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Evaluation of novel and traditional measures for vigor of laboratory-cultured termites, *Reticulitermes flavipes* (Kollar)

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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 biological molecules (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. Of the novel measures tested, lipid and body water percentage show promise in distinguishing weak from vigorous groups of termites, with body water percentage a more practical means of measurement. Low body water percentage was concluded to be an indicator of weak groups of termites.

Keywords: Vigor; Termites; *Reticulitermes*; Biological molecules; Body water

1. 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 La Fage, 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 groups of termites before use in bioassay have become too weak for the experiment to continue (Su and La Fage, 1984a, b). An ability to predict 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 movement of individual insects across a predetermined distance might be a means of describing vigor. If the health of 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 insects 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, 1972; Mauldin, 1977; Potrikus and Breznak, 1980; 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

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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; also, 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. Groups of heavier individuals are thought to be characteristic of older, declining populations (Su and La Fage, 1984a; Grace et al, 1995). If stored biomolecules, water content, weight, and running speed could be separated between "healthy" or "unhealthy" levels, each could potentially provide a predictive identifier of population health. The current study tested novel, predictive measures of termite population vigor.

2. Materials and methods

2.1. Field collection and rearing conditions of Reticulitermes flavipes workers

Logs infested with *R. flavipes* (Kollar) workers were collected in July 2003 from two sites at least 1 km apart at Whitehall Forest, Clarke County, GA, designated as Populations 1 and 2 (*P*1 and *P*2). Insects were also collected from an inspection port (*P*3) located next to the south wall of the University of Georgia Chapel in Athens, Clarke County, GA. 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).

2.2. Experimental design and laboratory rearing procedure

Experimental units consisted of Quikrete® sand (10 g) added to a plastic Petri dish (20 mm × 100 mm in diameter) and moistened with 2 ml of distilled water. A section of Tygon® tubing (19 mm × 10 mm in 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 *P*1, 12 Petri dishes were prepared; for *P*2 and *P*3, 15 dishes were set up due to a surplus of termites. Experimental units for the *P*2 population contained first and second instar larvae comprising approximately 5% of the total. Nymphs numbered 6% of the total for *P*1, 2% of *P*3, and were not present in the *P*2 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 × 19 cm × 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 10 workers above third instar were pooled from each population and weighed

Table 1
Mean food removal rate and percent change in cellulose removal rate from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date

Week	*P*1		*P*2		*P*3	
	Mean food removal rate (mg cellulose removed/g termite/day)	Percent change	Mean food removal rate (mg cellulose removed/g termite/day)	Percent change	Mean food removal rate (mg cellulose removed/g termite/day)	Percent change
0	N/A	N/A	N/A	N/A	N/A	N/A
2	25.3	0	34.8	0	33.6	0
4	25.1	−1	35.2	1	30.6	−9
6	20.9	−17	29.6	−15	29.9	−11
8	25.5	1	25.1	−28	23.1	−31
10	19.3	−24	22.3	−36	26.1	−22
12	20.8	−18	24.3	−30	23.3	−31
14	21.9	−13	16.7	−52	21.5	−36
16	23.1	−9	24.4	−30		
18	21.6	−15	24.8	−29		
20	20.8	−18	24.4	−30		
22	21.3	−16	25.1	−28		
24	21.3	−16	22.9	−34		

together for estimation of total lipid, uric acid, soluble proteins, glycogen, and body water percentage for one termite equivalent. 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 *P*1, week 24 for *P*2, and week 14 for *P*3. For determination of soluble protein content, five workers were pooled at weeks 22 and 24 for *P*1, and weeks 18, 20, and 22 for *P*2; three workers were pooled at week 24 for *P*2 and week 14 for *P*3. For lipid determination, five workers were pooled at week 24 for *P*1, weeks 22 and 24 for *P*2, and week 14 for *P*3. For determination of glycogen levels, five workers were pooled at weeks 22 and 24 for *P*1, and weeks 18, 20, and 22 for *P*2; three workers were pooled at week 14 for *P*3.

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 workers to move a distance of 6 cm, as well as food removal rate and

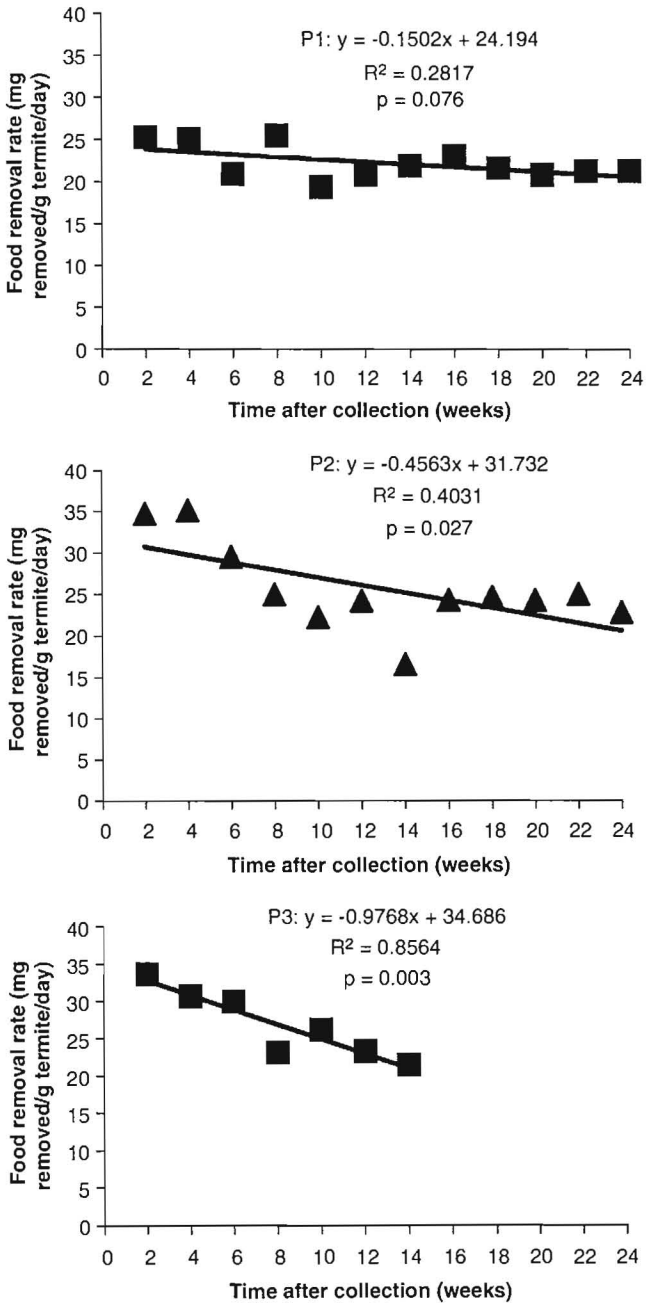

Fig. 1. Linear regression of change in food removal rate (mg cellulose removed/g termite/day) over time in captivity for *R. flavipes* workers from three field populations.

