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AN OPTIMIZATION APPROACH TO THE TWO-CIRCLE METHOD OF ESTIMATING GROUND-DWELLING ARTHROPOD DENSITIES

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Abstract

Information on ground-dwelling arthropod densities is important for efficient management in agro-ecosystems. A method of using paired pitfall traps with different inter-trap distances, called the two-circle method (TCM), was proposed recently for accurate and efficient estimation of arthropod densities. Using the numbers of individuals caught in paired traps and the inter-trap distances between the paired traps as input, the TCM can simultaneously estimate the effective trapping radius and the population density by fitting a nonlinear model. However, the previous fitting procedure (using the nonlinear least squares approach) provides the estimates and standard errors of only these two variables, and often suffers from its hypersensitivity to the initial values assigned in the nonlinear regression. To estimate the confidence intervals of these estimates and to assess the effects of the number of replications per distance class and the number of distance classes on the accuracy of density estimates, we provide a new procedure for fitting the model by using the optimization function. Evaluation based on simulated and field data suggests that the TCM could provide a reliable estimate of density by using at least 15 paired traps per distance class and at least 4 distance classes.

Key words: *nls* function, *optim* function, BC_a method, confidence interval, coefficient of variation

Resumen

Información sobre la densidad de artrópodos que habitan el suelo es importante para un manejo eficiente de agroecosistemas. Un método que utiliza trampas de caída emparejadas con diferentes distancias entre las trampas, llamado método de dos círculos (MDC) ha side propuesto para la estimación precisa y eficiente de las densidades de artrópodos. Usando los datos del número de individuos atrapados en trampas emparejadas y las distancias entre pares, el MDC puede estimar simultáneamente el radio de captura efectiva y la densidad de población mediante el ajuste de los datos a un modelo no lineal. Sin embargo, el procedimiento de ajuste anterior (utilizando mínimos cuadrados no lineales) proporciona las estimaciones y los errores estándar de sólo estas 2 variables, y a menudo sufre de su hipersensibilidad a los valores iniciales asignados en la regresión no lineal. Para calcular

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el intervalo de confianza de estas estimaciones y para evaluar los efectos de que el número de repeticiones por categoría de distancia y el número de categorías de distancias sobre la exactitud de las estimaciones de densidad, proveemos un nuevo procedimiento para ajustar el modelo mediante el uso de la función de optimización. Nuestra evaluación basada en datos simulados y de campo sugiere que por lo menos 15 réplicas por categoría de distancia y al menos 4 clases de distancias son suficientes para garantizar una estimación fiable de la densidad por el MDC.

Palabras Clave: función *nls*, función *optim*, método, intervalo de confianza, coeficiente de variación

Population density is a building block of ecology, and the mark-recapture method is widely used approach for estimating densities of many species in various phyla (Hutton & Woolhouse 1989; Lebreton et al. 1992; White et al. 2006; Matthews & Preisler 2010). However, the mark-recapture method has limitations in estimating the densities for most arthropods because of their small body size, short life cycle and high mortality. Different approaches for estimating densities of ground beetles have received attention recently (e.g., Kromp 1999; Davis et al. 2001; Ulrich & Zalewski 2006; Shrestha & Parajulee 2010; Hummel et al. 2012).

Perner & Schueler (2004) proposed to estimate the density of ground-dwelling arthropods via a nested-cross array design of pitfall traps. However, this approach could substantially underestimate the real densities of ground-dwelling arthropods (Zhao et al. 2013). A new method, called the two-circle method (TCM), has been proposed to resolve this issue and give more accurate estimates of population densities by setting paired pitfall traps with different inter-trap distances and simultaneously calculating the effective trapping radius of the pitfall trap (Zhao et al. 2013).

The TCM assumes homogenous density distribution and a common trapping area of paired traps. It requires a reliable nonlinear regression method to fit the relationship between the total individuals caught in paired traps and the inter-trap distance to an inverse trigonometric function of population density and effective trapping radius (equation 1; Zhao et al. 2013). This nonlinear regression is often produced using the least squares, for example, the *nls* function in R statistical software, which generally works well with the Gauss-Newton algorithm (Bates & Watts 1988). However, this function has required that initial values must be assigned to the model parameters, and it often fails to work when fitting a complex nonlinear model, such as the Sharpe-Schoolfield-Ikemoto model for describing the temperature-dependent developmental rates in insects and mites (Ikemoto 2005; Ikemoto et al. 2013; Shi et al. 2013b). Moreover, to calculate the confidence interval (CI) of a target parameter, a bootstrap method is often needed, which requires 2,000 replications to obtain a relatively stable 95% CI for a model parameter (Efron & Tibshirani 1994). This often breaks the procedure of nonlinear least squares as the initial values of model parameters need be adjusted.

