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Problems With the Interpretation of Mark-Release-Recapture Data in Subterranean Termites (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The Lincoln index (Peterson method) is frequently used to estimate animal population size in mark-release-recapture studies. We tested the accuracy of this method to estimate termite colony size using logs infested with termites that were maintained in the laboratory. Termites were fed paper towels dyed either with 0.05% or 0.1% (w/w) of the dye marker Nile blue and released into their host logs in the laboratory. Following recapture a week later, estimates of termite population size were approximately 10 times greater than the actual population size for termites dyed with 0.05% Nile blue, and were approximately 3 times greater for termites dyed with 0.1% Nile blue. Concentrations of 0.1% Nile blue are not used in field studies because they result in higher mortality than 0.05% in laboratory trials. However, our data suggest that greater accuracy may be obtained using the higher dye concentration. The triple-catch method is frequently used in place of the Lincoln index in order to reduce standard errors of the estimate. However, our standard errors were lower than those of many published studies using the triple-catch method. The assumptions governing the efficacy of mark-release-recapture may be violated because of potential model biases that result from developing marking techniques in the laboratory that are intended for field use.

INTRODUCTION

Estimating the foraging population of subterranean termite colonies is difficult because of their cryptic behavior (Forschler 1994). Termites can tunnel several meters below the surface of the soil (Watson 1960) and forage well over a thousand square meters (Grace *et al.* 1989, Grace 1990, Su *et al.* 1993). Although direct counts are sometimes possible (Howard *et al.* 1982, Nutting and Jones 1990), such destructive sampling techniques preclude further study of the termite colony. Using mark-release-recapture studies to estimate subterranean termite colony

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size allows future monitoring of population dynamics for pest management and ecological study purposes.

The Lincoln index (Peterson method) is commonly used to determine the size of field populations of subterranean termites (Grace *et al.* 1989, Grace 1990, Su *et al.* 1984). However, there is usually a large standard error associated with these estimates. Su and Scheffrahn (1988) and Su *et al.* (1993) used the triple-catch method with weighted means (Begon 1979) instead of the simple Lincoln index to measure termite colony size, thereby improving the precision of the population estimates by reducing the standard error of the estimate (Oi and Su 1994). However, estimates may be precise but not accurate. The accuracy of both techniques is based on the same five assumptions: 1) the population is closed, 2) all animals have the same chance of being caught, 3) marking does not affect their catchability, 4) animals do not lose their marks, and 5) all marks are reported in the recapture sample (Begon 1979, Krebs 1989). Estimate bias may arise if one or more of these assumptions is violated.

No studies have determined the accuracy of their estimates of termite population density. In the present study, we adapted the mesocosm concept (Odum 1984) to determine the accuracy and precision of the population estimates generated by the Lincoln index. Mesocosm refers to an experimental design which bridges the gap between the laboratory and the real world environment. In the laboratory, termites were maintained in their host logs collected from the field. The termite population for each log was first estimated by the Lincoln index, and then the termites were directly counted for comparison with the estimate. We tested two concentrations of the dye marker Nile blue: 0.05% which is routinely used in mark-release-recapture studies in *Reticulitermes* (Su *et al.* 1993, Haagsma and Rust 1995, Forschler and Henderson 1995) and a concentration of 0.1% for comparison. Previous laboratory studies have shown that termite mortality is greater with 0.1% Nile blue than at 0.05% (Haagsma and Rust 1995).

MATERIALS AND METHODS

Six pine logs infested with *Reticulitermes virginicus* (Banks) (Isoptera: Rhinotermitidae) were collected from southeastern Virginia, USA, from July, 1995 through September, 1995. Voucher specimens from each colony were deposited in the reference collection at Old Dominion University, Norfolk, Virginia. Each log was approximately 70 cm long x 25 cm in diameter and each was isolated in an 80-liter metal trash can. Termites were removed from a section approximately 1/8 the mass of each log and placed into a 4-liter plastic container. The containers were

first prepared by adding 200 ml of vermiculite, 100 ml of deionized water, and six paper towels dyed with either 0.05% or 0.1% (w/w) Nile blue. After five days, only dyed termites were counted and then released into their respective logs.

