Effects of starvation on survival, cannibalism, body mass, and intestinal protozoan profile in the subterranean termite *Reticulitermes lucifugus* "Sicily"

Concise title: Effects of starvation on *Reticulitermes lucifugus* "Sicily" and its protozoa

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Abstract

Scarcity or inadequate nutrition can affect biological and behavioural aspects of subterranean termites and their intestinal protozoan profile. The aim of this work was investigate changes in survival, cannibalism, body mass and protist community structure of *Reticulitermes lucifugus* Rossi subspecies "Sicily" following starvation, to understand the termite survival strategy under stressful conditions. In nature this termite consumes many food source and its feeding activity is continuous during the year. In the experiments, groups of 50 termites (worker/soldier ratio 49:1) were subjected to two diets, starvation and filter-paper diet (as control), kept for 35 days with 7-day intervals of inspection and compared with field collected termites. Under starvation termite survival decreased to 0% after 35 days for both workers and soldiers, cannibalism rate was on average 84% and 100% of workers and soldiers respectively, and body mass of workers decreased from 3.5 mg/worker (first day of test) to 2.05 mg/worker (last day of test). The community structure and abundance of the intestinal protozoa of workers changed to dependence on the diet. Starvation caused the loss of four, six and two protist species after 7, 14 and 28 days, respectively, persisting only one species after 35 days. In most inspection dates, results were significantly different from those of filter paper fed and field collected groups. This work suggests that under starvation or inadequate nutrition, *R. lucifugus* "Sicily" adopts behavioural mechanisms to maintain its social structure, and also the symbiotic intestinal community varies to adapt to different diets, in dependence on the division of labour among protist species in the lignocellulose digestion.

Key words: *behaviour changes, inadequate nutrition, protists, social insects, survivorship*

Introduction

Reticulitermes lucifugus (Rossi) (Blattodea, Termitoidae, Rhinotermitidae) is the most abundant species of *Reticulitermes* genus in Italy (Ghesini and Marini 2012). A taxon of subspecific level, has been identified in the Sicily region and named *R. lucifugus* ''Sicily'' (Luchetti et al. 2004; 2013). Colonies of this species are composed by numerous individuals (several tens of thousands) of which the soldiers represent a small quantity (Sbrenna and Micciarelli Sbrenna 2008). *R. lucifugus* consumes many food source in nature and causes mainly damages to wooden structures of buildings of the historical and artistic heritage. A previous work (Buchli 1958) reports that the nutrition has effects on caste differentiation of *R. lucifugus* because, if inadequate, it can suppress the formation of soldiers and neotenics in laboratory colonies. Many researches on subterranean termites showed that the scarcity and/or type of food sources can influence their survival, cannibalism, and body mass (Smythe and Maulding 1972; LaFage 1976; LaFage and Nutting 1978; Haverty and Howard 1979; Song et al. 2006; Hu et al. 2011). Starvation leads to an increase in the mortality rate of termites than those with a food supply. Cannibalism also increases when termites face long periods of starvation. Cannibalism can be caste-specific, when starved workers consume more soldiers before eating other workers (Su and LaFage 1986; Song et al. 2006). It is selective when weaker individuals are eliminated as a mechanism of survival strategy to maintain fittest members in the termite colony (Hu et al. 2011). It is well known that lower termites harbours in their hindgut a complex of microorganisms necessary for the digestion of woody materials, nitrogen fixation, recycling, vitamin production, and acetogenesis (Honigberg 1970; Breznak and Switzer 1986; Ohkuma 2008; Brugerolle and Radek 2006; Purdy 2007; Tartar et al. 2009; Scharf et al. 2011; Brune and Dietrich 2015). The role of the protozoan fauna is fundamental in the lignocelluloses digestion by termites and in maintaining the physical–chemical equilibrium inside the termite hindgut (Brune and Ohkuma 2011). This fact has been demonstrated by eliminating gut protists (Cleveland 1923; 1924). Literature reported that starvation or insufficient nutrition can cause changes on termite symbiotic protists. In fact, there may be numerical reduction or elimination of some protist species in relation to the presence or absence of different nutritional components of food (Cleveland 1924; Mannesmann 1973; Haverty and Howard 1979; Breznak and Brune 1994; Yoshimura 1995; Inoue et al. 1997; Belitz and Waller 1998; Cook and Gold 2000; Smith and Koehler 2007). *R. lucifugus* “Sicily” harbours 13 protist species (Grassi and Foà 1911; Lo Pinto et al. 2017) belonging to the families Pyrsonymphidae, Holomastigotoididae, Monocercomonadidae and Trichonymphidae. Termite foraging in nature is affected by natural factors that can lead to periods of starvation or inadequate nutrition and consequent loss of protist symbionts (Esenther 1969; Forschler and Henderson 1995; Cleveland 1925). We previously found protist species in the hindgut of workers and soldiers of *R. lucifugus* “Sicily” during the whole year, as result of continuous feeding activity in field (Lo Pinto et al. 2016). Therefore, we hypothesized that this termite could adopt a survival strategy under stressful conditions due to absence or scarcity of food and its protist community could change to enhance survival. In order to verify this hypothesis, the purpose of this work was to investigate effects of starvation (no source of cellulose offered to the termites) on survival and cannibalism rate, body mass loss and changes in the abundance of the intestinal protists species of *R. lucifugus* “Sicily”, in comparison with filter-paper fed and freshly field collected termites.

Materials and methods

Insects

To evaluate effects of starvation on *R. lucifugus* “Sicily”, individuals were collected from a field site of Palermo (38°6’25.72”N; 13°21’2.22”E) (Sicily, Italy). Blocks of ten fir tablets (each 10 cm x 10 cm x 1 cm), held together by an elastic band and installed inside open-bottomed buckets placed in the ground at 30 cm depth, approximately one meter from each other, were used. The blocks were covered with crocks to maintain the necessary moisture.

