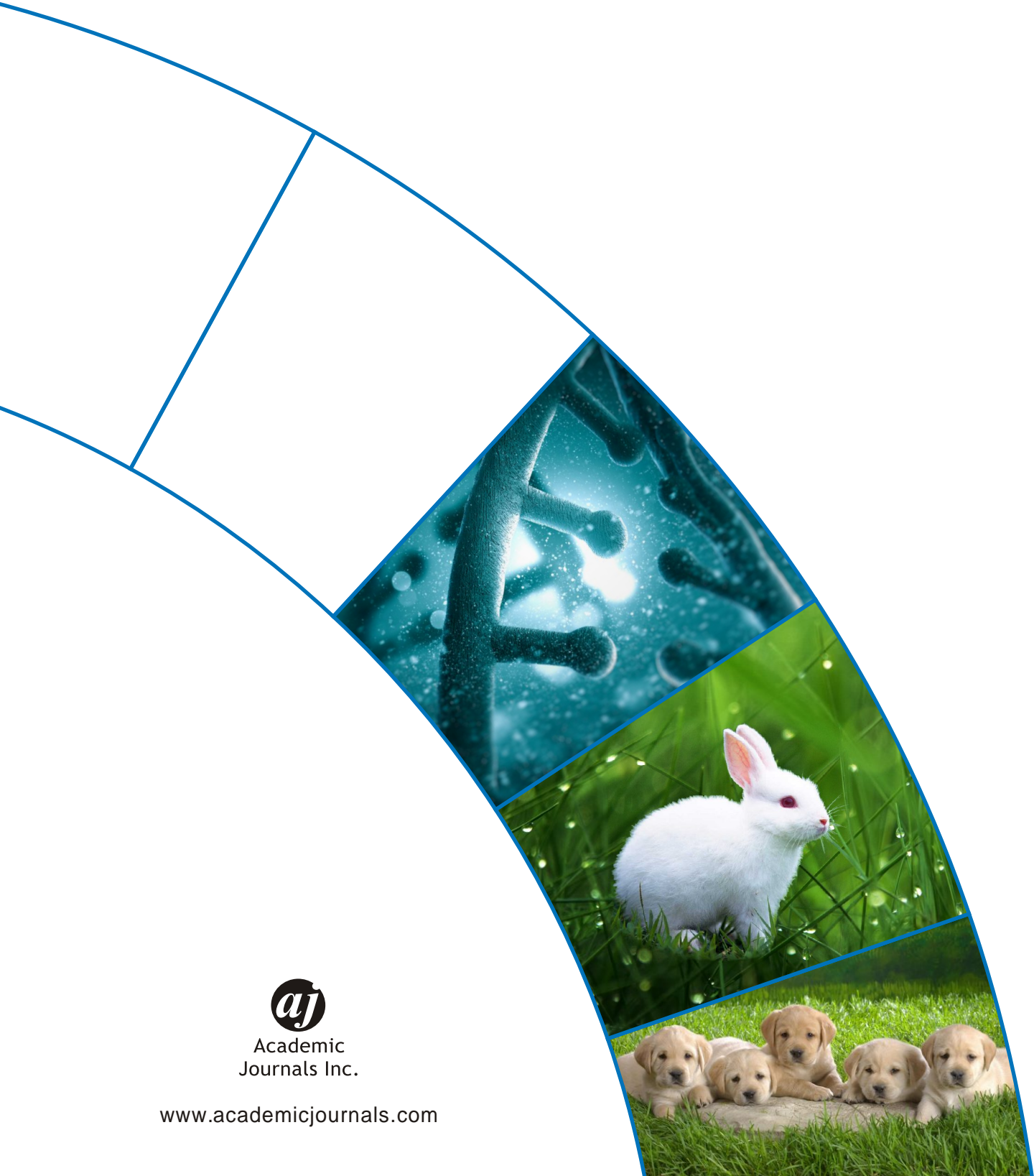


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## Research Article

# Estimation Methods of Rat Abundance Concerning Zoonotic Diseases in Gunung Kidul Regency, Yogyakarta, Indonesia

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### Abstract

**Background and Objective:** Several species of rats are commensal, the population spreads along with human settlements. Rat surveys mostly carried out as a precaution for various rat borne diseases. An accurate and precise estimation of the rat population is required to provide an overview of the abundance of rats in an area, during and after the eradication program. This study aims to demonstrate and compare the application of several methods and formulas for estimating rat abundance. **Materials and Methods:** The cross-sectional survey was conducted for 21 consecutive days at Wotgalih Hamlet, Pilangrejo Village, Nglipar District, Gunung Kidul Regency, Yogyakarta, Indonesia, using 200 single live trap units. The rat abundance estimation was calculated using the observation method, the method of removal and non-removal sampling. The calculation on the removal method uses the Trap Sukses; Hayne; Zippin and Maximum Weighted Likelihood formulae. The non-removal method uses the Lincoln-Petersen and Jolly Seber formulas. The observation method describes the number of houses with rats contain. **Results:** Trap success formulae; Zippin and Maximum Weighted Likelihood tends to get an underestimate abundance while the Lincoln-Petersen and Jolly Seber formulas tend to overestimate. **Conclusion:** The removal sampling method is more suitable for surveying residential rats, the Hayne formulae are easier to apply in rat surveillance concerning health problems and produce a more accurate rat abundance estimation.

**Key words:** Rat abundance, estimating methods, gunung kidul, jolly seber formulas, leptospirosis, *Rattus argentiventer*, murine typhus

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The existence of rats in the scattered human environment causes various problems. In agricultural land, they act as pests which often lead to reduced crop production<sup>1</sup>. For the health sector, rats are a reservoir for the transmission of various types of zoonotic diseases<sup>2</sup>. Aesthetically, the abundance of rats in a residential settlement illustrates the poor sanitation and hygiene conditions of the area<sup>3</sup>.

Many species of rats are commensal and ubiquitous, the populations spread throughout human settlements. The fluctuation of the rat population is greatly influenced by the presence of a food source that depends on the abundance of the human population in the habitat. Meanwhile, human activity can also act as a limiting factor for the rat population<sup>4</sup>. Eradication of rats is carried out to reduce economic losses and diseases caused by rats.