To have stable estimates of model parameters and their CIs, Ikemoto et al. (2013) and Shi et al. (2013a) suggested using an optimization function based on the Nelder-Mead algorithm (Nelder & Mead 1965); for example, the *optim* function in R statistical software has shown extreme resilience for regressing highly nonlinear models. Here, we applied the optimization function (based on the least residual sum of squares [RSS]) to the TCM for simultaneously calculating the arthropod densities and their 95% CIs, as well as the effective trapping radii. The performance of this procedure was evaluated using simulations and data from field experiments. In addition, a testing function of the validity of sample size for the TCM was also presented.

Materials and Methods

Two-circle Method

The two-circle method depicts the number of arthropods caught in paired pitfall traps (N) as a function of the inter-trap distance (d), effective trapping radius of the pitfall traps (r in m), and the density of ground-dwelling arthropods (D per m^{-2}) (Zhao et al. 2013):

$$N = \begin{cases} \left(2\pi r^2 - 2r^2 \arccos\left(\frac{d}{2r}\right) + d\sqrt{r^2 - \left(\frac{d}{2}\right)^2} \right) \cdot D & \text{if } d < 2r \\ 2\pi r^2 D & \text{if } d \geq 2r \end{cases} \quad (1)$$

In the above equation, N and d are measurements from the field experiment; r and D are the target variables to be estimated from the nonlinear regression. Here, we developed 3 R functions (see Supplementary Material online in Florida Entomologist 97(2) (2014) at http://purl.fcla.edu/fcla/entomologist/browse).

Specifically, the first function is named *optim.tcm* and it was designed to estimate variables r and D in the above equation based on the *optim* function in R statistical software. The *optim.tcm* function can be considered as a better parameter

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estimation approach than the *tcm* function in Zhao et al. (2013). The second function is named as *bca.tcm*, and it was designed to calculate the standard errors and confidence intervals of the estimated r and D; the bootstrap BC_a method was chosen to calculate the confidence interval of density estimate because this method has been shown to produce a better CI estimate than other methods such as the bootstrap percentile method or bootstrap-t method (Efron & Tibshirani 1994). Because the *optim* function could not directly provide the standard error of a target parameter, the jackknife method (Efron & Tibshirani 1994) was used to calculate the standard errors of estimated r or D in the *bca.tcm* function. The third function is named *sample.validity*, and it was designed to check the validity of sample size at each distance class and the number of distance classes needed for density estimation.

Evaluation

Due to weak aggregation of ground beetles and following Zhao et al. (2013), we considered 3 levels of the coefficient of variation (CV = standard error / mean × 100% = 5%, 10%, and 15%) in the following simulations. As population density (D) is the real concern in most research experiments, we fixed the effective trapping radius (r) to 1.5 m for simplicity, but simulated the 3 densities to D = 1.0, 1.5, and 2.0 m^{-2}. We simulated the number of arthropods using the *tc.points* function in Zhao et al. (2013) and evaluated the performance of the optimization approach under 15, 30, 60 pairs per distance class and 4, 6, 8 distance classes between 0 to 3 m. The *optim.tcm* and *bca.tcm* functions were used to estimate the densities of simulated arthropod populations and the corresponding 95% confidence intervals. For exhibiting the practicality of the optimization approach for real observations, we used the published field data of 6 species of ground-dwelling arthropods, which were collected in 2 kinds of habitats (desert steppe and corn field in northern China; see Zhao et al. (2013) for details). In these 2 habitats, 4 distance classes of 0, 1.5, 2, and 2.5 m were set, each distance class had 15 pairs of traps (i.e., 15 replications for every distance class). The *optim.tcm* and *bca.tcm* functions were also used to estimate the densities of 6 real species of ground-dwelling arthropods and the corresponding 95% confidence intervals.

