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Estimating the adult population size of ground beetles (Carabidae) using the removal method

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除去法を用いた地表徘徊性ゴミムシ類成虫の個体数推定　Salah Uddin Siddiquee・中村寛志（信州大学農学部）

長野県にある信州大学農学部構内の森林内とその近くの野菜畑において，除去法を用いた地表徘徊性ゴミムシ類成虫の個体数推定を行った．プラスチック製の境界で区切られた40 m^{2}の区画に，乳酸飲料を入れた15個のプラスチック・トラップをセットし，10日間毎日ゴミムシを回収した．調査は森林では2002年の9月末，野菜畑では2003年の10月初めに行われた．森林での優占3種は*Synuchus cycloderus, Pterostichus subovatus, Synuchus nitidus*で，野菜畑では*Harpalus griseus, Harpalus sinicus, Amara simplicidens*であった．全ゴミムシ類と優占3種の個体数および m^{2}当たりの密度は，いくつかある除去法の中で回帰法と最尤法を用いて行われた．森林内では合計250個体のゴミムシ類が捕獲され，回帰法による推定値は254個体であった．また野菜畑では176個体の採集で，推定値は180個体であった．最尤法による推定値は回帰法とほぼ同じ値で，10回の実際の採集個体数と推定値がほぼ等しかった．またある時点の捕獲個体数とその時点までの累積捕獲個体数の相関係数は－0.9以下であり，推定精度も0.12以下の値であった．ゴミムシ類の個体数推定に応用する上での除去法の前提条件や捕獲回数と推定精度の関係が議論された．

Adult population sizes of ground beetles (Carabidae) in a forest and vegetable field in Nagano Prefecture, Japan were estimated using the removal method. Removal collections using 15 pitfall traps with a lactic acid beverage were conducted at 40-m^2 survey sites enclosed by a thick plastic sheet for 10 days in September 2002 in the forest site and October 2003 in the field site. Dominant species were *Synuchus cycloderus, Pterostichus subovatus* and *S. nitidus* in the forest, and *Harpalus griseus, H. sinicus* and *Amara simplicidens* in the field. Population sizes within the 40-m^2 sites and the density (/m^2) of total carabid beetles and dominant species were estimated by the regression and maximum likelihood methods. A total of 250 and 176 carabid beetles were caught in the forest and field sites, and estimates by the regression method were 254 and 180 individuals, respectively. Estimates of dominant species and total carabid beetles by the maximum likelihood method were almost equal to those obtained by the regression method. The observed numbers caught from 10 trappings were almost the same as the estimated values. The correlation coefficients between the number of individuals captured during the *i*th trapping and the total number captured prior to the *i*th trapping were less than -0.9, and the precision level of the estimations was less than 0.12. The prerequisite for the removal method and appropriate number of trappings required for estimating carabid population size were discussed in relation to the precision level of the estimations.

Key words: Ground beetle (Carabidae), population estimation, removal method, regression method, maximum likelihood method, pitfall trap

Introduction

Because of their diversity, ground beetles (Carabidae) have been studied from taxonomical, biogeological and evolutional viewpoints, and recently, their role as potential predators in agroecosystems has been explored. The species composition, seasonal activity and spatial distribution of ground beetles have been studied globally (Yano *et al.*, 1995), and in Japan, important work in paddy fields (Habu and Sadanaga, 1970; Yahiro *et al.*, 1992), and a series of ground beetle studies have been conducted in various agroecosystems (Ishitani and Yano, 1994; Ishitani *et al.*, 1994). It has been established that ground beetles could be used as a biological control in pest management (Holland, 2002), and furthermore, with regards to environmental evaluation, some researches hope to develop ground beetles into a bio-indicator (Ishii *et al.*, 1996; Ishitani, 1996; Villa-Castillo and Wagner, 2002).

