

## Cuticular permeability of two species of *Coptotermes* Wasmann (Isoptera: Rhinotermitidae)

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### Abstract

Cuticular permeability (CP) values of worker and soldier castes of *Coptotermes formosanus* Shiraki and *Coptotermes vastator* Light were determined using gravimetric techniques. Comparisons were made between castes, species, and between colonies of *C. formosanus* and *C. vastator*. CP values did not differ among either species or colony, however, they did differ between castes. Data reported here and that of established data in the literature for *C. formosanus* are inconsistent, with the current report having values as small as half that of previously published data. Examining mean CP data by colony indicated that there is a range for *Coptotermes* spp. termites, from  $6.84 \pm 1.2$  to  $25.61 \pm 1.49$   $\mu\text{gH}_2\text{O cm}^{-2} \text{mmHg}^{-1} \text{h}^{-1}$  dependent upon caste. A novel method of examining intercaste CP variability is the use of worker:soldier CP ratios. Ratios for *C. formosanus* were consistent between the current study and previous reports. Mean percentage total body water (%TBW) lost at the time of death was  $33.75 \pm 1.15\%$  (soldiers) and  $54.24 \pm 1.43\%$  (workers) for *C. formosanus*, and  $37.69 \pm 3.31\%$  (soldiers) and  $52.87 \pm 3.65\%$  (workers) for *C. vastator*. Consistently, %TBW (and fresh mass) was greater in worker than in soldier castes. These data suggest the use of water storage mechanisms in worker termites, as shown for other rhinotermitids.

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**Keywords:** Cuticular permeability; Subterranean termites; Water balance; *Coptotermes formosanus*; *Coptotermes vastator*

### 1. Introduction

Physiological ecology of subterranean termites has taken a backseat to control research in the past 30 years. However, interest in basic biology has recently become increasingly popular among researchers of the Isoptera. Such efforts have included interest in intercolonial agonistic and foraging behavior (Thorne and Haverty, 1991; Husseneder and Grace, 2001; Puche and Su, 2001; Campora and Grace, 2001). There has also been a

resurgence of interest in physiology among termite researchers recently both from descriptive as well as experimental aspects. Research areas have been wide ranging, including transfer of foraged materials among colony members (Suárez and Thorne, 2000), water balance (Sponsler and Appel, 1990), glandular descriptions (Grube and Rudolph, 1999), surface area modeling (Haagsma et al., 1996), and even respiratory patterns (Shelton and Appel, 2001a,b).

Cuticular permeability (CP) is defined as  $\mu\text{gH}_2\text{O}$  lost per unit surface area ( $\text{cm}^{-2}$ ) per unit time ( $\text{h}^{-1}$ ), per unit saturation deficit ( $\text{mmHg}^{-1}$ ), and is a means of describing and comparing water loss from insects and other arthropods (Wiggles-

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Table 1

Mean CP (in  $\mu\text{gH}_2\text{O lost}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}\cdot\text{mmHg}^{-1}$ )  $\pm$  S.E.M. values, fresh and dry mass, and %TBW for *C. formosanus* and *C. vastator* by species, colony, and caste for the surface area formulae of Meeh (1897) (M) and Haagsma et al. (1996) (H)

