

Behavioural patterns and functional responses of a generalist predator revealed using automated video tracking

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Abstract

BACKGROUND: Tracking predator–prey interactions using automated image recording systems provides insights into novel patterns and mechanisms of predator–prey dynamics, thus these systems have the potential to evaluate biological control agents effectively. Using an automated video tracking system, we aimed to quantify the behavioural patterns of a generalist predator *Harmonia axyridis* in response to changing prey density. The effect of intraspecific interactions on foraging efficiency was evaluated. In addition, functional response parameters were compared between the observations and model predictions.

RESULTS: The associated behavioural component of prey consumption by *H. axyridis* was modified by prey density, especially for dual predator trials. Both individual and paired *H. axyridis* exhibited type II functional responses and a consistent cycle of behaviour. Interestingly, intra-specific interference did not affect overall prey consumption. Divergence between estimated and calculated functional response parameters was observed, which might due to the difficulty of separating foraging and non-foraging activity.

CONCLUSIONS: Interference interactions between *H. axyridis* conspecifics might alter their foraging patterns; however, the outcome of prey consumption was not affected by this behaviour. In conclusion, automated video tracking systems may be used to expose the detailed foraging behaviour of predators and could be used to evaluate a wide range of natural enemies.

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Keywords: automated image tracking; functional response; *Harmonia axyridis*; predator–prey interactions; generalist predator

1 INTRODUCTION

Predators play a key role in shaping the structure of the food web in agro-ecosystems. Understanding the essential factors that affect predator foraging efficiency is crucial to interpreting predator–prey dynamics.^{1,2} Insect predators are remarkably common and could provide ecosystem services, such as pest management. Therefore, the predator–prey dynamics of insect predators and their pest prey are important subjects for behavioural and population studies.^{3–5} The relationship between prey density and the killing rate of predators is described by the functional response, which provides insights into the mechanisms underlying their predator–prey dynamics.^{5–9} There are three basic types of functional response. The type I functional response indicates the constant consumption of prey until a certain threshold of prey density has been reached. In addition, predators that exhibit type II functional response cause maximum kill rates at low host densities. In comparison, the killing rate of the type III functional response increases at an accelerating rate at low prey densities, and then reduces at higher prey densities.¹⁰ The nature of the functional response determines whether or not a predator regulates the density of its prey.¹¹ Therefore, experiments on functional responses have long been used to quantify the strength of predator–prey interactions.⁵

The type of functional response largely depends on the shape and parameters of the functional response curve. There are two

key parameters in the functional response: (i) the prey handling time (T_h), which is the time required to capture and consume prey, and (ii) the attack rate (a), which reflects foraging efficiency for the prey.^{1,12} The attack rate is the instantaneous searching rate, while handling time refers to the mean handling time per prey. When handling time is not negligible, both the attack rate and the handling time determine the type of functional response. In the type II functional response, predators exhibit a constant attack rate per prey and a constant handling time, which produces an inversely density-dependent predation rate. However, if one or both of the attack rate per prey and handling time vary with prey density over a relatively short range of

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low prey densities, the response is classified as type III and produces positive density-dependent predation over at least a limited range of low prey densities.^{1,13} To determine a and T_h , functional response models are evaluated with foraging behaviour.¹ Integrating the observation of foraging behaviour with functional response models provides an insight into the mechanisms of functional response.^{1,14,15} Many studies combine functional response parameters with predation behaviour.^{16–21} However, the specific behavioural responses of predators to changing prey density during the experiments remains unknown.

Since the early 1990s, automated video tracking of animal behaviour^{22,23} has been used to study insect predator–prey dynamics. The tracking of functional responses using automated image recording systems has provided novel insights into the patterns and biological mechanisms of predator–prey interactions.²⁴ Characterizing predatory behaviour as a proxy for the functional response represents a promising approach, as the precise behavioural parameters for a predator (such as handling time, activity duration and distance moved) may be measured. This method provides an opportunity to interpret the ecological basis of the functional responses regarding the behavioural organization of predators, and the detailed time budget of a predator in response to changing prey densities. However, this approach is also challenging, as some technical problems must be solved, such as the small size of focal insects and their behavioural complexity (predators might exhibit behaviours that require manual recording even in a small controlled arena). Moreover, it is difficult to analyse nonlinear and complex behavioural data. Therefore, appropriate statistical and computational methods are essential for the analysis of behaviour data.²⁵

To advance our understanding of the behavioural mechanisms underlying the functional responses of a generalist predator, we established a predation system using the multi-colored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), and the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). We selected *H. axyridis*–*M. persicae* as our study system because it is easy to incorporate it with an automated video tracking system in the laboratory to assess the foraging behaviour of predators. In addition, *H. axyridis* is one of the most common aphidophagous coccinellid species globally,²⁶ while *M. persicae* is an important pest of various crops worldwide.²⁷ Thus, the results of this work have the potential to be adopted and developed in studies of common predator–prey systems.

