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Influence of acclimation time on laboratory-recorded social behaviors of the termite *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae)

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Abstract. Behavioral assays are widely used to investigate social interactions in termites; however, methodological factors such as acclimation time prior to observation remain poorly standardized. Handling and confinement may induce transient stress responses that bias behavioral measurements, yet empirical assessments of this effect are scarce. Here, we evaluated the influence of short-term acclimation time on the expression of social behaviors in the termite *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) under laboratory conditions. Groups of termites were observed after different acclimation periods (5, 30 and 60 min.), and the frequencies of antennation, vibration, grooming, and trophallaxis were video recorded for posterior quantification. Acclimation time significantly affected vibration behavior, which was more frequent immediately after handling and declined following longer acclimation periods. In contrast, antennation, grooming, and trophallaxis were not significantly influenced by acclimation time. These results indicate that vibration is particularly sensitive to handling-induced stress, whereas other social behaviors are comparatively robust to short-term experimental disturbance. Our findings highlight the importance of reporting and standardizing acclimation time in termite behavioral assays and provide practical guidance for improving methodological consistency and comparability across studies.

Keywords: termite behavior, handling stress, bioassay standardization, eusocial insects.

Known mainly for their lignocellulose-feeding habits, termites (Blattodea: Isoptera) are eusocial insects of considerable ecological importance (Bignell 2018). They play key roles in the breakdown of organic matter and nutrient cycling, thereby sustaining soil fertility and ecosystem functioning (Jouquet et al. 2011; Griffiths et al. 2019; Khan et al. 2018; Amogha et al. 2024; Kishore et al. 2024). On the other hand, several species thrive in anthropogenically modified environments and are considered serious pests, causing economic losses estimated at billions of dollars annually due to damage to wooden structures and agricultural systems (Rust & Su 2012; Chand et al. 2018; Mahapatro & Chatterjee 2018; Rana et al. 2021).

Behavioral assays are widely used to investigate interactions in termites. Extensive experimental research has examined termite ecology (e.g., Almeida et al. 2018; Silva et al. 2021; 2023), behavior (e.g., Hugo et al. 2020; Paiva et al. 2021), and biological or chemical control strategies (e.g., Chen et al. 2015; Ferreira et al. 2023). Accordingly, numerous protocols have been developed to improve the reliability of laboratory bioassays and minimize external sources of bias. These efforts include studies on collection and rearing methods (Chouvenc 2023), humidity regimes (Zukowski & Su 2017), and the use of color markers (Marins et al. 2023). Nevertheless, because handling-induced stress and other experimental factors may significantly affect social dynamics, additional caution is required when behavioral patterns are the primary focus.

In termites, disturbance commonly elicits transient increases in alarm signaling, vibrational activity, exploratory movement, and inspection or defensive behaviors, which may temporarily disrupt routine social interactions such as grooming or trophallaxis (Martin & Bateson 2007). Although longer acclimation periods may reduce these immediate alert responses and allow interaction patterns to stabilize, extended acclimation can also mask short-lived but ecologically relevant behaviors, alter contact rates and social organization under artificial conditions, and facilitate secondary processes (e.g., habituation or social transfer) that may influence behavioral outcomes (Martin & Bateson 2007). Thus, acclimation time represents a trade-off between minimizing handling artifacts and preserving biologically meaningful responses.

One particularly overlooked parameter is acclimation time, defined as the interval between handling or experimental manipulation and the onset of behavioral observation (Martin & Bateson 2007). In termite studies, acclimation time is often arbitrarily defined, inconsistently applied, or not reported at all (Cornelius & Osbrink 2009). This lack of standardization limits the comparability and reproducibility of behavioral data across studies. Short acclimation periods may capture transient stress or alert responses rather than baseline social behavior, whereas longer periods may allow groups to stabilize and resume normal interaction patterns (Roisin 2000; Jeanson & Weidenmüller 2014). Despite its potential importance, empirical evidence assessing the influence of acclimation time on termite behavior remains scarce.

Interactions among nestmates are essential for group cohesion and the transfer of information in eusocial insects (Bagnères & Hanus 2015). Termites primarily use inputs from social interactions or environmental cues to shape their behavioral repertoire. Several studies have demonstrated that termite behavior can be influenced by the presence of competitors and predators (e.g., Ishikawa & Miura 2012; Silva et al. 2023), as well as by exposure to insecticides and entomopathogens (e.g., de Mendonça et al. 2023; Ferreira et al. 2023).

