

## Breeding biology of the cavernicolous harvestman *Goniosoma albiscriptum* (Arachnida, Opiliones, Laniatores): sites of oviposition, egg batches characteristics and subsocial behaviour

RODRIGO H. WILLEMART and PEDRO GNASPINI\*

*Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo,  
Caixa Postal 11461, 05422-970, São Paulo, SP, Brazil  
email: gnaspini@ib.usp.br*

Received 11 June 2002; Accepted 28 February 2003

### Summary

We present field data on seasonality, mating behaviour, sites of oviposition, characteristics of egg batches, and parental care of a cavernicolous population of the Brazilian harvestman *Goniosoma albiscriptum*. We also tested if guarding females became more aggressive than non-guarding ones by allowing a potential predator to approach *G. albiscriptum* females. Field work was conducted in several visits to a cave, from September 1999 to April 2001. Reproductive activity was detected almost the whole year, with a minor peak in the spring (October–November) and a major peak in the summer (February–March). Average sex ratio throughout the year was female biased. Proportionally, guarding females were found significantly more often in exposed places than females without eggs. No significant difference was detected between the number of eggs laid in exposed and non-exposed places. The mean number of eggs per batch was  $69.58 \pm 26.96$ , and the average minimum time of embryonic development was  $33.26 \pm 9.78$  days. Twenty-nine females produced two egg batches, with no significant difference between the number of eggs laid in the first and second batch. Intervals between the two ovipositions ranged from 3 to 17 months. Laboratory tests showed that conspecifics and other cave inhabitants, such as other harvestmen species and crickets, preyed on *G. albiscriptum* eggs. Guarding females reacted aggressively to a non-conspecific approaching harvestman. Non-guarding females never reacted aggressively. Con-specific males near females during the day attacked conspecifics males but not males of another species. Once, a male was seen standing next to eggs and immatures, possibly guarding them.

**Key words:** Behaviour, egg-guarding, Gonyleptidae, offspring defence, parental care

### Introduction

Subsocial behaviour is defined as postovipositional parental care that promotes the survival, growth and development of offspring (see Tallamy and Wood,

1986). Offspring-guarding, one of the forms of subsocial behaviour (see Filippi et al., 2001), is known to occur in several taxa and has been shown to increase the survival of offspring (Clutton-Brock, 1991).

\*Corresponding author.

Several studies have demonstrated that parents behave aggressively towards intruders in the presence of eggs/immatures (for instance, arthropods; e.g., Saito, 1986; Kight, 1997; fishes, e.g., Ongarato and Snucins, 1992; Magnhagen and Vestergaard, 1993; birds, e.g., Wingfield and Lewis, 1993; Sjöberg 1994). Few studies have been done, however, to detect if parents become more aggressive in the presence of their offspring when compared to when their offspring are not present (but see Ayer and Whitsett, 1980; Wolff 1985; Koskela et al. 1997). In some predator presentation studies, it is not always possible to know if parents were protecting their immatures (and/or eggs) or themselves (see Saito, 1986). In harvestmen (Arachnida, Opiliones), although females and sometimes males remain close to their eggs (see Machado and Raimundo, 2001, and cited references), only two studies provided evidence that survivorship of offspring decreases in the absence of the parents (namely, Mora, 1990, and Machado and Oliveira, 1998). However, until now, no studies have been done to compare behaviour of parents towards intruders in the absence/presence of offspring.

Opiliones are represented by 4500–5000 species (Adis and Harvey, 2000). Specimens of the suborder Laniatores are abundant in the tropics, including species very diverse in form, size, colour, and behaviour. They are typically nocturnal, inhabiting moist forests or caves, although some are diurnal (Hoenen and Gnaspini, 1999) and others live in deserts (MacKay et al., 1992). Some laniatores species reproduce throughout the year (Juberthie, 1972; Matthiesen, 1975; Gnaspini, 1995; Machado and Oliveira, 1998), while others reproduce only once a year (Juberthie and Muñoz Cuevas, 1971). Although a few species display courtship behaviour (e.g., Mora 1990), the great majority of the studied species do not have any elaborate behaviour previous to copulation. Eggs are laid in groups or isolated (Machado and Raimundo, 2001). In several species, the female may cover the eggs with debris (Willemart, 2001) or stay close to the eggs (Machado and Raimundo, 2001). Paternal care has been reported in some laniatores species, and no case of strict biparental care is known, although Machado and Oliveira (1998) showed that males can take the place of females removed from egg batches (see Machado and Raimundo 2001; Hara et al., 2003).

The most well known group of the Laniatores, in terms of behaviour, is the subfamily Goniotosomatinae. Pinto-da-Rocha (1993) first reported the occurrence of maternal care in this group, based on observations of *Goniotosoma* aff. *baulum* C.L. Koch, 1839. Ramirez and Giaretta (1994) reported maternal care in *Acutisoma proximum* (Mello-Leiño, 1922). Gnaspini studied several aspects of the natural history of *G. spelaeum*

(Mello-Leiño, 1933), including reproduction, ecology and defence (Gnaspini, 1995, 1996, 1998, with Cavalheiro). These same aspects of the natural history were also studied in *G. longipes* (Roever, 1913) by Machado and Oliveira (1998) and Machado et al. (2000). Data on food competition in *Heteromitobates discolor* (Soerensen, 1884) were provided by Willemart (2001).

The present paper reports on the breeding biology of a cavernicolous population of *G. albiscrriptum* Mello-Leiño, 1932, mistakenly identified as *G. proximum* in Gnaspini and Trajano (1994). We present field data on seasonality, mating behaviour, sites of oviposition, characteristics of egg batches and parental care. With previous knowledge that all breeding females of *G. albiscrriptum* guard their eggs, we also tested if guarding females are more aggressive than non-guarding ones. The potential egg predators in the cave are also presented.

## Material and Methods

### Study area

The harvestmen were studied in the Quarta Divisão cave (23°38' S, 46°22' W), in Ribeirão Pires, state of São Paulo, southeastern Brazil (see Gnaspini and Trajano, 1994). Tropical rainforest surrounds this granitic cave, which is formed by a conduit of 130 m, crossed by a stream (~60 cm wide, <10 cm deep). Since the cave has many entrances, it has several regions that remain lighted during the day, although there are also some constantly shaded places. To allow a better understanding of the harvestmen distribution in the cave, we divided the cave into exposed and non-exposed places. A harvestman was considered to be on an exposed place when it was standing on a cave wall that faced an illuminated and open area (epigeal environment) or opening at the ceiling in the hypogean environment). Harvestmen in sites that did not fit this definition were considered to be in non-exposed places.