Results

For simulated data (Table 1), increasing the number of replications per distance class and/or increasing the number of distance classes can improve the accuracy of density estimates and reduce the width of the 95% CI. Specifically, increasing the number of replications per distance class dramatically reduced the standard error of the estimated density and the width of the 95% CI. Increasing the number of distance classes increased the accuracy of the density estimates. Fig. 1 shows the 95% CIs of the estimated densities with different numbers of replications for 4 distance classes when CV = 10%. It was apparent that the width of 95% CI became narrower with the increase of replications (n). When n > 250, the width of 95% CI was approximately 15% of estimated density; when n approximated 1000, this width was less than 10%. However, it would be impractical to design so many replications per distance class. For real arthropod data collected in 2 habitats (Table 2; Zhao et al. 2013), the estimates of density and effective trapping radius estimated by the *optim.tcm* function were nearly the same as those estimated by the previous *tcm* function. Fig. 2 shows the comparison between the investigated numbers of individuals caught in paired traps for *Anacolica mucronata* Reitter (Coleoptera: Tenebrionoidea) and the predicted values by using the *opitm.tcm* function. The cases of other five species were not showed for lack of space. However, most standard errors estimated by the jackknife method are greater than those estimated by the *nls* function. As the 95% CIs of densities for the 6 species of ground-dwelling arthropods are still too wide, more replications per distance class are needed. Overall, the *optim.tcm* function can provide an accurate estimate of the known density; we suggest having at least 15 replications per distance class and at least 4 distance classes for obtaining a reliable estimate of density.

Discussion

Normality of the Residuals

In using equation 1 to fit the data, we assumed that the residuals between the observed and predicted numbers of individuals caught in paired traps were normally distributed. If the residuals of N meet normality, it would be feasible to use the nonlinear least square regression or other standard fitting methods, like the least RSS. We simulated 5,000 population densities (i.e., setting D to 5,000 different values) that meet normally distributed densities with mean = 1.5 m^{-2} and standerd error = 0.15 (i.e., CV = 10%), assuming an effective trapping radius = 1.5 m and inter-trap distances ranging from 0 to 3 m at 1 m intervals. By using the *tc.points* function (Zhao et al. 2013), we were able to obtain the simulated numbers of individuals caught in paired traps (as known numbers) for different inter-trap distances. Then we used the *optim.tcm* function to fit these simulated numbers in order to obtain the corresponding predicted values. Fig. 3 displays

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TABLE 1. SIMULATIONS OF GROUND-DWELLING ARTHROPOD DENSITIES BY THE *TC.POINTS* FUNCTION AND PERFORMANCE OF THE NEW OPTIMIZATION APPROACH. FITTED RESULTS FOR THE SIMULATED DATA OF 4, 6 AND 8 DISTANCE CLASSES AND 15-60 REPLICATIONS[†].