For experimental tests, one of three blocks was taken from the ground and brought to the laboratory (25 °C, 60 % R.H.), and termites were collected from it. Workers with dark brown and distended abdomens were selected to ensure they had not recently molted whereas soldiers were chosen randomly. All termites were collected from the colony of field and used on the day of start of the experiment. Intact termites were used after their examination under a stereomicroscope to be sure that they were healthy and not missing body parts. Termites were subjected to two different diets: starvation (no source of cellulose offered to the termites) and filter paper with distilled water (as control). For experiments on body mass and intestinal protist species a further comparison was made with freshly field collected termites fed with natural source.

Experimental unit

The experimental units were Petri dishes (6.0 cm in diameter and 1.5 cm high) with 10 g of sterilized sand and 1.2 ml of distilled water to maintain the necessary moisture. In the control, also a piece of moistened filter paper (4.7 cm in diameter, Whatman # 541) was put in each Petri dish as food supply. Following the introduction of termites, Petri dishes were sealed with Parafilm (American National Can, Menasha, WI) and placed in a glass box (70 x 35 x 35 cm) darkened by black cardboard, with two deionized distilled water containers. This box was kept in a conditioned chamber at 24 ± 1°C and 70 ± 5% RH. In total, there were 60 experimental units (30 starved and 30 control groups).

Experiment 1: effects on survival and cannibalism

Termites collected from block of field were chosen in groups of 50 termites, in their natural worker/soldier ratio of 49:1, and introduced into the experimental Petri dishes. Observations were made at 7-day intervals for 35 days (5 inspections). At each inspection date, 6 Petri dishes from both starved and control groups were examined under a stereoscope and the number of survivor intact, survivor cannibalized, dead intact, and dead cannibalized termites was recorded. Cannibalized termites were classified if their appendages or body parts partially or completely were missing. Among dead cannibalised termites we also considered the missing termites. After each observation the Petri dishes with the remaining termites were eliminated.

Experiment 2: body mass loss

To evaluate the body mass loss, 3 groups of five intact live workers were randomly selected from freshly field collected (natural diet), starved and filter-paper fed termites and each group was weighed on an electronic analytical balance (M-AAA, ORMA, Eurotek, Milano, Italy). Groups from freshly field collection and those fed with filter paper were examined at the first day and 30 days of experiment, respectively, while groups from starved termites at 6, 8, 13, 15 days of experiment. However, the test on starved workers was considered finished at 13 days because only two alive termites were found at 15-day inspection date. There were 3 replicates of 3 groups per each diet category whit 9 weighs at each observation date, making a total of 45 experimental units.

Experiment 3: changes in the abundance of the intestinal protists species

For protist species counting, data were detected on the first day of the experiment in the termites freshly collected from the field, and at 7-day intervals up to 28th day of experiment in starved and paper-fed termites. At each dates of inspection, 18 live workers were randomly selected from each group (freshly field collected, starved and paper-fed termites) and the gut contents from 3 termites were pooled to form a sample, using then 6 samples. According to a previously described method (Lo Pinto et al. 2016) the last two abdominal segments of the body were removed gently using fine-tipped forceps. Gut contents were put into 60 μl salt solution (NaCl 0.8 g, KCl 0.02 g, CaCl2 0.02 g, NaHCO3 0.01 g in 100 ml distilled water) in which neutral red was dissolved (0.5 ml of 1 % aqueous neutral red solution into 10 ml salt solution). A small amount of samples was placed in a cell counting chamber (such as Burker's chamber HBG Germany), where two counting sections were randomly slected. Counting of protists was performed under a Leitz Dialux 20 EB microscope (phase contrast) at 400x magnification. The calculation of the average number of protists per hindgut (X_F) was made as follows: $X_F = G \times n/V \times 3$, where G is volume of solution containing the three hindguts (60 μl), n is mean of the two countings; V is volume of the counted area (in μl).

Statistical analysis

Differences in the survival and cannibalism rate from experiment 1, were analysed by two-way analysis of variance (ANOVA) ($P < 0.05$). Differences in body mass from experiment 2, and intestinal protozoa profile from experiment 3 were tested by one-way analysis of variance (ANOVA) ($P < 0.05$). Significant differences between the means were separated using Tukey's multiple comparison test. Before data analysis, survival and cannibalism proportional data were arcsine transformed and body mass and protist species data were square root transformed (x+0.5). However, untransformed data are provided in the figures and tables for their interpretation. Statistical analysis were performed using Statistica 7.0 for Windows Package (Stat Soft Inc. 2001).

Results

Effects on survival and cannibalism

After 35 days of starvation, for both workers and soldiers survival was 0% (Figure 1A; Figure 2A) whereas in the control group (filter paper diet) it was 83% and 66%, respectively (Figure 1B; Figure 2B). During the period of starvation, worker mortality increased gradually from 26% (first inspection) to 100% (last inspection) and cannibalism was on average 84% on dead workers and 1.7% on survivor workers. Survivor intact workers were found up to 28-day inspection, survivor cannibalised workers until 21-day inspection whereas dead intact workers and dead cannibalized workers were detected at each inspection date. Soldier mortality ranged from 33% (first two inspections) to 100% (subsequent inspections) with cannibalism of 100% at each inspection date. Survivor intact soldiers were found until 14-day inspection, whereas survivor cannibalized soldiers and dead intact soldiers were not observed (Figure 2A). In the control group, mortality of workers was 11% on average, ranging from 6% (third inspection) to 17% (last inspection) (Figure 1B), and that of soldiers was 33% (last two inspections) (Figure 2B). Cannibalism was 100% on dead individuals in both castes. Survivor intact workers and soldiers, and dead cannibalised workers were found at each inspection date, whereas dead cannibalized soldiers were detected from 28-day inspection to the last observation. Survivor cannibalised and dead intact workers and soldiers were not found (Figures 1B and 2B).

