*Vidyodaya J., Sci., Vol. 1, Nos. 1 & 2 — July 1989 — pp. 33-45*

# EFFECT OF TWO METHODS OF SAMPLING ON THE OOTHECAL PARASITES OF *PERIPLANETA AMERICANA* (L.)

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**Abstract**

Empty and live oothecae of *P. americana* found in houses were separately sampled to determine natural parasitism levels of the two oothecal parasites *E. appendigaster* and *T. hagenowii*. When a quarter of the live oothecae at a site was removed and sampled for 12 months, *T. hagenowii* was found to be the dominant parasite with a mean parasitism level of 30.5%, compared to *E. appendigaster* with a parasitism level of 8.1%. When all the empty oothecae were removed from the same sites and sampled in the following 12 months the two oothecal parasites gave equal levels of parasitism, where each species parasitized a third of the total oothecal population. When from two separate sites either empty or live oothecae were removed concurrently, parasitism levels obtained were similar to those recorded when each type of ootheca was removed from the same sites but in two different years. The study indicates that the removal of even a quarter of the live *P. americana* oothecae, which represents a sampling method with no replacement has adverse effects on *E. appendigaster* populations as compared with *T. hagenowii*. The adverse effect on *E. appendigaster* populations due to a sampling method with no replacement stems from the low fecundity of this species together with the habit of laying a single egg in an oothecae that yields only a single parasite at emergence.

## 1. Introduction

The American cockroach, *Periplaneta americana* (L) (Dictyoptera : Blattidae) is a well known household pest the world over. The control of this pest using insecticides has generally not been successful because of its habit of sheltering beneath wooden floors, manhole covers and other similar places not easily accessible to the application of insecticides.

The possibility of controlling *P. americana* using its natural enemies has been considered since 1925 by several workers. Cameron (1952) has listed 26 species of parasites of *P. americana*. Cameron (1952) and Roth & Wills (1954, 1960)

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in particular, worked on the general biology of two of the oothecal parasites of *P. americana*, *Tetrastichus hagenowii* (Ratz) (Hymenoptera : Eulophidae) and *Evania appendigaster* (L) (Hymenopters : Evaniidae). Their studies revealed a natural parasitism level of 0-68% by both *E. appendigaster* and *T. hagenowii*. Studies on the oothecal parasites of *Periplaneta americana* were initiated in Sri Lanka at the University of Sri Jayewardenepura in the mid-1970s. (Gamalath, 1980)

A preliminary survey conducted in Sri Lanka by Kumarasinghe[6] revealed only the two above mentioned oothecal parasites of *P. americana* in Sri Lanka. Further studies on the biology of these parasites revealed that *T. hagenewii* has an ability to parasitize the immature stages of *E. appendigaster* inside the oothecae (Kumarasinghe and Edirisinghe, 1987) Investigations into the role of *E. appendigaster* and *T. hagenowii* in the control of *P. americana* began with a study of the incidence of natural parasitism in houses harbouring cockroaches. To determine the level of natural parasitism of the two parasites sampling by the removal of live *P. americana* oothecae was carried out for a period of 12 months. Results of this sampling procedure showed that the level of parasitism by *E. appendigaster* was very low and in fact reached zero levels as sampling continued. No such effects were shown by *T. hagenowii* which always showed a higher level of parasitism. It was suspected at this stage that the removal of live oothecae during sampling was exerting an adverse effect on the population of *E. appendigaster* and therefore a different method of sampling based on empty oothecae was employed during the following year.

This paper reports the variation in the natural level of parasitism of the two oothecal parasites of *P. americana* in three regions of Sri Lanka, obtained by sampling live and empty oothecae.

## 2. Materials and Methods.

A total of six sites, two dwelling houses (separated by about 200 m) each in three regions or towns, Aluthgama, Pita Kotte and Gangodawila* in the low country wet zone Sri Lanka were chosen for sampling. At each sampling, the interior of the entire house (floor area 150 $m^2$) was carefully searched for oothecae both concealed (inside cupboards and containers) and unconcealed (on floors, walls and ceilings).