We used a video tracking system integrated with behaviour analysis software to investigate the functional responses of the foraging behaviour of individuals and pairs of *H. axyridis*. Our goals were to (i) elucidate the details of behavioural patterns of this generalist predator in response to changing prey density, (ii) interpret the effects of interspecific interactions on foraging behaviour and functional responses in *H. axyridis*, and (iii) compare the key functional parameters, including handling time (T_h) and attack rate (a), between direct behavioural observations and model predictions.

2 MATERIAL AND METHODS

2.1 Plant, insects and the study system

Young seedlings of *Capsicum annuum* var. *grossum* with 15–20 leaves were used to rear aphids. *Harmonia axyridis* and *M. persicae* were collected from an experimental farm (34° 17' 52" N, 108° 04' 18" E) at Northwest A&F University, Yangling, China. Adults and larvae of *M. persicae* were fed potted *C. annuum*. Larval *H. axyridis* were kept with an excess of *M. persicae* daily until pupation. The

adult females that emerged from these pupae were used in the experiments. All insect colonies were maintained in an insectary (25 ± 2 °C, 65–75% RH, L:D 16:8 h).

2.2 Functional response experiment using an automated video tracking system

Prior to the experiment, adult *H. axyridis* were individually transferred from the stock culture into Petri dishes (3.5 mm in diameter) and were provided with a piece of dental cotton wool soaked with distilled deionised water for 24 h. Leaf disks were used as an experimental arena, in which one leaf of sweet pepper was cut to the same size as the 3.5-mm Petri dish and placed on a thin layer (4 mm thick) of 1.0% agar at the bottom of the dish. Prey density was 4, 10, 20, 30, 60 and 100 aphids. Fourth instar aphids were used in all trials. The behavioural trials were conducted between 08:00 and 17:00. *Harmonia axyridis* were placed individually or as pairs in each arena. The activity of *H. axyridis* in two Petri dishes was recorded simultaneously for 1 h. All predators in each arena were tracked simultaneously. The experiment was replicated for 10–13 times for single predator treatment and 5–10 times for dual predator treatment. All trials were carried out within 2 weeks.

To differentiate between the patterns of *H. axyridis* foraging activity, the behaviour of adult female *H. axyridis* was recorded (15 frames s⁻¹) using a digital camera (Panasonic® Lumix DMC-GH4, China) together with an EthoVision® XT 12 (Noldus (Beijing) Information Technology Co. Ltd, Beijing, China) video tracking and analysis system. Room temperature was kept constant at 24 °C. For both individual and dual predator treatments, two trials were run concurrently with densities chosen at random, and predator behaviour was monitored for 1 h. Any *M. persicae* consumed by *H. axyridis* were not replaced during the trial period. We used live tracking to detect insects at a maximum resolution of 1280 × 960 pixels with 15 video frames per second. The centre-point detection and differencing were used as detection methods, with a dark contrast of 8–255. Subject size detection was limited to the range of 10–160 pixels. Pixel smoothing was set to medium. Moving thresholds started when the velocity of the predator exceeded 0.5 mm s⁻¹ averaged over 15 video frames s⁻¹ and stopped when it fell below 0.5 mm s⁻¹. The distance moved (mm) and movement velocity (mm s⁻¹) were recorded. Based on preliminary observations, eight and nine behaviours were distinguished for individual and paired *H. axyridis* treatments, respectively (Table 1). The categories were chosen to represent the clearest aims of the different behaviours. The frequency and duration of behaviours were determined from the video recordings using a manual event coding software application, Observer® XT 12 (Noldus (Beijing) Information Technology Co. Ltd, Beijing, China).

