintained at 25° C with saturated humidity. Petri dishes were checked at least weekly for maintaining moisture and food remaining ad libitum. Every 2 weeks for 24 weeks or until all termites from a population died, one petri dish was randomly removed from each population and destructively sampled. Each of the three petri dishes measured at a particular time interval was considered one replicate, while termites processed and frozen on the day of collection from the field served as control groups, i.e. insects that were not subjected to the artificial conditions of laboratory culture. At each biweekly test period, surplus termites from each petri dish were weighed and frozen at -70° C as backup samples.

At biweekly intervals, three groups of ten workers above third instar were pooled from each population and weighed together for estimation of total lipid, uric acid, soluble proteins,

glycogen, and body water percentage for one termite equivalent, requiring at least 120 live workers per experimental unit. In cases of low survivorship, either three or five workers were pooled for testing. For the uric acid bioassay and determination of body water percentage, five workers were pooled at week 24 for P1, week 24 for P2, and week 14 for P3. For determination of soluble protein content, five workers were pooled at weeks 22 and 24 for P1, and weeks 18, 20, and 22 for P2; three workers were pooled at week 24 for P2 and week 14 for P3. For lipid determination, five workers were pooled at week 24 for P1, weeks 22 and 24 for P2, and week 14 for P3. For determination of glycogen levels, five workers were pooled at weeks 22 and 24 for P1, and weeks 18, 20, and 22 for P2; three workers were pooled at week 14 for P3.

Results of biochemical assays are reported in units of µg biological molecule/mg termite, except for body water that is expressed as a percentage of the live weight. For each spectrophotometric test (uric acid, soluble proteins, and glycogen) an equation was devised that determined the content for each molecule in one termite equivalent. Other measures determined the mean time for 30 termites to move a distance of 6 cm, as well as food consumption rate and percent survivorship, two established techniques for monitoring the vigor of control insects in laboratory bioassay. Insects tested for uric acid and body water levels were oven-dried and reweighed before freezing at -70° C. All other insects were frozen immediately following determination of live weight, timed movement, and number of surviving insects.

Assay procedures

Percent survivorship and consumption rate. Percent survivorship was determined at each biweekly test period by dividing the number of surviving workers in each petri dish by 250. Remaining cellulose was weighed after drying at 85°C for 24 hours in a convection oven

(VWR). Consumption rate in units of mg food eaten/g termite/day was then determined (Su and LaFage, 1984b.

Estimation of live weight. The mean weight of one termite was estimated from six groups of 10 termites, ± the standard deviation, using pooled weights of termites destined for the uric acid and lipid assays. In cases of low survivorship, when less than 10 termites were pooled for either the uric acid or lipid assays, all pooled weights available were used to estimate the weight of one termite.

Uric Acid. Uric acid content was measured following the procedure of Potrikus and Breznak (1980a) using a diagnostic kit (Sigma 292). Termites were dried in a convection oven (VWR) at 85°C for 8 hours, and then held at room temperature in a desiccation chamber containing Drierite® crystals for 5 minutes before weighing. The dried termites were placed in a 1.5 ml centrifuge tube (Eppendorf) and ground into a powder with a plastic pestle, followed by addition of 1.5 ml lithium carbonate solution (0.6% w/v) (Sigma). Known amounts of uric acid (Sigma) were added to 1.5 ml lithium carbonate as a standard. Standards and unknown samples were suspended in a water bath at 60°C for 10 minutes, followed by centrifugation for 15 minutes at 3000x g. Supernatant (either 40 µl, or 400 µl for very dilute samples) was added to 1.5 ml centrifuge tubes containing 0.2 ml glycine buffer solution (0.7 M, pH 9.4) (Sigma 292) and 1.2 ml nanopure water, and pulsed for 10 seconds on an Eppendorf® microcentrifuge. Supernatant (0.6 ml) was added to 1.5 ml centrifuge tubes labeled "test" and "blank," followed by 10 µl of uricase (Sigma 292) added to the "test" vials, and 10 µl Nanopure® water to the "blank" vials. Tubes were vortexed and left at room temperature for 30 minutes to allow for complete digestion of uric acid in the tubes containing uricase. Both "test" and "blank" samples were read simultaneously at 292 nm (Spectronic® Genesys model 5, Spectronic Instruments).

The difference between "test" and "blank" absorbance values was used to determine uric acid concentration. The absorbance value for an unknown sample was defined as the fraction of termite extract in supernatant at the time the absorbance was measured. An initial 10 termite extract was diluted to an equivalent of 0.093 termite extract for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 5 µg of uric acid in solution, then the same absorbance reading for an unknown sample would mean there was 5 µl of uric acid for a 0.093 fraction of one termite. The result from expanding this fraction to give the amount of uric acid for one termite equivalent is 5.0 µg uric acid/0.093 termite equivalent = 53.8 µg uric acid/one termite equivalent.

Soluble Proteins. The Bradford method was used to determine levels of soluble proteins (Bio-Rad Laboratories) with a standard of bovine serum albumin. Termites were added to 1.5 ml centrifuge tubes (Eppendorf) containing 1 ml of distilled water, and then sonicated on ice (Branson sonifier model 250, VWR). After centrifuging at 14,000 rpm for 5 minutes, 0.8 ml of supernatant was added to empty centrifuge tubes followed by 0.2 ml of reagent. Sample supernatant, standards, and distilled water for a blank were added 175 µl per microplate well (Becton Dickinson) and protein concentration determined at 595 nm (Spectra Max 340 microspectrophotometer, Molecular Devices Corp., Sunnyvale, CA).

Following the above procedure, an initial 10 termite extract was diluted to an equivalent of 0.01 termite for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 1 µg of soluble protein in solution, then the same absorbance reading for an unknown sample would mean there was 1 µg of soluble protein for a 0.01 fraction of one termite. The result from expanding this fraction to give the amount of soluble protein for one termite

equivalent is 1.0 µg soluble protein/0.01 termite equivalent = 100 µg soluble protein/one termite equivalent.

Glycogen. Glycogen content of whole termites was determined based on a procedure by Van Handel (1965). Pooled groups of live termites were placed in 1.5 ml microcentrifuge tubes (Eppendorf) containing 0.4 ml of sodium sulfate solution (2% w/v) (J. T. Baker) and sonicated on ice (Branson sonifier model 250, VWR). To each homogenized termite sample was added 1 ml of 100% ethanol (J. T. Baker). Tubes were vortexed and samples frozen at -70° C for at least 24 hours to break cells and release glycogen. As termite samples thawed glycogen standards (Sigma) were prepared. Termite samples and glycogen standards were heated 10 minutes in a water bath at 60° C, and then centrifuged at 4000 rpm for 5 minutes. Supernatant was then poured off and discarded, with traces of liquid removed from around the remaining pellet with a pipetter. To each tube containing a homogenized termite pellet was added 750 µl of amyloglucosidase/sodium acetate solution (stock solution: 3.2 mg amyloglucosidase w/v (Sigma) mixed with a 5 ml of sodium acetate solution (0.2 M, pH 5.2) (Fisher Scientific)). To standard pellets, and 50 µl distilled water to be used as a blank for the spectrophotometer reading, was added 50 µl of the amyloglucosidase/sodium acetate solution. Microcentrifuge tubes were taped to rotators in a mini hybridization oven (Bellco Glass, Inc.) and spun at 55° C for 2 hours at medium speed. Following centrifugation for 5 minutes at 12,000 rpm, between 25 to 200 µl solution containing termite sample (depending on glycogen concentration) and 50 µl of blank and standard solution were transferred to empty microcentrifuge tubes. Following the addition of 0.5 ml glucose trinder solution (Sigma), tubes were vortexed and allowed to stand for 18 minutes at room temperature. Supernatant was transferred to microplate wells (0.15 ml per well) (Becton Dickinson) and absorbances determined at 505 nm (SpectraMax model 340).

Following the above procedure, if 50 µl of unknown sample was set aside for mixture with the color-developing glucose trinder solution, an initial 10 termite extract was diluted to an equivalent of 0.182 termite extract for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 5 µg of glycogen in solution, then the same absorbance reading for an unknown sample would mean there was 5 µg of glycogen for a 0.182 fraction of one termite. The result from expanding this fraction to give the amount of glycogen for one termite equivalent is 5.0 µg glycogen/0.182 termite equivalent = 27.5 µg glycogen/one termite equivalent.

Lipid content. Total lipids were extracted based on the procedure of Zera and Larsen (2001). Pooled groups of live termites were weighed on a 0.01 mg scale (model AB104, Mettler Toledo, Columbus OH), and placed in 1.5 ml microcentrifuge tubes containing 0.66 ml chloroform (J. T. Baker) with 0.05% butylated hydroxytoluene (BHT) (Sigma) w/v added. This was followed by addition of 0.33 ml methanol (J. T. Baker) containing 0.05% BHT (w/v). Termites were sonicated on ice (Branson sonifier model 250, VWR) and centrifuged 5 minutes at 14,000 rpm. All supernatant was transferred into empty 1.5 ml centrifuge tubes with a pipette, and the pellet discarded. Samples were vortexed after adding 0.34 ml aqueous KCL (Sigma) (0.88% w/v) to the supernatant, resulting in two liquid layers. Non-lipid contaminants, isolated in the upper hydrophilic layer, were suctioned off with an aspirator. Dissolved lipids remained in the lower chloroform layer. The chloroform with lipid was poured onto a pre-weighed aluminum foil bowl and evaporated overnight. Lipid content was determined from the difference in weight between the foil bowl with lipid residue and the initial weight of the bowl.

Water content. Insects dried for the uric acid assay were those used to determine water content. After weighing, groups of ten live termites were dried at 85°C for 8 hours in a

convection oven (VWR). Dried termites were held at room temperature in a desiccation chamber containing Drierite® crystals for 5 minutes before reweighing. Percent body water was determined by obtaining the difference between live and dry weights, divided by the live weight.

Running speed. A straight line was drawn on a sheet of 8.5" x 11" typing paper with a 1 cm diameter circle drawn at one end of the line. A perpendicular mark was drawn on the straight line 6 cm from the circle, and the sheet photocopied numerous times. The photocopied lines and circles were traced over with a red Papermate® pen. Termites are attracted to a component of Papermate® pen ink, 2-hydroxyphenol (Chen et al, 1998). Immediately after tracing photocopied lines one termite was gently tapped from the petri dish into a weighing boat, and then tapped from the weighing boat to inside of the ink circle. As soon as the termite started moving away from the circle along the straight line, a hand-held stopwatch was used to record the time to move 6 cm. Times were recorded only when termites moved 6 cm without stopping or straying from the straight line. After each test, the termite was tapped off the sheet of paper into a weighing boat resting on wet paper towels inside a plastic box (27 cm x 19 cm x 9.5 cm), and the box lid replaced. A new photocopied sheet was traced with ink for each termite tested. Running speeds of 30 termites were recorded per replicate group.

Statistical analysis. Regression analysis ($p \leq 0.05$) (Microsoft Excel®) was used to correlate changes in measures of vigor and increasing time in laboratory captivity. The Student's paired t-test determined significant differences between measurements taken either within or across populations. Consumption rate was calculated using a formula devised by Su and LaFage (1984b) in units of mg cellulose eaten/g termite/day.

Su and LaFage (1984a) concluded that when small groups of *Coptotermes formosanus* workers in captivity reach 80% survivorship, the insects should be regarded as unhealthy. This

rule was applied to the current study, with groups considered healthy if survivorship of an experimental unit was above 80%, and unhealthy below this point. Small groups of termites in captivity are assumed to die off at a linear rate (reviewed by Su and LaFage, 1984b), supported in the current study from significant negative correlations between survivorship and time for each population (Figure 2.2, $p \leq 0.05$). Linear survivorship curves were used to approximate the point when 80% survivorship was reached for each population, determined as 8 weeks for P1 and P2, and 6 weeks for P3 (Figure 2.2). Means for each novel measure of vigor were determined both above and below 80% survivorship, with significant differences between them determined using one-way ANOVA ($p \leq 0.05$, Table 2.10) and Tukey's HSD ($p \leq 0.0033$, Tables 2.11-2.13).

RESULTS

Populations collected from Whitehall forest (P1 and P2) had workers survive through the conclusion of the 24-week study (Table 2.2). Termites in all but one P3 experimental unit died between 12 and 14 weeks; the last petri dish with live workers had 20 percent of the original number at 14 weeks (Table 2.2). Overall consistent trends were not found for any novel measure of vigor between the three populations examined in the study (Tables 2.3a–2.9a). This highlights the variability inherent in subterranean termite vigor as it pertains to selecting laboratory test subjects. However, there were several findings worth noting including dissimilar changes in uric acid content between populations, decreasing water percentage, and up to five-fold increases in lipid content over time in captivity. This section will first discuss traditionally used, non-predictive measures of vigor, followed by a discussion of each potentially predictive measure of vigor in turn.

Consumption Rate

P1 consumption rates ranged between 19.3 to 25.3 mg cellulose eaten/g termite/day through 24 weeks, while P2 measured 16.7 to 35.2 mg/g/day through 24 weeks, and P3 21.5 to 33.6 mg/g/day through 14 weeks (Table 2.1a). Compared to initial consumption rates measured 2 weeks after field collection, each population tended to consume less cellulose later in captivity (Table 2.1a). Beyond 8 weeks cellulose consumption declined 16 $\pm$ 4% for P1, 34 $\pm$ 8% for P2, and 30 $\pm$ 7% for P3 (Table 2.1b). The mean of all consumption rates recorded below 80% survivorship was significantly lower than the rates above 80% survivorship (ANOVA, $p < 0.0001$) (Table 2.1b). This agreed with a past description of consumption rate decreasing in unhealthy termite groups in captivity (Su and LaFage, 1984b). P1 consumption rate was lower than P2 and P3 at each biweekly test period except for weeks 8 and 14 (Table 2.1a); however P1 ate at a consistent rate compared to P2 or P3 (Figure 2.1). This was illustrated by a significant negative correlation between consumption rate and time in captivity for P2 ($R^2 = 0.403$) and P3 ($R^2 = 0.856$), but not P1 ($R^2 = 0.282$) (Figure 2.1).

Survivorship

Survivorship decreased steadily in captivity for P1, decreased slowly at first for P2 followed by a sharper decline than P1, and wavered for P3 between 65 to 95% per experimental unit until most insects died between 12 to 14 weeks (Figure 2.2). Although 80% survivorship was estimated to have been reached at 8 weeks for P1 and P2, and 6 weeks for P3, each population had at least one experimental unit below 80% survivorship earlier than indicated by survivorship curves (Figure 2.2). For instance, P2 and P3 survivorship at 2 weeks was 74% and 79%, respectively (Table 2.2). A significant negative correlation was shown for each population

between survivorship and time in the laboratory (P1, $R^2 = 0.922$, $p < 0.0001$; P2, $R^2 = 0.822$, $p < 0.0001$; P3, $R^2 = 0.527$, $p = 0.042$) (Figure 2.2).

