ECOLOGY OF LADYBIRDS-

FACTORS INFLUENCING THEIR SURVIVAL

by

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ABSTRACT

Associated with the variability in aphid abundance, there is cannibalism and intra-guild predation (I. G. P.) in aphidophagous ladybird guilds, which is thought to increase ladybird larval survival when aphids are scarce. In this thesis, the factors that affect the survival of the three species of ladybirds, *Harmonia axyridis*, *Coccinella septempunctata brucki* and *Propylea japonica*, were determined based on two years of field research and laboratory experiments.

In the field, survival of *H. axyridis* and *C. s. brucki* varied from year to year, and that of *P. japonica* was always low. Laboratory experiments revealed that the larvae of *H. axyridis* were "top predators" in this ladybird guild, and their survival appeared to depend on the availability of the other species as intra-guild prey. Although, *C. s. brucki* was present in both years of the study, the likelihood of it being eaten by *H. axyridis* larvae depended on when it oviposited relative to the population dynamics of the aphid. In addition, although *P. japonica* larvae were highly likely to be eaten by *H. axyridis* larvae, the abundance of this prey varied from year to year. These results suggest that prey availability is variable even for an intra-guild predator.

The above results led to the suggestion that variation in the speed of development of larvae might facilitate their survival when food availability is both variable and uncertain. To test this, life history traits associated with speed of development were determined in the laboratory using the two spot ladybird, *Adalia bipunctata*. In general, the life history traits were associated positively with speed of development. Fast-developing larvae consumed more prey in a short period and were vulnerable to starvation when aphids were scarce than the slow-developing larvae. It is suggested that this inherited variation in the speed of development increases the chances of aphidophagous ladybird larvae surviving.

SECTION 1

GENERAL INTRODUCTION

All the predators and parasitoid that feed on aphids make up the aphidophagous guild (Sakuratani, 1977; Arakaki, 1992; Winder *et al.*, 1994; Dixon, 1998). Ladybirds are very voracious and abundant in terms of numbers of species and individuals, and are large; they are regarded as an important component of aphidophagous guilds.

A factor that directly affects their survival is food availability during their development. Several authors reported that low prey availability adversely affects the survival of larvae (Dimetry, 1976; Kawauchi, 1979). However, as the incidence of cannibalism and intra-guild predation (I. G. P.) increases when the relative abundance of aphids is low (Kawai, 1978; Mills, 1982; Takahashi, 1987; Osawa, 1989, 1992; Agarwala & Dixon, 1991, 1992; Yasuda & Shinya, 1997), these predators are themselves are an important cause of mortality in ladybird populations (Osawa 1989, 1992; Yasuda & Shinya, 1997).

As in some species of ladybirds, the first instar larvae develop relatively fast when fed conspecific eggs they are thought to be a better food for ladybirds than aphids (Kawai, 1978; Takahashi, 1987, Osawa, 1989, 1992; Agarwala & Dixon, 1991, 1992). In addition, the fourth instar larvae can survive equally well on a diet of conspecifics or aphid prey (Yasuda & Onuma, 2000). Therefore, nutritionally there are no penalties associated with eating conspecifics. On the other hand, eating the eggs and larvae of other species of ladybirds can result in reduced survival, as several species of ladybirds are known to be toxic to other ladybirds (e.g. Agarwala & Dixon, 1998). However, I. G. P. can be advantageous because it prolongs the duration of survival of starving larvae (Hemptinne *et al.*, 2000). Therefore, in the field, cannibalism and I. G. P. are possibly important when aphids are scarce (Osawa, 1991; Yasuda & Shinya, 1997).

Both eggs and young larvae are more vulnerable to cannibalism than older larvae; the victim is usually at a vulnerable stage in its development (Agarwala & Dixon, 1992; Dong & Polis, 1992; Stevens, 1992). In addition, generally the larger predator is likely to be the intra-guild

predator, and the smaller the extra-guild prey (Sengonca & Frings, 1985; Lucas *et al.*, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001). That is, in general, cannibalism and I. G. P. are more likely to occur when the potential predators and victims differ in size.

However, such predation may not occur for the following reason. Small species like *Adalia bipunctata* contain more alkaloid per unit weight than the large species, *Coccinella septempunctata* (De Jong *et al.*, 1991; Holloway *et al.*, 1991). Several authors report that small species appear to be well protected chemically against predation by larger species (Agarwala & Dixon, 1992; Agarwala *et al.*, 1998; Hemptinne *et al.*, 2000). That is, although small species are likely to be eaten by larger species (Sengonca & Frings, 1985; Lucas *et al.*, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001), defensive chemicals reduce the incidence of I. G. P. of small species by large species.

In addition, Winder (1990) suggests that the tendency of larvae to disperse when prey availability is low may reduce the probability of their encountering con- and hetero-specific larvae. Leaving rate depends on species (Schellhorn & Andow, 2000). That is, although the incidence of cannibalism and I. G. P. are likely to increase when prey availability is low (Kawai, 1978; Mills, 1982; Takahashi, 1987; Osawa, 1989, 1992; Agarwala & Dixon, 1991, 1992; Yasuda & Shinya, 1997), emigration of larvae from plants may reduce the incidence of cannibalism and I. G. P.. Consequently, the incidence of Cannibalism and I. G. P. are likely to vary between species.

However, the effects of chemical defense and emigration on the incidence of cannibalism and I. G. P. in the field are poorly understood. Therefore, in Section 2, survival of three species of ladybirds in relation to prey abundance was monitored in the field. In addition, in Section 3, the effect of chemical protection and leaving rate on the incidence of cannibalism and I. G. P. were studied in a semi natural environment.

In addition, if the incidence of cannibalism and I. G. P., which may increase the survival of

ladybirds when prey is scarce (Osawa, 1991; Yasuda & Shinya, 1997; Hemptinne *et al.*, 2000), varies between species, then the less cannibalistic or predatory species are more likely to be affected by prey availability than the more cannibalistic or predatory species.

Body size and duration of development in ladybird are determined by prey availability. For instance, the larvae of *Propylea japonica* tend to develop more slowly to the adult stage when food availability is low (Kawauchi, 1979). In addition, the duration of development is also likely to be linked to body size and prey consumption. Ueno (1994) reported that the duration of development varies between individuals fed equally well. In this study, the fast-developing larvae of *H. axyridis* tend to develop into large adults and the slow-developing larvae into small adults. Prey consumption is likely to vary between fast- and slow developing larvae. Rodriguez-Saona & Miller (1995) selected *Hippodamia convergens* for fast development over several generations and showed that the fast-developing larvae consumed more aphids per unit time and developed into larger adults than the more slowly developing individuals.

Dixon (2000) suggest that all the life history traits of predatory ladybirds, including both aphidophagous and coccidophagous species, are associated positively with speed of development. For instance, aphidophagous species develop faster and consume more prey than coccidophagous species. Accordingly, if all life history traits of ladybirds are associated with speed of development life history traits, such as food consumption, are likely to differ between fast- and slow developing individuals. Therefore, in Sections 4 and 5, life history traits of fast- and slow-developing were determined. In addition, if slow developing individuals consume less prey, they are less likely to be affected by low food availability. Therefore, the effect of different levels of food availability on the performance of fast- and slow-developing larvae were also determined (Section 4). Finally, in Section 6, the effect of variations in the availability of prey, including con- and hetero-specific ladybirds are discussed based on the results in Sections 2 and 3, and the

advantages of fast- and slow-development are discussed based on the results obtained in Sections 4 and 5.

SECTION 2

POPULATION DYNAMICS OF 3 LADYBIRDS IN THE FIELD

2-1 INTRODUCTION

The importance of various processes that structure communities and determine population sizes have been discussed for several decades. Intra-guild predation is often reported in insect populations, and prey abundance seems to be an important factor determining the frequency of this predation, which is thought to be an important force structuring insect communities (Polis *et al.*, 1989; Dong & Polis, 1992).

All the predators and parasitoids feeding on aphids make up the aphidophagous predator guild (Sakuratani, 1977; Arakaki, 1992; Winder *et al.*, 1994; Dixon, 1998). Ladybirds are very voracious, and abundant in terms of numbers of species and individuals, and are large; they are regarded as an important component of aphidophagous predator guilds.

Both cannibalism and I. G. P. of eggs has been observed in aphidophagous ladybirds (Kawai, 1978; Mills, 1982; Takahashi, 1987; Osawa, 1989, 1992; Agarwala & Dixon, 1991, 1992). Similarly, cannibalism and I. G. P. of larvae occur both in the laboratory and field, and, it is suggested that prey density affects the incidence of cannibalism and I. G. P. (Takahashi, 1987; Agarwala & Dixon, 1991, 1992; Yasuda & Shinya, 1997). Additionally, larval density affects the incidence of eggs cannibalism (Mills, 1982). However, what happens in the field is still poorly documented and less well understood.

There are few studies on the population dynamics of predatory ladybirds in the field (Osawa, 1993, Yasuda & Shinya, 1997). In these studies mortality of the larvae was relatively high compared to the other developmental stages; egg and pupa. Additionally, mortality of the latter larval stages tend to be high when prey density is low (Yasuda & Shinya, 1997). Osawa (1993) showed that the fourth instar larvae that prior to pupation moved furthest away from an aphid colony were the most likely to survive being cannibalized or parasitized. Larvae commonly leave a host plant when prey becomes scarce (Yasuda & Shinya, 1997). In fact, in some species of

ladybird, it is reported that 90% of the larvae leave a host plant prior to pupation (Lucas *et al.*, 2000), and that the leaving rate depends on species (Schellhorn & Andow, 1999). Therefore, for a better understanding of the population dynamics of ladybirds it is necessary to estimate larval dispersal.

Recently it was reported that the species composition of ladybird guilds in the field has changed over the last seventeen-years in United States (Elliott, 1990; Elliott *et al.*, 1996). A dominant introduced species, *Harmonia axyridis*, is currently invading ladybird guilds in North America (Gordon & Vandenberg, 1991; Day *et al.*, 1994; LaMana & Miller, 1996; McCorquodale, 1998; Michael & Miller, 1996; Brown & Miller, 1998; Colunga-Garcia & Gage, 1998). As the mechanism leading to the change in guild structure is unknown, there is a need to study the interaction between the species of ladybirds in such guilds.

In this section, the mortality and the dispersal of three species of ladybirds are studied in relation to aphid population dynamics over a period of two years in the field. The effect of these mortalities on the temporal structure of a ladybird guild is discussed.

2-2 MATERIALS AND METHODS

(1) Study site

The field research was done on the Yamagata University farm (38° 43'N, 139° 49'E), which is mainly used for cultivating experimental plants. About fifty young trees of *Hibiscus syridis* were planted in a line in 1990 and 1992. The cotton aphid, *Aphis gossypii*, occurs on these trees from the middle of spring, and forms large aggregations on young shoots. Over wintered adults of three species of aphidophagous ladybirds, *Harmonia axyridis, Coccinella septempunctata brucki* and *Propylea japonica,* exploit the aphid in spring. Body sizes of the first two species of ladybird are similar and about double that of the latter species. The ground around the trees was weeded at regular intervals in order to facilitate the finding of emigrating larvae.

(2) Population dynamics of ladybirds and aphid

Seasonal changes in the numbers of ladybirds in spring were monitored in 1995 and 1996. Ten and twelve trees, 1.5-2.5 m in height, were sampled in 1995 and 1996, respectively, and the fates of all developmental stages of the ladybird except for first instar larvae were monitored. The branches of the trees adjacent to the sample trees were tied up against their trunks, which prevented them from touching those of the sample trees. The trees were searched for eggs daily, and the number of eggs in each cluster of eggs was noted. Each egg cluster was marked with an elliptical plastic tag (4 cm length × 3 cm width). The tag was numbered and attached to the petiole of the leaf on which the eggs were laid. These clutches of eggs were observed daily, and the number that hatched and the fate of the rest was noted. The first instar larvae of *H. axyridis* and *C. s. brucki* are difficult to distinguish, whereas the later instars are easily identified. Therefore, the larvae were only assigned to a species from the second instar onwards. Pupae were also marked with plastic tags, and observed daily until adult emergence. When ladybirds were observed eating another ladybird the species of the prey and predator and their developmental stage, which included first instar larvae, were also noted.

Aphid abundance was estimated by randomly selecting ten leaves on two randomly selected twigs from each of the upper, middle and lower parts of each tree. This was done every other day until all the aphids disappeared.

(3) Identification of ladybird eggs

The eggs of *P. japonica* differ in appearance from those of the other two species. However, eggs of *H. axyridis* and *C. s. brucki* are similar in size, colour, number in a cluster and morphology, and this similarity makes species identification difficult. Therefore, a few eggs from each cluster of eggs were taken back to the laboratory. The larvae that hatched from these eggs were reared on an excess of cotton aphids and identified when they reached the second instar.

(4) Assessment of larval emigration

Species and instar of emigrants and the time, relative to the aphid peak in abundance, of larval emigration were assessed. In 1995, larvae found on the ground around the 10 trees, on which the ladybirds were being monitored, were collected and identified to species and developmental stage. In 1996, six trees, adjacent to the twelve trees used for the population study of ladybirds, were used to trap emigrating larvae. From before egg laying started these six trees were observed daily and any egg clusters were removed so there was no recruitment of ladybird larvae via eggs on these trees. In addition, all larvae found on the six trees were removed daily, and their species and developmental stage noted.

2-3 RESULTS

Seasonal changes in the percentages, relative to the peak abundances, of ladybird eggs, and larvae, and aphids present on the trees in 1995 and 1996 are given in Figure 2-1 and 2-2, respectively.

2-3-1 Life history (Eggs)

(1) Seasonal changes in the number of eggs

In both years, the oviposition periods of the three species of ladybird began with the emergence of aphids (Fig. 2-1, 2-2). The number of eggs laid by *H. axyridis* and *C. s. brucki* increased with increase in aphid abundance until conspecific larvae of the second instar onwards developed. Consequently the egg laying in *H. axyridis* and *C. s. brucki* peaked before the aphid peaked in abundance, and their oviposition period ended at the time of the peak in the number of conspecific larvae. In addition, the number of eggs of *H. axyridis* peaked when the aphid abundance exceeded 60% of the peak in both years. However, the aphid abundance when egg number of *C. s. brucki* peaked differed in the two years, it was 68% and 34% in 1995 and 1996, respectively. The number of eggs laid by *P. japonica* in 1995 increased until conspecific larvae

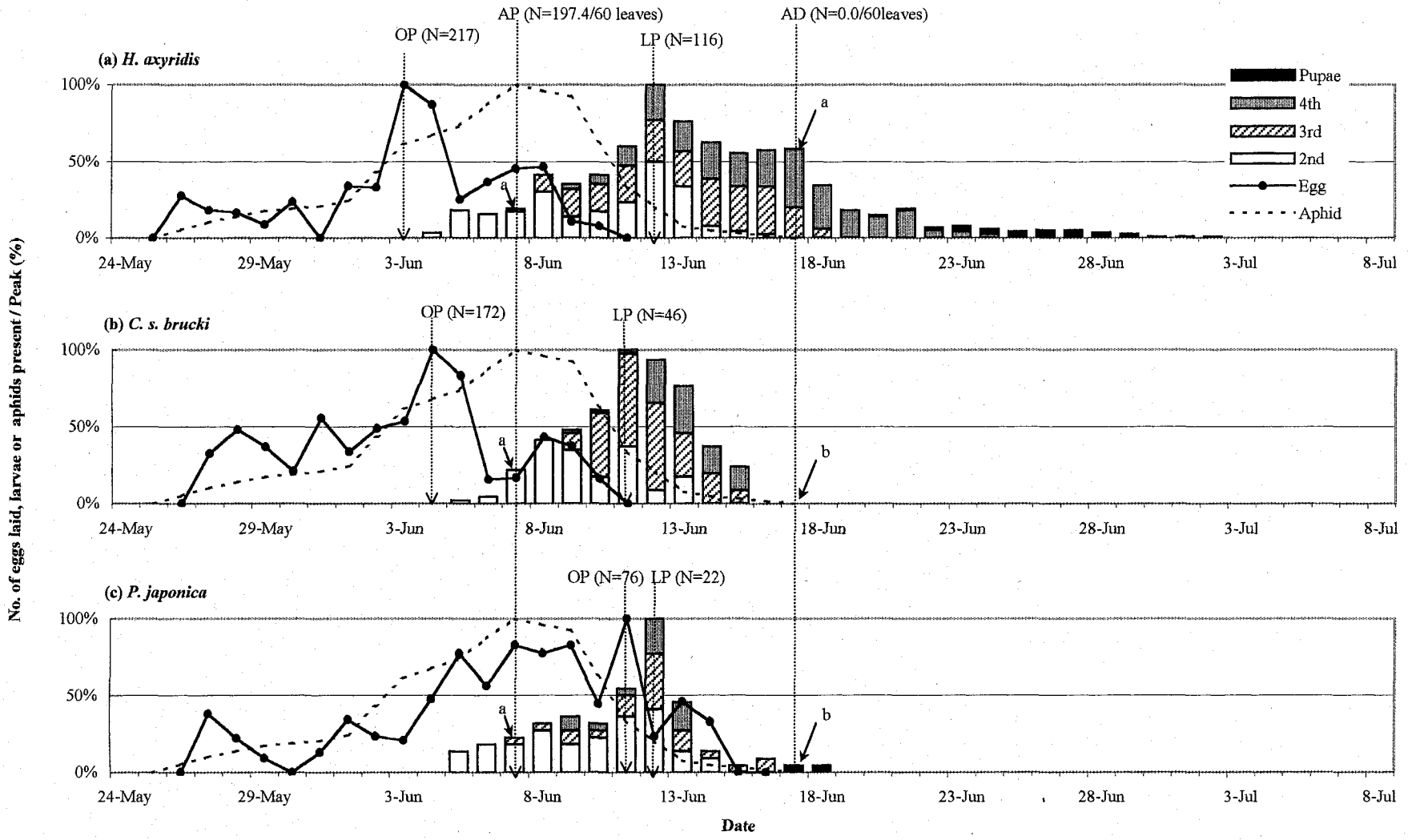


Figure 2-1. Seasonal changes in the number of eggs and larvae of ladybirds, and aphids on 10 trees in 1995, expressed as a percentage of the peak number in each case. (Peak numbers of eggs laid, larvae and aphids = 100%, OP: Oviposition peaked, LP: Ladybird larvae and pupae peaked, AP: Aphids peaked, AD: Aphids disappeared. Histograms with the same letter do not differ significantly between species, $P > 0.05$: χ^2 test.)

peaked in abundance, and their oviposition ended when the aphid became extinct (Fig. 2-1c). In 1996, eggs of *P. japonica* were found on only two days, just before and after the aphid peaked in abundance (Fig. 2-2c).

In 1995, the duration of the oviposition periods of *H. axyridis* (17 days) and *C. s. brucki* (16 days) were similar, and shorter than that of *P. japonica*, which was 20 days. In 1996, the duration of the oviposition period was 18 and 13 days in *H. axyridis* and *C. s. brucki*, respectively; and was longer in *H. axyridis* and shorter in *C. s. brucki*, than in 1995.

(2) Distribution of eggs

Numbers of eggs laid were compared in the two weeks prior to and the week following the peak in aphid abundance (Fig. 2-3).

In 1995, a total of 1133, 1105 and 624 eggs were laid by *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. Two weeks prior to the peak in aphid abundance, the percentages of eggs laid did not exceed 30% in all the species. In the week, prior to the peak, the percentages of eggs laid increased significantly in all the species ($P < 0.05$). After the aphid peaked in abundance, the percentages of eggs laid decreased significantly to less than 20% in *H. axyridis* (N=142) and *C. s. brucki* (N=166) ($P < 0.05$), but increased significantly to 49% (N=310) in *P. japonica* ($P < 0.05$). Consequently, the percentages of eggs laid by *H. axyridis* and *C. s. brucki* tended to increase in the weeks prior to the peak in aphid abundance and to decrease the following week. In contrast, the percentages of eggs laid by *P. japonica* tended to increase over the same period.

In 1996, a total of 2167, 2308 and 37 eggs were laid by *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. Percentages of eggs laid by *H. axyridis* was significantly increased in the week prior to the peak in aphid abundance ($P < 0.05$) and significantly decreased the following week ($P < 0.05$). Although, in *P. japonica* there was no significant difference in the percentages of eggs laid in the two weeks prior to and after the peak in aphid abundance ($P > 0.05$), the

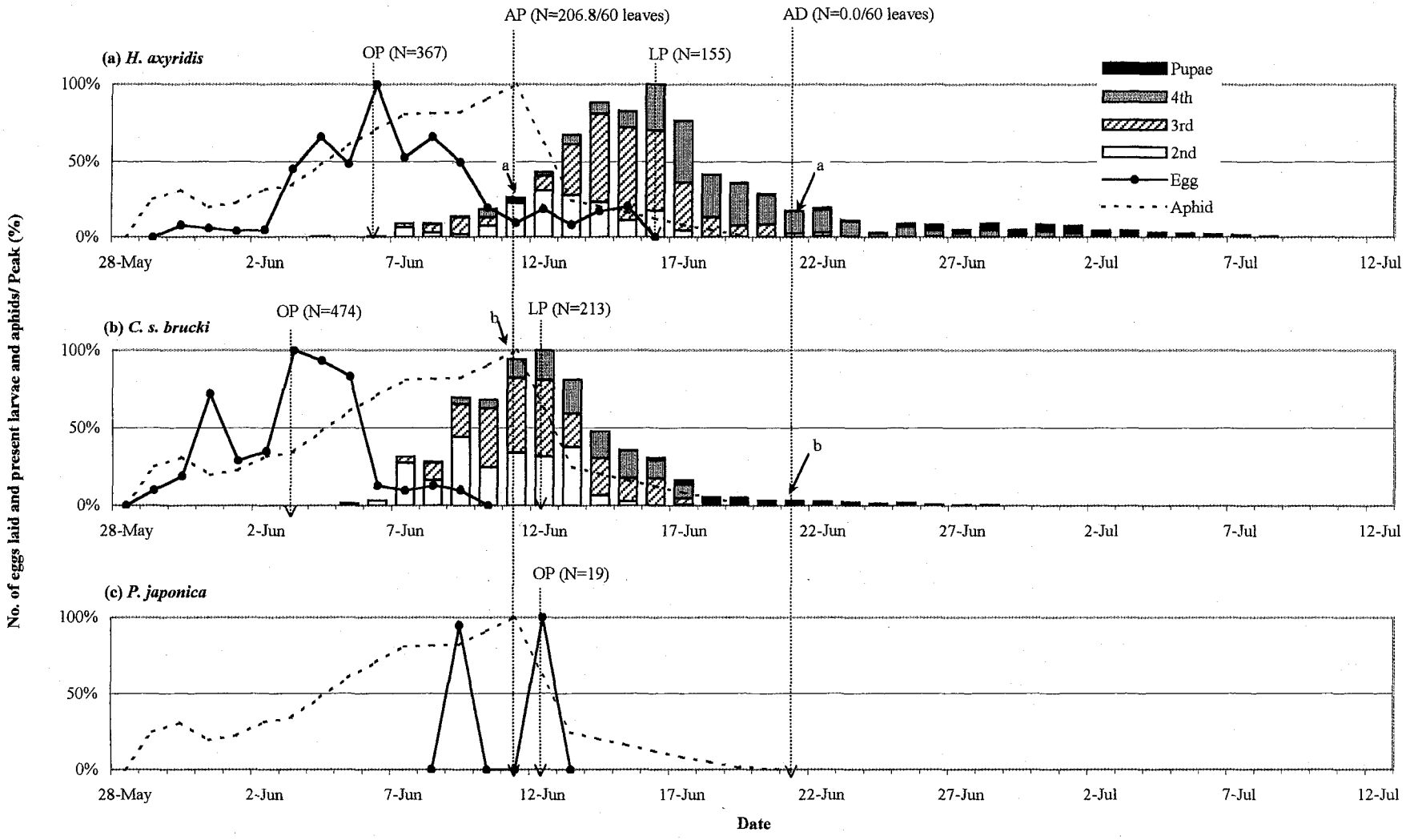


Figure 2-2. Seasonal changes in the number of eggs and larvae of ladybirds, and aphids on 12 trees in 1996, expressed as a percentage of the peak number in each case. (Peak numbers of eggs laid, larvae and aphids = 100%, OP: Oviposition peaked, LP: Ladybird larvae and pupae peaked, AP: Aphids peaked, AD: Aphids disappeared. Histograms with the same letter do not differ significantly between species, $P < 0.05$: χ^2 test.)

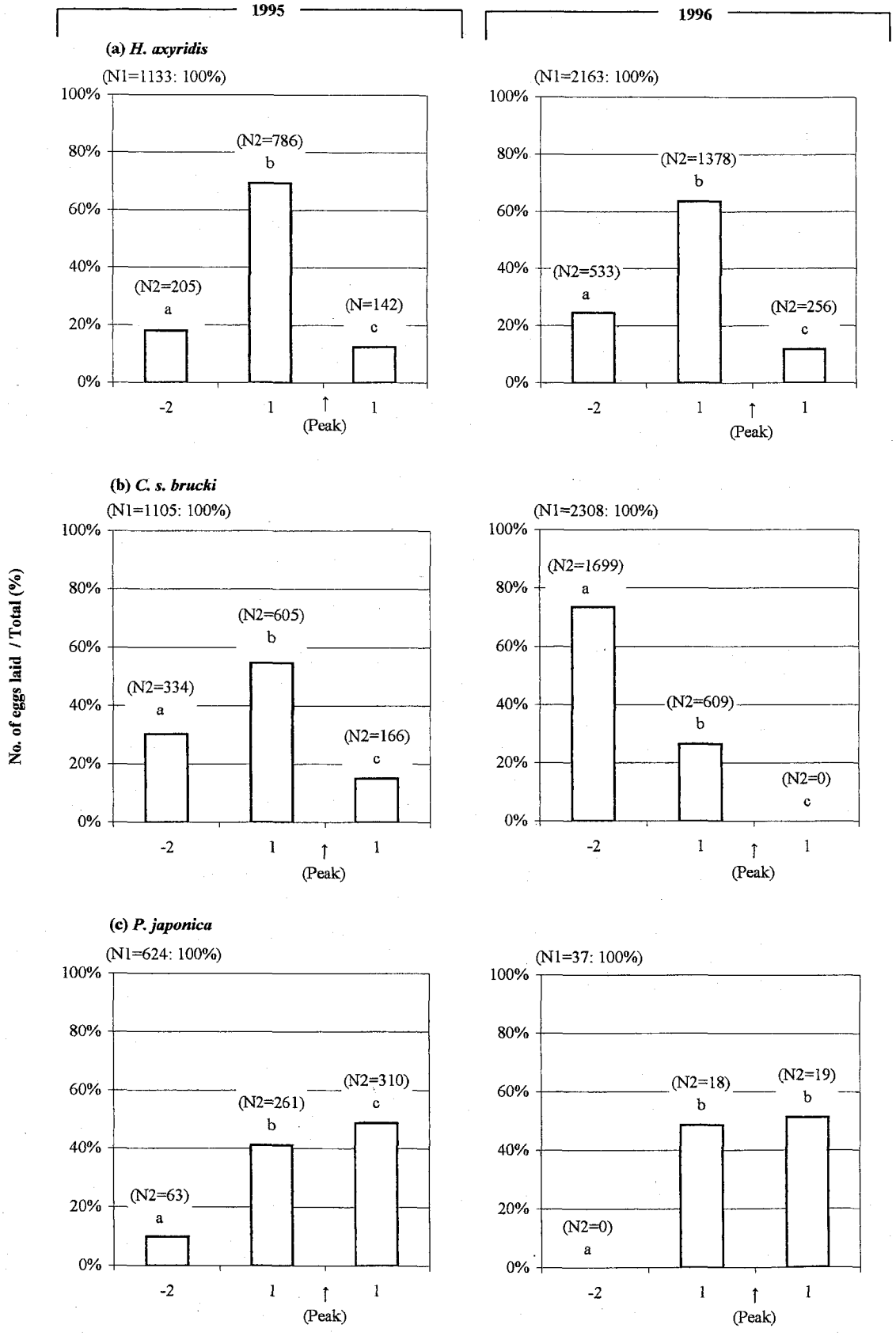


Figure 2-3. Percentages of eggs laid relative to the time of the peak in aphid abundance in 1995 and 1996. (N1 and N2 show the number of eggs laid in total and in each period, respectively. Histograms topped by the same letter do not differ significantly, $P > 0.05$: χ^2 test.)

percentages were significantly higher in the two weeks prior to the peak in aphid abundance ($P < 0.05$). Both *H. axyridis* and *P. japonica*, distributed their eggs in time in 1996 similarly to in 1995. However, *C. s. brucki* distributed its egg in 1996 differently from in 1995. The percentage of eggs laid in the two weeks prior to the peak in aphid abundance was 75% (N=1699), which was over double that in 1995 (30.2%), and decreased in the following two weeks ($P < 0.05$).

The percentages of eggs laid were compared in the three species (Fig. 2-4). In both years, in the two weeks prior to the peak in aphid abundance, the percentages of eggs laid was significantly higher in *C. s. brucki* than in the other two species ($P < 0.05$). In the week prior to the peak, the percentages of eggs laid was significantly higher in *H. axyridis* than in the other two species ($P < 0.05$). After the aphid peaked in abundance, the percentages of eggs laid was significantly higher in *P. japonica* than in the other two species ($P < 0.05$).

That is, in both years, the sequence of oviposition of the species was the same. However, in 1996, proportionally more of the eggs of *C. s. brucki* were laid early than in 1995, whereas that of the other two species did not differ in the two years.

(3) Duration of egg stage

In 1995, the average temperatures two and one week prior to the peak in aphid abundance and in the following week, were 16.8 ± 0.6, 17.2 ± 0.6 and 18.6 ± 0.5 ℃, respectively. These temperatures do not differ significantly ($P > 0.05$, F=3.0: One-Way ANOVA) (Fig. 2-5). In the period two weeks prior to the peak in aphid abundance, the average incubation period for clusters of *H. axyridis*, *C. s. brucki* and *P. japonica* eggs, was 6.8 ± 0.3, 6.8 ± 0.2 and 6.4 ± 0.5 days, respectively. In the following two weeks, the average incubation period significantly shortened to less than 5 days for all species ($P < 0.05$). Over the whole oviposition period, the average incubation periods for *H. axyridis* and *P. japonica* eggs were 4.8 ± 0.2 and 4.2 ± 0.3 days, which were significantly shorter than that for *C. s. brucki* (5.8 ± 0.2days, $P < 0.05$) (Fig. 2-6).

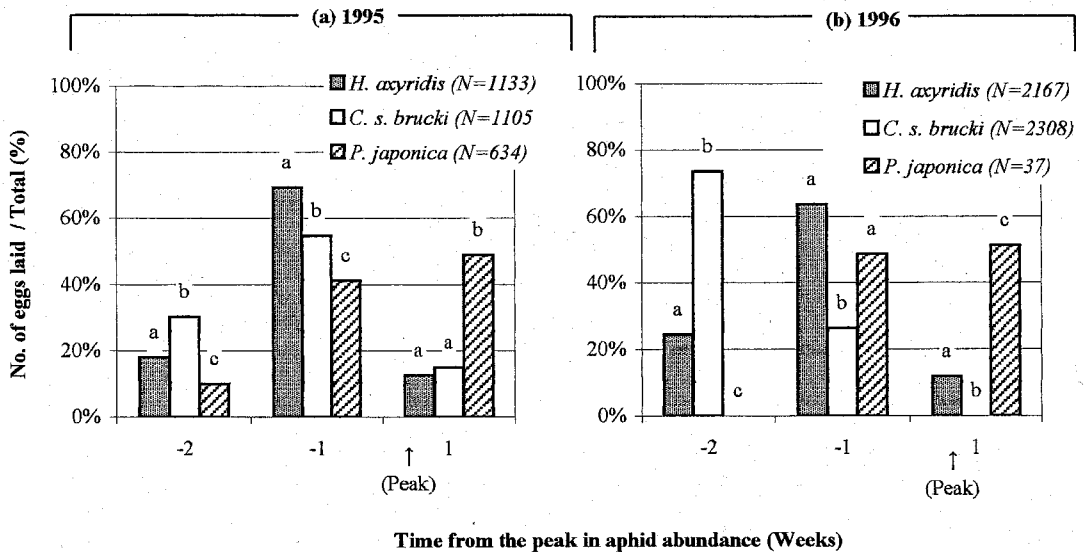


Figure 2-4. Comparison of percentages of egg laid relative to the peak in aphid abundance for the three species of ladybirds in (a)1995 and (b)1996. (Histograms for a species topped by the same letter do not differ significantly, $P > 0.05$: χ^2 test.)