To study the ecology of ground beetles and establish them as a predator or bio-indicator, much attention needs to be paid to population estimations in different habitats and seasons. However, population numbers per unit area have yet to be clearly reported, though the spatial distributions and seasonal activity of ground beetles represented by the number of insects collected per trap in various habitats have been previously analyzed (Ishitani *et al.*, 1997; Thomas *et al.*, 2002).

Many methods for estimating the population sizes of animals and insects have been presented. Mark and recapture methods have been mainly used to estimate the population sizes of insects because birth, death and migration occur during their short life spans. The removal method, another population estimation method, has been applied to estimates of the stable population size of rats (Leslie and Davis, 1939) and fish (DeLury, 1951), and involves a series of trapping or collecting without replacement. Inoda and Tsuzuki (2000) tried to estimate population sizes of two *Cybister* species using the removal method.

There are three different approaches to analyzing removal trapping data. In this study we tried to estimate the population density ($/\mathrm{m}^2$) of adult ground beetles at two different habitats in Nagano Prefecture, Japan, using the removal method, and then compared the estimates of three approaches.

Materials and Methods

1. Study sites

Two sites in Minamiminowa Village, Nagano Prefecture were selected to estimate the population size of carabid beetles using the removal method. One site was a small area of experimental forest in the Faculty of Agriculture Campus, Shinshu University (Site 1) dominated by Japanese larch, *Larix leptolepis* Gord., Japanese cypress, *Chamaecyparis obtuse* Endl., and some broadleaf trees. A playing field is located in the northern part of Site 1. The other site (Site 2) was located in a vegetable field on the eastern side of the campus (**Fig. 1**). Tomatoes, eggplants, beans and potatoes were the main crops of this plot.

Field surveys using pitfall traps were conducted in Sites 1 and 2 from September 20 to 29, 2002 and September 30 to October 9, 2003, respectively. Fifteen trap stations spaced 2 m apart lengthwise and 1 m apart widthwise were set in 40-m² areas (10 × 4 m) in both sites (**Fig. 2**). The survey areas were enclosed by thick plastic sheets 30 cm high above the ground and buried to a depth of 10 cm to protect against invasion of carabid beetles from the outside as well as escape from within.

The prerequisite for this method is that the population must remain stable during the trapping period, that is, there must be no significant natality, mortality or migration (Southwood, 1978). In this study, the adult carabid beetles could not enter or leave the site as a result of the plastic sheet boundary, because these beetles are almost unable to fly. As the surveys were conducted for only 10 days in autumn, the prerequisite mentioned above could be satisfied, even if new emergence and death occurred slightly.

Transparent plastic cups 13.5 cm deep and with an upper and lower diameter of 9 and 6 cm, respectively, were used as traps. Plastic covers were placed 10 cm above the traps to protect them from rainfall