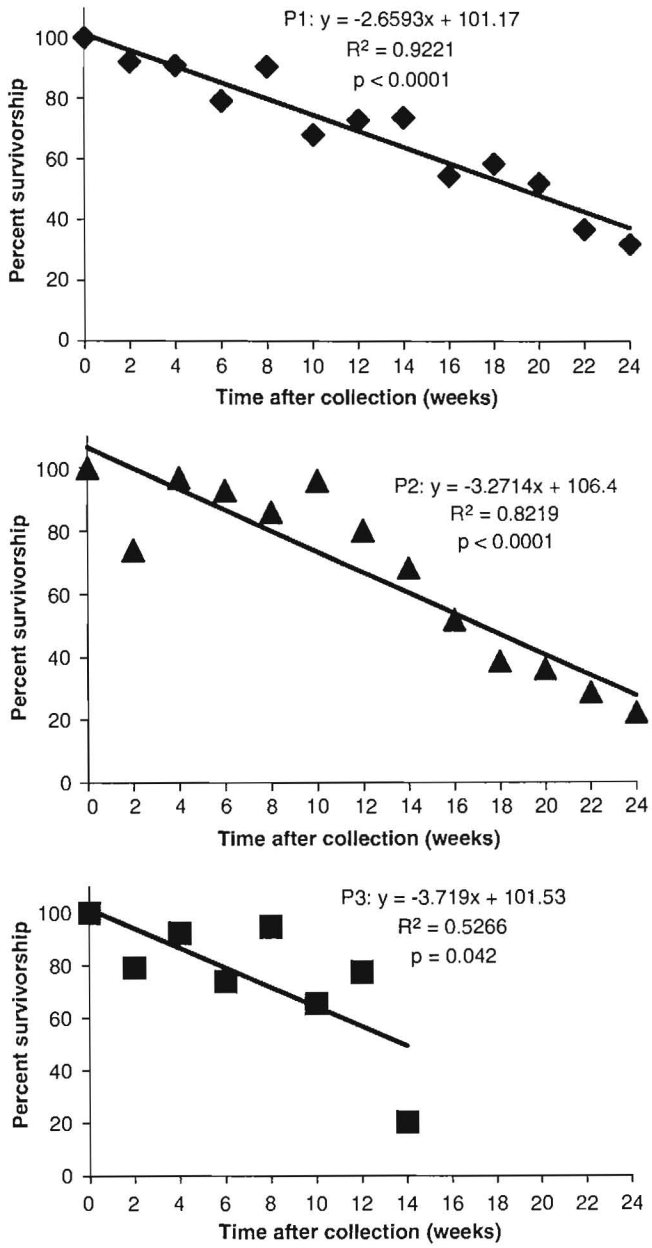

Fig. 2. Linear regression of change in percent survivorship over time in captivity for *R. flavipes* workers from three field populations.

percent survivorship, two established techniques for monitoring the vigor of control insects in laboratory bioassay. Workers 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 from the group surviving.

2.3. Novel measurements of vigor

2.3.1. Survivorship

Survivorship was determined at each biweekly test period by dividing the number of surviving workers in each Petri dish by the original number of insects, 250.

2.3.2. Food removal rate

Remaining cellulose in the section of Tygon® tubing after consumption or removal to other areas of the Petri dish was weighed after drying at 85 °C for 24 h in a convection oven (VWR). Food removal rate in units of mg cellulose removed/g termite/day was then determined using the method of Su and La Fage (1984b) for estimation of consumption rate.

2.3.3. Live weight

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

2.3.4. Uric acid

Uric acid content was measured following the procedure of Potrikus and Breznak (1980) using a diagnostic kit (Sigma 292).

2.3.5. Soluble proteins

The Bradford method (Bradford, 1976) was used to determine levels of soluble proteins (Bio-Rad Laboratories) with a standard of bovine serum albumin.

2.3.6. 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 h to break cells and release glycogen. As termite samples thawed glycogen standards (Sigma) were prepared. Termite samples and glycogen standards were heated 10 min in a water bath at 60 °C, causing glycogen to

Table 2
Mean survivorship percentage for three groups of laboratory-cultured *R. flavipes* workers by sampling date

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 3
Mean live weight (mg) and percent change in live weight from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

Week	P1		P2		P3	
	Mean live weight (mg)	Percent change	Mean live weight (mg)	Percent change	Mean live weight (mg)	Percent change
0	3.36±0.15	0	3.04±0.10	0	2.84±0.15	0
2	3.58±0.25	7	3.05±0.10	0	2.93±0.15	3
4	3.59±0.27	7	3.11±0.06	2	3.01±0.11	6
6	3.55±0.05	6	3.24±0.14	7	3.35±0.07	18
8	3.20±0.16	−5	3.30±0.11	9	3.10±0.08	9
10	3.51±0.15	4	3.24±0.06	7	3.35±0.03	18
12	3.55±0.13	6	3.14±0.21	4	3.35±0.20	18
14	3.62±0.18	8	3.42±0.09	13	3.54±0.13	25
16	3.56±0.09	6	3.27±0.15	8		
18	3.92±0.15	17	3.27±0.18	8		
20	3.39±0.10	1	3.41±0.19	12		
22	3.37±0.21	0	3.37±0.31	11		
24	3.33±0.25	−1	3.67±0.35	21		

precipitate, followed by centrifuging at 4000 rpm for 5 min, forming a pellet. Supernatant was then poured off and discarded, with traces of liquid removed from around the pellet with a pipetter. To each tube containing a glycogen pellet from homogenized termites 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 glycogen pellets, as well as 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 h at medium speed. Following centrifugation for 5 min at 12,000 rpm, between 25–200 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 min at room temperature. Supernatant was transferred to microplate wells (0.15 ml per well) (Becton Dickinson) and absorbances determined using a SpectraMax model 340 spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA) at 505 nm.

2.3.7. Lipid

Total lipids were extracted based on the procedure of Zera and Larsen (2001). Live termites were 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). Insects were sonicated on ice (Branson sonifier model 250, VWR) and centrifuged 5 min 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.

2.3.8. Body water

Insects dried for the uric acid assay were those used to determine body water content. After weighing, groups of 10 live termites were dried at 85 °C for 8 h in a convection oven (VWR). Dried termites were held at room temperature in a desiccation chamber containing Drierite® crystals for 5 min before reweighing. Percent body water was determined by obtaining the difference between live and dry weights, divided by the live weight.