Live Weight

P1 worker live weights were generally steady in captivity, while P2 and P3 weights tended to increase (Table 3.3b). Exceptions for P1 occurred at week 18, with 17% heavier weights compared to the day of collection; and at weeks 20 to 24, when weights were similar to week 0 (Table 2.3b). In contrast, P2 workers at weeks 20 to 24 were 10 to 20% heavier compared to the day of collection (Table 2.3b). P3 workers increased in weight faster in captivity than P1 or P2, with 18% heavier weights measured at 6 weeks compared to 6% for P1 and 7% for P2 (Table 2.3b), and 25% heavier weights at week 14 (Table 2.3b). Workers of all populations combined were significantly heavier below 80% survivorship compared to above (ANOVA, $p = 0.01$, Table 2.10). P1 did not show a positive correlation between weight change and time in the laboratory ($R^2 = 0.0001$, $p = 0.972$) as did P2 ($R^2 = 0.712$, $p = 0.0003$) and P3 ($R^2 = 0.837$, $p = 0.001$) (Figure 2.3).

Uric Acid

Mean uric acid content differed significantly between each population on the day of collection. P1 was significantly higher than P2 at week 0 (paired t-test, $p = 0.032$), while P3 was significantly higher than both P1 ($p = 0.0006$) and P2 ($p < 0.0001$) (Table 2.4a). At week 0, P3 uric acid content was four-fold higher than P1 and sixteen-fold higher than P2 (Table 2.4a).

Through 14 weeks, uric acid was generally low in P1 and P2. P3 workers were measured at 40 ± 2 µg uric acid/mg termite just after field collection, but declined as much as two-fold in uric acid content at week 10 (Table 2.4a). P1 levels tended to be low throughout the study, with an exception at 24 weeks with 600% higher levels than at week 0 (Table 2.4b). However, at

week 22 the P1 workers measured significantly lower for uric acid compared to week 0 (paired t-test, $p = 0.0013$) (Table 2.4a). From weeks 16 to 24, P2 uric acid content ranged from 44.5 to 108.8 µg uric acid/mg termite (Table 2.4a), or 1,500 to 4,200% higher than at week 0 (Table 2.4b).

P1 did not show a correlation between uric acid content and time in the laboratory ($R^2 = 0.191$, $p = 0.318$), P2 showed a significant positive correlation ($R^2 = 0.609$, $p = 0.001$), and P3 showed a weak negative correlation ($R^2 = 0.392$, $p = 0.01$) (Figure 2.4).

Soluble Proteins

P1 and P2 soluble protein levels remained within a narrow range of 45 to 60 µg protein/mg termite throughout the study (Table 2.5a). An exception occurred for P1 at week 18 when soluble protein content measured 78.6 µg protein/mg termite, significantly higher compared to the next highest reading recorded for the study (paired t test, $p<0.0001$) (Table 2.5a). For P2 exceptions occurred at weeks 18, 22, and 24 when readings were 17 to 38% lower compared to week 0 (Table 2.5b). Only three measurements were taken for P3 over 14 weeks ranging from 33 ± 3 to 54 ± 5 µg protein/mg termite (Table 2.5a). There was not a significant correlation between soluble protein levels and time in the laboratory for P1 ($R^2 = 0.104$) or P2 ($R^2 = 0.128$), nor was there a significant difference between P1 and P2 means above and below 80% survivorship (ANOVA, $p = 0.95$, Table 2.10). As no correlation was shown for change in soluble protein content over time, regression figures are not provided.

Glycogen

Glycogen levels increased 100 to 200% for all groups through 2 weeks in captivity (Table 2.6b). Workers of each population fluctuated within a narrow range for glycogen content throughout the study. Measurements of 5.4 ± 2.0 µg glycogen/mg termite were recorded for P1

between weeks 0 to 24, while P2 measured 5.5 $\pm$ 3.3 µg/mg between weeks 0 to 24, and P3 measured 8.5 $\pm$ 2.7 µg/mg between weeks 0 to 14 (Table 2.6a). Means of levels above and below 80% survivorship did not differ significantly (ANOVA, $p = 0.17$, Table 2.10). There was not a correlation between change in glycogen levels and time in captivity (P1, $R^2 = 0.043$; P2, $R^2 = 0.012$; P3, $R^2 = 0.052$). As no correlation was shown for change in glycogen content over time, regression figures are not provided.

Lipid

Lipid content increased two to three-fold for each population between the day of collection and 2 weeks in captivity (Table 2.7a). By 2 weeks, P1 and P2 lipid content increased 180%, and P3 140% (Table 2.7b). Each of these increases was significant (paired t-test, $p < 0.0001$) (Table 2.7a). Increase in lipid levels occurred in the P3 population despite two-fold higher readings at week 0 compared to P1 and P2 (Table 2.7a). Subsequently, P3 workers tended to have elevated lipid content compared to workers of the other two populations through 14 weeks in the laboratory (Table 2.7a). Means of all lipid levels measured below 80% survivorship were significantly higher compared to above (ANOVA, $p = 0.04$, Table 2.10).

Each population showed elevated lipid levels in captivity two to five-fold above week 0 readings (Table 2.7a). P1 lipid content, after an initial increase through 2 weeks, was generally steady through 14 weeks before increasing again, plateauing between 16 and 24 weeks (Table 2.7a). Toward the end of the study P1 lipid content was as much as 460% higher than at week 0 (Table 2.7b). Readings for P1 and P2 lipid content were similar through week 8, and throughout the study P2 levels fluctuated two to four-fold above the week 0 reading (Table 2.7a). P3 lipid content tended to be steady in captivity except for an increase to 167 $\pm$ 10 µg lipid/mg termite at

the last measurement at 14 weeks, the highest reading recorded for any group in the study (Table 2.7a).

P1 showed a significant positive correlation in lipid levels through 24 weeks ($R^2 = 0.805$, $p < 0.0001$), as did P3 through 14 weeks ($R^2 = 0.590$, $p = 0.027$). P2 lipid levels did not show a trend towards change in captivity ($R^2 = 0.009$, $p = 0.757$) (Figure 2.5).

Body Water

Body water just after field collection was measured at 78.6% for P1 (1 reading), 77.9% for P2, and 74.6% for P3. These were the highest readings recorded within each respective group throughout the study. Body water percentage declined significantly for each population through 6 weeks in the laboratory, with means of 72.2 $\pm$ 0.8% for P1 (paired t-test, $p = 0.0046$), 71.7 $\pm$ 1.1% for P2 ($p = 0.0096$), and 67.6 $\pm$ 0.6% for P3 ($p = 0.0032$) (Table 2.8a). Percent water declined steadily through 6 weeks regardless of differences in the week 0 readings between populations (P1, $R^2 = 0.96$, $y = -1.1x + 78.1$, $p = 0.02$; P2, $R^2 = 0.96$, $y = -1.1x + 78.0$, $p = 0.02$; P3, $R^2 = 0.96$, $y = -1.1x + 74.3$, $p = 0.02$). P1 and P3 each dropped steadily in percent water content throughout the study, with a final measurement of 66% for P1 at 24 weeks and 64% for P3 at 14 weeks (Table 2.8a). The lowest body water percentage recorded for P2 was 70%, measured at 20 weeks (Table 2.7a). P1 and P2 body water percentages were similar through 8 weeks, the point when 80% survivorship was reached for each population (Table 2.6, Figure 2.6). The mean of all body water measurements recorded below 80% survivorship were significantly lower than above 80% survivorship (ANOVA, $p = 0.01$, Table 2.10).

P1 and P3 each showed a significant negative correlation between percent body water and time in the laboratory (P1, $R^2 = 0.708$, $p = 0.0003$; P3, $R^2 = 0.915$, $p = 0.0002$), but P2 did not ($R^2 = 0.261$, $p = 0.074$) (Figure 2.6). P3 body water percentage declined at more than twice

the rate of P1 (Figure 2.6). While each group had a similar rate of decrease in percent water through 6 weeks, P3 continued declining until the last measurement at 14 weeks, while P1 readings stabilized. Subsequently, later P3 water percentages were the lowest measured in the study, and 12 to 15% lower compared to week 0 (Tables 2.8a and 2.8b).

Running Speed

P1 termites moved faster than 3.0 s/6 cm for almost half of the biweekly measurements, compared to one time each for P2 (week 8) and P3 (week 16) (Table 2.9a). Mean speeds of P2 workers were 3.9 s or slower for almost half of the measurements, while mean P1 and P3 running speed was slower than 3.9 s only one time (P1, week 22 and P3, week 0) (Table 2.9b). Through 24 weeks, one third of individual P1 termites moved 2.6 s or faster across a 6 cm distance, compared to 11% for P3 (14 weeks) and 7% for P2 (24 weeks). The fastest mean running speed of 30 termites was 2.55 $\pm$ 0.26 s, which occurred at 18 weeks for P1 (Table 2.9a). Overall there was not a significant correlation between running speed and time in captivity for any of the groups (P1, $R^2 = 0.119$, $p = 0.70$; P2, $R^2 = 0.20$, $p = 0.13$; P3, $R^2 = 0.444$, $p = 0.07$), nor was there a significant difference for all mean running speeds recorded above 80% survivorship compared to below (ANOVA, $p = 0.61$, Table 2.10). As no correlation was shown for change in running speed over time, regression figures are not provided.

DISCUSSION

This study aimed to identify ways to predict the vigor of small groups of termites using novel methods. Non-predictive methods for describing termite vigor have been described previously by Su and LaFage (1984a and 1984b) on the basis of changing survivorship and consumption rates of small groups of the insects in the laboratory. Each method monitors rather than predicts the performance of groups of termites in captivity. A predictor of termite vigor

would have to be independent of time, and valid across populations regardless of vigor level; such a measure might describe termites as healthy or unhealthy by a threshold in a particular level of a stored biological molecule.

Survivorship is assumed to decrease at a steady rate in small groups of termites in captivity (reviewed by Su and LaFage, 1984b). This was confirmed in the current study with significant R^2 values for linear decrease in survivorship of each population (Figure 2.2). Useful applications based on the assumption of steady termite mortality in the laboratory have been previously described. One such application used survivorship as the basis for a non-predictive measure of vigor. Su and LaFage (1984a) concluded that when survivorship of small groups of termites in captivity reaches 80% of the original number, the insects should be considered unhealthy. In a separate study Su and LaFage (1984b) used the assumption of consistent linear decrease in survivorship to devise an equation that closely approximates actual consumption rates of small termite groups. The consistency in which steady decrease in survivorship occurs and the fact that it has been applied as a measure of vigor allowed for its use as a point of reference against which the data collected from other measures for this study were compared. Applying Su and LaFage's (1984a) method for describing vigor from survivorship, termite groups of the current study were considered healthy above 80% survivorship, and unhealthy below 80% surviving. Data collected for novel measures of vigor for a group either above or below 80% survivorship was therefore regarded as occurring in healthy or unhealthy insects.

In order for a measure of termite vigor to work it must be consistent across populations, as is the case with steadily declining survivorship in captivity. Some measures considered for this study were excluded from further consideration when it was determined that they changed differently between populations. Among the measures excluded were soluble protein content,

glycogen content, and running speed, as changes for each did not correlate with time in captivity, as well as showing nonsignificant p-values for means of healthy and unhealthy levels from each population combined (ANOVA; soluble protein, $p = 0.95$; glycogen, $p = 0.17$; running speed, $p = 0.61$) (Table 2.10). Uric acid content, although significantly different between healthy and unhealthy levels across populations (ANOVA, $p = 0.01$, Table 2.10), had dissimilar trends toward change in captivity between populations (Figure 2.4), suggesting different physiological processes involving the molecule were occurring between the three groups. Therefore, uric acid was not considered as a potential predictor of vigor. Consumption rate has been established as a means of describing vigor in termites in captivity by placing insects with lower consumption rates into an unhealthy category. Even though this occurred across populations in the current study when using survivorship as a point of reference for termite health ($p < 0.0001$, Table 2.10), this method for describing vigor being non-predictive was not considered further. Live weight has been previously described as an indicator of health based on change over time in the laboratory (Su and LaFage, 1984a) as well as in the field (Grace et al, 1995), with heavier termites occurring in older, weaker field populations or laboratory groups. However, individual termite weights vary with the age of the field population (Grace et al, 1995). This was illustrated in the current study from the population that died early (P3) and therefore the least vigorous group but had lighter workers at week 0 than P1 or P2 (Table 2.3a). Since live weights are not similar across field populations, it could not be used to predict vigor in captivity based on a threshold of a particular weight.

Lipid content and percent body water each showed potential as predictive measures of vigor. Readings for each changed similarly across populations early in captivity, with lipid levels initially increasing and percent body water decreasing (Figure 2.5, Figure 2.6). Means for

lipid content and percent water above 80% survivorship were not significantly different between populations (ANOVA, lipid $p = 0.09$, water $p = 0.12$). Lipid content increased throughout the study for P1 ($R^2 = 0.81$, $p < 0.0001$) and P3 ($R^2 = 0.59$, $p = 0.03$), but not P2 ($R^2 = 0.009$, $p = 0.757$) (Figure 2.5). Percent body water decreased in captivity for P1 ($R^2 = 0.71$, $p < 0.0001$) and P3 ($R^2 = 0.91$, $p <0.0001$), while for P2 water percentage decrease over time was weak ($R^2 = 0.26$) and not significant ($p = 0.07$) (Figure 2.6).

Comparing water percentage and lipid content figures of P1 as well as P2, a "mirror image" is apparent (Figures 2.5, 2.6). All three populations show a correlation between body water percentage and lipid content (Figure 2.7). Such a correlation was also described in a survey of termites from 11 field populations (Chapter 3), and previously reported in a paper by Shelton and Appel (2001) for *R. flavipes* alates. The correlation suggests that one or the other molecule could be used in the same way as a measure of vigor, if found to be a reliable measure of vigor. However, each of these measures changed differently from each other early in captivity, so as measures of vigor each could not be used interchangeably. A two to three-fold increase in lipid content through 2 weeks in captivity measured across populations may have been due to the switch to an artificial diet of pure cellulose, as measurements of small termite groups fed wood in captivity did not change appreciably from week 0 readings (Mauldin, 1977). Lipid content stabilized after this initial rise across populations through weeks 8 to 12, while water percentages continued declining through weeks 6 to 12 (Figures 2.5 and 2.6). Steadily declining water percentages across populations as lipid levels stabilized indicates another factor besides change in lipid content influenced changes in percent water in termites of this study. One possible factor could be loss of body water. Measuring water percentage for termites in captivity fed different diets may demonstrate whether water loss is occurring, particularly from

reduction in water percentage in termites fed wood as such termites would be expected to maintain lipid content similar to levels from the field (Mauldin, 1977).