### Field work and marking methods

From December 1999 to March 2001, the cave was visited one day each month for the mark-recapture study. Additionally, from October 1999 to April 2001, the cave was visited on 10 more occasions (herein called intermediate visits) in between the marking visits, in order to make behavioural observations. Behavioural observations were opportunistic, making use of head-lamps covered with red filters (it is well

accepted among entomologists and arachnologists that red light does not affect the behaviour of most of these arthropods). Both in the monthly and in the intermediate visits, harvestmen behaviour was observed during periods of day and night.

In the monthly visits to the cave, we measured the temperature and relative humidity of the epigeal environment and in ten locations inside the cave at 14:30 h, using a portable whirling thermo-hygrometer. All adult females and adult/subadult males (which can be distinguished from each other by the degree of armature, colour and size; see Gnaspini, 1995) found in the cave, were captured, marked and then released in the exact place they were found. The location and sex of each individual were noted. To mark the harvestmen, we used enamel paint on the dorsum, femur and tibia of legs III and IV. Each animal had an individual mark (left legs and dorsum), which allowed individual recognition and a "first capture" mark (right legs), which corresponded to the month that the animal was captured for the first time (modified from Gnaspini, 1996).

Voucher specimens are deposited in the Museu de Zoologia da Universidade de São Paulo, São Paulo (MZSP).

### Egg batch location and characteristics

During each monthly visit, the egg batches were quantified in order to detect a possible seasonal variation in reproductive effort. The eggs of batches seen for the first time were counted (only for those in which no eggs had hatched). A manual calliper was used to measure egg batches (maximum length and width) and the size of the eggs (we measured three eggs per batch). The location of each egg batch and the individual mark of guarding females were also noted. To allow better localisation of the individuals and of the eggs, and as there was no intention of observing behaviour, searching was done with head lamps not provided with red filters.

### Test of female behavioural response to potential egg predators

To detect which cave animals could be potential egg predators, conspecifics and individuals of other species were taken to our laboratory and left with eggs of *G. albiscrriptum*. After 3 days, the eggs were checked to see if they were eaten or not.

To test if females become more aggressive when guarding eggs, the following test was performed in the field: an adult male of the harvestman *Promitobates* sp. (Gonyleptidae, Mitobatinae) was held by legs IV, and

was allowed to walk towards an egg batch guarded by a *G. albiscrriptum* female. To allow comparison, the approach of the newcomer was made in a way that it always faced the female of *G. albiscrriptum*, and the experiments were all conducted during the day. The first reaction of the female was noted. For a control, the same procedure was repeated with non-guarding females. After the first reaction, for non-guarding females, *Promitobates* sp. was still held until the female left the place; for guarding females, *Promitobates* sp. was held for 10 min more close to the eggs because the guarding female could return to the batch after leaving it. *Promitobates* sp. was chosen because it was common in the cave and it has been suggested that the parent's response must be interpreted with the use of natural predators to which they would normally respond (Montgomerie and Weatherhead, 1988). The potential predator was not a conspecific to exclude a sexual component (see Sandell and Smith, 1997). *Promitobates* sp. was used in all tests because it is known that parents may react differently to different predators (Winkler, 1992, and cited references).

### Statistical analysis

Analyses were conducted with SIGMASTAT statistical software version 2.0. This software tests for homoscedasticity and normality before analysing the data with parametric tests. The *P* value used to reject homoscedasticity and normality, and to conclude that there is a significant difference or correlation between groups, was 0.05. The tests used in each case are indicated in the text. Results are shown as mean  $\pm$  standard deviation when appropriate.

## Results

### Meteorological features and reproductive seasonality

Temperature and humidity variation throughout the year is presented in Figs. 1 and 2. Most of these meteorological parameters were not significantly correlated with the number of egg batches found in the monthly trips (Spearman Rank Order correlation: epigeal temperature:  $r_s = 0.472$ ;  $P = 0.117$ ;  $n = 12$ ; epigeal humidity:  $r_s = 0.300$ ;  $P = 0.329$ ;  $n = 12$ ; hypogean humidity:  $r_s = 0.700$ ;  $P = 0.124$ ;  $n = 12$ ). However, there was a significant correlation when hypogean temperature is concerned ( $r_s = 0.651$ ;  $P = 0.020$ ;  $n = 12$ ).

Eggs could be detected 3/4 of the year, with a minor peak in the spring (October–November) and a major peak in the summer (February–March). Eggs were not observed at the end of autumn or in the winter

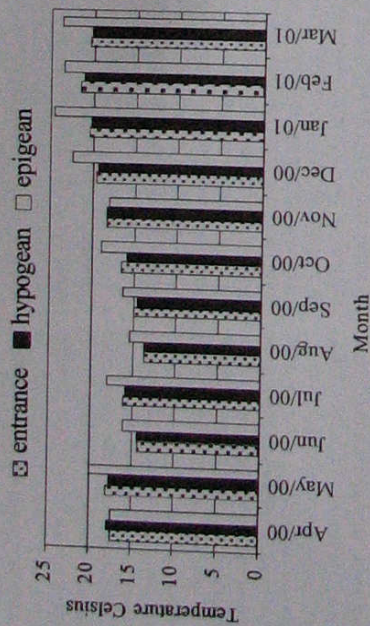


Fig. 1. Temperature variation in the epigeal environment, the main entrance of the study cave and the hypogean environment of the Gruta da Quarta Divisão, Ribeirão Pires, SP, Brazil.

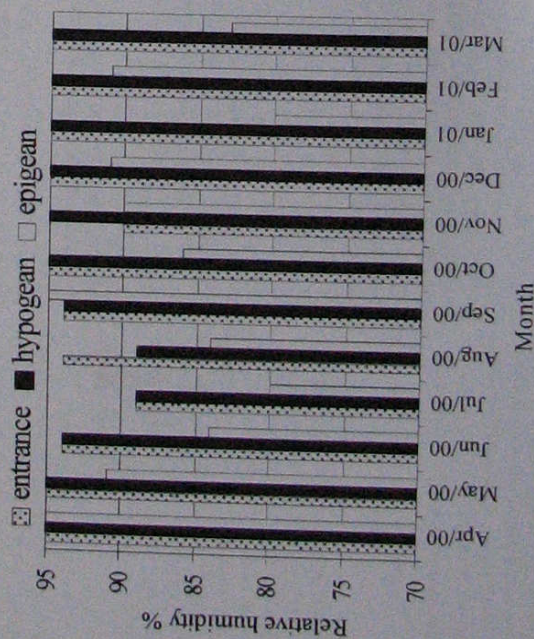


Fig. 2. Relative humidity variation in the epigeal environment, the main entrance of the cave and the hypogean environment.