Several rat surveys have been carried out during the last few years to mitigate diseases caused by a rat, including leptospirosis<sup>5</sup>, Hantavirus<sup>6</sup>, Plague<sup>7</sup>, rickettsia<sup>8,9</sup> and murine typhus<sup>10</sup>. Unfortunately, most of the programs and studies only include the number of rats caught which does not yet reflect the abundance of rats at the sites. Several study and rat surveillance regarding health program commonly used the number of trap success to determine the relative abundance of rats<sup>11,12</sup>.

Trap success is a percentage number resulting from dividing the number of rats captured by the number of traps installed and the number of days of capture<sup>13</sup>. This formula has weaknesses in describing the abundance: First, the catch success mostly depends on several technical factors such as trap conditions, suitability of bait and type of traps; second, the successful trap rate does not reflect the diversity of the rat caught.

Each rat species has a different behaviour pattern, even though they exist in the same place or habitat. *Rattus tanezumi* (house rat), tends to occupy the roof of the house by occasionally going down on foraging<sup>14</sup>. *Rattus norvegicus* (brown rat) is relative can't climb, they make nest holes around settlements and come out at night to find food in the human environment, it can even enter the houses<sup>15</sup>. *Rattus argentiventer* (Paddy-field rat) can also be found in people's homes to look for food. This usually occurs when the rice fields are in a "fallow" condition, there are not sufficient sources of feed in the rice fields which are their natural habitat<sup>16</sup>.

The literature on population size estimation methods is widely available in ecological study, but the application of the methods is mostly done in the natural environment. This study

aims to apply a method of estimating the abundance of rats in residential areas, significantly different characteristics. Information about the advantages and disadvantages of the method is very useful for determining a more accurate estimation method. For the rat's eradication program, information on the number of rats that have been captured and killed is useful, but data on the number of rats that have not been captured will be more useful in determining the next course of action.

## MATERIALS AND METHODS

**Study area:** The rat survey was conducted in Wotgalih Hamlet, Pilangrejo Village, Nglipar District, Gunung Kidul Regency, Yogyakarta, Indonesia, for 20 consecutive nights. This research project was conducted from July-August, 2019.

**Sample collection:** Rats were captured using 200 units of Single Live Trap, placed in resident's houses and paddy fields surrounding the settlement. The study area was calculated based on the placement and buffer zone of the Google Earth according to the home range of rats of 30 m<sup>17,18</sup>.

**Methodology:** The captured rats were being put in cloth bags every morning and then sedated for identifying process and labelling using a numbered plastic necklace attached to the neck. The width of the label collar is adjusted so that it does not easily slip off the rat's neck but does not cause the rat to choke. The conscious rats were released in the afternoon on the place they were trapped. The recording was carried out every day including the species of rat, the tagged rat that was caught again and the rat that was released again. The rat abundance estimation was calculated from the observations of the householder. The method of removal and non-removal. The removal method uses a trap success; Hayne; Zippin and Maximum Weighted Likelihood formulae. The non-removal method uses the Lincoln-Petersen and the Jolly Seber formulae. The abundance calculation for the removal sampling method was done by ignoring the number of marked rat that was recaptured in the non-removal sampling method.

## RESULTS AND DISCUSSION

The trapping was settled in houses and paddy fields/garden that 9.2 ha coverage area. The Houses clumped in the village surrounded and separated by paddy field and small teak forest to other villages, forming a kind of boundary

Table 1: Record of captured rats from July 16 through August 4 2019, at Wotgalih Hamlet, Pilangrejo Village, Nglipar District, Gunung Kidul Regency, Yogyakarta

Number of captured	Days																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Total captured	7	4	8	4	4	2	2	6	5	3	5	3	1	2	4	3	0	3	0	2	68
Marked	0	0	1	3	1	0	1	1	3	2	3	1	0	2	2	0	0	1	0	0	21
Unmarked	7	4	7	1	3	2	1	5	2	1	2	2	1	0	2	3	0	2	0	2	47
Released	7	4	8	4	4	2	1	6	5	3	5	1	1	2	4	3	0	3	0	0	63

Table 2: Record of unmarked rat captured during 20 days

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Number of captured	7	4	7	1	3	2	1	5	2	1	2	2	1	0	2	3	0	2	0	2
Cumulative	0	7	11	18	19	22	24	25	30	32	33	35	37	38	38	40	43	43	45	45

that makes this village separate from other settlements. A total of 28 houses are located in the village with an average distance of 20 m between houses. The rats were captured for 20 consecutive nights, the results are summarized in Table 1.

**Removal sampling methods**

**Trap success formulae:** Relative abundance calculations using the Trap Success formulae were carried out by focusing on the catch of unmarked rat, assuming that the tagged rat had been excluded from the population (removal sampling):

$$\text{Trap success} = \frac{47}{20 \times 200} \times 100\% = 1.18\%$$

If it is assumed that the effort to catch rats is carried out only for the first 3 days, then the results of a successful trap are:

$$\text{Trap success} = \frac{18}{3 \times 200} \times 100\% = 3\%$$

**Hayne formulae:** The regression method was introduced by Hayne (1949) as illustrated by Bord *et al.*<sup>19</sup>, The daily catch is plotted on the graph as the Y coordinate while the cumulative catch is the X coordinate. The plotting results are then used as the basis for making linear equations. The estimated number is obtained by assuming the Y value is zero.

The results of trapping mice for 20 nights, excluding tagged rats recaptured are shown in Table 2.

If it is assumed that the trapping periods was 4 nights (the common period of surveying rats in the health sector), then the graph obtained.