2.3 Data analysis

Statistical analyses were conducted by R.²⁸ Regression analysis using generalised linear models (GLMs) with binomial errors to test for a relationship between prey density and the proportion duration of each category of behaviour. In addition, GLMs were also used to determine the relationship between interference behaviour and other categories of behaviour in terms of both proportion duration and total frequency.

First-order Markovian transition matrices were used to determine the frequency of transition from one behaviour to another for a complete record of female behaviour at each prey density/arena combination. Based on the assumption that there were no significant differences among individuals, data were pooled within

Table 1. Behaviour categories of *Harmonia axyridis*

Event	Description
Attack prey (Ap)	Attack prey
Interference (Ac)†	Interference behaviour including slight contact, fighting for few seconds, or chasing with each other
Stationary (St)	Standing still without moving any part of the body
Grooming (Gr)	Preening any part of the body while standing still
Feeding (Fe)	Prey consumption
Walking (Wk)	Walking along the substrate
Struggling‡ (Ot)	Lying upside down and trying to recover the right body position
Open-wing‡ (Ot)	Any wing unfolding activity
Faeces‡ (Ot)	Excretion waste

†Paired predators only.
‡These behaviours were pooled as 'other' behaviour.

each prey density treatment. Because behaviours could not follow themselves in our observations, the principal diagonal elements of these matrices were set to 0. The expected values of the matrix cells were obtained using the 'catspec' package (version 0.97, date: 2015-02-19)²⁹ in R. The statistical significance of the overall table was evaluated using a log-likelihood ratio (G) test. Yates correction for continuity was applied throughout. As the deviations in the overall table were statistically significant, significant transitions were determined by collapsing the table into a 2×2 matrix around each transition and performing a G test. The significance of these individual tests was adjusted to a table-wide level of 5% using the sequential Bonferroni method.³⁰

Functional responses were analysed using model selection and hypothesis testing.³¹ Logistic regression of the proportion of prey consumed versus initial prey density was used to determine the type of functional responses fitted using a maximum likelihood (ML) procedure. Model fitting was then performed with ML. Significant negative or positive linear coefficients derived from the regression indicated type II or type III responses, respectively.³¹ For significant, negative linear terms, the data were fitted to a type II functional response curve using ML estimation of the random predator equation,³² which includes prey depletion:

$$N_e = N_0 [1 - \exp(-aT_h N_e - aT)] \quad (1)$$

where N_e is the number of prey consumed, N_0 is the given prey density, a is the attack rate, T_h is the handling time and T is the experimental duration.

To compare fitting of functional responses between different predator combinations, non-parametric bootstrapping ($n = 2000$) was used to generate 95% confidence intervals (CIs) around functional response curves and their associated parameters. Equation 1 was then fitted to the bootstrapped dataset using initial parameter values estimated from the original ML estimates. Divergence between CIs indicated that the functional responses and/or the corresponding parameters differed.

The estimated overall consumption of prey for paired *H. axyridis* was calculated based on a model proposed by Soluk,³³ in which the estimated total prey consumption does not exceed the given prey density:

$$C_{ab} = N_p (P_a + P_b - P_a P_b) \quad (2)$$

where C_{ab} is the estimated total consumption for a given initial prey density (N_0), and P_a and P_b are the probabilities of being consumed by each predator present, predator a and predator b, respectively. Data were acquired from single *H. axyridis* functional responses. All functional response analyses were conducted using the 'frair' package in R (version 0.5.100, date: 2017-03-26).³⁴

For each aphid density, the attack rate was calculated as:

$$a = \frac{E * P(\text{eaten}|\text{encounter})}{N} \quad (3)$$

where E represents the encounter rate between *H. axyridis* and *M. persicae*, $P(\text{eaten}|\text{encounter})$ is the probability of eating a prey upon encounter, and N is the prey density.¹⁸ Both E and $P(\text{eaten}|\text{encounter})$ were calculated from the video recording data. Prey handling time (T_h) was treated as the average duration of attack plus prey consumption. The mean attack rate and handling time were calculated for each aphid density. Differences in the calculated attack rate and handling time among aphid densities for both individual and paired predators were analysed using the Kruskal–Wallis rank sum test.