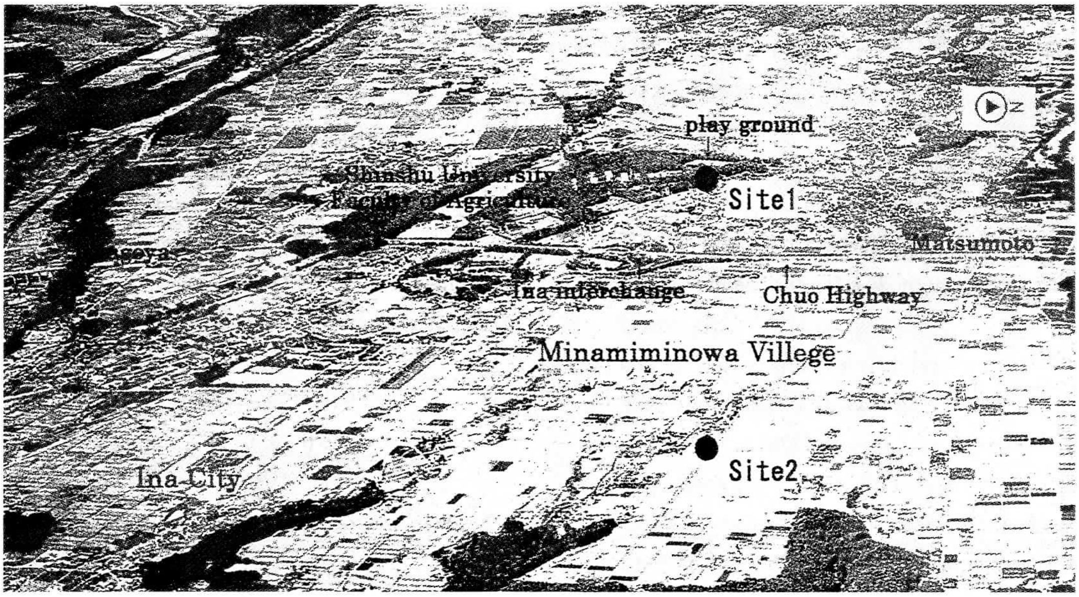

Fig. 1 Map of 2 survey sites in Minamiminowa Village.

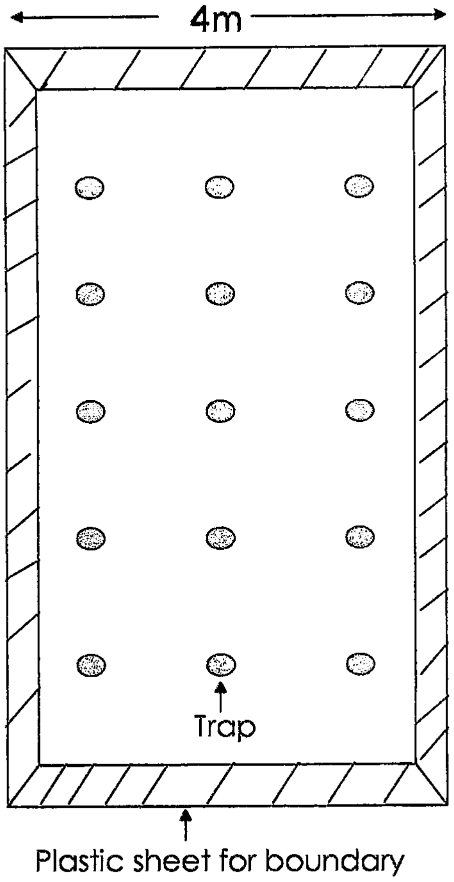

Fig. 2 Arrangement of traps in Site 1 and Site 2

and falling leaves. Inside the traps, lactic acid beverage (Culpis™, Culpis Co., Ltd., Tokyo) was used as bait. Beetle collections were made once a day for ten days incessantly at both sites.

2. Estimation methods using the removal collection data

There are several different approaches for analyzing removal trapping data. In this study we used the regression method (Leslie and Davis, 1939; DeLury, 1947, 1951) and maximum likelihood method (Moran, 1951; Zippin, 1956) to estimate the numbers of the three dominant carabid species and total number of carabid beetles within the 40-m^2 sites in the two distinguishable habitats. The density per one m^2 and variance were also estimated.

Regression method: The following liner relation is expected under random trapping:

$$C_i = b\,(N - T_i)$$

where C_i, T_i and N are the number of insects captured during the ith trapping, the total number captured prior to the ith trapping, and the population size, respectively, and b is a constant. As N is equal to T_i at $C_i = 0$, the population size is then estimated as:

$$\hat{N} = \overline{T} + \frac{\overline{C}}{b} \qquad b = -\frac{\sum_i^s T_i C_i - s\overline{TC}}{\sum_i^s T_i^2 - s\overline{T}^2}$$

where $\overline{T}$ and $\overline{C}$ are the mean values of C_i and T_i, respectively, and s is the number of trappings. The variance of this estimate $(\hat{N})$ is calculated as:

$$v(\hat{N}) = \frac{\hat{\sigma}^2}{b^2}\left\{\frac{1}{s} + \frac{(\hat{N} - \overline{T})^2}{\sum_i^s T_i^2 - s\overline{T}^2}\right\} \qquad \cdots\cdots (1)$$