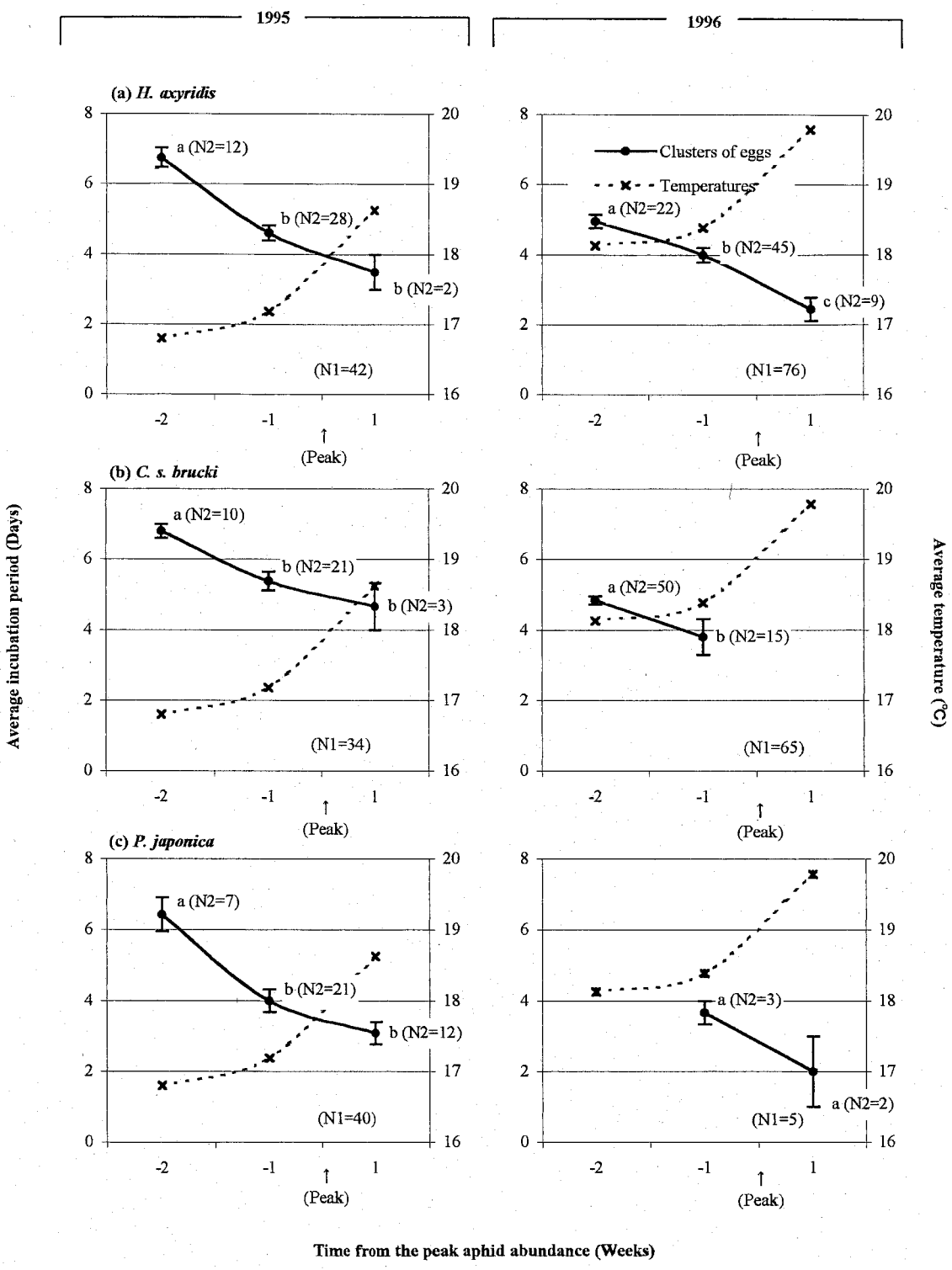


Figure 2-5. Average temperatures and duration of egg stage in the two weeks prior to and the week following the peak in aphid abundance in 1995 and 1996. (N1= Number of clusters of eggs that hatched in each period. Dots followed by the same letter do not differ significantly, $P > 0.05$: ONE-WAY ANOVA, Bonferroni test)

In 1996, as in 1995, the average temperature tended to increase with time, although there was no significant difference between the average temperature in the three weeks ($P > 0.05$, F=2.1: One-Way ANOVA) (Fig. 2-5). In addition, the incubation period tended to be shorter in the latter oviposition period in all species. Over the whole oviposition period, the average incubation periods for *H. axyridis* and *C. s. brucki* eggs were 4.2±0.2 and 4.5±0.2) days, which were significantly longer than that for *P. japonica* (2.8±0.7days) ($P < 0.05$) (Fig. 2-6). In this year, the average temperature over the oviposition periods was 18.8±0.4℃, which was significantly higher than in 1995 (17.5±0.4℃)($P < 0.05$, U=130.5: Mann-Whitney test). In addition, the average incubation period of egg clusters also tended to be short in 1996.

(4) Fate of eggs

During the research, 77 and 48 eggs (4%) in 1995, 163 (8%) and 119 eggs (5%) in 1996 of *H. axyridis* and *C. s. brucki*, respectively, were removed in order to identify the species. Egg mortality was categorized as: cannibalism by non sib-larvae or I. G. P., cannibalism by sib-larvae, or disappearance or destruction. The percentage of eggs lost to each of these mortalities and the percentage of eggs that hatched were compared in the three weeks and for the three species.

Cannibalism by non sib-larvae or I. G. P.

In 1995, the percentage of eggs eaten tended to be greater late in the oviposition period. It was 73%, 67% and 41% for *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively, in the week prior to the peak in aphid abundance (Fig. 2-7a). The percentages of *H. axyridis* and *P. japonica* eggs eaten in 1996 also tended to be greatest in the week following the peak in aphid abundance, and the trend is similar to that observed in 1995 (Fig. 2-7b). However, in 1996 *C. s. brucki* did not lay any eggs after the peak in aphid abundance. Percentage of egg mortality attributable to egg cannibalism by non sib-larvae or I. G. P. was compared in the three species (Fig. 2-12, p. 26). In both years, the percentage of eggs lost in this way was significantly greater in *H. axyridis* and *P.*

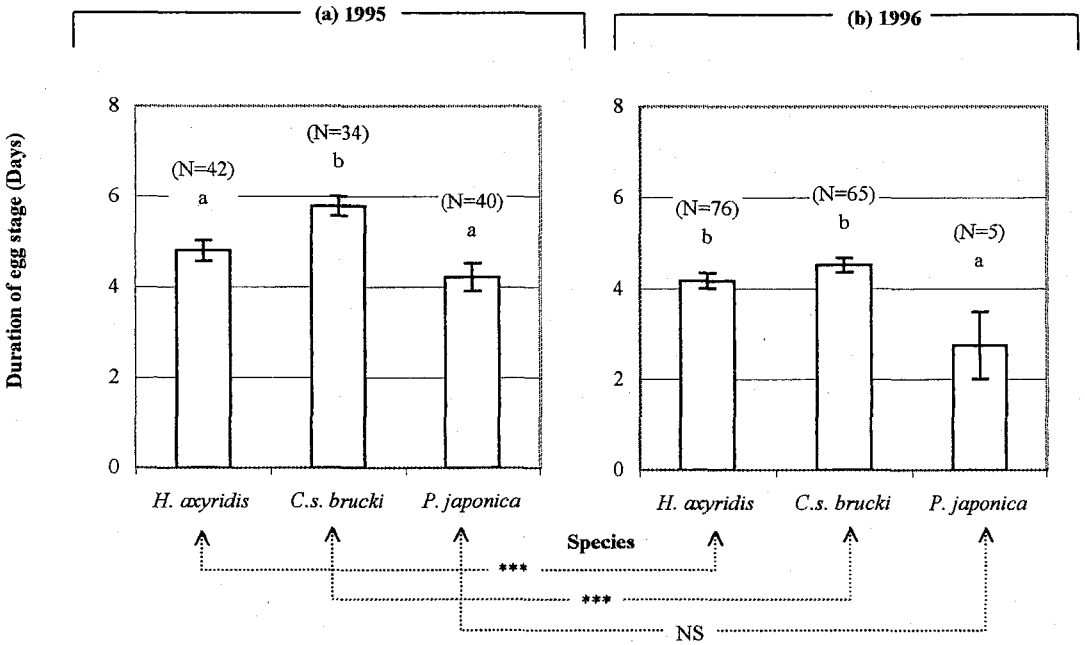


Figure 2-6. Average duration of egg stage of the three species of ladybird in (a)1995 and (b)1996. (Histograms with the same letter did not differ significantly, $P > 0.05$: ONE-WAY ANOVA, Turkey HSD test. ***shows significant difference between years, $P < 0.001$ and NS show no significant difference between years at $P > 0.05$: Mann-Whitney test.)

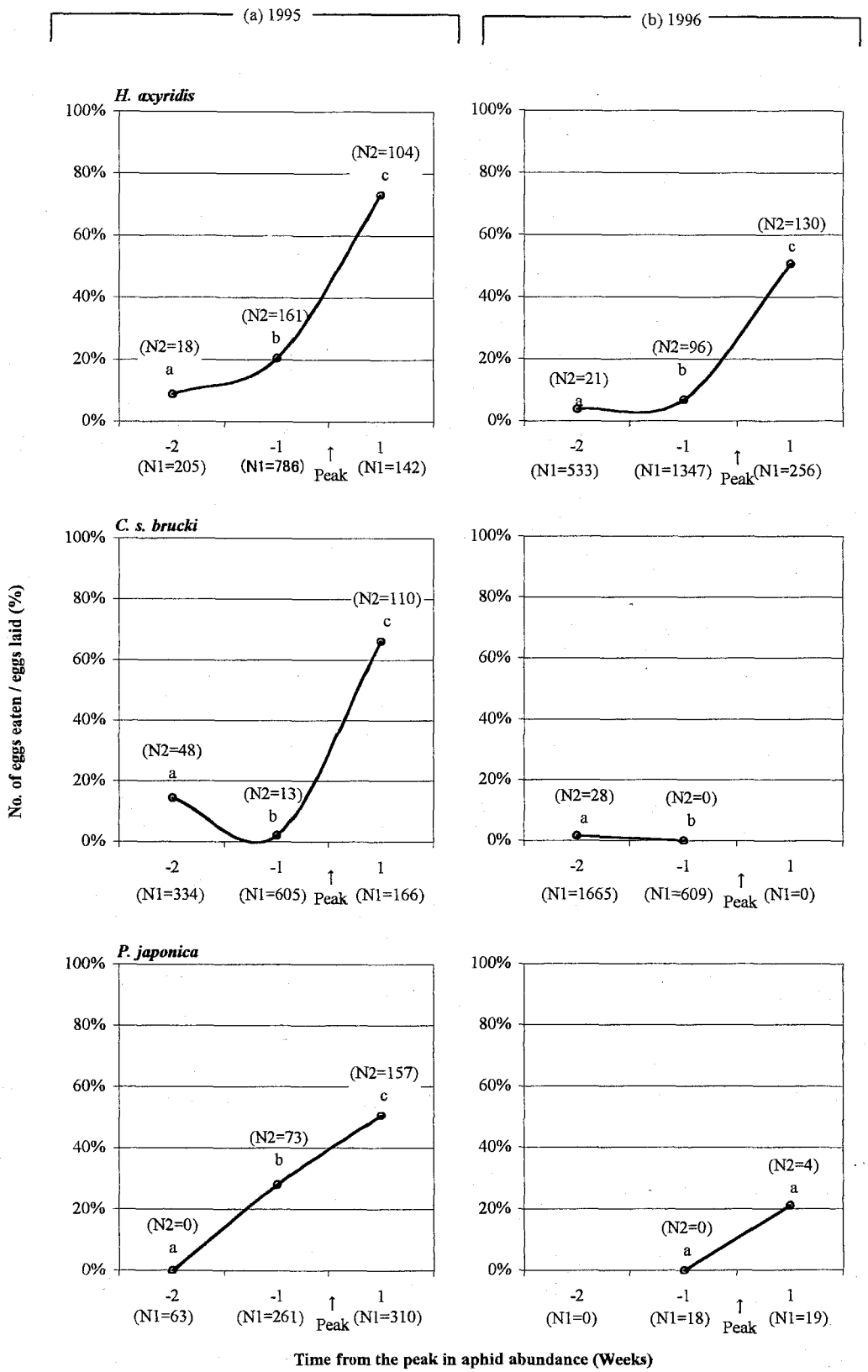


Figure 2-7. Percentages of eggs eaten by non-sib conspecific larvae or eaten by intra-guild predators relative to the time of the peak in aphid abundance in (a)1995 and (b)1996. (N1 and N2 show total number of eggs laid and eaten in each period, respectively. Dots topped by the same letter do not differ significantly between periods at $P > 0.05$: χ^2 test.)

japonica than in *C. s. brucki* ($P < 0.05$) (Fig2-12, p. 26).

Cannibalism by sib-larvae

In both years, the percentages of eggs eaten by sib-larvae did not exceed 20% in each of the species. It tended to decrease after the peak in aphid abundance in *H. axyridis* and *C. s. brucki* (Fig. 2-8). In *P. japonica*, the percentage did not differ significantly in the three periods ($P > 0.05$). In both years, although the total percentage egg mortality due to cannibalism by sib-larvae differed in the three species, the percentages were lower than for the other causes of egg mortality (Fig. 2-12, p. 26).

Disappearance or destruction

In 1995, percentages of eggs that disappeared or were destroyed was 32.2%, 42.2% and 54.0% for *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. In the two weeks prior to the peak in aphid abundance, the percentages were higher than in the following two weeks (Fig. 2-9a). The percentages of *H. axyridis* and *C. s. brucki* eggs that disappeared or were destroyed in 1996 also tended to be greatest two weeks prior to the peak in aphid abundance, as was observed in 1995. However, *P. japonica* did not lay any eggs in the two weeks prior to the peak in aphid abundance. In both years, a greater percentage of *C. s. brucki* than of *H. axyridis* eggs disappeared or was destroyed ($P < 0.05$) (Fig. 2-12, p. 26).

Importance of the three causes of egg mortality

Percentages of eggs lost in each week to the three mortality factors were compared (Fig. 2-10). Two weeks prior to the peak in aphid abundance, the percentages of eggs that disappeared or were destroyed tended to be greater than the losses attributable to the other two mortalities. In the week prior to the peak in aphid abundance, egg mortality generally was lower than in the other two periods. In the week following the peak in aphid abundance, percentage egg cannibalism by non-sib larvae or mortality due to intra-guild predation tended to be higher than that attributable

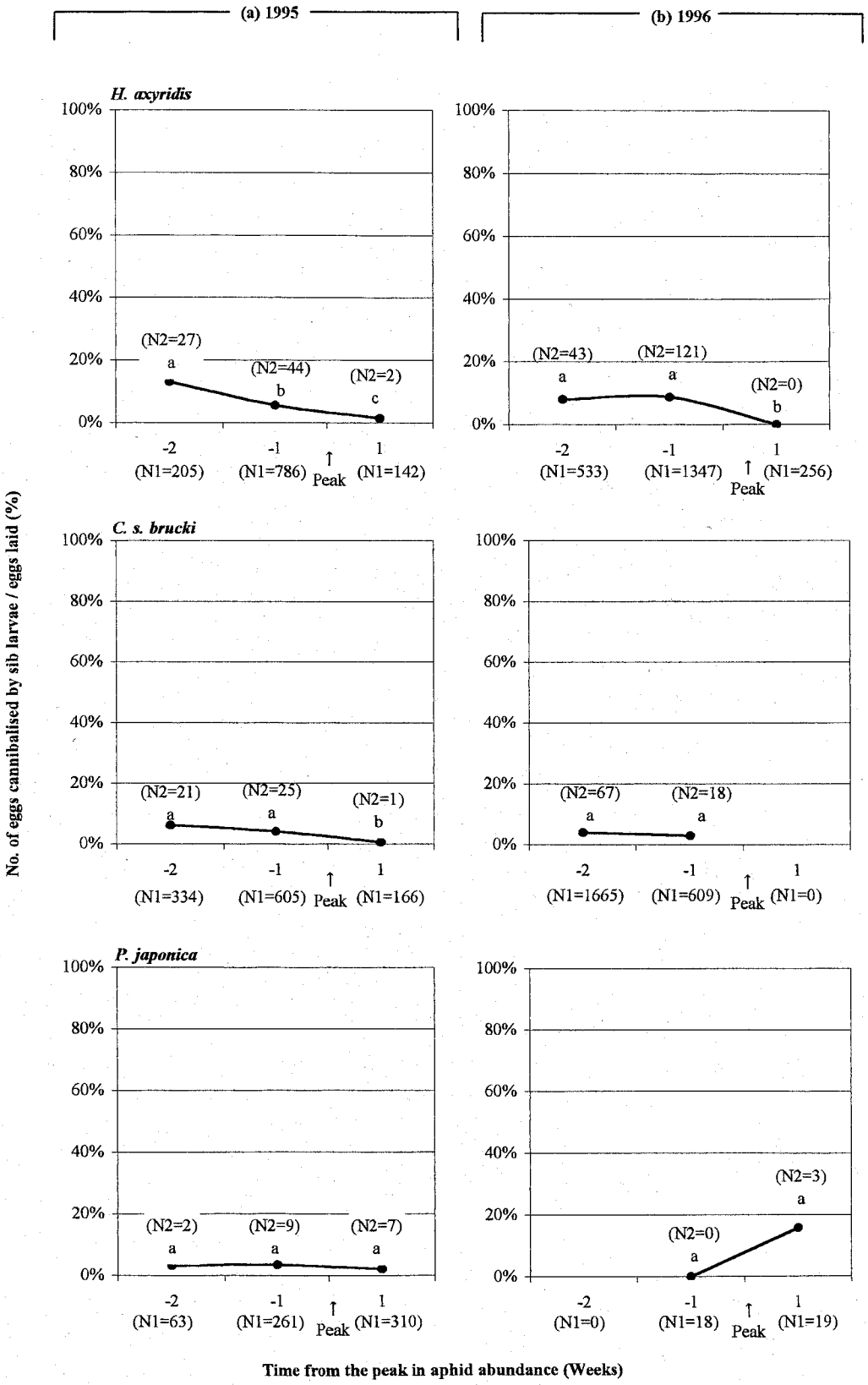


Figure 2-8. Percentages of eggs eaten by sib larvae relative to the time of the peak in aphid abundance in (a)1995 and (b)1996. (N1 and N2 show total number of eggs laid and eaten by sib larvae in each period, respectively. Dots topped by the same letter do not differ significantly between periods at $P > 0.05$: χ^2 test.)

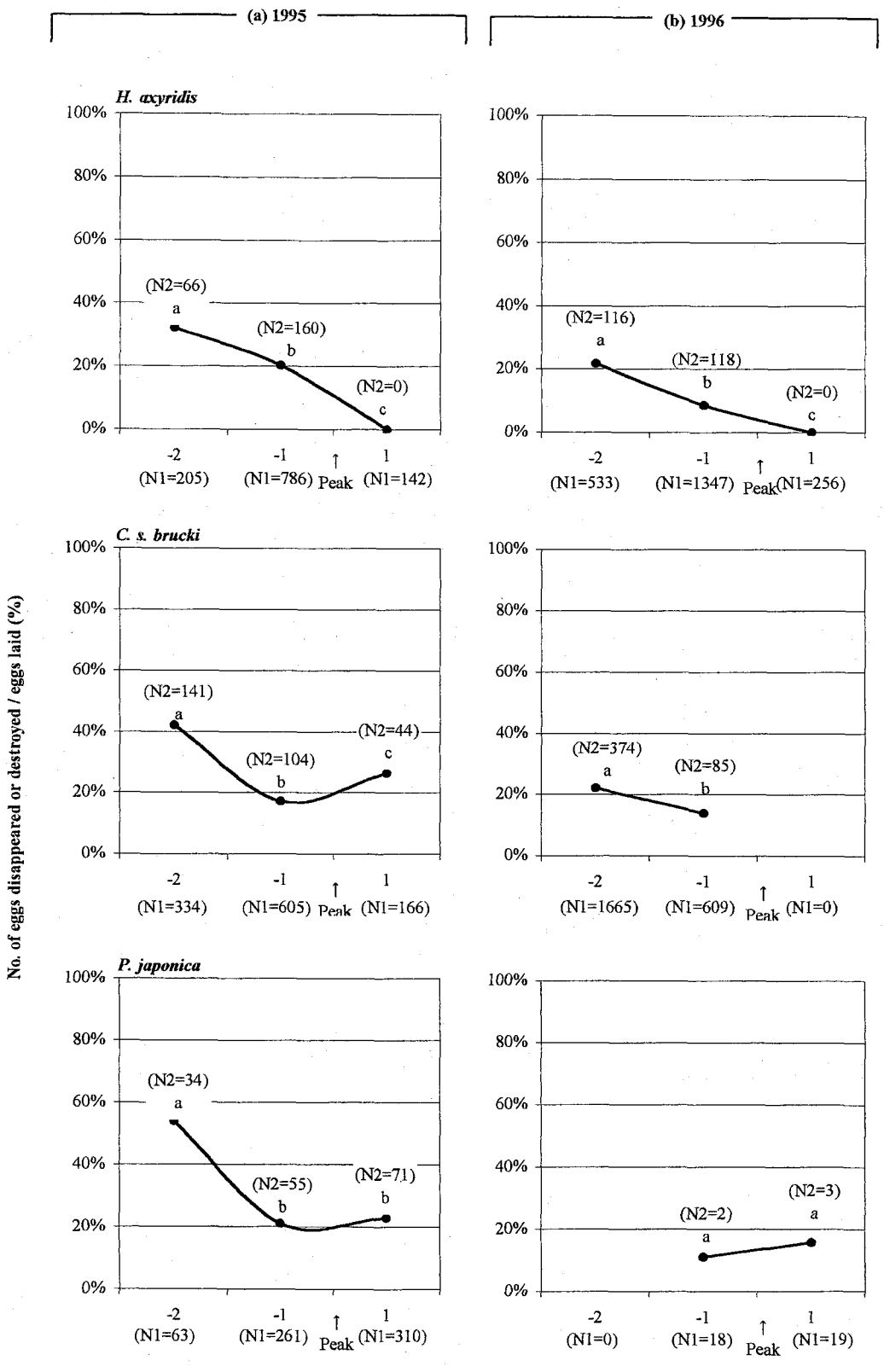


Figure 2-9. Percentages of eggs that disappeared or were destroyed relative to the time of the peak in aphid abundance in (a)1995 and (b)1996. (N1 and N2 show total number of eggs laid, and disappeared or were destroyed in each period, respectively. Dots topped by the same letter do not differ significantly between periods at $P > 0.05$: χ^2 test.)

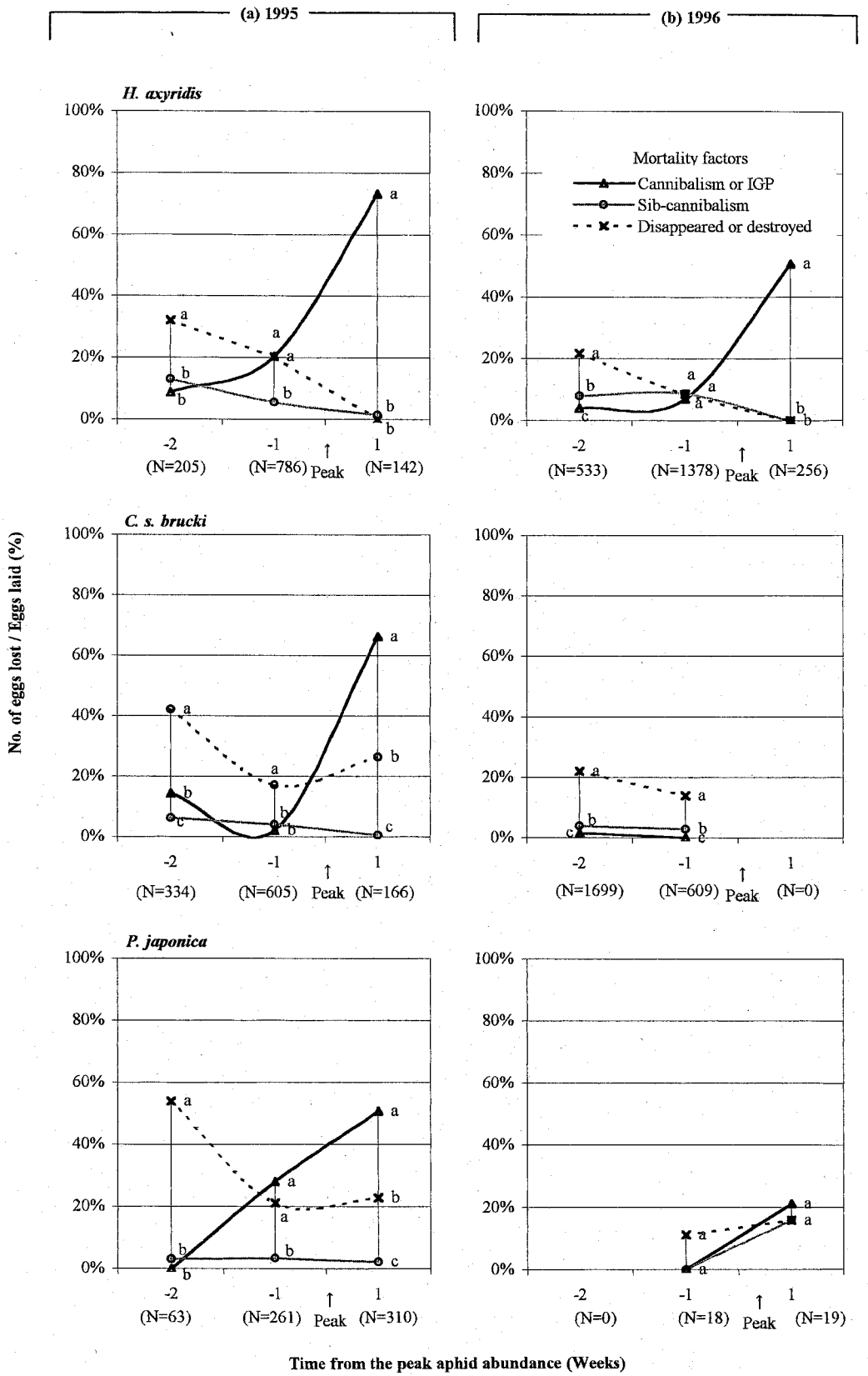


Figure 2-10. Comparison of percentages of eggs lost to the three mortality factors in (a)1995 and (b)1996. (Dots with the same letter do not differ significantly between mortality factors, $P > 0.05$: χ^2 test.)

to the other two causes of mortality.

Consequently, the percentages of eggs that hatched differed in the three weeks (Fig. 2-11). In 1995, two weeks prior to the peak in aphid abundance it was 34%, 34% and 43% in *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. In all species, the percentages that hatched tended to decrease significantly in the week following the peak in aphid abundance to 20%, 4% and 24% in *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively ($P < 0.05$). In 1996, the percentages of eggs that hatched was significantly greater in the week prior to the peak in aphid abundance than in the other weeks ($P < 0.05$), and was similar in trend to that observed in 1995. The percentage of *C. s. brucki* eggs that hatched tended to be higher than of the other two species in both years (Fig. 2-12, p. 26).

2-3-2 Life history (Larvae)

(1) Seasonal changes in the percentage of larvae and aphids

In 1995, larvae of all the three species of ladybird occurred in early June (Fig.2-1, p. 10). In this year, aphids peaked in abundance (N=197.4/60 leaves) on 7th of June. On that day, the percentages of larvae present did not differ significantly between the three species ($P > 0.05$), i.e., approximately 20% of the larvae were present for all the species. Four days after the peak in aphid abundance, 11th of June, the percentage of larvae of *C. s. brucki* peaked (N=46) whereas aphid abundance decreased to 33% of the peak (N=66/60 leaves). The percentages of larvae of the other two species peaked on the next day, 12th of June, when aphid abundance had decreased to 21% of the peak (N=41/60 leaves). In all species, the percentage of larvae peaked after the peak in aphid abundance, and there was only a difference of one day in the peaks in abundance of the three species. That is, in this year, larvae of all three species occurred about the same time. However, larvae decreased differently in the three species. Although no larvae of *C. s. brucki* or *P. japonica* were present when the aphid became extinct, 59% (N=68) of the larvae of *H. axyridis*

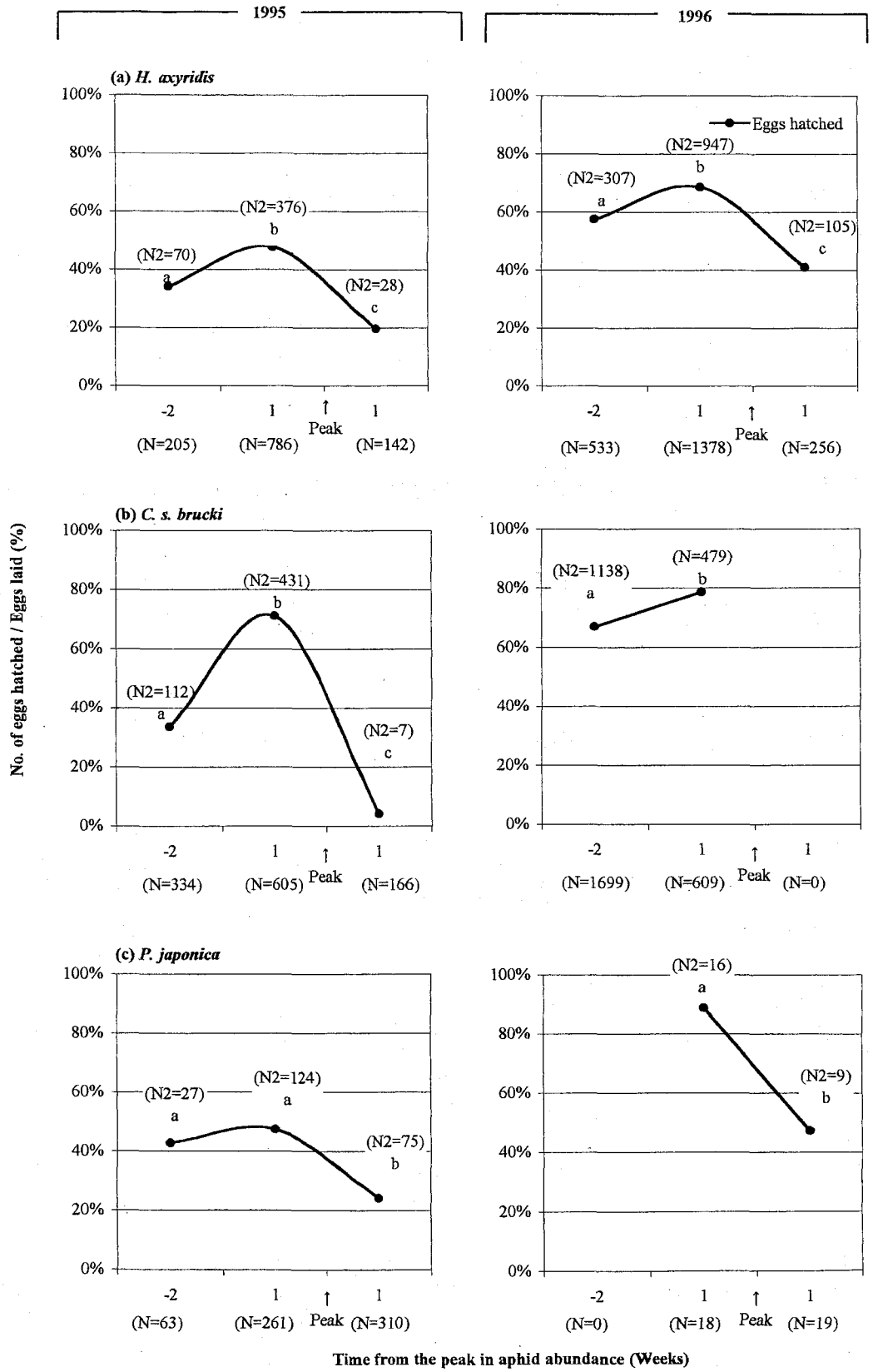


Figure 2-11. Percentages of eggs that hatched relative to the time of the peak in aphid abundance in 1995 and 1996. (N shows the number of eggs laid in total. Dots with by the same letter do not differ significantly between periods, $P > 0.05$: χ^2 test.)

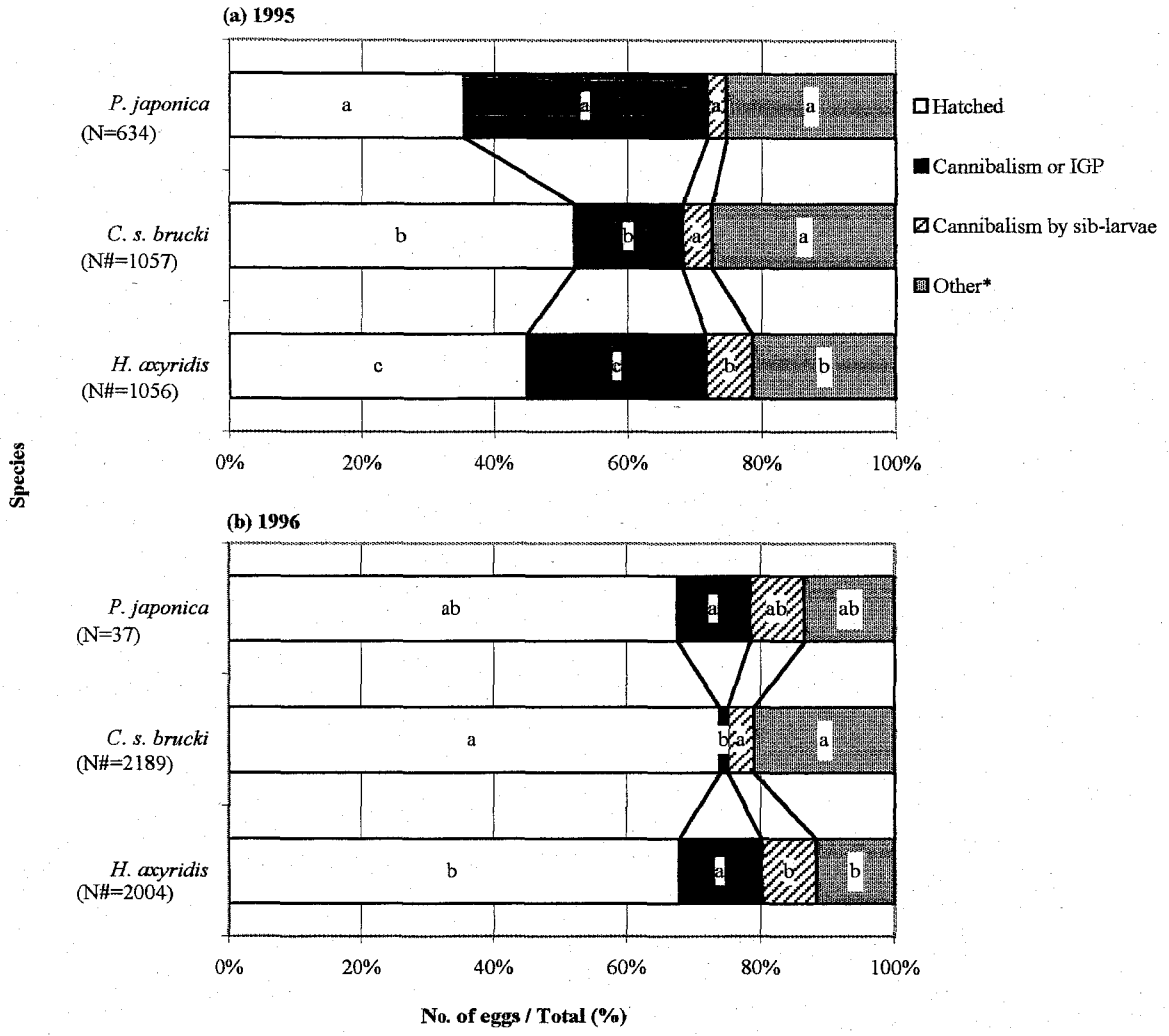


Figure 2-12. Fate of the eggs of the three species of ladybird in (a)1995 and (b)1996. (*Eggs disappeared or destroyed. Bars with the same letter do not differ significantly between species, $P > 0.05$: χ^2 test. #Eggs used to identify species were excluded.)

were still present ($P < 0.05$). That is, larvae of *C. s brucki* and *P. japonica* tended to disappear more rapidly than those of *H. axyridis*.