			4 distance classes		6 distance classes		8 distance classes	
CV	D	n	$\hat{D}$	95% CI	$\hat{D}$	95% CI	$\hat{D}$	95% CI
5%	1	15	1.02 ± 0.07	(0.87, 1.17)	1.03 ± 0.08	(0.86, 1.19)	0.98 ± 0.06	(0.86, 1.09)
		30	1.02 ± 0.06	(0.90, 1.13)	0.98 ± 0.06	(0.86, 1.09)	1.00 ± 0.05	(0.90, 1.09)
		60	0.99 ± 0.04	(0.92, 1.07)	1.00 ± 0.04	(0.92, 1.08)	1.01 ± 0.04	(0.93, 1.09)
	1.5	15	1.46 ± 0.12	(1.23, 1.70)	1.49 ± 0.08	(1.31, 1.66)	1.53 ± 0.08	(1.36, 1.68)
		30	1.51 ± 0.09	(1.33, 1.69)	1.49 ± 0.07	(1.33, 1.62)	1.49 ± 0.07	(1.35, 1.62)
		60	1.48 ± 0.06	(1.36, 1.60)	1.51 ± 0.06	(1.37, 1.63)	1.49 ± 0.04	(1.40, 1.57)
	2	15	1.98 ± 0.17	(1.66, 2.31)	2.01 ± 0.08	(1.85, 2.16)	1.98 ± 0.11	(1.75, 2.18)
		30	1.98 ± 0.11	(1.75, 2.21)	2.00 ± 0.12	(1.76, 2.24)	2.01 ± 0.09	(1.83, 2.18)
		60	2.01 ± 0.08	(1.83, 2.16)	1.99 ± 0.08	(1.82, 2.16)	1.99 ± 0.07	(1.85, 2.13)
10%	1	15	0.99 ± 0.19	(0.66, 1.38)	1.04 ± 0.09	(0.85, 1.24)	1.01 ± 0.09	(0.83, 1.20)
		30	1.00 ± 0.14	(0.75, 1.29)	1.02 ± 0.09	(0.84, 1.19)	0.98 ± 0.08	(0.81, 1.15)
		60	1.03 ± 0.08	(0.87, 1.18)	1.00 ± 0.08	(0.83, 1.17)	0.98 ± 0.07	(0.84, 1.13)
	1.5	15	1.54 ± 0.23	(1.10, 2.03)	1.49 ± 0.27	(1.00, 2.14)	1.51 ± 0.15	(1.21, 1.86)
		30	1.53 ± 0.22	(1.12, 1.98)	1.49 ± 0.15	(1.18, 1.79)	1.51 ± 0.16	(1.21, 1.86)
		60	1.48 ± 0.11	(1.28, 1.70)	1.51 ± 0.13	(1.25, 1.78)	1.51 ± 0.09	(1.33, 1.70)
	2	15	2.02 ± 0.34	(1.41, 2.71)	1.97 ± 0.24	(1.49, 2.51)	1.99 ± 0.29	(1.43, 2.69)
		30	2.06 ± 0.23	(1.59, 2.54)	2.00 ± 0.16	(1.68, 2.33)	2.01 ± 0.19	(1.64, 2.43)
		60	1.99 ± 0.16	(1.67, 2.31)	2.01 ± 0.12	(1.77, 2.24)	2.06 ± 0.13	(1.80, 2.31)
15%	1	15	0.97 ± 0.24	(0.58, 1.51)	0.93 ± 0.21	(0.58, 1.50)	0.96 ± 0.20	(0.61, 1.43)
		30	0.99 ± 0.17	(0.68, 1.33)	0.98 ± 0.23	(0.60, 1.58)	1.04 ± 0.15	(0.77, 1.42)
		60	0.99 ± 0.15	(0.71, 1.30)	1.02 ± 0.11	(0.80, 1.25)	1.02 ± 0.10	(0.83, 1.24)
	1.5	15	1.57 ± 0.30	(1.06, 2.22)	1.55 ± 0.39	(0.91, 2.57)	1.60 ± 0.23	(1.17, 2.16)
		30	1.48 ± 0.26	(1.01, 2.03)	1.43 ± 0.18	(1.07, 1.82)	1.51 ± 0.23	(1.06, 2.07)
		60	1.51 ± 0.19	(1.14, 1.91)	1.53 ± 0.15	(1.23, 1.84)	1.47 ± 0.14	(1.19, 1.78)
	2	15	1.89 ± 0.36	(1.26, 2.64)	2.02 ± 0.34	(1.36, 2.80)	1.98 ± 0.38	(1.30, 2.89)
		30	2.11 ± 0.41	(1.37, 3.03)	2.02 ± 0.32	(1.42, 2.75)	2.07 ± 0.26	(1.55, 2.69)
		60	1.98 ± 0.29	(1.46, 2.58)	2.00 ± 0.24	(1.53, 2.51)	2.00 ± 0.21	(1.59, 2.43)

[†]CV represents the coefficient of variation in density (D); n represents the number of replications (i.e., sample size) per distance class; D represents the known value of density; $\hat{D}$ represents the estimate of density.

Note: The number of arthropods were simulated using the *tc.points* function in Zhao et al. (2013) and the performance of the optimization approach was evaluated for 15, 30, 60 paired traps per distance class and 4, 6, 8 distance classes between 0 to 3 m.