(Fig. 3). The number of egg batches found each month was not significantly correlated with the average number of eggs per egg batch in the corresponding month (Pearson correlation:  $r = 0.501$ ;  $P = 0.169$ ;  $n = 9$ ).

#### Sex-ratio

The average observed adult sex ratio throughout the year was female biased (1:1.42), with one exception

#### Copulatory behaviour

Copulation was observed on four occasions and lasted only for a few seconds because the animals stopped copulation immediately after they were lit with our headlamps without red filters. As copulation had already begun in all observations, it was not possible to

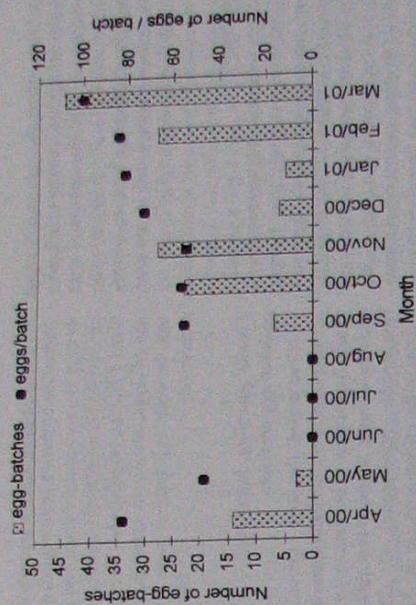


Fig. 3. Reproductive seasonality and number of eggs per batch of *Goniosoma albiscrriptum* in the study cave.

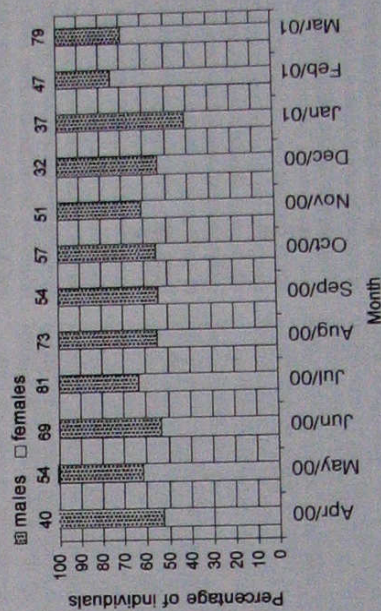


Fig. 4. Sex-ratio of the population of *Goniosoma albiscrriptum* inhabiting the study cave. The numbers above the bars correspond to the total of individuals observed each month.

detect if there was any pre-copulatory courtship. The position adopted was face-to-face with the anterior portion of the body raised, male and female in the same plane. In these four cases, the females were already guarding egg batches. In one case, we interrupted a mating of two marked individuals at 19:45 h. When we came back at 23:00 h, the same couple was copulating again.

#### Location and characteristics of egg batches

Egg batches were found only on the cave walls, either in vertical, oblique or horizontal places, and

never on the floor. Guarding and non-guarding females were found either on exposed (guarding;  $n = 38$ ; non-guarding;  $n = 12$ ) or non-exposed places (guarding;  $n = 84$ ; non-guarding;  $n = 154$ ). Proportionally, the guarding females were found significantly more often in exposed places than females without eggs ( $\chi^2 = 26.398$ ;  $d.f. = 1$ ;  $P < 0.001$ ). No significant difference was detected between the number of eggs/batch laid in exposed ( $n = 24$  batches) and non-exposed ( $n = 71$  batches) places ( $t$ -test:  $t = 0.896$ ;  $d.f. = 93$ ;  $P = 0.373$ ) (see Appendix).

The mean number of eggs per batch was  $69.58 \pm 26.96$  (range = 4–145;  $n = 97$ ). Although the density of

eggs in an egg batch varied, the result of "longest length × larger width" of egg batches was significantly and positively correlated with the number of eggs (Pearson correlation:  $r = 0.673$ ;  $P < 0.001$ ;  $n = 45$ ). There was no significant difference between the area occupied by the eggs in exposed and in protected places ( $t$ -test:  $t = 1.881$ ; d.f. = 41;  $P = 0.067$ ).

#### Embryonic development

Recently laid eggs were uniformly cream coloured and averaged  $1.52 \pm 0.18$  mm in diameter ( $n = 30$ ). Eggs about to hatch were darker, with irregularly distributed dark brown spots, and averaged  $1.97 \pm 0.09$  mm in diameter ( $n = 30$ ). The latter were significantly larger than the recently laid eggs (paired  $t$ -test,  $t = 14.52$ ; d.f. = 28;  $P < 0.001$ ). Hatching was not simultaneous for all eggs. The minimum average time of embryonic development necessary for egg-hatching that could be detected was  $33.26 \pm 9.78$  days (range = 24–60 days;  $n = 27$ ). From eggs hatched larvae, which can be distinguished from first instar immatures by their short and crooked legs and their apparent difficulty in walking. Larvae were sometimes seen among the eggs/first instar immatures. Larval stage duration could be calculated when one hatching egg was seen. Checking this at irregular intervals allowed us to know the minimum (21 min) and maximum (41 min) larval stage duration. Afterwards, larvae moulted again to become first instar immature, which remained aggressive with the rest of the offspring, next to the guarding female.

#### Females that produced more than one egg batch

Complete data are presented in the Appendix to allow future analysis. Twenty-nine females produced two egg batches (see Appendix). There was no significant difference between the number of eggs laid in the first and second egg batch detected (paired  $t$ -test:  $t = 0.41$ ; d.f. = 17;  $P = 0.686$ ). Among 20 females that laid the first egg batch in a non-exposed place, 17 also laid the second egg batch in a non-exposed place. Among nine females that laid the first egg batch in an exposed place, six also laid the second egg batch in an exposed place.

The intervals (from the first time each egg batch was detected) between the two ovipositions ranged from 3 to 17 months (see Appendix). There was no significant correlation between the numeric difference of eggs of the second egg batch and the number of eggs of the first egg batch in relation to the number of days between the two ovipositions (Pearson correlation:  $r = -0.121$ ;  $P = 0.611$ ;  $n = 20$ ).

Two females produced three egg batches during the study. One of them laid 88 eggs in an exposed place, 44 eggs in a non-exposed place, and finally 69 eggs in an exposed place (intervals between egg batches were 7 and 4 months, respectively). The other laid 114, 56, and 66 eggs, always in non-exposed places (intervals between egg batches were 4 and 5 months, respectively).

#### Female guarding behaviour — reaction against potential egg-predators

All arthropods that were captured in the cave and left with eggs in the laboratory, namely the male and female harvestmen *Pronitobates* sp. and *Discocyrtoides areolatus* B. Soares 1944 (Opiliones, Lamiales, Gonyleptidae, Mitobatinae), *G. albiscrriptum*, and females of the cricket *Strimitia* sp. (Ensifera, Phalangopsidae) consumed the eggs. Therefore, all of them are potential egg predators.