*P. americana* oothecae were sampled using two methods. In the first method live or unhatched oothecae were sampled i.e. oothecae that showed no evidence of either cockroach or parasite emergence. Every fourth oothecae found at a site was removed and brought into the laboratory. Removal of a quarter of the total number of live oothecae in this manner (leaving three quarters intact) was assumed to have the minimum effect on the remaining parasite population, and at the same time represent a sample size sufficiently

* Gangodawila is within 400m of the laboratory,
Pita Kotte is about 8km to the North and Aluthgama about 50km to the South.

large to give an accurate estimate of the level of parasitism. In the laboratory the live oothecae that were collected were kept separately in glass vials until emergence of nymphs or parasites and were then recorded as been unparasitized or parasitized by *E. appendigaster* or *T. hagenowii*. The level of parasitism by each of the parasites for the collected sample was then calculated and extrapolated to the entire live oothecal population.

In the second method, empty or hatched oothecae were sampled i.e. oothecae bearing emergence holes made either by cockroach nymphs or adult parasites. All the empty oothecae found at a sampling site were removed and brought into the laboratory, These oothecae were then recorded as either parasitized by *T. hagenowii* if a small emergence hole (diameter 0.25 mm) was observed, or as parasitized by *E. appendigaster* if a large emergence hole (diameter 3.20 mm) was present, or as unparasitized if a split was seen along the seam of the ootheca.

With each method sampling was carried out once a month for the period between oviposition and emergence, both for *P. americana* and for each of its parasites, is approximately four weeks (Kumarasinghe, 1984) This sampling frequency ensured that each generation was sampled once only. Sampling using the two methods had to be carried out in *two separate years*, since the nature of the two sampling methods does not permit the use of both methods concurrently at the same sampling sites. Hence, the first method of sampling was employed for 12 months during the year1980 and the second method for12 months during 1981, for the same six sites from the three towns. Thereafter, the two sampling methods were employed *concurrently* by having separate sites (about 500 m apart) for the two methods in only two of the three towns, namely, Aluthgama and Gangodawila. Of a total of six sites in Aluthgama and two sites in Gangodawila the first method of sampling was used at three of the sites at Aluthgama and one of the sites at Gangodawila. At the remaining three sites at Aluthgama and one site at Gangodawila the second method was employed. In this manner sampling using both methods was carried out concurrently once a month for five months from March — July 1982.

The levels of parasitism obtained were statistically analysed using either the t-test or were subjected to an analysis of variance.

## 3. Results

When empty oothecae were sampled in 1981 a higher overall level of parasitism was obtained than when live oothecae were sampled in 1980, at the same six sites (Table 1). On examination, of the level of parasitism in 1980 by each of the parasites separately, it was evident that, it is *T. hagenowii* which contributes significantly (four-fold) to the overall level of parasitism. However, in the following year the two parasites had contributed equally towards the total level of parasitism.

**Table 1**—Level of parasitism of *P. americana* oothecae, obtained by using a different method of sampling each year in the three regions.

| Parasite (year and method of sampling) | % Parasitism X — S.E. (range) Overall | Aluthgama | Pita Kotte | Gangodawila |
|---|---|---|---|---|
| *E. appendigaster* ... (1980—live oothecae) | 8.1+12.5 | 9.1+ 5.1 a (0 —16.7) | 10.8+ 9.2b (0 —26.7) | 4.3+ 4.4 c (0—9.9) |
| *T. hagenowii* ... (1980—empty oothecae) | 30.5+22.5 | 31.8+ 8.6 d (16.7—45.0) | 31.2+13.9d (0.48.5) | 28.4+ 9.7g (11.1—50.0) |
| *E. appendigaster* ... (1981—empty oothecae | 29.6+16.6 | 33.5+ 6.6f 20.7—43.3) | 28.7+ 9.3g 21.2—44.8) | 25.9+ 4.4 h (0—36.8) |
| *T. hagenowii* ... (1981—empty oothecae) | 31.9+17.2 | 25.8+ 6.1i 14.2—32.9) | 30.4+11.2j (15.3—46.7) | 39.7+7.0 k (29.8—50.3) |

$t_{22, 0.05} = 1.72$

Mean values followed by different letters indicate significant difference at P = 0.05

In each town too a similar trend was observed (Table 1) where in 1981 with the second method of sampling similar mean levels of parasitism for both parasites were recorded while in 1980 comparatively higher levels of parasitism by *T. hagenowii* only was recorded with the first method. Thus *T. hagenowii* consistently showed a higher monthly level of parasitism in all three regions when the first method was employed (Figs. I, II and III). while *E. appendigaster* on the contrary showed very low levels of parasitism including zero levels. However, in 1981 when the second method of sampling was used at the same sites in the three towns, *E. appendigaster* gave comparatively high levels of parasitism particularly in the month of July in Aluthgama (Fig. IV) and Pita Kotte (Fig. V) and in April at Gangodawila (Fig. VI), with no zero levels of parasitism been recorded.