3 RESULTS

3.1 Effects of prey density on foraging behaviour

For individual *H. axyridis* there was no significant relationship between prey density and the percentage duration of stationary behaviour ($X^2 = 1.66$, $P = 0.198$, Fig. 1(A)), feeding behaviour ($X^2 = 2.19$, $P = 0.139$, Fig. 1(B)), grooming behaviour ($X^2 = 1.04$, $P = 0.307$, Fig. 1(C)) and other minor behaviours ($X^2 = 0.03$, $P = 0.865$, Fig. 1(E)). However, the percentage duration of walking behaviour slightly decreased with the increase in aphid density with marginal statistical significance ($X^2 = 3.52$, $P = 0.061$, Fig. 1(D)).

When foraging in a pair, *H. axyridis* spent less time stationary as the increase of aphid density ($X^2 = 7.72$, $P = 0.005$, Fig. 2(A)). On the other hand, the percentage duration of feeding behaviour increased significantly with increasing aphid density ($X^2 = 4.08$, $P = 0.043$, Fig. 2(B)). This pattern was also observed for the percentage duration of grooming behavior, but with only marginal significance ($X^2 = 3.45$, $P = 0.063$, Fig. 2(C)). There was no relationship between prey density and the percentage duration of walking behaviour ($X^2 = 0.78$, $P = 0.371$, Fig. 2(D)), other minor behaviour ($X^2 = 0.23$, $P = 0.629$, Fig. 2(E)) and interference behaviour ($X^2 = 0.08$, $P = 0.775$, Fig. 2(F)).

There was no relationship between the percentage duration of interference behaviour and the percentage duration of stationary behaviour ($X^2 = 0.32$, $P = 0.573$, Fig. 3(A)), feeding behaviour ($X^2 = 0.62$, $P = 0.430$, Fig. 3(B)), grooming behaviour ($X^2 = 0.14$, $P = 0.709$, Fig. 3(C)) and other minor behaviours ($X^2 = 0.14$, $P = 0.706$, Fig. 3(E)). The percentage duration of walking behaviour increased with increasing interference behaviour but had only marginal significance ($X^2 = 3.01$, $P = 0.082$, Fig. 3(D)). In comparison, the frequency of stationary behaviour increased with increasing frequency of interference behaviour ($X^2 = 17.01$, $P < 0.001$, Fig. 4(A)), while the same pattern was observed for grooming ($X^2 = 12.82$, $P < 0.001$, Fig. 4(C)), walking ($X^2 = 326.96$, $P < 0.001$, Fig. 4(D)) and other minor behaviours ($X^2 = 13.35$, $P < 0.001$, Fig. 4(E)). Only the frequency of feeding behaviour decreased with an increase in the frequency of interference behaviour ($X^2 = 4.29$, $P = 0.038$, Fig. 4(B)).