Species	Colony	Caste	n	CP (M)	CP (H)	Fresh mass (mg)	Dry mass (mg)	%TBW	CP (M) ratio (worker:soldier)
<i>C. formosanus</i>	1	Worker	30	25.61 $\pm$ 1.49	37.83 $\pm$ 2.20	2.86 $\pm$ 0.04	0.65 $\pm$ 0.02	77.4 $\pm$ 0.41	2.41
	1	Soldier	30	10.63 $\pm$ 1.40	14.82 $\pm$ 1.96	2.29 $\pm$ 0.04	0.63 $\pm$ 0.02	72.4 $\pm$ 0.53	
	2	Worker	30	19.33 $\pm$ 1.17	30.31 $\pm$ 1.85	3.97 $\pm$ 0.07	0.99 $\pm$ 0.02	75.1 $\pm$ 0.41	2.13
	2	Soldier	30	9.07 $\pm$ 0.98	13.73 $\pm$ 1.48	3.25 $\pm$ 0.07	0.92 $\pm$ 0.03	71.7 $\pm$ 0.44	
	3	Worker	30	19.35 $\pm$ 1.75	29.45 $\pm$ 2.73	3.30 $\pm$ 0.07	0.77 $\pm$ 0.02	76.5 $\pm$ 0.57	2.83
	3	Soldier	30	6.84 $\pm$ 1.20	10.46 $\pm$ 1.86	3.35 $\pm$ 0.05	0.98 $\pm$ 0.04	70.7 $\pm$ 1.34	
<i>C. vastator</i>	A	Worker	30	17.10 $\pm$ 1.47	25.92 $\pm$ 2.26	3.25 $\pm$ 0.08	0.85 $\pm$ 0.03	73.7 $\pm$ 0.71	1.81
	A	Soldier	30	9.46 $\pm$ 0.89	13.93 $\pm$ 1.31	2.85 $\pm$ 0.06	0.90 $\pm$ 0.03	68.5 $\pm$ 0.47	
	B	Worker	30	11.06 $\pm$ 1.14	16.43 $\pm$ 1.70	2.95 $\pm$ 0.04	0.73 $\pm$ 0.02	75.3 $\pm$ 0.47	1.34
	B	Soldier	30	8.28 $\pm$ 0.79	11.95 $\pm$ 1.13	2.58 $\pm$ 0.03	0.78 $\pm$ 0.01	69.8 $\pm$ 0.31	
	C	Worker	30	14.82 $\pm$ 1.71	21.94 $\pm$ 2.54	2.82 $\pm$ 0.06	0.64 $\pm$ 0.01	77.1 $\pm$ 0.67	1.32
	C	Soldier	30	11.16 $\pm$ 1.25	15.65 $\pm$ 1.74	2.36 $\pm$ 0.04	0.72 $\pm$ 0.02	69.6 $\pm$ 0.53	

worth, 1945; Edney, 1977; Hadley, 1994). A CP value describes the species' most common ecological habitat (Hadley, 1994). The association is fairly straightforward, low values are associated with low levels of water loss indicating animals adapted to xeric environments, while hydrically adapted animals usually have much higher CP values. Examples from termites extend from very low CP values in drywood termite workers (1.5–2.3  $\mu\text{gH}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{mmHg}^{-1}\cdot\text{h}^{-1}$ ; Rust et al., 1979) to relatively high in dampwood termite alates (46.87  $\mu\text{gH}_2\text{O}\cdot\text{cm}^{-2}\cdot\text{mmHg}^{-1}\cdot\text{h}^{-1}$ ; calculated from Collins, 1969).

While descriptions of the CP of subterranean termites have been reported for many years (Collins and Richards, 1963, 1966; Collins, 1969; Sponsler and Appel, 1990), few studies have offered CP comparisons between different species in the same genus. Previous work by Collins and Richards (1963) examined members of the *Reticulitermes* Holmgren genus found in the southeastern US. Sponsler and Appel (1990) compared two subterranean termites; one endemic, *R. flavipes* (Kollar), and one recently introduced to the southeastern US, *Coptotermes formosanus* Shiraki.

The present study examined CP values of the Formosan subterranean termite (*C. formosanus*) and the Philippine milk termite, *Coptotermes vastator* Light, each from three colonies on the island of Oahu, Hawaii. Both species of *Coptotermes* Wasmann are introduced to Hawaii, with *C. formosanus* recorded in the islands for at least 150 years (Su and Tamashiro, 1987). *C. vastator* was first reported from a shipment of bananas in

Honolulu in 1918 (Kofoid, 1934). However, since then it has been reported only sporadically, most recently in the Barber's Point Naval Air Station in Western Oahu (Woodrow et al., 2001).

Worker and soldier castes of both species were examined. The purpose of the study was to examine the potential for co-habitation of these species in different environments. Sponsler and Appel (1990) also examined CP in different castes of *C. formosanus*, but did not account for possible colony variation. In addition to potential differences between the species, we also examined differences in CP between physical (as opposed to temporal) castes and among colonies.