The data of Fig. 1 shows the plotting of the Y, daily capture for 4 consecutive nights (7; 4; 7 and 1 rat caught) with X, cumulative values (0; 7; 11 and 18). The estimated rat abundance following Hayne formula<sup>19</sup> calculated based on the equations generated by the graph:

$$y = -0.2824x + 7.2912$$

if y = 0, with the result that:

$$0.2824x = 7.2912$$

$$x = 7.2912 / 0.2824$$

If the calculation considers all available data (20 days of capture), in the same way as before:

$$x = 25.82$$

Then, the graph:

$$x = 26$$

The data of Fig. 2 represents the daily catch results plotted against the cumulative value for 20 consecutive nights. It is calculated by Hayne formulae<sup>19</sup>, based on the equation generated by the graph in Fig. 2, then the estimated rat abundance:

$$y = -0.1115x + 5.6109$$

If y = 0; then:

$$0.1115x = 5.6109$$

$$x = 5.6109 / 0.1115$$

$$x = 50.32$$

$$x = 50$$

**Zippin formulae:** Zippin, as reviewed by Rodriguez de Rivera and McRea<sup>20</sup> presented an abundance estimation formulae based on the removal sampling method so that this study still used the data of the unmarked rats captured. Unfortunately, the Zippin method only accommodates capture periods of 3, 4, 5 and 7 times:

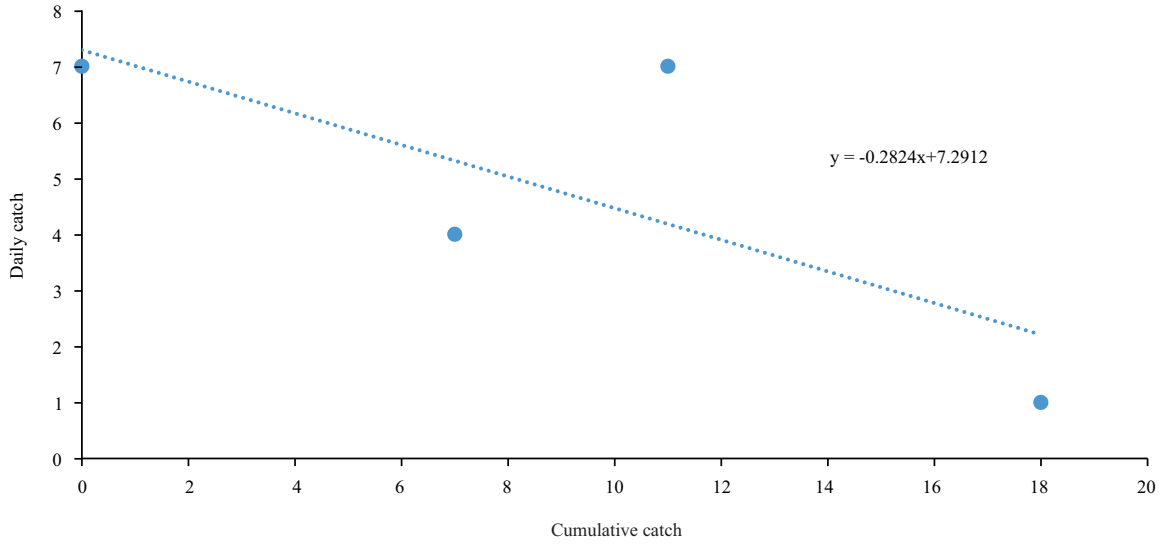


Fig. 1: Line equation results from plotting the rats capturing for 4 days

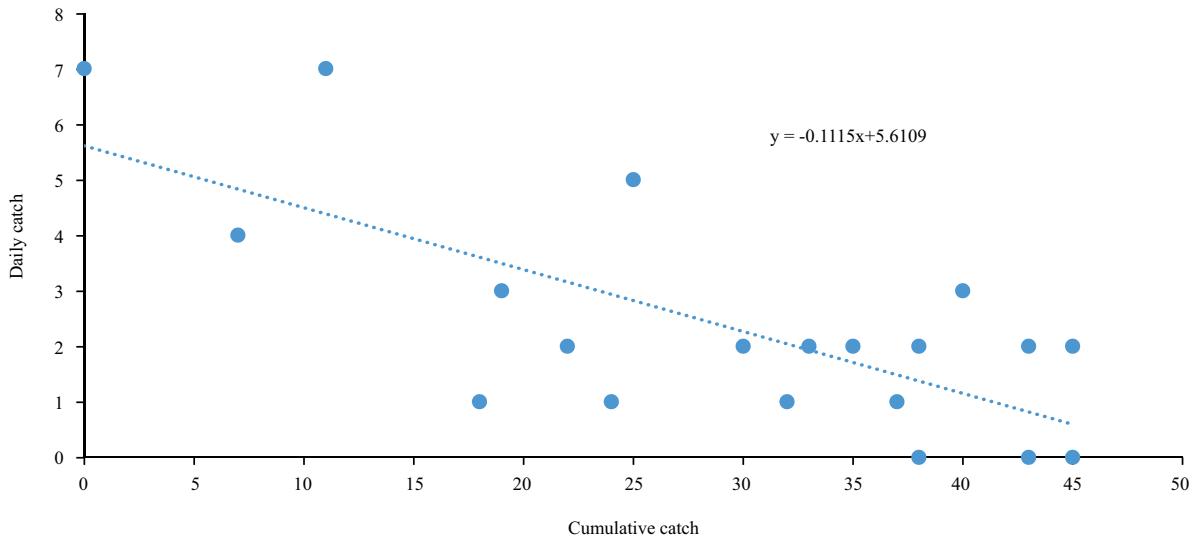


Fig. 2: Line equation results from plotting the rats capturing for 20 days

$$N = \frac{T}{1 - q^k}$$

So as:

$$R = \frac{y_2 + 2y_3 + 3y_4 + \dots + 19y_7}{y_1 + y_2 + y_3 + \dots + y_7}$$

where, N is the abundance estimation, T is the total caught of rat in all samples and  $1 - q^k$  is the estimation factor.

Estimation factor ( $1 - q^k$ ) calculate by R:

for  $k = 3$  (three night trapping periods), R value:

$$R = \frac{\sum_{i=1}^k (i-1)y_i}{T}$$

$$R = \frac{(0 \times 7) + (1 \times 4) + (2 \times 7)}{7 + 4 + 7}, R = 1$$

where, R is the captured probability ratio,  $y_i$  is the number of captured in day-i.

The data of Fig. 3a-d and Fig. 4a-d are paired, both of which are used to calculate the estimated density value based on the Zippin formula.