In 1996, larvae of *H. axyridis* and *C. s. brucki* were present in early June, but no second instar or older larvae of *P. japonica* developed (Fig. 2-2, p. 11). When the aphid peaked in abundance (N=206.8/ 60leaves) on 11th of June the percentages of larvae present were 26% (N=40) and 94% (N=201) for *H. axyridis* and *C. s. brucki*, respectively ($P < 0.001$), but there were no larvae of *P. japonica*. In addition, the percentage of *C. s. brucki* larvae present when the aphid peaked in abundance was four times grater than in 1995 ($P < 0.001$), whereas the percentage of *H. axyridis* larvae present at that time did not differ significantly in the two years ($P > 0.05$). On 12th of June, the percentage of larvae of *C. s. brucki* peaked (N=213: 100%), whereas the abundance of the aphid decreased to 62% of the peak (N=131.2/60 leaves) (Fig. 2-2, p. 11). The percentage of larvae of *H. axyridis* peaked (N=155: 100%) four days later, when the abundance of the aphid had decreased to 16% of the peak (N=25.8/60 leaves). The difference in the day on which the percentage of larvae peaked in these two species increased to four days compared to one day in 1995. That is, in this year, *C. s. brucki* larvae occurred predominantly earlier than in 1995, although the occurrence in time of *H. axyridis* larvae did not differ in the two years. Consequently, larvae of *C. s. brucki* occurred earlier than those of *H. axyridis*.

When the aphid became extinct on 21st of June there were no larvae of *C. s. brucki* present, although 18% (N=26) of the larvae of *H. axyridis* were still present ($P < 0.05$). However, the percentage of *H. axyridis* larvae present when the aphid became extinct was about one-third of that observed in 1995 (59%, N=68) ($P < 0.001$, χ^2 test). That is, in this year, the decrease in *C. s. brucki* larvae was similar to that observed in 1995, all larvae disappeared before the aphid became extinct. In both years, although *H. axyridis* larvae were present when the aphid became extinct, larvae decreased in abundance faster in 1996 than in 1995.

(2) Development of larvae

The developmental stage of the larvae present at the time of the peak in aphid abundance was compared in the three species. In 1995, the percentages of each instar present did not differ significantly in the three species ($P > 0.05$); the percentages of second instar larvae were not less than 80% in all species (Fig. 2-13a). The earliest larva to pupate was one of *P. japonica,* which pupated a day before the aphids became extinct (Fig. 2-1, p. 10). However, this pupa was eaten two days after pupation by a third-instar larva of *H. axyridis*. Five larvae of *H. axyridis* pupated after the aphid became extinct. Subsequently, four became adult, and one was eaten by a conspecific fourth-instar larva. No pupae of *C. s. brucki* were observed. That is, in this year, although larvae of all three species developed simultaneously, only those of *H. axyridis* became adults.

In 1996, the percentages of each instar present differed significantly in the two species ($P < 0.05$), with the percentage of second instar larvae greater in *H. axyridis,* and that of the third and fourth instar larvae greater in *C. s. brucki* (Fig. 2-13b). In addition, the percentages of each instar of *C. s brucki* differed significantly in two years ($P < 0.05$), more mature larvae developed in 1996 than in 1995. However, in *H. axyridis*, the percentages of each instar present did not differ significantly between two years ($P > 0.05$), more young larvae were present in both years. Ten larvae of *C. s. brucki* pupated before the aphid became extinct (Fig. 2-2, p. 11), of which eight became adults and two were eaten by fourth-instar larvae of *H. axyridis*. Fourteen larvae of *H. axyridis* pupated after the aphid became extinct, of which nine became adults and five were cannibalized by fourth instar larvae. That is, in 1996, development of *C. s. brucki* larvae was advanced compared to that in 1995, although that of *H. axyridis* larvae did not differ in the two years. Consequently, in 1996, larvae of *C. s. brucki* developed earlier than those of *H. axyridis*, and larvae of both species became adults.

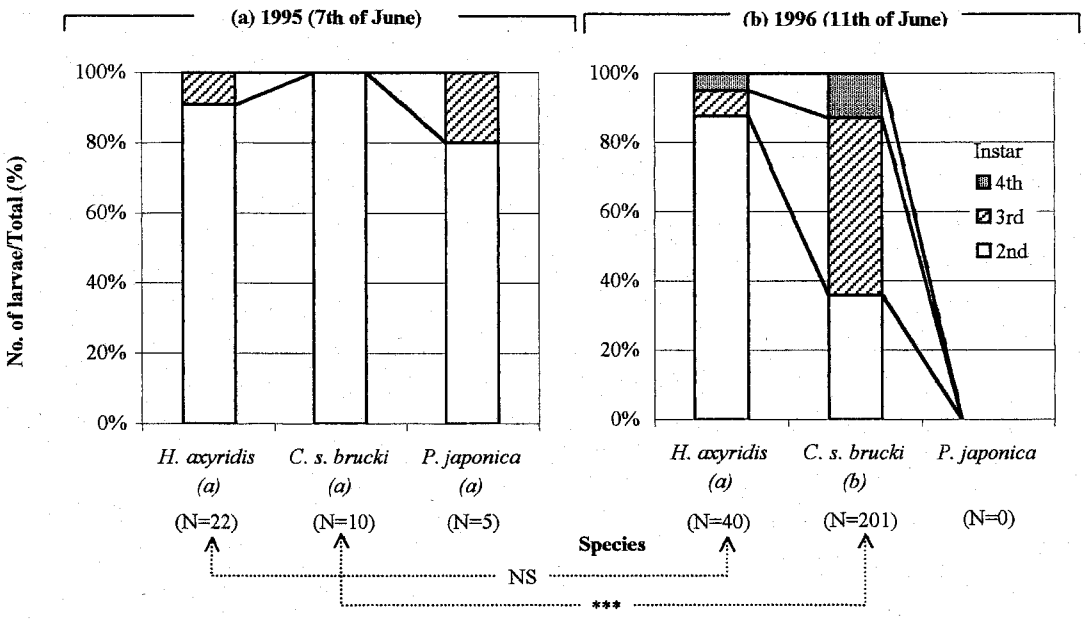


Figure 2-13. Instar composition of the larvae present when the aphid peaked in abundance in (a) 1995 and (b) 1996. (N=Total number of larvae when aphid peaked in abundance. Species followed by the same letter in parenthesis do not differ significantly in instar composition, $P > 0.05$: χ^2test. *** significant difference, $P < 0.001$ and NS no significant difference, $P > 0.05$ in two years: χ^2 test.)

(3) Survival of larvae

Initially in 1995, there were 474, 547 and 226 larvae of *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. In 1996, it was 1359, 1617 and 23 larvae of *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. Survival of these larvae, when they peaked in abundance and when they became adults were compared in the three species in both years.

In 1995, 24.5% (N=116), 8.4% (N=46) and 9.7% (N=22) of larvae of *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively, were present when they peaked in abundance (Fig. 2-14a). Percentage survival of *H. axyridis* larvae was more than double that of the other two species ($P < 0.05$). In 1996, the percentage of larvae present when they peaked in abundance was 11.4% (N=155), 14.2% (N=213) and 0%, respectively, for *H. axyridis*, *C. s. brucki* and *P. japonica*. Survival of *C. s. brucki* was significantly greater than that of the other two species ($P < 0.05$). In this year, the survival of *H. axyridis* larvae was less than half of that in 1995 ($P < 0.001$), while the survival of *C. s. brucki* larvae improved by 1.7 times compared with 1995 ($P < 0.001$). The survival of *P. japonica* larvae did not differ significantly in the two years ($P > 0.05$).

In 1995, the survival to the adult stage, expressed as the percentage of the hatchling larvae that became adults, was 0.8% (N=4) for *H. axyridis* (N=474), and 0% in the other two species (Fig. 2-14b). In 1996, the percentages were 0.7% (N=9) and 0.5% (N=8), respectively, for *H. axyridis* and *C. s. brucki* and 0% for *P. japonica*. In both years, the percentage survival to the adult stage did not differ significantly in the three species ($P > 0.05$), and did not exceed 1%. However, although the percentage survival of *C. s. brucki* in 1996, 0.7%, was greater than in 1995 (0.0%) ($P < 0.05$), it did not differ significantly in the other two species ($P > 0.05$).

(4) Emigration of larvae

Number emigrating

Emigration was expressed as the percentage of the larvae at the hatchling stage that emigrated. In

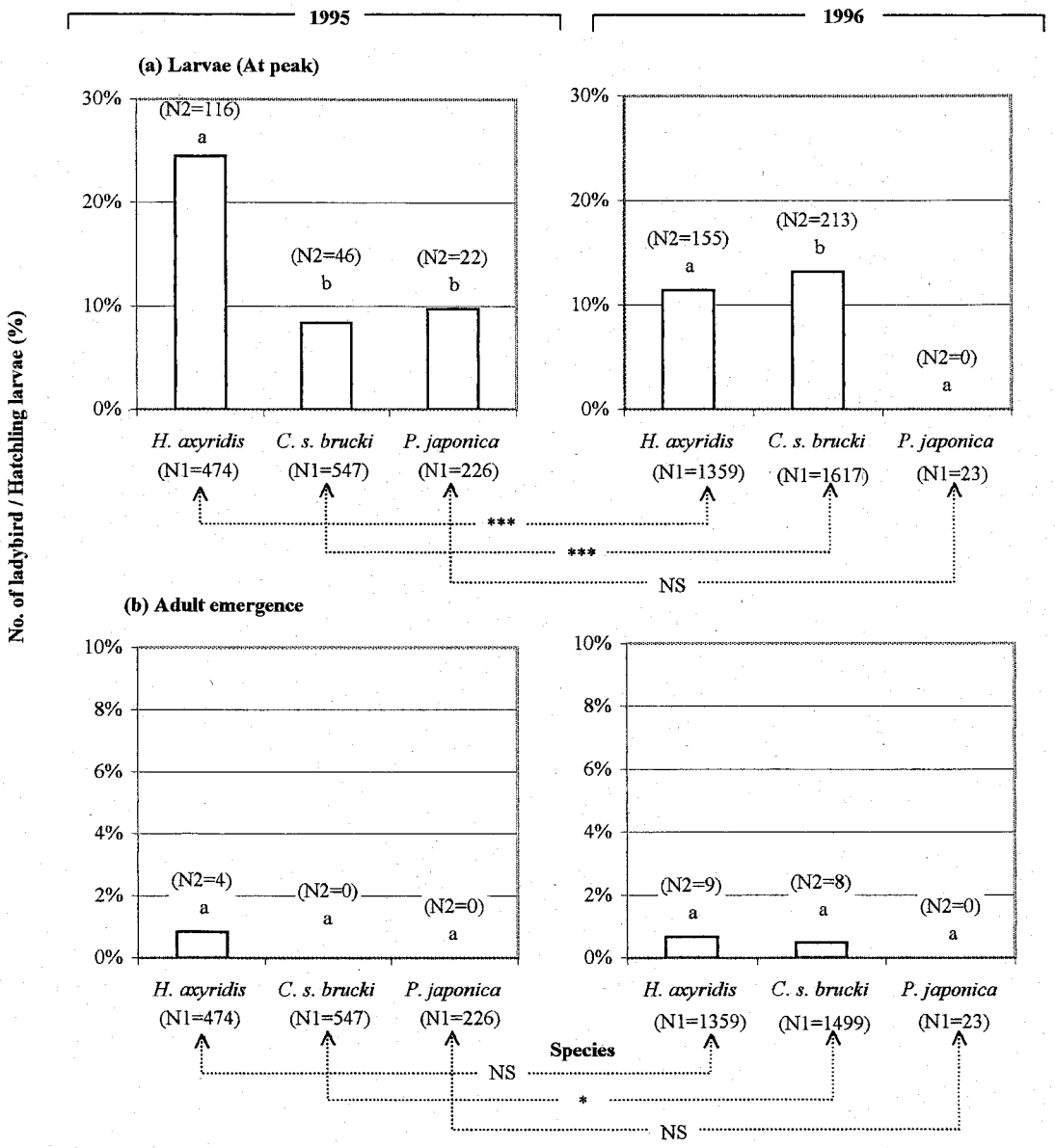


Figure 2-14. Percentage of (a) larvae present when they peaked in abundance and (b) at adult emergence in 1995 and 1996. (N1and N2 are total number of hatchling larvae and (a) larvae from 2nd instar onward when they peaked in abundance or (b) adults emerged. Histograms with the same letter do not differ significantly between species, $P > 0.05$: χ^2 test. *and *** significant difference at $P < 0.05$ and $P < 0.001$, respectively, and NS no significant difference at $P > 0.05$ between two years: χ^2 test.)

1995, 9.5% (N=45) of *H. axyridis* larvae emigrated, which was significantly greater than that for the other two species ($P < 0.05$) (Fig. 2-15a). In 1996, 21.3% (N=289) of the *C. s. brucki* larvae emigrated (Fig. 2-15b), which was significantly greater than for either of the other two species ($P < 0.05$). In both years, no larvae of *P. japonica* emigrated and emigration by this species was significantly lower than for the other two species ($P < 0.05$).

Time of emigration

In 1995, 45 and 27 larvae of *H. axyridis* and *C. s. brucki,* respectively, emigrated. Distribution in time of emigration in these species was compared relative to aphid abundance (Fig. 2-16a). The number of emigrants of *H. axyridis* tended to increase significantly with time ($P < 0.05$) with 87% (N=39) of the emigrants recorded after the aphid became extinct. In contrast, 93% (N=25) of emigrant *C. s. brucki* were observed during the period from the peak in aphid abundance to their extinction, and was significantly greater then than in other periods ($P < 0.05$).

In 1996, 289 and 367 larvae, respectively, of *H. axyridis* and *C. s. brucki* emigrated (Fig. 2-16b). As in 1995, the number of emigrants of *H. axyridis* tended to be greatest after the aphid became extinct (74%, N=213) ($P < 0.05$), and emigrants of *C. s. brucki* were mainly observed during the period when the aphid peaked in abundance and of their extinction ($P < 0.05$) (79%: N=291).

That is, in both years, larvae of *C. s. brucki* emigrated earlier than those of *H. axyridis*.

Instar composition

Percentages of the different larval instars that emigrated in *H. axyridis* and *C. s. brucki* were compared. In 1995, of the total (N=45) for *H. axyridis,* 2% (N=1), 33% (N=15) and 64% (N=29) were second, third and fourth instar larvae, respectively. In the same year, for *C. s. brucki,* it was 26% (N=7), 41% (N=11) and 33% (N=9) for second, third and fourth instars, respectively (Fig. 2-17a). Percentages of the three instars differed significantly in these two species ($P < 0.01$), the

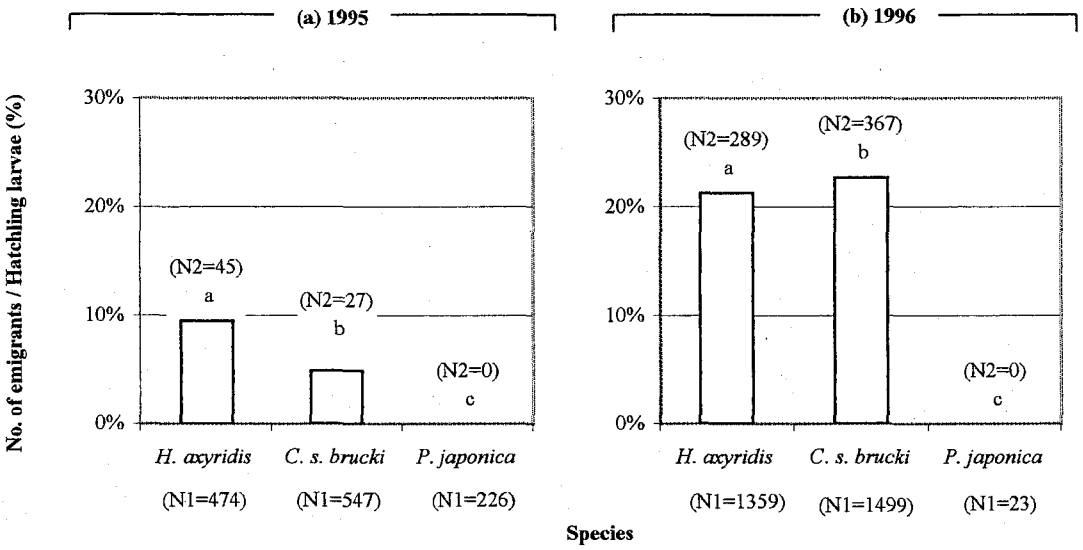


Figure 2-15. Percentage of larvae that emigrated in (a) 1995 and (b) 1996. (N1and N2 are the number of hatchling larvae and larvae that emigrated. Histograms with the same letter do not differ significantly between species, $P > 0.05$: χ^2 test.)

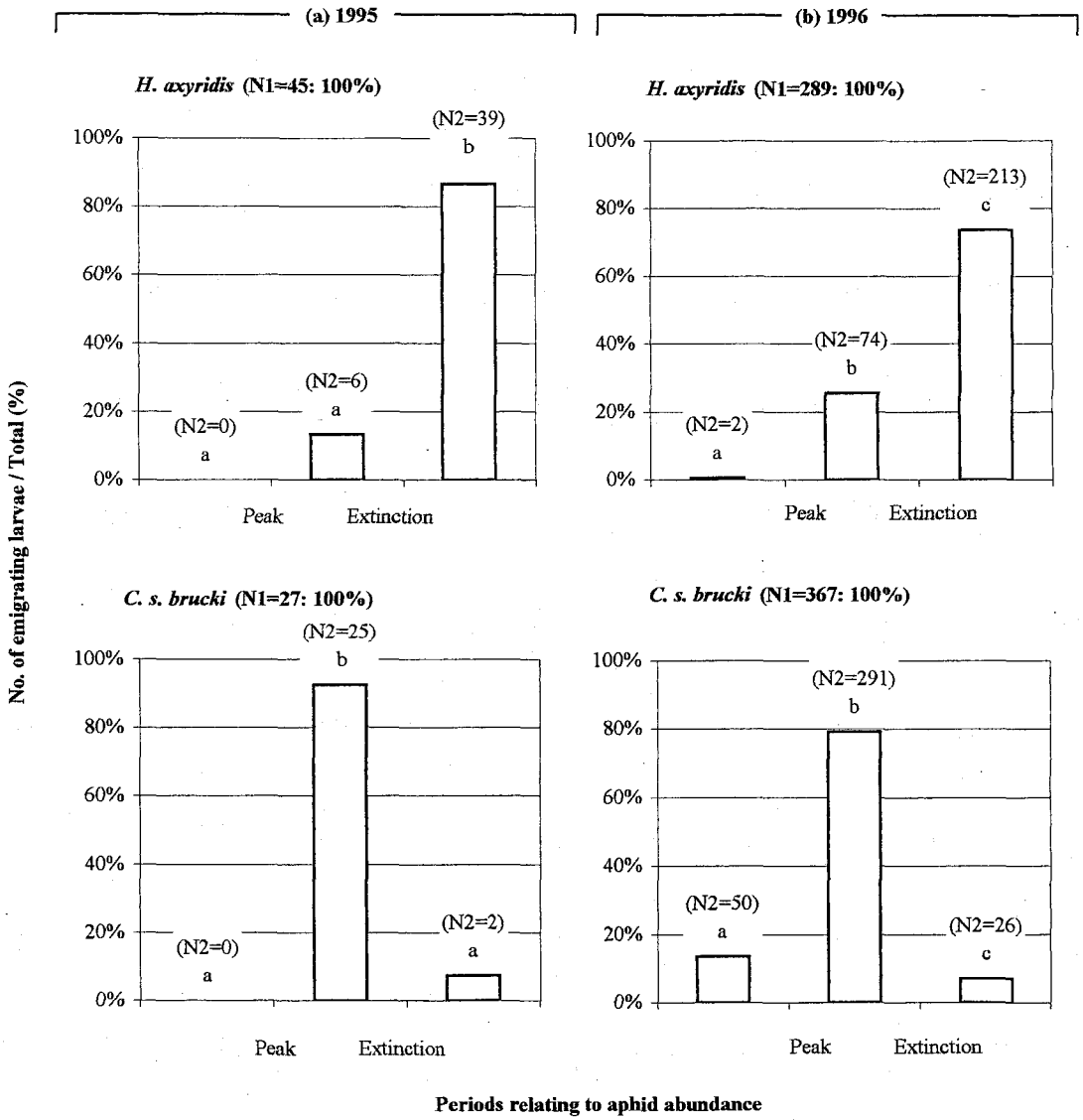


Figure 2-16. Percentage of the larvae that emigrated relative to aphid abundance in (a)1995 and (b)1996. (N1and N2 the number of larvae that emigrated in total and in each period, respectively. Histograms from each period with the same letter do not differ significantly, $P > 0.05$: χ^2test.)

percentages of fourth instar larvae tended to be higher in *H. axyridis* and that of second instar in *C. s. brucki*.

In 1996, the percentages of each instar that emigrated in *H. axyridis* was 13% (N=37), 34% (N=97) and 54% (N=155) for second, third and fourth instars, respectively (Fig. 2-17b). For *C. s. brucki*, it was 11% (N=41), 35% (N=127) and 54% (N=199) for second, third and fourth instars, respectively. In both species, the percentages emigrating tended to increase with instar, there was no significant difference in percentages of three instars between these two species ($P < 0.05$).

Percentages of each instar that emigrated were compared in the two years. In *H. axyridis*, the percentages of the three instars that emigrated did not differ significantly in the two years ($P > 0.05$). Instar composition of *C. s. brucki* larvae that emigrated differed significantly in the two years ($P < 0.05$), percentage of the fourth instar increased and of the younger two instars decreased in 1996.

(5) Aphid population dynamics

To determine whether the population dynamics of the aphid differed in the two years the percentages of aphids present before and after they peaked in abundance were compared between 1995 and 1996 (Fig. 2-18ab). Percentages present before the peak in aphid abundance were relatively higher in 1996 than in 1995 (Fig 2-18a). The number of aphids increased more rapidly in 1996 than in 1995. In contrast, percentage present over the three days following the peak in aphid abundance was significantly lower in 1966 than in 1995 ($P < 0.001$) (Fig. 2-18b). The number of aphids decreased more rapidly in 1996 than in 1995.

2-3-3 Dominance

In both years, the percentages of the aphidophagous guild made up of each the three species was compared throughout the developmental stages.

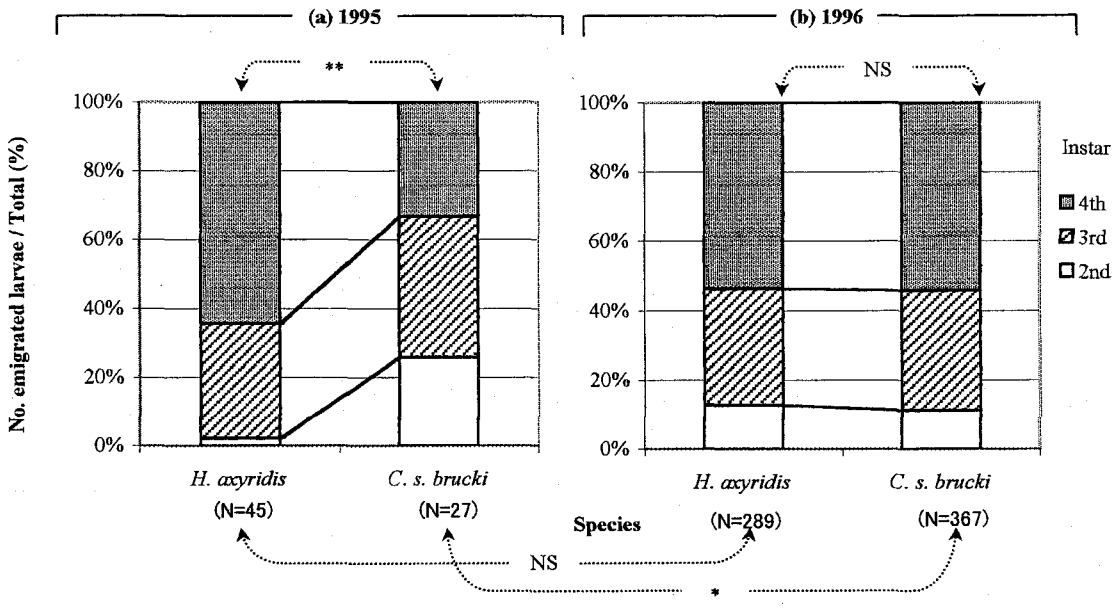


Figure 2-17. Instar composition of the larvae that emigrated in (a) 1995 and (b) 1996. (N=Total number of larvae that emigrated. * and ** significant difference between species or years, $P < 0.05$ and $P < 0.01$, respectively, and NS no significant difference, $P > 0.05$: χ^2 test.)

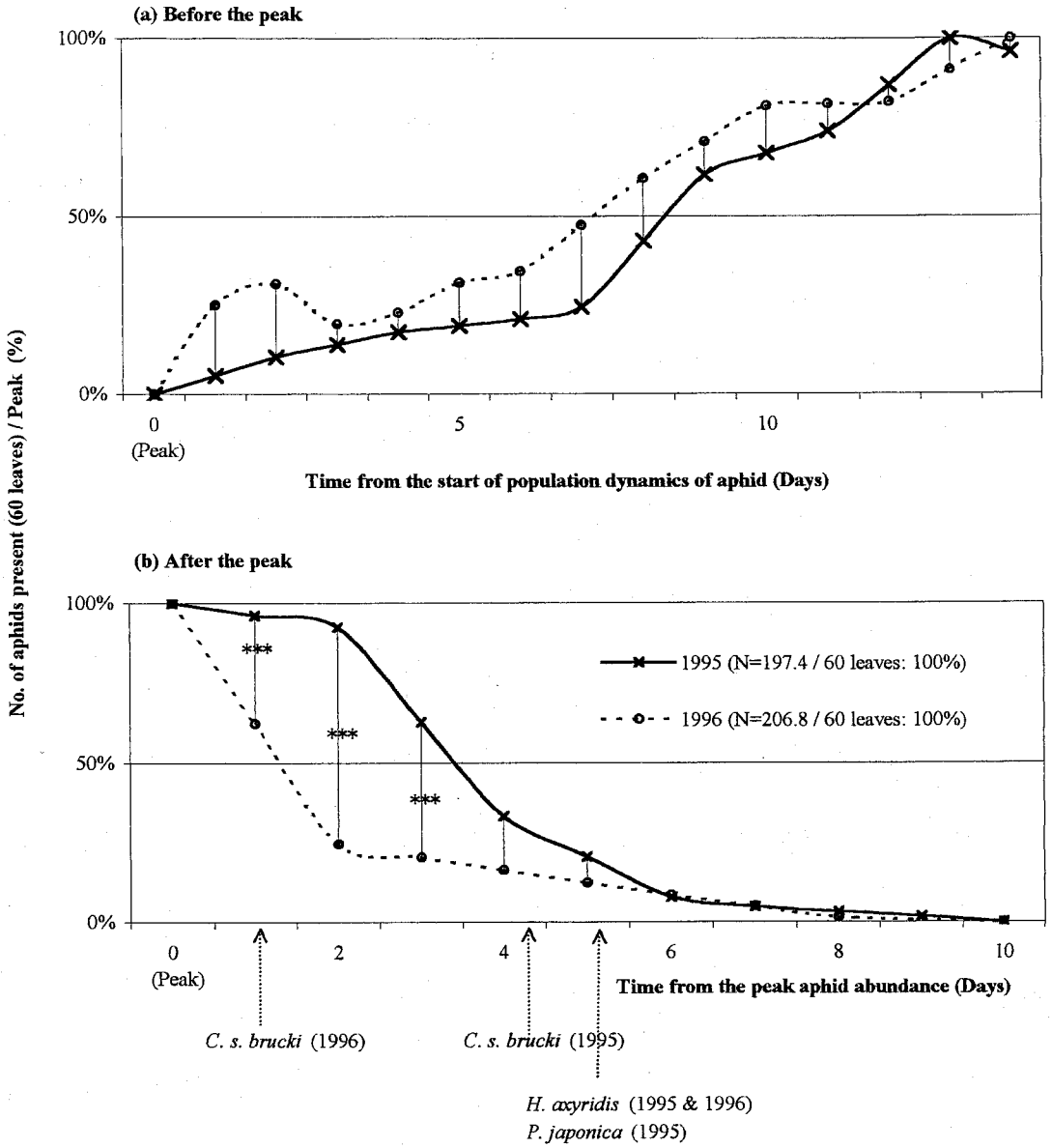


Figure 2-18. Percentages of aphids present each day (a) before and (b) after they peaked in abundance in 1995 and 1996. (Species names of ladybirds indicates when their larvae peaked in abundance. *** significant difference between years, $P > 0.001$: χ^2 test.)

(1) Guild structure in 1995

Egg stage

A total of 2862 ladybird eggs (100%) was recorded (Fig. 2-19a). Of these eggs, those of *H. axyridis* and *C. s. brucki* made up 39.4% (N=1133) and 38.5% (N=1105), respectively. That is, they laid a similar numbers of eggs ($P > 0.05$). The percentage of *P. japonica* eggs was 22.1% (N=634), less than that laid by the other two species.

Hatchling larval stage

The eggs gave rise to 1247 larvae (100%) (Fig. 2-19b). At this stage, the percentages of *H. axyridis*, *C. s. brucki* and *P. japonica* was 38.0% (N=474), 43.9% (N=547) and 18.1% (N=226), respectively. The percentage composition made up of *C. s. brucki* had increased by 5.4% compared to the egg stage, whereas that of *H. axyridis* and *P. japonica* had decreased by 1.4% and 4.0%, respectively. Consequently, at this stage, the percentages of each species had changed significantly from the egg stage ($P < 0.001$).

At peak in larval abundance

When the number of larvae peaked in abundance, there was a total of 184 larvae (100%) (Fig.2-19c). At this stage, the percentage of *H. axyridis*, *C. s. brucki* and *P. japonica* larvae was 63.0% (N=116), 25.0% (N=46) and 12.0% (N=22), respectively. The percentage of *H. axyridis* increased by 25% compared to the hatchling larval stage, and that of *C. s. brucki* and *P. japonica* decreased by 18.9% and 6.1%, respectively. Consequently, percentages of each species at this stage had changed significantly from that at the hatchling larval stage ($P < 0.0001$).

Adult stage

Four adults emerged; all of them were *H. axyridis* (Fig. 2-19d). Although percentages of each species at this stage did not differ significantly from that at the peak in larval abundance ($P > 0.05$), no larvae of *C. s. brucki* or *P. japonica* became adult. The guild structure changed

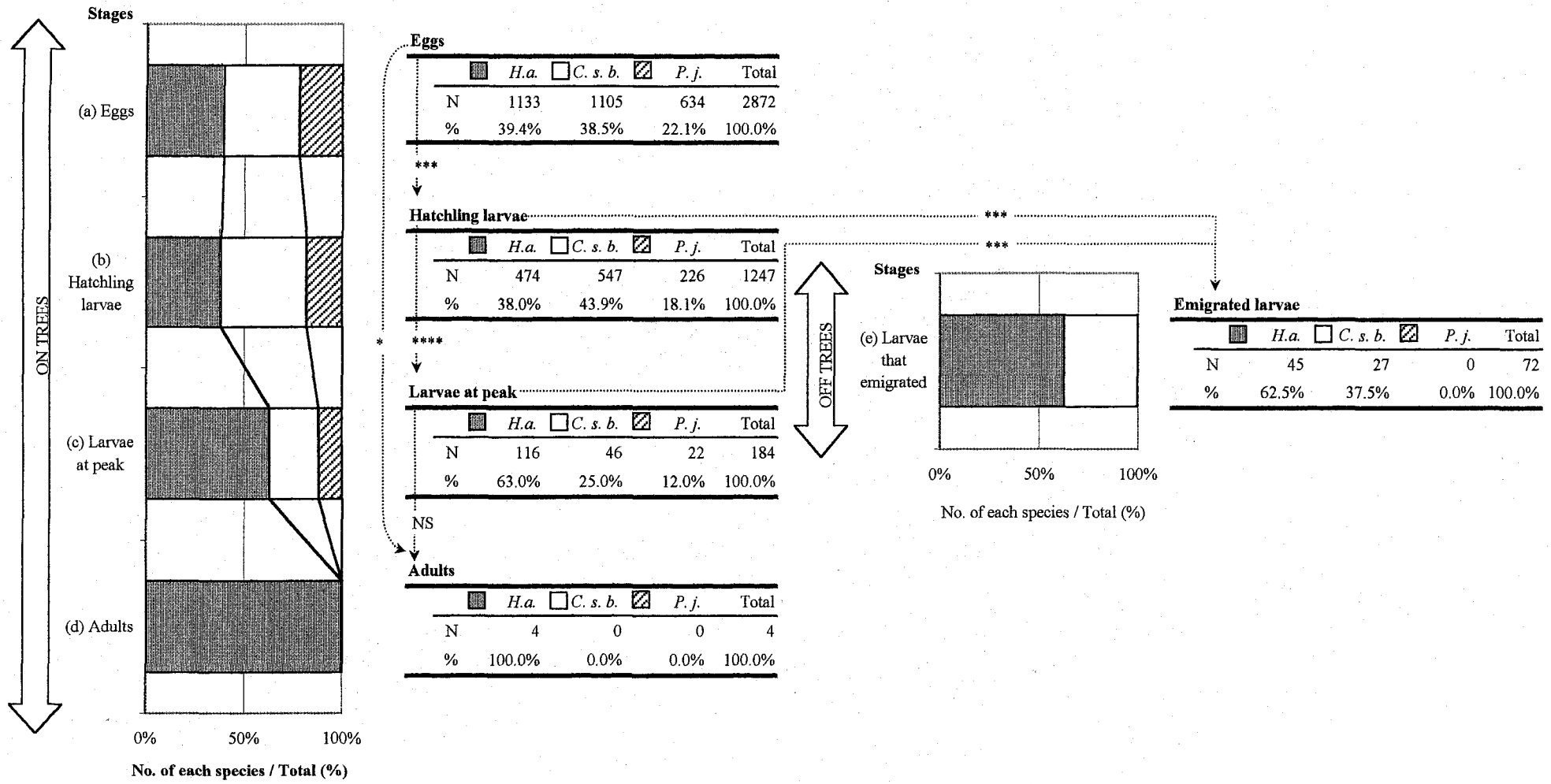


Figure 2-19. Percentage composition of the ladybird guild at the (a) egg, (b) hatchling larva, (c) peak larval abundance, (d) newly emerged adult stages and among the (e) larvae that emigrated in 1995. (*, **, ***, **** significant difference, $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively, and NS no significant difference, $P > 0.05$ between stages: χ^2 test.)

significantly during the course of the development of the ladybirds ($P < 0.05$), *H. axyridis* dominated the guild, and the other two species became extinct.