Maximum likelihood method: With random trapping, the probability of capturing C_i insects during the ith trapping, given that T_i insects were previously captured is:

$$P(C_i / T_i) = \binom{N - T_i}{C_i} p^{C_i} q^{N - T_i - C_i}$$

where $p = 1 - q$ is the probability of capturing during a single trapping. Based on the maximum likelihood of the joint probability of the catch samples in s trappings, Zippin (1956) showed that population size (N) and variance can be estimated as follows:

$$\hat{N} = \frac{\overline{T}}{(1 - \hat{q}^s)}$$

$$v(\hat{N}) = \frac{\hat{N}(1 - \hat{q}^s)\hat{q}^s}{(1 - \hat{q}^s)^2 - (\hat{p}s)^2 \hat{q}^{s-1}}$$

The estimates of $1 - q^s$ and p are given in Zippin (1956).

As the area of the survey sites in this study is 40 m^2, the estimates of density (m) per m^2 and variance($v(\hat{m})$) *are given as:*

$$\hat{m} = \left(\frac{1}{40}\right)\hat{N}$$

$$v(\hat{m}) = \left(\frac{1}{40}\right)^2 v(\hat{N}).$$

Results

1. Species composition

A total of 250 carabid beetles from 4 subfamilies and representing 14 species were caught in Site 1 (**Table 1**). Three species, *Synuchus cycloderus, Pterostichus subovatus* and *S. nitidus,* were most frequently caught accounting for 187 individuals, which was 74.8 % of the total carabid beetles caught in Site 1. Of these, *S. cycloderus* was most frequently caught, representing 34 % of the total.

A total of 176 carabid beetles from 4 subfamilies and representing 19 species were caught in Site 2 (**Table 1**). Three species, *Harpalus griseus, H.*

Table 1 Species and number of carabid beetles caught in Site 1 and Site 2

Species	No. of individuals	
	Site 1	Site 2
Leptocarabus procerulus (Chaudoir)	16	0
Patrobus flavipes Motschulsky	3	0
Trigonognatha cuprescens Motschulsky	0	11
Pterostichus planicollis (Motschulsky)	0	2
Pterostichus subovatus (Motschulsky)	63	0
Pterostichus microcephalus (Motschulsky)	0	2
Pterostichus nimbatidius Chaudoir	9	2
Dolichus halensis (Schaller)	1	1
Synuchus nitidus (Motschulsky)	39	0
Synuchus cycloderus (Bates)	85	1
Synuchus dulcigradus (Bates)	3	1
Synuchus arcuaticollis (Motschulsky)	17	0
Synuchus sp.	7	0
Amara simplicidens Morawitz	0	22
Amara mocronota ovalipennis Jedlicka	0	9
Anisodactylus signatus (Panzer)	0	6
Harpalus capito Morawitz	1	0
Harpalus jureceki (Jedlicka)	1	0
Harpalus griseus (Panzer)	3	62
Harpalus tridens (Morawitz)	0	14
Harpalus sinicus (Hope)	0	23
Harpalus niigatanus Schauberger	0	2
Harpalus platinotus Bates	0	3
Harpalus corporosus (Motschulsky)	0	5
Harpalus bungii Chaudoir	0	2
Harpalus tinctulus Bates	0	7
Harpalus discrepans Morawitz	2	0
Chlaenius naeviger Morawitz	0	1
Total carabid	250	176

sinicus and *Amara simplicidens*, were most frequently caught accounting for 107 individuals, which was 60.8 % of the total carabid beetles caught in Site 2. Of these, *H. griseus* was most frequently caught, representing 35.2 % of the total.

2. Daily change in the number of trapped individuals

Daily changes in the numbers of the 3 dominant species trapped are shown in **Fig. 3**. The numbers of beetles captured in Site 1 decreased abruptly on the second and third trappings but thereafter showed a gentle reduction (**Fig. 3A**). *S. cycloderus* was not trapped on the tenth trapping, though a total of 10 other carabid beetles were captured. The correlation coefficients between the number of *S. cycloderus*, *P. subovatus* and *S. nitidus* individuals captured during the ith trapping (C_i) and the total number captured prior to the ith trapping (T_i) were −0.947, −0.925 and −0.968, respectively.