2.3.9. Running speed

A straight line was drawn on a sheet of 8.5″ × 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-phenoxyethanol (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

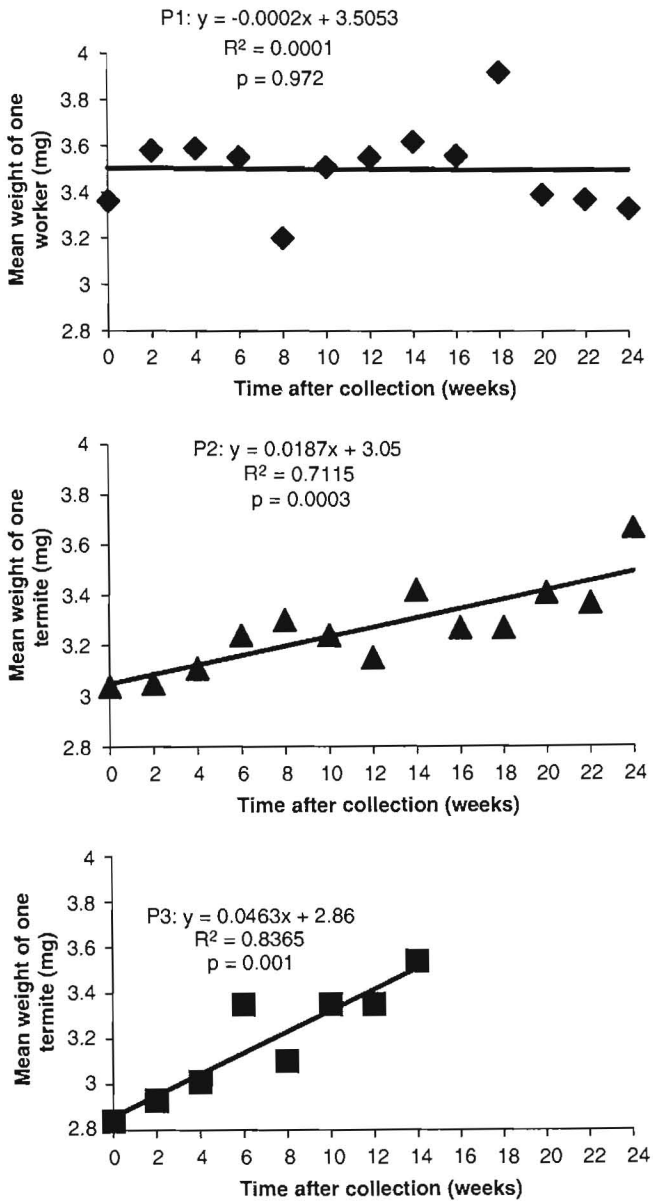

Fig. 3. Linear regression of change in live mass of one termite over time in captivity for *R. flavipes* workers from three field populations.

Table 4
Mean μg uric acid/mg termite and percent change in uric acid from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

Week	*P*1		*P*2		*P*3	
	Mean μg uric acid/mg termite	Percent change	Mean μg uric acid/mg termite	Percent change	Mean μg uric acid/mg termite	Percent change
0	9.6±1.0	0	2.5±0.3	0	40.4±2.1	0
2	7.8±2.7	−19	21.9±3.0	776	40.5±4.6	0
4	4.6±1.8	−52	4.7±5.7	88	27.2±3.9	−33
6	6.1±3.1	−36	1.3±1.1	−48	21.7±0.6	−46
8	1.0±1.7	−90	8.2±7.0	228	24.4±3.5	−39
10	26.6±4.2	177	5.5±1.6	120	19.9±2.9	−51
12	11.9±2.2	24	21.6±5.1	764	22.1±3.6	−45
14	11.5±2.7	20	10.7±2.9	328	31.1±14.9	−23
16	39.2±0.8	308	44.2±6.3	1668		
18	7.3±0.4	−24	108.8±15.2	4252		
20	4.5±3.4	−53	44.9±4.3	1696		
22	2.1±1.6	−78	66.8±18.4	2572		
24	68.7±4.0	616	72.8±17.4	2812		

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 insects 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 × 19 cm × 9.5 cm), and the box lid replaced. A new photocopied sheet was traced with ink for each termite tested. Running speeds of 30 workers were recorded per replicate group.

2.4. Statistical analysis

Regression analysis ($p<0.05$) (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. Cellulose removal rate was calculated using a formula devised by Su and La Fage (1984b) in units of mg cellulose eaten/g termite/day.

Su and La Fage (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 La Fage, 1984b), supported in the current study from significant negative correlations between survivorship and time for each population (Fig. 2, $p<0.05$). Linear survivorship curves were used to approximate the point when 80% survivorship was reached for each population, determined as 8 weeks for *P*1 and *P*2, and 6 weeks for *P*3 (Fig. 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 ($p<0.05$, Table 9) and Tukey's HSD ($p<0.0033$, Tables 2.10–2.11).

3. Results

3.1. Summary of findings

Populations collected from Whitehall forest (*P*1 and *P*2) had workers survive through the conclusion of the 24-week study (Table 2). Termites in all but one *P*3 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). Overall consistent trends were not found for any novel measure of vigor between the three populations examined in the study (Tables 3–8). 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.

3.2. Food removal rate

*P*1 food removal rates ranged between 19.3–25.3 mg cellulose removed/g termite/day through 24 weeks, while *P*2 measured 16.7–35.2 mg/g/day through 24 weeks, and *P*3 21.5–33.6 mg/g/day through 14 weeks (Table 1). Compared to initial rates measured 2 weeks after field collection, each

population tended to remove less cellulose later in captivity (Table 1). Beyond 8 weeks, cellulose removal declined by 16±4% for *P*1, 34±8% for *P*2, and 30±7% for *P*3 (Table 1). The mean of all food removal rates recorded below 80% survivorship was significantly lower than the rates above 80% survivorship (ANOVA, $p<0.0001$) (Table 1). This agreed with a past description of consumption rate decreasing in unhealthy termite groups in captivity (Su and La Fage, 1984b). *P*1 food removal rate was lower than *P*2 and *P*3 at each biweekly test period except for weeks 8 and 14 (Table 1); however *P*1 removed cellulose at a consistent rate compared to *P*2 or *P*3 (Fig. 1). This was illustrated by a significant negative correlation between food removal rate and time in captivity for *P*2 ($R^2 = 0.403$) and *P*3 ($R^2 = 0.856$), but not *P*1 ($R^2 = 0.282$) (Fig. 1).

3.3. Survivorship

Survivorship decreased steadily in captivity for *P*1, decreased slowly at first for *P*2 followed by a sharper decline than *P*1, and wavered for *P*3 between 65–95% per experimental unit until most insects died between 12–14 weeks (Fig. 2). Although 80% survivorship was estimated to have been reached at 8 weeks for *P*1 and *P*2, and 6 weeks for *P*3, each population had at least one experimental unit below 80% survivorship earlier than indicated by survivorship curves (Fig. 2). For instance, *P*2 and *P*3 survivorship at 2 weeks was 74% and 79%, respectively (Table 2). A significant negative correlation was shown for each population between survivorship and time in the laboratory (*P*1, $R^2 = 0.922$, $p<0.0001$; *P*2, $R^2 = 0.822$, $p<0.0001$; *P*3, $R^2 = 0.527$, $p = 0.042$) (Fig. 2).