After the tests of approaching a potential egg predator (*Pronitobates* sp.) in the field were conducted, the following behavioural categories of female behaviour towards the newcomer were recognised: (1) non-aggressive behaviour (always with palps flexed), which includes fleeing slowly, fleeing quickly remaining motionless and discreetly moving the body; (2) aggressive behaviour (always with palps extended), which includes body raising, and displacement towards the newcomer (see Table 1).

The extension of the palps by the female was considered to be an aggressive behaviour because (1) harvestmen extend the palps to bite aggressors or preys, and (2) in the tests done in this study, the extension of the palps was always followed by biting. As shown in Table 1, guarding females reacted aggressively significantly more often than non-guarding females (Fisher exact test,  $P < 0.001$ ). In fact, non-guarding females never reacted aggressively while guarding females reacted aggressively in more than 90% of the encounters.

In the 10 min following the first reaction, the non-guarding females, which had remained in the same place, always fled after some seconds. The guarding females continued to attack the newcomer. They bit with their chelicerae, and with the palps grasped different regions of the newcomer's body, such as the first and second pair of legs (holding it for up to 45 s) and the dorsal part of its body. Previously and during the fight, while biting, females tapped the newcomer's body with legs I and II. Occasionally, females stopped the attack, retreated, and after some minutes returned and attacked the newcomer again. No exudation of the scent glands nor of the mouth, which are com-

Table 1. Reaction of *Goniosoma albiscrriptum* females to the approach of a potential egg predator, a *Pronitobates* sp. male

	Guarding females (n = 30)	Non-guarding females (n = 20)
Non-aggressive (palps flexed) – total	2	20
Fleeing slowly (1a)	0	3
Fleeing quickly (1b)	0	4
Immobility (1c)	0	6
Discreet movement (1d)	2	7
Aggressive (palps extended) – total	28	0
Body raising (2a)	11	0
Displacement towards newcomer (2b)	17	0

mon behaviours in goniosomatine harvestmen when disturbed (see Gnaspini and Cavalheiro, 1998), was observed.

In addition to the tests made, some natural observations corroborate the above results. In the field, a wandering female was once observed passing next to a guarding female. The latter extended palps towards the newcomer, which did not react and continued to walk, moving further from the egg-guarding female, which returned to her initial position. In a second observation, an egg-guarding female attacked an approaching female, which ran back 15 cm and stopped. The guarding female returned to its initial position.

Aggressive behaviour of egg-guarding females towards males was observed twice. Both males retreated. It is not possible to determine if this aggressive behaviour should be interpreted as egg defence against predation or non-acceptance of copulation. It should be noted that egg-guarding females might accept approaching males and copulate, as previously mentioned.

Finally, guarding females were never seen leaving the cave to forage, and batches were never seen alone.

#### Potential male guarding behaviour

Among 120 guarding females observed during the day, on ten occasions (8.3%) there was an active male nearby. When a male *Pronitobates* sp. was held and approached towards the eggs, passing next to a *G. albiscrriptum* male, the male touched the newcomer with legs II and stood still, allowing the newcomer to approach the egg-guarding female, which reacted aggressively, as described above ( $n = 4$ ). When a conspecific male was approached towards the eggs, passing next to the guarding male, the latter attacked

the newcomer, grasping the newcomer legs with his palps and biting with his chelicerae ( $n = 3$ ). In one of these cases, the newcomer was released and the two males fought for no more than 2 s with their legs interlocked. They fell down from the cave wall and ran away from each other on the ground. In another case, the guarding male (a subadult) fled from the newcomer, but the latter was larger than the guarding male. However, we also observed during the day a male attacking a larger male, which fled. There were neither males nor females nearby.

On March 4th, 2001, at ~17:00 h, a marked male was observed close to four eggs in their last developmental phase. Several first instar immatures were near the male, but not aggregated as they typically behave when guarded by a female. After several photographs with a flash had been taken, the male abandoned the eggs by walking slowly away. After 1 h the place was checked and the male was back, and now the immatures were aggregated close to the male and the eggs. There were neither conspecifics nor other animals observed the male. This male had been previously observed alone, on April 30, 2000, June 03, 2000, and June 17, 2000, in the same region of the cave.

#### Discussion

##### Reproductive seasonality

Reproductive seasonality of *G. albiscrriptum* differed from that reported for *G. spelaeum* (Gnaspini, 1995) but is similar to that reported for *G. longipes* (Machado and Oliveira, 1998). Both *G. albiscrriptum* and *G. longipes* showed one peak at the end of spring and another one at the end of summer. Moreover, in both cases, few or no egg batches were found at the end of autumn/beginning of winter (May, June, July). Machado and Oliveira (1998) suggested that the largest peak in October would benefit the immatures because they would be hatching in January (rainfall peak), when arthropod prey are more abundant. This explanation would not apply to *G. albiscrriptum*, since it would fail to explain the larger number of ovipositions in March. The positive correlation between temperature inside the cave and reproductive activity may be related to ideal conditions for the embryonic development since temperature (Juberthie, 1972; Goodnight and Goodnight, 1976; Cokendolpher and Jones, 1991) is known to influence the duration of embryonic development. As we had no annual replication, the factors influencing seasonality in this species should be further explored.

It should also be noted that *G. albiscrriptum* and *G. longipes* are species that inhabit both epigeal and hypogean environments, and, in the latter case,

generally inside short caves. In turn, *G. spelaeum* should be considered a strict troglone (see Gnaschini, 1996), since it inhabits caves with large non-illuminated places. Therefore, different reproductive tactics might be explained by these ecological differences, but this remains to be tested.

#### Sex ratio

The biased sex ratio was apparently not related to meteorological variation. The sex ratio detected herein differed from the results obtained by Gnaschini (1995) for *G. spelaeum*, both concerning the average sex ratio (1:1 in *G. spelaeum*) and the variation throughout the year (see Fig. 4); *G. spelaeum* did not show a marked seasonality although the sex ratio seems to be female biased during winter.

#### Copulatory behaviour

The observed copulatory position was similar to the described in the literature for other species (Gnaschini, 1995 and references therein; Machado and Oliveira, 1998). We did not observe copulation in perpendicular planes, as described for the genus *Ischyropsalis* (Juberthie, 1964) and also observed in *G. spelaeum* (Gnaschini, 1995).