Stress is naturally produced during removal of termites from their nests and experimental units' assembly, which may have immediate impact on individual behaviors and, in turn, laboratory study results. Therefore, in this study, we evaluated the effect of acclimation resting time on the frequency of behavioral events exhibited by termites. We used groups of different sizes to do this, since experiments with termites tend to vary in group size. Specifically, we hypothesized that a minimum resting period is required to start behavioral bioassays without confounding effects of handling-induced stress during unit assembly. We used the termite *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) as a model species in order to answer these questions. Because of its extremely voracious behavior and ability to establish in both urban areas and agricultural environments, *N. corniger* is currently considered one of the most significant termite pest species in the Americas (Boulogne et al. 2017).

To test our hypothesis, three colonies of *N. corniger* were collected on the campus of the Universidade Federtal Rural de Pernambuco (UFRPE), localized in Recife, Pernambuco, Northeastern, Brazil. From each colony, experimental groups of 10 and 60 individuals were established (Fig. 1), with three replicates per group size. Groups consisted of workers and soldiers, maintaining the natural caste proportion of 30% soldiers (Thorne 1985). To standardize handling-induced stress and acclimation time, individuals were initially separated into plastic containers (3 L.) and subsequently transferred to Petri dishes (50 × 12 mm) lined with filter paper (Fig. 1). Behavioral recordings were performed after three resting intervals (five, 30, and 60 min.) (Fig. 1), under controlled conditions (25±2 °C, RH 70±5%, in low-intensity lighting). Videos were recorded for five minutes using a digital camera (Webookers WB®, Webcam USB Full HD 1080P WB) and later analyzed by two independent observers. The following behaviors were quantified: antennation, vibration, grooming, and trophallaxis, as defined in Tab. 1. A total of 54 videos were analyzed.

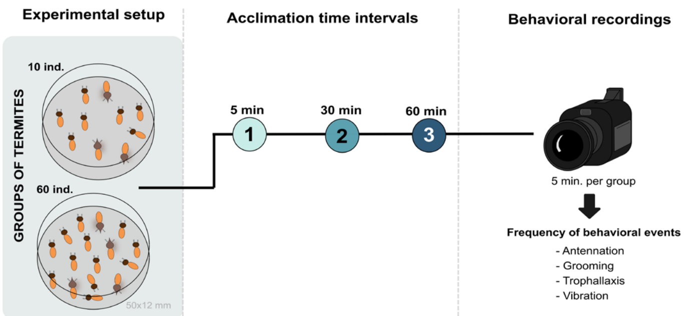

Figure 1. Experimental design and behavioral recording protocol. Termite groups (10 or 60 individuals) were placed in Petri dishes (50 × 12 mm) lined with filter paper. To standardize handling-induced stress, groups were subjected to one of three acclimation periods (5, 30, or 60 min) prior to recording. Behavioral activity was then recorded for 5 min using a video camera for posterior analysis. Videos were analyzed by two independent observers, and the frequency of antennation, vibration, grooming, and trophallaxis was quantified.

Table 1. Description of behaviors observed during bioassays with *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) groups.

Behavior	Description
Vibration	vibrating movement of the body with or without stimulation from another individual
Antennation	contact between the antennae or between the antennae and the body of a nest mate
Grooming	contact between an individual's mouth and their own body or the body of another individual
Trophallaxis	apparatus of one individual with the end of the abdomen of another individual, receiving proctodeal contents

Data was analyzed with Generalized Linear Mixed Models (GLMMs) with Poisson error distribution to assess the effect of acclimation time on the frequency of behaviors. Models included acclimation time as fixed factor and repetition nested in colony identity as random factors. Behaviors and group sizes were analyzed independently. All models were subjected to residual diagnostics to check the overdispersion of the models (*DHARMa* package). Differences between time intervals for each behavior were conducted using Estimated Marginal Means with false discovery rate adjustments (*EMmeans* package). All statistical analyses were performed in R (R Core Team 2025).