Comparisons between two groups (starved and control termites) showed that worker survival in the starved group was significantly lower than that in the control group during the experiment, except for 7-day inspection date (F= 9.47; P= 0.0002). Starved dead cannibalized workers differed significantly from those of control groups (F= 32.82; P= 0.0000). Survivor soldiers and dead cannibalized soldiers of starved group were statistically different from those of the control group from 21-day inspection to 35-day inspection (F= 20.25; P=0.0002 and F= 12.89; P= 0.0018 respectively). Comparisons among data from starved group showed significant differences between 7-day inspection and 21-day and 28-day inspections, and between 14-day inspection and 28-day inspection for survivor intact workers (F= 11.71; P= 0.0009), and between 7-day inspection and 35-day inspection for dead cannibalized workers (F= 3.51; P= 0.025). No significant differences were found between dates of inspection for survivor cannibalized workers (F= 0.96; P= 0.46) and dead intact workers (F= 0.67; P= 0.62). Significant differences were found between the first two dates (7-day and 14-day inspections) and the others remaining (21-day, 28-day and 35-day inspections) (F= 2.87; P=0.049) for survivor intact soldiers. No significant differences were found for dead cannibalized soldiers (F= 1.80; P= 0.17). Within the control group, both workers and soldiers did not differ significantly between dates of inspection (F= 2.31; P= 0.13 and F= 2.1; P= 0.15, respectively).

Body mass loss

The body mass of termites subjected to starvation decreased significantly during the whole period of observation from 3.5 mg/worker detected at the first day of experiment (data recorded on freshly field collected termites) to 2.05 mg/worker after 13 days of starvation (Figure 3). The body mass loss was 20.0 %, 28.6 % and 41.4 % at 6-day, 8-day and 13-day inspection, respectively. A decrease of weight up to 3.0 mg/worker was also observed in the group subject to filter-paper feeding for 30 days with 14.3 % of body mass loss. Comparing body mass data recorded on the examined groups (freshly field collected, starved and paper-fed termites), significant differences were found among inspection dates of the whole period, except for 30-day paper fed workers that did not differ from 6-day starved workers (F = 57.30; P = 0.0000).

Changes in the abundance of the intestinal protists species

Total protozoa population estimates differed significantly between field collected, paper fed and starved groups, but not within each group except for the first 7-day fed and 7-day starvation (F = 66.57; P = 0.0000) (Table 1). During the period of starvation, the protist species decreased gradually until reduced their population to zero, except for *D. gracilis* that had persisted. Specifically, four species (*S. flagellate*, *T. agilis*, *T. minor* and *P. major*) were eliminated after 7 days of starvation, six species (*D. fimbriata*, *M. hexamitoides*, *H. elongatum*, *S. kofoidi*, *P. flagellata* and *P. minor*) after 14 days, and two species (*D. exilis* and *M. termitis*) after 28 days. In paper fed test, all species were detected after 28 days from the start except for *P. Major*, that disappeared after 21 days. *D. exilis*, *D. gracilis*, *D. fimbriata* and *M. hexamitoides* were dominant over the other species both in field sample (natural diet) and 7-day filter-paper fed group, whereas after 7 days of starvation *P. minor* together *M. hexamitoides* and *D. gracilis* were the most abundant (Figure 4). Statistical analysis for comparisons among field collected, fed and starved groups, showed significant differences for all protist species (*D. fimbriata,* F = 27.61; P = 0.0000; *D. gracilis,* F = 21.35; P = 0.0000; *D. exilis,* F = 36.73; P = 0.0000; *M. termitis,* F = 4.34; P = 0.0006; *M. hexamitoides,* F = 29.83; P = 0.0000; *S. flagellate,* F = 31.16; P = 0.0060; *H. elongatum,* F = 4.80; P = 0.0002; *T. agilis,* F = 4.78; P = 0.0003; *T. minor,* F = 6.37; P = 0.0000; *S. kofoidi,* F = 5.85; P = 0.0000; *P. flagellate,* F = 8.97; P = 0.0000; *P. minor,* F = 20.64; P = 0.0000; *P. major,* F = 4.89; P = 0.0002). Nevertheless, filter paper diet offered to termites for the first 14 days did not affect significantly the numeric variation of most protist species in comparison with those of freshly collected termites, as well as observed at 7 days of starvation with respect to paper-fed group.

Discussion

Our results showed that survival of termites subjected to starvation decreased during the experiment by corroborating other works that indicate the mortality as a direct result of starvation (Smythe and Maulding 1972; Haverty and Howard 1979; Yoshimura et al. 1994; Song et al. 2006; Hu et al. 2011). Starved workers survived up to 28 days of the experiment, whereas starved soldiers up to 14 days. In the control, survivor termites were found for the whole period of observation, even if a certain rate of mortality was detected already from the first inspection for workers and at the last two dates of inspection for soldiers, and all dead termites were found cannibalised. This fact can be explained as mortality and cannibalism that normally occurs in laboratory experiments (Smythe and Carter 1970; Su and LaFage 1986; Osbrink and Lax 2002; Raina et al. 2004). Further, physiological and behavioural changes can be a result of using filter paper as a food of termites that lacking in some nutritional components (xylan, nitrogen content, etc.) can lead to cannibalism and death of termites (Cook and Scott 1933; LaFage 1976; Song et al. 2006; Smith and Koehler 2007). Previous works reported that cannibalism occurs in termites where the food source is low or absent (Buchli 1950; Weesner 1956; LaFage and Nutting 1978; Raina et al. 2004; Song et al. 2006; Hu et al. 2011). In this work we found dead cannibalized workers and soldiers in both starved and control groups and survivor cannibalized workers solely in the starved group, with significant differences between groups. This result shows that starvation of termites influences the cannibalism rate of both workers and soldiers. As starvation continued, the number of cannibalized termites increased. We found that the number of cannibalized workers was less than that of soldiers which were all cannibalized within the first 21 days of starvation. It is reported that, in the absence of food, soldiers may represent an emergency food ration for survival of the other members of the colony (Su and LaFage 1986; Song et al. 2006). In addition, cannibalism contributes to adjust the proportion of castes by eliminating soldiers and reproductives (selective cannibalism) (Stuart 1969). Controlled cannibalism is also a supply of nitrogen (Cook and Scott 1933; Buchli 1950; Hendee 1934), and provides cellulolytic symbionts to maintain the cellulose digesting ability (Seifert and Becker 1965). Furthermore, it may be a protein-conserving practice, because the natural diet of termites is low in this constituent (Moore 1969; Wilson 1971). In the filter-paper fed group (used as control), cannibalism of workers was less than 18% without significant variations throughout the period of the experiment, and that of soldiers about 30% at the last two dates of inspection. These rates of cannibalism may be a behavioural response to nutrient deficiency of the filter paper that is qualitatively different from field food sources (Smythe and Williams 1972; Mannesmann 1973; LaFage and Nutting 1978).