Considering lipid levels and water percentage as potential measures of vigor, lipid content does not in itself suggest an unhealthy condition, as it can be regarded as stored food, but decreasing water percentage could directly implicate a less healthy condition if water loss is occurring. *R. flavipes* workers die at a critical point of water loss (Shelton and Appel, 2001), so termites would be expected to weaken as the threshold for mortality from water loss is approached. Considering that the mean of P3 water percentage below 80% survivorship, 64.7%, was determined by Tukey's HSD to be significantly lower than the next lowest mean recorded in the study (70.6%, P1 below 80% survivorship, Table 2.13), similarly low body water percentages may indicate a population is near death. Even if measurement of lipid levels were better comparable with water percentages of termites, its measurement would be far less practical than for determining water percentage, and would therefore not be advantageous to perform.

In the current study a significant difference was shown between the mean for all body water readings measured above 80% survivorship (73.4%) compared to below 80% survivorship (70.4%) (ANOVA, $p = 0.01$, Table 2.10). From this, healthy and unhealthy termites could be separated on the basis of body water percentage, with low body water regarded as unhealthy. Assuming that midway between these means—72% body water—could be assigned as a threshold for separation of healthy and unhealthy groups, this value could be applied as a measure of vigor using readings for body water percentages recorded in captivity for this study, as well as water percentages determined just after field collection as in Chapter 3. P1 termites first measured 72% body water at 12 weeks, P2 at 6 weeks, and P3 at 2 weeks (Table 2.8a).

A 72% body water threshold for health may be set too low considering P3, a clearly unhealthy group measuring close to 75% body water at week 0, and significantly lower than P1 and P2 just after field collection (paired t-test, $p = 0.001$) (Table 2.8a). It is interesting to note that one of the three populations of the current study was considered unhealthy, measuring close to 75% body water from the field, while a similar proportion of field populations surveyed (3 of 11, or 27%) measured body water near 75% or lower (Chapter 3). Hypothesizing that low body water percentage correlates with unhealthy termites favors setting a threshold for health at 75% over the lower, statistically-derived threshold of 72%, as an actual population in captivity determined to be unhealthy had water percentage close to 75% at the time of field collection. A larger sampling of field groups could confirm that close to 30% of the general termite population measures close to 75% body water or lower. Further study could also confirm longer survivorship rates of termites in the laboratory with high initial percentages of body water over those with lower percent body water from the field.

Applying 75% body water as a threshold for health to the readings for field termites from Chapter 3, the population with significantly lower water percentage than the others (71.7%) (Duncan's multiple range test, $p \leq 0.05$) could be described as unhealthy. Field readings for body water well below 75% could justify excluding a termite group from use in bioassay. Additionally two populations measured 74.6 and 75.2% body water just after field collection. Although one of these readings was slightly above 75% body water, termites of each population were close to the threshold for health and therefore both could be considered unhealthy (Chapter 3).

In the current study all populations declined in body water percentage at the same rate through 6 weeks in captivity; P1 and P2 decreased below 75% body water between 2 to 4 weeks

(Table 2.8a). Applying a threshold of 75% body water alongside percent survivorship, all three populations of the current study were near 75% body water 5 to 6 weeks prior to the point that the non-predictive, 80% survivorship threshold for health was reached (Figure 2.6). This illustrates how body water could be used as a predictive measure of termite vigor.

As lowest body water percentages consistently occurred with highest lipid levels, and high lipid was recorded sooner for P3, termites from this group consistently had significantly higher lipid content than P1 and P2 (Tukey's HSD, Table 2.12). However lipid content and water percentages did not correlate early in the study for any of the groups, with body water percentages decreasing early in the study while lipid content was steady (Figures 2.5, 2.6). Therefore measurement of lipid would not serve to enhance body water readings as a measure of vigor; the complicated procedure for measuring lipid content compared to percent water makes its measurement unnecessary for determination of vigor. Further study could establish whether survivorship of termites in the laboratory with higher percentages of body water is greater than those with lower percent body water.

A hypothesis formed from this study is that measurement of percent body water could be used to predict the vigor of termites. Body water close to or below 75% should be regarded as a sign of unhealthy termites. Further study could confirm the reliability of this threshold in the selection of more vigorous termites either from the field or in long-term laboratory culture for use in bioassay.

Table 2.1a. Mean cellulose consumption rate (μg cellulose eaten/g termite/day) for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1	P2	P3
0	N/A	N/A	N/A
2	25.3	34.8	33.6
4	25.1	35.2	30.6
6	20.9	29.6	29.9
8	25.5	25.1	23.1
10	19.3	22.3	26.1
12	20.8	24.3	23.3
14	21.9	16.7	21.5
16	23.1	24.4	
18	21.6	24.8	
20	20.8	24.4	
22	21.3	25.1	
24	21.3	22.9	

Table 2.1b. Percent change in consumption rate from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1 percent change	P2 percent change	P3 percent change
0	N/A	N/A	N/A
2	0	0	0
4	-1	1	-9
6	-17	-15	-11
8	1	-28	-31
10	-24	-36	-22
12	-18	-30	-31
14	-13	-52	-36
16	-9	-30	
18	-15	-29	
20	-18	-30	
22	-16	-28	
24	-16	-34	

Table 2.2. Mean survivorship percentage for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	100	100	100
2	92	74	79.2
4	90.8	96.8	92.4
6	79.2	92.8	74
8	90.4	86	94.8
10	68	96	65.6
12	72.8	80.4	77.6
14	73.6	68.4	20.4
16	54.4	52	
18	58.4	38.8	
20	52	36.4	
22	36.8	28.8	
24	32	22.4	

Table 2.3a. Mean live weight for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	3.36 ± 0.15	3.04 ± 0.10	2.84 ± 0.15
2	3.58 ± 0.25	3.05 ± 0.10	2.93 ± 0.15
4	3.59 ± 0.27	3.11 ± 0.06	3.01 ± 0.11
6	3.55 ± 0.05	3.24 ± 0.14	3.35 ± 0.07
8	3.20 ± 0.16	3.30 ± 0.11	3.10 ± 0.08
10	3.51 ± 0.15	3.24 ± 0.06	3.35 ± 0.03
12	3.55 ± 0.13	3.14 ± 0.21	3.35 ± 0.20
14	3.62 ± 0.18	3.42 ± 0.09	3.54 ± 0.13
16	3.56 ± 0.09	3.27 ± 0.15	
18	3.92 ± 0.15	3.27 ± 0.18	
20	3.39 ± 0.10	3.41 ± 0.19	
22	3.37 ± 0.21	3.37 ± 0.31	
24	3.33 ± 0.25	3.67 ± 0.35	

Table 2.3b. Percent change in live weight from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1	P2	P3
	percent change	percent change	percent change
0	0	0	0
2	7	0	3
4	7	2	6
6	6	7	18
8	-5	9	9
10	4	7	18
12	6	4	18
14	8	13	25
16	6	8	
18	17	8	
20	1	12	
22	0	11	
24	-1	21	

Table 2.4a. Mean µg uric acid/mg termite for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	9.6 ± 1.0	2.5 ± 0.3	40.4 ± 2.1
2	7.8 ± 2.7	21.9 ± 3.0	40.5 ± 4.6
4	4.6 ± 1.8	4.7 ± 5.7	27.2 ± 3.9
6	6.1 ± 3.1	1.3 ± 1.1	21.7 ± 0.6
8	1.0 ± 1.7	8.2 ± 7.0	24.4 ± 3.5
10	26.6 ± 4.2	5.5 ± 1.6	19.9 ± 2.9
12	11.9 ± 2.2	21.6 ± 5.1	22.1 ± 3.6
14	11.5 ± 2.7	10.7 ± 2.9	31.1 ± 14.9
16	39.2 ± 0.8	44.2 ± 6.3	
18	7.3 ± 0.4	108.8 ± 15.2	
20	4.5 ± 3.4	44.9 ± 4.3	
22	2.1 ± 1.6	66.8 ± 18.4	
24	68.7 ± 4.0	72.8 ± 17.4	

Table 2.4b. Percent change in uric acid from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1	P2	P3
	percent change	percent change	percent change
0	0	0	0
2	-19	776	0
4	-52	88	-33
6	-36	-48	-46
8	-90	228	-39
10	177	120	-51
12	24	764	-45
14	20	328	-23
16	308	1668	
18	-24	4252	
20	-53	1696	
22	-78	2572	
24	616	2812	

Table 2.5a. Mean μg soluble proteins/mg termite for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	46.7 ± 3.4	44.7 ± 2.0	40.3 ± 2.7
2	46.4 ± 2.9	ND	ND
4	ND	48.1 ± 3.9	ND
6	54.2 ± 4.2	52.5 ± 2.6	ND
8	47.4 ± 0.9	59.3 ± 1.6	33.1 ± 4.7
10	53.6 ± 3.3	51.6 ± 2.0	53.9 ± 4.5
12	53.7 ± 4.0	45 ± 5.7	ND
14	51.3 ± 3.1	58.4 ± 2.7	ND
16	56 ± 5.5	61 ± 9.7	
18	78.6 ± 6.7	37 ± 1.8	
20	43.1 ± 2.9	44.8 ± 2.5	
22	58.1 ± 4.4	27.9 ± 4.6	
24	49.9 ± 5.8	32.8 ± 3.8	

Table 2.5b. Percent change in soluble protein from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1 percent change	P2 percent change	P3 percent change
0	0	0	0
2	-1	ND[1]	ND
4	ND	8	ND
6	16	17	ND
8	1	33	-18
10	15	15	34
12	15	1	ND
14	10	31	ND
16	20	36	
18	68	-17	
20	-8	0	
22	24	-38	
24	7	-27	

[1]ND = no data

Table 2.6a. Mean μg glycogen/mg termite for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	2.6 ± 1.3	3.1 ± 0.2	4.1 ± 0.6
2	6.3 ± 0.9	6.6 ± 1.9	12.2 ± 1.8
4	7.6 ± 1.4	7.9 ± 1.2	9.7 ± 1.7
6	6.3 ± 2.6	4.9 ± 1.3	11.7 ± 1.5
8	8.8 ± 1.4	3.0 ± 0.4	7.1 ± 1.1
10	2.5 ± 0.6	5.0 ± 1.9	8.8 ± 0.9
12	5.3 ± 1.2	6.6 ± 2.6	7.6 ± 4.2
14	3.2 ± 0.8	1.5 ± 0.4	7.0 ± 2.1
16	4.5 ± 0.8	4.8 ± 1.3	
18	4.8 ± 0.9	2.0 ± 1.0	
20	5.2 ± 2.1	4.3 ± 2.7	
22	2.4 ± 6.6	14.3 ± 6.5	
24	2.6 ± 3.5	7.1 ± 0.3	

Table 2.6b. Percent change in glycogen from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1 percent change	P2 percent change	P3 percent change
0	0	0	0
2	142	113	198
4	192	155	137
6	142	58	185
8	238	-3	73
10	-4	61	115
12	104	113	85
14	23	-52	71
16	73	55	
18	85	-35	
20	100	39	
22	-8	361	
24	0	129	

Table 2.7a. Mean µg lipid /mg termite for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	24.5 ± 6.5	26.7 + 3.5	51.9 ± 10.4
2	70.9 ± 14.1	76 + 6.8	122.9 ± 11.3
4	65.2 ± 13.1	74.6 ± 5.4	129.3 ± 21.3
6	82.3 ± 8	91.7 ± 2.5	125 ± 1.6
8	85.3 ± 13.8	67.6 ± 4.4	142.6 ± 6.4
10	57.9 ± 12.4	115.3 ± 32.2	136.7 ± 16.7
12	87.8 ± 13.6	53.1 ± 4.4	126.6 ± 10.0
14	107.4 ± 8.7	107 ± 9.1	167 + 9.5
16	132.9 ± 10.8	62.6 ± 16.8	
18	141.7 ± 3.5	44.9 ± 3.8	
20	112 ± 32.4	76.6 ± 10	
22	131.9 ± 20.8	79.7 ± 20	
24	138.6 ± 10.2	66.5 ± 12.5	

Table 2.7b. Percent change in lipid from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1 percent change	P2 percent change	P3 percent change
0	0	0	0
2	184	181	137
4	160	178	148
6	228	241	140
8	240	152	175
10	132	326	163
12	252	96	144
14	328	296	221
16	432	133	
18	464	67	
20	348	185	
22	432	196	
24	456	144	

Table 2.8a. Mean percent body water/termite for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	78.6	77.9 ± 0.9	74.6 ± 0.5
2	75.6 ± 0.9	76.4 ± 0.4	71.3 ± 0.6
4	73.2 ± 0.5	72.9 ± 0.5	70.6 ± 1.1
6	72.2 ± 0.8	71.7 ± 1.1	67.6 ± 0.6
8	73.7 ± 0.6	73.7 ± 0.7	65.5 ± 0.2
10	73.1 ± 1.4	71.7 ± 0.8	65.3 ± 1.0
12	73.0 ± 0.3	76.2 ± 0.3	63.7 ± 0.6
14	72.1 ± 0.3	72.0 ± 0.3	64.4 ± 0.8
16	71.2 ± 1.9	74.1 ± 0.8	
18	68.1± 1.4	75.1 ± 1.0	
20	73.5 ± 1.1	70.2 ± 0.6	
22	68.0 ± 0.5	71 ± 0.8	
24	66.0 ± 0.9	73.1 ± 1.8	

[1]P1, week 0: Reading of one sample.