## 2. Materials and methods

### 2.1. Experimental animals

Three colonies each of *C. formosanus* and *C. vastator* were located on the island of Oahu, Hawaii. Colonies of *C. formosanus* were located in the Manoa valley, near buildings on the campus of the University of Hawaii (colonies 1, 2 and 3). These colonies were each more than 1000 m apart, having previously been designated as separate colonies by mark-release-recapture (MRR) studies (JKG, unpublished data), agonistic studies (Shelton and Grace, 1997), and multilocus DNA fingerprinting analysis (Husseneder and Grace, 2001). Colony size as estimated using triple MRR for each colony were: 0.5 (colony 1), 1.7 (colony 2), 1.3 (colony 3) million workers (all colony size estimates were made within the past 10 years;

JKG, unpublished data). Two colonies of *C. vastator* were found in separate former Navy housing units in the Kalaeloa area of western Oahu (colonies A and B). Triple MRR data was only available for colony A of *C. vastator*, indicating an estimated 0.2 million workers (in 2000; JKG, unpublished data). A single *C. vastator* colony was located on the outskirts of a Keawe forest near the Barber's Point Naval facility horse stables in Kalaeloa (colony C). All *C. vastator* colonies were separated by more than 1000 m.

Termites were extracted from infested Douglas fir (*Pseudotsuga menziesii* Dougl. ex. Laws.) box traps according to the methods of Tamashiro et al. (1973). The animals were collected less than 48 h prior to the start of each replicate study, and placed into an unlit incubator at  $28 \pm 1$  °C and ~90% relative humidity (RH) in 9.0 cm diameter glass Petri dishes lined with a single piece of filter paper (Whatman #2, Whatman International Ltd, Maidstone, England) moistened with <1 ml of distilled water.

Immediately prior to initiating each replicate, 6 workers and 6 soldiers from each colony tested were moved individually from the dishes using fine sable brushes (preventing possible injury from aspiration) into 40 ml glass shell vials. Only intact animals were used; soldiers that secreted defensive secretion (Quennedey and Deligne, 1975) were replaced. Since the amount secreted could not be measured exactly, the defensive secretion emitted would represent an uncontrollable error in the calculations of total body water (TBW) and dry mass for soldier caste termites. To avoid this bias, only those soldiers that did not secrete defensive fluid upon movement to the vials were used.

Initial masses were determined for each individual to the nearest 0.01 mg using a digital balance (Mettler model AE163, Mettler Instruments AG, Switzerland). Weighed termites were then placed in individual vials and placed in an 11-l glass desiccator containing a  $100 \times 150$  mm<sup>2</sup> plastic Petri dish (360 ml volume) filled with fresh (either new or newly reconstituted after 1+ h at 200 °C) indicating Drierite (anhydrous CaSO<sub>4</sub>; W.A. Hammond Drierite Company, Xenia, OH). Also prior to each replication, new stopcock grease (Lubriscal stopcock grease, Thomas Scientific, Swedesboro, NJ) was applied to the desiccator lid, and vacuum tested to ensure sealing. Thus, desiccators contained a RH value of 0–2% during the experiment. Desiccators were maintained in a drying oven

(Isotemp Oven, model 655F, Fisher Scientific, Fairlawn, NJ) at  $30 \pm 1$  °C. This combination of temperature and RH resulted in a saturation deficit of 31.824 mmHg within the desiccator.

At 2, 4, 6, 8, 10, 12 and 24 h after placing the vials in the desiccator (and the desiccator into the oven), the vials were removed briefly in groups of 6, and individual termite masses were determined to the nearest 0.01 mg before being returned to the desiccator. Time of death was noted when the termite could no longer be induced to show any movement by gently tapping the side of the vial. Percentage TBW lost at the time of death was determined for those termites dying within the first 12 h, since no recordings were made between 12 and 24 h. CP values were calculated using the difference between the initial and 2 h masses (in µg) as µg of H<sub>2</sub>O lost (Edney, 1977;  $CP = \mu\text{gH}_2\text{O lost} \times \text{surface area (in cm}^{-2}) \times \text{saturation deficit (in mmHg}^{-1}) \text{ h}^{-1}$ ).

Following the 24 h mass determination, the oven temperature was changed to 50 °C, and the desiccator (with vials) was returned. The bodies were dried to constant mass under these conditions for 72 h. Dry masses were recorded to the nearest 0.01 mg, and used in the calculation of percentage total body water

$$\%TBW = [(\text{initial mass} - \text{dry mass}) / \text{initial mass}] \times 100.$$

## 2.2. Statistics

Percentage TBW loss at each time period was determined, and subjected to linear regression analysis to examine the relationships with time (from 0 to 2, and 2 to 24 h). CP values were calculated using the surface area formulae of Meeh (1897) and Haagsma et al. (1996) (for termites). Although there are problems in applying the formula of Haagsma et al. (1996) to very large or very small termites (Shelton and Appel, 2001a), CP values calculated with this formula are included for comparative value. These data, as well as initial and dry masses, and %TBW were subjected to nested ANOVA procedures using SAS (SAS Institute, Inc., 1985).