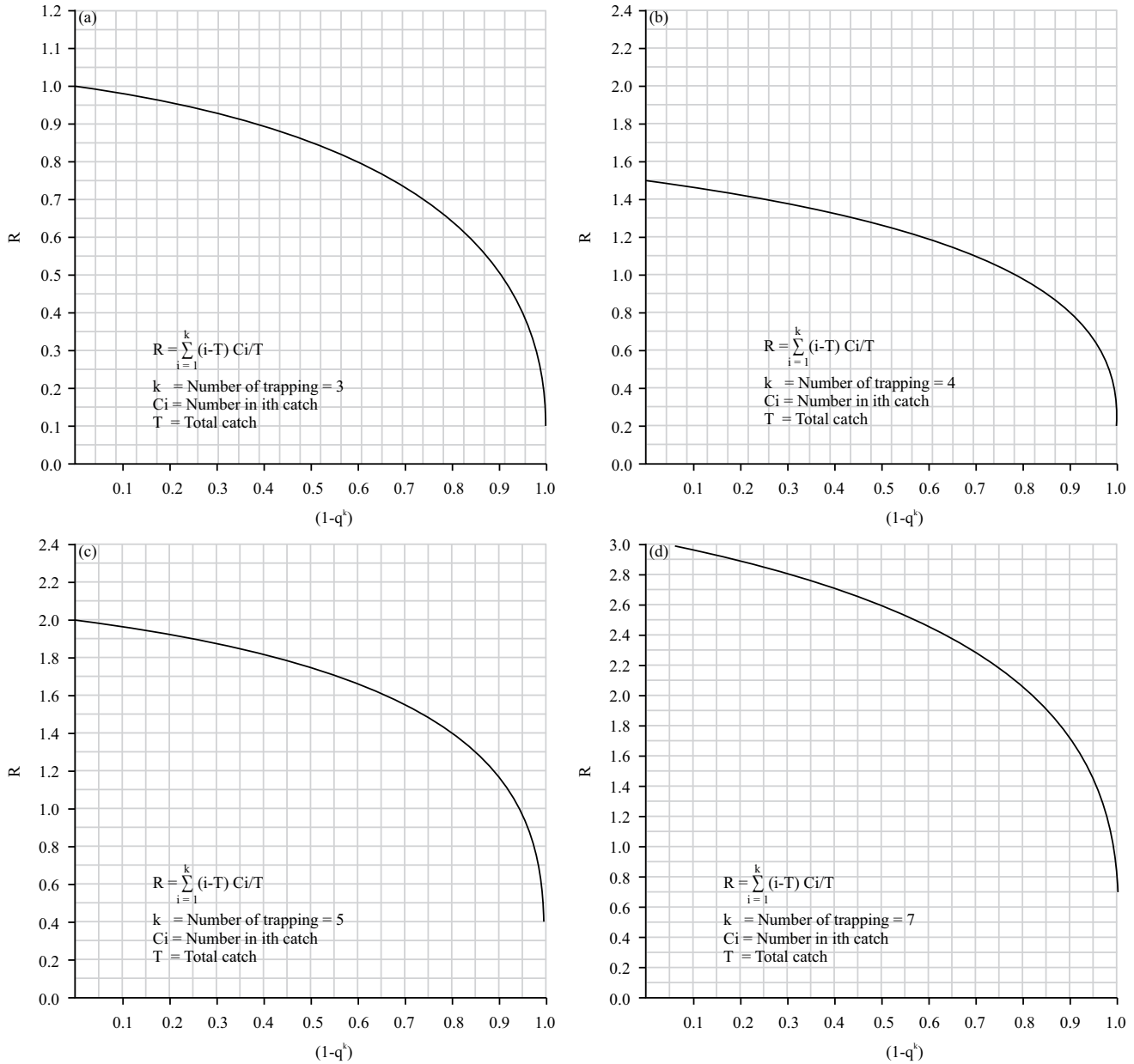


Fig. 3(a-d): Zippin chart 1, removal sampling method for abundance estimation, to determine the value of  $1-q^k$   
 (a) Graph for estimation of  $1-q^k$  from R, for 3 consecutive catching, (b) Graph for estimation of  $1-q^k$  from R, for 4 consecutive catching, (c) Graph for estimation of  $1-q^k$  from R, for 5 consecutive catching and (d) Graph for estimation of  $1-q^k$  from R, for 7 consecutive catching

By the use of Zippin charts (Fig. 3a) for  $k = 3$  and  $R = 1$ , the value of  $1-q^k = 0$ , so N cannot be determined.

For  $k = 4$ , R value:

$$R = \frac{(0 \times 7) + (1 \times 4) + (2 \times 7) + (3 \times 1)}{7 + 4 + 7 + 1}$$

$R = 1,105$ ; plotting in Zippin charts (Fig. 3b), with the result  $1-q^k = 0.8$ .

So the abundance estimation:

$$N = \frac{T}{(1-q^k)}$$

$$N = \frac{19}{0.7}$$

$$N = 24$$

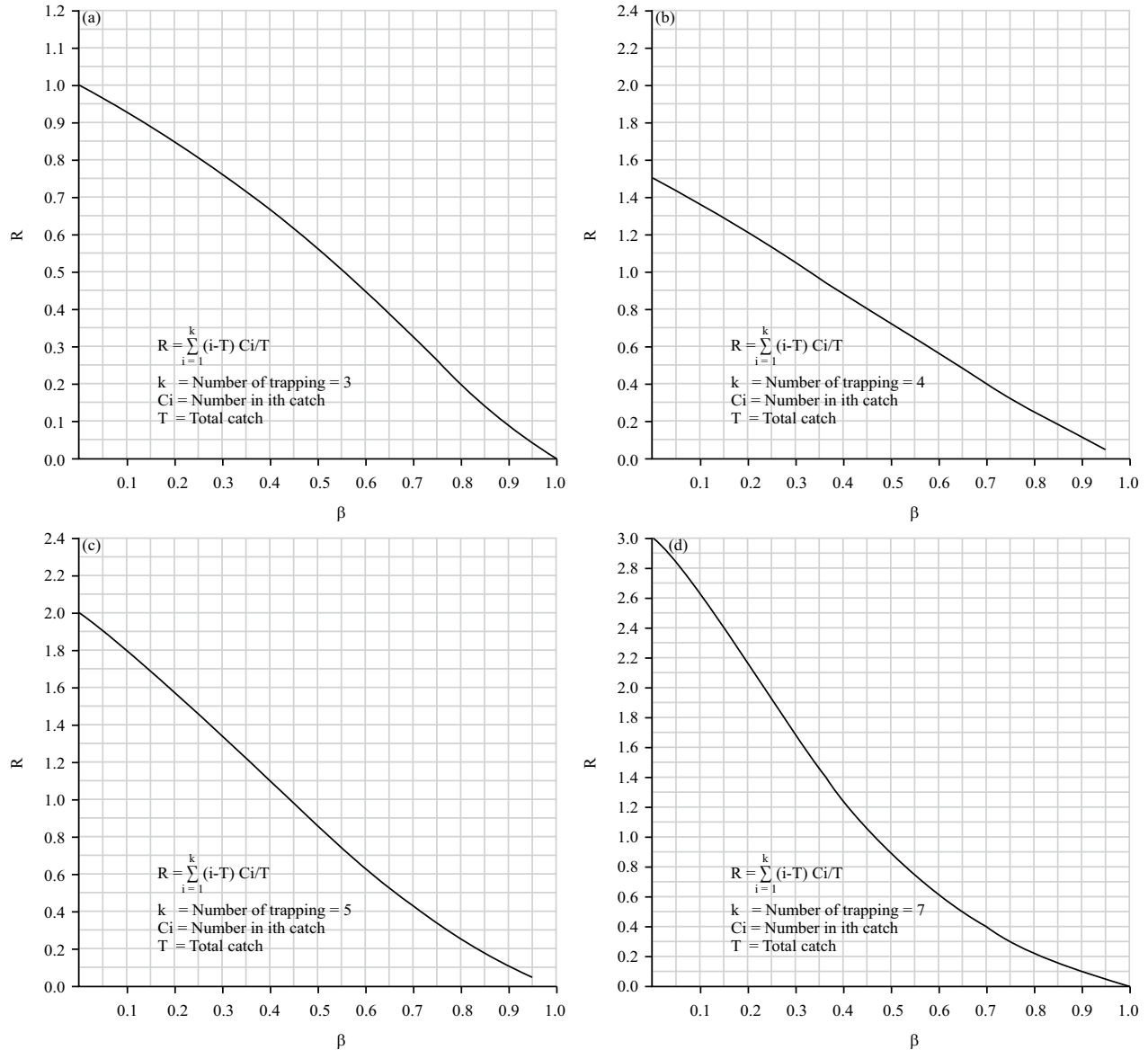


Fig. 4(a-d): Zippin chart 2, removal sampling method for abundance estimation, to determine the value of  $\beta$   
 (a) Graph for estimation of  $\beta$  from  $R$ , for 3 consecutive catching, (b) Graph for estimation of  $\beta$  from  $R$ , for 4 consecutive catching, (c) Graph for estimation of  $\beta$  from  $R$ , for 5 consecutive catching and (d) Graph for estimation of  $\beta$  from  $R$ , for 7 consecutive catching

Standard error calculated with the formulae:

$$SE = \sqrt{\frac{N(N-T)T}{T^2 - N(N-T)([kp]^2 / [1-p])}}$$

Rat's captured probability  $p$  obtained from Zippin charts 2 on Fig. 4a based on  $R$  and  $k$ . For  $R = 1.105$  and  $k = 4$  (Fig. 4b); then  $\beta = 0.3$ ; so the result:

$$SE = \sqrt{\frac{24(24-19)19}{19^2 - 24(24-19)([4 \times 0.3]^2 / [1-0.3])}} SE = 4.6$$

The abundance estimation of rats at survey area on 4 consecutive night period of trapping, at 95% significance level:

$$N \pm 1.96 (SE) = 24 \pm 1.96 (4.6) = 24 \pm 9$$

It has been explained that the use of Fig. 3a is followed by the use of Fig. 4a to calculate SE. Likewise, Fig. 3b will be followed by Fig. 4b. The count for the 5 and 7 night trapping period was shortened, because the method is the same as the previous one, but only the use of a different figure:

For 5 nights capture uses Fig. 3c to calculate N and Fig. 3d for 7 nights capture. Further, Fig. 4c are used to determine SE number for 5 nights captures and Fig. 4d to determine SE of 7 nights. For brevity, with the same method described previously, the calculation of abundance estimation for 5 and 7 consecutive night:

$$k = 5 \rightarrow N \pm 1.96 (SE) = 29 \pm 1.96 (10.4) \\ = 29 \pm 20$$

$$k = 7 \rightarrow N \pm 1.96 (SE) = 29 \pm 1.96 (1.4) \\ = 29 \pm 3$$

The data of Fig. 3 had copied from zippingmethods<sup>20</sup>, it was used to determine the value of  $1-q^k$ , based on R-value. Furthermore, this study cites the Hedger *et al.*<sup>21</sup> method which also uses the Zippin method in its study to calculate the density of fish populations, it uses the graph in Fig. 4 to determine the precision of the calculation results. The graphs was used to determine  $\beta$  value based on R and k value.

**Maximum weighted likelihood formulae:** Carle and Strub, as illustrated by Hedger *et al.*<sup>21</sup>, developed the "Maximum Weighted Likelihood" formulae which are claimed to better describe the population size through removal methods. Abundance estimates are calculated through the following asymptotic expression:

$$\left( \frac{N+1}{N-T+1} \right) \sum_{i=1}^k \left( \frac{kN - M - T + 0.5k}{kN - M + 1 + 0.5k} \right)^k \leq 1$$

The estimated abundance value is obtained through the largest integer number that satisfies the model above.

For 3 days trapping period, then:

$$k = 3; T = 7 + 4 + 7 = 18$$

The value of M counted:

$$M = \sum_{i=1}^k (k-i)c_i$$

so:

$$M = (3-1) 7 + (3-2) 4 + (3-3) 7 \\ = 14 + 4 + 0 \\ = 18$$

Further calculate from model; if N = 27:

$$\left( \frac{27+1}{27-18+1} \right) \sum_{i=1}^k \left( \frac{3 \times 27 - 18 - 18 + 0.5 \times 3}{3 \times 27 - 18 + 1 + 0.5 \times 3} \right)^3 \leq 1$$

$$\left( \frac{28}{10} \right) \left( \frac{46.5}{65.5} \right)^3 \leq 1$$

$$2.8 \times 0.358 \leq 1$$

$$1.0024 \leq 1 \text{ (not correct)}$$

if N = 28, by calculating the same:

$$0.995 \leq 1$$

Then, we get the value of N (abundance estimation) is 28. For the value of SE by the same formulae as Zippin methods:

$$SE = \sqrt{\frac{N(N-T)T}{T^2 - N(N-T)([kp]^2 / [1-p])}}$$

$$SE = \sqrt{\frac{28(28-18)18}{18^2 - 28(28-18)([3 \times 0.25]^2 / [1-0.25])}} SE = 6.65$$