Table 2.8b. Percent change in body water/termite from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1 percent change	P2 percent change	P3 percent change
0	0.0	0.0	0.0
2	-3.8	-1.9	-4.3
4	-6.9	-6.4	-5.4
6	-8.1	-8.0	-9.4
8	-6.2	-5.4	-12.2
10	-7.0	-8.0	-12.5
12	-7.1	-2.2	-14.6
14	-8.3	-7.6	-13.8
16	-9.4	-4.9	
18	-13.4	-3.6	
20	-6.5	-9.9	
22	-13.5	-8.9	
24	-16.0	-6.2	

Table 2.9a. Mean running speed (seconds) for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

week	P1	P2	P3
0	3.72 ± 1.16	3.30 ± 0.66	5.71 ± 2.05
2	3.38 ± 0.79	3.61 ± 0.75	3.85 ± 0.83
4	3.06 ± 0.80	3.30 ± 0.52	3.60 ± 1.15
6	3.10 ± 0.52	3.23 ± 0.63	3.19 ± 0.60
8	2.83 ± 0.40	3.20 ± 0.50	2.95 ± 0.36
10	2.66 ± 0.43	3.24 ± 0.30	3.04 ± 0.54
12	2.77 ± 0.48	4.95 ± 1.78	3.38 ± 0.58
14	2.70 ± 0.37	4.14 ± 1.26	3.43 ± 0.53
16	3.15 ± 0.43	2.96 ± 0.51	
18	2.55 ± 0.26	3.9 ± 0.76	
20	2.96 ± 0.50	3.89 ± 0.68	
22	3.98 ± 1.30	3.98 ± 0.61	
24	3.06 ± 0.63	4.09 ± 0.90	

Table 2.9b. Percent change in mean running speed (seconds) from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

week	P1	P2	P3
	percent change	percent change	percent change
0	0	0	0
2	-9	9	-33
4	-18	0	-37
6	-17	-2	-44
8	-24	-3	-48
10	-28	-2	-47
12	-26	50	-41
14	-27	25	-40
16	-15	-10	
18	-31	18	
20	-20	18	
22	7	21	
24	-18	24	

Table 2.10. Measurements for potential measures of vigor from healthy and unhealthy *R. flavipes* workers

P1, Week	Healthy	Unhealthy	Consumption	Survivorship	Live Weight	Uric Acid	Soluble Protein	Lipid	Percent Water	Glycogen	Running Speed
0	x		ND[1]	100	3.36	9.6	46.7	24.5	78.6	2.6	3.72
2	x		25.3	92.0	3.58	7.7	46.4	70.9	75.6	6.3	3.38
4	x		25.1	90.8	3.59	4.6	ND	65.2	73.2	7.6	3.06
6	x		20.9	79.2	3.55	6.1	54.2	82.3	72.2	6.3	3.10
8	x		25.5	90.4	3.20	1.0	47.4	85.3	73.7	8.8	2.83
10		x	19.3	68.0	3.51	26.5	53.6	57.9	73.1	2.5	2.66
12		x	20.8	72.8	3.55	11.9	53.7	87.8	73.0	5.3	2.77
14		x	21.9	73.6	3.62	11.7	51.3	107.4	72.1	3.2	2.70
16		x	23.1	54.4	3.56	39.1	56.0	132.9	71.2	4.5	3.15
18		x	21.6	58.4	3.92	7.3	78.6	141.7	68.1	4.8	2.55
20		x	20.8	52.0	3.39	3.9	43.1	112.0	73.5	5.2	2.96
22		x	21.3	36.8	3.37	2.7	58.1	131.9	68.0	2.4	3.98
24		x	21.3	32.0	3.33	68.7	49.9	138.6	66.0	2.6	3.06
OVERALL MEAN:			22.2	69.3	3.50	15.4	53.3	95.3	72.2	4.8	3.07
P1 HEALTHY MEAN (±SD):			24.2 ± 2.2		3.5 ± 0.2	5.8 ± 3.3	48.7 ± 3.7	65.6 ± 24.4	74.6 ± 2.5	6.3 ± 2.3	3.2 ± 0.3
P2 UNHEALTHY MEAN:			21.3 ± 1.1		3.5 ± 0.2	21.5 ± 22.7	55.5 ± 10.4	113.8 ± 29.1	70.6 ± 2.9	3.8 ± 1.6	3.0 ± 0.5
P2, Week	Healthy	Unhealthy	Consumption	Survivorship	Live Weight	Uric Acid	Soluble Protein	Lipid	Water	Glycogen	Running Speed
0	x		ND	100	3.04	2.5	44.7	26.7	77.9	3.1	3.30
2	x		34.8	74.0	3.05	21.9	ND	76.0	76.4	6.6	3.61
4	x		35.2	96.8	3.11	1.9	48.1	74.6	72.9	7.9	3.30
6	x		29.6	92.8	3.24	1.2	52.5	91.7	71.7	4.9	3.23
8	x		25.1	86.0	3.30	8.2	59.3	67.6	73.7	3.0	3.20
10		x	22.3	96.0	3.24	5.5	51.6	115.3	71.7	5.0	3.24
12		x	24.3	80.4	3.15	21.6	45.0	53.1	76.2	6.6	4.95
14		x	16.7	68.4	3.42	10.7	58.4	107.0	72.0	1.5	4.14
16		x	24.4	52.0	3.27	44.2	61.0	62.6	74.1	4.8	2.96
18		x	24.8	38.8	3.27	108.7	37.0	44.9	75.1	2.0	3.90
20		x	24.4	36.4	3.41	42.7	44.8	76.6	70.2	4.3	3.89
22		x	25.1	28.8	3.37	66.8	27.9	79.7	71.0	14.3	3.98
24		x	22.9	22.4	3.67	84.3	32.8	66.5	73.1	7.1	4.09
OVERALL MEAN:			25.8	67.1	3.27	32.3	46.9	72.5	73.5	5.5	3.68
P2 HEALTHY MEAN:			31.2 ± 4.8		3.2 ± 0.1	7.1 ± 8.7	51.2 ± 6.3	67.3 ± 24.4	74.5 ± 2.6	5.1 ± 2.2	3.3 ± 0.2
P2 UNHEALTHY MEAN:			23.1 ± 2.8		3.4 ± 0.2	48.1 ± 36.4	44.8 ± 11.9	75.7 ± 24.7	72.9 ± 2.1	5.7 ± 4.0	3.9 ± 0.6
P3, Week	Healthy	Unhealthy	Consumption	Survivorship	Live Weight	Uric Acid	Soluble Protein	Lipid	Water	Glycogen	Running Speed
0	x		ND	100	2.84	40.3	40.3	51.9	74.6	4.1	5.71
2	x		33.6	79.2	2.93	40.4	ND	122.9	71.3	12.2	3.85
4	x		30.6	92.4	3.01	27.2	ND	129.3	70.6	9.7	3.60
6	x		29.9	74.0	3.35	21.7	ND	125.0	67.6	11.7	3.19
8		x	23.1	94.8	3.10	24.4	33.1	142.6	65.5	7.1	2.95
10		x	26.1	65.6	3.35	19.9	53.9	136.7	65.3	8.8	3.04
12		x	23.3	77.6	3.35	22.0	ND	126.6	63.7	7.6	3.38
14		x	21.5	20.4	3.54	31.1	ND	167.0	64.4	7.0	3.43
OVERALL MEAN:			26.9	75.5	3.18	28.4	ND[2]	125.3	67.9	8.5	3.64
P3 HEALTHY MEAN:			31.4 ± 2.0	86.4 ± 11.9	3.0 ± 0.2	32.4 ± 9.5	ND	107.3 ± 37.0	71.0 ± 2.9	9.4 ± 3.7	4.1 ± 1.1
P3 UNHEALTHY MEAN:			23.5 ± 1.9	64.6 ± 31.8	3.3 ± 0.2	24.3 ± 4.9	ND	143.2 ± 17.2	64.7 ± 0.8	7.6 ± 0.8	3.2 ± 0.2
MEAN OF ALL HEALTHY DATA:			**28.7 ± 4.7**		**3.2 ± 0.2**	**13.9 ± 14.0**	**48.8 ± 5.6**	**78.1 ± 32.4**	**73.6 ± 2.9**	**6.8 ± 3.1**	**3.5 ± 0.7**
MEAN OF ALL UNHEALTHY DATA:			**22.5 ± 2.2**		**3.4 ± 0.2**	**32.7 ± 29.2**	**49.4 ± 12.1**	**104.4 ± 35.8**	**70.4 ± 3.8**	**5.3 ± 2.9**	**3.4 ± 0.6**
SIGNIFICANTLY DIFFERENT BY ANOVA?			**YES**		**YES**	**YES**	**NO**	**YES**	**YES**	**NO**	**NO**
			p < 0.0001		**p = 0.01**	**p = 0.03**	**p = 0.97**	**p = 0.04**	**p = 0.01**	**p = 0.17**	**p = 0.61**

[1]ND = No data.
[2]Mean not determined.

TABLE 2.11. Tukey's HSD for significant differences in uric acid content among healthy and unhealthy P1, P2, and P3 workers

The GLM Procedure
Least Squares Means

Pop.	Healthy/ Unhealthy[1]	LSMEAN	Standard Error	Pr > |t|	Table Designation (i,j)
P1	Healthy	5.6533333 [2]	5.2803995	0.2870	P1,H[3]
P1	Unhealthy	20.8583333	4.1745223	<.0001	P1,U
P2	Healthy	7.7000000	5.2803995	0.1480	P2,H
P2	Unhealthy	46.9125000	4.1745223	<.0001	P2,U
P3	Healthy	32.4333333	5.9036661	<.0001	P3,H
P3	Unhealthy	24.3750000	5.9036661	<.0001	P3,U

Least Squares Means for effect pop.*health
Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	P1,H	P1,U	P2,H	P2,U	P3,H	P3,U
P1,H		0.0262	0.7846	<.0001	0.0010	0.0201
P1,U	0.0262		0.0535	<.0001	0.1127	0.6278
P2,H	0.7846	0.0535		<.0001	0.0024	0.0379
P2,U	<.0001	<.0001	<.0001		0.0480	0.0024
P3,H	0.0010	0.1127	0.0024	0.0480		0.3369
P3,U	0.0201	0.6278	0.0379	0.0024	0.3369	

Values $\leq$ 0.0033 are significantly different. (Critical value for significance determined from 0.05 level of probability/15 pairwise comparisons = 0.0033).

[1] "Healthy" termites regarded as those from an experimental unit with higher than 80% survivorship, and "unhealthy" below 80% survivorship. Su and LaFage (1984a) defined 80% survivorship as a threshold for separating healthy from unhealthy termites, using small groups of *C. formosanus* workers in captivity.
[2] Units of µg uric acid/mg termite.
[3] H = "healthy," above 80% survivorship; U = "unhealthy," below 80% survivorship.

TABLE 2.12. Tukey's HSD for significant differences in lipid content among healthy and unhealthy P1, P2, and P3 workers

The GLM Procedure
Least Squares Means

Pop.	Healthy/ Unhealthy[1]	LSMEAN [2]	Standard Error	Pr > |t|	Table Designation (i,j)
P1	Healthy	65.600000	7.024121	<.0001	P1,H[3]
P1	Unhealthy	113.500000	5.672490	<.0001	P1,U
P2	Healthy	67.313333	7.024121	<.0001	P2,H
P2	Unhealthy	75.562500	5.553056	<.0001	P2,U
P3	Healthy	106.966667	7.853206	<.0001	P3,H
P3	Unhealthy	142.750000	7.853206	<.0001	P3,U

Least Squares Means for effect pop.*health
Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	P1,H	P1,U	P2,H	P2,U	P3,H	P3,U
P1,H		<.0001	0.8634	0.2687	0.0002	<.0001
P1,U	<.0001		<.0001	<.0001	0.5017	0.0033
P2,H	0.8634	<.0001		0.3592	0.0003	<.0001
P2,U	0.2687	<.0001	0.3592		0.0015	<.0001
P3,H	0.0002	0.5017	0.0003	0.0015		0.0017
P3,U	<.0001	0.0033	<.0001	<.0001	0.0017	

Values $\leq$ 0.0033 are significantly different. (Critical value for significance determined from 0.05 level of probability/15 pairwise comparisons = 0.0033).

[1] "Healthy" termites regarded as those from an experimental unit with higher than 80% survivorship, and "unhealthy" below 80% survivorship. Su and LaFage (1984a) defined 80% survivorship as a threshold for separating healthy from unhealthy termites, using small groups of *C. formosanus* workers in captivity.
[2] Units of µg lipid/mg termite.
[3] H = "healthy," above 80% survivorship; U = "unhealthy," below 80% survivorship.

TABLE 2.13. Tukey's HSD for significant differences in body water percentage among healthy and unhealthy P1, P2, and P3 workers

The GLM Procedure
Least Squares Means

Pop.	Healthy/ Unhealthy[1]	LSMEAN	Standard Error	Pr > |t|	Table Designation (i,j)
P1	Healthy	74.0461538 [2]	0.6413499	<.0001	P1,H[3]
P1	Unhealthy	70.6000000	0.4720207	<.0001	P1,U
P2	Healthy	74.5200000	0.5970643	<.0001	P2,H
P2	Unhealthy	72.5909091	0.4930096	<.0001	P2,U
P3	Healthy	71.0333333	0.6675381	<.0001	P3,H
P3	Unhealthy	64.7083333	0.6675381	<.0001	P3,U

Least Squares Means for effect pop.*health
Pr > |t| for H0: LSMean(i)=LSMean(j)

i/j	P1,H	P1,U	P2,H	P2,U	P3,H	P3,U
P1,H		<.0001	0.5900	0.0753	0.0016	<.0001
P1,U	<.0001		<.0001	0.0044	0.5974	<.0001
P2,H	0.5900	<.0001		0.0145	0.0002	<.0001
P2,U	0.0753	0.0044	0.0145		0.0637	<.0001
P3,H	0.0016	0.5974	0.0002	0.0637		<.0001
P3,U	<.0001	<.0001	<.0001	<.0001	<.0001	

Values $\leq$ 0.0033 are significantly different. (Critical value for significance determined from 0.05 level of probability/15 pairwise comparisons = 0.0033).

[1] "Healthy" termites regarded as those from an experimental unit with higher than 80% survivorship, and "unhealthy" below 80% survivorship. Su and LaFage (1984a) defined 80% survivorship as a threshold for separating healthy from unhealthy termites, using small groups of *C. formosanus* workers in captivity.
[2] Percent body water/termite.
[3] H = "healthy," above 80% survivorship; U = "unhealthy," below 80% survivorship.