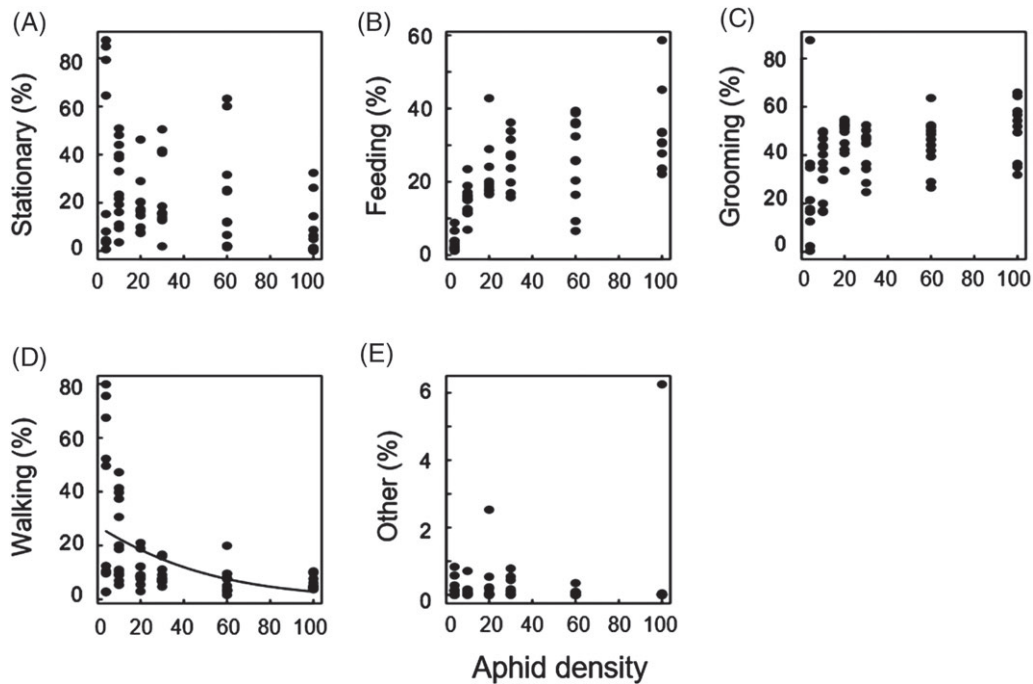


Figure 1. Relationship between aphid densities (4, 10, 20, 30, 60 and 100 aphids) and the percentage duration of stationary behaviour (A), feeding behaviour (B), grooming behaviour (C), walking behaviour and other minor behaviours (E) of individual *Harmonia axyridis*.

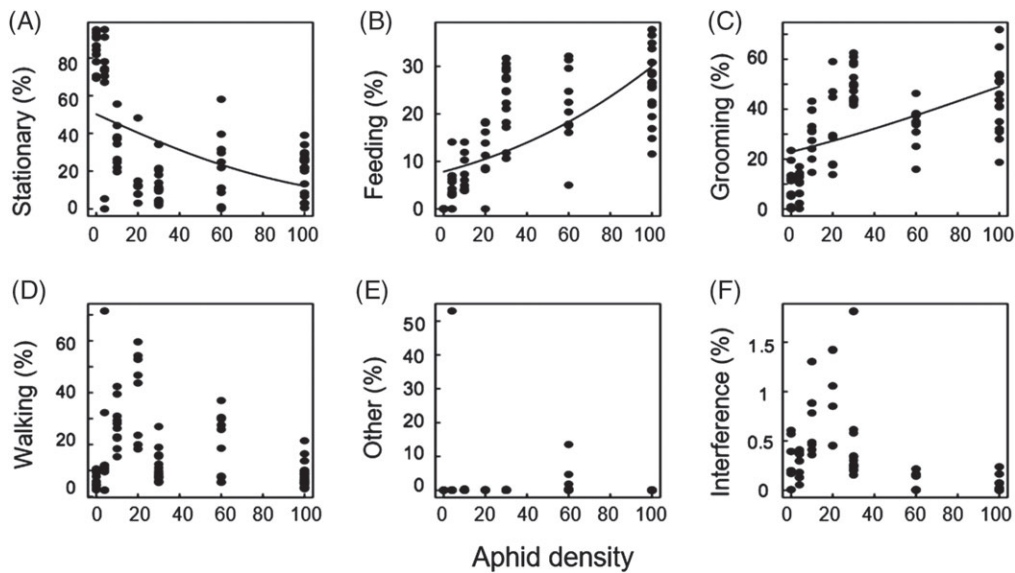


Figure 2. Relationship between aphid densities (4, 10, 20, 30, 60 and 100 aphids) and the percentage duration of stationary behaviour (A), feeding behaviour (B), grooming behaviour (C), walking behaviour (D), other minor behaviour (E) and interference behaviour (F) of dual *Harmonia axyridis*.

3.2 Predator foraging patterns

The EthoVision system successfully tracked the movement of individual *H. axyridis*. However, it could not efficiently distinguish between paired *H. axyridis* foraging in the same arena. This was because the image tracking system could not identify when one or both paired *H. axyridis* were moving upside down or twisting their bodies. The sophisticated wing colour pattern also made it difficult to distinguish between them. Therefore, the location intensity, moving distance and velocity of individual predators only were analysed (Fig. 5(A–C)). In brief, the location intensity maps indicated that individual *H. axyridis* walked in a random

pattern and along the edge of the arena at low aphid densities (four aphids). Subsequently, when aphid density was ten or more, *H. axyridis* exhibited area-restricted foraging within a specific area (Fig. 5(A)). Individual predators moved more frequently and faster at low prey densities than at high prey densities. *Harmonia axyridis* was most active when aphid density was four, with a body velocity of 9–10 mm s⁻¹ and frequency of over 5000. Subsequently, both the peak of body velocity and frequency decreased as prey density increased (Fig. 5(B)). In addition, both moving velocity and total distance decreased as prey density increased (Fig. 5(C)).