When the monthly levels of parasitism obtained by the two methods were subjected to an analysis of variance a significant difference due to (a) methods (year) was obtained for *E. appendigaster* and not for *T. hagenowii* (b) Months for *T. hagenowii* and not for *E. appendigaster* (c) Regions (R) for *E. appendigaster* and not for *T. hagenowii* (d) Method x Months for *T. hagenowii* and not for *E. appendigaster* (e) Months x Regions for *T. hagenowii* and not for *E. appendigaster* (Tables II and III). None of the remaining interactions were significantly different.

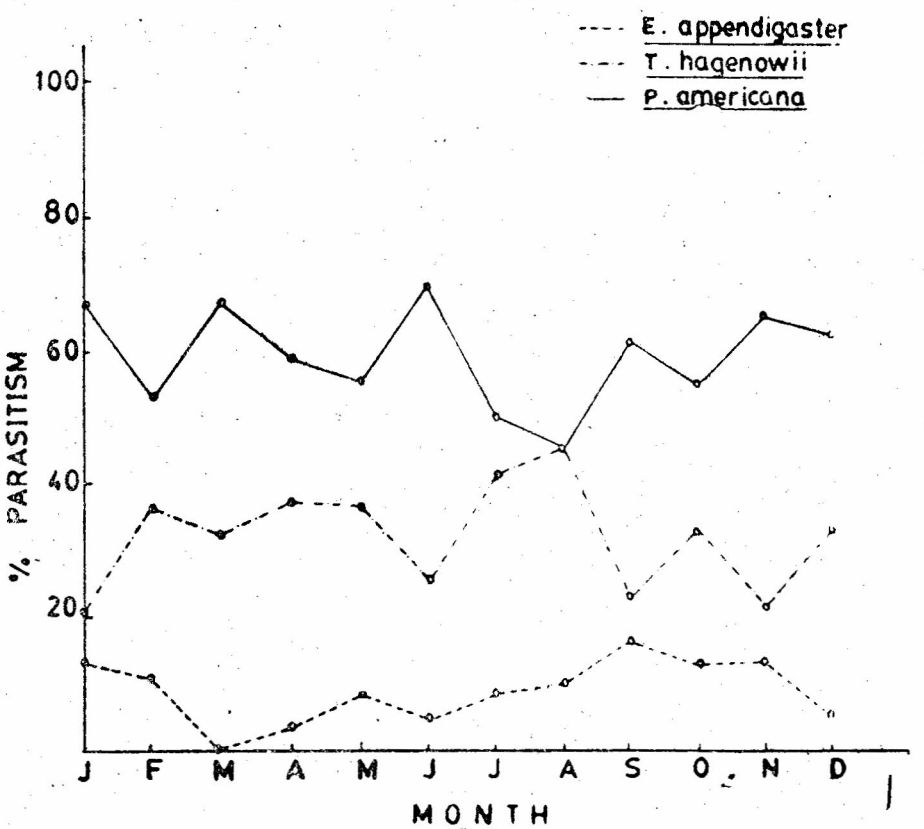


Fig: 1 Variation in monthly levels of Parasitism of *P. americana* obtained by sampling live oöthecae at Aluthgama.