3.4. Live weight

*P*1 worker live weights were generally steady in captivity, while *P*2 and *P*3 weights tended to increase (Table 3). Exceptions for *P*1 occurred at week 18, with 17% heavier weights compared to the day of collection; and at weeks 20–24, when weights were similar to week 0 (Table 3). In contrast, *P*2 workers at weeks 20–24 were 10–20% heavier compared to the day of collection (Table 3). *P*3 workers increased in weight faster in captivity than *P*1 or *P*2, with 18% heavier weights measured at 6 weeks compared to 6% for *P*1 and 7% for *P*2, and 25% heavier weights at week 14 (Table 3). Workers of all populations combined were significantly heavier below 80% survivorship compared to above (ANOVA, $p = 0.01$, Table 9). *P*1 did not show a positive correlation between weight change and time in the laboratory ($R^2 = 0.0001$, $p = 0.972$) as did *P*2 ($R^2 = 0.712$, $p = 0.0003$) and *P*3 ($R^2 = 0.837$, $p = 0.001$) (Fig. 3).

3.5. Uric acid

Mean uric acid content differed significantly between each population on the day of collection. *P*1 was significantly higher than *P*2 at week 0 (paired *t*-test, $p = 0.032$), while *P*3 was significantly higher than both *P*1 ($p = 0.0006$) and *P*2 ($p<0.0001$) (Table 4). At week 0, *P*3 was four-fold higher than *P*1 and sixteen-fold higher than *P*2 (Table 4).

Through 14 weeks, uric acid content was generally low for *P*1 and *P*2. *P*3 workers were measured at 40±2 μg uric acid/mg termite just after field collection, but declined as much as two-fold at week 10 (Table 4). *P*1 levels tended to be low throughout the study, with an exception at 24 weeks with 600% higher levels than at week 0 (Table 4). However, at week 22 the *P*1 workers measured significantly lower compared to week 0 (paired *t*-test, $p = 0.0013$) (Table 4). From weeks 16–24, *P*2 uric acid content ranged from 44.5 to 108.8 μg uric acid/mg termite (Table 4), or 1500–4200% higher than at week 0 (Table 4).

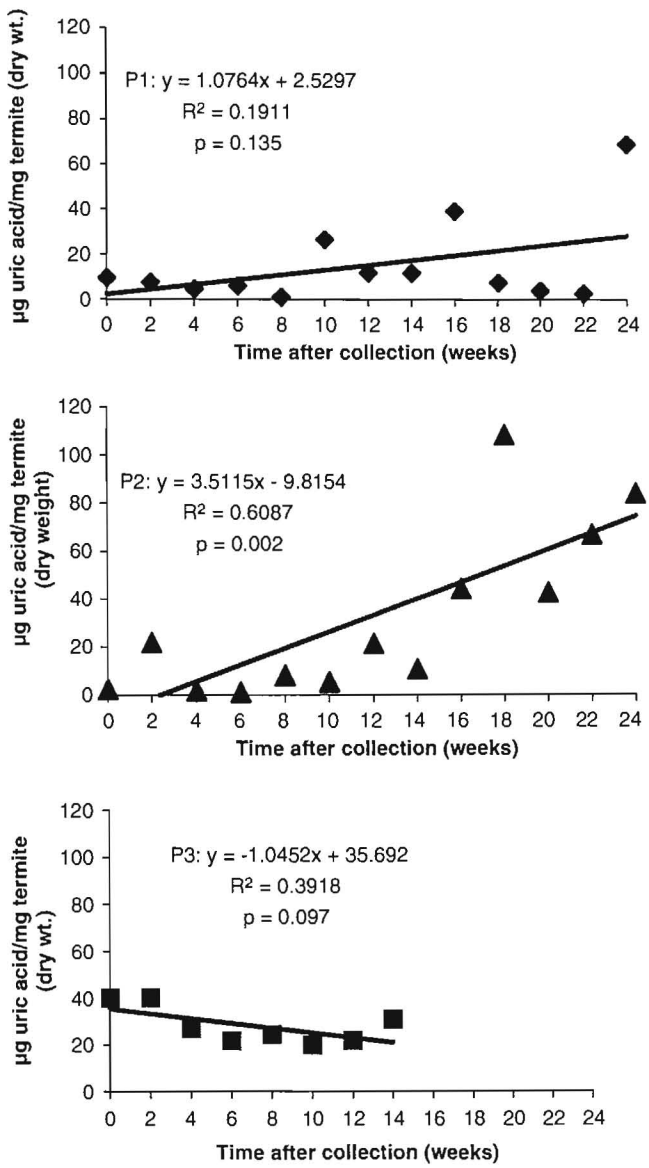

Fig. 4. Linear regression of change in uric acid content over time in captivity for *R. flavipes* workers from three field populations.

*P*1 did not show a correlation between uric acid content and time in the laboratory ($R^2 = 0.191$, $p = 0.318$), *P*2 showed a significant positive correlation ($R^2 = 0.609$, $p = 0.001$), and *P*3 showed a weak negative correlation ($R^2 = 0.392$, p = 0.01) (Fig. 4).

3.6. Soluble proteins

*P*1 and *P*2 soluble protein levels remained within a narrow range of 45–60 μg protein/mg termite throughout the study (Table 9). An exception occurred for *P*1 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 9). For *P*2 exceptions occurred at weeks 18, 22, and 24 when readings were 17–38% lower compared to week 0. Only three measurements were taken for *P*3 over 14 weeks ranging from 33 to 54 μg protein/mg termite (Table 9). There was not a significant correlation between soluble protein levels and time in the laboratory for *P*1 ($R^2 = 0.104$) or *P*2 ($R^2 = 0.128$), nor was there a significant difference between *P*1 and *P*2 means above and below 80% survivorship (ANOVA, $p = 0.95$, Table 9).

