

# A comparative study of larval cannibalism in three species of ladybird

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**Abstract.** 1. Cannibalism was studied under laboratory conditions in three species of Coccinellidae (Coleoptera): *Cycloneda sanguinea*, *Olla v-nigrum*, and *Harmonia axyridis*. Larval cannibalism varied among species, *C. sanguinea* > *H. axyridis* ≥ *O. v-nigrum*.

2. Larvae of all species cannibalised more in response to reduced food availability (14 h starvation daily) than in response to reduced food quality (dry *Ephestia* eggs).

3. Larvae of *H. axyridis* cannibalised siblings at lower rates than non-siblings, but larvae of *C. sanguinea* and *O. v-nigrum* did not. Rates of cannibalism increased significantly with increasing size disparity among larvae of all three species.

4. Cannibalism in *C. sanguinea* and *H. axyridis*, but not in *O. v-nigrum*, increased with larval density, suggesting that not all attacks on conspecifics are driven by hunger.

5. Costs, not benefits, were the predominant effects of exclusively and partially cannibalistic diets. A diet of conspecific eggs yielded survival equivalent to the *Ephestia* egg diet for *H. axyridis* and *O. v-nigrum*, but developmental time was extended and adults were smaller. Larvae of *C. sanguinea* had superior survival and faster development on conspecific eggs than on the *Ephestia* egg diet. Only *H. axyridis* larvae survived as well on diets comprised exclusively or partially of conspecific larvae as on *Ephestia* eggs, although they developed more slowly.

**Key words.** Cannibalism, *Cycloneda sanguinea*, *Harmonia axyridis*, *Olla v-nigrum*.

## Introduction

Cannibalism may represent an important survival tactic for many insect species. It has been documented in at least 10 insect orders (Elgar & Crespi, 1992) and probably remains undescribed in many others. Predatory insects seem particularly prone to cannibalise (Polis, 1981) and the behaviour can be problematic in the mass production of beneficial species for biological control purposes. For example, Duelli (1981) reported that cannibalism occurred to varying degrees in all 11 species of North American Chrysopidae that he examined. Michaud (2001a) found that commercially packed larvae of *Chrysoperla plorabunda* (Fitch) cannibalised at rates that reduced their numbers by 20% per day after hatching, even in the presence of a food source in the form of *Sitotroga* sp. eggs. Ecological theory

identifies many factors that can influence cannibalism, including food availability, food quality, size disparity, degree of relatedness, and population density. The work reported here was initiated to test the relative importance of these factors to rates of larval cannibalism in three species of predaceous Coccinellidae, and to explore the developmental consequences of exclusively and partially cannibalistic diets.

Cannibalism has long been studied in the Coleoptera, as evidenced by Crombie's (1944) study of competitive interactions in the grain beetle *Rhizopertha dominica* (F.). At least 12 predaceous coccinellid species are known to be cannibalistic (Stevens, 1992). Cannibalism and interspecific predation can be among the most important mortality factors for juvenile coccinellids (Wright & Laing, 1982; Schellhorn & Andow, 1999). Obrycki and Kring (1998) identified cannibalism as a major problem in the mass-rearing of coccinellid species for augmentative biological control programmes, however there appears to be considerable variation among coccinellid species in their propensity to cannibalise.

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For example, efforts in Florida to mass rear *Cycloneda sanguinea* L. have been thwarted by high rates of larval cannibalism, while colonies of *Harmonia axyridis* Pallas and *Olla v-nigrum* (Mulsant) suffer minimal losses to cannibalism under identical rearing conditions (J. P. Michaud, unpublished). While cannibalism has been well studied in *H. axyridis* (Joseph *et al.*, 1999; Wagner *et al.*, 1999; Snyder *et al.*, 2000), neither *C. sanguinea* nor *O. v-nigrum* has been examined in this regard.

There are inherent risks associated with cannibalism, primarily because a conspecific is probably a more dangerous adversary than a typical prey item (Elgar & Crespi, 1992). In order for cannibalism to persist in a population over evolutionary time, it must yield fitness benefits, directly or indirectly, that are sufficient to offset these costs. Cannibalism may permit survival and/or reproduction when either the quantity or quality of normal food is inadequate. Consequently, cannibals that are able to kill conspecifics and assimilate their corpses effectively can survive and/or reproduce when non-cannibals perish or fail to reproduce. In this case, the net benefit equals the food value of the corpse minus the costs of dispatching the victim; however cannibalism may also increase individual fitness indirectly through the elimination of intraspecific competitors. These two types of benefit are not mutually exclusive and could act in an additive manner to favour the evolution of cannibalism.

If cannibalism were triggered by hunger, rates of cannibalism would be expected to be affected strongly by degree of satiation. Individuals also require the ability to assimilate conspecific flesh or there is no nutritional benefit of cannibalism. If benefits are realised through reduced intraspecific competition, however, cannibalism might increase with the rate of encounter between conspecifics, in which case the benefits are indirect and independent of the nutritional value of the conspecific corpse. In either scenario, it would seem beneficial for individuals to avoid consuming related individuals because of the attendant loss of inclusive fitness.

If risk of injury is a significant cost of cannibalism, cannibals may target more vulnerable individuals preferentially. Dixon (2000) suggested that size, developmental stage, and hunger are probably key determinants of the relative vulnerability of coccinellid larvae to cannibalism. If decisions to cannibalise are risk sensitive, and the risk decreases with the relative size of the victim, the frequency of cannibalism should increase with increasing size disparity among individuals confined together.