Of emigrating larvae

A total of 72 larvae emigrated (Fig. 2-19e). Of these, the percentage made up each species was 62% (N=45), 37.5% (N=27) and 0.0% (N=0) for *H. axyridis, C. s. brucki* and *P. japonica*, respectively. The percentage of *H. axyridis* increased by 23.1% compared to the composition at the egg stage on the trees, and the percentages of *C. s brucki* and *P. japonica* decreased by 1.0% and 22.1%, respectively. Consequently, the percentage of each species amongst the emigrating larvae was significantly different from that at the egg stage on the trees ($P < 0.0001$); off the trees, *H. axyridis* dominated and *P. japonica* was absent.

(2) Guild structure in 1996

Egg stage

In 1996, a total of 4512 eggs (100%) were laid (Fig. 2-20a). Of these eggs, those of *H. axyridis* and *C. s. brucki* made up 48.0% (N=2136) and 51.2% (N=2208), respectively; that is, they laid a similar number of eggs. However, the percentage made up of *P. japonica* eggs was only 0.8% (N=37).

Hatchling larval stage

The eggs gave rise to a total of 3001 larvae (100%) (Fig. 2-20b). Of these larvae, the percentages that were *H. axyridis, C. s. brucki* and *P. japonica* was 47.1% (N=1359), 52.0% (N=1499) and 0.8% (N=25), respectively. The percentage of *H. axyridis* decreased by 2.7% compared to the egg stage, and that of *C. s. brucki* increased by 2.7%. The percentage of *P. japonica* did not differ from that at the egg stage. Consequently, the percentages of each species at this stage had changed significantly compared to that at the egg stage ($P < 0.05$).

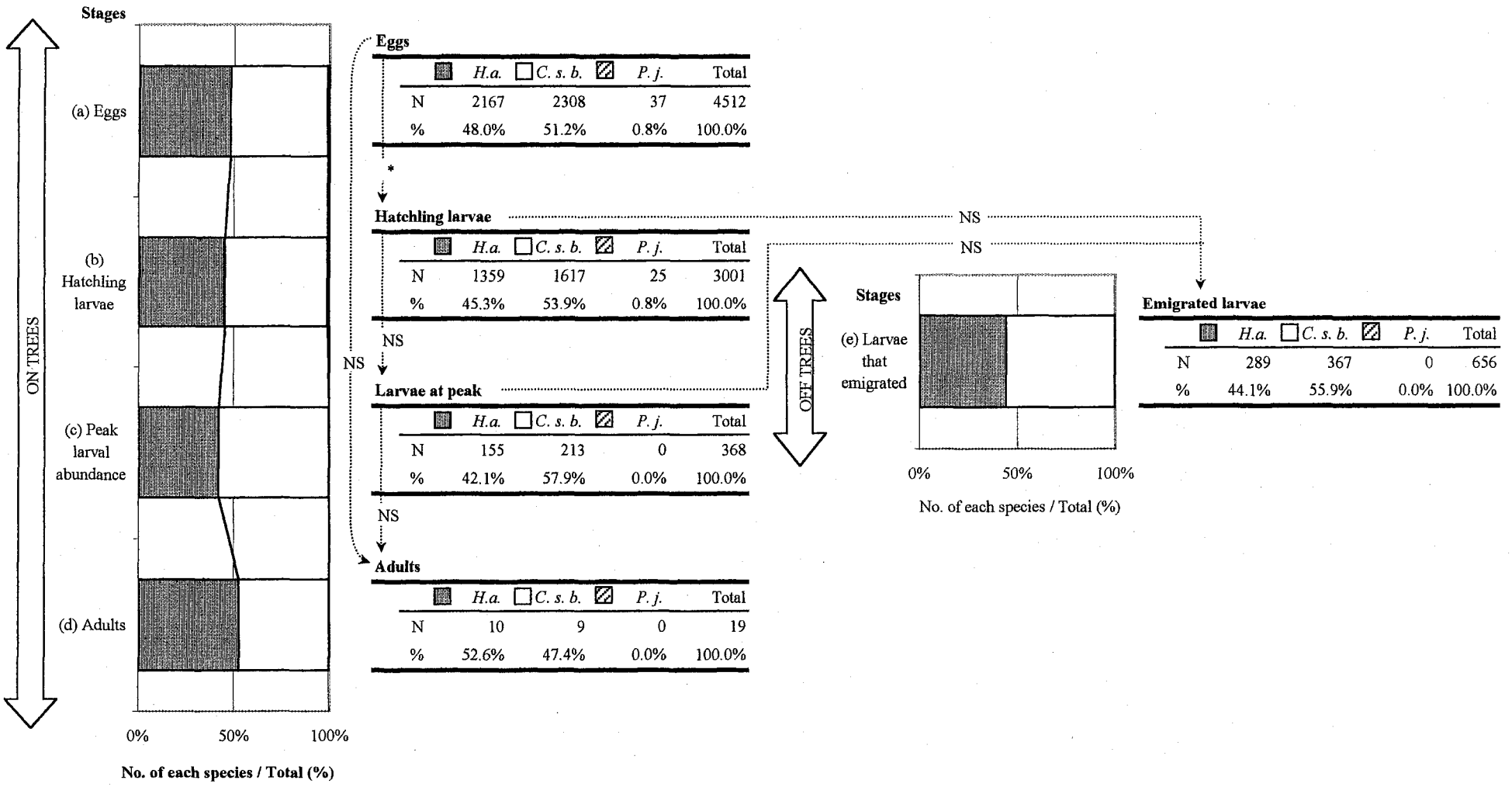


Figure 2-20. Percentage composition of the ladybird guild at the (a) egg, (b) hatchling larva, (c) peak larval abundance, (d) newly emerged adult stages and among the (e) larvae that emigrated in 1996. (* and ** show significant difference, $P < 0.05$ and $P < 0.01$, respectively, and NS no significant difference, $P > 0.05$ between stages: χ^2 test.)

At peak in larval abundance

When the total number of larvae peaked, there were 368 larvae (100.0%) (Fig. 2-20c). Of these larvae, those of *H. axyridis* and *C. s. brucki* made up 42.1% (N=155) and 57.9% (N=213), respectively. No larvae of *P. japonica* reached this stage. The percentage composition of *H. axyridis* decreased by 3.2% compared to the previous stage, and that of *C. s. brucki* increased by 4.0%. However, the percentage composition of each species did not differ significantly from that at the hatchling larval stage ($P > 0.05$).

Adult stage

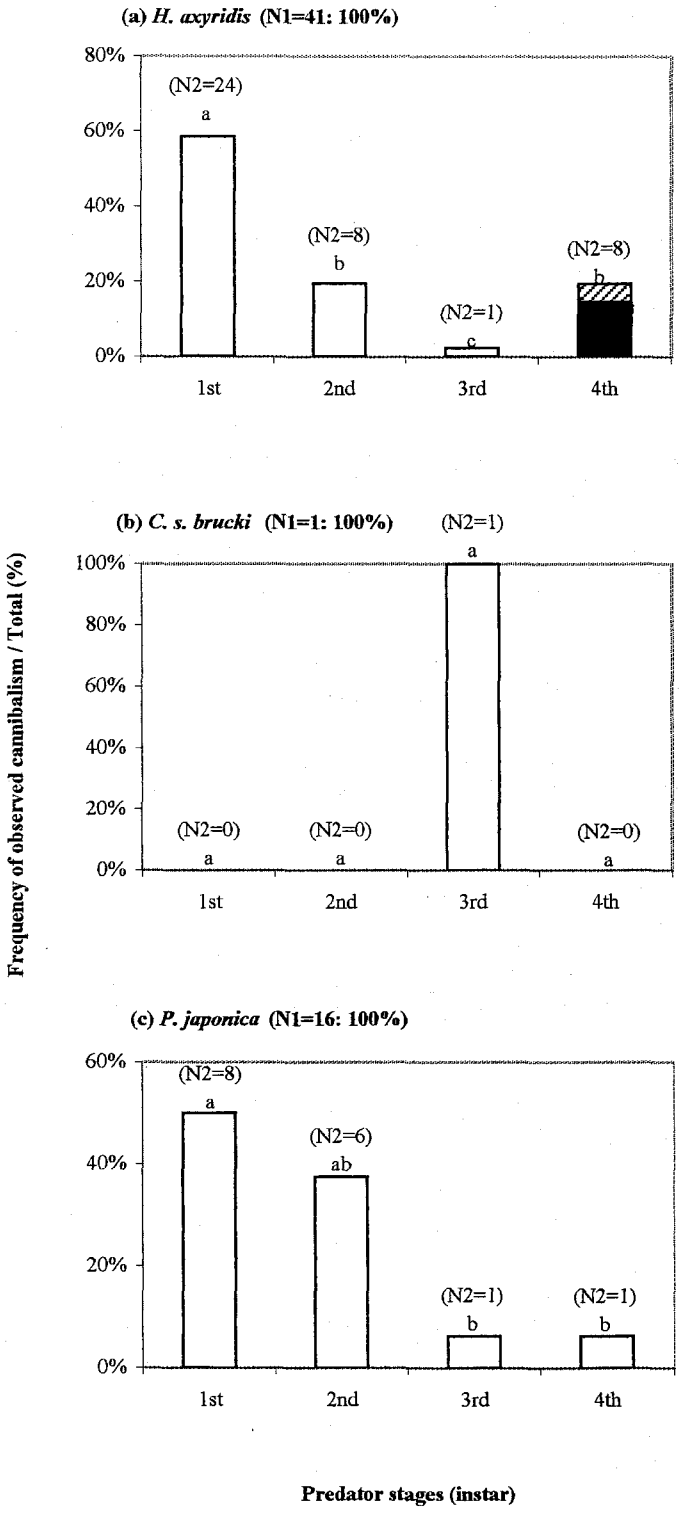
A total of 19 adults (100.0%) emerged (Fig. 2-20d). Of these adults, the percentages made up of *H. axyridis* and *C. s. brucki* were 52.6% (N=10) and 47.4% (N=9), respectively. The percentage composition at this stage did not differ significantly from that at the peak in larval abundance and the eggs stage ($P > 0.05$). That is, the guild structure did not change significantly from egg to adult.

Of emigrating larvae

A total of 656 larvae (100.0%) emigrated (Fig. 2-20e). Of these larvae, the percentage made up of each species differed significantly ($P < 0.05$), it was 44.1% (N=289), 55.9% (N=367) and 0.0% (N=0), respectively, for *H. axyridis, C. s. brucki* and *P. japonica*. The percentage composition of *H. axyridis* decreased by 3.9% compared to the egg stage on the tree, and that of *C. s. brucki* increased by 4.7%. No *P. japonica* larvae were observed emigrating. Consequently, the percentage composition of each species changed significantly from that at the egg stage on the tree ($P < 0.01$); the guild structure was co-dominated by *H. axyridis* and *C. s. brucki.*

2-3-4 Cannibalism and intra-guild predation of larvae

The different stages eaten by larvae, and their frequency of occurrence in each species in the two years are given in figure 2-21. Eggs were the most frequently observed conspecific prey for all



Prey stage	N	(%)
Eggs	33	(80.5%) a
Larvae (3rd instar)	2	(4.9%) b
Prepupae & pupae	6	(14.6%) b
Total	41	(100.0%)

Prey stage	N	(%)
Eggs	1	(100.0%)
Total	1	(100.0%)

Prey stage	N	(%)
Eggs	16	(100.0%)
Total	16	(100.0%)

Figure 2-21. Frequency of cannibalism by non-sibling larvae in 1995-1996. (N1and N2, respectively, show frequency of observation. Histograms or proportions in parenthesis topped or followed by the same letter do not differ significantly between instars or prey stages, $P > 0.05$: χ^2 test.)

species of larvae. In *H. axyridis* and *P. japonica*, most egg cannibalism was by young larvae, especially the first instar. Egg cannibalism in *C. s. brucki* was observed only once, and involved a third instar larva. Larval (3^{rd} instar) or pupal cannibalism were recorded only in *H. axyridis*, where the cannibal was a fourth instar larva in both cases.

The intra- or extra-guild prey, other than aphids and conspecific prey, consumed by ladybird larvae, and the frequency they were observed eating these prey items in the two years are given in Figure 2-22. The number of such prey eaten by each of the ladybirds varied. *H. axyridis* was the most voracious, and was observed eating insects other than aphids on 28 occasions, made up of eight species, including two extra-guild species. The highest frequency of predation was recorded for the fourth instar larvae, but all instars indulged in intra-guild predation. Of the eight prey species, *P. japonica* was most frequently eaten by *H. axyridis*, although very few were present in 1996. In *C. s. brucki*, intra-guild predation was observed twice, in the third instar. In *P. japonica*, neither intra- or extra-guild predation were observed.

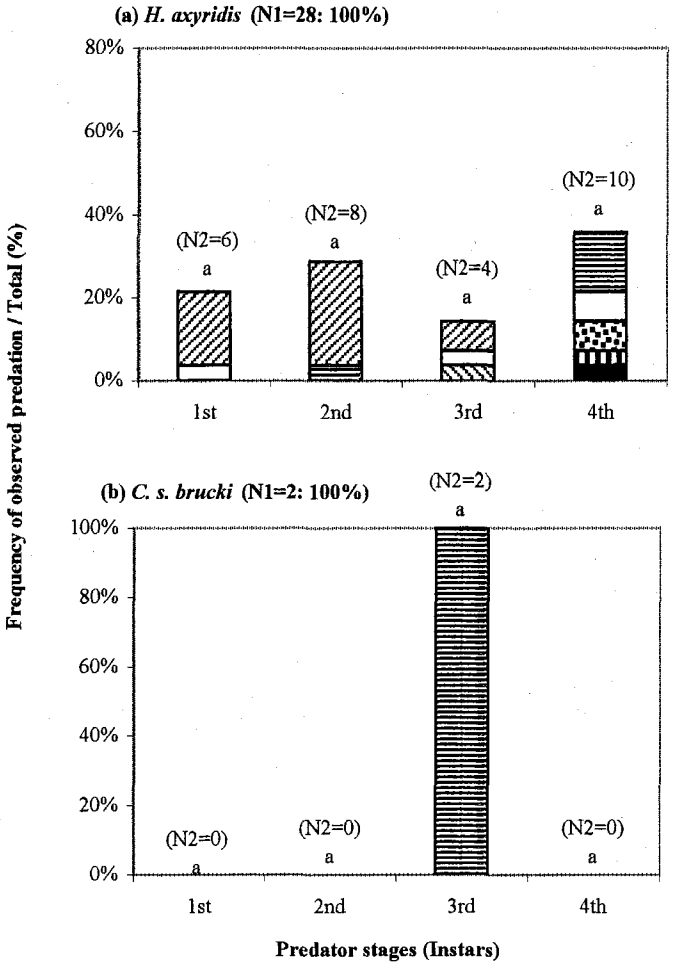
2-4 DISCUSSION

(1) Mortality of eggs

As causes of mortality during the egg stage, both sibling cannibalism (e.g., Dixon, 1959; Kawai, 1982; Kawauchi, 1985; Osawa, 1989) and non-sibling cannibalism (e.g., Banks, 1955; Mills, 1982; Osawa, 1989) have been reported. In the present study, other than these causes of mortality, eggs also disappeared or were destroyed. The incidence of these three causes of mortality varied in time and between species. In the first part of the discussion, the factors that affect these causes of mortality, and the consequences for guild structure during the egg stage, are considered.

Sib cannibalism

Sibling cannibalism is a consequence of asynchronized hatching and to presence of infertile eggs (Osawa, 1989). In general, the incidence of sib cannibalism was more marked early in



Intra- or extra-guild prey species	(Stage)	N	(%)
P. japonica	(Eggs and pupa)	14	(50.0%) a
Syrphidae	(Larvae)	5	(17.9%) b
C. s. brucki	(Eggs and prepupa)	4	(14.3%) b
Lepidoptera*	(Larvae)	2	(7.1%) b
Chrysopidae	(Eggs)	1	(3.6%) b
C. silvestri	(Prepupa)	1	(3.6%) b
S. hoffmanni	(Larva)	1	(3.6%) b
Total		28	(100.0%)

Intra- or extra-guild prey species	(Stage)	N	(%)
Syrphidae	(Larvae)	2	(100.0%)
Total		2	(100.0%)

Figure 2-22. Frequency of predation on prey other than aphids and conspecifics in 1995-1996. (N1and N2, respectively, show frequency of observation. Histograms or proportions in parenthesis topped or followed by the same letter do not differ significantly between instars or prey species, $P > 0.05$: χ^2 test. * Lepidoptera larvae were dead before being eaten by ladybirds.)

oviposition, when temperature was relatively low. In some ladybirds, each cluster of eggs contains both fast and slow developing individuals (Section 4). In addition, the incidence of sib cannibalism is correlated with the delay between the hatching of the first and the last eggs in a cluster (Kaddou, 1960). Therefore, it is suggested that at low temperature there are large differences in duration of development of the eggs in a cluster, and as a consequence the incidence of sib cannibalism is likely to be high.

The incidence of sib cannibalism differed between the three species of ladybirds, which also differed in the size of the clusters of eggs they produced. However, there is no correlation between the incidence of sib cannibalism and cluster size (Kaddou, 1960; Dixon & Guo, 1993). The difference in the incidence of sib-cannibalism is likely to be due to the male-killer disease, which is known to vary in incidence between populations both within and between species (Majerus & Hurst, 1997).

In general, egg mortality due to sib cannibalism was relatively low compared to the other two causes of mortality, and difference in incidence in sib cannibalism between species was small. Therefore, the effect this kind of mortality has in changing guild structure during the egg stage is likely to be less important than the other two causes of mortality.

Disappearance or destruction

Takahashi (1995) reported that *C. s. brucki* occasionally lays eggs on dried leaves and stones on the ground, where it is relatively warm in early spring. In the present study, early in the oviposition period when temperature was relatively low eggs were occasionally found inside dried remains of flowers from the previous year. The remains of the flowers were located at the tips of the branches, and therefore the inside of these flowers could be warmer due to the heating effect of the sun. However, as the remains of the flowers are only weakly attached to branches they are easily dislodged by wind. In addition, even eggs laid on leaves, are more likely to

disappear or be destroyed if the duration of the egg stage is prolonged. In the present study, egg mortality due to disappearance or destruction was higher in 1995, when temperature was relatively low, than in 1996, when temperature was relatively high. As high winds increase this cause of mortality, factors that increase the duration of incubation are also likely to increase the frequency of this kind of mortality. In both years, the incidence of this form of egg mortality was higher in *C. s. brucki* than in *H. axyridis* and *P. japonica*. That is, *C. s. brucki* is more adversely affected by this form of mortality than the other species. However, overall survival of *C. s. brucki* eggs tended to be higher than that of the other two species. Therefore, this is unlikely to be an important mortality factor determining guild structure during the egg stage.

Non-sib cannibalism or I. G. P.

Non-sib egg cannibalism in *C. s. brucki* is rare if the larvae are provided with an abundance of aphids (Takahashi, 1987), however, *H. axyridis* larvae prefer their own eggs to aphids (Kawai, 1978). In the present study, this form of cannibalism was frequently observed in *H. axyridis* and *P. japonica*, but rarely in *C. s. brucki*. Therefore, it appears that the larvae of both *H. axyridis* and *P. japonica* are more cannibalistic than those of *C. s. brucki*. The larvae of *H. axyridis* are polyphagous (Hodek & Honek, 1988). In the present study, intra-and extra guild predation by larvae of *H. axyridis* was frequently observed, but rarely for larvae of *C. s. brucki* and *P. japonica*. That is, larvae of both *C. s. brucki* and *P. japonica* appear to be less polyphagous than those of *H. axyridis*.

Agarwala & Dixon (1992) showed that the eggs of some species of ladybirds appear to be more protected chemically from predation than those of other species. The eggs of *H. axyridis* are well protected from predation. The eggs of *H. axyridis* appear to be highly toxic for *C. s. brucki* (Section 3). In addition, no larvae of *P. japonica* were observed eating eggs of *H. axyridis* in the present study. Therefore, it is likely that the eggs of *H. axyridis* are chemically protected from

predation by the larvae of both these species. That is, although *H. axyridis* laid most of its eggs late on in the development of the aphid population mortality was mainly due to sib cannibalism, or they disappeared or were destroyed, and non-sib cannibalism, than to I. G. P. by larvae of the other two species.

In *C. s. brucki*, when the oviposition period was relatively long (1995) a high incidence of egg mortality due to non-sib cannibalism or I. G. P. were recorded. The presence of a density dependent species-specific oviposition-deterring pheromone, produced by conspecific larvae, causes ladybirds to cease laying eggs (Dixon 2000). In 1995, the abundance of *C. s. brucki* larvae was relatively low. Therefore, it is assumed that the deterrent effect of the conspecific larvae was weak, and resulted in oviposition ending later. In contrast, the abundance of *C. s. brucki* larvae was relatively high in 1996, and the assumed deterrent effect of conspecific larvae relatively strong, which resulted in an early end to oviposition. Consequently, in this year, oviposition ceased before the increase in *H. axyridis* larvae, and as a consequence there was a low incidence of I. G. P. by *H. axyridis* larvae. As *P. japonica* is not regarded as a polyphagous species, and *C. s. brucki* is regarded as less cannibalistic than *H. axyridis*, i.e. the eggs of *C. s. brucki* as well as suffering from sib cannibalism, disappearance/destruction are also likely to suffer from I. G. P. by larvae of *H. axyridis*. In general, as *C. s. brucki* tended to lay more of its eggs early in the oviposition period than either of the other two species, the incidence of I. G. P. it suffers is likely to be low. Consequently, the decrease in abundance of this species during the egg stage was the smallest of the three species in this ladybird guild.

The eggs of *P. japonica* were frequently eaten by the larvae of *H. axyridis* and conspecific larvae, but not by *C. s. brucki*. That is, the egg mortality in *P. japonica* in addition to that due to sib cannibalism and disappearance/destruction is likely to be from both non-sib cannibalism and I. G. P. by larvae of *H. axyridis*. In addition, as their oviposition period continued after conspecific

and *H. axyridis* larvae peaked in abundance, the incidence of egg predation in this species was higher than in the other two species. Consequently, the decrease in abundance of this species during the egg stage was the largest for the three species.

The incidence of non-sib egg cannibalism in the middle and later part of the oviposition period is mainly due to the high density of conspecific larvae relative to that of aphids (Osawa, 1992), and the large size and mobility of the larvae at that time (Dixon, 2000). In the present study, when the survival of *H. axyridis* larvae was low and aphids abundant (1996), egg mortality due to non-sib cannibalism or I. G. P. was low for all species compared to 1995, when there was a good survival of *H. axyridis* larvae and aphid abundance was low. Therefore, in all species, egg mortality due to factors other than sib cannibalism, or disappearance/destruction was affected by the relative abundance of *H. axyridis* larvae during the oviposition period. In addition, the incidence of egg mortality due to non-sib cannibalism or I. G. P. was inversely related to species survival. Therefore, this form of egg mortality is likely to be the most effective factor changing the structure of this ladybird guild during the egg stage, and the relative abundance of *H. axyridis* larvae is likely to be the key factor determining the incidence of this mortality.

(2) Factors determining the decrease in larval abundance

I. G. P. is often reported in insects, and prey abundance appears to be an important factor determining its frequency, and is thought to be an important force structuring insect communities (Polis *et al*, 1989; Dong & Polis, 1992). In the present study, the structure of a ladybird guild changed markedly during the larval period, when all species occurred simultaneously (1995), but less so when their occurrence is not well synchronized (1996). That is, the frequency of I. G. P. differed in the two years. In this part of the discussion, the effect of cannibalism, I. G. P. and emigration, on changes in guild structure are considered.

Cannibalism and I. G. P.

As all stages of ladybirds contain similar concentrations of alkaloids (Pasteels *et al.*, 1973), it can be assumed that larvae of *H. axyridis* are also protected chemically form predation by larvae of *C. s. brucki* and *P. japonica*. That is, as in the egg stage, it is suggested that the mortality of *H. axyridis* larvae is more likely to be due to cannibalism than I. G. P., and that of larvae of *C. s. brucki* and *P. japonica* to both cannibalism and I. G. P.. In general, the larger predator is likely to be the intra-guild predator, and the smaller the extra-guild prey (Sengonca & Frings, 1985; Lucas *et al.*, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001). All stages of *C. s. brucki* are similar in size to those of *H. axyridis*, and all stages of these two species are larger than those of *P. japonica*. Therefore, in this ladybird guild, *P. japonica* larvae are more likely to be the intra-guild prey of *H. axyridis* larvae than are *C. s. brucki* larvae. In addition, the morphology of *H. axyridis* larvae differs from that of *C. s. brucki*; their spiny back and large anal disc enable them to defend themselves against predators, and their large mandibles make it easier for them to catch intra-guild prey. Therefore, although these two species of ladybirds are similar in size, *C. s. brucki* larvae are an easier prey for *H. axyridis* than are conspecific larvae.

In 1995, although aphid abundance was relatively low, the percentage survival of *H. axyridis* larvae was high. In this year, there was more *P. japonica* larvae than in 1996. In addition, as all the species were developing simultaneously and each consisted of several stages of larvae, both young and/or small *C. s. brucki* larvae were available as intra-guild prey for the older and/or larger *H. axyridis* larvae. That is, in this year, although prey abundance was low for larvae of both *C. s. brucki* and *P. japonica*, the abundance of intra-guild prey for the larvae of *H. axyridis* was high. Consequently, the frequency of asymmetric I. G. P. of larvae of *C. s. brucki* and *P. japonica* by larvae of *H. axyridis* increased, and *H. axyridis* dominated the ladybird guild on the trees in 1995.

In contrast, in 1996, although aphid abundance was relatively high, the survival of *H. axyridis* larvae was lower than in 1995. In this year, there were also initially no *P. japonica* larvae that could be exploited as intra-guild prey by *H. axyridis*. In addition, the early occurrence of *C. s. brucki* resulted in them being more advanced developmentally than the *H. axyridis* larvae. Therefore, there were very few small larvae of *C. s brucki* that could be exploited as intra-guild prey by *H. axyridis* larvae. That is, in this year, prey abundance including intra-guild prey for *H. axyridis* larvae was low. In addition, as most *C. s. brucki* larvae occurred earlier than those of *H. axyridis*, they monopolized the aphids before the *H. axyridis* larvae increased in size and abundance. Consequently, the survival and development of *C. s brucki* was improved, whereas that of *H. axyridis* larvae was depressed, and both species were equally dominant on the trees in 1996. However, only a small number of larvae completed their development on the trees in both years. As a relatively large number of emigrants were observed in both years, it is necessary to know the fate of these larvae, in order to better understand the changes in guild structure in ladybirds.

Emigration

Emigration is dependent on prey abundance (Yasuda & Shinya, 1997), and the leaving rate differs between species (Schellhorn & Andow, 1999). In the present study, *C. s. brucki* emigrated earlier than *H. axyridis*, and emigration in both species occurred after the aphid declined in abundance. In addition, in both species, young larvae emigrated earlier than older larvae. As there were no trees or plants with high numbers of aphids at the study site at the time of emigration, survival of emigrants is likely to be low. However, 20% of the larvae pupated after feeding for one day after moulting to the fourth instar (Personal observation). Therefore, it is likely that some of the emigrants completed their development, and the probability increased with the maturity of emigrants. In some species of ladybird, it is reported that 90% of the larvae leave a host plant

prior to pupation (Lucas *et al.*, 2000), and the leaving rate differs between species (Schellhorn & Andow, 2000). In addition, Osawa (1992) showed that the fourth instar larvae that prior to pupation moved furthest away from an aphid colony, were the most likely to survive. That is, it is suggested that emigration not only enables them to search for prey on another host plant, but also enabled them to avoid cannibalism or I. G. P.. Therefore, the percentage of larvae that emigrate should be lower in species A that is eaten frequently by other species than in species B that is eaten rarely. In the present study, when the ladybirds occurred simultaneously (1995) the percentage of larvae of *H. axyridis* that emigrated was higher than of *C. s. brucki*. In addition, the emigrants of *H. axyridis* were more mature than those of *C. s. brucki*. Therefore, the likelihood of the emigrants surviving was probably higher in *H. axyridis* than in *C. s. brucki*.

When *C. s. brucki* occurred and developed earlier than *H. axyridis* (1996), the percentage of larvae of *C. s. brucki* that emigrated was higher than of *H. axyridis*. In this year, more *C. s. brucki* emigrated than *H. axyridis*. Therefore, although, the maturity of the emigrants was similar in both species, more emigrants of *C. s. brucki* were likely to survive than *H. axyridis*. In both years, no emigrants of *P. japonica* were observed; frequency of the larvae of this species being eaten was likely to be highest in this ladybird guild. Overall, the hypothesis that the species that are most vulnerable should show the highest incidence of emigration is not supported by the above, especially in the case of *P. japonica*.

That is, when the frequency of I. G. P. by *H. axyridis* larvae appears to be high the structure of the ladybird guild changed markedly. Therefore I. G. P. by *H. axyridis* larvae is likely to be an important force structuring this ladybird guild. However, in this section, although I. G. P. and emigration are assumed to be important factors causing the decrease in larval abundance, actual losses due to these factors are poorly understood. Therefore, in Section 3, the effects of I. G. P. and emigration on larval survival are studied further.

(3) Conclusion

In both the egg and larval stages, i.e., during the whole development, I. G. P. is likely to be an important force structuring ladybird guilds, and the occurrence of larvae in time and the timing of oviposition appear to be key factors in the survival of ladybirds. In this part of the discussion, the occurrence of *P. japonica* and the timing of oviposition by *C. s. brucki* in the two years were discussed.

In several species of ladybirds that have overlapping habitat preferences, the smaller species appear to be better protected against predation by larger species (Agarwala & Dixon, 1992; Agarwala *et al.*, 1998; Hemptinne *et al.*, 2000). If generally true then it may imply that the main habitat of *P. japonica* is different from that of *H. axyridis*, as they were the most frequent intra-guild prey of *H. axyridis* in the present study. In fact, several studies show that *P. japonica* is abundant in field crops (e.g., Kawauchi, 1990). Aphids occur first on trees, and then on field crops. Occurrence of aphids is strongly affected by temperature and precipitation (Moritsu, 1954; Nozato & Abe, 1988), and increase in aphid density is positively affected by increase in temperature (Powell & Parry, 1976). Therefore, it is suggested that aphid development in the main habitat of *P. japonica* was delayed in 1995, when temperature was relatively low. This delay in the development of their preferred aphid prey may have made the aphids on trees more attractive to *P. japonica*, which is not its main habitat. Takahashi (1995) reported that high temperatures induce oviposition in *C. s. brucki* even when aphid abundance is low. Therefore, low temperatures early in the oviposition period (1995) may have delayed oviposition by *C. s. brucki*. Consequently, although, the occurrence and oviposition period of *H. axyridis* did not differ in the two years, its survival was affected by the occurrence of *P. japonica* and the timing of oviposition of *C. s. brucki*.

SECTION 3

IS *HARMONIA AXYRIDIS* A TOP PREDATOR?

3-1 INTRODUCTION

Larvae of *H. axyridis* are polyphagous (Hodek & Honek, 1988). Several authors report that the larvae of *H. axyridis* feed on and complete their development on other species of ladybird (e.g. Cottrell and Yeargan, 1998; Phoofolo & Obrycki, 1998; Yasuda & Ohnuma, 2000). In the field, larvae of *H. axyridis* appeared to be more polyphagous and voracious than those of the other two species, *C. s. brucki* and *P. japonica*, and dominated the ladybird guild in 1995 (Section 2). Therefore, it is suggested that asymmetric I. G. P. by *H. axyridis* may result in it dominating ladybird guilds, i.e., it is a top predator. In general, the incidence of cannibalism or I. G. P. by larvae is affected by the relative abundance of larvae to prey (Takahashi, 1987; Agarwala & Dixon, 1991, 1992; Yasuda & Shinya, 1997). However, the rates at which larvae leave plants differs between species (Schellhorn & Andow, 1998). In addition, the tendency of larvae to disperse when prey is scarce may further reduce the probability of encountering con- and heterospecific larvae on a plant (Winder, 1990). That is, in the field, the tendency of *C. s. brucki* larvae to emigrate early (Section 2) is likely to reduce the probability of it encountering the "top predator", *H. axyridis*, and so decrease its mortality due to I. G. P.. Therefore, for a better understanding of the affect of I. G. P. on the structure of ladybird guilds, it is necessary to determine the relationship between larval emigration and survival.