#### Location and characteristics of egg batches

As in all other cavernicolous populations of goniosomatines (see Pinto-da-Rocha, 1993; Gnaschini, 1995; Machado and Oliveira, 1998), *G. albiscriptum* eggs are laid on cave walls. However, *G. spelaeum* was reported to lay eggs on "protected regions, far inside the caves" (Gnaschini, 1995) and *G. longipes* to "oviposit in chambers protected from external wind and light" (Machado and Oliveira, 1998). In contrast, several but not all females of *G. albiscriptum* laid eggs in open and lighted areas. Knowing that, as mentioned above, temperature — but also humidity (Juberthie 1965; Edgar, 1971) — greatly influences the duration of embryonic development, and the eggs of *G. albiscriptum* must tolerate the climatic conditions of exposed areas, despite being in a cavernicolous environment. It should be stressed that even inside a cave there may be climatic variations to which organisms may be sensitive. Thus, areas that are more protected and/or close to rivers inside a cave may favour hydration of harvestmen eggs but also of fungi development (see Machado and Oliveira, 1998).

It was discussed above that the offspring of *G. albiscriptum* are capable of withstanding climatic conditions not typical of caves because females were found

guarding eggs in lighted and open areas of the cave. But why were guarding females found significantly more often in exposed places than females without eggs? A possible explanation is that this would be a way to avoid conspecific egg-predation, since males and non-guarding females of *G. albiscriptum* were rarely found in exposed areas of the cave but often found in non-exposed places. We should stress that our tests in the laboratory showed that male and female *G. albiscriptum* are indeed potential egg-predators. In addition, intra-specific predation was also reported for another goniosomatine species by Machado and Oliveira (1998).

Another important point is to test if there was an "individual preference" concerning the site of oviposition, i.e. females that laid their first batch in an exposed place would also lay their second egg batch in an exposed place, and females that laid their first batch in a non-exposed place would also lay the second batch in a non-exposed place. Our results (exposed places [9/29 × 6/9] — see results: six females among nine laid their second egg batches again in exposed places):  $\chi^2 = 0.89$ , d.f. = 1,  $P = 0.35$ ; non-exposed places [20/29 × 17/20]:  $\chi^2 = 2.31$ , d.f. = 1,  $P = 0.13$ ) showed, however, that there was no statistical "individual preference" for oviposition sites.

#### Embryonic development

The development of the eggs followed the pattern known for other harvestmen in which the eggs become larger and darker the closer they are about to hatch. The duration of embryonic development was similar to the recorded in *G. spelaeum* and *G. longipes* and longer than for other lamiate species (Willemart, 2001, and references therein). Egg size is very similar to that of *G. longipes* (see Machado and Oliveira, 1998) but smaller than that of *G. spelaeum* eggs (Gnaschini, 1995).

#### Females that produced more than one egg batch

From September 1999 to March 2001, 29 females produced two egg batches and two produced three egg batches (see Appendix). Among the other studied goniosomatines, no *G. spelaeum* female laid two egg batches (Gnaschini, 1995), and only six *G. longipes* females oviposited twice (Machado and Oliveira, 1998). The number of eggs of these two species, however, was higher (*G. longipes* lays twofold more eggs per batch than *G. albiscriptum*). Similarly to *G. albiscriptum*, the number of eggs laid by *G. longipes* females did not differ from the first to the second oviposition (Machado and Oliveira, 1998).

Another interesting fact about the females that produced two egg batches is the short interval between the two ovipositions of some females (see Appendix). The shortest intervals were 5 months ( $n = 5$ ), 4 months ( $n = 8$ ), and 3 months ( $n = 2$ ). These females spent about 2 months guarding their offspring, without eating. In other words, after having produced ca. 70 eggs, and passing through a ca. 60-day fasting period (ofspring guarding), they must have matured an additional set of ca. 70 eggs only some weeks or days later. It is thus possible that females increase their foraging activity to pass through the fasting period, as noted for *G. spelaeum* (Gnaschini, 1995). It is not known whether they have to copulate again to produce another batch.

#### Causes of egg mortality

Flatworms, ants, reduviid bugs, staphylinid beetle larvae, crickets and conspecifics have been reported to prey on harvestmen eggs (Capocasa and Brunotrezza, 1964; Juberthie and Muñoz-Cuevas, 1971; Mora, 1990; Gnaschini, 1995; Machado and Oliveira, 1998). The tests in our laboratory showed that, in addition to conspecifics and crickets, other harvestmen species should be added to the list of potential egg predators. This was expected because of the opportunistic feeding habits of harvestmen (Gnaschini, 1996; see also Sabino and Gnaschini, 1999; and Willemart, 2002, for food stealing).

Fungi are another source of egg mortality since females are not able to remove them, as observed by Machado and Oliveira (1998). Gnaschini (1996) and Machado et al. (2000) observed that the marsupial *Philander opossum* (Didelphidae) is an important predator of *Goniosoma* harvestmen, preying on up to ten harvestmen in one visit to a cave (see also Pellegatti-Franco and Gnaschini, 1996). Although we did not observe any case of egg or adult predation in our study, this marsupial was often seen in the cave at night. If egg-guarding females were killed, the eggs would be left unguarded. Machado and Oliveira (1998) provided evidence that unguarded batches of *G. longipes* are almost totally destroyed after 2 weeks (see also Gnaschini, 1995). Thus, although indirectly, *Philander opossum* may also increase egg mortality for cavernicolous *Goniosoma* populations.

#### Female guarding behaviour — reaction against potential egg-predators

Females of *G. albiscriptum* never reacted aggressively to newcomers when not guarding (Table 1). In contrast, 93.3% of the guarding females reacted aggressively. Two main conclusions can be

taken from these results: (1) it is not only the physical presence of the guarding female that repels predators — the females actively and aggressively protect their eggs; and (2) there is a change in female behaviour when guarding eggs, from non-aggressive to aggressive. Conclusion (1) was also found for birds (Anderson et al., 1980; Greig-Smith 1980; Regelmann and Curio, 1983; Bretzsch 1988; Wingfield and Lewis, 1993; Sjöberg 1994; Whitam and Leonard, 2000, and references therein); fishes (Keenleyside et al., 1985; Rudgway, 1987; Lavery and Keenleyside, 1990; Ongarato and Snucins, 1992; Maghagen and Vestergaard, 1993); and arthropods (Saito, 1986; Knight, 1997). Conclusion (2) was similar to that reported for some rodents in which lactating females responded more aggressively to intruders than non-lactating ones (Ayer and Whitsett, 1980; Wolff, 1985; Koskela et al., 1997). Attacking intruders is certainly costly and in some cases even risky (see below) and would probably not be justified if there were no important resource involved. In turn, when guarding eggs, the aggressive behaviour is justified by the possibility of growing and eventual mating success of the mother progeny (Clutton-Brock, 1991).