The numbers of beetles captured in Site 2 decreased almost linearly till the fifth trapping and showed a gentle reduction thereafter (**Fig. 3B**). *H. sinicus* was not captured after the sixth trapping, and *H. griseus* and *A. simplicidens* were not trapped on the tenth. The correlation coefficients between the C_i and T_i of *H. griseus*, *H. sinicus* and *A. simplicidens* were −0.977, −0.990 and −0.982, respectively.

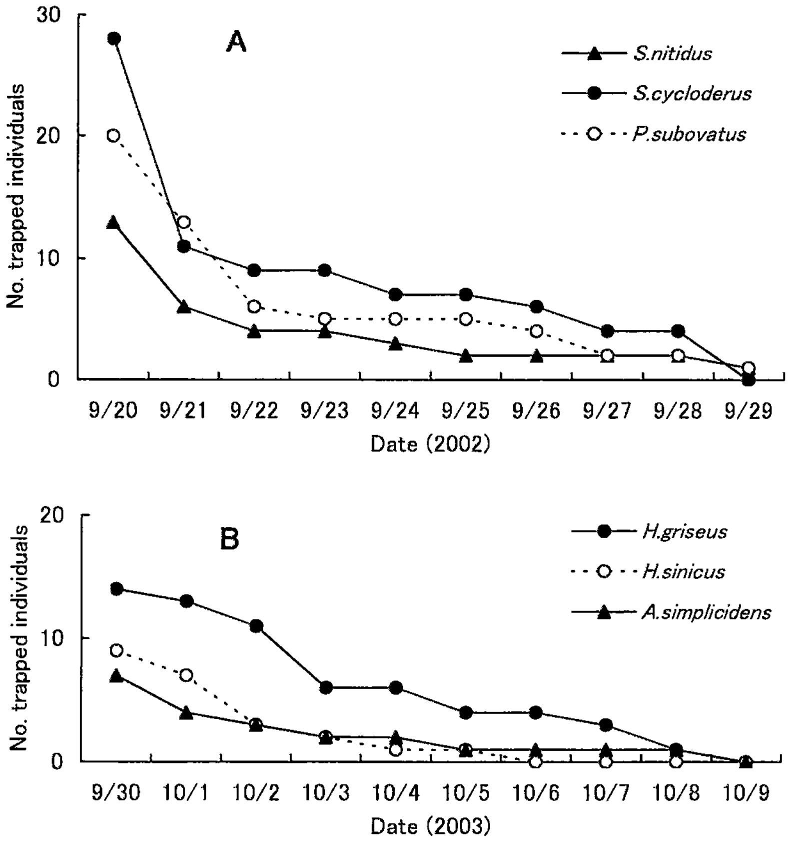

Fig. 3 Daily changes of trapped individuals of 3 dominant species in Site 1 (A) and Site 2 (B).

Daily changes in the total numbers of carabid beetles caught by 15 pitfall traps are shown in **Fig. 4**. A decreasing tendency similar to that of the 3 dominant species in Sites 1 and 2 was observed. No carabid beetles were captured on the tenth trapping in Site 2. The accumulated numbers of individuals captured up till the third and fifth trappings were 56 % and 73.2 % of the total in Site 1, respectively, and 66.5 and 84.7 % in Site 2, respectively.

The correlation coefficients between the C_i and T_i of the total carabid beetles in Sites 1 and 2 were -0.925 and -0.991, respectively.