Both *C. sanguinea* and *O. v-nigrum* are aphidophagous coccinellids indigenous to Florida (Gordon, 1985), whereas *H. axyridis* is an invasive species that has recently become prevalent in the citrus ecosystem (Michaud, 2000). All three species are important generalist predators that contribute to the biological control of many pests in the Florida citrus ecosystem (Michaud, 2000, 2001b). Cannibalism of eggs and larvae by adults and larvae of *C. sanguinea* and *H. axyridis* has been observed directly in the field (J. P. Michaud, unpublished). Michaud and Belliure (2000)

monitored colonies of the aphid *Toxoptera citricida* (Kirkaldy) in the field and reported cannibalism as the primary recognisable source of mortality among juvenile coccinellids, mainly *C. sanguinea*, observed on aphid colonies. Here, a series of experiments is presented that compared these three species with respect to their intrinsic propensity to cannibalise, their responses to experimental manipulation of the factors hypothesised to influence cannibalism, and the developmental consequences of consuming conspecific eggs and larvae.

## Materials and methods

Stock colonies were established by collecting adults from citrus trees in Polk County, Florida (28.1°N, 81.4°W) in March 2001 (*C. sanguinea* and *H. axyridis*) and in St Lucie County, Florida (26.2°N, 81.1°W) in April 2001 (*O. v-nigrum*). Stock colonies and all experimental insects were kept on benches in a laboratory at  $24 \pm 1^\circ\text{C}$  and a L:D 16:8 h period. Adult beetles were maintained in 1 litre, wide-mouth glass jars filled with shredded wax paper and covered with a fine mesh. Beetles in jars were fed a combination of bee pollen and frozen eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), with water available continuously on a cotton wick. Ovipositing females were removed from jars, isolated in plastic Petri dishes 5.5 cm diameter  $\times$  1.0 cm tall, and provided with frozen *Ephestia* eggs supplemented with bee pollen and water encapsulated in polymer beads as required (referred to hereafter as the control diet). It could be argued that different food sources (aphids, psyllids, etc.) could generate different results in cannibalism experiments as a function of their relative palatability etc., and that *Ephestia* eggs may not be an equally acceptable food for all three species, however there is considerable variation among these ladybirds with respect to their ability to utilise all natural food sources, even various aphid species (Michaud, 2000), making it difficult to select a diet that would be equally acceptable and suitable for all three. Although the relative palatability of *Ephestia* eggs might vary among these ladybirds, it was the only diet available known to support both development and reproduction in all three species.

All experiments were performed in the same size plastic Petri dishes (as above). Most cannibalism events were easily recognisable as they resulted in complete consumption of the victim, but apparently intact corpses were examined under a low power stereo-microscope to determine whether death was passive or the result of attack by a conspecific. Data from all experiments were collected between 07.00 and 09.00 hours daily. Larval developmental time was measured from day of egg hatch to formation of a pre-pupa. Adult dry weights were determined by transferring newly eclosed adults to individual glass vials, drying them in an oven at  $50^\circ\text{C}$  for 3 days, and weighing them on an analytical balance.

*Experiment 1: comparative cannibalism*

Newly hatched larvae (<12 h old) taken from the egg clusters of different females were placed together in Petri dishes ( $n=80$  for each species) in groups of three and provided with an excess of the control diet. Adequate fresh food and water beads were provided every 2 days so that larvae were never without food or water. Each replicate was examined daily for mortality, cannibalism, or pupation. The number of replicates with one or more cannibalism events, and the number of replicates with two cannibalism events, were compared between species using  $3 \times 2$  and  $2 \times 2$  contingency tables and the Chi-squared test (SAS Institute, 1998).

*Experiment 2: cannibalism as a function of food availability and quality*

Newly hatched larvae (<12 h old) taken from the egg clusters of different females were placed in Petri dishes in groups of three ( $n=40$  for each species) and provided with an excess of the control diet. Larvae in treatment 1 (controls) received additional frozen *Ephestia* eggs every day and water beads were replaced as required. Larvae in treatment 2 (reduced food quality) were treated the same as those in treatment 1 except that they received stale *Ephestia* eggs (eggs that had been left to dry in Petri dishes on the laboratory bench for 48 h). Larvae in treatment 3 (reduced food availability) were starved daily for a 14-h period (overnight) during which they received water beads only; an excess of frozen *Ephestia* eggs and bee pollen were provided following each starvation period. All replicates were examined daily for mortality, cannibalism, or pupation. Data were square-root transformed and analysed using a three-factor ANOVA (species, treatment, time) with repeated measures on the last factor (Littell *et al.*, 1991). A Least Significant Difference test was used to separate means at  $P < 0.05$ . Data for larval developmental time and adult weight were compared using one-way ANOVA (SPSS, 1998). Data on cannibalism events were compared using  $3 \times 2$  and  $2 \times 2$  contingency tables and the Chi-squared test (SAS Institute, 1998).

*Experiment 3: cannibalism of siblings vs non-siblings*

To test whether relatedness influences the frequency of cannibalism, pairs of sibling or non-sibling larvae were confined together in Petri dishes within 24 h of hatching. Sibling larvae hatched from the same egg cluster; non-sibling larvae were from egg clusters collected from different females. All replicates were fed an excess of the control diet every 2 days (food was never consumed completely) and water beads were replaced as required. Each replicate was examined daily for mortality. Replicates in which one larva died from causes other than cannibalism were excluded

from the analysis. The frequency of cannibalism events was compared between siblings and non-siblings by means of a Chi-squared Goodness-of-fit test.