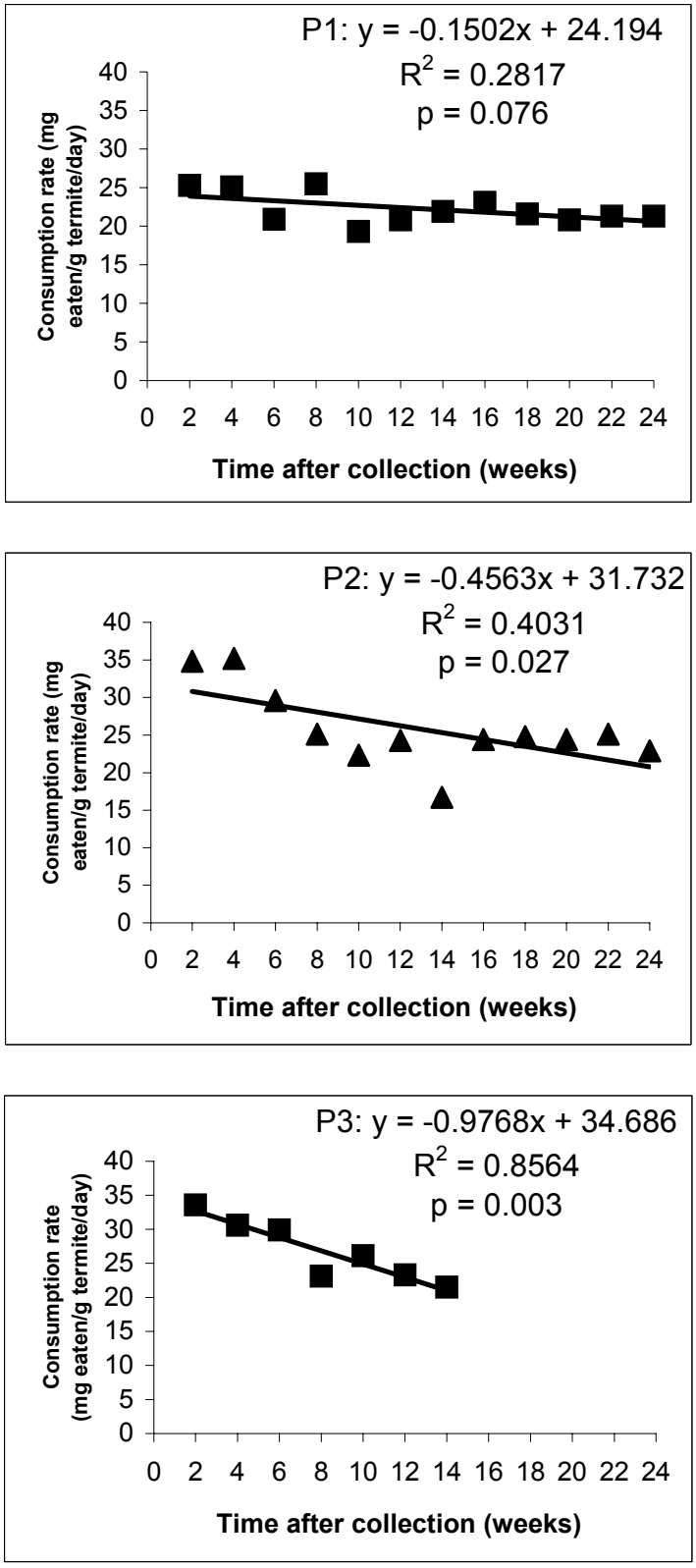


Figure 2.1: Linear regression of change in consumption rate (mg eaten/g termite/day) over time in captivity for *R. flavipes* workers from three field populations

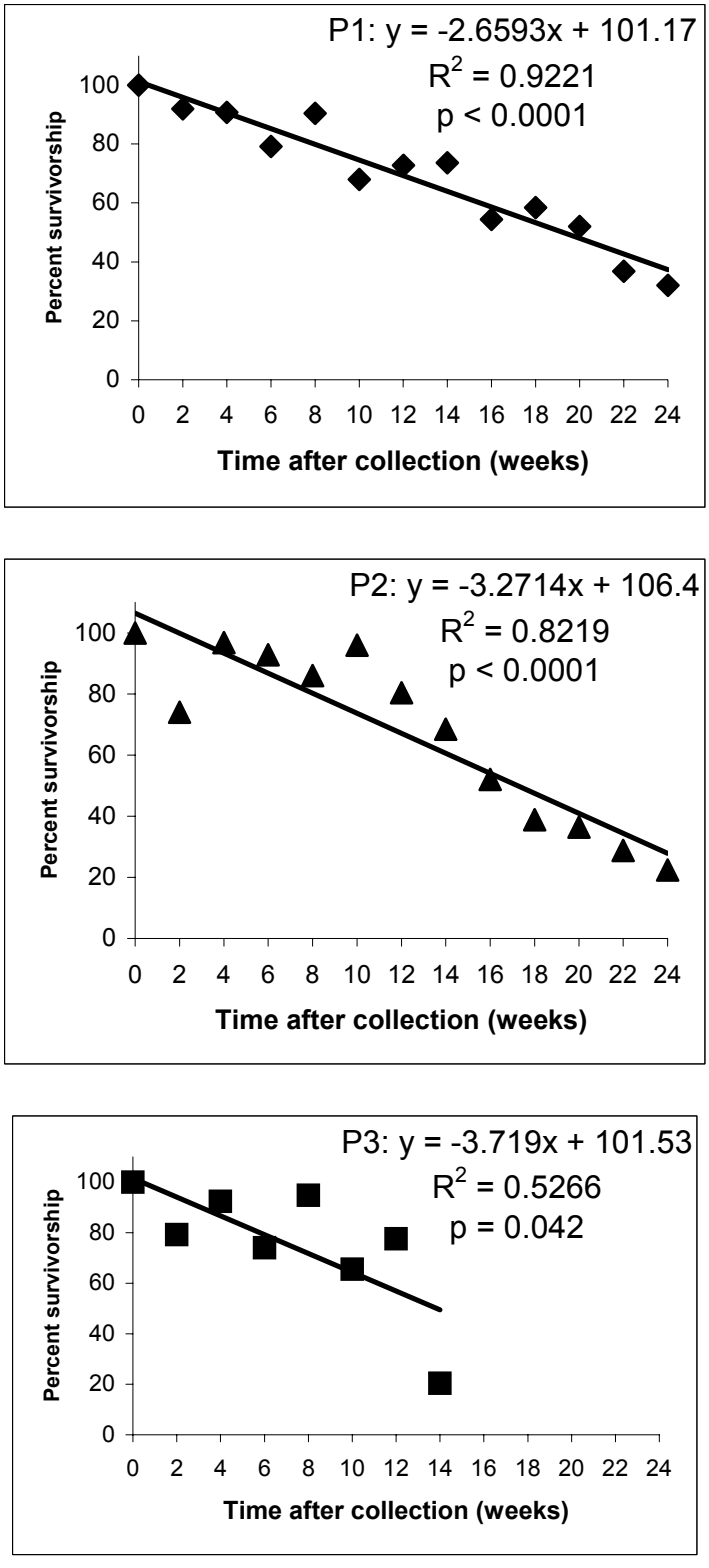
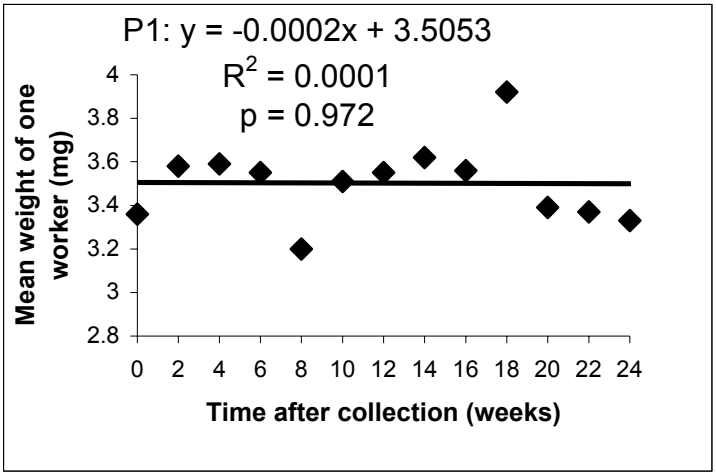
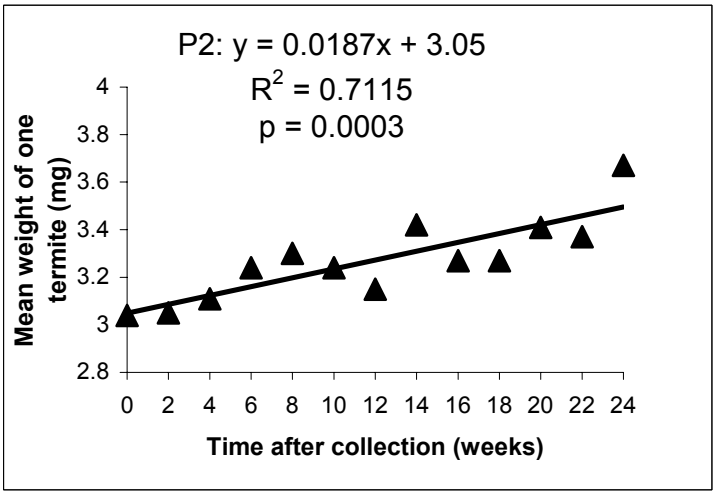


Figure 2.2: Linear regression of change in percent survivorship over time in captivity for *R. flavipes* workers from three field populations





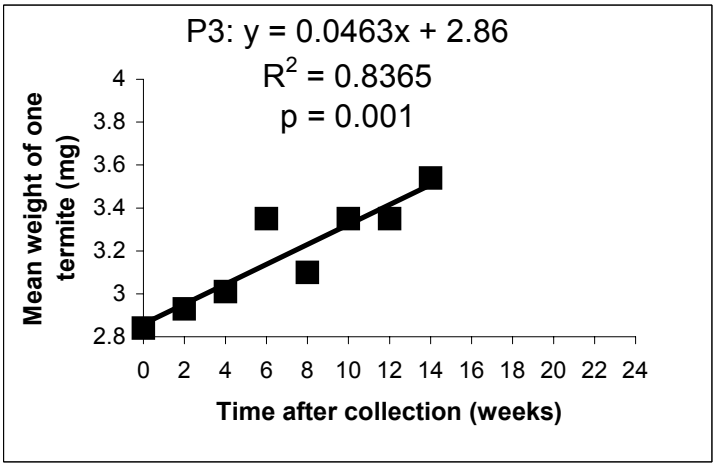
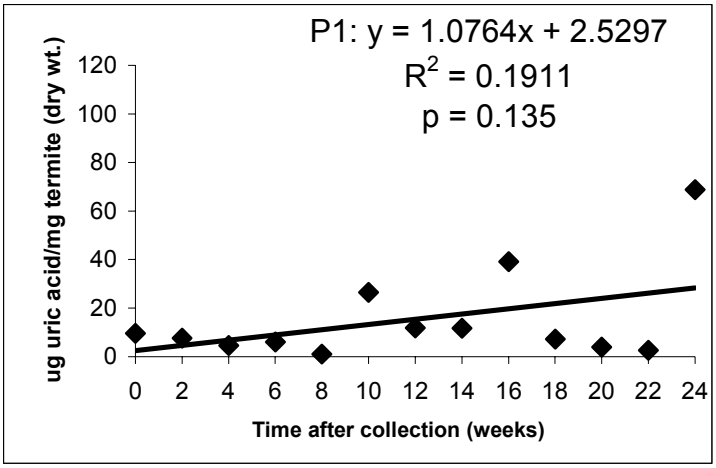
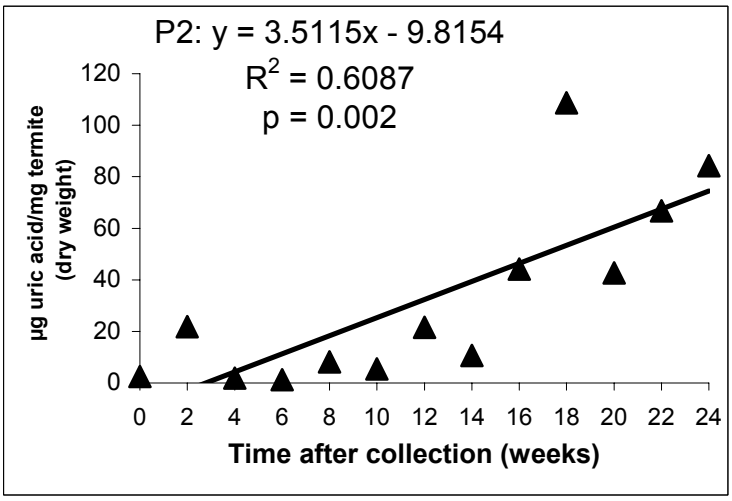


Figure 2.3: Linear regression of change in live mass of one termite over time in captivity for *R. flavipes* workers from three field populations





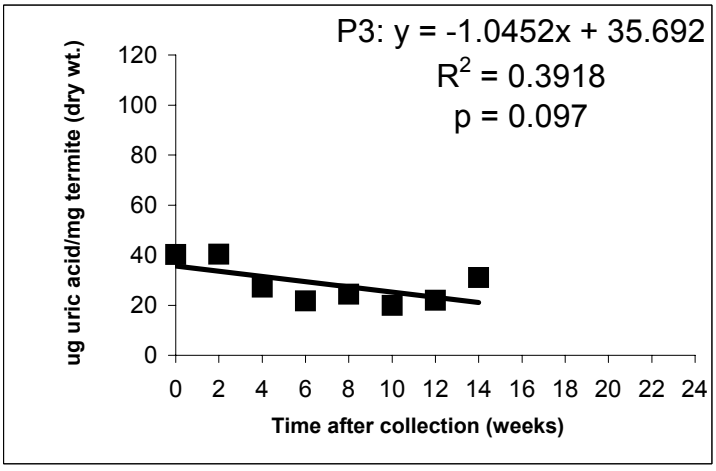
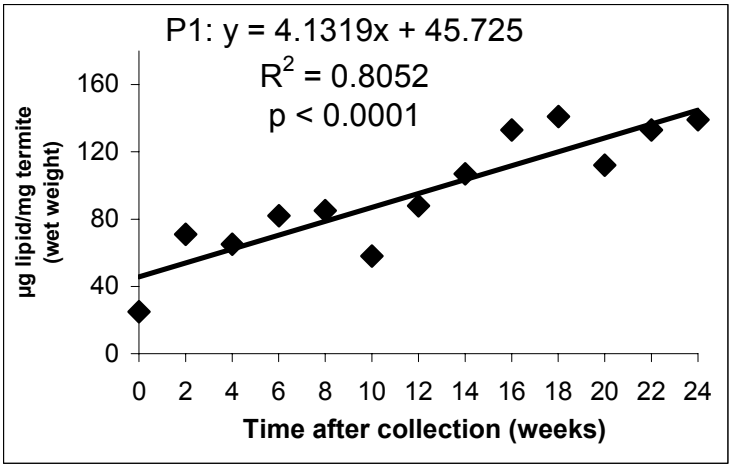
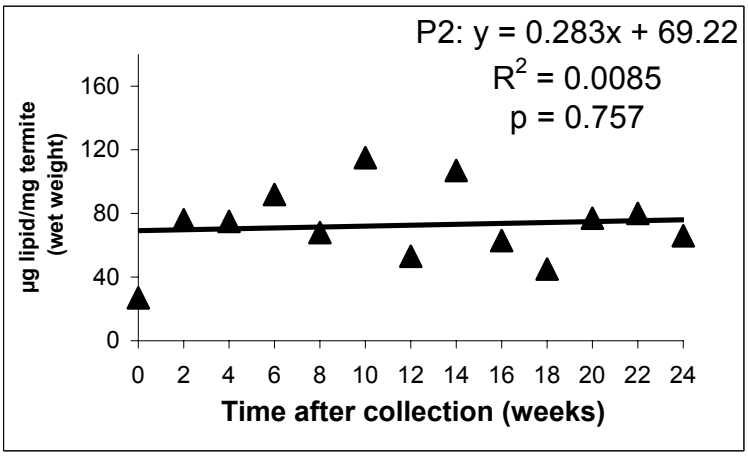