One week later, another 1/8 section of the log was removed and the marked and unmarked termites were counted. Immediately following this recapture count, the remaining portion of the log was broken and all termites were removed using a wire-mesh sifter with an aperture size of 3 mm. Their total volume was measured, and 10% of the total volume of termites was counted in three equal replicates, each 3.33% of the total volume. All blue termites were counted directly. Termite population size was determined by multiplying the total volume of termites by the average number of termites per milliliter of the three replicates.

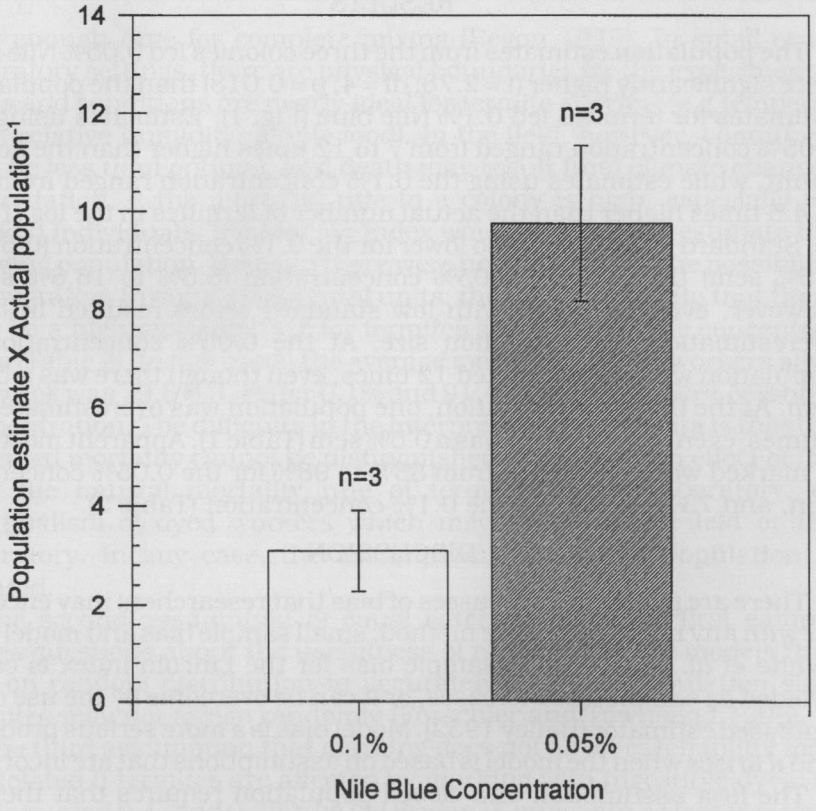

Fig. 1. Lincoln index population size estimates of *Reticulitermes virginicus* (Banks) using 0.05- and 0.1 % (w/w) Nile blue. Units of the y-axis are given as population estimate X actual population^{-1}.

Table 1. Lincoln index estimates of termite colony population size and the actual population size determined by direct count for both concentrations of Nile blue.

Nile blue conc. (w/w)	Colony	Population estimate*	sem**	% sem	Actual population	estimate X actual^{-1}
0.05%	1	82845	13727	16.6%	11705	7
	2	527449	29169	5.5%	42030	12
	3	95747	11914	12.4%	9953	9.6
0.1%	4	131799	2245	1.7%	78107	1.6
	5	62586	4688	7.5%	13798	4.5
	6	203595	998	0.5%	66970	3

*Lincoln index estimate
**Standard error associated with the Lincoln index estimate