*Experiment 4: cannibalism as a function of size disparity*

To test whether a larva's probability of being cannibalised increases with its vulnerability (inferior size), a series of three treatments was conducted for each species. In the first treatment, non-sibling conspecific larvae (harvested from the egg clusters of different females) were confined together in groups of three when they were 48–72 h old (early third instar). In the second treatment, one larva 48–72 h old was confined with two unrelated, conspecific larvae <24 h old (first instar). In the third treatment, one larva 96–120 h old (fourth instar) was confined with two unrelated, conspecific larvae <24 h old. All replicates were fed an excess of the control diet every 2 days (food was never consumed completely) and water beads were replaced as required. Data were square-root transformed and analysed using a three-factor ANOVA (species, treatment, time) with repeated measures on the last factor (Littell *et al.*, 1991). A Least Significant Difference test was used to separate means at  $P < 0.05$ .

*Experiment 5: cannibalism as a function of larval density*

To test whether the rate of cannibalism increases as a function of larval density (i.e. encounter rates between larvae), newly eclosed larvae of each species taken from the egg clusters of different females were held together in groups of two ( $n=40$ ), four ( $n=40$ ), and eight ( $n=40$ ) larvae per dish. Each replicate was fed the control diet daily and water beads were replaced as required. Each replicate was examined daily for mortality. The incidence of cannibalism was compared across treatments using  $3 \times 2$  and  $2 \times 2$  contingency tables and the Chi-squared test (SAS Institute, 1998).

*Experiment 6: development on conspecific eggs*

Newly hatched larvae of each species were divided randomly into two groups. Larvae in the treatment group ( $n=12$ ) were isolated in dishes with water beads and fed only freshly laid eggs (<24 h old) of their respective species. Larvae in the control group ( $n=12$ ) were fed the control diet. Food for both treatment groups was refreshed daily. All events were recorded daily, including mortality, pupation, and eclosion of adults. Data for survival were compared using a Chi-squared Goodness-of-fit test; data for larval developmental time and adult weight were compared using one-way ANOVA (SPSS, 1998).

### Experiment 7: development on conspecific larvae

Newly hatched larvae of each species were divided randomly into two groups. Larvae in the treatment group ( $n=12$ ) were isolated in dishes, provisioned with water beads, and fed only frozen conspecific corpses of their respective species that had been reared previously on the control diet and stored frozen. Larvae in the control group ( $n=12$ ) were fed the control diet. Food for both treatment groups was replaced daily. All events were recorded daily, including mortality, pupation, and eclosion of adults. Data for survival were compared using a Chi-squared Goodness-of-fit test; data for larval developmental time and adult dry weight were compared using one-way ANOVA (SPSS, 1998).

The experiment was replicated with identical procedures except that treatment groups received an alternating diet: control diet on the first day, frozen larval corpses on the second day, control diet on the third day, and so on until pupation.

## Results

### Comparative cannibalism (expt 1)

All three species exhibited some cannibalism (Table 1) despite the fact that sufficient food was provided for all three larvae to complete development in each replicate. The rate of cannibalism varied significantly among species, with *C. sanguinea* having significantly more replicates with both single and double acts of cannibalism than either of the other two species.

### Limitation of food availability and quality (expt 2)

The results of the repeated-measures ANOVA for number of larvae alive per replicate over the course of the experiment are reported in Table 2. The main effects for all three factors (species, treatment, day) were significant. There was a significant species–treatment interaction, and interaction terms for day  $\times$  species, day  $\times$  treatment, and time  $\times$  species  $\times$  treatment were also significant. An examination of the results for individual species indicated

that all three species responded to reduced food availability by increasing their rate of cannibalism relative to larvae in control groups (Fig. 1), but there was no significant change in the rate of cannibalism in response to reduced food quality (stale *Ephestia* eggs) in any species. Species differed with respect to the first date on which the starvation treatment had significantly fewer larvae alive per dish than controls: *O. v-nigrum* = *H. axyridis* < *C. sanguinea*. Larval development was extended significantly in all three species as a result of both the daily starvation treatments and the reduced food quality treatments (Table 3). The adults resulting from the starvation treatment weighed significantly less than control adults in all three species. The adults resulting from the reduced food quality treatment weighed significantly less than controls in *C. sanguinea* and *O. v-nigrum* but not in *H. axyridis*.

### Cannibalism of siblings vs non-siblings (expt 3)

Cannibalism was observed among sibling and non-sibling larval pairs of all three species (Table 4), but only *H. axyridis* larvae showed a significantly lower incidence of cannibalism among siblings. There was no difference in the mean number of days to a cannibalism event between related and unrelated larvae for any species (*C. sanguinea*: mean  $\pm$  SEM =  $4.1 \pm 0.39$  and  $3.4 \pm 0.26$  respectively,  $F = 1.812$ , 1,71 d.f., NS; *H. axyridis*: mean  $\pm$  SEM =  $3.7 \pm 0.32$  and  $4.1 \pm 0.30$  respectively,  $F = 0.855$ , 1,51 d.f., NS; *O. v-nigrum*: mean  $\pm$  SEM =  $5.6 \pm 0.37$  and  $6.2 \pm 0.34$  respectively,  $F = 1.333$ , 1,32 d.f., NS).