3.7. Glycogen

Glycogen levels increased 100–200% for all groups through 2 weeks in captivity (Table 5). Workers of each

Table 5
Mean μg glycogen/mg termite and percent change in glycogen from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ±the standard deviation

Week	*P*1		*P*2		*P*3	
	Mean μg glycogen/mg termite	Percent change	Mean μg glycogen/mg termite	Percent change	Mean μg glycogen/mg termite	Percent change
0	2.6±1.3	0	3.1±0.2	0	4.1±0.6	0
2	6.3±0.9	142	6.6±1.9	113	12.2±1.8	198
4	7.6±1.4	192	7.9±1.2	155	9.7±1.7	137
6	6.3±2.6	142	4.9±1.3	58	11.7±1.5	185
8	8.8±1.4	238	3.0±0.4	−3	7.1±1.1	73
10	2.5±0.6	−4	5.0±1.9	61	8.8±0.9	115
12	5.3±1.2	104	6.6±2.6	113	7.6±4.2	85
14	3.2±0.8	23	1.5±0.4	−52	7.0±2.1	71
16	4.5±0.8	73	4.8±1.3	55		
18	4.8±0.9	85	2.0±1.0	−35		
20	5.2±2.1	100	4.3±2.7	39		
22	2.4±6.6	−8	14.3±6.5	361		
24	2.6±3.5	0	7.1±0.3	129		

Table 6
Mean μg lipid/mg termite and percent change in lipid from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ±the standard deviation

Week	*P*1		*P*2		*P*3	
	Mean μg lipid/mg termite	Percent change	Mean μg lipid/mg termite	Percent change	Mean μg lipid/mg termite	Percent change
0	24.5±6.5	0	26.7+3.5	0	51.9±10.4	0
2	70.9±14.1	184	76+6.8	181	122.9±11.3	137
4	65.2±13.1	160	74.6±5.4	178	129.3±21.3	148
6	82.3±8	228	91.7±2.5	241	125±1.6	140
8	85.3±13.8	240	67.6±4.4	152	142.6±6.4	175
10	57.9±12.4	132	115.3±32.2	326	136.7±16.7	163
12	87.8±13.6	252	53.1±4.4	96	126.6±10.0	144
14	107.4±8.7	328	107±9.1	296	167±9.5	221
16	132.9±10.8	432	62.6±16.8	133		
18	141.7±3.5	464	44.9±3.8	67		
20	112±32.4	348	76.6±10	185		
22	131.9±20.8	432	79.7±20	196		
24	138.6±10.2	456	66.5±12.5	144		

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 *P*1 between weeks 0–24, while *P*2 measured 5.5±3.3 μg/mg between weeks 0–24, and *P*3 measured 8.5±2.7 μg/mg between weeks 0–14 (Table 5). Means of levels above and below 80% survivorship did not differ significantly (ANOVA, $p = 0.17$, Table 9). There was not a correlation between change in glycogen levels and time in captivity (*P*1, $R^2 = 0.043$; *P*2, $R^2 = 0.012$; *P*3, $R^2 = 0.052$).

3.8. Lipid

Lipid content increased two to three-fold for each population between the day of collection and 2 weeks in captivity (Table 6). By 2 weeks, *P*1 and *P*2 lipid content increased 180%, and *P*3 140% (Table 6). Each of these increases was significant (paired *t*-test, $p<0.0001$) (Table 6). Increase in lipid levels occurred in the *P*3 population despite two-fold higher readings at week 0 compared to *P*1 and *P*2 (Table 6). Subsequently, *P*3 workers tended to have elevated lipid content compared to workers of the other two populations through 14 weeks in the laboratory (Table 6). Means of all lipid levels measured below 80% survivorship were significantly higher compared to above (ANOVA, $p = 0.04$, Table 9).

Each population showed elevated lipid content in captivity two to five-fold above week 0 readings (Table 6). *P*1, after an initial increase in lipid content through 2 weeks, was generally steady through 14 weeks before increasing again, plateauing between 16 and 24 weeks (Table 6). Toward the end of the study *P*1 lipid content was as much as 460% higher than at week 0 (Table 6). Readings for *P*1 and *P*2 were similar through week 8, and throughout the study *P*2 fluctuated two to four-fold above the week 0 reading (Table 6). *P*3 workers tended to be steady in lipid content in captivity except for an increase to 167±10 μg lipid/mg termite at the last measurement at 14 weeks, the highest reading recorded for any group in the study (Table 6).

*P*1 showed a significant positive correlation in lipid levels through 24 weeks ($R^2 = 0.805$, $p<0.0001$), as did *P*3 through 14 weeks ($R^2 = 0.590$, $p = 0.027$). *P*2 lipid levels did not show a trend towards change in lipid content in captivity ($R^2 = 0.009$, $p = 0.757$) (Fig. 5).

3.9. Body water

Body water just after field collection was measured at 78.6% for *P*1 (1 reading), 77.9% for *P*2, and 74.6% for *P*3. 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±0.8% for *P*1 (paired *t*-test, $p = 0.0046$), 71.7±1.1% for *P*2 ($p = 0.0096$), and 67.6±0.6% for *P*3 ($p = 0.0032$) (Table 7). Percent water declined steadily through 6 weeks regardless of differences in the week 0 readings between populations (*P*1, $R^2 = 0.96$, $y = -1.1x+78.1$, $p = 0.02$; *P*2, $R^2 = 0.96$, $y = -1.1x+78.0$, $p = 0.02$; *P*3, $R^2 = 0.96$, $y = -1.1x+74.3$, $p = 0.02$). *P*1 and *P*3 each dropped steadily in percent water content throughout the study, with a final measurement of 66% for *P*1 at 24 weeks and 64% for *P*3 at 14 weeks (Table 7). The lowest body water percentage recorded for *P*2 was 70%, measured at 20 weeks (Table 7). *P*1 and *P*2 body water percentages were similar through 8 weeks, the point when 80% survivorship was reached for each population (Table 7, Fig. 6). The mean of all body water measurements recorded below 80% survivorship were significantly lower than above 80% survivorship (ANOVA, $p = 0.01$, Table 9).

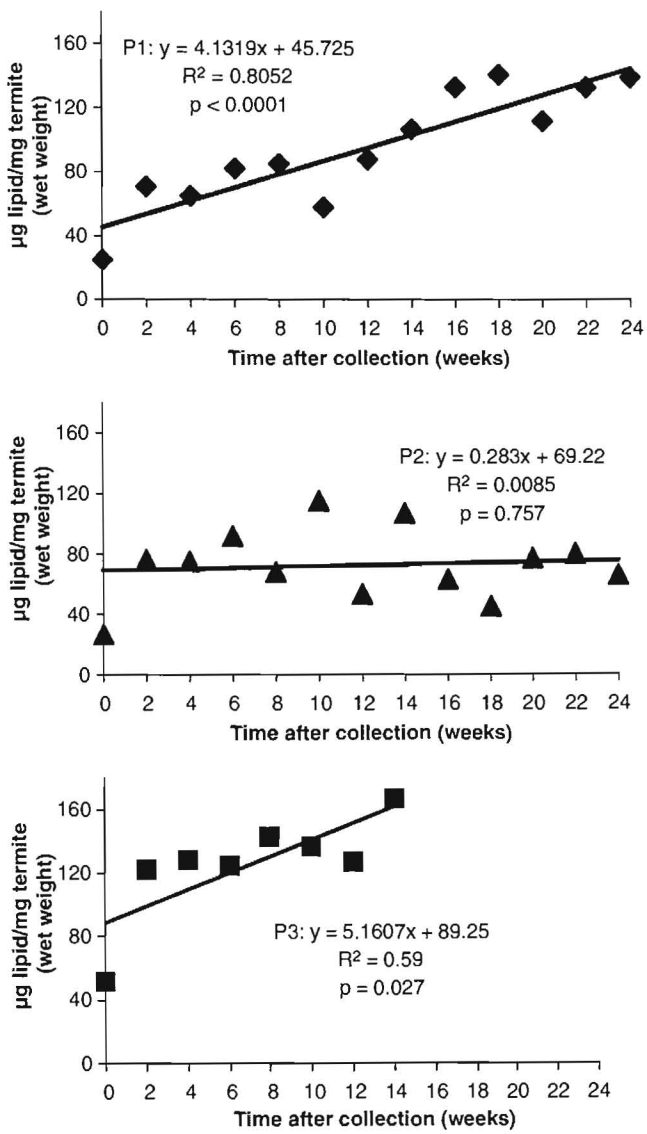

Fig. 5. Linear regression of change in lipid content over time in captivity for *R. flavipes* workers from three field populations.