Several studies show that *H. axyridis* occurs relatively late compared to *C. s. brucki* (Takahashi, 1987; Yasuda & Shinya, 1997), with the oviposition peak of *H. axyridis* occurring when aphid abundance is relatively high (Section 2). As hatchling larvae have limited powers of dispersal, it is suggested that laying eggs when aphids are abundant is advantageous as it improves the survival of the hatchling larvae. However, eggs and young larvae are vulnerable to predation by larger predators (Sengonca & Frings, 1985; Lucas *et al.*, 1997, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001), and any delay in oviposition is likely to result in a high

incidence of egg or larval predation by the species of ladybirds that oviposit first, as they are likely to be more advanced developmentally. Therefore, if *H. axyridis* is adapted to oviposition late in the development of an aphid population, then, its eggs and larvae should be protected from I. G. P. by species like, *C. s. brucki*. In some species of ladybirds, species-specific alkaloids are thought to protect them against intra-guild predators (e.g. Agarwala & Dixon, 1998). Therefore, it is suggested that *H. axyridis* is also likely to be protected chemically against predation by *C. s. brucki*.

In this section, the effect of the presence of other species on the incidence of I. G. P. and emigration is assessed by factorial experiments, using a combination of three species of ladybird; *H. axyridis*, *C. s brucki* and *P. japonica*. In addition, the toxicity of the two co-occurring species of ladybird, *H. axyridis* and *C. s. brucki* to one another (Section 2), is also determined.

3-2 MATERIALS AND METHODS

(1) Ladybird larval survival in, and emigration from mixed species populations feeding on aphids on trees

The effect of other species of ladybird on larval survival and emigration was studied in a greenhouse. Young trees of *Hibiscus syriacus*, 70 cm in height, were planted singly in flowerpots (20 cm in depth × 24 cm in diameter) and each fixed to a steel stake, 60 cm in length. To trap larvae that dropped from each tree a rectangular tray (10 cm in depth × 40 cm in width × 70 cm in length) filled with water was placed under each flowerpot. To trap emigrating larvae the inside of the rim of the flowerpots was sprayed with a sticky material.

Each tree was infested with cotton aphids, *A. gossypii* on several occasions. This was done by placing leaves infested with the aphid on the trees. The experiment was started when the number of aphids on each tree reached seven hundred. There were two treatments: single and mixed species, using a combination of species. Nine larvae of one of three species, *H. axyridis*, *C.*

s. brucki and *P. japonica*, or three larvae each of the three species were placed on a tree in the single and mixed species treatments, respectively. That is, the total number of larvae per tree was the same in both treatments. The number of larvae on each tree and their developmental stages were noted daily. The developmental stage of the larvae that emigrated or became trapped was noted, and then they were removed. When a larva is eaten the anterior parts of its body, such as the head and prothorax are left. These larval remains were identified to species and developmental stage. The number of aphids on the trees was also noted daily. Second instar larvae that were kept singly and starved for less than 12 hours after moulting, were used to initiate all experiments. Single species populations of *H, axyridis* and *P. japonica* were replicated 4 times, all other populations were replicated 5 times. The experiments were continued until all the larvae completed their development, were eaten or emigrated.

(2) Toxicity of eggs

Male and female pairs of field collected adults of two species of ladybird, *H, axyridis* and *C. s. brucki*, were kept in 9 cm diameter Petri dishes, each containing a piece of corrugated filter paper. These ladybirds were fed daily with an excess of pea aphids, *Acyrthosiphon pisum* (Harris). Any eggs they laid were removed and placed in 9 cm diameter Petri dishes, and the larvae that hatched from these eggs were transferred within 24 hours of hatching individually to 2.5 × 1.0 cm plastic tubes. The base of each tube had previously been half filled with Plaster of Paris, which was moistened daily with water. To facilitate ventilation there was a hole in the lid of each tube covered with muslin. The four treatments were; the larvae of both *H, axyridis* and *C. s. brucki* were offered daily either five of their own or the other species eggs. Each day the number of eggs eaten was noted, and the eggs and egg-remains from the previous day were removed and replaced. In order to obtain a more exact measurement of larval survival observations were made at 12-hour intervals, and continued until the larvae moulted to the second-instar or died. All

experiments were conducted at 20 ℃ and a 16-hour photoperiod, and each treatment was replicated twenty times.

3-3 RESULTS

3-3-1 Population dynamics of larvae

(1) Survival

The percentages of larvae present when the aphids became extinct and the experiments ended were compared between the three species of ladybirds in the single and mixed species populations (Fig. 3-1a).

In the single species populations, when the aphid became extinct the percentages of larvae present were 89.9% (N=32), 20.0% (N=9) and 86.1% (N=31), for *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. The percentage for *C. s. brucki* was less than one-fourth of that for the other two species ($P < 0.05$), the number of *C. s. brucki* larvae tended to decrease more rapidly than that of the other two species. At the end of the experiment, 27.8% (N=10) of *P. japonica* larvae completed their development, whereas all larvae of the other two species disappeared ($P < 0.05$). That is, in the single species population, although the number of both larvae of *H. axyridis* and *P. japonica* tended to decrease relatively slowly compared to those of *C. s. brucki*, only *P. japonica* larvae survived to maturity.

In the mixed species populations, all the larvae of *H. axyridis* were present when the aphid became extinct, whereas the percentages of larvae of *C. s. brucki* and *P. japonica* present at this time was 20.0% (N=3) and 40.0% (N=6), respectively ($P < 0.05$)(Fig.3-1a). The percentages of larvae present at the end of the experiment did not differ significantly in the three species ($P > 0.05$)(Fig.3-1a). However, 20.0% of *H. axyridis* larvae finally completed their development, whereas no larvae of *C. s. brucki* or *P. japonica* survived to maturity.

To determine the effect of the presence of other species on the survival of larvae, the

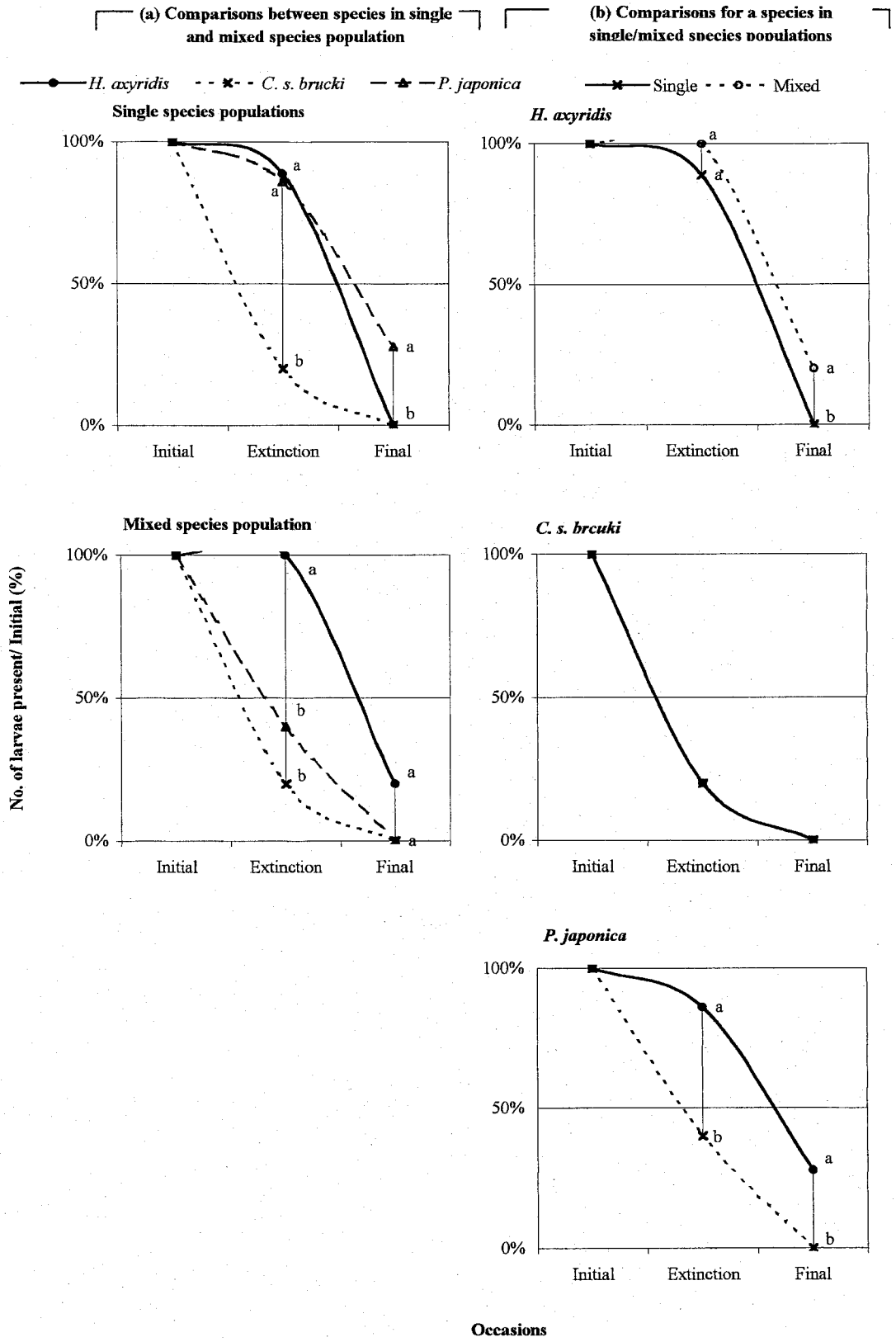


Figure 3-1. Comparison of percentages of the larvae present prior to the extinction of the aphid and at the end of the experiment (a) between species in single or mixed species populations and (b) for a species in single and mixed species populations. (Initial = Start of experiment, Extinction = Extinction of aphid, Final = End of experiment. Dots joined by a vertical line and with the same letter do not differ significantly at $P > 0.05$: χ^2 test.)

percentages of larvae present were compared between the single and mixed species populations (Fig.3-1b). The percentage of larvae of *H. axyridis* surviving at the end of the experiment differed significantly ($P < 0.05$); their survival was improved by the presence of the other two species. In contrast, the survival of larvae of *P. japonica* was decreased significantly in the mixed species populations, their survival worsened when the other two species were present. Interestingly, the percentage of *C. s. brucki* larvae that survived was the same in both single and mixed species populations ($P > 0.05$), their survival was not improved or worsened by the presence of the other two species, as none survived in either treatment.

(2) Factors that decreased survival

The percentages of larvae lost due to predation (Cannibalism or I. G. P.) and emigration, prior to the extinction of the aphid, and at the end of the experiment were compared for the three species of ladybirds in single and mixed species populations.

Cannibalism or I. G. P.

In the single species populations, predation accounted for one death, or 2.8% mortality, of the larvae of *P. japonica* prior to the extinction of aphids, but no deaths of larvae of the other two species (Fig. 3-2a). The percentages of larvae lost due to predation at the end of the experiments was 25% (N=9) and 13.9% (N=5) for *H, axyridis* and *P. japonica*, respectively; and was significantly higher than that for *C. s. brucki* ($P < 0.05$). There was no cannibalism of larvae of *C. s. brucki* in this experiment.

In the mixed species populations, there was predation and the percentage loss due to predation differed in the three species. The percentages lost due to predation before the extinction of the aphid was 26.7% (N=4) for *P. japonica*, which is significantly greater than for the other two species, where it was 0.0% ($P < 0.05$) (Fig. 3-2a). The percentage of *P. japonica* larvae lost by this stage was 23.9%, significantly greater than that recorded in the single species populations

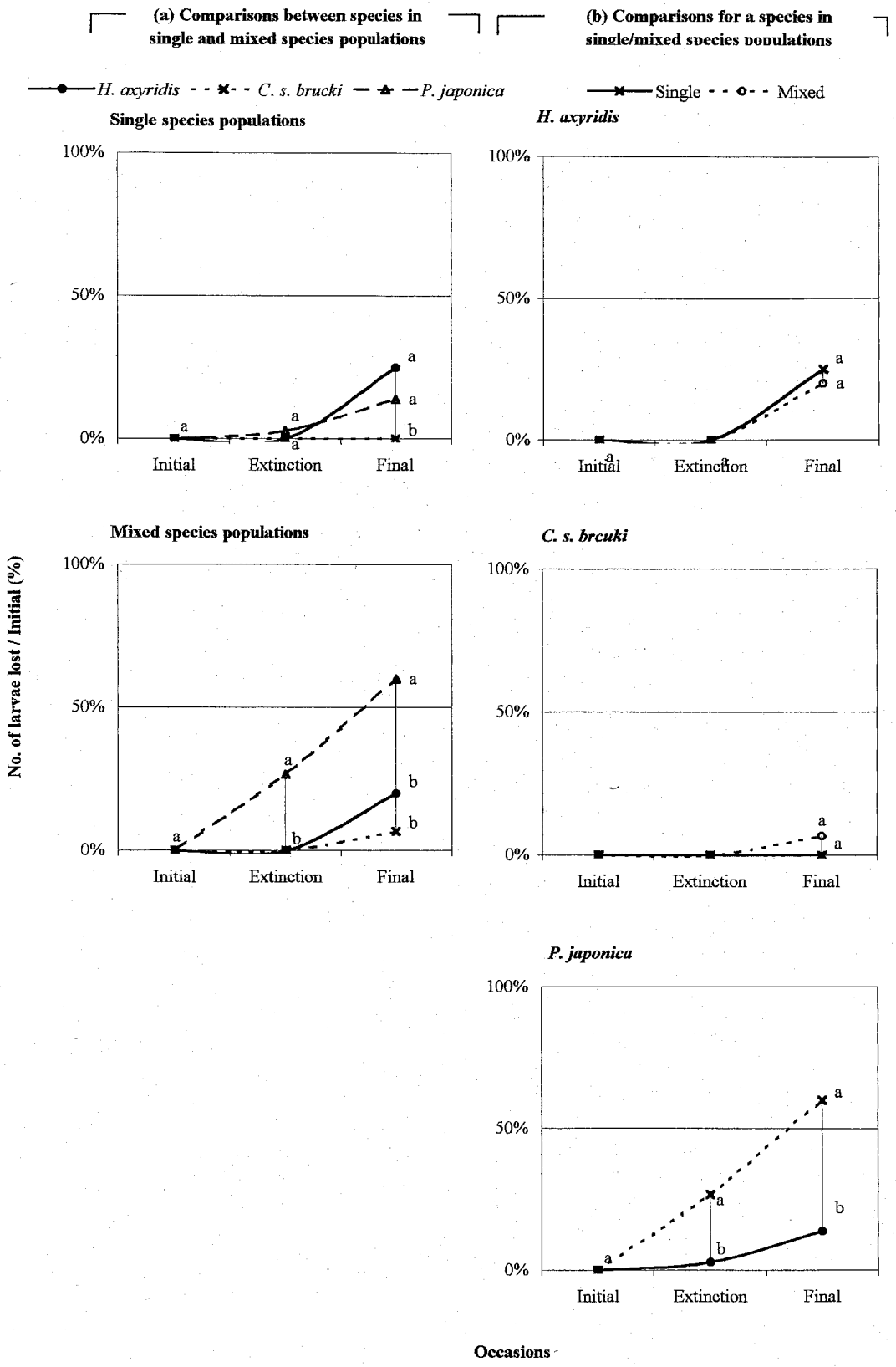


Figure 3-2. Comparison of percentages of the larvae lost due to cannibalism or IGP prior to the extinction of the aphid and at the end of the experiment (a) between species in single and mixed species populations and (b) for a species in single and mixed species populations. (Initial = Start of experiment, Extinction = Extinction of aphid, Final = End of experiment. Dots joined by a vertical line and with the same letter do not differ significantly at $P > 0.05$: χ^2 test.)

($P < 0.05$), although the percentages for the other two species did not differ significantly in the two types of populations ($P > 0.05$) (Fig.3-2b).

The percentage of larvae of *P. japonica* lost due to predation prior to the end of the experiment was 60.0% (N=9), which was significantly greater than for the other two species, where it did not exceed 20% (Fig. 3-2a). In addition, the percentage for *P. japonica* increased significantly by 23.3% compared to that in the single species population ($P < 0.05$), whereas the percentages for the other two species did not differ significantly in the two types of populations ($P > 0.05$) (Fig. 3-2b). That is, in the mixed species populations, larvae of all the species were subject to predation, but *P. japonica* larvae suffered the highest incidence of predation. Although predation of larvae of *H. axyridis* and *C. s. brucki* only occurred after the aphid became extinct, that of *P. japonica* larvae occurred throughout the experiment.

Emigration

In the single species populations, the percentage of larvae that emigrated prior to the extinction of the aphid was 80.0% (N=36) for *C. s. brucki*, which was significantly greater than for the other two species, where it was 20% (N=4) ($P < 0.05$) (Fig.3-3a). The percentages that emigrated prior to the end of the experiment was 75.0% (N=27), 100.0% (N=45) and 80.8% (N=21), for *H. axyridis*, *C. s. brucki* and *P. japonica*, respectively. The percentage for *C. s. brucki* was significantly greater than that for the other two species ($P < 0.05$).

In the mixed species populations, the percentage of larvae that emigrated prior to the extinction of aphid was 80.0% (N=12) for *C. s. brucki*, which was significantly greater than that for *P. japonica* (30.3%, N=5) ($P < 0.05$). No larvae of *H. axyridis* emigrated before the aphid became extinct ($P < 0.05$). The percentages prior to the end of experiments were 60.0% (N=9), 93.3% (N=14) and 40.0% (N=6), respectively. A greater percentage of larvae of *C. s. brucki* emigrated than of the other two species ($P < 0.05$). Overall, the percentage of larvae that

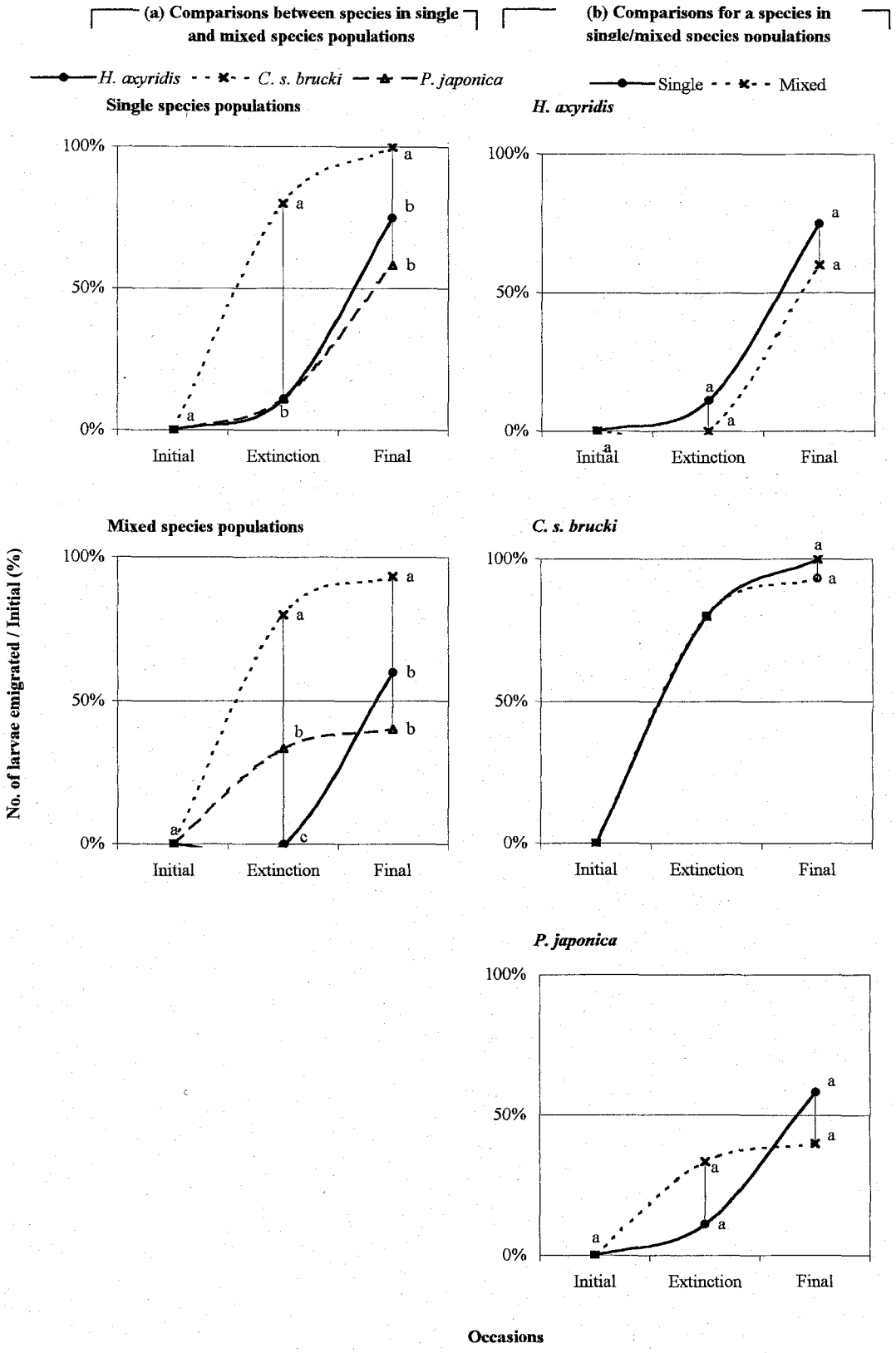


Figure 3-3. Comparison of percentages of the larvae that emigrated prior to the extinction of the aphid and at the end of the experiment (a) between species in single and mixed species populations and (b) for a species in single and mixed species populations. (Initial = Start of experiment, Extinction = Extinction of aphid, Final = End of experiment. Dots joined by a vertical line and with the same letter do not differ significantly at $P > 0.05$: χ^2 test.)

emigrated did not differ in the two types of populations, for each of the three species ($P > 0.05$). Larvae of *C. s. brucki* tended to emigrate earlier than those of the other two species in both single and mixed species populations (Fig3-3b).

(3) Development

The percentages of larvae present at each developmental stage were compared in the single and mixed species populations.

In the single species populations, the percentage of larvae present at the third instar was 100.0% (N=36), 97.8% (N=44) and 100.0% (N=36), respectively, for *H. axyridis*, *C. s. brucki* and *P. japonica*; and did not differ significantly in three species ($P < 0.05$) (Fig.3-4a). The percentage of *P. japonica* larvae present at the fourth instar was 97.2% (N=35), which was significantly greater than in *H. axyridis* and *C. s. brucki*, where it was 41.7% (N=15) and 15.6% (N=7), respectively ($P < 0.05$). Survival of *C. s. brucki* was the lowest of the three species ($P < 0.05$). Ten larvae (27.8%) of *P. japonica* pupated, and all of these became adults. None of the *H. axyridis* or *C. s. brucki* larvae pupated, which is significantly less than for *P. japonica* ($P < 0.05$).

The proportions of larvae that developed to the next developmental stage in the mixed species treatment is given in Figure 3-4b. The percentages of larvae present at the third instar in *H. axyridis*, *C. s. brucki* and *P. japonica* was 100% (N=15) and 80.0% (N=12), 93.3% (N=14), respectively. The percentage of larvae present at the fourth instar in *C. s. brucki* was 20.0% (N=3), which is significantly lower than for the other two species, where it was over 65% ($P < 0.05$). Twenty percent of the larvae of *H. axyridis* pupated, whereas none of fourth-instar larvae of the other two species survived to pupate, but because of the low numbers this difference is not significantly different.

The percentages of larvae of *H. axyridis* that developed to the fourth instar, pupated and became adults were significantly greater in the mixed species treatment than in the single species

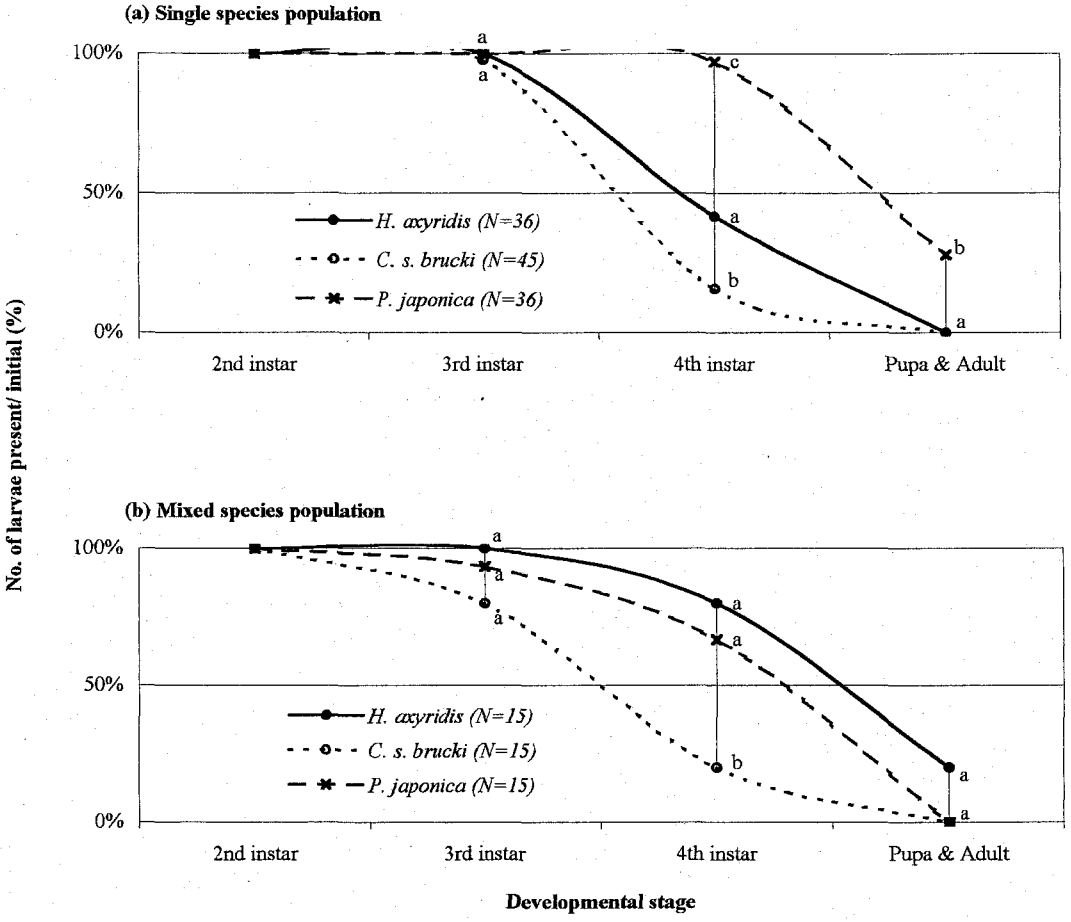


Figure 3-4. Comparisons of the percentages of larvae present at each developmental stage in the three species of ladybirds in (a) single and (b) mixed species populations. (Dots joined by a vertical line and with the same letter do not differ significantly at $P > 0.05$: χ^2test.)

treatment ($P < 0.05$) (Fig.3-5a). The proportion of larvae of *C. s. brucki* that developed to the third instar decreased significantly when the other two species were present ($P < 0.05$). However, in the following stages, their survival did not differ significantly in the two types of populations ($P > 0.05$). For *P. japonica* the proportion of larvae that developed to the fourth instar, pupated and became adult decreased significantly when the other two species were present ($P < 0.05$); none of the larvae survived beyond the fourth instar.

(4) Dominance

In both types of populations, the percentage of the aphidophagous guild made up of each of the three species, at each developmental stage, are given in Figure3-6. In both types of populations, the percentage of larvae of each species that reached the second and third instar did not differ significantly ($P > 0.05$). In the single species populations, there were a total of 56 larvae at the fourth instar stage (100%) (Fig.3-6a). Of these, the percentage that consisted of *P. japonica* was 62.9% (N=35), and was significantly greater than for the other two species, where it was less than 30% ($P < 0.05$). Ten adults emerged: all of them were *P. japonica*. That is, in the single species populations, only *P. japonica* reached the adult stage.

In the mixed species populations, when larvae had reached the fourth instar there were a total of 25 larvae (100%) (Fig.3-6b). Of these, the percentage made up of *H. axyridis* and *P. japonica* larvae was 48.0% (N=12) and 40% (N=10), respectively, which was significantly greater than the 12% (N=3) ($P < 0.05$) made up of *C. s. brucki* larvae. That is, in both types of populations, all *C. s. brucki* larvae disappeared by the fourth instar stage. Three adults emerged: all of them were *H. axyridis*. That is, as only *H axyridis* reached the adult stage, the ladybird guild was dominated by *H. axyridis* in the mixed species populations.

(5) Asymmetric predation

Cannibalism occurred in the single species populations of *H. axyridis* and *P. japonica*. The

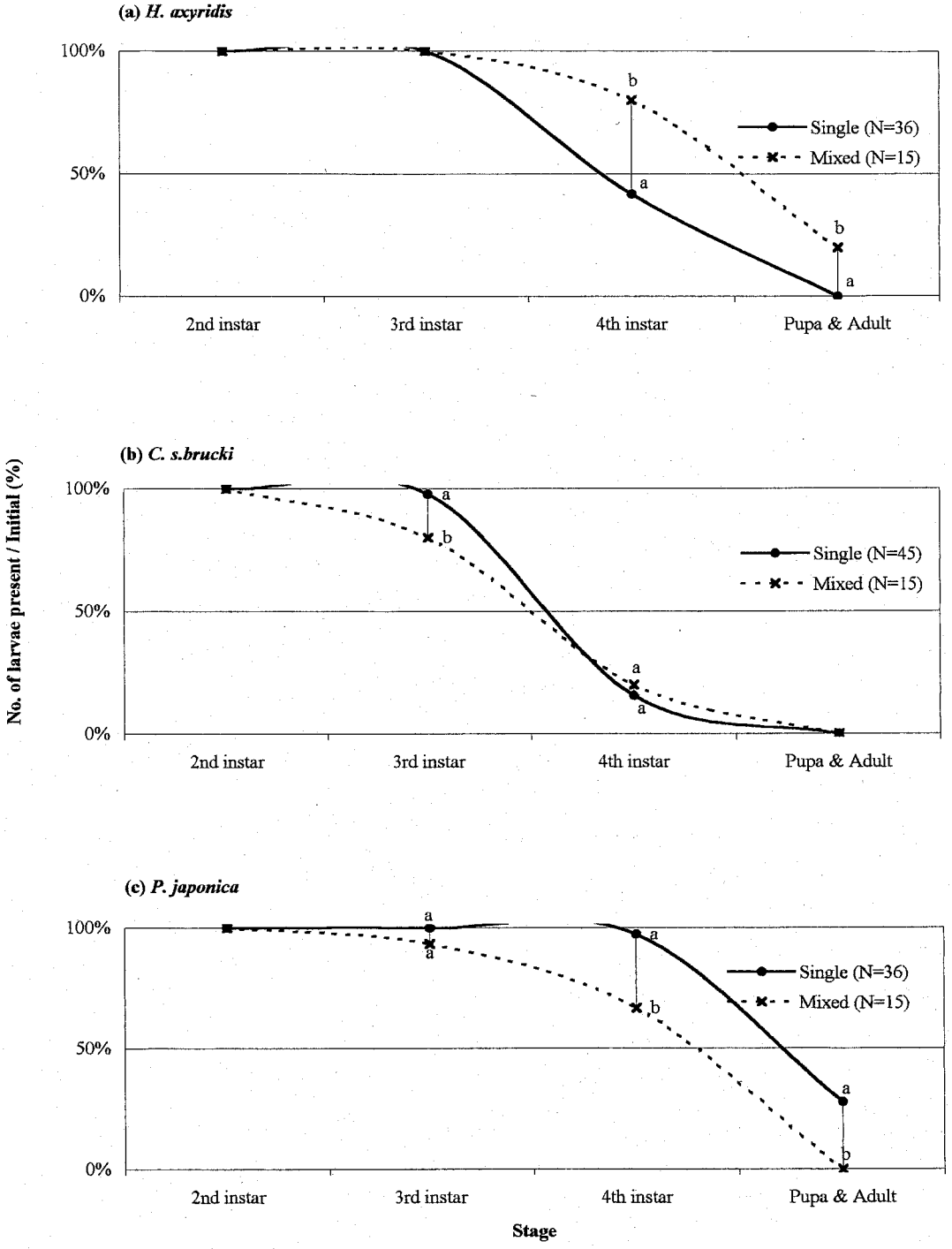


Figure 3-5. Comparisons of the percentages of individuals present of (a) *H. axyridis*, (b) *C. s. brucki* and (c) *P. japonica* at each developmental stage in single and mixed species populations. (Dots joined by a vertical line and with the same letter do not differ significantly at $P < 0.05$: χ^2 test)

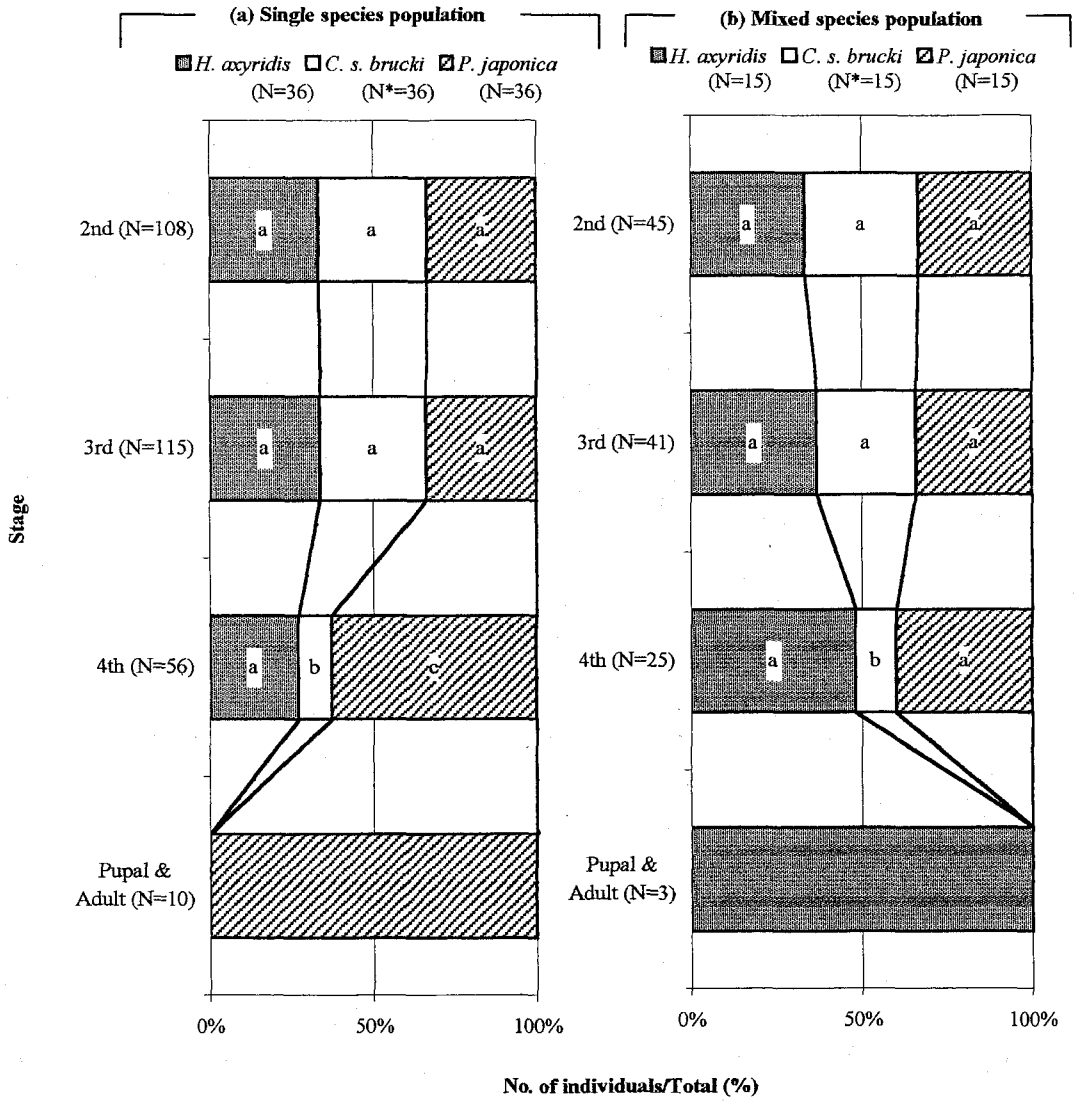


Figure 3-6. Percentage composition of three species of ladybird at each developmental stage in (a) single and (b) mixed species populations. (*Initial number of *C. s. brucki* in the single species populations was converted from 45 into 36. Percentages with the same letter do not differ significantly, $P > 0.05$: χ^2 test)

frequency of cannibalism and the age structure of the populations are given in Figure3-7. In total, for both populations, 14 individuals were lost due to cannibalism. Of these, when the populations consisted of a single developmental stage 3 individuals (21.0%) were eaten, whereas 11 individuals (79.0%) were eaten when the population consisted of two developmental stages. Cannibalism occurred more frequently when there was more than one developmental stage ($P < 0.01$), cannibals were fourth-instar larvae and the victims were either third-instar larvae or prepupae on every occasion.