So, abundance estimation of rats on the field, during 3 consecutive night of survey, calculated by MWL formulae, at 95% significance level:

$$N \pm 1.96 (SE) = 28 \pm 1.96 (6.65) \\ = 28 \pm 13$$

Furthermore, with the same calculation method for 4, 5 and 7 consecutive night trapping periods:

$$k = 4; T = 19; M = 36; \text{ then } N = 22 \pm 4$$

$$k = 5; T = 22; M = 55; \text{ then } N = 25 \pm 4$$

$$k = 7; T = 25; M = 101; \text{ then } N = 27 \pm 4$$

### Non-removal sampling methods

**Lincoln-Petersen formula:** Abundance estimation of rats using Lincoln-Petersen Formula, corrected by Seber and demonstrated in Schmalenbach *et al.*<sup>22</sup> study:

$$\frac{N}{M+1} = \frac{C+1}{R+1}$$

where, N is the population size, M is the animals marked and released, C is the size of the second sample and R is the marked animals recaptured.

Based on the capture data shown in Table 1, adjusted to the Lincoln-Petersen method resulted in the recapitulation shown in Table 3.



Table 3: Lincoln-Peterson data for rats abundance estimation

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
M+1	8	12	20	24	28	30	32	38	43	46	51	54	55	57	61	64	64	67	67	69
C+1	5	9	5	5	3	3	7	6	4	6	4	2	3	5	4	1	4	1	3	1
R+1	1	2	4	2	1	2	2	4	3	4	2	1	3	3	1	1	2	1	1	1
N	40	54	25	60	84	45	112	57	57	69	102	108	55	95	244	64	128	67	201	69

Average N = 87. Standard error = 12. Min-Max: 25-244. Estimation of N at 95% significance level =  $N \pm 1.96 (SE)$ , =  $87 \pm 1.96 \times 12$ , =  $87 \pm 24$ , (63-111)

Table 4: Capture history for abundance estimation by Jolly Seber formulae

Time of last capture	Time of capture																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1		0	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0
2			1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
3				1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0
4					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6							0	0	1	1	0	0	0	0	0	0	0	0	0	0
7								0	0	0	0	0	0	0	0	0	0	0	0	0
8									1	1	2	0	0	0	0	0	0	0	0	0
9										0	0	0	0	0	0	0	0	0	0	0
10											0	0	0	0	0	0	0	0	0	0
11												0	0	0	0	0	0	0	0	0
12													0	0	0	0	0	0	0	0
13														1	1	0	0	0	0	0
14															0	0	0	0	0	0
15																0	0	0	0	0
16																	0	0	0	0
17																		0	0	0
18																			0	0
19																				0
20																				0
Total marked ( $m_t$ )	0	0	1	3	1	0	1	1	3	2	3	1	0	2	2	0	0	1	0	0
Total unmarked ( $u_t$ )	7	4	7	1	3	2	1	5	2	1	2	2	1	0	2	3	0	2	0	2
Total caught ( $n_t = m_t + u_t$ )	7	4	8	4	4	2	2	6	5	3	5	3	1	2	4	3	0	3	0	2
Total released ( $s_t$ )	7	4	8	4	4	2	1	6	5	3	5	5	1	2	4	3	0	3	0	0

**Jolly Seber formulae<sup>23</sup>:**

$$\alpha_t = \frac{m_t + 1}{n_t + 1}$$

$$M_t = \frac{(s_t + 1)Z_t}{R_t + 1} + m_t$$

$$N_t = \frac{M_t}{\alpha_t}$$

where,  $m_t$  is the number of marked animals caught in sample t.  $u_t$  is the number of unmarked animals caught in sample t.  $n_t$  is the total number of animals caught in sample t =  $m_t + u_t$ .  $s_t$  is the total number of animals released after sample t.  $r_t$  is the accidental deaths or removals.  $R_t$  is the number of the  $s_t$  individuals released at sample t and caught again in some later sample.  $Z_t$  is the number of individuals marked before sample t, not caught in sample t, but caught in some sample after sample t.

The data of Table 4 presents the plotting results of the data in Table 1 to calculate the value of Total marked ( $m_t$ ); Total unmarked ( $u_t$ ); Total caught ( $n_t$ ); dan Total released ( $s_t$ ). Furthermore, these values are used to calculate the number of population fluctuation based on the formula that has been mentioned, presented in Table 5.

The estimation of the rat's population size using the Jolly Seber formulae obtained fluctuating results on every trapping day with a very wide range of numbers (Min = 1; Max = 90). At the 95% confidence level, the estimated value has a very wide range also ( $29 \pm 43$ ).

The study also tried to estimate the abundance of rats at the study site by interviewing residents about the presence of rats in their respective houses, according to the assumption that the occupants of the house are the ones who know best about the presence of rats in their house, as well as the limitations of the research to make direct observations about the presence of rats in all houses in the study location (28 units). The results of the interviews are shown in Table 6.

Table 5: Results for the Jolly Seber model

Sample	Proportion marked ( $\alpha_i$ )	Size of the marked population ( $M_i$ )	Population estimate ( $N_i$ )	Probability of survival ( $\phi_i$ )	Number joining ( $B_i$ )
1	0.000	0.0	NA	0.714	NA
2	0.200	5.0	25.0	1.111	17.2
3	0.222	10.0	45.0	1.647	-39.1
4	0.800	28.0	35.0	1.241	46.6
5	0.400	36.0	90.0	0.179	4.8
6	0.333	7.0	21.0	2.778	-20.8
7	0.667	25.0	37.5	0.432	22.0
8	0.286	10.8	37.8	3.228	-45.5
9	0.667	51.0	76.5	0.491	-2.9
10	0.750	26.0	34.7	0.778	4.5
11	0.667	21.0	31.5	0.391	5.7
12	0.500	9.0	18.0	0.103	0.6
13	0.500	1.3	2.7	3.429	-1.1
14	1.000	8.0	8.0	0.875	4.7
15	0.600	7.0	11.7	0.444	10.8
16	0.250	4.0	16.0	0.143	-1.3
17	1.000	1.0	1.0	1.000	1.0
18	0.500	1.0	2.0	0.000	0.0
19	1.000	0.0	0.0	NA	NA
20	0.333	NA	NA	NA	NA