Figure 2.4: Linear regression of change in uric acid content over time in captivity for *R. flavipes* workers from three field populations





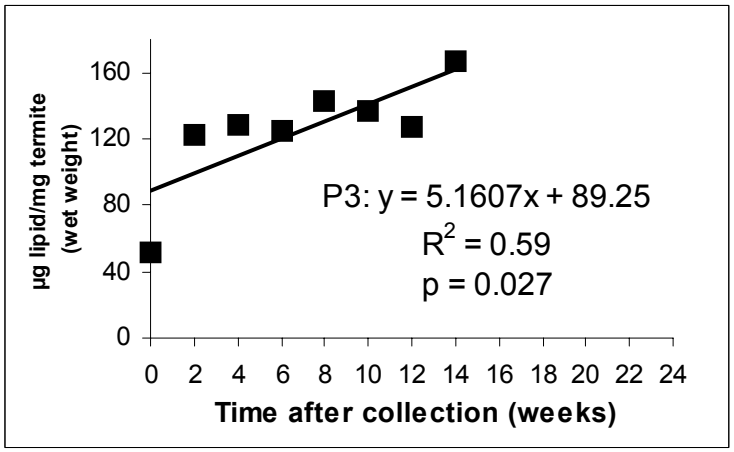
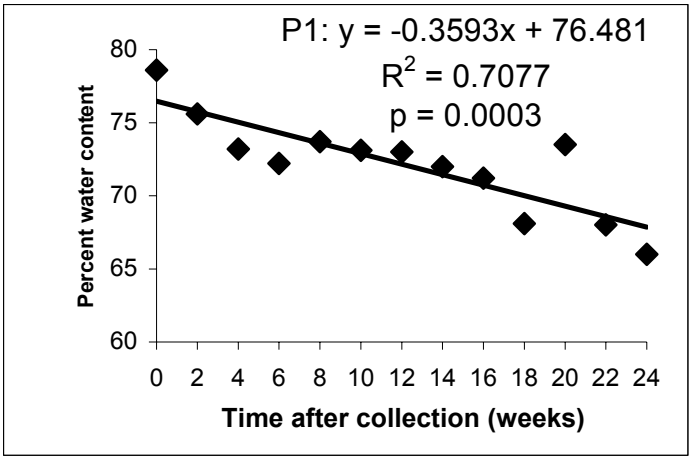
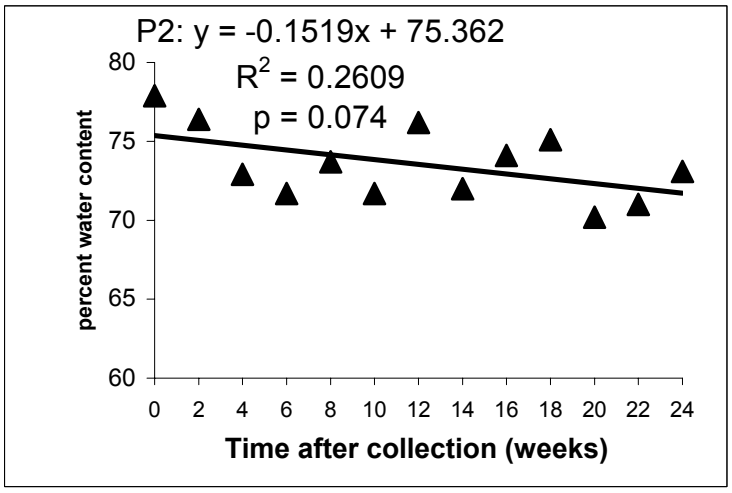


Figure 2.5: Linear regression of change in lipid content over time in captivity for *R. flavipes* workers from three field populations





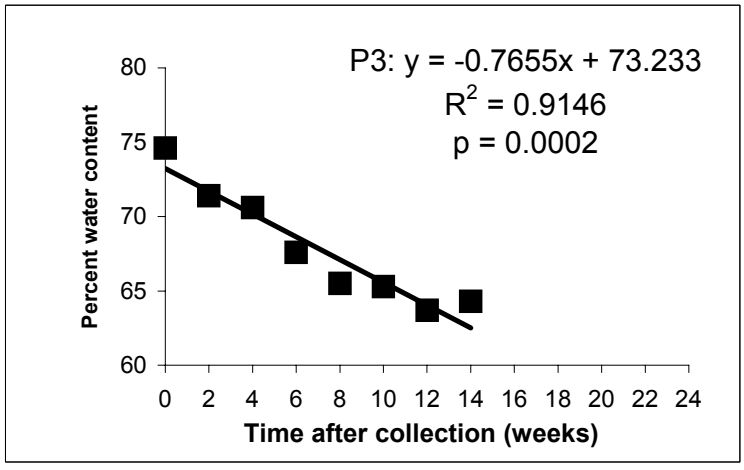
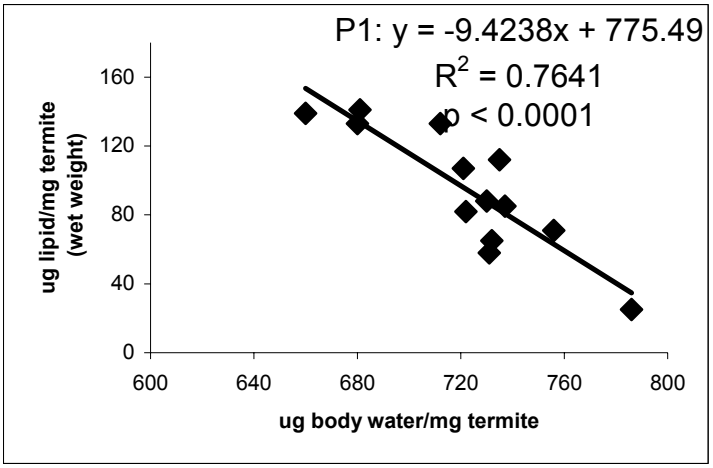
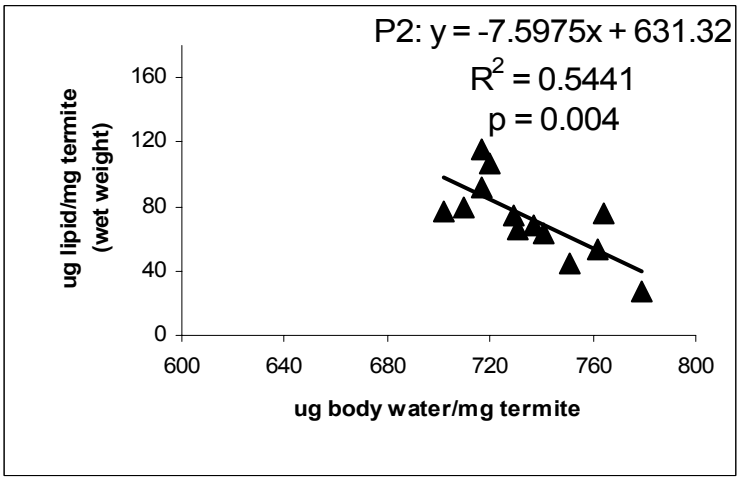


Figure 2.6: Linear regression of change in percent body water/termite over time in captivity for *R. flavipes* workers from three field populations





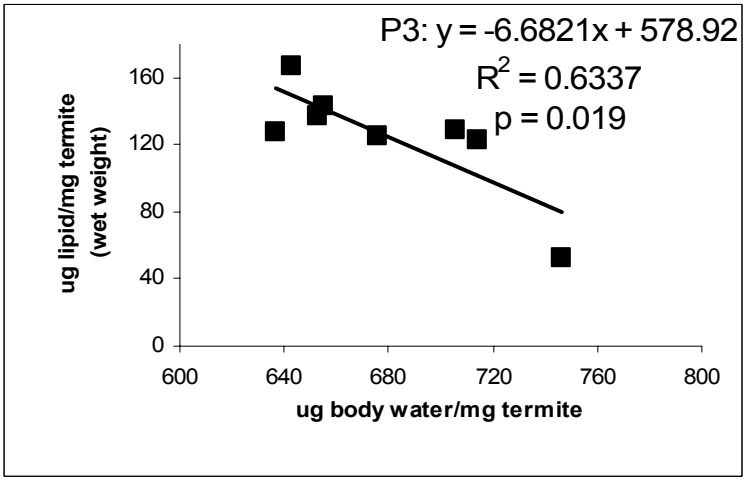


Figure 2.7**:** Linear regression for body water and lipid content in Captivity for *R. flavipes* workers from three field populations

CHAPTER 3

NOVEL MEASURES OF VIGOR APPLIED TO FRESHLY COLLECTED AND LABORATORY-CULTURED TERMITES, *RETICULITERMES FLAVIPES* (KOLLAR)[1]

[1]Arquette, T.A. and B.T. Forschler. To be submitted to *Journal of Insect Physiology*.

ABSTRACT

Total levels of uric acid, soluble proteins, lipid, glycogen, and body water were determined for *Reticulitermes flavipes* (Kollar) workers 24 to 72 hours after field collection. In addition, levels of biological molecules were compared for termites collected from the same inspection ports within 24 hours after field collection, and after laboratory captivity of 6 or 9 months. Uric acid content, described in a past study as steadily accumulating in *R. flavipes* termites in captivity, was found to be low or undetectable in workers tested after 6 or 9 months in the laboratory. Vigor of workers just collected from the field is described in terms of body water percentage, which was identified as an indicator of termite vigor in Chapter 2.

INDEX WORDS: vigor, uric acid, soluble proteins, lipid, glycogen, body water.

INTRODUCTION

Termites are periodically collected from inspection ports on Sapelo Island, Georgia as part of long-term studies being conducted by the Household and Structural Entomology Research Program at the University of Georgia, Athens. The termites are cultured at room temperature in petri dishes containing wet filter paper, stacked inside of a clear plastic box with a lid. Several hundred *Reticulitermes flavipes* (Kollar) termites can be maintained in petri dishes with wet filter paper for months at a time without other food resources (Becker, 1969). However, termites in such conditions decline in vigor soon after field collection (Su and LaFage, 1984), with changes in metabolism occurring from artificial diet and the laboratory environment (Chapter 2).

This study determined levels of uric acid, soluble proteins, glycogen, lipid, and body water as they occur in *R. flavipes* workers immediately after field collection. These results can be compared with past reports for levels of these molecules of *R. flavipes* and other termite species (Mauldin and Smythe, 1973; Mauldin, 1977 and 1978; Potrikus and Breznak, 1980a; Nazcarzuk et al, 1981; Lovelock et al, 1985; Sponsler and Appel, 1990; Waller and Curtis, 2003), as well as the results from Chapter 2 which identified body water percentage as a measure of the vigor of termite populations. This study also determined amounts of stored biological molecules both at the time of collection and after 6 or 9 months in captivity, using termites collected from the same inspection ports. Results of this study will compliment findings of Chapter 2 that described changing levels of biological molecules in *R. flavipes* workers in captivity.

MATERIALS AND METHODS

Reticulitermes flavipes (Kollar) workers were collected from different areas of Georgia in July, August, and November 2003 (Table 3.1). Collection sites were four inspection ports in the

yard of the Reynolds Mansion, Sapelo Island, designated BH10 ("BH" short for "Big House"), BH18, BH28, and S (Sapelo); inspection ports from Morehouse College, Atlanta (MH) and next to the Univerity of Georgia chapel, Athens (CH); and from pine logs and bark found at Mistletoe state park (MT), near Tallulah Gorge and Fort Mountain state parks (TG and FM), and two sites at Whitehall Forest, University of Georgia, Athens (WH1 and WH2) (Table 3.1). Termites were frozen within 24 hours after collection at -70°C, except for MH workers which were frozen within 72 hours. Termites were also collected in February and May 2003 from BH10, BH18, and BH28 inspection ports. These termites were cultured in the laboratory in petri dishes (100 x 20 mm) containing damp filter paper for 6 months (BH28) or 9 months (BH10 and BH18) before freezing (Table 3.3). Levels of each biological molecule were determined for one termite equivalent from three pooled groups of 10 termites from each population. Units of μg biomolecule/mg termite were used except for body water content which was expressed as a percentage of total live body weight. The mean weight of one termite was estimated from six groups of 10 termites per population, ± the standard deviation.

Assay procedures

Uric Acid. Uric acid content was measured following the procedure of Potrikus and Breznak (1980a) using a diagnostic kit (Sigma 292). Termites were dried in a convection oven (VWR) at 85°C for 8 hours, and then held at room temperature in a desiccation chamber containing Drierite® crystals for 5 minutes before weighing. The dried termites were placed in a 1.5 ml centrifuge tube (Eppendorf) and ground into a powder with a plastic pestle, followed by addition of 1.5 ml lithium carbonate solution (0.6% w/v) (Sigma). Known amounts of uric acid (Sigma) were added to 1.5 ml lithium carbonate as a standard. Standards and unknown samples were suspended in a water bath at 60°C for 10 minutes, followed by centrifugation for 15

minutes at 3000x g. Supernatant (either 40 µl, or 400 µl for very dilute samples) was added to 1.5 ml centrifuge tubes containing 0.2 ml glycine buffer solution (0.7 M, pH 9.4) (Sigma 292) and 1.2 ml nanopure water, and pulsed for 10 seconds on an Eppendorf® microcentrifuge. Supernatant (0.6 ml) was added to 1.5 ml centrifuge tubes labeled "test" and "blank," followed by 10 µl of uricase (Sigma 292) added to the "test" vials, and 10 µl Nanopure® water to the "blank" vials. Tubes were vortexed and left at room temperature for 30 minutes to allow for complete digestion of uric acid in the tubes containing uricase. Both "test" and "blank" samples were read simultaneously at 292 nm (Spectronic® Genesys model 5, Spectronic Instruments).