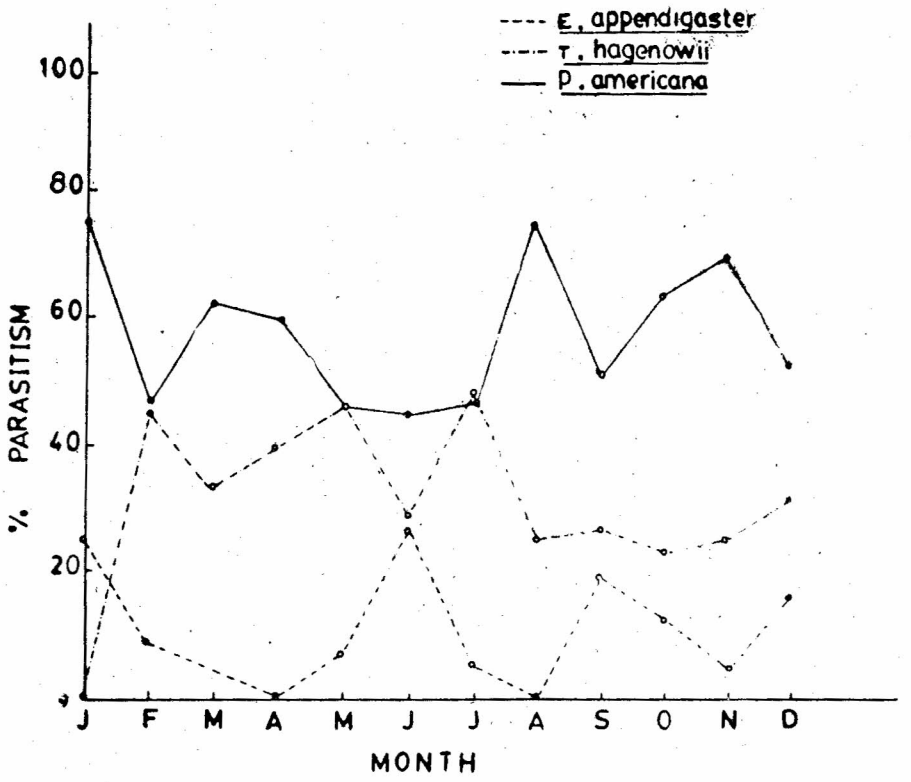


Fig: 2 Variation in monthly levels of parasitism of *P. americana* obtained by sampling live oöthecae at Pita Kotte.

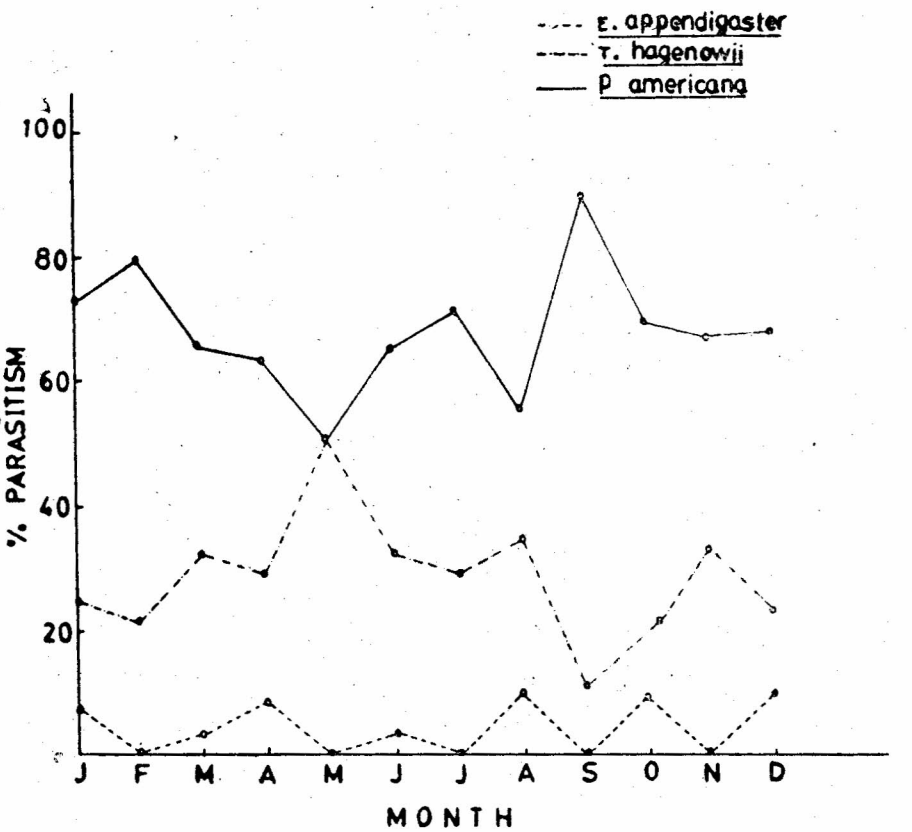


Fig: 3 Variation in monthly levels of parasitism of *P. americana* obtained by sampling live oöthecae at Gangodawila.