In our study, starvation affected significantly body mass of the survivors that lost about 41 % of their mass after 13 days. This result could be a peculiarity of *R. lucifugus* “Sicily”, that seems differs from other species: works on subterranean termites subject to starvation for 40 days, reported that *Coptotermes formosanus* lost a small percentage of body weight (Song et al. 2006) and *R. flavipes* had approximately the same body mass of those fed with filter paper and freshly collected from field (Hu et al. 2011). Interestingly, in our experiment, filter-paper fed group significantly differed from field collected group (14.3 % mass loss). This was also observed in *R. grassei* subjected to cellulose diet that reached significantly higher mass loss values when compared to natural diet (Duarte et al. 2017). The mass loss shown by filter-paper diet may indicate that it is a poorer lignocellulosic source than natural diet.

Many researches showed that host starvation affects the hindgut protozoa of lower termites not only influencing the ingestion of the wood particles by microorganisms but also the abundance of the protist species (Yoshimura 1995;

Inoue et al. 1997; Belitz and Waller 1998; Hu et al. 2011). Our results showed that during 28 days of experiment, starvation affected the community structure and abundance of the intestinal protozoa of workers that differed significantly from the paper fed and freshly field collected (natural diet) groups. Starving termites exhibited the most depleted flagellate protist fauna in terms of abundance. In addition, some differences were highlighted when filter-paper fed termites were compared with those of freshly field collected (natural diet). In fact food sources may affect hindgut protist communities of termites (Cleveland 1924; Hungate 1943; Mannesmann 1972; Smythe and Mauldin 1972; Mauldin et al. 1972; Mauldin et al. 1981; Lai et al. 1983; Yoshimura et al. 1993; 1994; 1996; Cook and Gold 2000), and filter paper is a food source with low nutritional quality. Previous studies reported that protist species have different roles in the metabolism of termites and they are involved in separate steps of cellulose digestion depending on whether they are cellulolytic or non-cellulolytic species (Mauldin et al. 1972; Smythe and Mauldin 1972; Grosovsky and Margulis 1982; Lai et al. 1983; Yoshimura 1995; Yoshimura et al. 1993; 1994; 1996; Cook and Gold 2000; Wheeler et al. 2007; Watanabe and Tokuda 2010; Ohkuma and Brune 2011; Tsukagoshi et al. 2014). In our results *S. flagellata*, *T. agilis*, *T. minor* and *P. major* were eliminated after 7 days of termite starvation, meaning that they were strongly affected by absence of food. *T. agilis* is reported to be cellulolytic species and considered one of the most important protists in all *Reticulitermes* species being essential for the survival of termites (Cleveland 1925; Mauldin et al. 1981). A previous study showed that 13 days of termite starvation had reduced the *T. agilis* population to zero (Haverty and Howard 1979). However, Hu et al. (2011) reported that this protist had persisted longer after the termites were starved. *Pyrsonympha* spp. are reported as facultative cellulolytic protists and some of them can use dead *T. agilis* cells as food source (Haverty and Howard 1979; Grosovsky and Margulis 1982; Cook and Gold 2000). In our experiment, *P. major* behaved as cellulolytic species whereas *P. minor* and *P. flagellata* as not cellulolityc species because they persisted after 7 days of starvation without particular differences with the fed group. Furthermore, *P. major* is missing at the 28-day paper fed inspection, suggesting that longer this species requires other nutritional components not present in filter paper. *S. flagellata* is considered not cellulolytic (Grosovsky and Margulis 1982), even if in our study disappeared early following starvation. In this study, *D. gracilis*, *D. exilis*, and *M. termitis* were less affected by starvation than other species and *D. gracilis* was the only species that had persisted at the end of experiment whereas *D. exilis*, and *M. termitis* were found until 21 days of starvation. *M. hexamitoides*, *P. minor* and *D. gracilis* after 7 days of starvation were clearly dominant over the other species present representing together over 60% of the flagellate protists, while in the field collected termites (natural diet) these three species represented less than 40%. Similar observation are reported on *R. grassei* after 6 days of starvation (Duarte et al. 2017). The majority of protist species in starved termite did not differ significantly from those of the fed group in the first 7 days. This might be explained by the fact these protist species are not considered crucial for host survival being not cellulolytic (Kirby 1924; Smythe and Mauldin 1972;

Grosovsky and Margulis 1982; Yoshimura et al. 1993). *Monocercomonas* spp. are reported not associated with wood ingestion or cellulose degradation and its population increases following the elimination and reduction of other species that foster its proliferation (Borge et al. 2007). Considering our results, the comparison among freshly field collected data (natural diet) and those detected at the end of the laboratory experiment (28-day paper fed and 28-day starved termites) showed significant differences between paper fed and field collected groups except for 5 protist species (*S. flagellata*, *H. elongatum*, *T. agilis*, *T. minor* and *S. kofoidi*). These results are consistent with a study on hindgut protists of *R. flavipes* 40-day starved (Hu et al. 2011) as regards all species common to *R. lucifugus*, except for *D. fimbriata* and *S. kofoidi* that persisted throughout the period of starvation whereas in our study they disappeared after 14 days of starvation. Inoue et al. (1997) reported that *T. agilis*, *H. elongatum* and *D. exilis* from *R. speratus* disappeared after 20 days of termite starvation. In our study this was confirmed for *D. exilis* but not for *T. agilis* and *H. elongatum* that disappeared during the first two weeks of starvation. This may be explained because the majority of intestinal protists are considered to be autochthonous and probably coevolving with termite species (Hongoh et al. 2005; Noda et al. 2007). Therefore, protist species are responding to termite starvation conditions in dependence of host species in which they evolve.