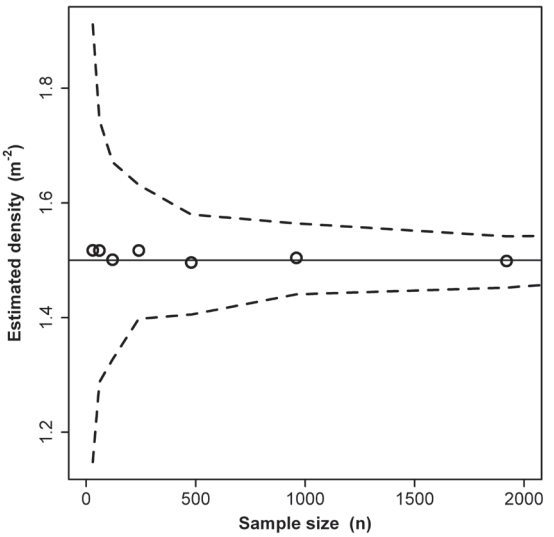


Fig. 1. Effect of sample size per distance class on the 95% CI of the estimated population density of about 1.5 beetles/m^2. The open circles represent the estimates for different sample sizes; the dashed lines represent the 95% CI of the estimated densities The inter-trap distances were 0, 1, 2, and 3 m, respectively. Here, r = 1.5, D = 1.5, CV = 10%, where r is the effective trapping radius, D is the density of beetles, and CV is the coefficient of variation in density.

the frequency of the residuals between the simulated and predicted densities, which was normally distributed. When we reduced or increased the simulated number for D, we found that the standard error in a normal density function was rather stable. Consequently, we simulated additional cases by letting r vary from 0.05 to 3 at an interval of 0.05 m and letting D vary from 0.05 to 3 at an interval of 0.05 beetles m^{-2}. Accordingly, we obtained Fig. 4, which shows the contour plot of the standard errors of the residuals between the simulated and predicted densities by 5,000 simulations. When the CV in density was set to be larger, the consequence was that the standard errors of the residuals of N increased. However, the residuals of N for each case were normally distributed and the mean values of the residuals were all equal to zero. Moreover the inter-trap distance gradients did not affect the stability of fitted standard error according to the simulations, which are not shown here.

Curvilinear Characteristics of TCM

The shape of the curve for describing the number of individuals caught in the paired traps (i.e., equation 1) - obtained by fitting the simulated and field data - appeared to be slightly protuberant in the range of 0 to 2r (Zhao et al. 2013). However, Zhao et al. (2013) did not demonstrate its concavity and convexity. In the present study, we

TABLE 2. FITTED RESULTS FOR THE FIELD DATA OF ARTHROPOD COLLECTION IN TWO HABITATS (DESERT STEPPE AND CORN FIELD)[†].

	OPTIM.TCM					TCM		SSM
Species	$\hat{D}$	95% CI	$\hat{r}$	95% CI	R^2	$\hat{D}$	$\hat{r}$	R^2	D_{real}
Desert steppe									
Anacolica mucronata	1.28 ± 0.30	(0.77, 2.03)	1.59 ± 0.21	(1.21, 2.13)	0.81	1.28 ± 0.29	1.58 ± 0.21	0.81	1.23 ± 0.12
Blaps femoralis	1.46 ± 0.26	(1.02, 2.02)	1.42 ± 0.14	(1.18, 1.74)	0.86	1.46 ± 0.29	1.42 ± 0.16	0.86	1.51 ± 0.12
Argiope bruennichi	1.57 ± 0.53	(0.76, 3.41)	1.19 ± 0.21	(0.79, 1.76)	0.62	1.57 ± 0.41	1.19 ± 0.17	0.62	1.56 ± 0.13
Corn field									
Chlaenius bioculatus	1.11 ± 0.38	(0.58, 2.02)	1.28 ± 0.25	(0.91, 1.86)	0.73	1.11 ± 0.28	1.28 ± 0.19	0.73	1.12 ± 0.09
Teleogryllus mitratus	1.69 ± 0.26	(1.28, 2.49)	1.62 ± 0.15	(1.29, 1.89)	0.89	1.69 ± 0.27	1.62 ± 0.15	0.89	1.71 ± 0.19
Pardosa astrigena	1.44 ± 0.34	(0.99, 1.99)	1.34 ± 0.18	(1.13, 1.66)	0.86	1.44 ± 0.28	1.34 ± 0.15	0.86	1.42 ± 0.13

[†]The habitat information could be found in Zhao et al. (2013). The real density (D_{real}) was estimated from samples taken by the suction sampling method (SSM).