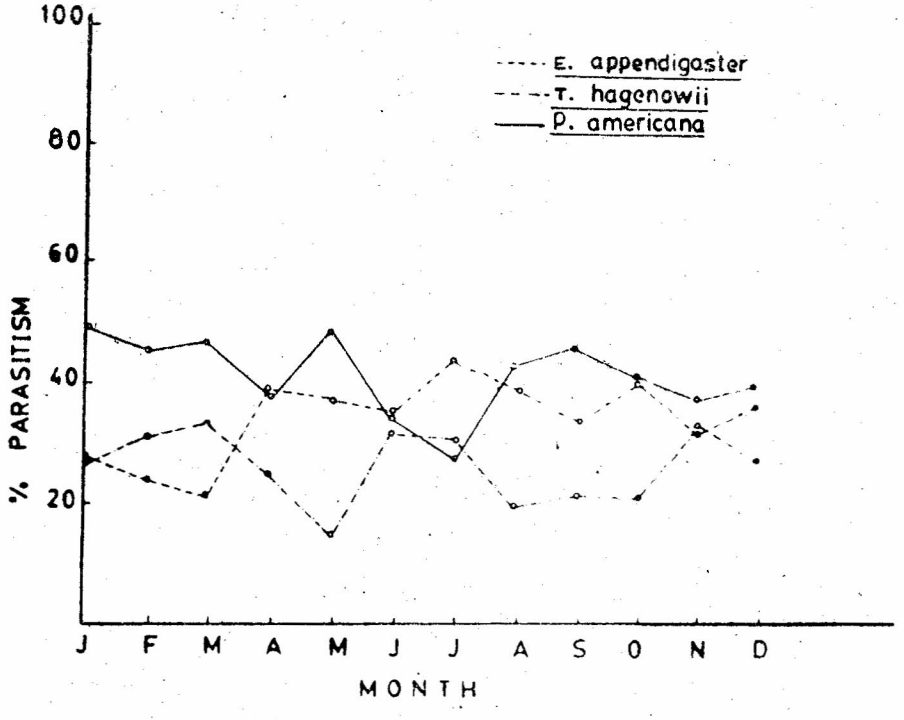


Fig: 4- Variation in monthly levels of parasitism of *P. americana* obtained by sampling empty oöthecae at Aluthgama.

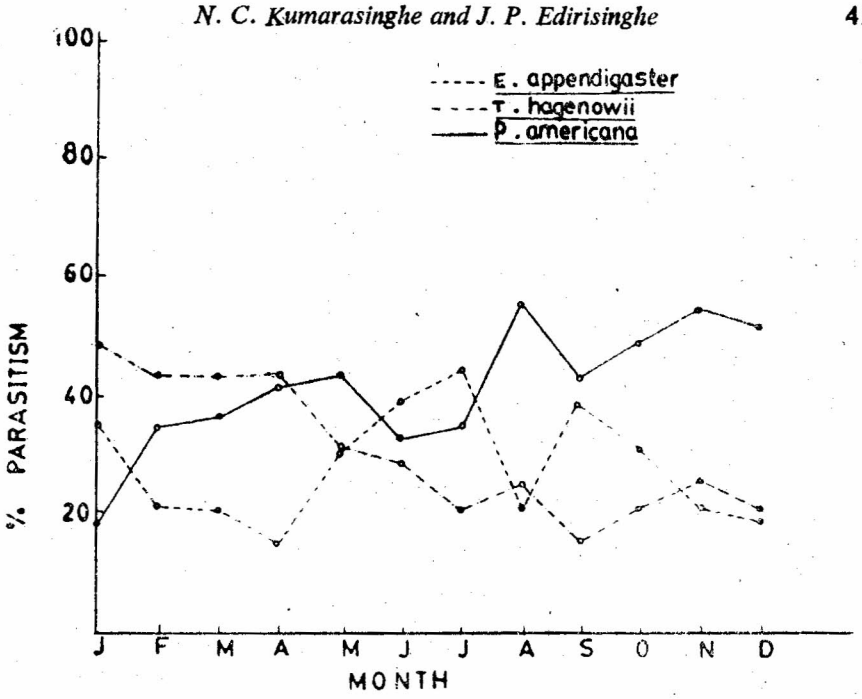


Fig: 5 Variation in monthly levels of parasitism of *P. americana* obtained by sampling empty oöthecae at Pita Kotte.