We think that our findings contribute to enhancing the understanding of termite survival strategies and protozoan symbiosis in response to stressful conditions: *R. lucifugus* “Sicily” likely adopts behavioural mechanisms, as cannibalism, to maintain its social structure, and the symbiotic intestinal community varies adapting to different diets, in dependence on the division of labour among protist species in the lignocellulose digestion.

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Figure legends

Fig. 1 Mean proportions of survived workers (intact or cannibalized) and dead workers (intact or cannibalized) of *R. lucifugus* during the 35-day experiment (A starved, B paper fed control)

Fig. 2 Mean proportions of survived soldiers (intact or cannibalized) and dead soldiers (intact or cannibalized) during the 35-day experiment (A starved, B paper fed control)

Fig. 3 Body mass of *R. lucifugus* workers (mean ± SD) subjected to natural diet (freshly field collected termites), starvation and filter-paper diet detected during 30 days of experiments, weighting natural and filter-paper fed termites at first day and 30 days, respectively, and starved termites at 6, 8, 13 days.

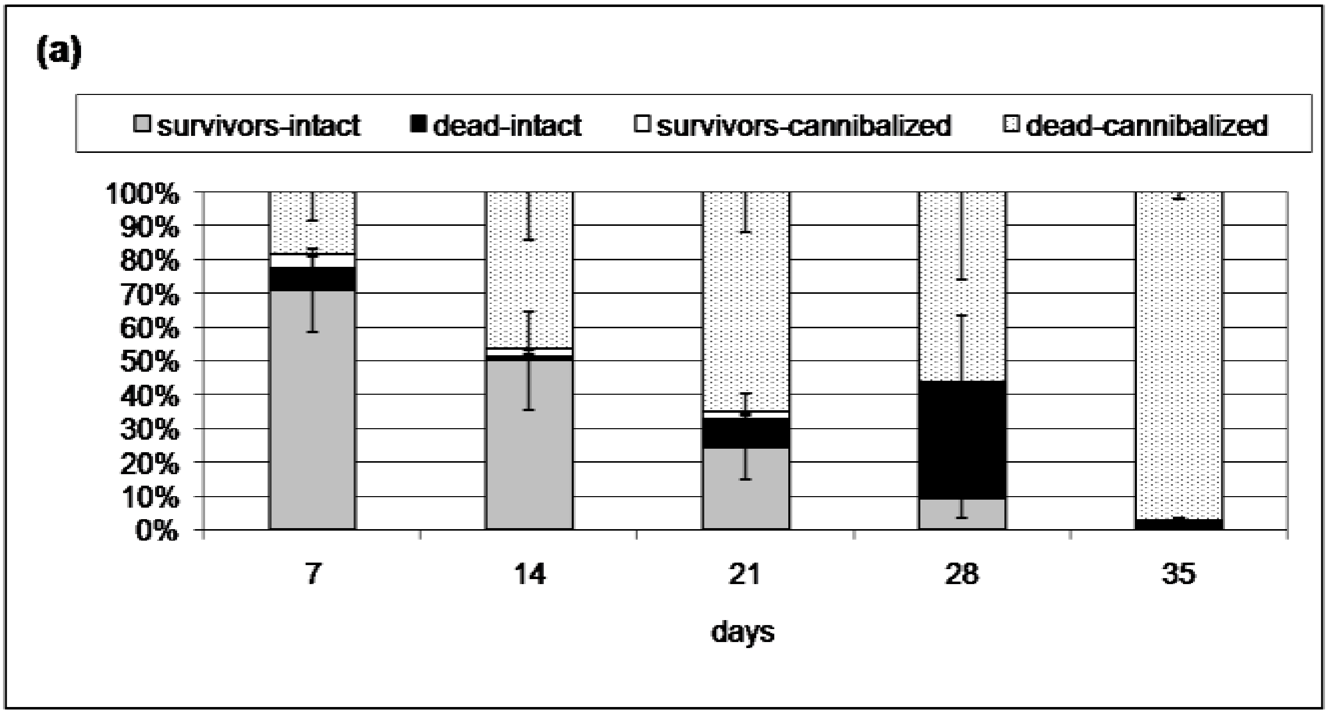
Fig. 4 Web chart representing the abundance of the protist species from termites subjected to natural diet (freshly field sample), filter-paper diet and starvation detected in laboratorial experiments for 28 days

Table 1 - Protozoa population estimate (mean ± SE) in the hindgut of *R. lucifugus* from different groups, subjected to natural diet (freshly field-collected), filter-paper fed and starved termites, detected in field collected workers in the first day of experiment and in paper fed and starved workers at 7-day intervals for 28 days in laboratory.