Mean: 29.0, Var: 610.9, SD: 24.7, Min: 0.0, Max: 90.0, SE: 22,  $N \pm 1.96 (SE) = 29 \pm (1.96 \times 22) = 29 \pm 43$

Table 6: Occurrence of rats in every house of the study area obtained from house holder report

House number	Occurrence of rats		Number of estimation
	Yes	No	
1	1		1
2	1		3
3	1		1
4	2		-
5	2		-
6	1		1
7	2		-
8	2		-
9	1		3
10	1		3
11	2		-
12	1		2
13	1		5
14	2		-
15	1		5
16	1		3
17	2		-
18	2		-
19	1		4
20	1		3
21	2		-
22	2		-
23	1		2
24	1		1
25	1		3
26	2		-
27	1		10
28	1		3
Total	17	11	53

Min: 1 rat. Max:10 rats. House indexes:  $17/28 \times 100\% = 60.7\%$

Estimation of rat abundance of the interview method was used to estimate the number of rats in residential areas. This estimate utilizes the knowledge of the occupant of the house about the presence of rats in their respective houses so that it can only estimate the presence of rats in the house, while rats outside the village head or in other environments cannot be estimated. Interviews were conducted with 28 householders that were set up with traps during the study. A total of 17 respondents admitted that there were rats in their house and 11 houses were thought to have no rats. The estimated rat abundance per household that claims to have rats is 1-10 per household.

As a summary, Table 7 presents a recapitulation of the calculation results, facilitating the comparison of the calculated numbers from the various methods above.

The research location in Wotgalih Hamlet, Pilangrejo Village, Nglipar District, Gunung Kidul Regency, Yogyakarta Province, Indonesia, is a rural settlement. The distance between houses is relatively far, the hamlet environment is rainfed rice fields which at the time of this research were being left in a fallow state or not being planted with rice because it was the dry season. There are several cassavas planted at the boundaries that can be a source of food for rats.

Only 3 species of rats were captured during the study: *Rattus tanezumi*, *Bandicota indica* and *Rattus tiomanicus*. Abundance estimates using several methods showed different results, but all of the methods indicated that the rat population in the study area was relatively low. The method

Table 7: Summary of rats abundance estimation results

Methods and formulae	Rats abundance estimation	Interval at 95% significance level
Trap success for 20 days	1.18%	NA
Trap success for first 3 days	3%	NA
Hayne for the first 4 days	26	NA
Hayne for 20 days	50	NA
Zippin, first 3 days	NA	NA
Zippin, first 4 days	24	15<N<33
Zippin, first 5 days	29	9<N<49
Zippin, first 7 days	29	26<N<32
MWL, first 3 days	28	15<N<41
MWL, first 4 days	22	18<N<26
MWL, first 5 days	25	21<N<29
MWL, first 7 days	27	23<N<31
Lincoln-Petersen	87	63<N<111
Jolly Seber	29	0<N<72
Observation	53	NA

most commonly used to describe rat abundance in residential settings is trap success. This method is easiest to apply with a cut-off point value of 7%. The successful trap rate in this study was 3% for a 4-day holding period (the number of days commonly used for surveying rats related to rat-borne disease). If the successful trap rate for 20 days is used, the success trap rate decreases to 1.18%. The weakness of the successful trap method is that it only describes the relative abundance at the study site, besides that the trap used must always use a single live trap. The neophobic nature of rats, the type of bait used and the rat's learning behaviour towards dangerous goods also influenced trap success.

The Hayne formulae application is still quite easy to apply. This formula only calculates an estimate of the abundance of rat based on the line equation generated from trapping rat over several days. In this study, the estimated number of 4trapping days was 26. This number increases to 50 if the data analyzed from 20 days of trapping. When the number compared with the total, the second estimate is the one that makes more sense.

The ideal Hayne formulae are used if the daily catch is decreasing relatively stable so that the resulting line equation will also be more precise<sup>19</sup>. In this study, the catch results were fluctuating in the first four days and on the fourth day not approaching the minimum number, this is affecting the bias in the estimation results which tends to result in a figure below the actual figure ("underestimate"). In 20 days, the minimum catch results have been achieved so that the resulting line equation is more precise and the estimated figure is also more accurate.

Compared to the Trap Success rate, the Zippin formulae provides an estimated number that better describes the number of rats in the study site. Zippin estimation requires a

maximum fishing time of 7 days to get the estimated results so that it is still relevant to the trapping period concerned public health that has been carried out so far.

In this study, the largest estimate using the Zippin method was 27 rats (23<N<31), but the total catch for 20 days was 47, indicating that the Zippin method resulted in an "underestimation" number. Related the reason that Removal Method requires several conditions<sup>19</sup>:

- Performed in closed populations, the effects of migration, births and mortality should be a minimum during the sampling period
- The number of individuals caught per capture period should significantly reduce the number of individuals in the population
- The probability of an individual being caught must always be constant
- The probability of an individual being caught must be the same for each individual in the population
- The population must not be so large that the capture of one individual interferes with the capture of another

However, in a common environment, these conditions are very difficult to fulfil. Surveys of rats are almost always carried out in open areas so it is very difficult to minimize migration, mortality and births during the survey period. The characteristics of the survey location in itself will determine the level of population dynamics. Rats relatively have a long roaming range, but in conditions where food sources are easily obtained, they tend to reduce their range. For the case of rats in residential areas, the home range of rats is quite low because the food source is always available by utilizing food scraps from humans. The location of this research is also a rural environment where the distance between houses is relatively far, the location of the hamlet is also limited to forests and rice fields so that the chances of migration out or entering the research location can be assumed to be minimal.

Rats have a gestation period of 21 days so that the survey period below that number of days is still possible to minimize the effect of births on population size. The life span of the rats is also relatively long, reaching up to 3 years, so we can assume the effect on population dynamics during the period the rat survey was conducted might be considered low.

On the second requirement, the rat survey is highly dependent on the effectiveness of the trapping method used. Types of traps; baits; the presence of other food sources and individual factors for the rat in recognizing hazards greatly influence the success of trapping. In this case, the number of rats caught is often not linearly decreasing each day, even

often fluctuates. This shows that the effectiveness of trapping has not been able to cover most members of the population at the beginning.