One of the costs of actively defending young from predators is the risk of physical injury or even death (Tallamy and Wood, 1986). It should be therefore stressed that our results are valid for a species of potential egg predator (*Promitobates* sp.) that does not threaten the much larger and well armed adult *G. albiscriptum* (see Montgomerie and Weatherhead, 1988). Therefore, *G. albiscriptum* females were not taking severe injuries when attacking individuals of *Promitobates* sp. This probably influenced the reaction of guarding females towards *Promitobates* sp., its physical characteristics probably assessed by the guarding females when they tapped the newcomer's body with legs I and II.

Two egg-guarding females did not react aggressively. It should be considered that defence can have different costs for different individuals and for the same individual at different times (Montgomerie and Weatherhead, 1988). As mentioned above, *G. albiscriptum* females apparently do not feed when guarding offspring. The amount of reserves a female has at the moment an egg-predator attack may thus influence the female reaction, and could be one possible explanation for the reason why these females did not react aggressively.

The use of scent gland secretions by harvestmen varies among species, and it is still not clear which situations lead to exudation of the compounds and what are the reasons for these differences (see Machado et al., 2000, and references therein). It is

therefore important to stress that the guarding females never released secretions — neither from their scent glands nor from the mouth — despite the stressful situation of defending the brood for up to ten consecutive minutes. In a completely different situation, but in the sole described case involving exudation in an interaction between two harvestmen, it was reported that *Neosadocus variabilis* (Mello-Leitão, 1935) (Gonyleptidae) released scent gland secretions when fighting for food against *H. discolor* (Willemart, 2002).

*G. albiscapitulum* guarding females were seen repelling conspecifics, and we expect them to use similar behaviour (tapping the newcomer with legs I and II, biting with chelicerae and palps) against the other cave inhabitants and potential egg predators.

#### Mate guarding and potential male guarding behaviour

It has been suggested that the largest part of aggression among conspecifics can be interpreted as competition (Wilson, 1975). Males were sometimes found next to guarding females; they did not react aggressively against approaching harvestmen of another species (potential egg-predators/not sexual competitors) but attacked conspecific approaching males (potential egg-predators/potential sexual competitors). It should be stressed that attacking a conspecific is probably more risky than attacking a male *Promitobates* sp. Goniostomatinae harvestmen have strong chelicerae, capable of causing severe damage in other harvestmen. Willemart (2002) reported that an adult male of *H. discolor* bit and amputated some legs of a male and female conspecific; droplets of hemolymph appeared in the amputated region of each damaged leg and these two animals died within a few days. The aggressor stayed alive. In our study, despite the strength and potential danger offered by conspecific newcomers, the guarding males readily attacked them. These results suggest that the males found close to egg-guarding females were guarding the female, and not guarding the eggs, which was done by the female. Most probably, these males were trying to copulate with the egg-guarding females and/or preventing other males from copulating, and for that reason did not allow the approach of competing conspecific males. In fact, all four copulations observed involved egg-guarding females. Gnaschini (1995) also observed *G. spelaeum* males close to egg-guarding females and copulating with egg-guarding females, and Machado and Oliveira (1998) observed that *G. longipes* males may stay for up to 2 months close to a group of up to five females with which they had

copulated. If one of these females were experimentally removed, in some cases the males stayed near the unguarded egg batch (Machado and Oliveira, 1998). In our study, a male was once seen close to the eggs and first instar immatures, and no female was observed in the surroundings. Although the mere presence of a male next to the offspring does not allow us to state that he was actually guarding them (see Tallamy, 2001), one comment on our observations must be made. After being disturbed, the male fled and the immatures dispersed, but the male returned to his initial place and the immatures aggregated next to him. This is exactly what happens if egg-guarding females are disturbed to the point that they leave the batch. Therefore, it seems that this male was actually guarding the offspring, as was previously recorded by Machado and Oliveira (1998).

#### Acknowledgements

We are deeply grateful to G. Machado and R. Macías-Ordóñez for several helpful suggestions on the manuscript. Two referees also provided helpful comments. We would also like to thank Mr. Almeida for allowing access to the cave, and all the friends that helped in the field, particularly M.C.C. da Inês and M.V. Nakamura. R. Pinto-da-Rocha and M.B. da Silva are thanked for identifying the harvestmen. A.B. Kury provided information about the identity of *Heteromitobates discolor*. This study was supported by a grant from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) #99/07943-9 to the senior author and partially by grant #00/04686-4 to the junior author. The junior author has a research fellowship from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) #300326/94-7.

#### References

- Adis, J. and Harvey, M.S., How many Arachnida and Myriapoda are there world-wide and in Amazonia? *Stud. Neotrop. Fauna Environm.*, 35 (2000) 139–141.
- Andersson, M., Wiklund, C.G. and Rundgren, H., Parental defence of offspring: a model and an example. *Anim. Behav.*, 28 (1980) 536–542.
- Ayer, M.L. and Whitsett, J.M., Aggressive behaviour of female prairie deer mice in laboratory populations. *Anim. Behav.*, 28 (1980) 763–771.
- Breitwiesch, R., Sex differences in defence of eggs and nestlings by northern mockingbirds, *Mimus polyglottos*. *Anim. Behav.*, 36 (1988) 62–72.
- Capoccase, R. and Bruno-Trezza, L., Biologia de *Acanthopachylus aculeatus* (Kirby, 1819) (Opiliones: Pachylinae). *Rev. Soc. Uruguaya Ent.*, 6 (1964) 19–32.