## 3. Results

### 3.1. Percentage TBW

Table 1 presents mean ( $\pm$ S.E.M.) %TBW for each species, colony, and caste combination. Nest-

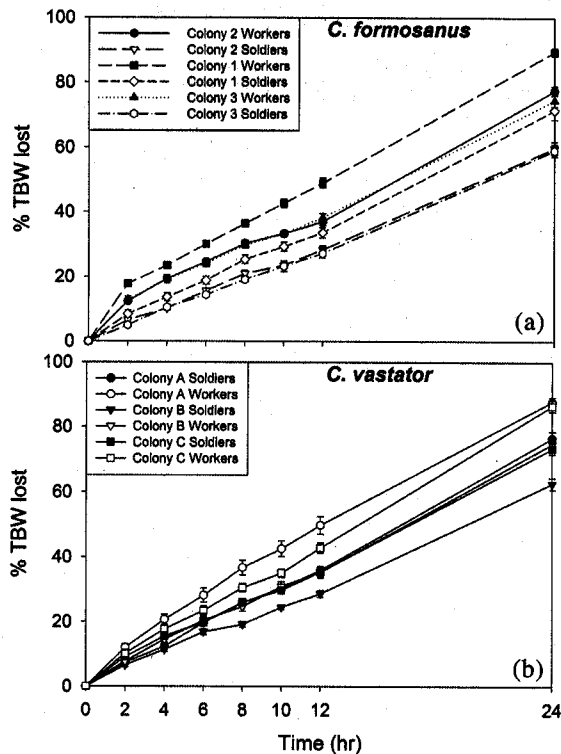


Fig. 1. Percentage TBW lost over time by workers and soldiers of *C. formosanus* (A) and *C. vastator* (B).

ed ANOVA analysis indicated that %TBW were significantly different only between castes (d.f. = 1;  $F=115.04$ ;  $P=0.0004$ ), with no significant differences between species or among colonies (d.f. = 1, 4;  $F=4.24, 1.95$ ;  $P=0.1087, 0.1013$ , respectively).

Percentage TBW loss over time is illustrated in Fig. 1 for each species. The origins for the data in Fig. 1 are constrained to zero, as no water loss occurred at the beginning of the experiment (time of initial mass). Linear regression of the %TBW data from 2 to 24 h indicated that for all castes, colonies, and species water was lost in a linear fashion.  $P$ -values for all slopes were  $<0.0001$ , although not all intercepts were significantly different from 0. However, when the initial mass is considered as a loss of zero, loss over time follows a two-step pattern (Fig. 1). The slope of %TBW from 0 to 2 h for both species combined was  $4.84 \pm 0.0004$ , while that of %TBW from 2 to 24 h was  $2.92 \pm 0.101$ . Loss of %TBW did vary among caste and colony as indicated in Fig. 1. Percentage TBW at the time of death ranged from

17.12% for workers of *C. vastator* to 63.33% for workers of *C. formosanus*. Mean %TBW at time of death was  $33.75 \pm 1.15\%$  for soldiers ( $n=37$ , mean time of death =  $10.7 \pm 0.31$  h), and  $54.24 \pm 1.43\%$  for workers ( $n=13$ , mean time of death =  $10.31 \pm 0.63$  h) of *C. vastator*. For *C. formosanus*, mean %TBW at time of death was  $37.69 \pm 3.31\%$  for soldiers ( $n=6$ , mean time of death =  $11.0 \pm 0.68$  h), and  $52.87 \pm 3.65\%$  for workers ( $n=4$ , mean time of death =  $12.0 \pm 0.0$  h).