Table 7
Mean percent body water/termite and percent change from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ± the standard deviation

Week	*P*1[a]		*P*2		*P*3	
	Mean percent body water/termite	Percent change	Mean percent body water/termite	Percent change	Mean percent body water/termite	Percent change
0	78.6	0.0	77.9±0.9	0.0	74.6±0.5	0.0
2	75.6±0.9	−3.8	76.4±0.4	−1.9	71.3±0.6	−4.3
4	73.2±0.5	−6.9	72.9±0.5	−6.4	70.6±1.1	−5.4
6	72.2±0.8	−8.1	71.7±1.1	−8.0	67.6±0.6	−9.4
8	73.7±0.6	−6.2	73.7±0.7	−5.4	65.5±0.2	−12.2
10	73.1±1.4	−7.0	71.7±0.8	−8.0	65.3±1.0	−12.5
12	73.0±0.3	−7.1	76.2±0.3	−2.2	63.7±0.6	−14.6
14	72.1±0.3	−8.3	72.0±0.3	−7.6	64.4±0.8	−13.8
16	71.2±1.9	−9.4	74.1±0.8	−4.9		
18	68.1± 1.4	−13.4	75.1±1.0	−3.6		
20	73.5±1.1	−6.5	70.2±0.6	−9.9		
22	68.0±0.5	−13.5	71±0.8	−8.9		
24	66.0±0.9	−16.0	73.1±1.8	−6.2		

[a] *P*1, week 0: Reading of one sample.

*P*1 and *P*3 each showed a significant negative correlation between percent body water and time in the laboratory (*P*1, $R^2 = 0.708$, $p = 0.0003$; *P*3, $R^2 = 0.915$, $p = 0.0002$), but *P*2 did not ($R^2 = 0.261$, $p = 0.074$) (Fig. 6). *P*3 body water percentage declined at more than twice the rate of *P*1 (Fig. 6). While each group had a similar rate of decrease in percent water through 6 weeks, *P*3 continued declining until the last measurement at 14 weeks, while *P*1 readings stabilized. Subsequently, later *P*3 water percentages were the lowest measured in the study, and 12–15% lower compared to week 0 (Table 7).

3.10. Running speed

*P*1 termites moved faster than 3.0 s/6 cm for almost half of the biweekly measurements, compared to one time each for *P*2 (week 8) and *P*3 (week 16) (Table 8). Mean speeds of *P*2 workers were 3.9 s or slower for almost half of the measurements, while mean *P*1 and *P*3 running speed was slower than 3.9 s only one time (*P*1, week 22 and *P*3, week 0) (Table 8). Through 24 weeks, one third of individual *P*1 termites moved 2.6 s or faster across a 6 cm distance, compared to 11% for *P*3 (14 weeks) and 7% for *P*2 (24 weeks). The fastest mean running speed of 30 termites was 2.55+0.26 s, which occurred at 18 weeks for *P*1 (Table 8). Overall there was not a significant correlation between running speed and time in captivity for any of the groups (*P*1, $R^2 = 0.119$, $p = 0.70$; *P*2, $R^2 = 0.20$, $p = 0.13$; *P*3, $R^2 = 0.444$, $p = 0.07$), nor was there a significant difference for all mean running speeds recorded above 80% survivorship compared to below (ANOVA, $p = 0.61$, Table 9).

4. Discussion

4.1. Evaluation of novel measures of vigor for R. flavipes workers

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 La Fage (1984a, b) 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 La Fage, 1984b). This was confirmed in the current study with significant R^2 values for linear decrease in survivorship of each population (Fig. 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 La Fage (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 La Fage (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

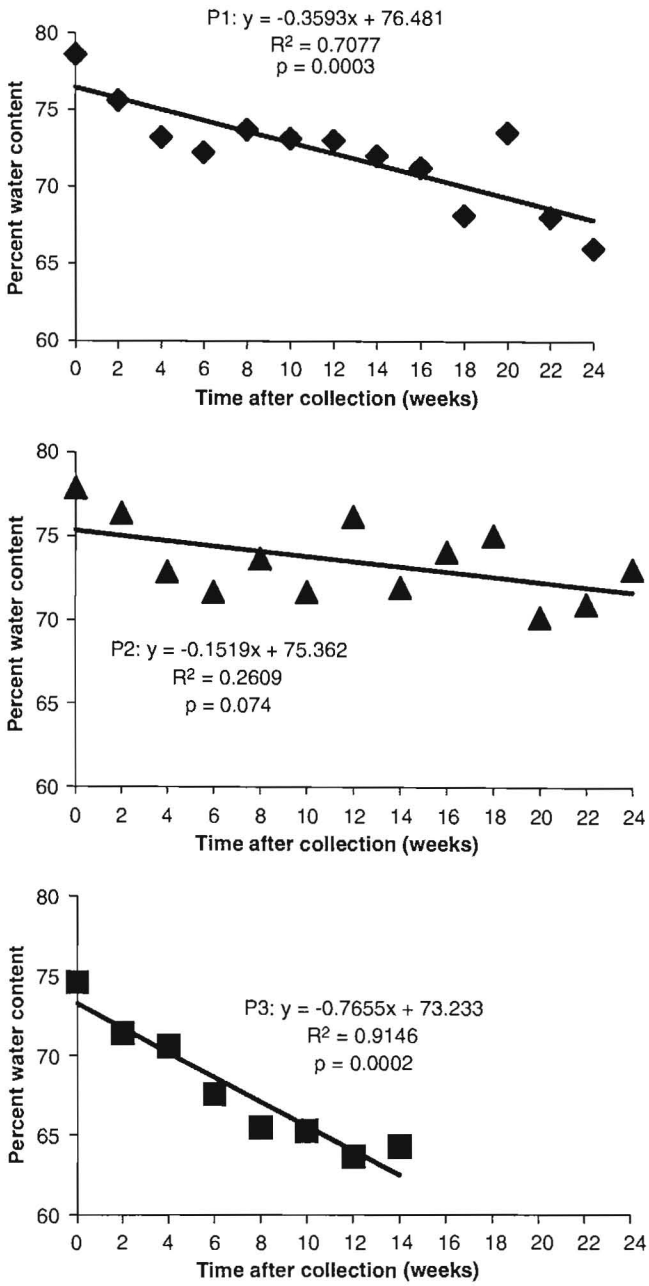

Fig. 6. Linear regression of change in percent body water/termite over time in captivity for *R. flavipes* workers from three field populations.