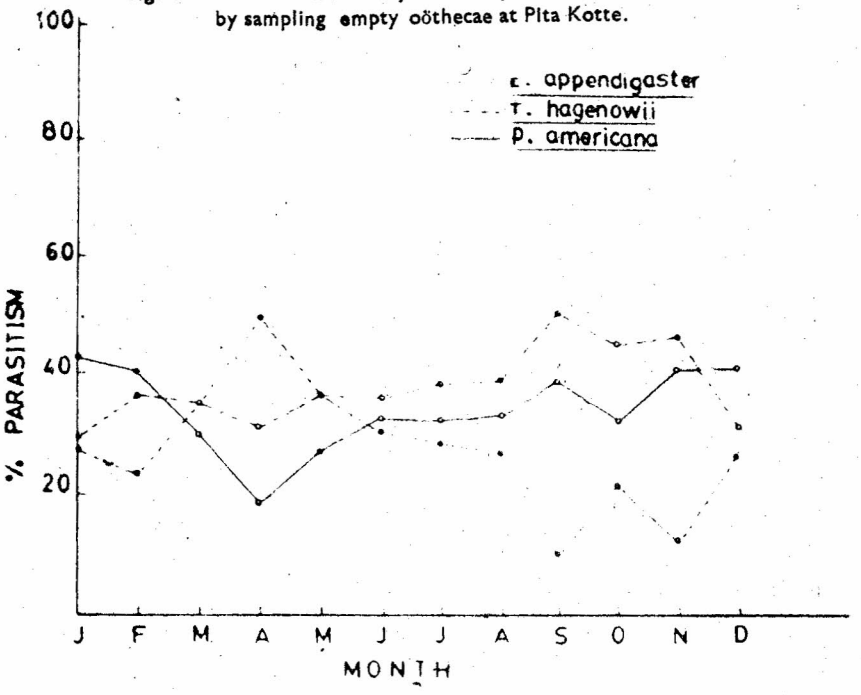


Fig: 6 Variation in monthly levels of parasitism of *P. americana* obtained by sampling empty oöthecae at Gangodawila.

**Table II**

Analysis of variance of percent parasitism by *E. appendigaster* obtained using a different method of sampling each year.

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| Methods (year)(M) | 1 | 4.44 | 4.44 | 119.80* |
| Month (T) | 11 | 0.35 | 0.03 | 0.88 |
| Region (R) | 2 | 0.28 | 0.14 | 3.86 |
| M x T | 11 | 0.30 | 0.03 | 0.75 |
| M x R | 2 | 0.06 | 0.03 | 0.87 |
| T x R | 22 | 1.02 | 0.05 | 1.25 |
| M x T x R | 22 | 0.29 | 0.01 | 0.36 |
| Error | 72 | 2.67 | 0.04 | — |
| Total | 143 | 8.41 | 4.77 | |

$F^{1}_{72,\ .05} = 3.98$

*significantly different.

**Table III**

Analysis of variance of peroent parasitism by *T. hagenowii* obtained using a different method of sampling each year.

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| Method (year)(M) | 1 | 0.04 | 0.04 | 2.44 |
| Months (T) | 11 | 0.59 | 0.05 | 2.90 |
| Regions (R) | 2 | 0.07 | 0.03 | 1.99 |
| M x T | 11 | 0.55 | 0.05 | 2.71 |
| M x R | 2 | 0.24 | 0.12 | 6.47 |
| T x R | 22 | 0.40 | 0.02 | 0.96 |
| M x T x R | 22 | 0.64 | 0.03 | 1.58 |
| Error | 72 | 1.33 | 0.02 | |
| Total | 143 | 2.86 | 0.36 | |

When sampling was carried out with the first and second methods concurrently at each of the four separate sites, mean parasitism levels of 7.67± 2.8% and 23.1 ± 1.5% respectively were obtained for *E. appendigaster*. Also zero levels of parasitism by *E. appendgaster* was recorded from all four sites with the first method. Analysis of the monthly levels of parasitism obtained using the two methods concurrently. (Table IV and V) indicated a significant difference between the methods of sampling for both parasites. In the case of *E. appendigaster*, monthly levels of parasitism, as well as the interactions between Methods x Months, showed a significant difference (Table IV).

**Table IV**

Analysis of variance of percent parasitism by *E. appendigaster* obtained concurrently using a different sampling method at four of the eight sites.