### Cannibalism as a function of size disparity (expt 4)

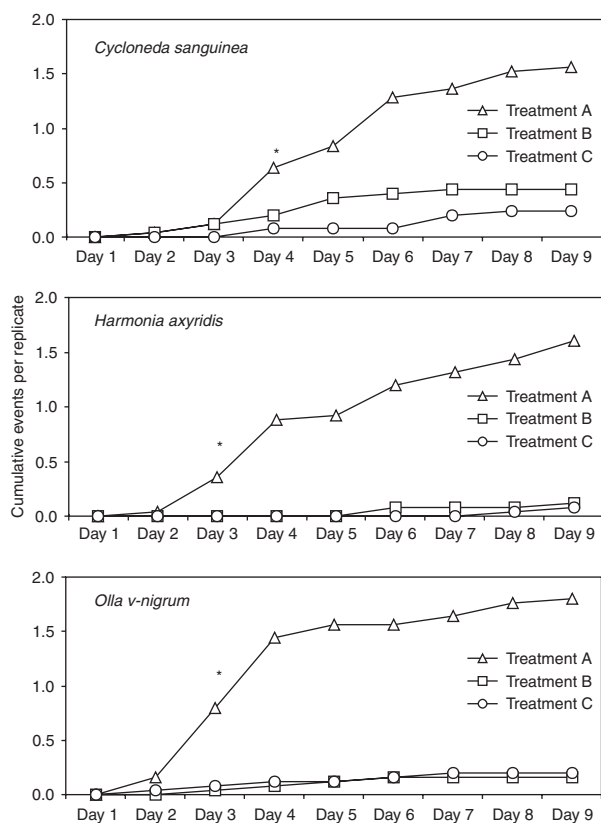
The results of the repeated-measures ANOVA for number of larvae alive per replicate over the course of the experiment are reported in Table 5. The main effects for all three factors (species, treatment, day) were significant. The species–treatment interaction was not significant, indicating that the three species responded to the treatments in a similar manner. There were significant day  $\times$  species, day  $\times$  treatment, and day  $\times$  treatment  $\times$  species interactions. The rate of cannibalism per replicate increased with

**Table 1.** The incidence of cannibalism among unrelated larvae of *C. sanguinea*, *H. axyridis*, and *O. v-nigrum* reared at three per Petri dish. Insects were maintained at  $24 \pm 1^\circ\text{C}$  with continuous access to water and fed an excess of frozen *Ephestia* eggs and bee pollen every second day. Values followed by the same letter were not significantly different in contingency table analysis ( $3 \times 2$  followed by  $2 \times 2$ ) at  $P = 0.05$ .

Species	<i>n</i>	Number of replicates with at least one cannibalism event	Number of replicates with two cannibalism events
<i>Cycloneda sanguinea</i>	80	44 <sup>b</sup>	10 <sup>b</sup>
<i>Harmonia axyridis</i>	80	29 <sup>a</sup>	5 <sup>a</sup>
<i>Olla v-nigrum</i>	80	19 <sup>a</sup>	1 <sup>a</sup>
Chi-squared		16.745	8.170
<i>P</i>		0.001	0.017

**Table 2.** Repeated-measures ANOVA for numbers of coccinellid larvae alive per dish in expt 2. Larvae of each species were reared at three per Petri dish ( $n=25$ ) in each of three treatments: 14 h starvation daily followed by frozen *Ephestia* eggs + bee pollen, stale *Ephestia* eggs + bee pollen daily, and frozen *Ephestia* eggs + bee pollen daily.

Source of variation	d.f.	Mean square	F	P
<b>Between subjects</b>				
Species	2	0.484	6.01	0.0029
Treatment	2	16.417	203.75	0.0001
Species $\times$ treatment	4	0.436	5.42	0.0004
Error	216	0.081		
<b>Within subjects</b>				
Day	8	1.546	172.09	0.0001
Day species	16	0.041	4.60	0.0001
Day treatment	16	0.815	90.66	0.0001
Day treatment $\times$ species	32	0.023	2.52	0.0013
Error (day)	1728	0.009		



**Fig. 1.** Cumulative number of cannibalism events per replicate for three coccinellid species, each receiving three different treatments ( $n=25$  per treatment). Treatment A (reduced food availability): larvae starved for 14 h daily (overnight) then fed frozen *Ephestia* eggs and bee pollen; treatment B (reduced food quality): larvae fed stale *Ephestia* eggs (2 days old) and bee pollen daily; treatment C (controls): (larvae fed frozen *Ephestia* eggs and bee pollen daily). Asterisks indicate the first day on which treatment A was significantly different from treatments B and C in a three-way ANOVA for repeated measures followed by a Least Significant Difference test for separation of means ( $P < 0.05$ ).

increasing size discrepancy among larvae confined together, becoming significantly different among treatments on the first day of the experiment in all three species (Fig. 2).

#### Cannibalism as a function of larval density (expt 5)

Comparing total numbers of cannibalism events among species, the order was *C. sanguinea*  $>$  *H. axyridis*  $>$  *O. v-nigrum* (overall: Chi-squared = 37.403,  $P < 0.001$ ; *O. v-nigrum* vs *H. axyridis*: Chi-squared = 5.486,  $P < 0.05$ ; *H. axyridis* vs *C. sanguinea*: Chi-squared = 13.871,  $P < 0.001$ ). The number of cannibalism events per larva

**Table 3.** Mean developmental times ( $\pm$ SEM) and adult dry weights ( $\pm$ SEM) for beetles of three species (confined three per Petri dish) receiving one of three treatments ( $n=25$  in all treatments): treatment A = 14 h starvation daily followed by frozen *Ephestia* eggs + bee pollen, treatment B = stale *Ephestia* eggs + bee pollen daily, treatment C = frozen *Ephestia* eggs + bee pollen daily. Values followed by the same letter did not differ significantly among treatments (within species) in a one-way ANOVA followed by Least Significant Difference ( $P < 0.05$ ).