In the mixed species populations, larval loss due to cannibalism or I. G. P. was recorded for all species. The frequency of these losses and the species structure of the populations are given in Figure3-8. When the populations consisted of all three species, eight individuals (73.0%) were eaten. The victims were either larvae of *C. s. brucki* or *P. japonica*; no larvae of *H. axyridis* were eaten. Three individuals (27.0%) of *H. axyridis* were eaten by conspecific larvae after the other two species became extinct. That is, cannibalism or I. G. P. tended to occur more frequently when the population consisted of more than one species ($P < 0.05$). In addition, no larvae of *C. s. brucki* or *P. japonica* ate larvae of *H. axyridis*.

3-3-2 Effects of egg cannibalism and predation on larval survival and development

The consequence of eating each other's eggs differed between the two species (Table3-1). All of the first instar larvae of *C. s. brucki* developed to the second instar after eating conspecific eggs, but none survived after eating several eggs of *H, axyridis*. On the other hand, the survival of first instar larvae of *H, axyridis* was not significantly adversely affected by eating eggs of *C.s. brucki*, although three larvae died ($P < 0.05$). However, the duration of the first instar of *H, axyridis* was extended significantly when fed eggs of *C. s. brucki* ($P < 0.05$). The total number of eggs eaten was not significantly different between treatments ($P > 0.05$). Of the larvae fed the eggs of the other species, three of *H, axyridis* and twenty of *C. s. brucki* died and of the larvae that died those

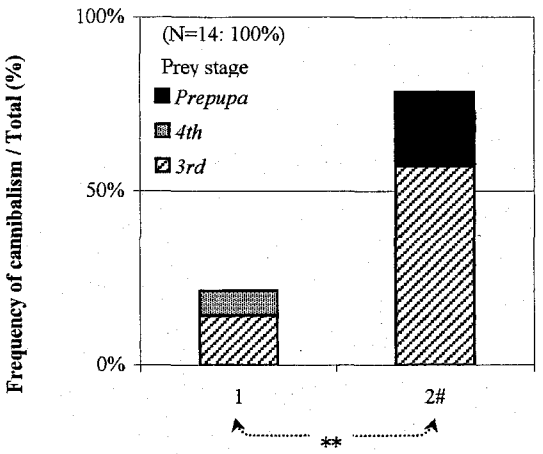


Figure 3-7. Frequency of cannibalism relative to the number of developmental stages present in single species populations. (#Predators were 4th instar larvae in all cases of cannibalism. **shows a significant difference in frequencies at $P < 0.01$: χ^2 test.)

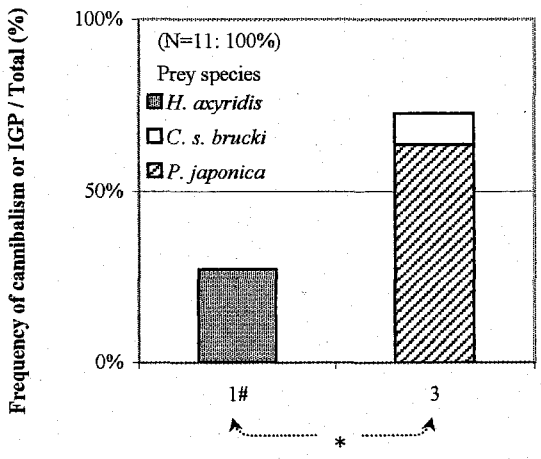


Figure 3-8. Frequency of cannibalism or IGP relative to the number of species present in mixed species populations. (#After *C. s. brucki* and *P. japonica* became extinct. * shows significant difference in frequencies at $P < 0.05$: χ^2 test.)

Table 3-1. Effect of egg predation on survival and development of the 1st instar larvae of 2 species of ladybird.

Predator: 1st instar of	***H. axyridis***						***C. s. brucki***					
Prey: Eggs of	***H. axyridis***			***C. s. brucki***			***H. axyridis***			***C. s. brucki***		
N	20			20			20			20		
% of larvae that moulted to 2nd instar larvae*	100.0%	(N=20)	(a)	85.0%	(N=17)	(a)	0.0%	(N=0)	(a)	100.0%	(N=20)	(b)
Mean duration (±SE) of 1st instar in days**	2.9±0.1		(a)	3.4±0.1		(b)	-			3.1±0.1		(a)
Mean number (±SE) of eggs eaten**	4.5±0.3		(a)	4.9±0.3		(a)	-			5.0±0.2		(a)
% of larvae that died before moulting	0.0%	(N=0)		15.0%	(N=3)		100.0%	(N=20)		0.0%	(N=0)	
Mean duration (±SE) of survival in days***	-			2.3±0.9		(a)	7.3±0.5		(b)	-		
Mean number (±SE) of eggs eaten***	-			0.9±0.9		(a)	2.6±0.2		(a)	-		

Figures in rows followed by the same letter in parentheses do not differed significantly at $P < 0.05$: *χ^2 test,**One-way ANOVA & Bonferroni method, ***Mann-Whitney U-test.

of *C. s. brucki* survived for seven days on average, significantly longer than the two days recorded for *H, axyridis* ($P < 0.05$). Two of the three larvae of *H, axyridis*, which died during the first instar, did not eat any eggs of *C. s. brucki*, while all the larvae of *C. s. brucki* ate several eggs of *H, axyridis*. There was no significant difference in the number of heterospecific eggs eaten by each of the species ($P > 0.05$).

3-4 DISCUSSION

I. G. P. is thought to be an important force structuring predatory insect communities (Polis *et al.*, 1989; Dong & Polis, 1992). In section 2, it was concluded that I. G. P. by *H axyridis* appears to be a force structuring ladybird guilds that include the two species, *C. s. brucki* and *P. japonica*. In the present experiment, the incidence of I. G. P. increased when the three species interacted with each other, and *H. axyridis* dominated the ladybird guild. When reared on its own under otherwise similar conditions, *H. axyridis* did not complete its development. Therefore, it is suggested that *H. axyridis* is an inter-guild predator. Larvae of *H. axyridis* are polyphagous (Hodek & Honek, 1988). In fact, although *H. axyridis* fed on several intra-guild prey and survived, none of the prey species could feed on *H. axyridis* and survive (e.g. Cottrell and Yeargan, 1998; Phoofolo & Obrycki, 1998; Yasuda & Ohnuma, 2000). In addition, the morphology of *H. axyridis* is also likely to be important in its role as a top predator. Lucas *et al* (1998) report that lacewing larvae eat coccinellid larvae and suggest that the lacewing's superiority could be attributed to its greater aggressiveness or shape of its mouthparts. *H. axyridis* larvae have relatively large mandibles and an anal disk, which may make them more effective predators.

If *H. axyridis* is a top predator, then it should be protected from I. G. P. in some way, especially as they are more exposed to I. G. P. because they tend to oviposit later than the other species. *H. axyridis* and *C. s. brucki* co-occur in spring (Section2), and all stages of these two

species are similar in size, but the two species differ in their periods of oviposition. *C. s. brucki* occurs (Takahashi, 1987) and lays its eggs (Yasuda & Shinya, 1997; Section 2) earlier than *H. axyridis*. Consequently, the development of *H. axyridis* larvae is likely to lag behind that of *C. s. brucki*. Both eggs and young larvae are more vulnerable to cannibalism than are older larvae (Agarwala & Dixon, 1992). In addition, small species are more vulnerable to I. G. P. than large species (Sengonca & Frings, 1985; Lucas *et al*, 1997, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al*., 2001). Therefore, it is suggested the latter occurring species, *H. axyridis,* would appear to be more at risk of predation than *C. s. brucki*, which will be relatively more mature and larger than *H. axyridis*.

In several species of ladybirds, which have overlapping habitat preferences, a better chemical protection of the smaller species against predation by the larger species has been reported (Agarwala & Dixon, 1992; Agarwala *et al*., 1998; Hemptinne *et al*., 2000). That is, it is suggested that a species-specific alkaloid is an important protection against predation by an intra-guild predator. In the present experiment, the eggs of *H. axyridis* were more protected against predation by first instar larvae of *C. s. brucki,* because of their toxicity, than *vice versa*. Therefore, the toxicity of *H. axyridis* eggs is likely to result in few of their eggs being eaten by *C. s. brucki* larvae, which is what was observed in the field (Section 2). In addition, as all stages of ladybirds contain similar concentrations of alkaloids (Pasteels *et al*., 1973), it can be assumed that the larvae of *H. axyridis* are also toxic to larvae of *C. s. brucki.* Therefore, although *H. axyridis* starts ovipositing after *C. s. brucki*, the eggs and larvae of *H. axyridis* are well protected chemically from predation by larvae of *C. s. brucki*. These results may also indicate that *H. axyridis* is a top predator in this ladybird guild. In the field, *H. axyridis* oviposits later because it is a "top predator", i.e., to oviposit later is adaptive if you can eat other species of ladybird.

C. s. brucki is thought to be less cannibalistic and polyphagous than *H. axyridis* (Section 2).

That is, larvae of *C. s. brucki* are not likely to be intra-guild predators. Is *C. s. brucki* an intra-guild prey of *H. axyridis* ? In the present experiment, the eggs of *C. s. brucki* were not toxic to the first instar larvae of *H. axyridis*. As all stages of ladybirds contain similar concentrations of alkaloids (Pasteels *et al*., 1973), it can be assumed that all stages of *C. s. brucki* are vulnerable to predation by *H. axyridis* larvae. Yasuda & Ohnuma (2000) reported that fourth instar larvae of *H. axyridis* fed on *C. s. brucki* larvae developed as well as those fed on aphids. That is, for *H. axyridis*, it is suggested that there is no penalty associated with eating *C. s. brucki*, and it is likely to be an intra-guild prey of *H. axyridis*. However, in the mixed species experiment, mortality of *C. s. brucki* larvae due to cannibalism or I. G. P. was low. In general, the incidence of cannibalism increased after the aphid became extinct. However, almost all of the *C. s. brucki* larvae had emigrated by that time, irrespective of which of the other species were present. Consequently, survival *C. s. brucki* was not affected by the presence of *H. axyridis*. As leaving plants when prey become scarce may further reduce the probability of encountering con- and hetero-specific larvae (Winder, 1990), their early emigration seems to reduce the incidence of I. G. P. of *C. s. brucki* by *H. axyridis*. In addition, in the field, the early oviposition by *C. s. brucki* may also be an important way of avoiding I. G. P. by *H. axyridis*. Although the eggs of *C. s. brucki* are not toxic to *H. axyridis* larvae, they hatch before those of *H. axyridis*. In addition, as large species are less vulnerable to predation than small species (e.g. Sengonca & Frings, 1985), it is suggested that the early oviposition by *C. s. brucki* enables it to achieve an advanced stage of development, and large body size, and thus reduce the likelihood of being eaten by the latter hatching and smaller *H. axyridis* larvae. However, in the field, the oviposition period of *C. s. brucki* relative to that of *H. axyridis* varies from year to year (Section 2). When the oviposition periods of these two species overlap the incidence of I. G. P. of *C. s. brucki* by *H. axyridis* is likely to increase. As a ladybird population is usually made up of individuals in different developmental stages, then when prey

becomes scarce young larvae of *C. s. brucki* are likely to be eaten by mature larvae of *H. axyridis*. That is, for *C. s. brucki*, to avoid I. G. P. by *H. axyridis* it is advantageous for *C. s. brucki* to lay as many eggs as possible before *H. axyridis* starts egg laying.

P. japonica is a small species, which is more vulnerable to I. G. P. than the larger species (e.g. Sengonca & Frings, 1985). Therefore, it can be assumed that it is more vulnerable to I. G. P. by *H. axyridis* than *C. s. brucki*. However, small species are more protected chemically from predation by larger species than are large species (e.g. Agalwara & Dixon, 1992). Therefore, *P. japonica* should be well protected chemically from predation by large species like, *H. axyridis*, if their habitat preferences overlap. In the field, *P. japonica* was the most frequently observed intra-guild prey of *H. axyridis* (Section 2). In the mixed species experiment, survival of *P. japonica* worsened when the other species were present, while that of *H. axyridis* improved. In addition, as almost all of the *C. s. brucki* larvae had emigrated when the incidence of I. G. P. of *P. japonica* increased, this predation is most likely to be attributable to *H. axyridis*. Therefore, it is likely *P. japonica* larvae are not well protected chemically against predation by the larger species, *H. axyridis*. That is, the habitat preferences of *P. japonica* and *H. axyridis* are likely to differ. Two small species of ladybird in Europe, *Adalia bipunctata* and *Adalia decempunctata,* are more toxic to the larger *Coccinella septempunctata* than *vice versa* (Agarwala & Dixon, 1992; Hemptinne *et al.*, 2000). However, *Adalia bipunctata* are not more toxic to the two large species in Japan; *H. axyridis* and *C. s. brucki*, than *vice versa* (Sato, unpublished). This may imply that the chemical protection of a small species against predation by a large species is unlikely to occur when the habitat preferences of the two ladybirds, small and large, differ.

The reason why a species of ladybird stays on or leaves a plant when its prey becomes scarce is unknown. It is likely that the two species have different habitat preferences and associated behaviours. Although several field studies have reported *H. axyridis* and *C. s. brucki*

co-occurring in the same habitat (Takahashi, 1987; Yasuda & Shinya, 1997; Section 2), the habitat preferences of these two species are likely to differ. Osawa (1991) suggested that *H. axyridis* prefers shrubs, while *C. s. brucki* prefer grassland, consequently, where one species is abundant the other species is likely to be scarce and *vice versa*.

Although Winder (1990) suggested that larvae leaving the plants are vulnerable to predation by ground predators such as carabid beetles, the risk associated with emigration may differ in the two habitats, shrub and grassland. In shrubby habitats, the size of the plants is generally larger than in grassland, and the between plant distance greater in shrubby habitats than in grassland. The greater the distance between plants the greater the risk of predation by ground beetle during emigration. In addition, in a shrubby habitat, as no plants were heavily infested with aphids when ladybird larvae emigrated (Yasuda & Shinya, 1997), emigrants are less likely to find other aphid colonies. To disperse when prey is scarce may further reduce the probability of encountering con- and heterospecific larvae (Winder, 1990). Accordingly, the incidence of cannibalism or I. G. P. of *H. axyridis*, which tend to stay rather than disperse when aphids are scarce, are likely to increase, as was observed in the present experiments. However, larvae of *H. axyridis* can complete their development before emigrating, as they are well protected from I. G. P. and used other species of ladybird as a food resource as well as aphids. That is, when plants are widely spaced it may be advantageous for larvae of polyphagous and chemically protected species like *H. axyridis* to stay on the plants if they are not already mature.

C. s. brucki can mature if they occur early in the development of an aphid colony. However, the early occurrence of *C. s. brucki* in spring depends on their emerging from hibernation early compared to *H. axyridis* (Takahashi, 1995). Therefore, after both *H. axyridis* and *C. s. brucki* emerge from hibernation, i.e. in spring, the shrubby habitat is unlikely to be suitable for *C. s. brucki,* as it cannot lay eggs earlier than *H. axyridis*, which is important for their survival.

In grassland, plant size is relatively small; and aphid number/plant is also likely to be small. Therefore, larvae may occasionally need to migrate to other plants in order to complete their development. However, as *H. axyridis* tend to stay longer on plants, the numbers of their larvae are likely to decrease due to cannibalism. That is, grassland is not likely to be a suitable habitat for *H. axyridis*. In fact, the displacement of *C. septempunctata*, which is a sub species of *C. s. brucki*, by *H. axyridis* in United States is reported on apple trees (Brown & Miller, 1998), i.e., in a shrubby habitat, and in a semi boreal habitat (LaMana & Miller, 1996). In contrast to *H. axyridis* larvae, those of *C. s. brucki* tend to emigrate soon after aphids become extinct; therefore, their loss due to cannibalism is likely to be low, which may result in a high pupal recruitment of *C. s. brucki* in grassland. In fact, Evans (2000) reported that the introduced species, *C. septempunctata*, increased rapidly in abundance in alfalfa fields in the United States He also revealed a large variance in body size in this species compared to native species. Therefore, although early emigration may be advantageous for avoiding cannibalism or I. G. P., the low frequency of cannibalism in *C. s. brucki* may result in low availability of aphid prey for fourth instar larvae, which affects their adult body size (Yasuda & Dixon, 2000).

Although the habitat preferences of *P. japonica* are poorly understood, several studies imply that they are abundant on field crops (e.g. Sakuratani, 1977; Kawauchi, 1990). In single species populations, only this species completed their development. That is, it is suggested that 700 aphids/9 second instar larvae, which was offered in the present experiments, were not enough for the larger two species, *H. axyridis* and *C. s. brucki*, to complete their development. Yasuda & Dixon (2000) reported that large sized adults of *Adalia bipunctata* require a relatively higher density of aphid for mating than small sized adults, and small sized females of this species are more fecund than large sized female when aphid abundance is low. Accordingly, it is suggested that the small species, *P. japonica*, can occur in a habitat where aphids are scarce and where the

large species are unlikely to occur. In addition, their oviposition site is likely to differ from those of the large species, *H. axyridis* and *C. s. brucki*. Eggs of *P. japonica* are more likely to be laid when aphid abundance is relatively low compared to the other two species (Sato, 1994). That is, *P. japonica* appears to prefer habitats with a low abundance of aphids, which is not suitable for survival of the large species.

SECTION 4

EFFECT OF FOOD AVAILABILITY ON THE PERFORMANCE OF FAST- AND SLOW-DEVELOPING LARVAE

4-1 INTRODUCTION

Many predatory ladybirds eat aphids, the number of which change in time and space (Kindlmann & Dixon, 1993; Yasuda & Shinya, 1997; Dixon, 1998; Osawa, 2000), and the timing of ladybird oviposition relative to the population dynamics of aphids affects the survival of their larvae (Section 2). If eggs are laid too late, then the aphids are likely to decrease in abundance before the larvae complete their development. Consequently, the incidence of cannibalism or IGP, which is an important cause of larval mortality (Section 3), increases with decrease in prey abundance and survival of larvae is likely to be low. However, if eggs are laid too early then the population density of the aphid is likely to be too low for the first instar larvae of the ladybird to be able to catch sufficient aphids to survive as hatchling larvae have poor powers of dispersal (Dixon, 1959). Therefore, to maximize the survival of their offspring ladybird should lay a few eggs early in the development of an aphid patch (Dixon, 2000).

However, the trends in aphid abundance can be changed by various factors, such as temperature and abundance or timing of oviposition of ladybirds (Section2). For instance, although egg laying by *H. axyridis* in relation to the peak in aphid abundance occurred at a similar time in the two years (1995/1996), aphids decreased in abundance rapidly when *C. s. brucki* occurred and started feeding earlier than *H. axyridis* (1996). Consequently, in 1996, survival of *H. axyridis* larvae was relatively low because of the low prey abundance during their larval development. In the field, all the predators feeding on aphids make up the aphidophagous predator guild (e.g. Sakuratani, 1977: Arakaki, 1992: Winder *et al.*, 1994, Dixon, 1998), and the peak in aphid abundance and its timing can be changed by these predators (Dixon, 2000). Therefore, although there may be species-specific oviposition period for each species of ladybird, the trends in aphid abundance after ladybird oviposition is likely to vary. Consequently, females cannot be certain about the future availability of prey for their larvae. Can ladybirds cope with

this uncertainty?

In *H. axyridis*, genetically, longer duration of development and slower growth rate are possibly connected to a smaller body size, and shorter development period and faster growth rate to larger body size (Ueno, 1994). Prey consumption is also likely to vary and be negatively associated with the duration of development. Rodriguez-Saona & Miller (1995) selected for fast development in *Hippodamia convergens* over several generations. The fast developing larvae consumed more aphids per unit time and developed into larger adults than the more slowly developing individuals. That is, if prey consumption is determined by the speed of development, then, slow developing larvae are less likely to be affected by poor food availability compared to fast developing individuals.

The objectives of this section were to: (1) determine the differences in food consumption of fourth instar larvae from fast and slow developing strains of the two spot ladybird, *A. bipunctata*; and (2) examine the effect of the availability of food on their development and survival.

4-2 MATERIALS AND METHODS

All experiments described in this section, were done at 20°C and a 16-hour photoperiod. Two sizes of Petri dish were used; they were either large or small, that is, 9 or 3 cms in diameter, respectively. Plastic tubes, 2.5 × 1.0 cm in size, were also used. The lids of these tubes were pierced with a hole, which was covered with muslin to facilitate ventilation. The lower half of each tube was filled with Plaster of Paris, which was moistened daily with water during experiments. In addition, the Plaster of Paris reduced the capacity of each tube. That is, ladybirds were able to find aphids more easily and expenditure of energy searching for prey was minimized. The ladybirds were weighed on a microbalance. They were weighed at least twice, and an average of the weights recorded. Adult body weight was measured within 12 hours of emergence from pupae and before they started feeding. After emergence from pupae adults were kept

individually in small Petri dishes and sexed after they had hardened completely. To determine the duration of each developmental stage they were observed at 12-hour-intervals. In order to prevent injury hatchling larvae were not handled until they were 24 hours old. Egg cannibalism by larvae accelerates their development. Therefore, only larvae from clusters of eggs that did not experience egg cannibalism were used.

(1) Ladybird cultures

A culture of the ladybird, *Adalia bipunctata*, was started using adults collected on the campus of the University of East Anglia in the spring. Several pairs of adults were kept in each sandwich box, and fed daily an excess of the pea aphid, *Acyrthosiphum pisum*. To facilitate ventilation the centre of the lids of each sandwich box was cut out and replaced with muslin. Corrugated paper towel was placed in the bottom of each sandwich box and the underside of the lid lined with tissue paper. To supply water a piece of folded and moistened tissue paper was placed in the corner of each box. The corrugated paper towel and paper tissues were renewed daily, and clusters of eggs were collected. The sandwich boxes were cleaned daily. The clusters of eggs were placed in the large Petri dishes. Hatchling larvae were fed an excess of pea aphid daily, when aphid remains and excreta from the previous day were also removed. To prevent prepupae from being eaten by other larvae they were transferred to another large Petri dish. In addition, adults were occasionally collected from the field during spring and autumn, and added to the ladybird culture in order to keep it genetically diverse.

(2) Selection for fast or slow development

As a preliminary experiment, the general relationship between duration of development and adult body size was determined. Clusters of eggs obtained from the ladybird culture were kept in large Petri dishes until egg hatch. Larvae were reared individually in plastic tubes on an excess of pea aphids. Aphid remains and excreta of the larvae were removed daily. The times of hatching,

moulting, pupation, adult emergence and adult body weight were noted. There was a significant negative relationship between duration of development and adult body size in both sexes ($P < 0.0001$) (Fig. 4-1). In addition, there was a significant relationship between the time it took to develop from egg to start of the fourth instar and the total duration of development in both sexes in all generations ($P < 0.0001$) (Fig. 4-2). That is, it is possible to identify fast or slow developing individuals at the end of the third instar. Therefore, the speed of development was identified based on the time that elapsed between oviposition and moulting to the fourth instar. Clusters of eggs were obtained from the ladybird cultures. Hatchling larvae were fed an excess of pea aphid daily, and aphid remains and excreta from the previous day were removed. These larvae were monitored every 12 hours, and when they moulted to the fourth instar they were removed and kept individually. The time of the moult to the fourth instar was noted. Those that developed faster or slower than the average were categorized as fast or slow, respectively, and used in the following two experiments. In total, 516 larvae were reared (Fig. 4-3). The average of duration from oviposition to start of fourth instar of these larvae was 12.6±0.1 days, and the frequency distribution of the duration skewed to right (Skewness = 1.92).

(3) Prey consumption and growth

Total number and rate of prey consumed, and increase in body weight during the fourth instar of fast and slow developing larvae were assessed. This experiment consisted of observing pea aphid consumption over a 24-hour period by fourth instar larvae of the fast and slow developing strains. Larvae were selected at random from the fast and slow developing strains. They were kept individually in small tubes with an excess of aphids of different stages, which had previously been weighed. Each dish contained a broad bean leaf, which was kept turgid by a piece of moistened sponge. After 24 hours, the weight of aphids in the container and that of the larvae were measured. This was continued until the larvae pupated. Weights of pupae and adults and

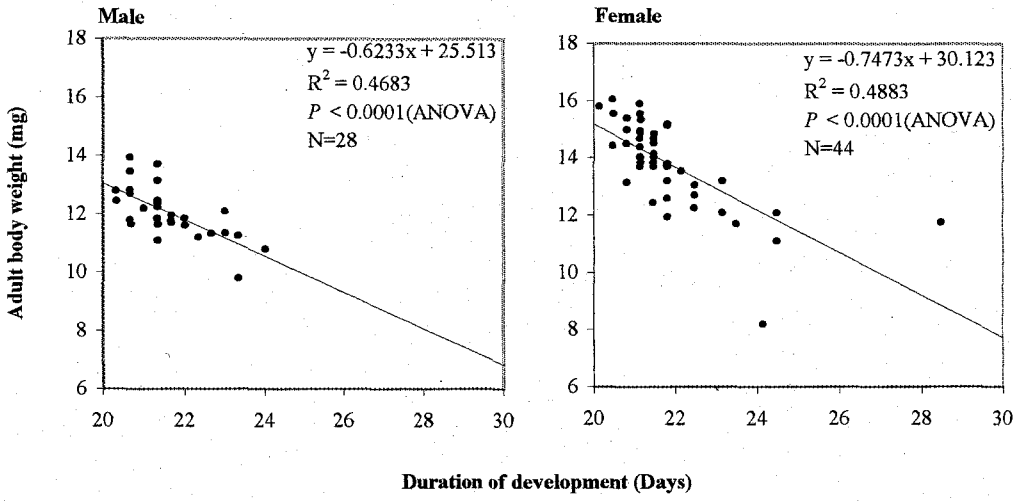


Figure 4-1. The relationship between duration of development and adult body weight.

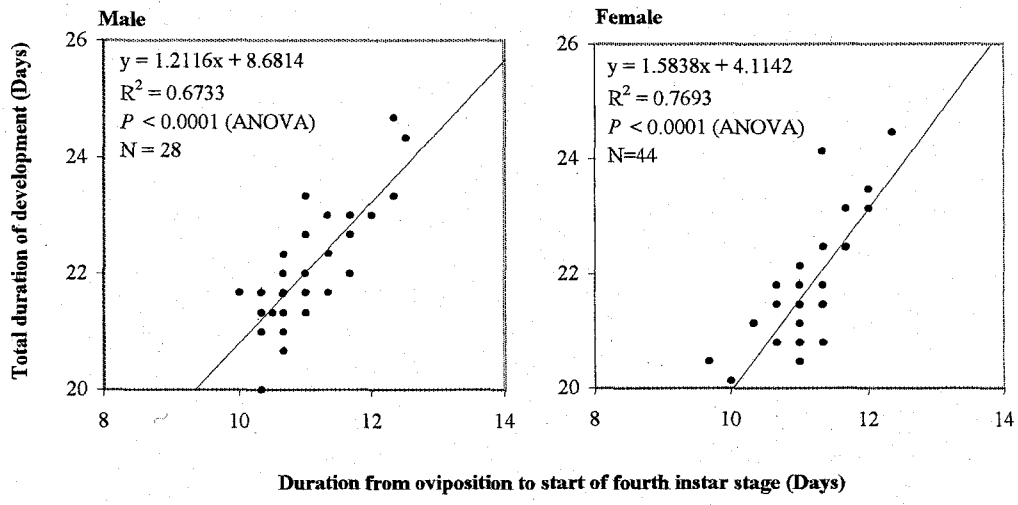


Figure 4-2. The relationship between the period from oviposition to start of fourth instar and total duration of development.

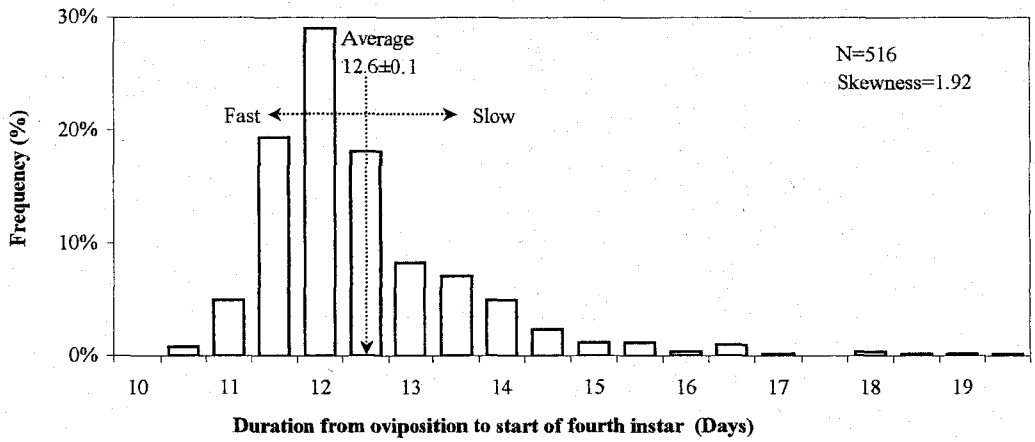


Figure 4-3. Frequency distribution of the duration in days from oviposition to start of fourth instar.

their sex were recorded. Total aphid consumption was the sum of the daily aphid consumptions during the fourth instar. The time of the peak aphid consumption differed for larvae of the fast and slow strains, therefore, maximum aphid consumption was the average of the maximum aphid consumptions of each individual. Average aphid consumption was the total aphid consumption divided by the duration of fourth instar. Increase in body weight was the final body weight of a fourth instar larva minus its initial body weight. Rate of production was the increase in body weight from the start to the end of the fourth instar divided by the duration of the fourth instar. Relative increase in body weight was the bodyweight at the end of fourth instar divided by the body weight at the beginning of the fourth instar.

(4) Effect of poor food supply on survival and development

Development and survival of individuals of the fast and slow developing strains when starved were determined. The fourth instar larvae were kept individually in small tubes. These larvae were supplied either one aphid (Adults only) every two days (0.5 aphids per day) or daily until they died or developed into pupae. Larvae were weighed daily, and the survival and time to pupation and emergence of adults monitored at 12-hour-intervals. Sex was also noted.