Vibration was the only behavior significantly affected by acclimation time in both group sizes (10 ind.: Chisq= 50.60, *d.f.*= 2, $P < 0.0001$; 60 ind.: Chisq= 514.11, *d.f.*=2, $P < 0.0001$). In groups of 10 individuals, the highest frequency of vibrations occurred after five minutes of rest, with no significant difference between 30 and 60 minutes (P= 0.12) (Fig. 2A). A similar pattern was observed in groups of 60 individuals, in which vibration frequency was highest at 5 minutes and decreased thereafter, with no difference between the 30- and 60-minute intervals (P= 0.18) (Fig. 3A). Acclimation time did not significantly affect antennation (10 ind.: Chisq= 5.66, *d.f.*= 2, P= 0.06; 60 ind.: Chisq= 1.13, *d.f.*= 2, P= 0.56), grooming (10 ind.: Chisq= 3.58, *d.f.*= 2, P= 0.16; 60 ind.: Chisq= 2.17, *d.f.*= 2, P= 0.33), or trophallaxis (10 ind.: Chisq= 0.93, *d.f.*= 2, P= 0.62; 60 ind.: Chisq= 4.02, *d.f.*= 2, P= 0.13) in either group size (Figs. 2B-D and 3B-D).

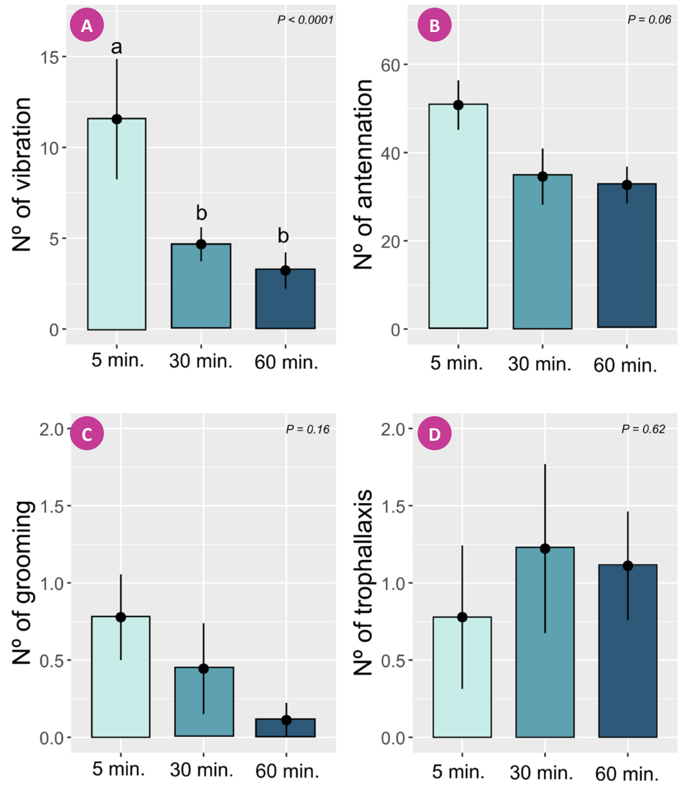

Figure 2. Effect of acclimation time on behaviors in *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) groups with 10 individuals. Asterisk indicates significant differences among acclimation times ($P < 0.05$). Note the different y-axis scales between panels.

By experimentally isolating the effect of acclimation time, our results clarify which social behaviors are most sensitive to short-term laboratory disturbance. The results of this study demonstrate that acclimation time can differentially influence the expression of social behaviors in *N. corniger*, with clear methodological implications for laboratory-based behavioral assays. Among the behaviors evaluated, vibration was the only one significantly affected by acclimation time, indicating that this alarm-related response is particularly sensitive to handling-induced disturbance. In contrast, antennation, grooming, and trophallaxis remained stable across acclimation periods, suggesting that these routine social interactions are comparatively robust to short-term experimental stress. These behaviors are essential for maintaining group cohesion (Bagnères & Hanus 2015), and their stability suggests that the alert state induced by handling does not interfere with activities unrelated to alarm responses.

Vibration behavior in termites is widely associated with alarm signaling and rapid communication in response to disturbances (Cristaldo et al. 2015). The elevated frequency of vibration immediately after handling likely reflects a transient alert state induced by manipulation and confinement rather than baseline social activity. As acclimation time increased, vibration levels declined, indicating that groups progressively stabilized and resumed normal interaction patterns. This finding highlights that short acclimation periods may lead to overestimation of alarm-related behaviors, potentially confounding interpretations in studies that aim to assess social communication or stress responses.