	Treatment	Developmental time (days)	Adult dry weight
<i>Cycloneda sanguinea</i>	A	11.5 $\pm$ 0.18 <sup>c</sup>	3.4 $\pm$ 0.11 <sup>a</sup>
	B	10.8 $\pm$ 0.12 <sup>b</sup>	3.7 $\pm$ 0.07 <sup>b</sup>
	C	10.3 $\pm$ 0.16 <sup>a</sup>	4.2 $\pm$ 0.08 <sup>c</sup>
	F	11.815	25.520
<i>Harmonia axyridis</i>	A	13.2 $\pm$ 0.16 <sup>c</sup>	8.4 $\pm$ 0.23 <sup>a</sup>
	B	10.8 $\pm$ 0.08 <sup>b</sup>	8.8 $\pm$ 0.14 <sup>a</sup>
	C	10.2 $\pm$ 0.15 <sup>a</sup>	9.9 $\pm$ 0.17 <sup>b</sup>
	F	101.051	18.743
<i>Olla v-nigrum</i>	A	10.9 $\pm$ 0.18 <sup>c</sup>	5.1 $\pm$ 0.19 <sup>a</sup>
	B	10.1 $\pm$ 0.09 <sup>b</sup>	6.0 $\pm$ 0.14 <sup>b</sup>
	C	9.1 $\pm$ 0.10 <sup>a</sup>	6.4 $\pm$ 0.14 <sup>c</sup>
	F	54.697	15.032

**Table 4.** Incidence of cannibalism among pairs of larvae arising from the same egg cluster (siblings) or from different egg clusters (non-siblings). Larvae were reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water and fed frozen *Ephestia* eggs and bee pollen every second day.

	Cannibalism events/ $n = \%$	Chi-squared	<i>P</i>
<i>Cycloneda sanguinea</i>			
Siblings	38/140 = 27.1		
Non-siblings	36/128 = 28.1	0.037	NS
<i>Harmonia axyridis</i>			
Siblings	19/196 = 11.2		
Non-siblings	33/169 = 19.5	5.939	<0.02
<i>Olla v-nigrum</i>			
Siblings	17/96 = 17.7		
Non-siblings	17/98 = 17.3	0.009	NS

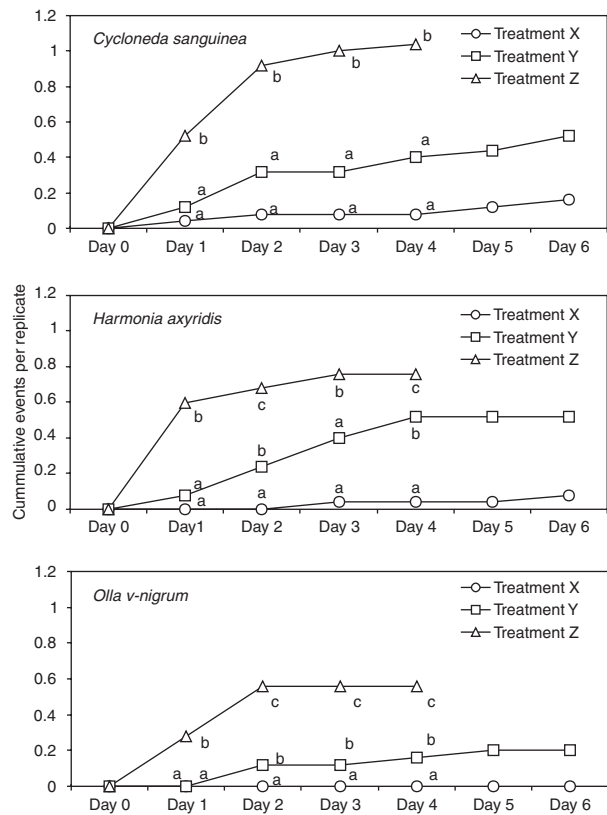
increased as a function of larval density (number of larvae per dish) in *C. sanguinea* and *H. axyridis* but not in *O. v-nigrum* (Table 6).

#### Development on conspecific eggs (expt 6)

Larvae of *H. axyridis* and *O. v-nigrum* developed as well on conspecific eggs as they did on the control diet, with no significant differences in developmental time, although adult dry weight was reduced slightly (Table 7). In contrast, *C. sanguinea* had higher survival on conspecific eggs than on the control diet and developed significantly faster, although there was no difference in the dry weight of resulting adults.

**Table 5.** Repeated-measures ANOVA for numbers of coccinellid larvae alive per dish in expt 4. In the first treatment, conspecific larvae harvested from the egg clusters of different females were confined together in groups of three when they were 48–72 h old (early third instar). In the second treatment, one larva 48–72 h old was confined with two conspecific larvae <24 h old (first instar). In the third treatment, one larva 96–120 h old (fourth instar) was confined with two unrelated, conspecific larvae <24 h old. Larvae were reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water and fed frozen *Ephestia* eggs and bee pollen every second day.