4-3 RESULTS

(1) Survival

Survival of larvae when fed at different rates is given in Figure 4-4. Although, there was no significant differences in the percentages of larvae that survived in the two strains in the three treatments ($P > 0.05$), a greater percentage of larvae tended to survive in the fast strain than in slow strain when fed an excess of aphids daily. In addition, although over 70% of the larvae survived in the 1.0 and excess aphid treatments, over 90% of the larvae died in the 0.5 aphid treatment in both strains. This difference is significant ($P < 0.001$). Therefore, the following three life history traits of larvae, **(2) Duration of development**, **(3) Bodyweight** and **(5) Growth**, are

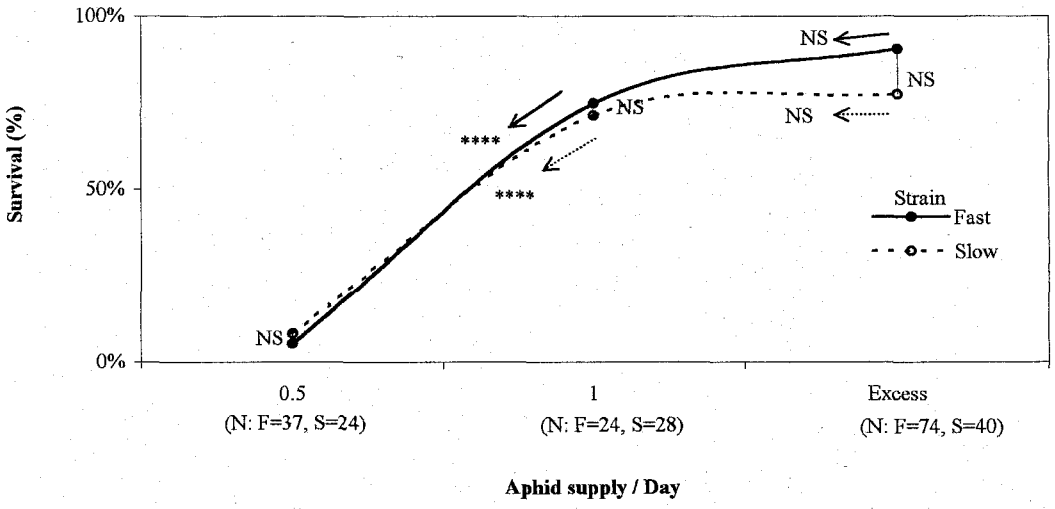


Figure 4-4. Percentage of fourth instar larvae of fast and slow developing strains that survived to the adult stage when fed 0.5, 1 or an excess of aphids daily. (**** significant difference between strains, $P < 0.001$, NS no difference between fast and slow strains, $P > 0.05$: χ^2 test.)

shown only for the 1 aphid and excess aphid treatments.

Duration of survival

Although almost all of the larvae died when fed 0.5 aphid/day, the larvae of the fast developing strain tended to survive for a shorter period than those of the slow developing strain (Fig. 4-5). On the 14th day, only 5% of the fast developing strain still survived, which was significantly less than that of the slow developing strain ($P < 0.0001$). In addition, there was a significant difference in average duration of survival of the two strains (Mann-Whitney test: $P < 0.001$), with larvae of the fast developing strain surviving for 9.8±0.5 days, and those of the slow developing strain for 17.0±1.3 days

(2) Duration of development

Duration of the fourth instar was compared in the fast and slow developing strains (Fig 4-6a). In the excess aphid treatment, duration of the 4th instar of the fast developing strain was about 3.5 days for both sexes, respectively, which was about 0.7 days shorter than that of the slow developing strain (Male and Female: $P < 0.001$). In the 1aphid treatment, there was also a significant difference in the duration of the 4th instar in the two strains for both sexes ($P < 0.001$); the difference increased to about 2 days.

(3) Bodyweight

Larval weight on the first, second, third, and final day of the fourth instar in the fast and slow strains when fed 1aphid and an excess of aphids /day are given in Figure 4-7 and 4-8, respectively. In both treatments, larval bodyweight on the first day differed significantly (e.g. Male: $P < 0.001$, Female: $P < 0.001$ in the excess aphid treatment), with the larvae of the fast strain over 1.2 times heavier than those of the slow strain in both sexes and both treatments. In the excess aphid treatment, the fast developing larvae were heavier than the slow developers on the second, third and final day of the fourth instar (Male: $P < 0.0001$, 0.0001 and 0.01, Female: $P < 0.001$,

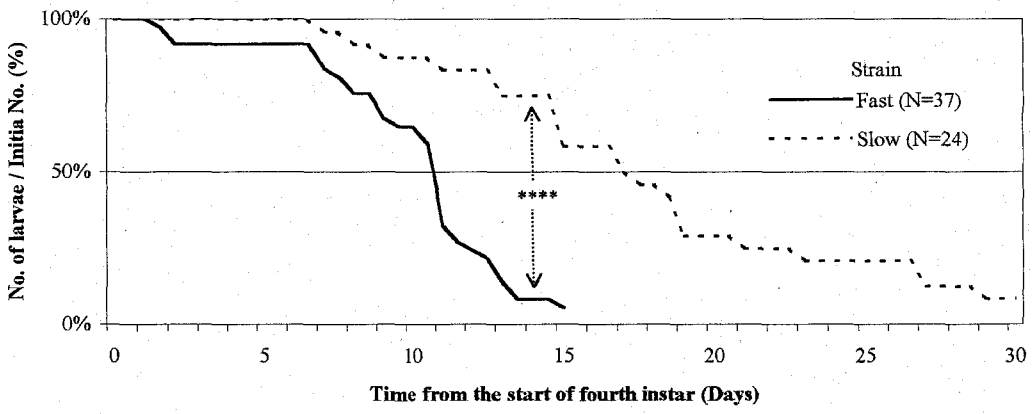


Figure 4-5. Survival of 4th instar larvae of the fast and slow developing strains when fed 0.5 aphids/day. (**** significant difference between strains, $P < 0.0001$: χ^2 test.)

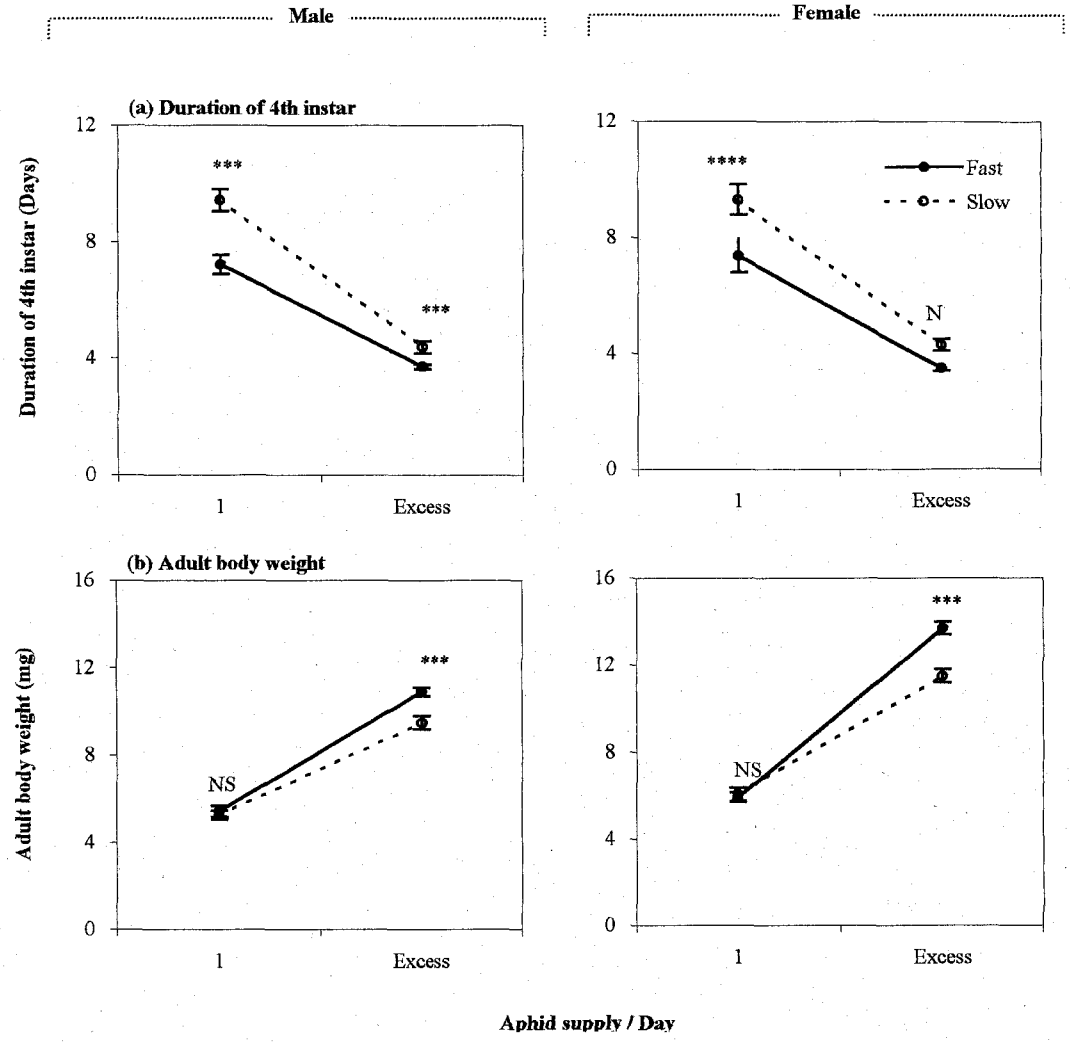


Figure 4-6. The duration of development of 4th instar larvae and average adult body weight of fast and slow developing strains when fed one or an excess of aphids daily. (*** and **** significant difference between strains, $P < 0.001$ and 0.0001, respectively. NS no difference between strains $P > 0.05$: Mann-Whitney U-test.)

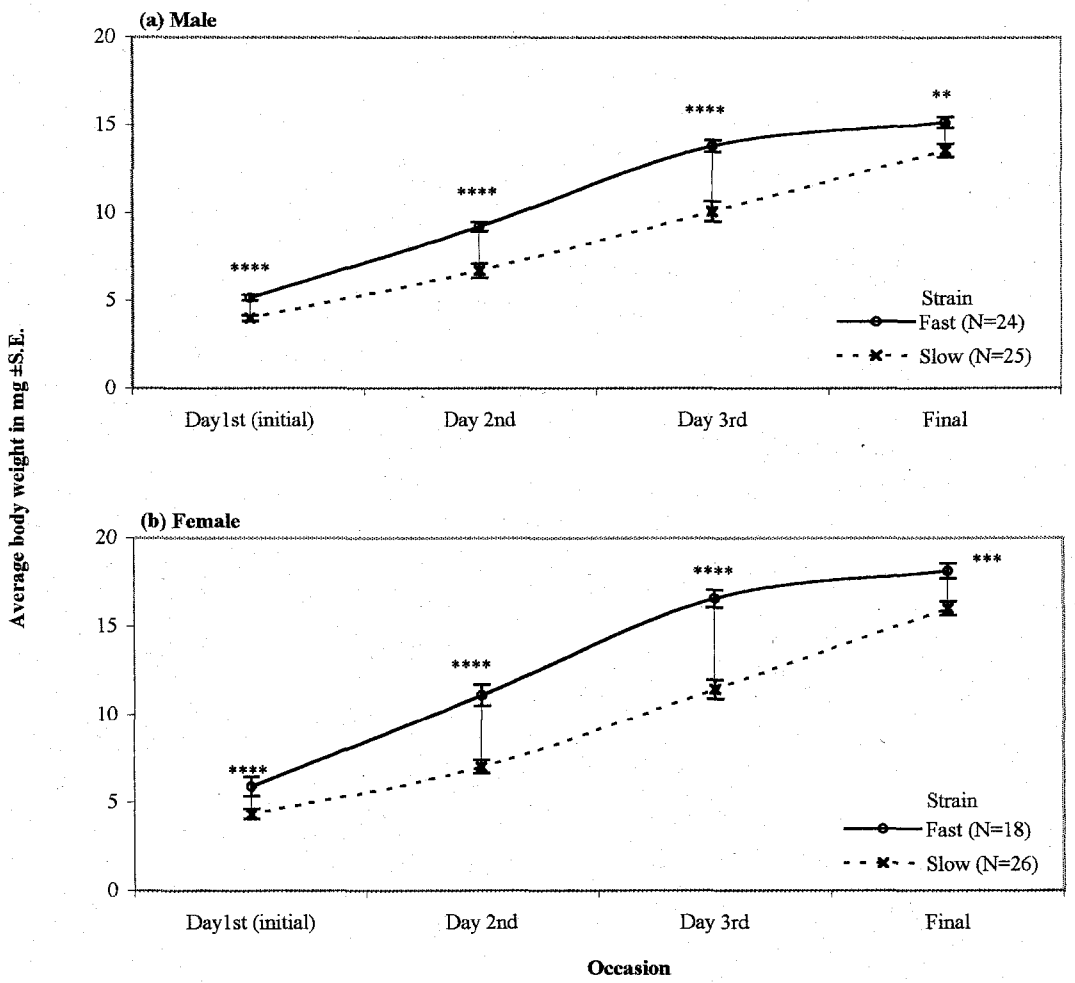


Figure 4-7. The average weight on the 1st, 2nd, 3rd and final day of the 4th instar larvae, and of the pupae of the fast and slow strains when fed an excess of aphids/day. (**, *** and **** significant differences between strains, $P < 0.01$, 0.001 and 0.0001. NS no difference between strains, $P > 0.05$: Mann-Whitney U test.)

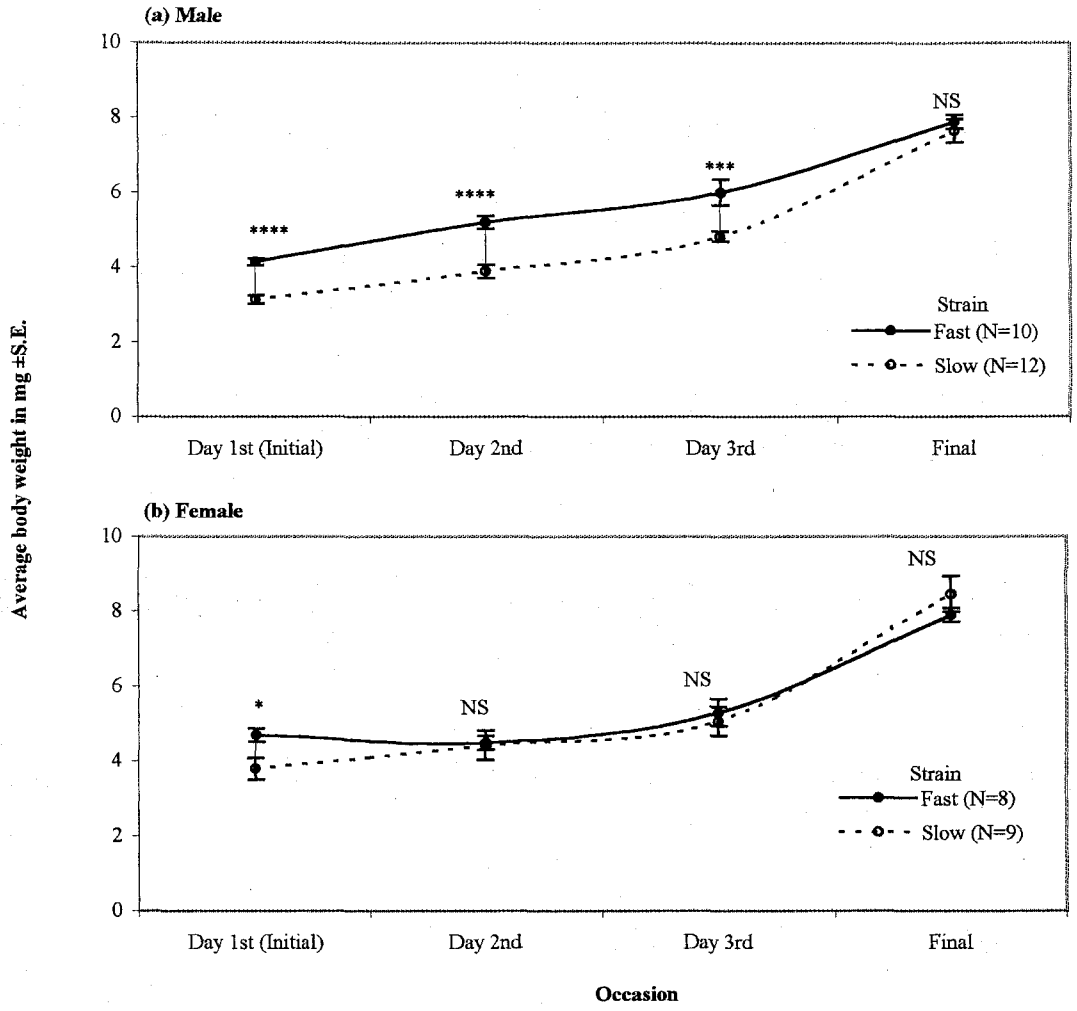


Figure 4-8. The average weight on the 1st, 2nd, 3rd and final day of the 4th instar larvae of the fast and slow strains when fed 1 aphid daily. (*, *** and **** significant difference between strains, $P < 0.05$, 0.001 and 0.0001, respectively. NS no difference between strains, $P > 0.05$: Mann-Whitney U-test.)

0.001and 0.01 on 2nd, 3rd and final days, respectively) (Fig. 4-7). However, the difference decreased from 30 % on day 1 to about 10% on the final day in both sexes. Consequently, adult body weight also differed significantly in the two strains for both sexes (Male and Female: $P <$ 0.001) (Fig. 4-6b). In the 1aphid treatment, although male larvae of the fast strain were heavier than those of the slow developing strain on the second and third day of the fourth instar ($P <$ 0.0001 and 0.001 on 2nd and 3rd days, respectively), there was no significant difference in female larval weight on those days in the two strains ($P > 0.05$) (Fig. 4-8). In both sexes, larval weight on the final day of the fourth instar did not differ significantly in the two strains ($P > 0.05$). Consequently, adult body weight of both sexes also did not differ significantly in the two strains (Male and Female: $P > 0.05$) (Fig. 4-6b).

(4) Food consumption

Aphid consumption during the fourth instar by fast and slow developing strains, when fed an excess of aphids daily is given in Figure 4-9. There was a significant difference in the average aphid consumption of both sexes on the first and second days in the two strains (Male: $P < 0.05$, 0.0001, Female: $P < 0.01$, 0.0001 on the day 1st and 2nd, respectively). Aphid consumption by male larvae in both strains and female larvae in the fast strain peaked on the second day, the fast developing larvae consumed 9.0 and 14.4 mg more aphids than the slow developing larvae, for males and females, respectively. Aphid consumption by slow developing female larvae peaked on the third day, and there was no significant difference in the aphid consumption of two strains, for both sexes, on that day (Male: $P > 0.05$, Female: $P > 0.0.5$). Although fast-developing larvae completed their feeding in four days, slow developers continued feeding until day seven of the fourth instar.

Maximum consumption of aphids differed in the two strains (Male and Female: $P < 0.0001$) (Table 4-1), with the male and female fast developers consuming 6.5 and 9.9 mg more aphids

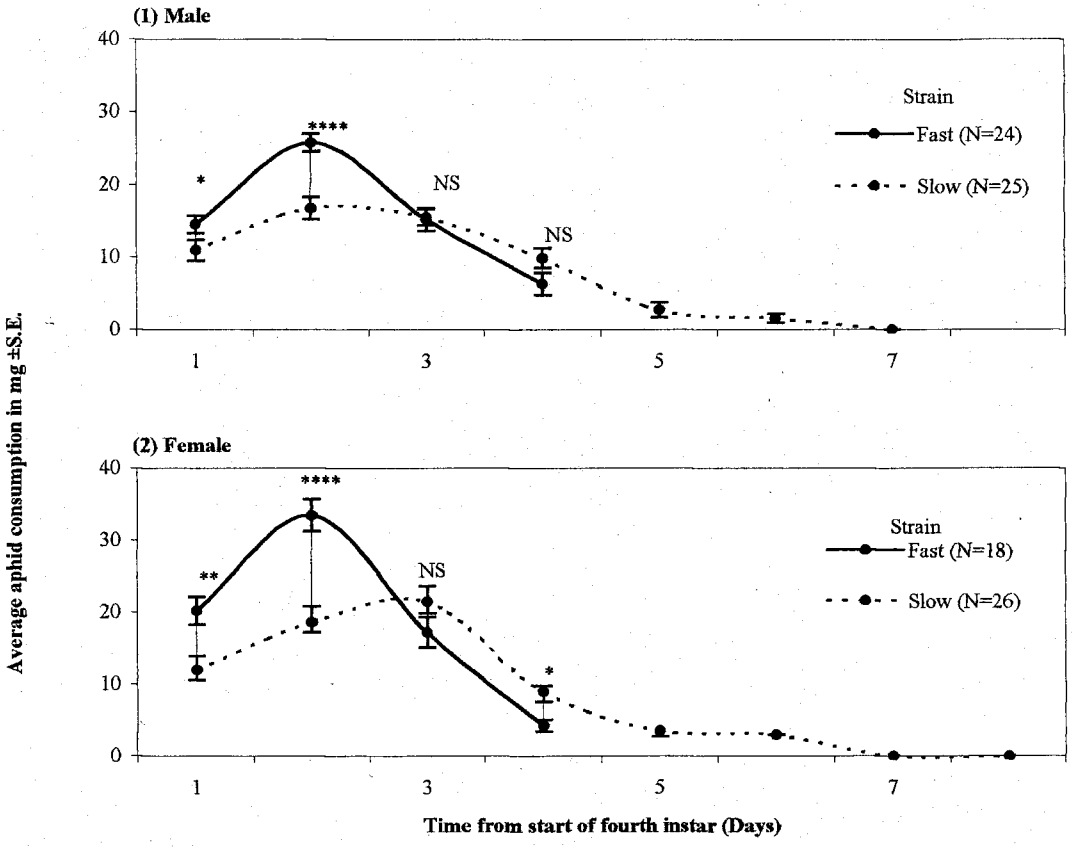


Fig. 4-9. Temporal changes in the average aphid consumption by fourth instar larvae of the fast and slow developing strains. (*,**and *** significant difference between strains, $P < 0.05$, 0.01 and 0.0001, respectively. NS no difference at $P > 0.05$: Mann-Whitney U-test.)

Table 4-1. The average aphid consumption in the fourth instar of fast and slow developing individuals.

(1) Male

Developmental		Mean aphid consumption in mg ±S.E.		
Strain	**N**	**Max.**	**Average/day**	**Total**
Fast	24	26.3 ± 1.2	16.2 ± 0.8	58.3 ± 2.4
Slow	25	19.8 ± 1.1	13.0 ± 1.1	54.7 ± 3.5
U		113	178	248
P <		0.0001	0.05	NS

(2) Female

Developmental		Mean aphid consumption in mg ±S.E.		
Strain	**N**	**Max.**	**Average/day**	**Total**
Fast	18	35.0 ± 1.8	21.5 ± 1.2	73.2 ± 3.2
Slow	26	25.1 ± 1.3	15.1 ± 0.8	62.5 ± 2.9
U		72	72.5	138
P <		0.0001	0.0001	0.05

NS no difference between fast and slow strains at $P < 0.05$: Mann-Whitney *U*- test.

than the slow developers, respectively. There was a significant difference in the average aphid consumption (Male: $P < 0.05$, Female: $P < 0.0001$), with the male and female fast developers consuming 3.2 and 6.4 mg more aphids than the slow developers, respectively. In terms of total aphid consumption, there was no significant difference between the two strains for males ($P > 0.05$), but the females of the fast developing strain consumed 10.7mg more aphids than the slow developers ($P < 0.05$).

(5) Growth

Larval growth in the fourth instar when fed 1 aphid or an excess of aphids/day was compared in the two strains (Fig. 4-10). In the excess aphid treatment, average increase in body weight was about 10.0 and 12mg, for males and females in both strains, respectively, and there was no significant difference between the strains for either sex (Male and Female: $P > 0.05$) (Fig. 4-10a). In the 1aphid treatment, although, average increase in body weight did not differ significantly in the two strains for males ($P > 0.05$), it tended to be higher in the slow than in the fast developers. This tendency was more obvious in females ($P < 0.01$).

In the excess aphid treatment, there was a significant difference in the average rate of increase in bodyweight in the two strains in both sexes (Male and Female: $P < 0.001$) (Fig. 4-10b). The male and female fast developers had a daily increase of 0.6 and 0.8 mg greater than the slow developers, respectively. In the 1 aphid treatment, there was no significant difference in average rate of increase in body weight in the two strains in both sexes; it was about 0.5mg (Male and Female: $P > 0.05$).

In the excess aphid treatment, the average relative increase in body weight of the slow developers was 350 and 390% for males and females, respectively, which is significantly greater than that of the fast developers, which were 300 and 330%, respectively (Male and Female: $P < 0.01$) (Fig. 4-10c). Similarly, in the 1aphid treatment, the average relative increase in body weight

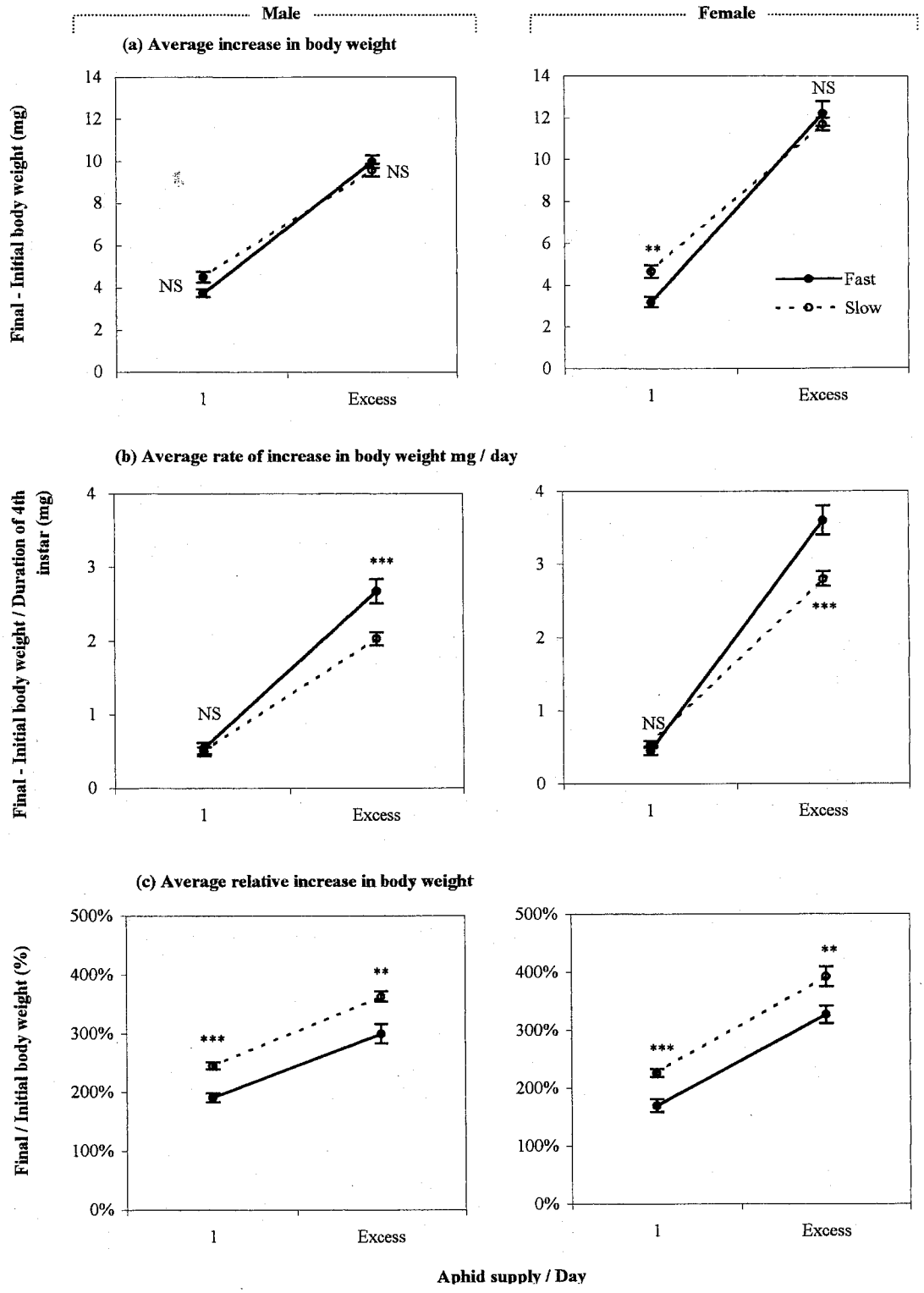


Figure 4-10. The average increase, rate of increase/day and relative increase in body weight/day of 4th instar larvae of fast and slow developing strains when fed one individual or an excess of aphids daily. (** and *** significant difference between strains, $P < 0.01$ and 0.001, respectively. NS no difference between strains, $P > 0.05$: Mann-Whitney U-test.)

was significantly higher in slow developers than in fast developers (Male and Female: $P < 0.001$).

4-4 DISCUSSION

In general, population growth is faster in aphids than in coccids. Therefore, Dixon (2000) suggests that aphidophagous ladybirds have been more strongly selected for fast development than coccidophagous species, as fast development optimises their harvesting of aphids (Kindlmann & Dixon, 1993). In the present experiment most larvae developed relatively fast, which also suggests that *A. bipunctata* have been selected for fast development.

After selecting *Hippodamia convergens* for fast development over several generations, fast-developing larvae consumed more aphids per unit time and developed into larger adults than more slowly developing individuals (Rodriguez-Saona & Miller, 1995). In the present experiments, the same tendency was observed in fast- and slow-developing strains of *A. bipunctata*. When food availability was unlimited larvae of a fast-developing strain consumed more aphids per day in total than those of a slow-developing strain. Consequently, the fast developing larvae gained more body weight and developed into larger adults than the slow developing strain. Many life history traits such as fecundity and mating success are positively related to adult body size. That is, when food is unlimited fast development is likely to be advantageous.

However, in the field, as aphid abundance changes in time and space (Kindlmann & Dixon, 1993; Yasuda & Shinya, 1997; Dixon, 1998; Osawa, 2000; Section2), ladybirds are often food limited. Several authors report that food availability adversely affects developmental rate (1/Duration of development) and body size in ladybirds (Kawauchi, 1979; Mills, 1979). Although larval survival did not decrease significantly when fed 1 aphid/day in this study, increase in body weight decreased significantly in both fast and slow strains. That is, they showed the same trend

in life history traits. However, the effect of poor food supply differed in the fast- and slow-developing strains. Although the rate of increase in body weight/day was similar in both the fast and slow strains, the longer duration of development of slow-developing strains enabled them to gain more body weight than fast-developing strains. Consequently, although larvae of fast-developing strain were larger than those of the slow-developing strain at the start of fourth instar, their body weights converged during fourth instar. Small larvae are more vulnerable to IGP than large larvae (Sengonca & Frings, 1985; Lucas *et al.*, 1997, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001). However, when food is limiting fast-developing larvae are unlikely to survive to reach a large size and become a threat to slow-developing larvae. Larval survival was significantly decreased in both fast- and slow-developing strains when fed 0.5 aphids/day. Therefore, at this level of food availability, larvae do not appear to be able to avoid starving to death. Large larvae are more likely to starve to death at a particular mean food availability, because their maintenance costs are higher (Tenhumberg *et al.*, 2000). In the present experiment, although almost all individuals of both the fast and slow strains starved to death when fed 0.5 aphids/day, the larvae of the slow-developing strain survived for longer than those of the fast-developing strain.

Generally, cannibalism is associated with an asymmetry in size or activity between cannibal and victim (Dixon, 2000). In the field, the most frequently reported cannibalism is of eggs and pupae (Section2). In section 3, although the same instar of larva was used to initiate the experiments, the duration of development of the larvae differed. Consequently, the slow-developing larvae often ate fast-developing individuals, which were in the prepupal stage when the aphid became extinct. That is, when food availability is low, slow development can be advantageous because it gives them more time to search for aphid prey, and the slow-developing individuals are more likely to eat the faster developing individuals than *vice versa*.

In conclusion, although aphidophagous ladybirds are likely to be selected for fast development this is only advantageous if aphids are abundant. That the speed of development of individuals from the same cluster of eggs varies (Section 5) makes it more likely that some larvae will survive even when aphids become scarce.

SECTION 5

LIFE HISTORY TRAITS IN THE SLOW-FAST CONTINUUM

5-1 INTRODUCTION

Most predatory ladybirds are either aphidophagous or coccidophagous and their life history traits differ. For instance, in general, aphidophagous species develop and consume their prey at a relatively fast rate compared to coccidophagous species. Their prey also differs in its speed of development and abundance; aphids develop faster and appear to be more abundant than coccids. Therefore, Dixon (2000) suggests that the pace of life of aphidophagous and coccidophagous ladybirds reflect that of their prey, and the life history traits, such as speed of development, of these two groups of ladybirds are likely to be associated with their pace of life.

In section 4, it was shown that fast-developing larvae of *A. bipunctata* consumed more aphids per day and in total, and developed into larger adults than slow-developing individuals. Interestingly, the differences in duration of development, body size and food consumption rate are positively related to their speed of development even within a species. Accordingly, if in general the speed of development is associated with the life history traits of predatory ladybirds, the life history traits of adults that develop fast or slow are also likely to differ.

In the field, the availability of aphids varies during the larval period (Kindlmann & Dixon, 1993; Yasuda & Shinya, 1997; Dixon, 1998; Osawa, 2000, Section 2). Therefore, larvae often face low food availability, which has adverse affects on their adult body size. Several authors have reported that life history traits, such as fecundity and longevity (Yasuda & Dixon, 2000), egg and cluster size (Dixon & Guo, 1993) are associated with adult size. However, whether the life history traits of adults are associated with their speed of development is still unknown.

The objectives of this section were to determine the longevity, fecundity, egg and cluster sizes of fast- and slow-developing individuals of the two spot ladybird, *A. bipunctata*.

5-2 MATERIALS AND METHODS

(1) Experiment 1: Reproduction and longevity

Fecundity and longevity of unmated adults of the fast and slow developing strains were compared. Fast- and slow-developing fourth instar larvae were selected at random (Materials and Methods, Section 4). These larvae were kept individually in 3cm-diameter Petri dishes and supplied an excess of pea aphid daily until they pupated. Time of pupation and adult emergence and the sex of the adults were noted. All adults were also kept singly in 3cm diameter Petri dishes, and fed an excess of pea aphids every 2 days, and the number of eggs laid and adult survival were recorded. This procedure was continued until all individuals died. Longevity was recorded for both the males and females.