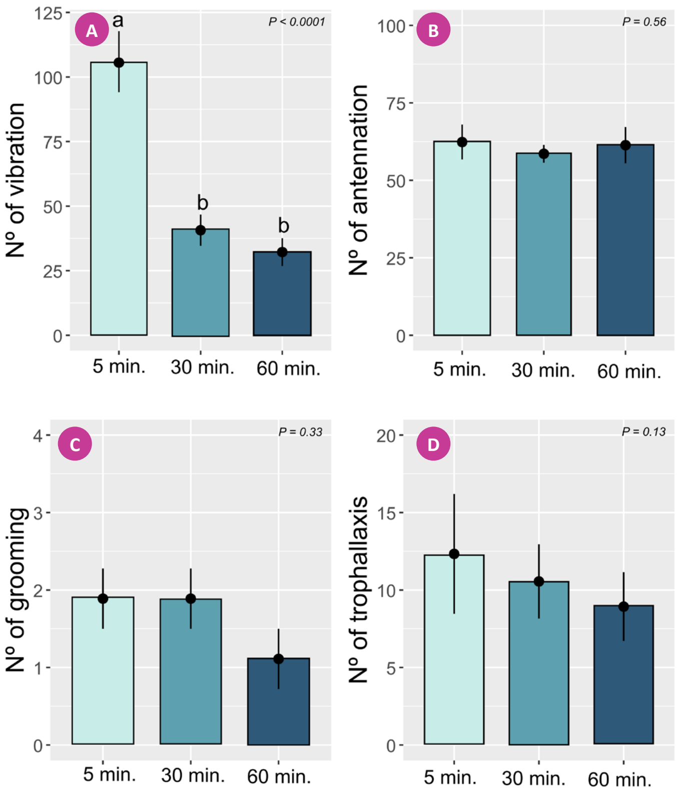

Figure 3. Effect of acclimation time on behaviors in *Nasutitermes corniger* (Motschulsky, 1855) (Termitidae: Nasutitermitinae) groups with 60 individuals. Asterisk indicates significant differences among acclimation times ($P < 0.05$). Note the different y-axis scales between panels.

In contrast, the absence of acclimation effects on antennation, grooming, and trophallaxis suggests that these behaviors are less sensitive to short-term disturbance. Antennation is a fundamental component of social interaction mediating nestmate recognition (Bagnères & Hanus 2015), while grooming and trophallaxis are central to colony maintenance and cohesion. The stability of these behaviors across acclimation times indicates that they can be reliably quantified even shortly after experimental setup, provided that handling intensity is comparable. This distinction among behavioral categories underscores the importance of considering the functional context of each behavior when designing and interpreting laboratory assays.

From a methodological perspective, our findings emphasize that acclimation time should not be treated as a trivial or interchangeable parameter in termite behavioral studies. Variation in acclimation protocols among studies may contribute to inconsistencies in reported behavioral frequencies, particularly for alarm-related responses. Explicitly reporting acclimation time and adopting standardized resting periods can improve the reproducibility and comparability of behavioral data across laboratories and experimental contexts.

It is important to note that this study focused on short-term acclimation under controlled laboratory conditions and on a single termite species. While *N. corniger* is a well-established model with pronounced social behaviors, the sensitivity of vibration to handling-induced stress may vary among species or experimental setups. Future studies could extend this approach to other termite taxa, group compositions, or environmental conditions to further refine methodological recommendations.

In conclusion, our results show that acclimation time selectively affects alarm-related behavior in *N. corniger*, while leaving routine social interactions largely unaffected. By identifying which behaviors are sensitive to experimental disturbance, this study provides practical guidance for the design and interpretation of termite behavioral assays. These findings contribute to improved experimental rigor in termite behavioral research.

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Authors' Contributions

SYMW: methodology, investigation, data curation, data analysis, & writing. DL: investigation & writing. LQLF: investigation & writing; PFC: conceptualization, methodology, editing & supervision.

Conflict of Interest Statement

The authors declare no conflict of interest

Ethical Approval

Not applicable

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Generative AI Statement

Not applicable.

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