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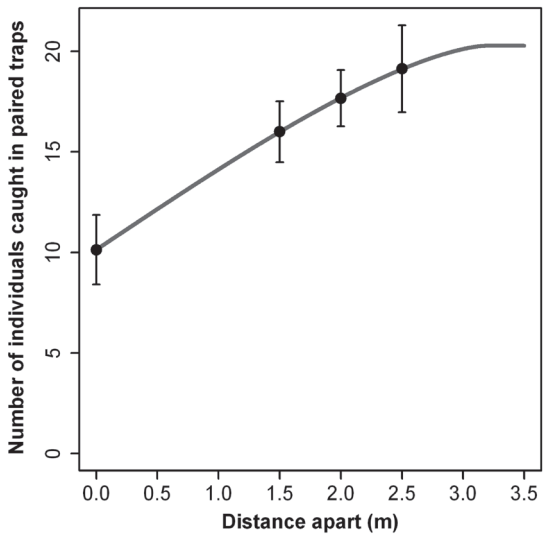


Fig. 2. Comparison between the investigated numbers of individuals (data points) caught in paired traps for *Anacolica mucronata* Reitter (Coleoptera: Tenebrionoidea) in desert steppe habitat (Zhao et al. 2013) and the predicted values (thick gray curve) by using the *optim.tcm* function. At every distance class, 15 pairs of traps were used. Every point represents the mean of 15 numbers of individuals caught in paired traps at a given distance class, and the corresponding standard error is expressed by the vertical bar passing by that point.

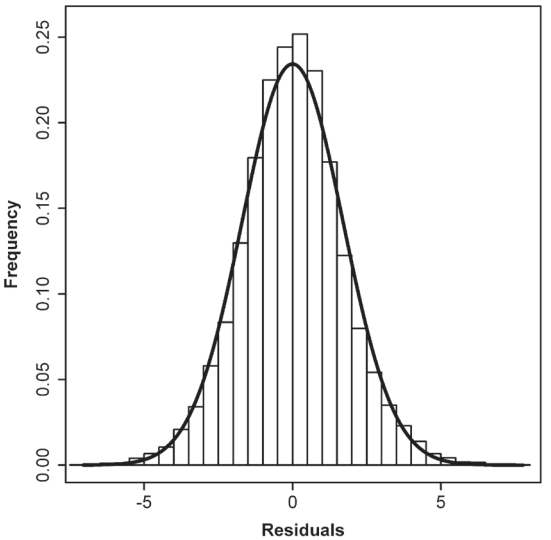


Fig. 3. Normality of the residuals between the simulated and predicted numbers of individuals caught in paired traps. The line represents the fitted normal density function. The fitted mean = 0, and the fitted standard error = 1.7.

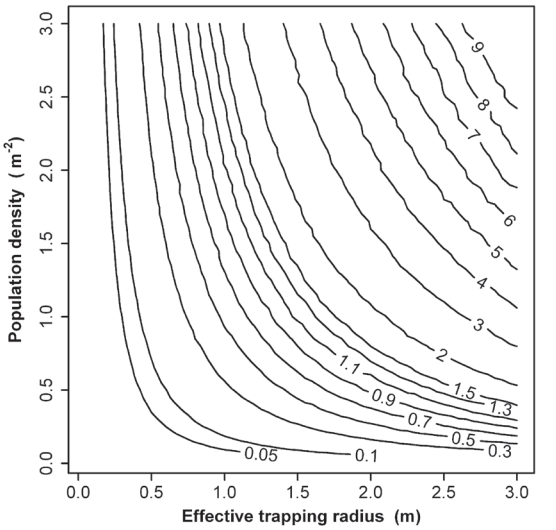


Fig. 4. Contours of the standard errors of the residuals between the simulated and TCM-estimated densities at various effective trapping radii. The distance classes ranged from 0 to 3 m at an interval of 1 m, with 5,000 replications for simulated paired traps per distance class. The coefficient of variation (CV) in density was assumed to be 10%.

provided the first-order and second-order derivatives of equation 1. In addition, we tested whether the curve could be linearly approximated based on the first-order derivative at the point of (d_c, N_c), where $d_c = r$, and N_c is the number at d_c according to equation 1. The first-order derivative of equation 1 is:

$$N'(d)=\begin{cases} -D\left(\dfrac{d^2}{4\sqrt{r^2-\left(\frac{d}{2}\right)^2}}-\sqrt{r^2-\left(\frac{d}{2}\right)^2}-\dfrac{r^2}{\sqrt{r^2-\left(\frac{d}{2}\right)^2}}\right) & \text{if } d<2r; \\ 0 & \text{if } d\geq 2r. \end{cases} \quad (2)$$