Source of variation	d.f.	Mean square	<i>F</i>	<i>P</i>
<b>Between subjects</b>				
Species	2	0.499	4.08	0.0182
Treatment	2	8.114	33.11	0.0001
Species treatment	4	0.124	1.02	0.3995
Error	216	0.122		
<b>Within subjects</b>				
Day	3	0.236	41.64	0.0001
Day species	6	0.017	2.98	0.0197
Day treatment	6	0.049	8.64	0.0001
Day treatment $\times$ species	12	0.016	2.75	0.0060
Error (day)	648	0.006		



**Fig. 2.** Cumulative number of cannibalism events per replicate for three coccinellid species, each receiving three different treatments ( $n = 25$  per treatment). Treatment X: three third-instar larvae confined together; treatment Y: two first-instar larvae confined with one third-instar larva; treatment Z: two first-instar larvae confined with one fourth-instar larva. Larvae were all fed frozen *Ephestia* eggs and bee pollen daily. Data points bearing the same letter were not significantly different on a particular day in a three-way ANOVA for repeated measures followed by a Least Significant Difference test for separation of means ( $P < 0.05$ ).

#### Development on conspecific larvae (expt 7)

Larvae of both *H. axyridis* and *O. v-nigrum* experienced extended developmental time and reduced adult dry weight on a diet of conspecific larval corpses when compared with the control diet (Table 8); only a single *C. sanguinea* larva completed development on conspecific corpses, which precluded comparisons. Whereas survival was significantly lower for *C. sanguinea* and *O. v-nigrum* fed larval corpses when compared with larvae fed the control diet, larvae of *H. axyridis* survived equally well.

Larvae of *H. axyridis* that fed as cannibals every second day were significantly heavier than control larvae fed the control diet daily, and there was no difference in survival or developmental time (Table 9); however larvae of *C. sanguinea* and *O. v-nigrum* both had significantly lower survival than controls when fed conspecific larval corpses every second day, and *O. v-nigrum* larvae were also significantly smaller and took longer to develop.

**Table 6.** Per capita rate of cannibalism among non-sibling larvae of *C. sanguinea*, *H. axyridis*, and *O. v-nigrum* confined at different densities: two per dish ( $n=40$ ), four per dish ( $n=40$ ), and eight per dish ( $n=40$ ). Insects were reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water and fed an excess of frozen *Ephestia* eggs and bee pollen daily. Values followed by the same letter within rows did not differ significantly in a contingency table analysis ( $3 \times 2$  followed by  $2 \times 2$ ) followed by the Chi-squared test with  $P=0.05$ .

Species	Number of larvae per Petri dish		
	2	4	8
<i>Cycloneda sanguinea</i>	0.000 <sup>a</sup>	0.050 <sup>b</sup>	0.122 <sup>c</sup>
<i>Harmonia axyridis</i>	0.000 <sup>a</sup>	0.019 <sup>ab</sup>	0.056 <sup>b</sup>
<i>Olla v-nigrum</i>	0.125 <sup>a</sup>	0.006 <sup>a</sup>	0.022 <sup>a</sup>

## Discussion

The first experiment yields comparative estimates of the relative intrinsic rate of cannibalism in each of the three species in the presence of an adequate food supply. *Cycloneda sanguinea* demonstrated the highest rate of cannibalism, with *O. v-nigrum* and *H. axyridis* demonstrating lower rates that were not significantly different from each other (Table 1). Wagner *et al.* (1999) demonstrated significant heritability of cannibalism behaviour in *H. axyridis* and significant variation among lineages. Although *C. sanguinea* and *O. v-nigrum* are closely related species (Gordon, 1985), they had different rates of cannibalism in this experiment, suggesting that the propensity to cannibalise can vary among species in a manner independent of their degree of relatedness. Furthermore, it may be concluded that food limitation by itself is not a complete explanation of larval cannibalism in these species, given that larvae in the experiments had access to sufficient food to complete development without resorting to cannibalism.

Wagner *et al.* (1999) found that cannibalism by larvae of *H. axyridis* could reduce developmental time under

conditions of limited prey availability, although there were no measurable benefits when food was abundant. In the present study, rates of cannibalism increased for all three species when access to food was limited to 10 h per day (Fig. 1), indicating that increasing hunger levels increases aggression between conspecifics. Similarly, Agarwala and Dixon (1992) demonstrated that both egg and larval cannibalism by *Adalia bipunctata* (L.) were related inversely to food supply; however the cannibalism response of *C. sanguinea* was slower than that of the other two species and not significantly different from controls until day 4 of the experiment. Therefore, the intrinsic rate of cannibalism expressed when food is available does not appear to reflect the rapidity of a species' cannibalism response when confronted with food limitation. Larvae of all three species receiving the daily starvation treatment took longer to complete development than did controls or larvae fed stale food, and the resulting adults weighed significantly less (Table 3). Thus, larvae were unable to compensate for the periods of food deprivation that they experienced, either by cannibalism or by compensatory consumption during periods of food availability.

There are substantial benefits of larval cannibalism to *H. axyridis* when food quality is low (Snyder *et al.*, 2000). Larvae fed prey of intermediate quality developed faster when conspecific larvae were included in their diet than when they were not, and larvae fed low quality prey did not complete development unless their diet included conspecifics. The reductions in adult dry weight and increases in developmental time relative to controls in all three species receiving stale *Ephestia* eggs indicate that this treatment did represent a reduction in food quality relative to the freshly defrosted eggs, but in no case did this reduction affect the rate of cannibalism (Fig. 1). These results would suggest that cannibalism is driven more by reduced food availability than by reduced food quality, although reducing food quality in other, more pronounced ways might have had different effects.