The difference between "test" and "blank" absorbance values was used to determine uric acid concentration. The absorbance value for an unknown sample was defined as the fraction of termite extract in supernatant at the time the absorbance was measured. An initial 10 termite extract was diluted to an equivalent of 0.093 termite extract for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 5 µg of uric acid in solution, then the same absorbance reading for an unknown sample would mean there was 5 µl of uric acid for a 0.093 fraction of one termite. The result from expanding this fraction to give the amount of uric acid for one termite equivalent is 5.0 µg uric acid/0.093 termite equivalent = 53.8 µg uric acid/one termite equivalent.

Soluble Proteins. The Bradford method was used to determine levels of soluble proteins (Bio-Rad Laboratories) with a standard of bovine serum albumin. Termites were added to 1.5 ml centrifuge tubes (Eppendorf) containing 1 ml of distilled water, and then sonicated on ice (Branson sonifier model 250, VWR). After centrifuging at 14,000 rpm for 5 minutes, 0.8 ml of supernatant was added to empty centrifuge tubes followed by 0.2 ml of reagent. Sample supernatant, standards, and distilled water for a blank were added 175 µl per microplate well

(Becton Dickinson) and protein concentration determined at 595 nm (Spectra Max 340 microspectrophotometer, Molecular Devices Corp., Sunnyvale, CA).

Following the above procedure, an initial 10 termite extract was diluted to an equivalent of 0.01 termite for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 1 µg of soluble protein in solution, then the same absorbance reading for an unknown sample would mean there was 1 µg of soluble protein for a 0.01 fraction of one termite. The result from expanding this fraction to give the amount of soluble protein for one termite equivalent is 1.0 µg soluble protein/0.01 termite equivalent = 100 µg soluble protein/one termite equivalent.

Glycogen. Glycogen content of whole termites was determined based on a procedure by Van Handel (1965). Pooled groups of live termites were placed in 1.5 ml microcentrifuge tubes (Eppendorf) containing 0.4 ml of sodium sulfate solution (2% w/v) (J. T. Baker) and sonicated on ice (Branson sonifier model 250, VWR). To each homogenized termite sample was added 1 ml of 100% ethanol (J. T. Baker). Tubes were vortexed and samples frozen at -70° C for at least 24 hours to break cells and release glycogen. As termite samples thawed glycogen standards (Sigma) were prepared. Termite samples and glycogen standards were heated 10 minutes in a water bath at 60° C, and then centrifuged at 4000 rpm for 5 minutes. Supernatant was then poured off and discarded, with traces of liquid removed from around the remaining pellet with a pipetter. To each tube containing a homogenized termite pellet was added 750 µl of amyloglucosidase/sodium acetate solution (stock solution: 3.2 mg amyloglucosidase w/v (Sigma) mixed with a 5 ml of sodium acetate solution (0.2 M, pH 5.2) (Fisher Scientific)). To standard pellets, and 50 µl distilled water to be used as a blank for the spectrophotometer reading, was added 50 µl of the amyloglucosidase/sodium acetate solution. Microcentrifuge

tubes were taped to rotators in a mini hybridization oven (Bellco Glass, Inc.) and spun at 55° C for 2 hours at medium speed. Following centrifugation for 5 minutes at 12,000 rpm, between 25 to 200 µl solution containing termite sample (depending on glycogen concentration) and 50 µl of blank and standard solution were transferred to empty microcentrifuge tubes. Following the addition of 0.5 ml glucose trinder solution (Sigma), tubes were vortexed and allowed to stand for 18 minutes at room temperature. Supernatant was transferred to microplate wells (0.15 ml per well) (Becton Dickinson) and absorbances determined at 505 nm (SpectraMax model 340).

Following the above procedure, if 50 µl of unknown sample was set aside for mixture with the color-developing glucose trinder solution, an initial 10 termite extract was diluted to an equivalent of 0.182 termite extract for reading on the spectrophotometer. If a standard curve absorbance of 0.500 was determined for 5 µg of glycogen in solution, then the same absorbance reading for an unknown sample would mean there was 5 µg of glycogen for a 0.182 fraction of one termite. The result from expanding this fraction to give the amount of glycogen for one termite equivalent is 5.0 µg glycogen/0.182 termite equivalent = 27.5 µg glycogen/one termite equivalent.

Lipid content. Total lipids were extracted based on the procedure of Zera and Larsen (2001). Pooled groups of live termites were weighed on a 0.01 mg scale (model AB104, Mettler Toledo, Columbus OH), and placed in 1.5 ml microcentrifuge tubes containing 0.66 ml chloroform (J. T. Baker) with 0.05% butylated hydroxytoluene (BHT) (Sigma) w/v added. This was followed by addition of 0.33 ml methanol (J. T. Baker) containing 0.05% BHT (w/v). Termites were sonicated on ice (Branson sonifier model 250, VWR) and centrifuged 5 minutes at 14,000 rpm. All supernatant was transferred into empty 1.5 ml centrifuge tubes with a pipette, and the pellet discarded. Samples were vortexed after adding 0.34 ml aqueous KCL (Sigma)

(0.88% w/v) to the supernatant, resulting in two liquid layers. Non-lipid contaminants, isolated in the upper hydrophilic layer, were suctioned off with an aspirator. Dissolved lipids remained in the lower chloroform layer. The chloroform with lipid was poured onto a pre-weighed aluminum foil bowl and evaporated overnight. Lipid content was determined from the difference in weight between the foil bowl with lipid residue and the initial weight of the bowl.

Water content. Insects dried for the uric acid assay were those used to determine water content. After weighing, groups of ten live termites were dried at 85°C for 8 hours in a convection oven (VWR). Dried termites were held at room temperature in a desiccation chamber containing Drierite® crystals for 5 minutes before reweighing. Percent body water was determined by obtaining the difference between live and dry weights, divided by the live weight.

Statistics used. Levels of biological molecules measured from BH termites in captivity as well as just after collection from the same inspection ports were compared for significant differences using the Student's paired t-test and one-way ANOVA (Microsoft Excel®). Duncan's multiple range test was used to identify significant differences in levels of biological molecules between all field populations. Correlation analysis was used for comparison of lipid content and body water percentage of all field populations (Figure 3.1).

RESULTS

CH and MH termites had significantly higher levels of uric acid compared to other populations (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). Readings of 40.4 ± 2.1 µg uric acid/mg termite were recorded for CH, 51.3 ± 2.0 µg/mg for MH, and between 1 to 10 µg/mg for remaining populations (Table 3.2). CH and MH respectively measured forty to fifty-fold higher for uric acid content than BH18, the group with the lowest levels (paired t-test, $p = 0.001$ for BH18 vs. either CH or MH). Uric acid content of BH termites was low both from the field and

after 6 or 9 months in captivity, with no significant difference in levels between field and laboratory termites (paired t-test). In captivity BH10 workers measured 1.0 µg uric acid/mg termite, while 3.5 µg/mg was recorded from BH18, and 0.07 µg/mg from BH28 (Table 3.3). Uric acid was too low to measure for BH28 from two of three pooled samples.

Among the different measurements determined for this study, soluble protein levels were the most consistent across populations, differing less than two-fold with readings between 24.7 $\pm$ 3.2 to 46.7 $\pm$ 3.4 µg protein/mg termite (Table 3.2). The difference between these high and low extremes was significant (paired t-test, $p = 0.02$). Sapelo Island termites had significantly lower soluble protein content than the two Whitehall Forest populations (ANOVA, $p < 0.0001$, Table 3.2). Protein levels of BH10, BH18, and BH28 workers were similar just after collection compared to those from the laboratory. The BH groups measured 24.7 $\pm$ 3.2 to 32.5 $\pm$ 3.4 µg/mg just after collection from the field, and 29.3 $\pm$ 2.6 to 38.2 $\pm$ 4.2 µg protein/mg termite after 6 or 9 months in captivity (Table 3.3).

Glycogen content varied up to ten-fold among field termites, measuring between 2.6 $\pm$ 1.3 to 25.4 $\pm$ 6.8 µg glycogen/mg termite (Figure 3.2). Workers collected from Sapelo Island had significantly higher glycogen levels than those from other areas (ANOVA, $p < 0.0001$), measuring 9.0 $\pm$ 0.5 to 25.4 $\pm$ 6.8 µg glycogen/mg termite compared with 2.6 $\pm$ 1.3 to 4.1 $\pm$ 0.6 µg/mg from other sites (Table 3.2). BH10 and BH18 termites had significantly more glycogen just after collection than any other field group (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). BH10, BH18, and BH28 termites in captivity also had high glycogen levels, ranging between 22.7 $\pm$ 1.8 to 29.4 $\pm$ 4.6 µg glycogen/mg termite. Glycogen content of BH termites in captivity was not significantly different compared to BH field termites (paired t-test, $p = 0.07$, Table 3.3).

Lipid content ranged between 22.7 ± 8.5 to 62.3 ± 13.4 µg lipid/mg termite just after collection (Table 3.2). Readings were lowest for workers from Sapelo Island and Whitehall Forest, ranging between 22.7 ± 8.5 to 29.7 ± 3.2 µg lipid/mg termite; termites from remaining sites measured significantly higher at 42.7 ± 5.5 to 62.3 ± 13.4 µg lipid/mg termite (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). Mean lipid content of BH10, BH18, and BH28 was up to six-fold higher in captivity compared to freshly collected workers (Table 3.3). BH 10 measured 29.7 ± 3.2 µg lipid/mg termite from the field, compared to 140.3 ± 9.5 µg/mg in captivity ($p = 0.004$); BH18, 23.7 ± 4.0 µg/mg vs. 145.3 ± 18.5 µg/mg ($p = 0.009$); and BH28, 22.7 ± 8.5 µg/mg vs. 127.3 ± 8.5 µg/mg ($p = 0.002$) (paired t-test, Table 3.3).

Body water percentage was highest in workers collected from Sapelo Island and Whitehall Forest, measuring 77.9 ± 0.8 to 80.8 ± 0.5%. MH, CH, and FM readings were significantly lower, with readings of 71.7 ± 0.8% for MH, 74.6 ± 0.8% for CH, and 75.2 ± 1.0% for FM (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). Intermediate levels were determined for termites collected from the remaining areas, 76.1 ± 0.9% for TG and 76.8 ± 0.4% for MT (Table 3.2). Termites in the laboratory 6 or 9 months measured 12 to 15% lower for body water compared to field termites collected from the same sites, with the mean of all BH field termites significantly different than those in captivity (paired t-test, $p < 0.0001$, Table 3.3).

DISCUSSION

This chapter reports total levels of lipid, uric acid, soluble proteins, glycogen, and body water of *R. flavipes* workers from up to 11 field sites (Table 3.2). There was a fifty-fold range in uric acid content among field groups, a ten-fold range in glycogen, and a three-fold range in lipid. Soluble protein levels were similar among the groups surveyed, ranging less than two-

fold. A difference of 11% separated the highest and lowest body water measurements (Table 3.2).

A surprising finding was that uric acid levels were low or absent in workers in captivity (Table 3.3). This is contrary to past studies that reported uric acid consistently accumulating in termites in laboratory culture (Potrikus and Breznak, 1980a; Nazcarzuk et al, 1981; Lovelock et al, 1984). Potrikus and Breznak (1980a) reported that uric acid content of *R. flavipes* workers increased at a steady rate from 1.3 to 45% worker dry weight over 18 months. Insects measured for Potrikus and Breznak's study were extracted from a log stored in a laboratory in a metal trash container. The current study, in contrast, found two of three pooled groups of BH28 termites did not have detectable levels of uric acid after 6 months in a petri dish with filter paper (Table 3.3). BH10 and BH18 uric acid content was low after 9 months in captivity, measuring 1.0 and 3.5 ug uric acid/mg termite, respectively (Table 3.3). The assumption from earlier studies that uric acid consistently or regularly accumulates in termites in captivity clearly needs to be reconsidered.

This study also showed uric acid accumulations from field termites, as reported previously by Chappell and Slaytor (1993). Freshly collected CH and MH workers measured 40.4 ± 2.1 and 51.3 ± 2.0 µg uric acid/mg termite, respectively, compared to 1 to 10 µg uric acid/mg termite for the nine other field groups, and 13 µg/mg reported by Potrikus and Breznak (1980a) for freshly collected *R. flavipes* workers. Uric acid accumulation in termites has been described as resulting from starvation (Slaytor and Chappell, 1994; Korb and Lenz, 2004), perhaps as a result of digestion of body proteins when food is not available (Slaytor and Chappell, 1994).

CH and MH termites, besides showing uric acid accumulation significantly higher than other field groups (Duncan's multiple range test, $p \leq 0.05$), also had significantly lower body

water percentages than other populations just after collection (Table 3.2). In addition lipid levels were significantly higher for MH than all other populations except CH. Body water percentage near or below 75% was hypothesized in Chapter 2 to be an indication of unhealthy termites. Both MH and CH workers measured below this threshold with 71.7% body water for MH and 74.6% for CH. Accompanying high levels of uric acid in MH and CH support low water percentage as an indication that termites of these populations were weak. FM workers showed body water levels of 75.2%, just above the threshold for identifying weak termites, and with low uric acid content. However, as the highest measurements for body water were close to 80%, FM water percentage was low in comparison, so FM could justifiably be placed into a less healthy category.

Glycogen levels have not been described for termite workers previously. This is understandable as flying insects are ordinarily used for glycogen studies (reviews by Chapman, 1998 and Nation, 2002). Termites collected from Sapelo Island had high glycogen content compared to other field termites. BH28 measured significantly higher for glycogen than all other groups except for BH10, which was significantly higher than BH28 (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). Levels were also high for BH10, BH18, and BH28 termites in captivity (Table 3.3). Glycogen content has been reported as 15 mg/g for blowflies, a strong flyer, as well as the American cockroach, a weak flyer, with levels depleted in blowflies after flight (reviewed by Downer, 1982). In comparison, termites collected in November from Sapelo Island measured 12.7 to 25.4 mg glycogen/g termite. An increase in glycogen levels in flying insects has been attributed to conversion from trehalose during resting periods (Downer, 1982). As termites in late fall are less active than in the summer, higher glycogen levels for BH10, BH18, and BH28 termites could have resulted from low activity level. Further study could

establish whether seasonality plays a role in levels of glycogen or other biomolecules stored by termites.