And the second-order derivative of equation 1 is:

$$N''(d)=\begin{cases} -D\left(\dfrac{d^3}{16\left(r^2-\left(\frac{d}{2}\right)^2\right)^{3/2}}+\dfrac{3d}{4\sqrt{r^2-\left(\frac{d}{2}\right)^2}}-\dfrac{r^2 d}{4\left(r^2-\left(\frac{d}{2}\right)^2\right)^{3/2}}\right) & \text{if } d<2r; \\ 0 & \text{if } d\geq 2r. \end{cases} \quad (3)$$

Based on equation 1, we can obtain

$$N_c=\frac{1}{6}Dr^2\left(3\sqrt{3}+8\pi\right). \quad (4)$$

Based on equation 2, we can obtain the slope of the tangent of equation 1 at $d_c = r$:

$$N'\big|_{d=r}=\sqrt{3}(rD). \quad (5)$$

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Thus, the tangent equation of equation 1 passing by the points of (d_c, N_c) is

$$y = \sqrt{3}(rD)(x - r) + \frac{1}{6}Dr^2(3\sqrt{3} + 8\pi). \quad (6)$$

It can be easily demonstrated that $N'(d) > 0$ and $N''(d) < 0$ when $0 < d < 2r$. Thus, the shape of the curve in equation 1 was protuberant (Department of Applied Mathematics, Tongji University 2007, pages 149-150). Because $N''(d) < 0$ when $0 < d < 2r$, $N'(d)$ was a descending function (Department of Applied Mathematics, Tongji University 2007, page 146), which means that the instantaneous rate of increase for equation 1 gradually decreased in this range. Fig. 5 exhibits the curve shapes of $N'(d)$ and $N''(d)$, and it also illustrates the approximate linearity of equation 1 when $d < 2r$ (i.e., equation 6).

Values of the Two Circle Method (TCM) and Its Applied Scope

Agroecosystems often contain a rich list of ground-dwelling arthropods, dominated by Carabid beetles and spiders (Elliott et al. 2006; Gardiner et al. 2010). These species differ widely in their body size, abundance, feeding habits, and seasonal activities (Schmidt et al. 2005; Tscharntke et al. 2007). Ground-dwelling arthropods have