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 La Fage'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 non-significant 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 9). Uric acid content, although significantly different between healthy and unhealthy levels across populations (ANOVA, $p = 0.01$, Table 9), had dissimilar trends toward change in captivity between populations (Fig. 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 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 9), 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 La Fage, 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 (*P*3) and therefore the least vigorous group but had lighter workers at week 0 than *P*1 or *P*2 (Table 3). 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.

4.2. Overview of potentially predictive measures of vigor for *R. flavipes* workers

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 (Figs. 5 and 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 *P*1 ($R^2 = 0.81$, $p<0.0001$) and *P*3 ($R^2 = 0.59$, $p = 0.03$), but not *P*2 ($R^2 = 0.009$, $p = 0.757$) (Fig. 5). Percent body water decreased in captivity for *P*1 ($R^2 = 0.71$, $p<0.0001$) and *P*3 ($R^2 = 0.91$, $p<0.0001$),

Table 8
Mean running speed (s) and percent change from week 0 for three groups of laboratory-cultured *R. flavipes* workers by sampling date, ±the standard deviation

Week	*P*1		*P*2		*P*3	
	Mean running speed (s)	Percent change	Mean running speed (s)	Percent change	Mean running speed (s)	Percent change
0	3.72±1.16	0	3.30±0.66	0	5.71±2.05	0
2	3.38±0.79	−9	3.61±0.75	9	3.85±0.83	−33
4	3.06±0.80	−18	3.30±0.52	0	3.60±1.15	−37
6	3.10±0.52	−17	3.23±0.63	−2	3.19±0.60	−44
8	2.83±0.40	−24	3.20±0.50	−3	2.95±0.36	−48
10	2.66±0.43	−28	3.24±0.30	−2	3.04±0.54	−47
12	2.77±0.48	−26	4.95±1.78	50	3.38±0.58	−41
14	2.70±0.37	−27	4.14±1.26	25	3.43±0.53	−40
16	3.15±0.43	−15	2.96±0.51	−10		
18	2.55±0.26	−31	3.9±0.76	18		
20	2.96±0.50	−20	3.89±0.68	18		
22	3.98±1.30	7	3.98±0.61	21		
24	3.06±0.63	−18	4.09±0.90	24		

while for *P*2 water percentage decrease over time was weak ($R^2 = 0.26$) and not significant ($p = 0.07$) (Fig. 6).

Comparing water percentage and lipid content figures of *P*1 as well as *P*2, a "mirror image" is apparent (Figs. 5 and 6). All three populations show a correlation between body water percentage and lipid content (Fig. 7). Such a correlation was also described in a survey of termites from 11 field populations (Arquette, 2005), and previously reported in a paper by Shelton and Appel (2001) for *R. flavipes* alates, as well as for insects in general (reviewed by Hadley, 1994). 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, these measures changed differently from each other early in captivity, so as measures of vigor each could not be used interchangeably. Lipid content stabilized after this initial rise across populations through 6 weeks or longer, while water percentages continued declining through 6 or more weeks (Figs. 5 and 6). This indicates another factor besides change in lipid content influenced change in body water percentage. Loss of body water may have been an additional factor.

Various factors influence long-term water loss in insects. For example, insects that burrow through sand, the substrate used in this study, can be expected to have abrasion of the waterproofing wax layer, leading to water loss (Hadley, 1994). Also, body water loss has been hypothesized to increase with age from loss of control of the spiracular closing mechanism (Hadley, 1994). If this occurs it may have been a factor for the low water percentage of *P*3 workers just after field collection, and a steep decline in water percentage of this group even as lipid levels were generally constant (Figs. 5 and 6).

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 about 50% body 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 *P*3 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%, *P*1 below 80% survivorship, Table 10), 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 9). 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 (Arquette, 2005). *P*1 termites first measured 72% body water at 12 weeks, *P*2 at 6 weeks, and *P*3 at 2 weeks (Table 7).

Table 9
Measurements for potential measures of vigor from healthy and unhealthy *R. flavipes* workers

	Healthy	Unhealthy	Food removal	Survivorship	Live weight	Uric Acid	Soluble protein	Lipid	Percent water	Glycogen	Running speed
*P*1, *Week*											
0	x		ND[a]	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
*P*1 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
*P*2 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
*P*2, *Week*											
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
*P*2 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
*P*2 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
*P*3, *Week*											
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[b]	125.3	67.9	8.5	3.64
*P*3 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
*P*3 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
			$p<0.0001$		$p=0.01$	$p=0.03$	$p=0.97$	$p=0.04$	$p=0.01$	$p=0.17$	$p=0.61$

[a]ND = No data.
[b]Mean not determined.

Table 9
Measurements for potential measures of vigor from healthy and unhealthy *R. flavipes* workers

	Healthy	Unhealthy	Food removal	Survivorship	Live weight	Uric Acid	Soluble protein	Lipid	Percent water	Glycogen	Running speed
*P*1, *Week*											
0	x		ND[a]	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
*P*1 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
*P*2 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
*P*2, *Week*											
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
*P*2 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
*P*2 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
*P*3, *Week*											
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[b]	125.3	67.9	8.5	3.64
*P*3 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
*P*3 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
			$p<0.0001$		$p=0.01$	$p=0.03$	$p=0.97$	$p=0.04$	$p=0.01$	$p=0.17$	$p=0.61$

[a]ND = No data.
[b]Mean not determined.