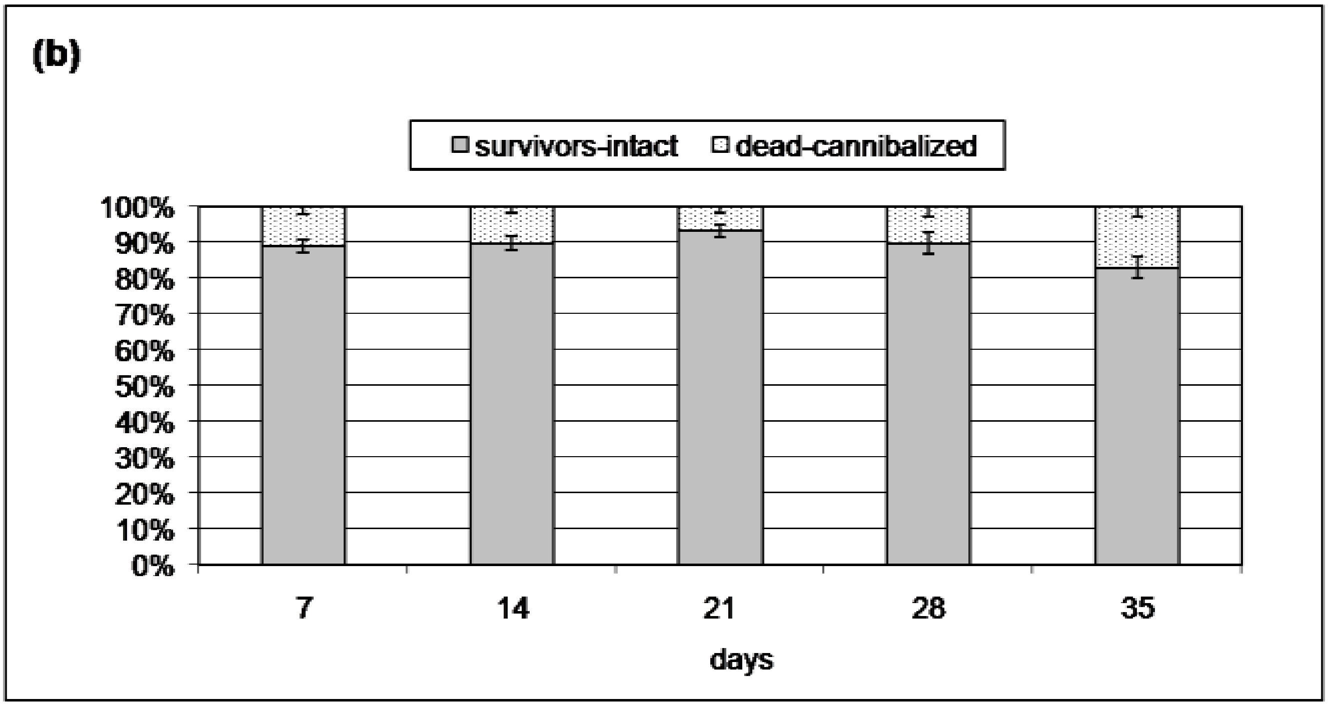
Protis species	Field collected	7-day paper fed	14-day paper fed	21-day paper fed	28-day paper fed	7-day starved	14-day starved	21-day starved	28-day starved
Dinenympha fimbriata Kirby (Pyrsonymphidae)	7950 ±1495 a	7800 ± 859 a	2700 ± 569 b	2100 ± 684 b	1500 ± 444 b	900 ± 328 b	0 ± 0 c	0 ± 0 c	0 ± 0 c
D. gracilis Leidy (Pyrsonymphidae)	16200 ± 2883 a	7050 ± 1194 b	5700 ± 300 b	5850 ± 1032 b	6600 ± 1348 b	3300 ± 444 b	750 ± 429 c	500 ± 228 c	975 ± 640 c
D. exilis Koidzumi (Pyrsonymphidae)	31050 ± 5512 a	14100 ± 1824 b	5100 ± 948 c	3750 ± 969 c	4050 ± 557 c	2250 ± 307 c	1200 ± 553 c	1600 ± 306 c	0 ± 0 d
Pyrsonympha flagellata Grassi (Pyrsonymphidae)	2850 ± 588 a	600 ± 300 b	1050 ± 429 ab	450 ± 201 b	900 ± 402 b	1800 ± 402 ab	0 ± 0 c	0 ± 0 c	0 ± 0 c
P. minor Powell (Pyrsonymphidae)	7650 ± 1179 a	3900 ± 501 ab	3450 ± 818 ab	2850 ± 882 b	2400 ± 600 b	4650 ± 1421 ab	0 ± 0 c	0 ± 0 c	0 ± 0 c
P. major Powell (Pyrsonymphidae)	1650 ± 429 a	750 ± 588 ab	300 ± 189 b	600 ± 300 b	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c
Monocercomonas termitis (Grassi) (Monocercomonadidae)	3750 ± 674 a	1050 ± 361 ab	1050 ± 429 ab	300 ± 189 b	1050 ± 588 b	2550 ± 588 ab	1200 ± 553 ab	900 ± 402 ab	0 ± 0 c
Microjoenia hexamitoides Grassi (Holomastigotoidae)	9450 ± 1006 a	9450 ± 761 a	6600 ± 1444 ab	5100 ± 1200 ab	3300 ± 1244 b	7950 ± 1323 a	0 ± 0 c	0 ± 0 c	0 ± 0 c
Spirotrichonympha flagellata (Grassi) (Holomastigotoidae)	600 ± 189 a	1050 ± 276 a	1050 ± 488 a	600 ± 189 a	450 ± 201 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
Spironympha kofoidi Koidzumi (Holomastigotoidae)	1050 ± 361 a	1350 ± 385 a	1050 ± 276 a	1200 ± 379 a	900 ± 232 a	1050 ± 276 a	0 ± 0 b	0 ± 0 b	0 ± 0 b
Holomastigotes elongatum Grassi (Holomastigotoidae)	1500 ± 379 a	1200 ± 501 a	750 ± 276 a	450 ± 201 a	300 ± 300 a	1350 ± 385 a	0 ± 0 b	0 ± 0 b	0 ± 0 b
Trichonympha agilis Leidy (Trichonymphidae)	900 ± 464 a	1350 ± 307 a	300 ± 189 a	450 ± 201 a	900 ± 402 a	0 ± 0 b	0 ± 0 b	0 ± 0 b	0 ± 0 b
T. minor Grassi & Foà (Trichonymphidae)	450 ± 307 a	2250 ± 687 b	900 ± 232 ab	900 ± 464 ab	900 ± 464 ab	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c
total population	85050 ± 7758 a	51900 ± 4437 b	30000 ± 2584 c	24600 ± 4332 c	23250 ± 3610 c	25800 ± 1925 c	3450 ± 488 d	4350 ± 785 d	975 ± 640 d

Means (±SE) with the same letter in a row do not differ significantly at P<0.05 (GLM with a quasi-Poisson distribution)

Fig 1A


(a)
survivors-intact
dead-intact
survivors-cannibalized
dead-cannibalized
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
7
14
21
28
35
days

2A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survivors-intact
dead-cannibalized
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
7
14
21
28
35
days