- Clutton-Brock, T.H., *The Evolution of Parental Care*. Princeton University Press, Princeton, 1991.
- Cokendolpher, J.C. and Jones, S.R., Karyotype and notes on the male reproductive system and natural history of the harvestman *Vonones sayi* (Simon) (Opiliones: Cosmetidae). *Proc. Entomol. Soc. Wash.*, 95 (1991) 86–91.
- Edgar, A.L., Studies on the biology and ecology of Michigan Phalangida (Opiliones). *Misc. Publ. Mus. Zool. Univ. Mich.*, 144 (1971) 1–64.
- Filippi, L., Hironaka, M. and Nomakuchi, S., A review of the ecological parameters and implications of subsociality in *Parasarchia japonensis* (Hemiptera: Cydnidae), a semelparous species that specializes on a poor resource. *Popul. Ecol.*, 43 (2001) 41–50.
- Giribet, G., Edgecombe, G.D., Wheeler, W.C. and Babitt, C., Phylogeny and systematic position of Opiliones: A combined analysis of chelicerate relationships using morphological and molecular data. *Cladistics*, 18 (2002) 5–70.
- Gnaschini, P., Reproduction and postembryonic development of *Goniosoma spelaeum*, a cavernicolous harvestman from southeastern Brazil (Arachnida: Opiliones: Gonyleptidae). *Invert. Repr. Develop.*, 28 (1995) 137–151.
- Gnaschini, P., Population ecology of *Goniosoma spelaeum*, a cavernicolous harvestman from south-eastern Brazil (Arachnida: Opiliones: Gonyleptidae). *J. Zool.*, 239 (1996) 417–435.
- Gnaschini, P. and Cavalheiro, J.A., Chemical and behavioral defenses of a Neotropical cavernicolous harvestman: *Goniosoma spelaeum* (Opiliones: Lamiales: Gonyleptidae). *J. Arachnol.*, 26 (1998) 81–90.
- Gnaschini, P. and Trajano, E., Brazilian cave invertebrates, with a checklist of troglomorphic taxa. *Rev. Bras. Entomol.*, 38 (1994) 549–584.
- Goodnight, M.R. and Goodnight, C.J., Observations on the systematics, development and habits of *Erginulus clavifimbriatus* (Opiliones, Cosmetidae). *Trans. Amer. Microsc. Soc.*, 95 (1976) 654–664.
- Greig-Smith, P.W., Parental investment in nest defence by stonechicks (*Saxicola torquata*). *Anim. Behav.*, 28 (1980) 604–619.
- Hara, M., Gnaschini, P. and Machado, G., Male egg guarding behavior in the neotropical harvestman *Ampheles leucopneus* (Mello-Leitão 1922) (Opiliones, Gonyleptidae). *J. Arachnol.*, 31 (2003) 441–444.
- Hoenen, S. and Gnaschini, P., Activity rhythms and behavioral characterization of two epigeic and one cavernicolous harvestmen (Arachnida, Opiliones, Gonyleptidae). *J. Arachnol.*, 27 (1999) 159–164.
- Juberthie, C., Recherches sur la biologie des Opiliones. *Annales de Spéologie*, Moulins, 19 (1964) 5–238.
- Juberthie, C., Données sur l'écologie, le développement et la reproduction des Opiliones. *Rev. Ecol. Biol. Sol.*, 2 (1965) 377–396.
- Juberthie, C., Reproduction et développement d'un opilion Cosmetidae, *Cynorta cubana* (Banks), de Cuba. *Ann. Spéol.*, 27 (1972) 773–785.
- Juberthie, C. and Muñoz-Cuevas, A., Sur la ponte de *Pachylus quitamavuitensis* (Opilion, Gonyleptidae) (Bull. Soc. Hist. Nat. Toulouse, 107 (1971) 468–474.
- Keenleyside, M.H.A., Rangeley, R.W. and Kuipers, B.U., Female mates choice and male parental difference behaviour in the cichlid fish *Cichlasoma nigrofasciatum*. *Can. J. Zool.*, 63 (1985) 2489–2493.
- Kight, S.L., Factors influencing maternal behaviour in a burrower bug, *Sehirus cinctus* (Heteroptera: Cydnidae). *Anim. Behav.*, 53 (1997) 105–112.
- Koskela, E., Mappes, T., and Ylonen, H., Territorial behaviour and reproductive success of bank vole *Clitellionomys glareolus* females. *J. Anim. Ecol.*, 66 (1997) 341–349.
- Lavery, R.J. and Keenleyside, M.H.A., Parental investment of a biparental cichlid fish, *Cichlasoma nigrofasciatum*, in relation to brood size and past investment. *Anim. Behav.*, 40 (1990) 1128–1137.
- Machado, G. and Oliveira, P.S., Reproductive biology of the neotropical harvestman (*Goniosoma longipes*) (Arachnida, Opiliones: Gonyleptidae): mating, oviposition behaviour, brood mortality, and parental care. *J. Zool.*, 246 (1998) 359–367.
- Machado, G. and Raimundo, R.L.G., Parental investment and the evolution of subsocial behaviour in harvestmen (Arachnida Opiliones). *Ethol. Ecol. Evol.*, 13 (2001) 133–150.
- Machado, G., Raimundo, R.L.G. and Oliveira, P.S., Daily activity schedule, gregariousness, and defensive behaviour in the Neotropical harvestman *Goniosoma longipes* (Opiliones: Gonyleptidae). *J. Nat. Hist.*, 34 (2000) 587–596.
- MacKay, W.P., Grimsley, C. and Cokendolpher, J.C., Seasonal changes in a population of desert harvestmen, *Trachyrhinus marmoratus* (Arachnida, Opiliones), from Western Texas. *Psyche*, 99 (1992) 207–213.
- Magnhagen, C. and Vestergaard, K., Brood size and offspring age affect risk-taking and aggression in nest-guarding common gobies. *Behaviour*, 125 (1993) 233–243.
- Martens, J., Hoheisel, U. and Götz, M., Vergleichende Anatomie der Legegrößen der Opiliones als Beitrag zur Phylogenie der Ordnung (Arachnida). *Zool. Jb. Anat.*, 105 (1981) 13–76.
- Mathiesen, F.A., Sobre a postura de *Discocyrtus peñi-nifemur* Mello-Leitão, 1937 (Opiliones, Gonyleptidae). *Ciênc. Cult.*, 27 (1975) suppl. 372.
- Montgomerie, R.D. and Weatherhead, P.J., Risk and rewards of nest defence by parent birds. *Q. Rev. Biol.*, 63 (1988) 167–187.
- Mora, G., Paternal care in a neotropical harvestman, *Zigopachylus albomarginatus* (Arachnida, Opiliones: Gonyleptidae). *Anim. Behav.*, 39 (1990) 582–593.
- Ongarato, R.J. and Snavets, E.J., Aggression of guarding male smallmouth bass (*Micropterus dolomieu*) towards potential brood predators near the nest. *Can. J. Zool.*, 71 (1992) 437–440.
- Pellegati-Franco, F.P. and Gnaschini, P., Use of caves by *Philander opossum* (Mammalia: Didelphidae) in Southeastern Brazil. *Pap. Avul. Zool.*, 39 (1996) 351–364.
- Pinto-da-Rocha, R., Invertebrados cavernícolas da porção meridional da Província Espeleológica do Vale do Ribeira, sul do Brasil. *Rev. Bras. Zool.*, 10 (1993) 229–255.