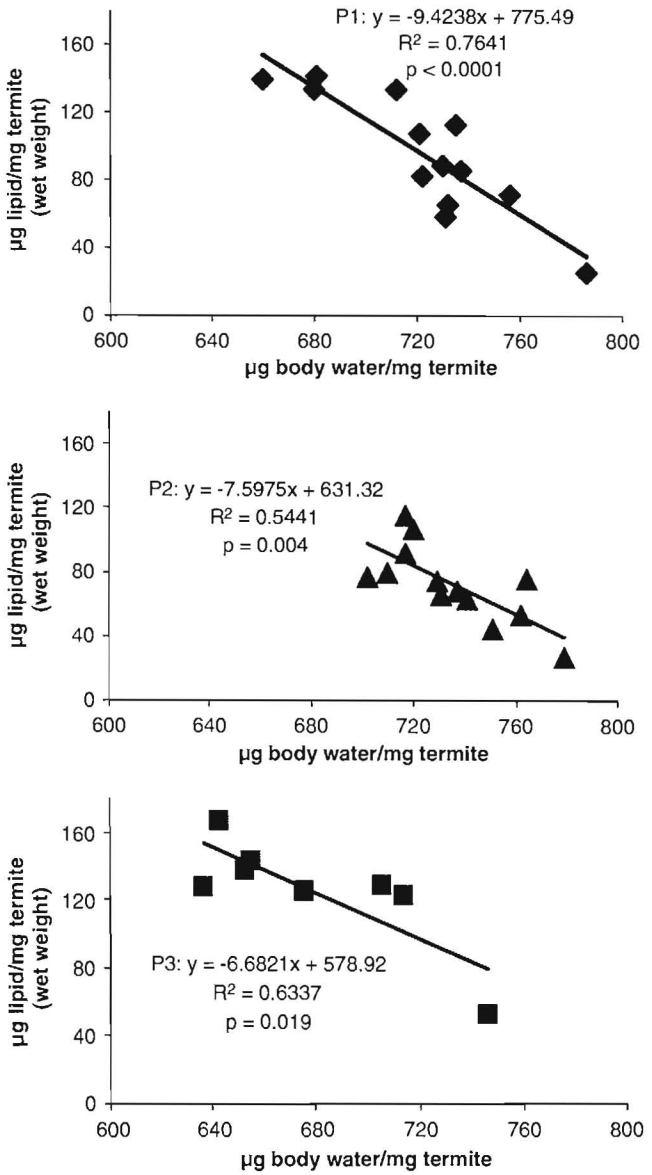

Fig. 7. Linear regression for body water and lipid content in captivity for *R. flavipes* workers from three field populations.

A 72% body water threshold for health may be set too low considering *P*3, a clearly unhealthy group measuring close to 75% body water at week 0, and significantly lower than *P*1 and *P*2 just after field collection (paired *t*-test, $p = 0.001$) (Table 7). 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 (Arquette, 2005). 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 Arquette (2005), the population with significantly lower water percentage than the others (71.7%) (Duncan's multiple range test, $p < 0.05$) 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 (Arquette, 2005).

In the current study all populations declined in body water percentage at the same rate through 6 weeks in captivity; *P*1 and *P*2 decreased below 75% body water between 2–4 weeks (Table 7). Applying a threshold of 75% body water alongside percent survivorship, all three populations of the current study were near 75% body water 5–6 weeks prior to the point that the non-predictive, 80% survivorship threshold for health was reached (Fig. 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 *P*3, termites from this group consistently had significantly higher lipid content than *P*1 and *P*2 (Tukey's HSD, Table 11). 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 (Figs. 5 and 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.

4.3. Summary

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 long-term laboratory culture for use in bioassay.

Table 10
Tukey's HSD for significant differences in body water percentage among healthy and unhealthy *P*1, *P*2, and *P*3 workers

The GLM procedure
Least squares means

Pop	Healthy/Unhealthy[a]	LSMean	Standard error	Pr > |t|	Table designation (*i*,*j*)
*P*1	Healthy	74.0461538 [b]	0.6413499	<0.0001	*P*1,H[c]
*P*1	Unhealthy	70.6000000	0.4720207	<0.0001	*P*1,U
*P*2	Healthy	74.5200000	0.5970643	<0.0001	*P*2,H
*P*2	Unhealthy	72.5909091	0.4930096	<0.0001	*P*2,U
*P*3	Healthy	71.0333333	0.6675381	<0.0001	*P*3,H
*P*3	Unhealthy	64.7083333	0.6675381	<0.0001	*P*3,U

Least squares means for effect pop × health
Pr > |*t*| for H0: LSMean(*i*) = LSMean(*j*)

i/*j*	*P*1,H	*P*1,U	*P*2,H	*P*2,U	*P*3,H	*P*3,U
*P*1,H		<0.0001	0.5900	0.0753	0.0016	<0.0001
*P*1,U	<0.0001		<0.0001	0.0044	0.5974	<0.0001
*P*2,H	0.5900	<0.0001		0.0145	0.0002	<0.0001
*P*2,U	0.0753	0.0044	0.0145		0.0637	<0.0001
*P*3,H	0.0016	0.5974	0.0002	0.0637		<0.0001
*P*3,U	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

Values ≤ 0.0033 are significantly different. (Critical value for significance determined from 0.05 level of probability/15 pairwise comparisons = 0.0033).
[a]"Healthy" termites regarded as those from an experimental unit with higher than 80% survivorship, and "unhealthy" below 80% survivorship. Su and La Fage (1984a) defined 80% survivorship as a threshold for separating healthy from unhealthy termites, using small groups of *C. formosanus* workers in captivity.
[b]Percent body water/termite.
[c]H = "healthy," above 80% survivorship; U = "unhealthy," below 80% survivorship.

Table 11
Tukey's HSD for significant differences in lipid content among healthy and unhealthy *P*1, *P*2, and *P*3 workers

The GLM procedure:
Least squares means

Pop.	Healthy/ Unhealthy[a]	LSMean	Standard Error	Pr > |t|	Table designation (*i*,*j*)
*P*1	Healthy	65.600000[b]	7.024121	<0.0001	*P*1,H[c]
*P*1	Unhealthy	113.500000	5.672490	<0.0001	*P*1,U
*P*2	Healthy	67.313333	7.024121	<0.0001	*P*2,H
*P*2	Unhealthy	75.562500	5.553056	<0.0001	*P*2,U
*P*3	Healthy	106.966667	7.853206	<0.0001	*P*3,H
*P*3	Unhealthy	142.750000	7.853206	<0.0001	*P*3,U

Least squares means for effect pop. × health
Pr > |*t*| for H0: LSMean(*i*) = LSMean(*j*)

i/*j*	*P*1,H	*P*1,U	*P*2,H	*P*2,U	*P*3,H	*P*3,U
*P*1,H		<0.0001	0.8634	0.2687	0.0002	<0.0001
*P*1,U	<0.0001		<0.0001	<0.0001	0.5017	0.0033
*P*2,H	0.8634	<0.0001		0.3592	0.0003	<0.0001
*P*2,U	0.2687	<0.0001	0.3592		0.0015	<0.0001
*P*3,H	0.0002	0.5017	0.0003	0.0015		0.0017
*P*3,U	<0.0001	0.0033	<0.0001	<0.0001	0.0017	

Values ≤ 0.0033 are significantly different. (Critical value for significance determined from 0.05 level of probability/15 pairwise comparisons = 0.0033).
[a]"Healthy" termites regarded as those from an experimental unit with higher than 80% survivorship, and "unhealthy" below 80% survivorship. Su and La Fage (1984a) defined 80% survivorship as a threshold for separating healthy from unhealthy termites, using small groups of *C. formosanus* workers in captivity.
[b]Units of μg lipid/mg termite.
[c]H = "healthy," above 80% survivorship; U = "unhealthy," below 80% survivorship.

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