### 3.2. Cuticular permeability

Mean CP data ( $\pm$ S.E.M.) are presented in Table 1 for each species, colony, and caste combination. These data are presented using both Meeh's (1897) general surface area model, as well as that specifically for termites by Haagsma et al. (1996). In all cases, Haagsma et al. (1996) formula produced lesser surface areas, reflected in the data as greater CP values. For CP using Meeh's (1897) surface area equation, nested ANOVA procedures resulted in significant differences among the castes (d.f. = 1;  $F=73.0$ ;  $P=0.001$ ), as well as a significant species by caste interaction (d.f. = 1;  $F=15.22$ ;  $P=0.0175$ ). However, colony, both within species (colony (species)) and as colony by caste (species), did not significantly influence CP (d.f. = 4, 4;  $F=3.58, 1.80$ ;  $P=0.1221, 0.1274$ , respectively). Likewise, species (with all other variables combined) were not significantly different (d.f. = 1;  $F=2.73$ ;  $P=0.1741$ ). Nested ANOVA procedures using CP data calculated using the surface area formulae of Haagsma et al. (1996) resulted in the same trends (and corresponding levels of significance) presented for the data calculated using Meeh's (1897) formula.

### 3.3. Fresh and dry mass

Mean fresh masses ( $\pm$ S.E.M.) as well as dry masses ( $\pm$ S.E.M.) are reported in Table 1. Nested ANOVA indicated that species did not have different fresh masses (d.f. = 1;  $F=1.20$ ;  $P=0.3346$ ). However, fresh masses differed among colonies (by caste within species: d.f. = 4;  $F=13.44$ ;  $P<0.0001$ ) and between castes within species (d.f. = 1;  $F=12.05$ ;  $P=0.0256$ ). For dry masses, no significant differences were found between species or castes (d.f. = 1, 1;  $F=0.23, 1.55$ ;  $P=0.6542, 0.2809$ , respectively). However colonies (by caste,

within species) were significantly different (d.f. = 4;  $F=9.24$ ;  $P<0.0001$ ).

#### 4. Discussion

Individuals of both species, when considered separately by caste and colony showed a typical two-compartment model for mass loss (assumed to be water loss) over the first 24 h of desiccation. Thus the animals lost water at a greater rate during the first 2 h of desiccation than during the other time periods (during which %TBW loss was linear). These data are consistent with prior information from the literature for other species with two-compartment water loss models (beetles and blood-feeding Hemiptera, Wigglesworth, 1945; cockroaches, Appel et al., 1983; Nymphalidae, Mazer and Appel, 2001). These models assume that water is lost first from the exterior surface of the integument (the highest rate of loss), and then lost from the interior of the animal during the second phase or compartment (the lower rate of loss) (Wigglesworth, 1945).

Our methods were unable to differentiate among the components of total water loss: cuticular (or true CP), defecation, and respiratory water loss. CP values given in this paper represent only total water loss, however these values may only slightly overestimate true CP. In the Western drywood termite, *Incisitermes minor* (Hagen), cuticular water loss represented the major water loss route, accounting for 89.1–93.5% of total water loss depending on temperature (Shelton and Appel, 2000). However, drywood termites generally have much lower CP values than rhinotermitids (Collins and Richards, 1963, 1966; Collins, 1969). In *R. flavipes* alates, ~95% of the total water loss is non-respiratory (peak vs. valley water loss; Shelton and Appel, 2001a) during cyclic respiration. However, subterranean termite workers (*C. formosanus* and *R. flavipes*) do not exhibit cyclic respiration (Shelton and Appel, 2001b). It is difficult to argue that CP should not account for the majority of the total water loss in subterranean termites, however the exact relationship is unknown. Future work on *Coptotermes* spp. water balance should provide these answers.

Differences in %TBW lost at time of death were mainly between castes rather than between species. When separated by caste, these values for each species were within 1 S.E.M. of the other. However, the number of individuals surviving to 12 h

under the desiccating conditions were widely different. In *C. formosanus*, only 10 individuals (0.06%; 6 soldiers, 4 workers) died prior to 12 h in the study, while 50 individuals (27.8%; 37 soldiers, 13 workers) of *C. vastator* fell into this category. For both species, %TBW lost at time of death was well within the range for soldiers and workers of *C. formosanus* reported previously (35.30 and 50.52%, respectively; Sponsler and Appel, 1990). This five-fold difference in survival to 12 h under desiccation is contrary to what might be predicted from their CP values (Table 1). This points out that while CP estimates transpiration rates across the cuticle, it is not necessarily predictive of animal survival under desiccation that may involve other mechanisms. While both species are tropical, *C. vastator* occurs in locations that are more equatorial (such as the Philippine Islands; Kofoid, 1934), while *C. formosanus* occurs in areas that are more subtropical (Japan, China, US; Su and Tamashiro, 1987).