- Ramires, E.N. and Giaretta, A.A., Maternal care in a neotropical harvestman, *Acutisoma proximum* (Opiliones, Gonyleptidae). *J. Arachnol.*, 22 (1994) 179–180.
- Regelmann, K. and Curio, E., Determinants of brood defence in the great tit *Parus major* L. *Behav. Ecol. Sociobiol.*, 13 (1983) 131–145.
- Ridgway, M.S., Developmental stage of offspring and brood defense in smallmouth bass (*Micropterus dolomieu*). *Can. J. Zool.*, 66 (1988) 1722–1728.
- Sabino, J. and Gnaspini, P., Harvestman (Opiliones, Gonyleptidae) takes prey from a spider (Araneae, Ctenidae). *J. Arachnol.*, 27 (1999) 675–678.
- Saito, Y., Biparental defence in a spider mite (Acari: Tetranychidae) infesting *Saxa bamboo*. *Behav. Ecol. Sociobiol.*, 18 (1986) 377–386.
- Sandell, M.I. and Smith, H.G., Female aggression in the European starling during the breeding season. *Anim. Behav.*, 53 (1997) 13–23.
- Shultz, J.W., Phylogeny of Opiliones (Arachnida): an assessment of the "Cyphopalpatores" concept. *J. Arachnol.*, 26 (1998) 257–272.
- Sjöberg, G., Factors affecting nest defence in female Canada Geese *Branta canadensis*. *Ibis*, 136 (1994) 129–135.
- Tallamy, D.W., Evolution of exclusive paternal care in arthropods. *Ann. Rev. Entomol.*, 46 (2001) 139–165.
- Tallamy, D.W. and Wood, T.K., Convergence patterns in subsocial insects. *Ann. Rev. Entomol.*, 31 (1986) 369–390.
- Whitam, R.M. and Leonard, M.L., Characteristics of predators and offspring influence nest defence by arctic and common terns. *Condor*, 102 (2000) 301–306.
- Willemart, R.H., Egg covering behavior of the neotropical harvestman *Promitobates ornatus* (Opiliones, Gonyleptidae). *J. Arachnol.*, 28 (2001) 249–252.
- Willemart, R.H., Cases of intra- and inter-specific food competition among Brazilian harvestmen, in captivity (Opiliones, Lamiales, Gonyleptidae). *Rev. Arachnol.*, 14 (2002) 49–58.
- Wilson, E.O., *Sociobiology: The New Synthesis*. Belknap Press, Cambridge, MA, 1975.
- Wingfield, L.C. and Lewis, D.M., Hormonal and behavioural responses to simulated territorial intrusion in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Anim. Behav.*, 45 (1993) 1–11.
- Winkler, D.W., Causes and consequences of variation in parental defence behavior by tree swallows. *Condor*, 94 (1992) 502–520.
- Wolff, J.O., Maternal aggression as a deterrent to infanticide in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Anim. Behav.*, 33 (1985) 117–123.

**Appendix**  
Data on egg-guarding females of *Goniosoma albiscryptum* observed throughout the study. Females 18 and 19 laid a third egg batch (see text)

#	First egg batch			Second egg batch		
	Date	No of eggs	Non-exposed (ns)/exposed (e)	Date	No. of eggs	Non-exposed/exposed
1	10/10/1999	12	ne	05/11/2000	72	ne
2		69	ne			
3		51	ne	30/7/2000	85	ne
4		48	ne	28/11/2000	95	ne
5		39	ne	01/3/2000	73	ne
6		62	ne			
7		88	ne	01/3/2000	imm	ne
8		77	ne	04/3/2001	imm	ne
9		28	e	01/3/2000	imm	e
10		40	ne			
11		imm	e	28/11/2000	104	e
12	28/11/1999	imm	e			
13		38	ne			
14		imm	e			
15		imm	ne			
16		51	ne			
17	05/11/2000	73	ne	30/4/2000	37	ne
18		114	ne	30/4/2000	56	ne
19	28/11/2000	88	e	30/8/2000	44	ne
20		115	ne			
21	01/3/2000	104	e			
22		75	ne	30/8/2000	35	ne
23		88	ne	30/9/2000	99	ne
24		imm	e	05/11/2001	59	e
25		94	ne	30/8/2000	4	ne
26		99	e	28/10/2000	87	ne
27		imm	ne	30/9/2000	35	ne
28		94	e	06/2/2001	79	ne
29		70	ne			
30	02/4/2000	60	ne	06/2/2001	65	ne
31		imm	ne			
32		110	ne	30/9/2000	80	ne
33		imm	ne	28/10/2000	imm	ne
34		74	e	28/10/2000	65	ne
35		imm	e			
36		imm	e	06/2/2001	imm	e
37		imm	e	05/11/2001	78	e
38	30/8/2000	57	ne			
39		55	ne			
40		4	ne			
41		55	ne			
42	30/9/2000	54	ne	06/2/2001	83	e
43		72	ne			
44		44	ne			
45		60	ne	06/2/2001	95	ne
46		11	ne			
47		39	ne			

## Appendix, continued

#	First egg batch			Second egg batch			
	Date	No of eggs	Non-exposed (ne)/ exposed (e)	Date	No. of eggs	Non-exposed/ exposed	Interval (months)
48		49	ne	04/3/2001	imm	e	5
49		41	ne	-	-	-	-
50		61	ne	06/2/2001	57	ne	4
51		62	ne	04/3/2001	93	ne	5
52		47	ne	-	-	-	-
53		79	ne	-	-	-	-
54		69	ne	-	-	-	-
56	28/10/2000	imm	ne	-	-	-	-
57		78	e	06/2/2001	93	e	3
58		30	ne	04/3/2001	imm	e	4
59		20	e	-	-	-	-
60		50	ne	-	-	-	-
61		51	ne	-	-	-	-
62	03/12/2000	imm	e	-	-	-	-
63		imm	ne	-	-	-	-
64		73	ne	-	-	-	-
65	05/1/2001	69	e	-	-	-	-
66		59	e	-	-	-	-
67		119	ne	-	-	-	-
68	06/2/2001	61	ne	-	-	-	-
69		53	e	-	-	-	-
70		imm	e	-	-	-	-
71		75	e	-	-	-	-
72		95	ne	-	-	-	-
73		112	ne	-	-	-	-
74		115	ne	-	-	-	-
75		93	ne	-	-	-	-
76		111	ne	-	-	-	-
77		78	e	-	-	-	-
78		91	ne	-	-	-	-
79		39	e	-	-	-	-
80		72	ne	-	-	-	-
81		78	e	-	-	-	-
82		93	e	-	-	-	-
83		123	e	-	-	-	-
84	04/3/2001	imm	ne	-	-	-	-
85		97	e	-	-	-	-
86		109	ne	-	-	-	-
87		96	ne	-	-	-	-
88		84	e	-	-	-	-
89		115	ne	-	-	-	-

imm = presence of first instar immatures (i.e., all or some eggs already hatched).