(2) Experiment 2: Effect of female size on their offspring

Egg and cluster size

Five females, which differed in body weight (6.5 mg, 10.5 mg, 13.4 mg, 14.6 mg and 16.1 mg) were selected from the ladybird culture, described in Section 4. Each female was kept with a male in a Petri dish (3 cm diameter), and fed daily an excess of pea aphids. Unfertilised eggs are usually smaller than fertilized eggs (Personal observation). Therefore, to avoid using unfertilised eggs, the first few clutches of eggs were kept to determine whether they were fertile. Once it was established that the females were laying fertile eggs, clusters of eggs were collected and the eggs were separated from one another using a damp paintbrush. The eggs were allowed to dry for a few hours, and then each egg was weighed at least twice. The average of these measurements was recorded. Number of eggs/cluster for each female was also recorded for a week after they started to lay fertilised eggs.

Offspring size and their duration of development

Of the eggs obtained in Experiment 2, 68 from four females (6.5 mg: N=15, 10.5 mg: N=19, 14.6

mg: N=19, 16.1 mg: N=15) were selected. The larvae that hatched from these eggs were kept individually in plastic tubes (Section 4) and the larvae each fed an excess of pea aphids daily until they pupated. Aphid remains and larval excreta were removed daily. Time taken to develop from oviposition to adult emergence, sex and the fresh weight of each adult at emergence were recorded.

5-3 RESULTS

(1) Reproduction and longevity

Average fecundity of the fast- and slow-developing individuals was compared (Table 5-1). The total egg production of the fast developing individuals, which averaged 140.3±19.4 eggs, was 2 times greater than that of the slow developing individuals (63.2±15.5 eggs) ($P < 0.01$). There is a significant negative relationship between the duration of development and fecundity (R = 0.407, $P < 0.001$): the shorter the duration of development the greater the fecundity. Rate of egg production was also different in the fast and slow developing individuals also differed ($P < 0.01$), the fast developers tended to lay a greater number of eggs per day than the slow developers.

Although, the pre-oviposition period did not differ significantly in the fast and slow developers ($P > 0.05$), the 19.8 days of the fast developers was 30 days shorter than that of the slow developers (Table 5-1). There was a significant positive relationship between duration of development and pre-oviposition period (R = 0.436, $P < 0.001$): shorter the duration of development the shorter the pre-oviposition period

There was no significant difference in the average duration of survival of individuals of the fast and slow developers, which was about 95 and 120 days for males and females, respectively ($P > 0.05$) (Table 5-1).

However, temporal changes in survival of adults differed (Fig. 5-1). The initial survival of males of the slow developers was poorer than those of the fast developers. It was 67% on the

Table 5-1. Adult life history traits of fast and slow developing individuals.

Sex	**Male**					**Female**				
Speed of development	**Fast (N=23)**	**Slow (N=29)**	***U***	***P***		**Fast (N=34)**	**Slow (N=35)**	***U***	***P***	
Duration of development in day (±S.E.)	21.5 ± 0.1	23.4 ± 0.2	0.0	0.0001		21.9 ± 0.1	24.6 ± 0.3	0.0	0.0001	
Body weight in mg (±S.E.)	9.5 ± 0.7	8.3 ± 0.2	131.0	0.001		11.2 ± 0.3	9.5 ± 0.3	284.0	0.001	
Duration of survival in day (±S.E.)	94.2 ± 9.7	94.0 ± 14.2	314.5	NS		125.4 ± 11.2	118.4 ± 12.8	572.5	NS	
Pre-oviposition period in day (±S.E.)	-	-	-	-		19.8 ± 2.7	45.6 ± 10.6	278.0	NS	
Total number of eggs laid (±S.E.)	-	-	-	-		140.3 ± 19.4	63.2 ± 15.5	359.5	0.01	
Number of eggs laid / day (±S.E.)	-	-	-	-		1.1 ± 0.2	0.5 ± 0.1	365.0	0.01	

NS no significant difference between strains at $P > 0.05$: Mann-Whitney U-test.

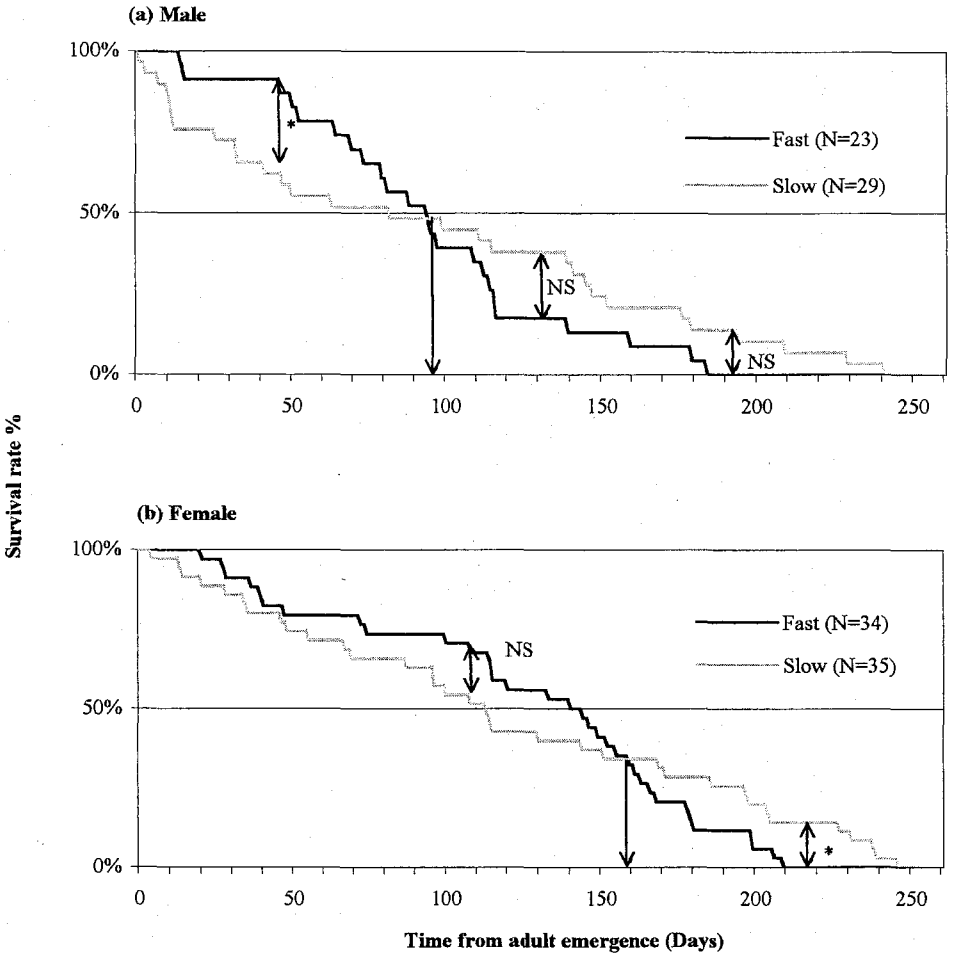


Figure 5-1. Temporal changes in percentage survival of adults of the fast and slow developing individuals. (Females and males were not mated. * significant differences between strains, $P < 0.05$. NS no differences between strains, $P > 0.05$: χ^2 test.)

40th day after emergence, 24% lower than that for the fast developers ($P < 0.05$). Subsequently, survival in the fast developers decreased more rapidly than in the slow developers. Consequently, all of the fast developers died by the 190th day, whereas some of the slow developers survived for over 240 days. Similarly, the same trend was observed in females, of which a greater percentage of the slow developers survived beyond the 160th day than of the fast developers..

(2) Egg and cluster size

Effect of female size on their egg and cluster size

Figure 5-2a and 5-2b show that average egg weight and cluster size, respectively, of five females that differed in body weight. Both average egg and cluster size differed significantly in the females ($P < 0.05$) and is positively associated with female body size (Egg size: R = 0.98, $P < 0.0001$, Cluster size: R = 0.997, $P < 0.0001$). To determine the strength of this association the egg and cluster sizes of the five females is expressed as a percentage of those of the smallest female (Fig. 5-3). Average cluster size tended to increase more dramatically than egg size with increase in female body size, the percentage increase in egg weight and cluster size produced by the largest female (16.1 mg) relative to the smallest female (6.5 mg) were 129.2% and 451.1%, respectively. That is, although both average egg and cluster sizes tended to increase with increase in female size, the effect of female size was much less on egg weight than on cluster size.

Effect of egg size on duration of development and adult size

Average duration of development and adult weight on emergence of the offspring of the five females was compared (Fig. 5-4). Male offspring obtained from the two smaller females, which were 6.5 and 10.5 mg in weight, took significantly longer to develop than those of the two largest females, 14.6 and 16.1 mg ($P < 0.05$). Female offspring of the smallest females also generally took significantly longer to develop than those of the largest females ($P < 0.05$), the average duration of development of those of the smallest female (6.5 mg) was 1.1 times longer than that

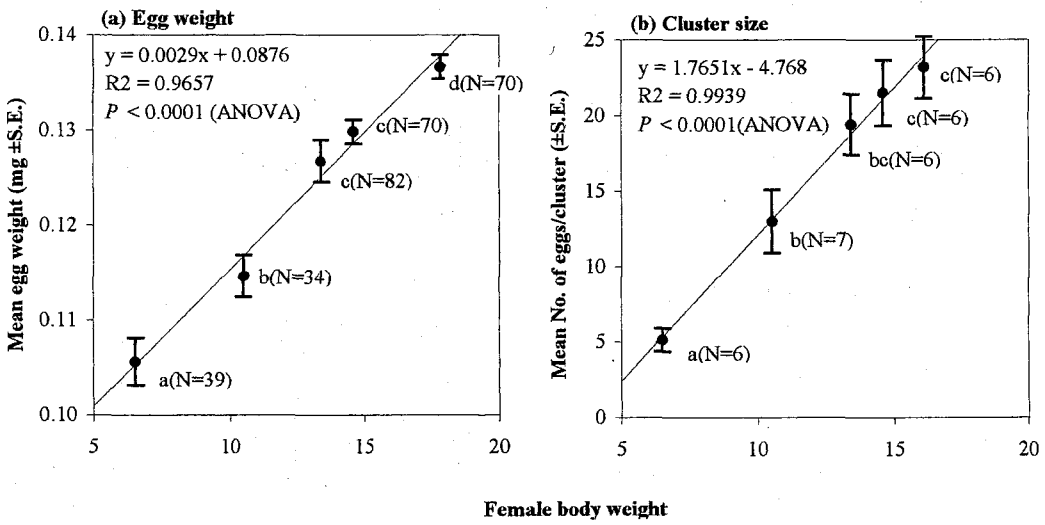


Figure 5-2. The relationship between female body weight and their average (a) egg weight and (b) cluster size. (Dots followed by the same letter do not differ significantly, $P > 0.05$: Mann-Whitney U test.)

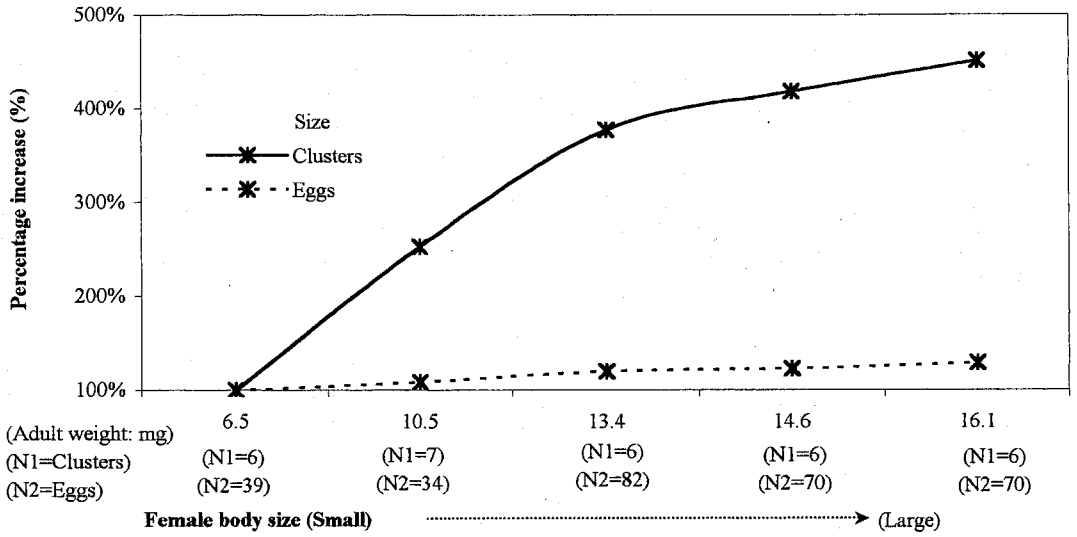


Figure 5-3. Average egg and cluster size of females of a range of weight expressed as a percentage increase over that produced by the smallest females (6.5mg). (Egg and cluster of the smallest female (6.5mg) = 100%.)

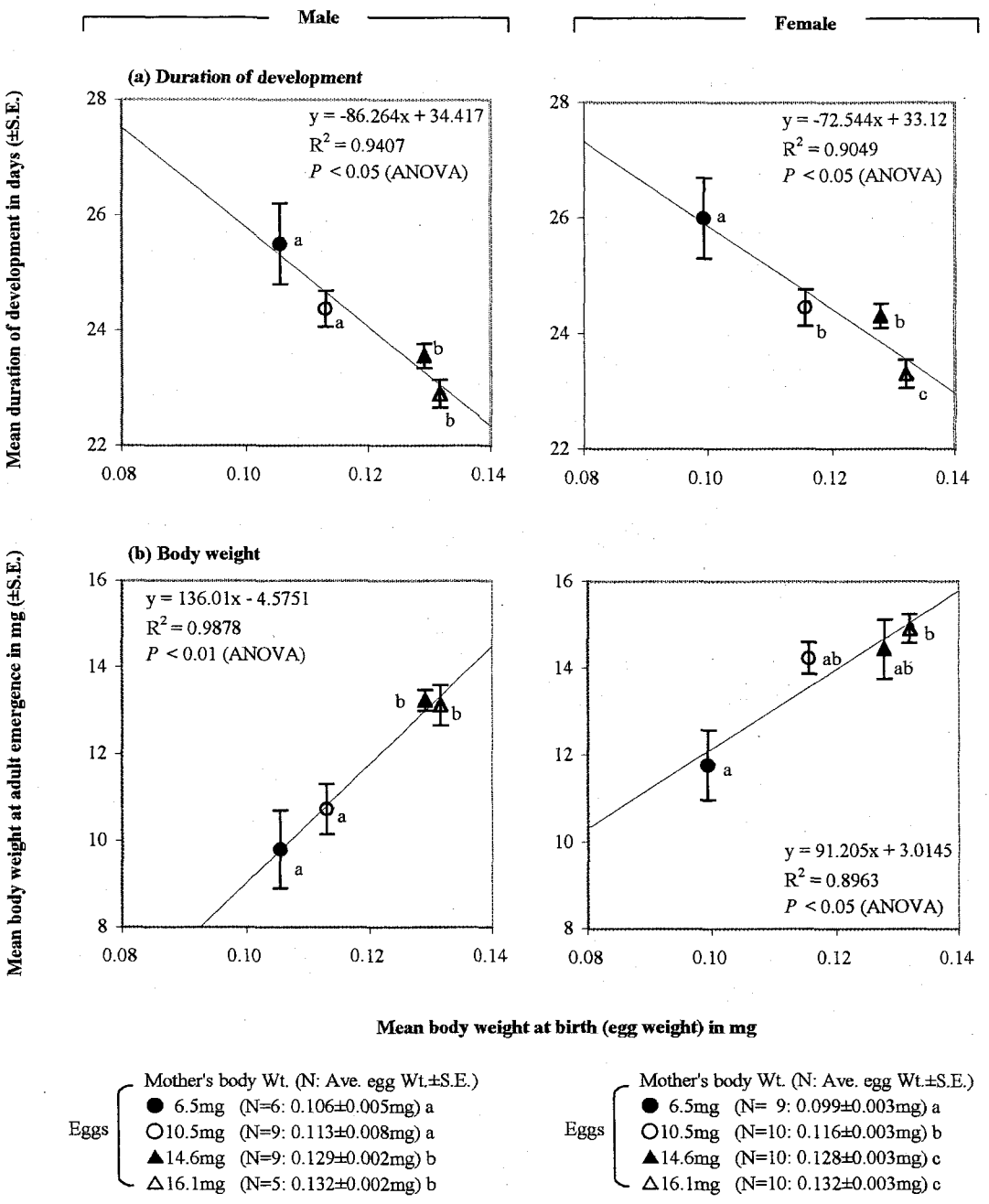


Figure 5-4. The relationship between average offspring weight at birth and (a) their average duration of development and (b) average body weight at adult emergence. (Dots or numerals followed by the same letter do not differ significantly, $P > 0.05$: Mann-Whitney U test.)

of the largest female (16.1 mg) for both sexes. There was a significant negative relationships between average egg size and average duration of development (Male offspring: R = 0.973, $P < 0.05$, Female offspring: R = 0.970, $P < 0.05$).

Offspring size of the different sized females differed significantly at maturity. Those produced by the smallest female (6.5 mg) was less than 80% of the weight of those produced by the largest female (16.1 mg) for both sexes ($P < 0.05$) (Fig. 5-4b). There was a significant positive relationship between egg and subsequent adult size (Male offspring: R = 0.994, $P < 0.05$, Female offspring: 0.947. $P < 0.05$).

5-4 DISCUSSION

Many predatory ladybirds eat aphids the numbers of which change in time and space (Kindlmann & Dixon, 1993; Yasuda & Shinya, 1997; Dixon, 1988; Osawa 2000, Section 2). These ladybirds are often food limited during their development, which adversely affects their adult size. Many life history traits of adults are associated with body size, and several authors have reported that food availability during the larval period affects the life history traits of the adults (Dixon & Guo, 1993; Yasuda & Dixon, 2000). In these studies, the life history traits of large and small adults that experienced high and low food availability during their development, respectively, were reported. In this study the fast- and slow-developing adults differed in body size, but they did not experience limited food availability in their immature stages (Section4). That is, size was possibly more genetically than nutritionally determined.

In *A. bipunctata,* large females are more fecund than small females (Yasuda & Dixon, 2000). In this study, the fast-developing large adults laid more eggs per day and in total than slow-developing small adults. In *C. septempunctata,* ovariole number is positively related to female body size (Dixon & Guo, 1993). Therefore, the different fecundities of the fast- and slow-developing adults was probably due to their body size.

The survival of large and small adults of *A. bipunctata* is similar when fed similarly (Yasuda, personal communication). However, in this study, the fast-developed large females on average survived for a shorter period than the slow-developed small individuals. Life history theory predicts that reproductive activity should shorten adult life (Roff, 1992). Therefore, the earlier death of fast-developed large adults could be a consequence of the relatively high reproductive rate compared to the slow-developed small adults. However, although males from both the fast- and slow-developing individuals were not mated, the fast-developed males also died earlier than the slow-developed individuals. This may imply that speed of development also affected their longevity.

Although females used in experiment 2 were not food limited during their development (Materials and Methods, Section 4), their body size varied from 6.5-16.1 mg. As in general, adult body size of this species is negatively related to their duration of development when food is unlimited (Section 4), it likely that the large and small females used in this experiment were fast- and slow-developers, respectively. The average egg size of these females was positively related to their body size. However, in size-manipulated females of *C. septempunctata*, i.e. different sized females produced by manipulating food availability, egg size is not related to size of mother (Dixon & Guo, 1993). Therefore, it is suggested that egg size is determined genetically, and eggs size determines the speed of development rather than female body size directly.

Offspring size at adult emergence is positively related to size at birth (i.e. egg size). As egg size is positively related to size of mother, offspring adult size reflects their mother's size. In fact, there was a significant positive relationship between average offspring size and that of their mothers, although average male offspring size was not related significantly to that of their mothers (Male: $P > 0.05$, Female: $P < 0.001$) (Fig. 5-5). However, this may be due to the relatively few observations. As large and small mothers are likely to be fast and slow developers,

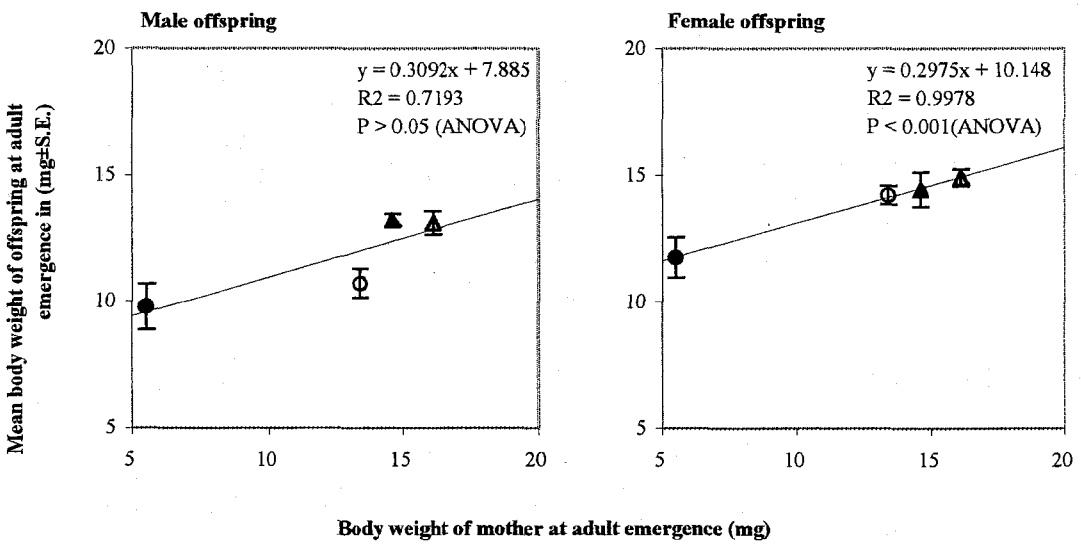


Figure 5-5. The relationship between body weight of four mothers and the average body weight of their offspring at adult emergence.

respectively, the speed of development of the offspring is also likely to be related to that of their mothers.

Table 5-2 gives a summary of the life history traits of the fast- and slow-developing strains used in section 4 and this section, and of the general life history traits of aphidophagous and coccidophagous species (Dixon, 2000). The slow and fast individuals of *A. bipunctata* show the same differences in life history traits as coccidophagous and aphidophagous species of ladybirds (c.f. Dixon, 2000). That is, the speed of development is likely to be a key factor determining the other life history traits of predatory ladybirds.

Several authors have reported that eggs obtained from the same female ladybirds vary in size (Dixon & Guo, 1993). Eggs obtained from single females in the present study also varied in size (e.g. eggs laid by a 6.5 mg- and 13.4 mg-female, Fig. 5-6). If speed of development is associated with egg size, which is suggested by the results presented here, then the life history traits of the offspring are also likely to vary. Fast-developing larvae appear to be less well adapted to low food availability than slow developing larvae (Section4). Therefore, the variation in speed of development could be advantageous because an ovipositing ladybird is unlikely to be able to predict future availability of prey for their offspring. In such circumstances a variable speed of development of the offspring might be the best strategy for aphidophagous ladybirds.

Table 5-2. Summary of the differences in the life history traits of fast and slow developing strains of *A. bipunctata* and of aphidophagous versus coccidophagous species of ladybirds.

	Speed of dvelopment of *A. bipunctata*			Species of ladybirds*[1]		
Life history traits	**Fast**	**Differences**	**Slow**	**Aphidophagous**	**Differences**	**Coccidophagous**
Larvae*[2]						
Rate of development*[3]		>			>	
Relative growth rate		>			>	
Food consumption		>			>	
Adults						
Fecundity		>			>	
Rate of ageing*[4]		>			>	
Egg and cluster size		>			>	

*[1] Differences between aphidophagous and coccidophagous species of ladybirds (Dixon, 2000)

*[2] Differences between fast and slow developing strains of larvae (Section 4)

*[3] Rate of development = 1/Duration of development

*[4] Rate of ageing = 1/ Longevity or pre-oviposition period

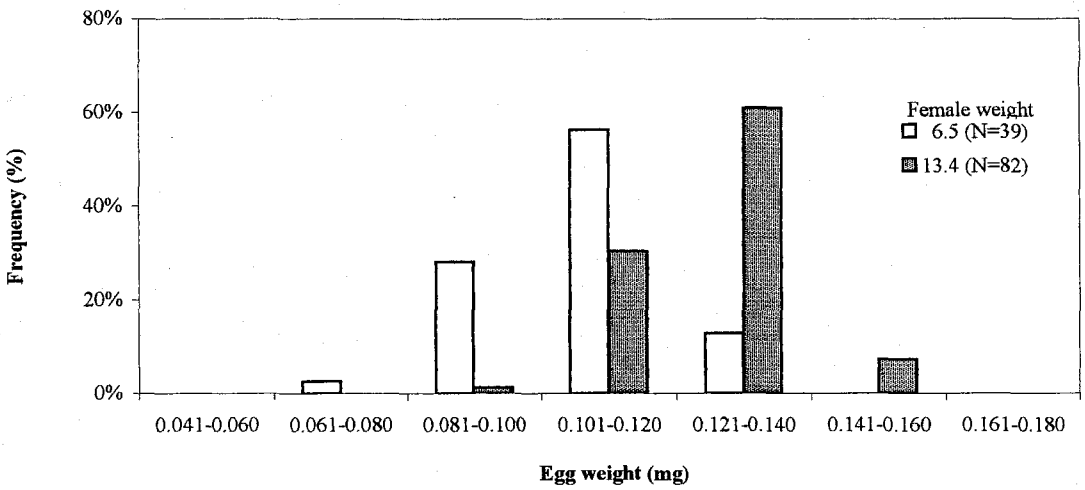


Figure 5-6. Frequency distribution of the eggs weights of two females, 6.5mg and 13.4 mg in weight, respectively.

SECTION 6

GENERAL DISCUSSION

In general, the incidence of cannibalism and I. G. P. are likely to increase when aphids are scarce relative to the abundance of ladybirds (e.g. Takahashi, 1987; Agarwala & Dixon.1991, 1992; Yasuda & Shinya, 1997). As cannibalism and I. G. P. more commonly occur when prey become scarce, they are possibly important for the survival when prey availability is low (Osawa, 1991; Yasuda & Shinya).

This discussion will be presented in three sections. In the first, subsection 6-1, variation in the incidence of cannibalism in ladybirds is discussed based on the results presented in Sections 2 and 3. This is followed by a discussion of the possible effects of the availability of intra-guild prey on the survival of an intra-guild predator in subsection 6-2. Finally, how variation in prey availability might affect survival is discussed in subsection 6-3 based on the results presented in Sections 4 and 5.

6-1 Cannibalism

Egg cannibalism in *C. s. brucki* is rare if aphid prey is abundant (Takahashi, 1987), *H. axyridis* larvae prefer their own eggs to aphids (Kawai, 1978). In section 2, it is reported that egg cannibalism occurred more frequently in *H. axyridis* and *P. japonica* than in *C. s. brucki*. Therefore, the incidence of egg cannibalism may depend the species.

In addition, the preferred oviposition site may also affect the percentage egg loss due to cannibalism. Osawa (1989) shows that incidence of egg cannibalism is high if eggs are too close to aphids, as conspecific larvae and adults, which are potential cannibals, tend to aggregate in patches of aphid (e.g. Nakamuta, 1985; Carter & Dixon, 1982). Therefore, differences in their oviposition sites may also reflect the incidence of egg cannibalism in the three species. Females of *C. s. brucki* appear to lay their eggs further from aphid colonies than either of *H. axyridis* and *P. japonica* (Sato, 1994). However, as their preferred oviposition site is still unknown, further work on this is needed if we are to achieve a better understanding of egg cannibalism in these

species.

Winder (1990) suggested that the tendency of larvae to disperse when prey is scarce may reduce the probability of their encountering con- and hetero-specific larvae. In addition, the leaving rate depends on species (Schellhorn & Andow, 1998). These studies suggest that the incidence of larval cannibalism varies between species.

In Section 3, it is recorded that the mortality of larvae due to cannibalism was low for *C. s. brucki* because they tended to emigrate soon after the aphid became extinct, when the incidence of cannibalism is likely to increase. However, as the larvae of *H. axyridis* and *P. japonica* stayed on the plants after the aphids became extinct, the larvae of these species suffered a higher mortality due to cannibalism than the larvae of *C. s. brucki*. Cannibalism may result in the survival of some larvae when prey is scarce (Osawa, 1991). In the present study, some larvae of *P. japonica* ate conspecific individuals and completed their development after prey became extinct. Although *H. axyridis* larvae also ate conspecifics, no larvae of this species became adult.

In marked contrast, the incidence of larval cannibalism in *C. s. brucki* was low even when prey became scarce. As larvae of *C. s. brucki* dispersed when aphid prey was scarce, the availability of potential conspecific victims decreased and the incidence of cannibalism was relatively low in this species compared to the other two species.

6-2 I. G. P.

In general, I. G. P. is associated with an asymmetry in size; small species generally are more vulnerable to I. G. P. than large species (Sengonca & Frings, 1985; Lucas *et al.*, 1997, 1998; Phoofolo & Obrycki, 1998; Hindayana *et al.*, 2001). However, in several species of ladybirds, which have overlapping habitat preferences, chemical protection of the smaller species from predation by the larger species has been reported (Agarwala & Dixon, 1992; Agarwala *et al.*, 1998; Hemptinne *et al.*, 2000).

Several studies indicate that *H. axyridis* and *C. s. brucki* commonly co-occur in early spring (Takahashi, 1987; Yasuda & Shinya, 1997; Section 2). Although these two species are similar in body size, their timing of occurrence differs. The latter occurring species is vulnerable, as it is likely to be at an earlier stage of development than the other species (Section 2). However, the latter occurring species, *H. axyridis* is toxic to *C. s. brucki* (Section 3). That is, although *H. axyridis* appears to be vulnerable to predation by *C. s. brucki*, it is unlikely to be eaten by *C. s. brucki* larvae because it is toxic, which is what was reported by Yasuda & Onuma (2000).

On the other hand, *C. s. brucki* is suitable intra-guild prey for *H. axyridis*. Yasuda and Onuma (2000) reported that larvae of *H. axyridis* develop equally well when fed either aphids or larvae of *C. s. brucki*. In the field, larvae of *H. axyridis* were observed eating eggs, larvae and pupae of the other species, but no larvae of *C. s. brucki* or *P. japonica* were observed eating any stages of *H. axyridis* (Section 2). Therefore, it is suggested that *H. axyridis* is an intra-guild predator in this ladybird guild. In addition, although none of the larvae of *H. axyridis* completed their development when reared on their own, some of them developed to the adult stage when larvae of the other species were present (Section 3). These results suggest that larvae of *H. axyridis* are adapted to eat other species.

For some species of ladybirds, it is reported that the females react to the larval tracks of other species and reduce their egg production (Růžička, 2001). Therefore, as larvae of *H. axyridis* appear to require the larvae of other species in order to complete their development (Section 3), females of *H. axyridis* may react to the larval tracks of other ladybird species and prefer to lay their eggs in prey patches already being exploited by larvae of the other species, such as *C. s. brucki* and *P. japonica*.

In conclusion, *H. axyridis* is possibly a "top predator", and more likely to be able to survive when aphids are scarce, as they can exploit both extra- and intra-guild preys. However, Section 2 and 3

revealed that when *C. s. brucki* oviposit markedly earlier than *H. axyridis* the eggs and larvae of *C. s. brucki* were less likely to suffer from predation by larvae of *H. axyridis*. In addition, the abundance of *P. japonica*, which is the most frequently observed intra-guild prey of *H. axyridis*, varied from year to year. The timing of the oviposition of *C. s. brucki* and the abundance of *P. japonica* are likely to be affected by temperature (Section 2 and 3), which varies from year to year (Section 2). Therefore, prey availability is uncertain even for a "top predator" like *H. axyridis*. To cope with this uncertainty of prey availability variation in speed of development is likely to be advantageous, which was discussed in a next subsection.

6-3 Fast and slow development

All the life history traits of predatory ladybirds, including both aphidophagous and coccidophagous species, are associated positively with speed of development (Dixon, 2000). In section 4 and 5, it is reported that the life history traits of fast- and slow-developing individuals differed even in the same species.

As fast-developing larvae are potentially capable of consuming more aphids and developing into larger adults than slow-developing larvae, they are well adapted to high food availability (Section 4). A short duration of development may reduce the incidence of cannibalism or I. G. P.. In addition, developing into large adults is advantageous because large adults are more fecund than small adults (Section 5). Therefore, it could be assumed that fast development is advantageous when prey availability is high. However, as reported in subsections 6-1 and 6-2, availability of both intra- (Con- and hetero-specific) and extra-guild prey (Aphids) for ladybird larvae is likely to be uncertain whether they are "top predators" or not.

Fast-developing larvae are more adversely affected by low food availability (Section 4). For instance, fast-developing larvae starved to death sooner than those of slow-developing individuals when food availability was low. Therefore, if prey

availability is low during their development, the fast-developing larvae are less likely to complete their development than the slow-developing individuals. In addition, although prey availability is high during their development, large adult size of the fast-developing individuals can be disadvantageous if food is scarce for the adults. Yasuda & Dixon (2000) reported that a large male is less likely to copulate when food is scarce. Therefore, it is suggested that fast-developed adults are also more likely affected by low prey availability than the slow-developed adults.

On the other hand, as the slow-developing larvae potentially require fewer prey to complete their development than fast-developing individuals, their survival is less affected by low food availability than that of the fast-developing larvae (Section 4). In addition, slow-developing larvae tended to live longer than fast-developing individuals when prey was scarce. This longer duration of survival may enable them to search for food resources for longer than the fast-developing individuals. In addition, small males are more likely to copulate when aphids is scarce (Yasuda & Dixon, 2000). The slow developing larvae tended to develop into small adults (Section 4). That is, it is suggested that slow development is advantageous when prey availability is low.

As in the field aphid abundance is affected by many factors (Dixon 2000; Section 2), the availability of prey for the larvae is likely to be uncertain. If so, variation in the speed of development of the offspring could be advantageous because it increases the chances of ladybird larvae surviving when food availability is low.

In conclusion, this thesis suggested that flexibility of ladybird due to variation in the speed of development is important for their survival when prey availability is variable. Although the speed of development of ladybird potentially varied between individuals (Section 4), the actual effect of this life history trait on their survival has been largely ignored. Therefore, there is a need

for this area of study to be further confirmed for a better understanding of the population dynamics of ladybirds.

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