3. Population estimations

Estimations using the regression and maximum likelihood methods were conducted using the numbers of the 3 dominant species and total carabid beetles trapped at both sites. **Table 2** shows the total number ($\hat{N}$) estimates for the two study sites, the density ($\hat{m}$) per m^2 and 95 % confidence limits. All data from the 10 trappings were used to calculate the estimates using the regression method. However, with the maximum likelihood method estimates were calculated using only the data sets from the first to the seventh trappings, because the graphs for

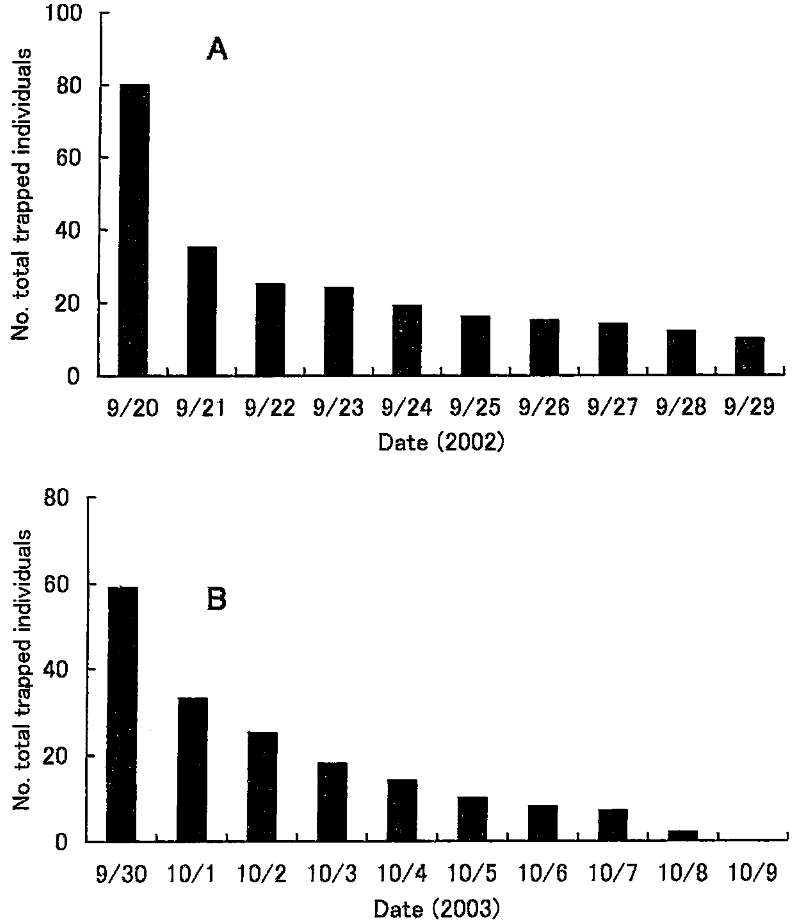

Fig. 4 Daily changes of total trapped individuals of total carabid beetles in Site 1 (A) and Site 2 (B).

Table 2 Estimats of the number of adult carabid beetles within 40 m^2 and the density per m^2 by two removal methods using trapping data

	species	Regression method					Maximum likelihood method				
		$\hat{N}$	$v(\hat{N})$	$\hat{m}$ (/m^2)	±95% limits	D	$\hat{N}$	$v(\hat{N})$	$\hat{m}$ (/m^2)	±95% limits	D
Site 1	*S. cvcloderus*	87.7	36.4	2.2	0.30	0.07	91.7	73.8	2.3	0.42	0.09
	P. subovatus	63.9	6.9	1.6	0.13	0.04	66.7	33.4	1.7	0.28	0.09
	S. nitidus	39.1	4.5	1.0	0.10	0.05	37.8	12.3	0.9	0.17	0.09
	Total carabid	253.9	309.1	6.3	0.86	0.07	246.0	123.1	6.1	0.54	0.05
Site 2	*H. griseus*	68.1	6.9	1.7	0.13	0.04	69.0	55.6	1.7	0.37	0.11
	H. sinicus	23.5	0.2	0.6	0.02	0.02	23.7	1.4	0.6	0.06	0.05
	A. simplicidens	22.5	0.5	0.6	0.03	0.03	22.2	7.2	0.6	0.13	0.12
	Total carabid	180.3	14.2	4.5	0.18	0.02	185.6	60.4	4.6	0.38	0.04

estimating $1-q^s$ and p were only given for $s=3$, 4 and 7 trappings (Zippin, 1956).