Fig 1B

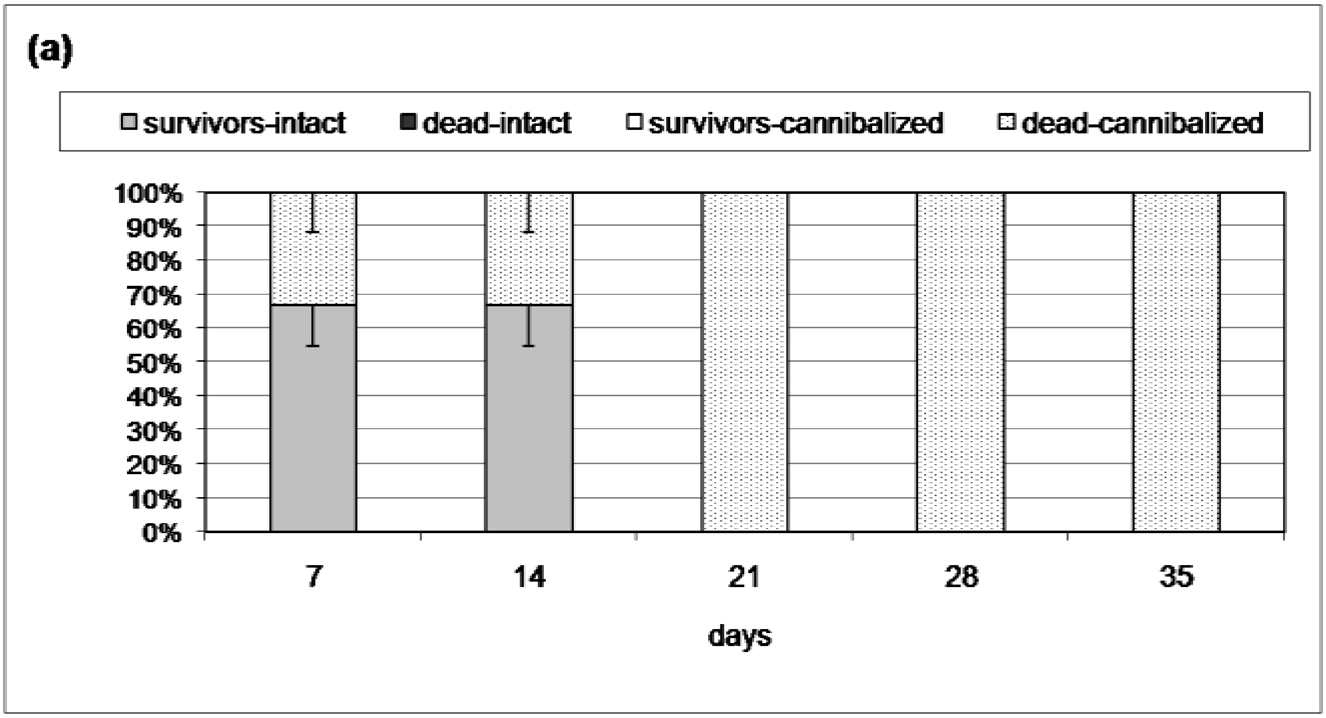

(a)
survivors-intact
dead-intact
survivors-cannibalized
dead-cannibalized
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
7
14
21
28
35
days