**Table 7.** Comparative performance of coccinellid larvae fed a diet of conspecific eggs ( $n=12$ ) vs *Ephestia* eggs and bee pollen ( $n=12$ ). Insects were reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water and fed daily.

	Diet		Statistic	d.f.	P
	<i>Ephestia</i> eggs + bee pollen	Conspecific eggs			
<b><i>Cycloneda sanguinea</i></b>					
Survival (%)	58.3	91.7	$\chi^2 = 12.165$	1	<0.001
Developmental time (days)	$10.1 \pm 0.26$	$9.2 \pm 0.12$	$F = 14.068$	1,16	0.002
Adult dry weight (mg)	$4.0 \pm 0.31$	$4.2 \pm 0.23$	$F = 0.284$	1,16	NS
<b><i>Harmonia axyridis</i></b>					
Survival (%)	100.0	100.0	$\chi^2 = 0.0$	1	NS
Developmental time (days)	$10.5 \pm 0.15$	$10.8 \pm 0.27$	$F = 1.158$	1,22	NS
Adult dry weight (mg)	$10.3 \pm 0.41$	$8.4 \pm 0.37$	$F = 11.886$	1,22	0.002
<b><i>Olla v-nigrum</i></b>					
Survival (%)	91.7	100.0	$\chi^2 = 0.689$	1	NS
Developmental time (days)	$8.7 \pm 0.21$	$9.0 \pm 0.12$	$F = 0.522$	1,21	NS
Adult dry weight (mg)	$6.2 \pm 0.21$	$5.4 \pm 0.17$	$F = 8.076$	1,21	0.010

**Table 8.** Comparative performance of coccinellid larvae reared on a diet of frozen conspecific larval corpses ( $n = 12$ ) vs *Ephestia* eggs and bee pollen ( $n = 12$ ). Insects were fed daily and reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water.

	Diet		Statistic	d.f.	P
	<i>Ephestia</i> eggs + bee pollen	Conspecific larvae			
<b><i>Cycloneda sanguinea</i></b>					
Survival (%)	91.7	8.3	$\chi^2 = 75.851$	1	<0.001
Developmental time (days)	$9.7 \pm 0.24$	$16.8 \pm 1.11$	$F = 89.848$	1,13	<0.001
Adult dry weight (mg)	$4.1 \pm 0.19$	2.9	Insufficient data		
<b><i>Harmonia axyridis</i></b>					
Survival (%)	100.0	91.7	$\chi^2 = 0.689$	1	NS
Developmental time (days)	$10.4 \pm 0.26$	$13.6 \pm 0.67$	$F = 92.807$	1,21	<0.001
Adult dry weight (mg)	$10.6 \pm 0.60$	$8.6 \pm 0.40$	$F = 7.233$	1,21	0.014
<b><i>Olla v-nigrum</i></b>					
Survival (%)	83.3	58.3	$\chi^2 = 7.530$	1	<0.01
Developmental time (days)	$8.2 \pm 0.13$	$12.0 \pm 0.73$	$F = 28.202$	1,18	<0.001
Adult dry weight (mg)	$6.7 \pm 0.23$	$3.9 \pm 0.31$	$F = 54.050$	1,18	<0.001

Dong and Polis (1992) considered population density to be 'a key factor for the population dynamics of cannibalism', and studies demonstrating density-dependent cannibalism in diverse taxa were reviewed by Fox (1975) and Polis (1981). Mills (1982) showed convincingly that egg cannibalism by *A. bipunctata* in the field was density dependent. The results of the fourth experiment reveal that larval cannibalism is a density-dependent behaviour in both *C. sanguinea* and *H. axyridis*, in that cannibalism events increased with the rate of encounter between conspecific larvae, independent of the food supply. Although a larva can presumably assess its own hunger level, it cannot assess the hunger level of conspecifics and the attendant risks of being cannibalised. Larvae of cannibalistic species might benefit from responding to the risk of being cannibalised, a risk that will be directly proportional to a larva's encounter

rate with conspecifics. Assuming that the attacker has some advantage in larval conflicts, pre-emptive attacks on conspecifics might be of selective advantage under conditions of high population density if they reduce the risk of becoming a victim. In this scenario, the corpse may still be consumed because of the nutritional resource it represents, but hunger is no longer a prerequisite for eliciting an attack.

In the field, Osawa (1989) found that 25% of *H. axyridis* eggs were killed by sibling cannibalism compared with 36% killed by non-sibling cannibalism. Joseph *et al.* (1999) demonstrated that *H. axyridis* larvae cannibalise kin with lower frequency than non-kin and the present results support this finding. It is therefore of interest that neither *C. sanguinea* nor *O. v-nigrum* has apparently developed sibling recognition mechanisms, however recognition of kin may have no adaptive value if most larval encounters,

**Table 9.** Comparative performance of coccinellid larvae reared on an alternating diet (*Ephestia* eggs and bee pollen first day, frozen conspecific larval corpses the next day, etc.,  $n = 12$ ) vs the control diet alone (*Ephestia* eggs and bee pollen,  $n = 12$ ). Insects were fed daily and reared at  $24 \pm 1^\circ\text{C}$  with continuous access to water.