The population size estimates of the 3 dominant species and total carabid beetles obtained using the regression method were almost equal to those obtained using the maximum likelihood method, and there were no significant differences judging by the 95 % confidence limits.

The standard deviation ($\sqrt{v(\hat{m})}$) to density ($\hat{m}$) ratio ($D=\sqrt{v(\hat{m})}/\hat{m}$) was used to represent the precision level of the estimations (Kuno, 1971). This ratio was less than 0.1 for almost all species. The regression method showed better precision than the maximum likelihood method except for the total number of carabid beetles in Site 1. The observed numbers caught by 10 trappings were almost the same as the estimated values (**Tables 1 & 2**).

Discussion

In this study we used the removal method to estimate the population size of carabid beetles to determine whether or not this method could be applied successfully. There are 3 different approaches to estimating population size with removal trapping data, namely, the regression method (Leslie & Davis, 1939; DeLury, 1947, 1951), the maximum likelihood method (Moran, 1951; Zippin, 1956) and time-unity collecting (Kono, 1953).

The high correlation coefficients of C_i and T_i, and small D values observed here suggest the reliability of the estimates obtained by the regression method. We calculated the 95 % confidence limits using the variance of $\hat{N}$ using Eqn. (1). However, the confidence limits of the regression method can be given more precisely using a solution (x_1, x_2) of the following quadratic equation with cases of less than 10 trappings (Kuno, 1986):

$$\left[b^2 - \frac{\hat{\sigma}^2 t_{s-2}{}^2(\alpha)}{\sum_{i}^{s}\left(T_i-\bar{T}\right)^2} \right] x^2 - 2\bar{C}bx + \bar{C}^2 - \frac{\hat{\sigma}^2 t_{s-2}{}^2(\alpha)}{s} = 0$$

Where $t_{s-2}(\alpha)$ is the critical value of t distribution ($df=s-2$) for ($1-\alpha$) % confidence. The lower and upper limits of ($1-\alpha$) % confidence are ($\bar{T}+x_1$, $\bar{T}+x_2$). According to the above, the 95 % confidence limits of the total carabid beetles trapped in Sites 1 and 2 were (225.8, 307.0) and (173.5, 188.5), respectively. These confidence limits of Site 2 were nearly equal to those obtained by Eqn. (1), but the precision level of the estimates for Site 1 was lower. This seems to be related to the fact that the correlation coefficient between the C_i and T_i of Site 1 (−0.925) was lower than that of Site 2 (−0.991).

The maximum likelihood method is the most accurate of the 3 approaches (Southwood, 1978), though it strictly requires the chance of being caught and effort of catching to be equal during the trapping period. Our trapping surveys were conducted using identical trapping intervals and identical shapes and numbers of pitfall traps. The fact that the estimates obtained by the maximum likelihood method were not significantly different from those obtained with the regression method, even though only 7 data sets were used, shows that the prerequisites were met and the propriety of this method.

Zippin (1956) showed the relationship between the precision level of the estimates and proportion of individuals removed from a population, and suggested that to obtain a precision level of 0.1, 75 % of a population would have to be removed when the population size was less than 300. Turner (1962) pointed out that for this reason it is impractical to estimate populations of insects caught in pitfall traps when the catching efficiency of these traps is very low. In this study, we used a high trap density (0.375 traps per m^2) and attractive bate so that more than 80 % of the beetles used to estimate population size were removed by the 7th trapping and the precision levels of these estimates with the maximum likelihood method were high (D<0.12) (**Table 2**).