RESULTS

The population estimates from the three colonies fed 0.05% Nile blue were significantly higher ($t = 2.78$; $df = 4$; $p = 0.018$) than the population estimates for termites fed 0.1% Nile blue (Fig. 1). Estimates using the 0.05% concentration ranged from 7 to 12 times higher than the actual count, while estimates using the 0.1% concentration ranged from 1.6 to 4.5 times higher than the actual number of termites in the log (Table 1). Standard errors were also lower for the 0.1% concentration (0.5% to 7.5% sem) than for the 0.05% concentration (5.5% to 16.6% sem). However, even estimates with low standard errors resulted in large overestimations of population size. At the 0.05% concentration, a population was overestimated 12 times, even though there was a 5.5% sem. At the 0.1% concentration, one population was overestimated by 3 times, even though there was a 0.5% sem (Table 1). Apparent mortality of marked workers ranged from 85% to 98% for the 0.05% concentration, and 73% to 82% for the 0.1% concentration (Table 2).

DISCUSSION

There are generally two classes of bias that researchers may encounter with any mark-recapture method: small sample bias and model bias (White *et al.* 1982). Small sample bias for the Lincoln index is easily avoided as sample size increases, or it can be overcome by the use of an unbiased estimator (Bailey 1952). Model bias is a more serious problem, and it arises when the model is based on assumptions that are incorrect.

The first assumption of a closed population requires that there is neither emigration nor death in the population. Therefore, the time between release and recapture should be kept to a minimum, allowing

Table 2. Number of termites marked and released (M), number of individuals in recapture sample (C), number of individuals in recapture sample that are marked (R), actual number of marked individuals in the log at the time of the estimate (AM), and mortality of marked individuals (MM).

Dye conc.	Colony	M	C	R	AM	MM*
0.05%	1	484	1026	5	12	98%
	2	2406	3945	17	186	92%
	3	433	1768	7	67	85%
0.1%	4	1570	4868	57	428	73%
	5	1647	493	12	269	84%
	6	4026	10113	199	712	82%

*The value given for the mortality of marked individuals (MM) may be inflated due to factors other than the death of the termites (e.g. complete loss of dye mark or indistinguishable dye mark).

only enough time for complete mixing (Begon 1979). In small-scale laboratory settings, there are physical boundaries for the experimental units and conditions are nearly ideal for termite survival (e.g temperature, relative humidity, ample food). In the field, however, conditions may be less than optimal, and death may result from climate changes or predation. If the mortality rate in a colony is high, especially for marked individuals, the Lincoln index would tend to overestimate the foraging population. Because there were no predators or the possibility of emigration in our experimental units, the results indicate that there may be a high mortality rate for termites at both Nile blue concentrations (Table 2). In our units, the average mortality of dyed workers after one week was 79.6% 3.4% for 0.1% and 91.4% 3.7% for 0.05% Nile blue concentration. The difficulty in the interpretation of the data is that the observed mortality cannot be distinguished from either an effect of the dye, the natural mortality rate of termites in the laboratory, or cannibalism of dyed workers which may occur in the field or the laboratory. In any case, the assumption of a closed population is violated.

The second assumption of equal catchability in the first sample raises questions about the usefulness of mark-recapture models that rely on random distribution to accurately estimate population size; termites may not forage randomly (Forschler and Townsend 1996).

The third assumption that marking does not affect catchability may be violated if termites are affected by marking with ingestible dyes. *R. virginicus* fed 0.1- and 0.25% (w/w) Sudan IV appear to be less vigorous than controls (Oi and Su 1994). Grace and Abdallay (1989) found *R. flavipes* fed significantly less on papers dyed with Sudan Red 7B (Fat

Red 7B) than controls over 15 days. Furthermore, the synergism of two or more dyes may affect termite behavior or survivability. Synergistic effects have not been investigated, but in some field studies termites were marked with a different color dye after they had been previously marked (Haagsma and Rust 1995). Because there is a dose response mortality in termites fed single dye markers (Su *et al.* 1991), feeding termites multiple dyes may intensify this response. If a sublethal dose of dye marker affects the behavior or metabolic processes of the termites, then their catchability may also be affected which could lead to an inaccurate population estimate.