	Diet		Statistic	d.f.	P
	Control	Alternating			
<b><i>Cycloneda sanguinea</i></b>					
Survival (%)	91.6	66.7	$\chi^2 = 6.768$	1	<0.010
Developmental time (days)	$9.7 \pm 0.30$	$10.5 \pm 0.27$	$F = 3.315$	1,17	NS
Adult dry weight (mg)	$3.5 \pm 0.16$	$3.5 \pm 0.15$	$F = 0.001$	1,17	NS
<b><i>Harmonia axyridis</i></b>					
Survival (%)	91.6	100.0	$\chi^2 = 0.770$	1	NS
Developmental time (days)	$10.4 \pm 0.15$	$10.9 \pm 0.26$	$F = 3.216$	1,21	NS
Adult dry weight (mg)	$8.5 \pm 0.39$	$10.2 \pm 0.30$	$F = 12.466$	1,21	0.002
<b><i>Olla v-nigrum</i></b>					
Survival (%)	100.0	75.0	$\chi^2 = 6.250$	1	<0.020
Developmental time (days)	$8.8 \pm 0.13$	$10.0 \pm 0.17$	$F = 35.924$	1,19	<0.001
Adult dry weight (mg)	$6.9 \pm 0.23$	$5.1 \pm 0.33$	$F = 17.997$	1,19	<0.001



and opportunities for cannibalism, occur between siblings in nature. For ladybirds that normally occur at relatively low population densities and lay their eggs in clusters (as do all of these species), most encounters would be between sibling larvae, and opportunities to cannibalise non-siblings might be rare. In this context, cannibalism of related individuals may be the norm for surviving conditions of prey scarcity, leading to selection against sibling recognition. On the other hand, *H. axyridis* is apparently a very dominant species with a highly developed ability to survive as an intra-guild predator (Dixon, 2000). It can complete development on an exclusive diet of the eggs of *Coleomegilla maculata* DeGeer (Cottrell & Yeargan, 1998) or the larvae of other coccinellids such as *Coccinella septempunctata* L. (Yasuda & Ohnuma, 1999) and *C. sanguinea* (Michaud, 2002). A strong interspecific competitor with broad dietary capabilities might afford the luxury of avoiding sibling cannibalism better than species with fewer dietary options.

It is well recognised that conspecific eggs represent a relatively nutritious diet for larvae of predaceous coccinellids (Pienkowski, 1965; Dixon, 2000). All three species examined here appear to be well adapted as egg cannibals and survived equally well on an exclusive diet of eggs of their own species, although there was a cost in terms of reduced adult size for *H. axyridis* and *O. v-nigrum* (Table 7). These results are comparable with those of Dimetry (1974) for *A. bipunctata*. Agarwala and Dixon (1992) found that weight gain by *A. bipunctata* larvae was higher on a diet of conspecific eggs than on an equivalent amount of *Acyrtosiphon pisum* Harris, suggesting relatively low suitability of this aphid as food for *A. bipunctata*. Similarly, the higher survival and faster development of *C. sanguinea* on a diet of conspecific eggs than on the control diet may indicate that this diet is not as suitable for *C. sanguinea* as it is for the other two species, although it does support successful development and reproduction.

Conspecific eggs were a better food source for the growth and development of all three species than were conspecific larvae. Yasuda and Ohnuma (1999) showed that fourth-instar *H. axyridis* larvae that were switched to a diet of conspecific larvae survived as well as those that continued development on an aphid diet. Of the three species tested here, only *H. axyridis* survived as well on an exclusive diet of conspecific larval corpses as on the control diet, although developmental time was extended and the resulting adults weighed less than controls. When the diet alternated between conspecific larval corpses and *Ephestia* eggs plus bee pollen, however, *H. axyridis* adult dry weights were higher than controls and there were no costs in terms of increased developmental time (Table 9). Thus some degree of cannibalism may actually serve to improve nutrition in this species, even though an exclusively cannibalistic diet is detrimental.

Some of the costs of cannibalism were alleviated for *C. sanguinea* on the alternating diet (adult weights and developmental times were no different from controls) but survival was still reduced significantly. On the other hand, the alternating diet did not appear to alleviate any of the

costs of cannibalism for larvae of *O. v-nigrum*, suggesting that this species is the least adapted cannibal of the three. The assumption of Dixon (2000) that 'there appear to be no physiological costs associated with cannibalism compared to eating their usual prey' is not supported by these results, assuming that *usual prey* are at least as suitable as *Ephestia* eggs. The absence of any measurable benefits of larval cannibalism for either *C. sanguinea* or *O. v-nigrum* would suggest that this behaviour is disadvantageous for both species unless suitable prey are scarce or benefits accrue through the elimination of conspecific competitors.

*Harmonia axyridis* stands out as the most highly adapted cannibal of the three species in this study, having the best capacity to utilise conspecific larvae for growth and development, and mechanisms for avoiding sibling cannibalism. Dixon's (2000) inference that coccinellid species well adapted to cannibalism should be powerful intra-guild competitors would seem to be supported by these results; *H. axyridis* is a highly aggressive interspecific competitor that appears to be displacing native ladybirds from arboreal habitats in various regions of North America (Hoebeke & Wheeler, 1996; Lamana & Miller, 1996; Brown & Miller, 1998; Colunga-Garcia & Gage, 1998; Michaud, 2002). Cannibalism appears to be a strategic behaviour worthy of study in other species of predaceous Coccinellidae, both in light of its theoretical implications for population dynamics and for its potential ramifications on the effectiveness of these beetles as biological control agents.

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