Soluble protein levels were mostly similar in both field and laboratory-cultured termites (Table 3.2). Exceptions were the two groups collected from Whitehall Forest, which were significantly higher in protein content than each of the groups collected from Sapelo Island (Duncan's multiple range test, $p \leq 0.05$, Table 3.2). Protein levels for Sapelo Island termites in captivity versus the field were not significantly different (paired t-test, Table 3.3). No description for determination of vigor based on protein levels was given in Chapter 2, as there was not a trend toward change in levels in captivity. However, the P2 population of that study showed decreasing protein content when uric acid levels were highest, agreeing with Slaytor and Chappell (1994) who concluded body proteins are digested in starving termites, resulting in uric acid accumulation. Sapelo Island termites, which had lower protein content than other field groups, had low or absent amounts of uric acid, and therefore were not showing signs of starvation (Table 3.3).

Lipid content ranged from 22.7 ± 8.5 µg lipid/mg termite for BH28 to 62.3 ± 13.4 µg lipid/mg termite for MH (Table 3.2). Within this range were levels reported by Mauldin (1977) of 53 µg lipid/mg termite for *R. flavipes* workers. In the same study, Mauldin reported defaunated workers dropping to as low as 13 µg lipid/mg termite, well below the lowest reading for the current study. Lipid content increased in BH termites up to six-fold after 6 or 9 months in captivity, comparable to results reported in Chapter 2 with lipid levels in captivity increasing up to five-fold. In each case lipid increase may have resulted from an artificial diet of pure cellulose or filter paper, as termites fed wood in Mauldin's 1977 study did not increase in lipid content from field levels.

As previously mentioned workers collected from CH, MH, and FM showed a low body water percentage (Table 3.2). Workers at the opposite extreme for percent body water came from Whitehall Forest and Sapelo Island, with readings significantly higher than the three groups with the lowest percentages (Table 3.2). Water content from field termites ranged from 71.7% for MH to 80.8% for the S group (Table 3.2). In previous studies, 75% to 76% water content was noted for freshly collected *R. flavipes* workers (Sponsler and Appel, 1990; Waller and Curtis, 2003). Mean water percentage was 12 to 15% higher for BH field termites compared to BH groups in captivity for 6 or 9 months (Table 3.3). This similarly was seen in Chapter 2 with up to 16% lower body water readings in captivity. In Chapter 2, body water percentage correlated with lipid levels for each of the three populations of the study, as previously described for *R. flavipes* alates by Shelton and Appel (2001). Field termites for this study also showed a correlation between water percentage and lipid content ($r^2 = 0.822$, Figure 3.1). However, in Chapter 2 lipid content and percent water changed differently early in captivity. In that study lipid levels of each population increased two to three-fold by 2 weeks in captivity and then stabilized through 8 to 12weeks, while water percentage steadily declined across populations through 6 to 12 weeks. Therefore an additional effect besides change in lipid content was apparent for declining water percentage. As a switch in diet to pure cellulose may have been responsible for the initial increase in lipid content from week 0 levels, further study could establish whether water percentage declines for termites fed different foods, which would provide evidence of water loss rather than simply occurring as an effect of increased lipid levels.

Body water percentage as an indicator of termite vigor would be useful information for laboratory personnel who currently must set up extra replicates for their laboratory experiments in the event that a population dies before a bioassay ends. Pest control operators could also

benefit from having a simple method for describing the health of termites by having an ability to identify more vigorous populations around structures to target for treatment. As demonstrated from small standard deviations for means of water readings from pooled samples (Table 3.2), body water measurement is accurate. Although this study allowed 8 hours for oven-drying termites for precise determination of body water levels, a close approximation should be obtainable in a shorter period of time, allowing for a fast, practical method for determining termite vigor.

Geographical region and environment influence the vigor of termite field populations (Lenz, 1982), and each factor may similarly influence levels of biological molecules for individual insects. Termites measured for this study were collected from different parts of Georgia in varying environmental conditions (Table 3.1). Similar readings were measured for termites from CH and MH, with insects collected from inspection ports in landscaped areas of urban college campuses. Also, comparable readings were seen for the two populations of termites collected from Whitehall Forest as well as the four from Sapelo Island, a barrier island well south of other collection sites (Table 3.2). Future study involving collection and measurement of a larger sampling of termites from different geographic areas, field environments, and time of year could determine how each influences levels of biological molecules in termites.

Table 3.1: Natural environments of termites used in this study

Collection site	Abbreviation	Environment	Where found	When collected	Additional Information
Whitehall Forest, site 1	WH1	Woods	Log	July, 2003	
Whitehall Forest, site 2	WH2	Woods	Log	July, 2003	
UGA Chapel	CH	Urban, landscaped	Inspection port	July, 2003	Cardboard bait
Morehouse College	MH	Urban, landscaped	Inspection port	August, 2003	Cardboard bait
Fort Mountain State Park	FM	Woods	Log	August, 2003	5 miles NW of park
Tallulah Gorge State Park	TG	Woods	Bark	August, 2003	3 miles N of park
Mistletoe State Park	MT	Woods	Log	August, 2003	
Sapelo Island big house	S	Yard of house	Inspection port	August, 2003	Wood block bait
Sapelo Island big house, site 10	BH10	Yard of house	Inspection port	November, 2003	Wood block bait
Sapelo Island big house, site 18	BH18	Yard of house	Inspection port	November, 2003	Wood block bait
Sapelo Island big house, site 28	BH28	Yard of house	Inspection port	November, 2003	Wood block bait

Table 3.2. Levels of biomolecules and water content measured from workers of freshly-collected *R. flavipes* field populations, ± the standard deviation

Termite population	Soluble protein[1]	Lipid[1]	Glycogen[1]	Water content[2]	Uric Acid[1]
WH1[3]	46.7 ± 3.4a[4]	24.7 ± 1.0c	2.6 ± 1.3e	78.6bc[5]	9.6 ± 0.9c
WH2	44.7 ± 2.0a	26.7 ± 3.2c	3.2 ± 0.1e	77.9 ± 0.81cd	2.5 ± 0.3d
CH	40.2 ± 2.7ab	52.0 ± 10.6ab	4.1 ± 0.6de	74.6 ± 0.81g	40.4 ± 2.1b
MH	39.6 ± 3.9ab	62.3 ± 13.4a	3.3 ± 0.5e	71.7 ± 0.8h	51.3 ± 2.0a
FM	38.7 ± 6.7ab	45.3 ± 1.0b	4.0 ± 1.0de	75.2 ± 1.0fg	4.5 ± 1.2cd
TG	37.6 ± 8.4abc	49.0 ± 7.1b	ND[6]	76.1 ± 0.9ef	2.4 ± 1.5d
MT	ND	42.7 ± 5.5b	3.9 ± 0.6de	76.8 ± 0.4de	ND
S	28.2 ± 3.4cd	29.0 ± 2.8c	9.0 ± 0.5cd	80.8 ± 0.5a	ND
BH10	24.7 ± 3.2d	29.7 ± 3.2c	25.4 ± 6.8a	78.7 ± 0.8bc	6.1 ± 7.9cd
BH18	32.5 ± 3.4bcd	23.7 ± 4.0c	12.7 ± 1.7c	78.4 ± 0.1bc	1.1 ± 0.6d
BH28	30.8 ± 7.9bcd	22.7 ± 8.5c	19.5 ± 4.3b	79.6 ± 0.6ab	5.8 ± 0.2cd

[1]μg of uric acid, soluble protein, lipid, or glycogen per mg of termite.
[2]Percentage of live weight.
[3]Indicates collection site: WH1 and WH2 = Whitehall forest sites 1 and 2; CH = UGA Chapel; MH = Morehouse College; FM = Fort Mountain park; TG = Tallulah Gorge park; MT = Mistletoe park; SAP = Sapelo Island, Reynolds mansion, August 2003;
BH10, BH18, and BH28 = Reynolds mansion, November 2003.
[4]Means followed by the same letter within each column are not significantly different from each other by Duncan's multiple range test at the 0.05 level of probability.
[5]One reading only.
[6]ND = No data.

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Table 3.3: Levels of biological molecules from *R. flavipes* workers measured within 24 hours of collection or after 6 or 9 months in captivity, ± the standard deviation

	Field	Captivity
Test	**BH10[1]**	**BH10[2]**
uric acid	6.1 ± 7.9a[4]	1.0 ± 0.1b
soluble proteins	24.7 ± 3.2a	36.8 ± 3.2a
lipid	29.7 ± 3.2a	140.3 ± 9.5b
glycogen	25.4 ± 6.8a	22.7 ± 1.8a
water content	78.7 ± 0.78a	68.0 ± 1.7b
	BH18	**BH18[2]**
uric acid	1.1 ± 0.6a	3.5 ± 2.8a
soluble proteins	32.5 ± 3.4a	38.2 ± 4.2a
lipid	23.7 ± 4a	145.3 ± 18.5b
glycogen	12.7 ± 1.7a	22.9 ± 3.9b
water content	78.4 ± 0.1a	67.7 ± 0.4b
	BH28	**BH28[3]**
uric acid	5.8 ± 0.2a	0.07 ± 0.1b
soluble proteins	30.8 ± 7.9a	29.3 ± 2.6a
lipid	22.7 ± 8.5a	127.3 ± 8.5b
glycogen	19.5 ± 4.3a	29.4 ± 4.6b
water content	79.6 ± 0.6a	70.2 ± 1.0b

[1]BH = "Big House" inspection port at Reynolds Mansion, Sapelo Island
[2]Measured after 9 months in the laboratory.
[3]Measured after 6 months in the laboratory.
[4]Measurements followed by the same letter across a row are not significantly different by the Student's paired t-test.

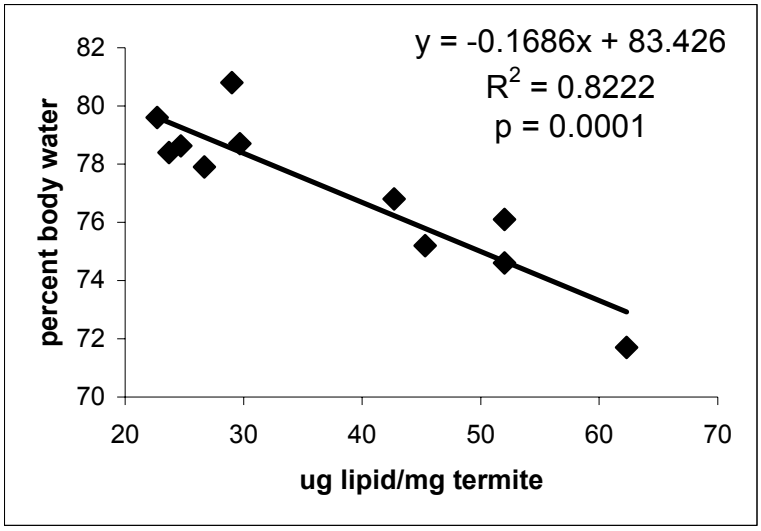

Figure 3.1: Linear regression for body water percentage and lipid content (µg lipid/mg termite) measured from 11 field populations of *R. flavipes* workers

CHAPTER 4

CONCLUSIONS

The objective of this study was to obtain and compare levels of stored biological molecules from *R. flavipes* workers held in captivity under conditions similar to bioassay, as well as from different field populations immediately after collection. The data collected was used to identify potential methods for determining termite vigor predictably. Previously established methods for determining vigor of small termite groups in captivity, percent survivorship and consumption rate, are not predictive measures of vigor.

Running speed, glycogen content, and soluble protein content were excluded from further consideration as being potential predictors of termite vigor because none showed a significant correlation for change over time in the laboratory; also, values were not significantly different above and below 80% survivorship, a threshold for separation of healthy from unhealthy termites (Su and LaFage, 1984a). Uric acid content was expected to increase steadily in *R. flavipes* workers in captivity (Potrikus and Breznak, 1980a), which would allow for the identification of a threshold level of uric acid for separation of healthy from unhealthy termites. However, levels of the molecule changed differently between populations of the current study, and in some cases between experimental units of the same population. As consistent changes did not occur across populations uric acid was not considered as potentially predictive of vigor. Termite live weight has previously been reported to be two-fold heavier in an older, declining *C. formosanus* population compared to measurements taken from the same population 16 years earlier (Grace et al, 1995). However, since the current study used workers of different instars, and specific weights of termites from younger or older *R. flavipes* field populations is unknown, it would not be possible to categorize a specific termite live weight as a threshold for separation of vigorous termite groups from weaker ones.

Body water percentage, determined to decrease consistently and significantly across populations above the 80% survivorship threshold for health, was hypothesized to be a predictor of vigor (Chapter 2). Combining all water percentages recorded above as well as below 80% survivorship and comparing means by one-way ANOVA showed termites had significantly lower percent water below 80% survivorship. The midpoint between means of each of these categories, 72% body water, was suggested in Chapter 2 as a threshold for separating healthy from weak groups of termites.

Of 11 field groups measured for percent body water, workers from one group measured below the 72% threshold defined as the limit for healthy termites. Readings of 74.6% and 75.2% obtained for two other groups were higher than the healthy threshold for water percentage, but also well below the population with the highest field body water reading of 80.8%. As both of the lower readings were close to 72% body water, each could be considered a weak group of termites compared to those measuring near 80% body water. In Chapter 2, two populations with body water levels close to 80% from the field survived for 24 weeks in captivity, while termites from a third population that measured less than 75% body water at week 0 died by 14 weeks. The earlier mortality of insects from the group with lower percent body water demonstrated that it was weaker than the other two groups, and suggests that the threshold for acceptably low water should be adjusted so that termites just above 72% water could also be regarded as unhealthy. Therefore 72% body water should be regarded as an absolute lower limit for acceptable health, and insects measuring 74 to 75% should be considered less ideal for use in laboratory bioassay compared to those with body water readings close to 80%. Further study could confirm that termites with higher percent body water survive longer in the laboratory, and therefore are more vigorous.

In a previous study uric acid was reported to increase steadily in workers of two *R. flavipes* populations in captivity (Potrikus and Breznak, 1980a). Workers from one of these populations increased in uric acid at a rate of 2 to 3% per month, comprising 45% of the dry weight of the insects after 18 months. However, results of the current study show that uric acid levels of termites should not be assumed to increase steadily in the laboratory. Uric acid levels were low or undetectable after 6 and 9 months in captivity (Chapter 3), and decreased by 50% in termites showing accumulation from the field (Chapter 2). The population showing decreasing uric acid content in captivity had significantly lower levels below 80% survivorship than above (Tukeys HSD, $p = 0.001$, Table 2.11). This provides evidence that the molecule was being mobilized from fat body to the hindgut, the only site where uric acid digestion could occur in *R. flavipes* as uricolytic bacteria are present there (Breznak, 2000). Uric acid accumulation is a sign of starvation in termites (Slaytor and Chappell, 1994; Korb and Lenz, 2004), and termites showing elevated uric acid levels should be regarded as weakened from starvation whenever it occurs.

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