For the third requirement, the probability of capture must always be constant as likely to be fulfilled as long as the trap used is assumed to capture all members of the population in the area. In this study, 200 traps were used, which are estimated to be able to catch all rats in that location. A single live trap is only able to catch a rat that is actively looking for food. These traps are not designed to catch immature and young rats that are not actively foraging. This means that the probability of rats being caught is not the same for all rats.

The rat population required by the Zippin method should not be too large. In this study, the number of rats in the study location was still relatively small, so it was assumed that they still met these requirements. However, it will be difficult to assume this if the survey was conducted at another time and place. Because it may take some kind of preliminary survey to get a rough idea of the number of rats in the location, which of course needs extra time, effort and cost.

Another formula used to estimate the abundance of rat in the removal method is the Maximum Weighted Likelihood (MWL) formulae. The estimation results with these formulae are in the range of 22-28 individuals, which is not significantly different from the methods of Zippin results. Like the Zippin, this formulae is effective if the catch probability requirement for all individuals in the population remains constant<sup>21</sup>, which in this study was difficult to fulfil due to various reasons previously described.

The rat's abundance estimation was also carried out using the non-removal method, the rats that were caught were marked and then returned to the population. This method has the advantage over the removal method, it has a better accuracy; includes the population dynamics during the survey periods and is very well suited to natural populations.

The disadvantage of this method is that it is relatively difficult to implement for target animals that are unlikely to be returned to the population. The rat survey to mitigating the zoonotic potential aims to minimize the risk of disease's transmission so that it is impossible for rats caught to be returned into the population. Besides, the survey location is a residential area, so there is potency for refusal from residents.

This study was conducted in a free rat-borne disease case location, so it was assumed that the zoonotic potential from rats in that place was relatively low. Besides, before the implementation of the research, socialization was carried out to residents regarding the technical research and requests for approval from residents whose houses would be trapped in.

After the arrest took place for several days, one resident expressed his discomfort because the rats caught in his house were always released back.

The Lincoln-Petersen estimate abundance of the rat in the study location was 87 ( $63 < N < 111$ ), significantly higher than the result of the removal method. The Lincoln-Petersen estimation formulae tend to be a higher result than the total (Overestimate)<sup>22</sup>. The probability of bias is influenced by several variables used to calculate abundance. The variable R (marked animal caught in the second arrest) has the most effect on the bias because it acts as the denominator in the formulae. R values that tend to be small will lead to larger estimation results. In the case of this study, the estimated subjects are rat where they are known to have trap shyness or deterrent properties of traps or bait deterrents so that the effectiveness of the traps for the second catch, especially for rats that have been caught, will tend to decrease so that in the end it produces an abundance estimate figure that is larger than the actual.

The Jolly Seber formulae used to estimate the abundance of rat at the study site obtained the mean rat abundance of 29 ( $0 < N < 72$ ). At first glance, this figure is quite close to the abundance calculation result in the removal method, but if we look at the SE figures, the estimated range is very wide so that the precision is very weak. During the 20 days of capture, the estimated number fluctuated greatly, with the lowest estimated value being 0 and the highest being 90 rats. This figure is very doubtful considering that the population can't drop drastically to 0.

Another drawback of the Jolly Seber formulae is its complicated application<sup>23</sup>. Surveys of rats in the context of mitigating rat-borne diseases are usually carried out by holders of animal-based disease surveillance programs at local and central government health authorities. A large number of tasks or other workloads makes the rat survey a more practical method and does not require a lot of time, but the results still answer the objectives of the survey.

The non-removal sampling method is used to calculate the estimated value of animals by considering population dynamics. In closed populations, the dynamics are affected only by births and deaths. Meanwhile, the open population is also affected by migration. In practice, this method is suitable if the arrest is carried out over a relatively long duration (influenced by the subject's life period, gestation time and age of maturity). For example, catching and tagging for hoofed animal abundance estimates are carried out throughout one month<sup>24</sup>; bimonthly for geckos population<sup>25</sup>; mallard population dynamics are calculated annually<sup>26</sup>. The period for abundance calculations that take into account population size

fluctuations should be longer than the gestation period and period between newborns to maturity, but a shorter life span (estimated mean life span from natural birth to death). In this research survey, the trapping time span is one day, where population dynamics fluctuate less in this time span, so that the non-removal method seems less suitable to be applied in daily surveys.

Abundance estimation was also carried out using direct observation methods. The method used is by direct interviews with the occupant of the house regarding the presence of rats in each house and the estimated number. In this case, the house holder is the observer who knows the best about the condition of his house because they live in it every day, so they can be considered a "static observer". This method was very easy to apply and only requires a small cost and time. However, the bias or estimation error persists mainly due to the home range of the rat that can easily move between houses so that it is possible for an individual rat to be repeatedly observed by different observers and ultimately lead to "overestimation".

At least, the interview method can provide information on the number of houses encroached on by rats ("rat positive"), which illustrates the potentially zoonotic risk in the community. This figure is similar to the "House Index" function which is commonly used in the *Aedes aegypti* mosquito survey for the mitigation of dengue fever. The potential bias for rat positive houses with this method is relatively small because the householder only identified the presence of rats without having to estimate the number<sup>27</sup>.

The results of calculations using the Zippin and MWL formulas tend to underestimate, as well as the Hayne formulae on the 4-day catch. On the contrary, the Lincoln-Petersen formula tends to be overestimated and less applicable to cross-sectional studies. Only the Hayne formulae for 20 days of capture and the method of observation is closest to the true abundance value at the study site.

## CONCLUSION

The method that allows being carried out in rat surveys to mitigate zoonotic diseases is the removal method. The Hayne formulae for estimating rat abundance in cross-sectional surveys can provide information on the estimated number of rats at the survey site. However, the application of these formulae should consider the trends in daily catch fluctuations. The observation method can be applied to obtain an overview of the abundance of rats at the survey site, the results of the observations are used to evaluate the effectiveness of the catch at the final survey period.

## SIGNIFICANCE STATEMENT

Several methods have been proposed by zoological researchers to estimate rat density. Based on this, the study proposes the most appropriate method to be used to obtain a more precise method for calculating density in surveys conducted for rat control. Estimates of the number of rats remaining after the implementation of the program can be determined so that it is useful for determining the next steps. The results of this study also help researchers, governments and companies engaged in pest control to determine the effectiveness of their control methods. This study also proposes an interview method that can be used to complement the existing.

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