Kono (1953) presented a formula for estimating population size using time-unity collecting data based on the exponential relationship between the number collected and time. Where n_1, n_2 and n_3 are the accumulated numbers of collected individuals at three time points (t_1, t_2 and t_3), such that $(t_1+t_2)/2 = t_3$, $\hat{N}$ is estimated as:

$$\hat{N} = \frac{n_3^{\,2} - n_1 n_2}{2n_3 - (n_1 + n_2)}.$$

As trappings were carried out daily in this study, n_1, n_2 and n_3 are T_2, T_6 and T_4, respectively, on t_1 = the 2nd trapping day, t_2 = the 6th trapping day and $t_3 = (2+6)/2$ = the 4th trapping day. The total carabid beetle population sizes in Sites 1 and 2 according to the above formula were 291.8 and 188.4, respectively. The Site 1 value was overestimated slightly in comparison to the estimate obtained by the regression method, but there was no difference in the Site 2 value, although the variance of the estimate was not given with Kono's method (Kono, 1953).

In this study we estimated population size using the regression and likelihood methods with data from 10 and 7 trappings, respectively. To determine the appropriate numbers of trappings required, the estimates and precision levels were shown in relation to the number of trappings used for the regression method (**Table 3**). The precision level became lower with decreasing trapping times and about half the D values were more than 0.1 when population size was estimated by data from less than 5 trappings. From **Table 3** it can be suggested that at least 5 trappings will give an estimate of carabid beetle population size with a precision level of less than 0.1 using the regression method.

It is still questionable whether the number of carabid beetles caught in pitfall traps (activity-density) accurately reflects the population size (absolute density) in an immediate area (Thomas *et al.*, 2002). Several researchers tried to overcome this problem by additional mark-recapture studies using pitfall traps with barriers (Thomas *et al.*, 1998). In contrast to the capture and recapture method, which is widely used for population estimations, it is not possible to estimate the parameters of population dynamics, such as birth and death rates, from the

Table 3 Estimates of population size and the precision level (D) in relation to the number of trappings

	Sopecies		Number of trappings							
			3	4	5	6	7	8	9	10
Site 1	*S. cycloderus*	$\hat{N}$	53.7	62.6	69.3	76.3	82.2	85.4	88.6	87.7
		D	0.12	0.14	0.12	0.12	0.11	0.10	0.09	0.07
	P. subovatus	$\hat{N}$	48.8	50.4	53.8	57.9	61.1	62.2	63.4	63.9
		D	0.09	0.05	0.06	0.07	0.07	0.06	0.05	0.04
	S. nitidus	$\hat{N}$	26.5	30.1	32.5	33.8	35.2	36.7	38.3	39.1
		D	0.07	0.10	0.09	0.07	0.06	0.06	0.06	0.05
	Total carabid	$\hat{N}$	159.7	181.2	197.5	210.8	223.5	235.5	245.7	253.9
		D	0.09	0.11	0.10	0.09	0.08	0.08	0.07	0.07
Site 2	*H. griseus*	$\hat{N}$	128.2	74.7	74.0	71.2	72.0	72.1	70.1	68.1
		D	0.19	0.22	0.13	0.09	0.07	0.05	0.04	0.04
	H. sinicus	$\hat{N}$	25.5	24.7	24.2	24.3	23.9	26.1	24.3	23.5
		D	0.19	0.09	0.06	0.04	0.03	0.31	0.14	0.02
	A. simplicidens	$\hat{N}$	18.6	19.3	20.8	21.0	21.4	22.0	22.6	22.5
		D	0.09	0.05	0.06	0.04	0.04	0.04	0.04	0.03
	Total carabid	$\hat{N}$	153.5	163.3	170.6	174.7	178.2	181.6	181.4	180.3
		D	0.10	0.06	0.05	0.04	0.03	0.03	0.02	0.02

prerequisite of the removal method. Furthermore, precise estimates need a large part of the population to be removed. This is a critical obstruction for life-table studies. However, it is easier to estimate the population size of ground beetles or other small animals using the removal method because it does not require marking and recapture, which takes time as well as hard labor. The removal method can be used to easily estimate the density of a population per unit area as shown in this study. The removal method using pitfall traps might therefore be useful in quantitatively evaluating whether carabid beetles could be used as a predator or for measuring their biomass.

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