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| Method (M) | 1 | 0.83 | 0.83 | 83* |
| Months (T) | 4 | 0.17 | 0.04 | 4* |
| Sites (S) | 3 | 0.05 | 0.02 | 2 |
| M x T | 4 | 0.14 | 0.04 | 4* |
| M x S | 3 | 0.04 | 0.01 | 1 |
| T x S | 12 | 0.17 | 0.01 | |
| Error | 12 | 0.18 | 0.01 | |

$F^1_{12,\ .05} = 4.75$ $F^4_{12,\ .05} = 3.26$
*significantly different.

**Table V**

Analysis of variance of percent parasitism by *T. hagenowii* obtained concurrently using a different sampling method at four of the eight sites.

| SV | DF | SS | MS | F |
|---|---|---|---|---|
| Method (M) | 1 | 0.08 | 0.08 | 29.33* |
| Month (T) | 4 | 0.32 | 0.08 | 2.67 |
| Sites (S) | 3 | 0.08 | 0.03 | 1.00 |
| M x T | 4 | 0.02 | 0.005 | 0.16 |
| M x S | 3 | 0.05 | 0.02 | 0.67 |
| T x S | 12 | 0.33 | 0.03 | 1.00 |
| Error | 12 | 0.33 | 0.03 | |
| Total | 39 | 1.21 | 0.75 | |

$F^1_{12,\ .05} = 4.75$ $F^4_{12,\ .05} = 3.26$
*significantly different.

## 4. Discussion

A marked difference in the natural level of parasitism of the two oothecal parasites was seen with the two methods of sampling. Since the two sampling methods were initially used at the same sites but in two different years, the observed differences in the level of parasitism using the two methods could be attributed to the monthly/annual fluctuations in the population levels of *E. appendigaster* and *T. hagenowii*. However, the analysis of variance tables II and III ruled out this possibility. Furthermore, for *E. appendigaster*, but not for *T. hagenowii*, significantly different levels were obtained when the two sampling methods were employed *concurrently* and this further rules out the possibility of fluctuations in parasite populations (Tables IV & V). The only other reason for this difference in the natural parasitism level recorded would be the difference in the two sampling techniques employed in the two years. The method employed in the first year was equivalent to sampling without replacement as in this method a quarter of the total number of live oothecae found at any site was removed. The method employed in the following year was equivalent to sampling with replacement, as this method was based on empty oothecae. The removal of empty oothecae would not affect the parasite population in any way unlike the removal of live oothecae. This difference in the natural level of parasitism with the two sampling methods was reflected mostly in case of *E. appendigaster*.

The differential response of the two parasites to the sampling method with no replacement could be attributed to their innate capacity for increase which is a reflection of their fecundity. *E. appendigaster* (Kumarasinghe, 1984) is a parasite with a significantly low fecundity of $11 \pm 3.0$ compared to *T. hagenowii* (Gamalath, 1980)which has a fecundity of $64 \pm 10.1$. The higher fecundity of *T. hagenowii* would permit this species to increase at a much faster rate thereby replacing any losses to its population, unlike in the case of *E. appendigaster*.

Moreover, a single ootheca parasitized by *T. hagenowii* would yield about 40-60 adults of both sexes. On the contrary, a parasitized ootheca of *E. appendigaster* would result in only a single adult of either sex (Cameron, 1955; Kumarasinghe 1984) Thus, any parasitized live ootheca of *T. hagenowii* left at the site following sampling(by the removal of live oothecae as in the first method) would more than compensate for losses incurred to its population due to sampling unlike in *E. appendigaster*.

From the study it is evident that employment of a sampling method without replacement has detrimental effects particularly on the population of *E. appendigaster*. Hence, it is important to study the impact of a sampling method on a population prior to its use in the assessment of that population.

**Acknowledgements**

This work was funded by a grant from the Natural Resources, Energy and Science Authority of Sri Lanka. We are grateful to Professor Winston E. Ratnayake, Head, Department of Zoology, University of Sri Jayawardenepura, for providing facilities for the above work. The authors are also thankful to Professor A. C. J. Weerakoon, formerly of the Department of Biological Sciences, University of Sri Jayawardenapura, Sri Lanka, for his helpful comments on the manuscript. Dr. A. D. Austin of the C.I.E. London is acknowledged for identifying the parasites.

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