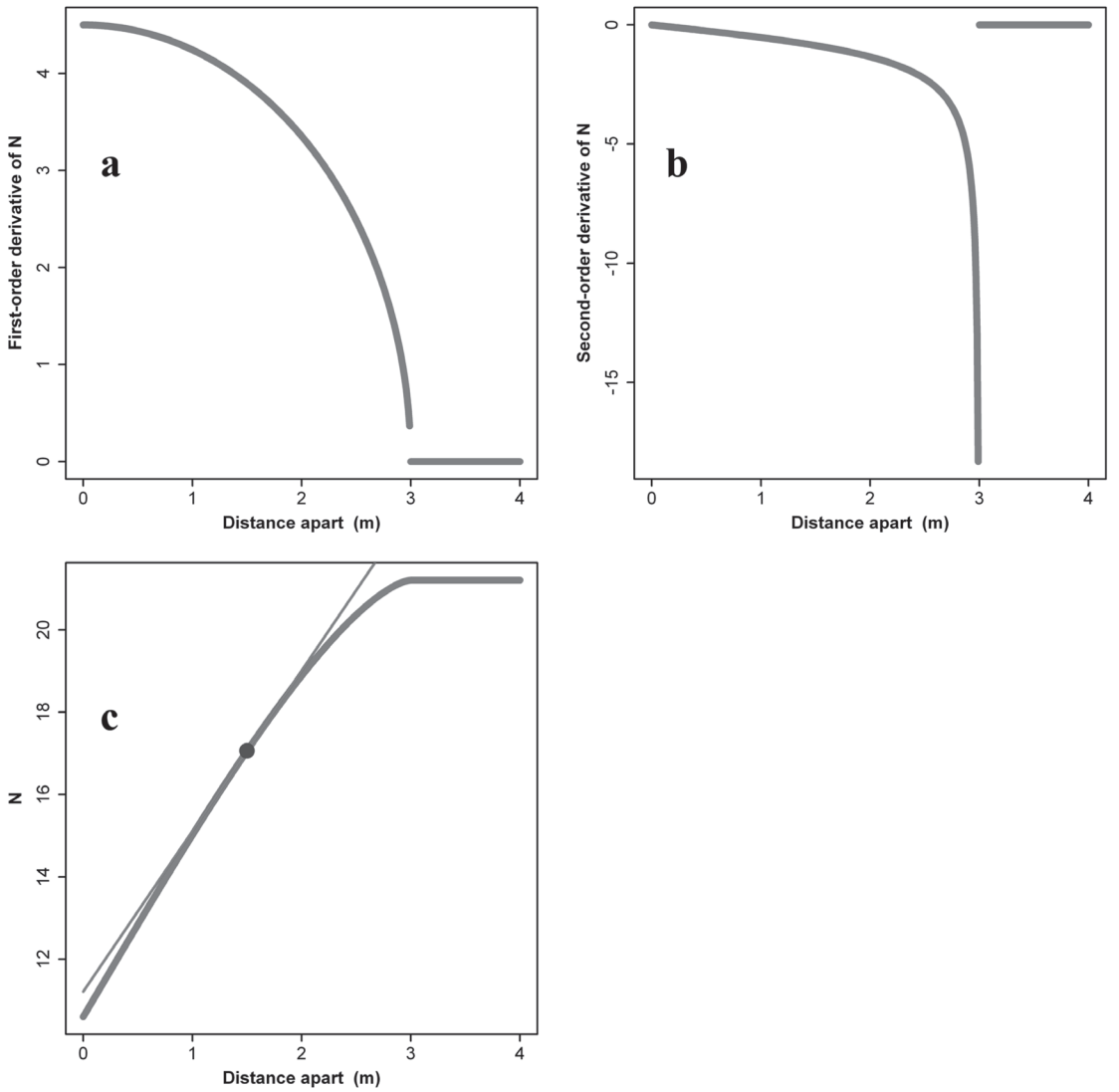


Fig. 5. Curve characteristics of the number of individuals (N) caught in paired traps. (a) The first-order derivative of N. (b) The second-order derivative of N. (c) Approximate linearity of N. The straight line is the tangent of N at the point of (d_c, N_c), where d_c represents $d = r$.

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important ecological functions, such as controlling pests and maintaining food-chain robustness due to their high diversity and abundance in crop fields and in semi-natural habitats across the world (Zhao et al. 2013; Elliott et al. 2002; Schmidt et al. 2005).

Pitfall traps are widely used for collecting ground-dwelling arthropods (Schmidt et al. 2008; Anjum-Zubair et al. 2010). However, pitfall trapping is sensitive to a wide variety of abiotic and biotic factors, such as semiochemical composition (attractant liquids such as propylene glycol and alcohol), trap distribution and shape, as well as the dispersal abilities of insects (Melnychuk et al. 2003; Lange et al. 2011). As such, density estimates from pitfall trapping often do not reflect the real population densities (Roschewitz et al. 2005; Perdikis et al. 2011).

As species respond to attractants differently, the species caught in pitfall traps thus reflect a biased local community profile (Zhao et al. 2013). It is challenging to have an unbiased and accurate density estimate for ground-dwelling arthropods (Anjum-Zubair et al. 2010). Some methods could provide more accurate density estimates than pitfall trapping, such as the D-vac suction sampling within enclosures followed by hand collection of the plants and soil (Elliott et al. 2006). However, D-vac suction is expensive and difficult to handle. To have an unbiased profile of local arthropod community (species composition and abundance), we have proposed the two-circle method (TCM) through designing multiple paired traps with different inter-trap distances (Zhao et al. 2013). Here we provided the method for calculating confidence intervals and a better approach to handling the problems encountered in nonlinear regression in the TCM. Obviously, the TCM can also be applied for density estimation of other insects. For example, the population density of Noctuids (trapped by black-light) could be simulated through designing multiple paired light traps in different distances apart. Likewise, the insects trapped by sex attractants could be also estimated accurately by the same way. We believe that the two-circle method can be widely used to estimate population densities of multiple insect species.

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