The most striking difference observed in our data is the difference in CP values between the castes of both species. In both *C. formosanus* and *C. vastator*, the soldier caste had significantly reduced CP values compared with the workers. The values measured here also differ greatly from those published on *C. formosanus* from other geographical areas (Sponsler and Appel, 1990). Sponsler and Appel's (1990) study showed a significantly greater CP value in workers (mean =  $37.49 \pm 2.32$ ) than in soldiers (mean =  $15.22 \pm 0.93$ ). However, CP values in our study are nearly half those of Sponsler and Appel (1990) (workers, colony 2:  $19.33 \pm 1.17$ ; soldiers, colony 3:  $6.84 \pm 1.2$ ; Table 1). Their study only used a single colony for workers and soldiers. While colony did not significantly influence CP value (see below), there was a range of CP for various colonies of both *C. formosanus* and *C. vastator* (Table 1). Perhaps this inconsistency with the previous data reflects a larger range of CP value for colonies of *Coptotermes* spp. Another consideration is the difference in rearing methods between the two studies. The current study used field-collected termites taken directly from feeding stations (within 48 h), while Sponsler and Appel (1990) used termites maintained in an incubator for at least 1 week. Perhaps the termites in the previous study had become acclimated, and therefore differed in their measured CP values, as with

*Periplaneta fuliginosa* (Serville) cockroaches (Appel and Rust, 1985).

One way to examine the variability in these data is to look at the ratio of worker to soldier CP values. When considered as a ratio, we find that Sponsler and Appel's (1990) (*C. formosanus*: 2.46) data come closer to our values (Table 1). By comparison, *C. vastator* (Table 1) has a significantly lower ratio than *C. formosanus* (paired *t*-test; d.f. = 3;  $T=3.74$ ;  $P=0.03$ ). The ratio concisely reflects the caste differences observed in Table 1 for each species, with *C. vastator* worker and soldier caste CP values being much more similar than in *C. formosanus*.

While *C. formosanus* and *C. vastator* did not have significantly different CP values, our data suggest that there is considerable variability in both species for this value. While the variation between colonies was fairly high, CP values did not differ significantly with colony affiliation. Table 1 indicates that similar castes among different colonies have variable CP values over a range, with very little overlap even between colonies of the same species. In general, our data suggest that *C. vastator* does have a lower CP for worker caste individuals (but not soldiers) than *C. formosanus*. However, due to the overlap of colony A with colonies 2 and 3, this was not statistically evident. Soldier CP values for the two species appeared to be quite similar (Table 1). Considering that soldiers normally constitute no more than 25% of the colony in *Coptotermes* spp. (Haverty, 1977), a trend of reduced permeability of *C. vastator* workers compared with *C. formosanus* may indicate greater xeric adaptation in the former species. Since soldiers typically act to block breaks in the gallery system with their bodies, as well as moving out of the gallery entirely to defend the colony from attacking ants (Prestwich, 1984), an overall greater degree of sclerotization and concomitant reduced CP values in this defensive caste is not unexpected.

Both species in this study showed variability in fresh and dry masses (Table 1). However, the species themselves were not significantly different for these values. Fresh mass was significantly different between castes, but not dry mass. This indicates that the castes differed in their %TBW, which is supported by the data from %TBW (Table 1). In all colonies, soldiers contained a lesser percentage of water than workers, perhaps an indication of increased muscle percentage (or

increased sclerotization) in their fresh masses. However, there was no difference between dry masses, which suggests that the muscle masses were equivalent. These data are more consistent with the idea that the workers simply contain more water than soldiers, or store more water perhaps by using water sacs as in *R. santonensis* De Feytaud (Grube and Rudolph, 1999).

The low level of differences in CP values between these two species of *Coptotermes* is consistent with Chown's (2001) recent review of variability in basic physiological parameters of insects. He argues that these variables are more consistent at the species level, and more variable at higher taxonomic levels. Our data also indicated differences among colonies for fresh and dry mass, and %TBW. Fresh and dry masses of colonies should change over time as the colony ages and the age class distribution of its members moves toward larger individuals (Grace et al., 1995). Exact ages for the colonies used in this study are not known, although all three *C. formosanus* have been under observation for at least 10 years (colony 1 for nearly 30 years; JKG, unpublished observations). Our data indicate that colony source should be considered when physiological values are estimated. However, colony does not always have a significant influence on physiological parameters as with CO<sub>2</sub> emission rates (Shelton and Appel, 2001b).

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