The fourth and fifth assumptions that marks are not lost and that all marks are reported have particular relevance to the present study. Our population estimates of termites fed 0.1% Nile blue were on average 3 times the actual population, and those for 0.05% Nile blue averaged 10 times greater. Su and Scheffrahn (1988) reported the population of *Coptotermes formosanus* Shiraki, using the triple-catch weighted-mean method, to be 10 times greater than direct counts previously reported for that species. In our study, termites fed 0.05% Nile blue dyed paper were noticeably lighter in color than those stained with 0.1% Nile blue immediately following the marking step. Over the week between release and recapture of the dyed termites, their color faded considerably. One explanation for the overestimation is that the color faded in the 0.05% colonies to a point which rendered some of them undetectable upon recapture. If termites lose their marks in the field, then the estimate would be larger than the actual population. Although in previous laboratory studies termites fed 0.1% (w/w) Nile blue suffered greater mortality than those fed 0.05%, we realized greater accuracy with the higher concentration marker, perhaps because the mark lasted longer.

We fed our termites on dyed paper for 5 days, but the length of time that termites are allowed to feed on dyed papers varies from 3 to 10 days (Forschler and Henderson 1995, Grace *et al.* 1989, Grace *et al.* 1995, Haagsma and Rust 1995, Oi and Su 1994, Su and Scheffrahn 1988, Su *et al.* 1991, Su *et al.* 1993). Subterranean termites that have been fed 0.05% and 0.25% (w/w) Nile blue for three days have been reported to be distinguishable from controls for up to 15 days (Su *et al.* 1991). However, those termites were compared to termites fed white filter paper and maintained in petri dishes following the marking step. In our experiment, termites were returned to their host logs following marking. Perhaps the dye dissipates faster under the more natural conditions inside the log, or perhaps there is decreased visible contrast between termite guts with dyed paper mixed with wood particles compared to those with dyed paper mixed with undyed paper. Thorne *et al.* (1996)

showed that *Reticulitermes* lose their marks faster when fed wood following the marking step than termites fed white filter paper. This would make it more difficult to distinguish marked from unmarked individuals in the field resulting in fewer marked individuals being recorded. This may also explain the high "apparent mortality" observed.

Although the triple-catch weighted-mean method (Begon 1979) can sometimes reduce the standard error of population estimates, its accuracy is dependent on the assumptions made. In our study, the simple Lincoln index provided lower standard errors than those reported by Su and Scheffrahn (1988), Su *et al.* (1993), Forschler and Henderson (1995), and Haagsma and Rust (1995) using the triple-catch weighted mean method (Table 1). However, the accuracy of our estimate was as much as 12 times greater than the actual count of termites (Table 1). Therefore, the estimate is precise but not accurate. The validity of the population estimate of the triple-catch weighted-mean method is based on the same assumptions as the simple Lincoln index (Begon 1979), and therefore it is possible to produce precise but inaccurate population estimates due to model bias using either method (White *et al.* 1982).

Many dye markers have been laboratory tested for their usefulness in termite mark-release-recapture studies, but only a few have been accepted by researchers (Su *et al.* 1983, Su *et al.* 1988, Su *et al.* 1991, Grace and Abdallay 1990). Nile blue has been recommended for studies on *Reticulitermes* (Su *et al.* 1984, Su *et al.* 1991, Haagsma and Rust 1995). However, mortality rates have been measured only under laboratory conditions. The difficulty of measuring termite mortality in field populations is obvious and may require further mesocosm approaches to approximate field conditions to understand the population dynamics of subterranean termites. Subterranean termites are involved in biogeochemical cycles (Thompson 1984, Pandey *et al.* 1992), and some species also produce greenhouse gases such as methane (Zimmerman and Greenberg 1983, Brauman *et al.* 1992). Understanding the importance of termites in ecosystems relies on accurate estimates of their population size. If the assumptions governing the accuracy of the techniques are violated, then the validity of the estimates should be questioned.

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