Fig 2B

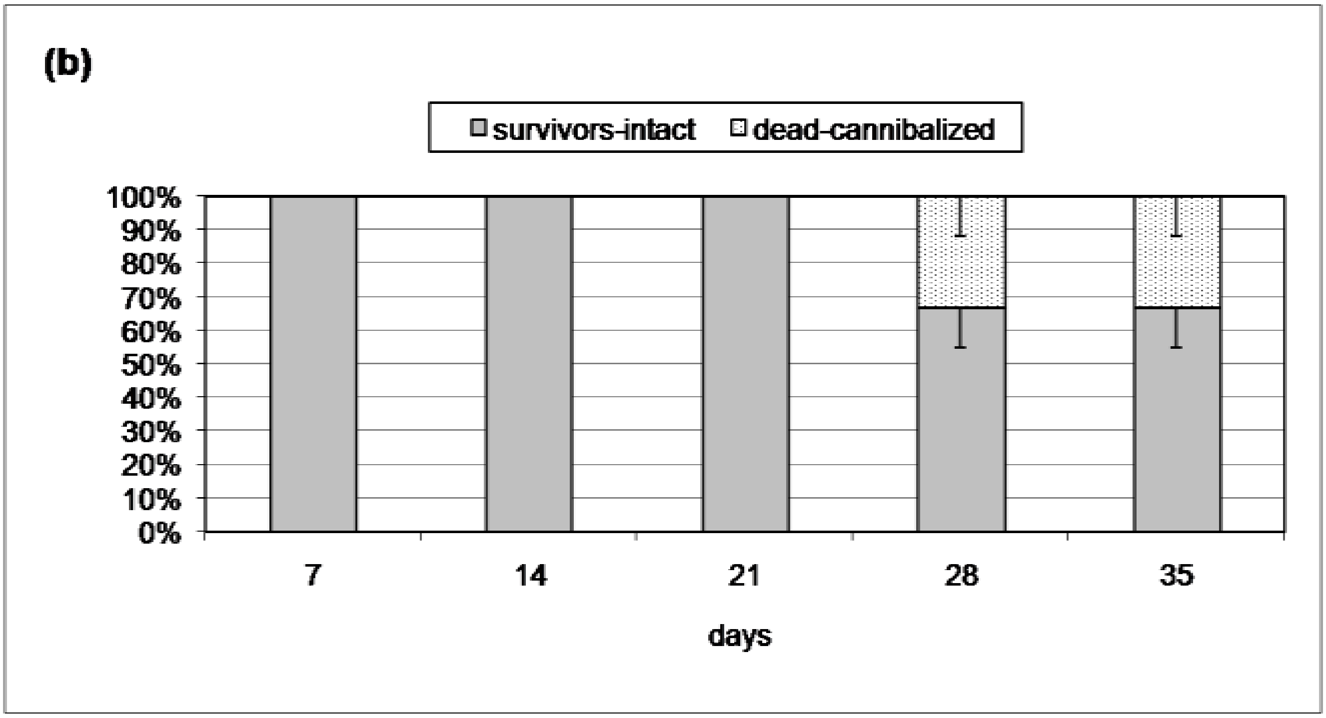
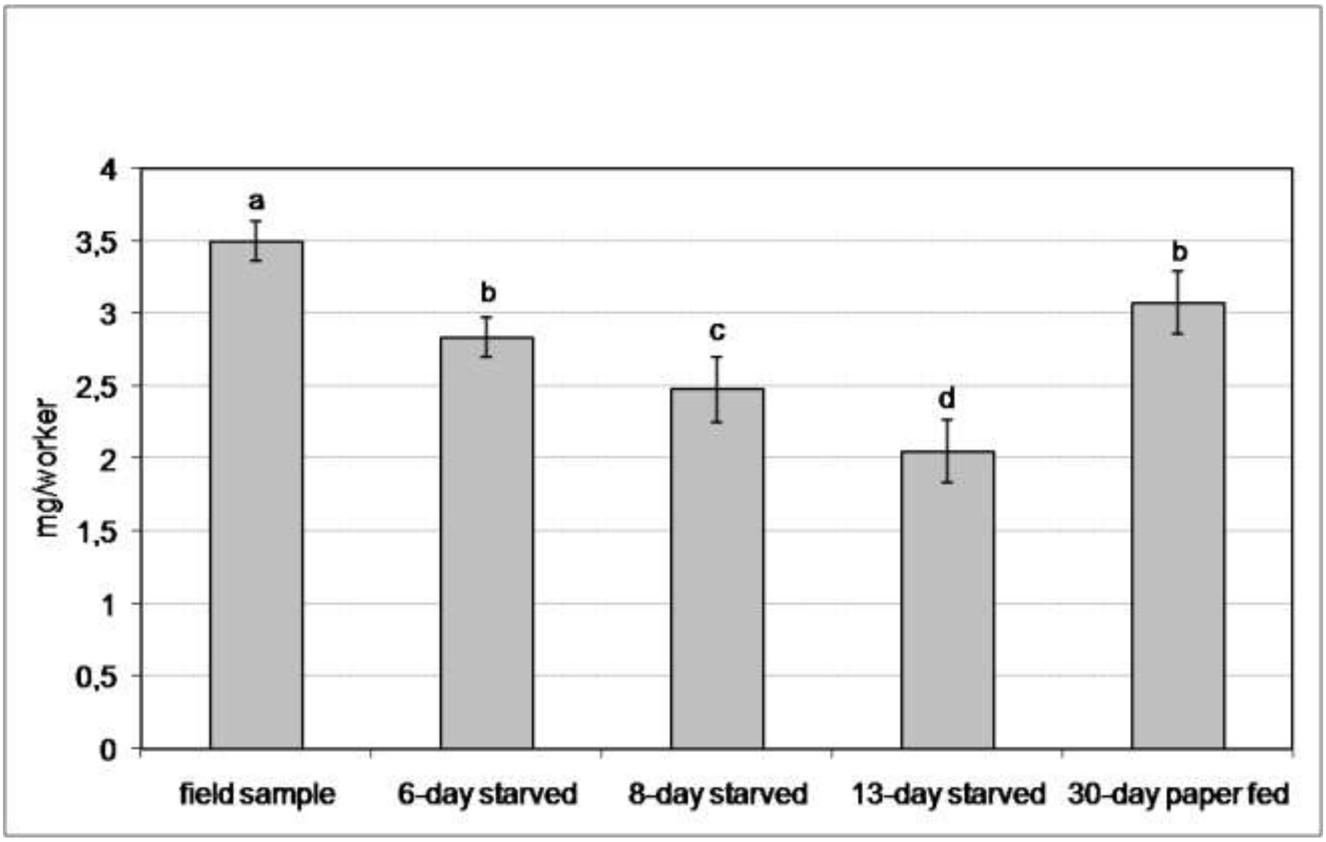

(b)
survivors-intact
dead-cannibalized
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
7
14
21
28
35
days

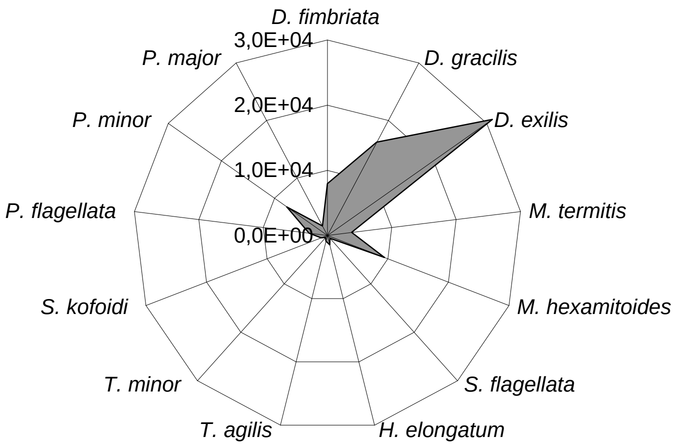
Fig 3



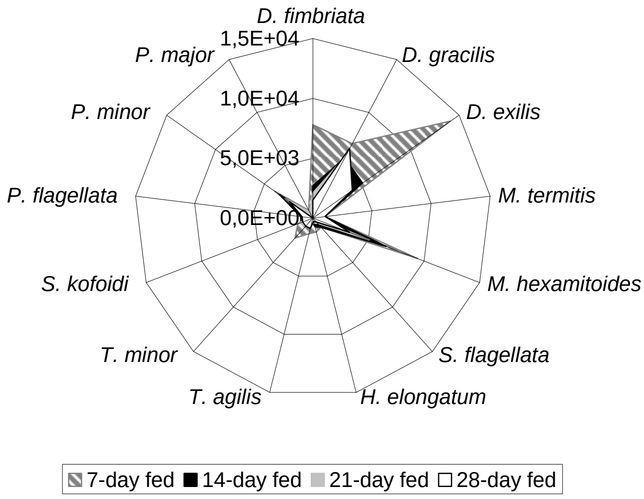
mg/worker
4
3,5
3
2,5
2
1,5
1
0,5
0
a
b
c
d
b
field sample
6-day starved
8-day starved
13-day starved
30-day paper fed

Fig 4

Field sample



Filter-paper fed



Starved termites