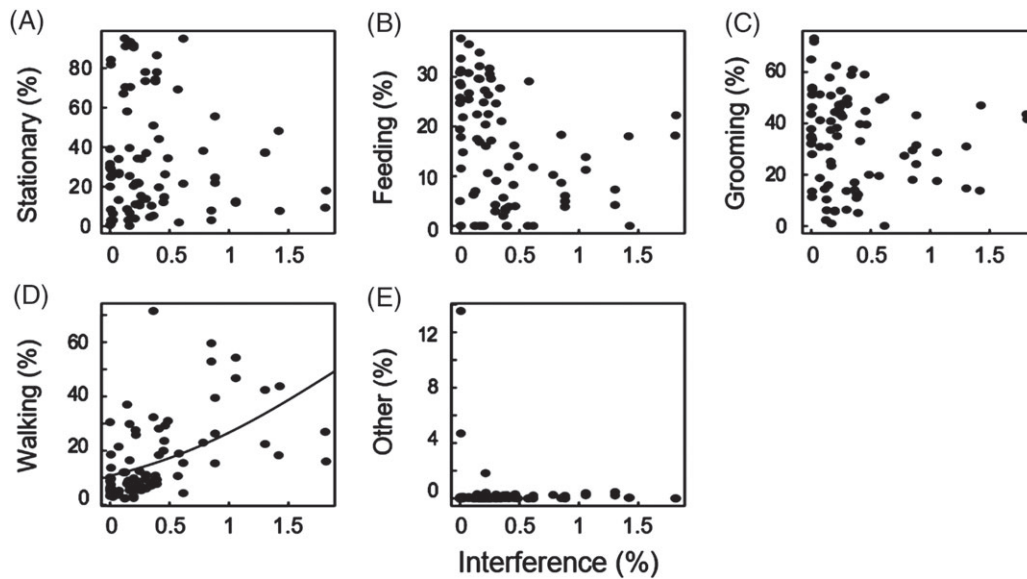


Figure 3. Relationship between the percentage duration of interference behaviour and percentage duration of stationary behaviour (A), feeding behaviour (B), grooming behaviour (C), walking behaviour (D) and other minor behaviour (E).

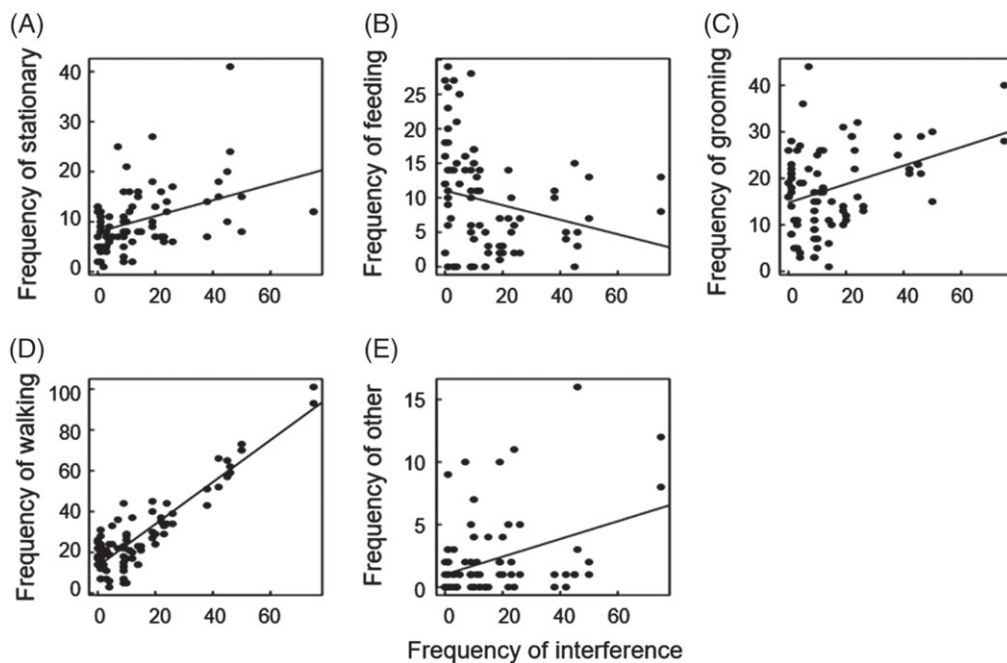


Figure 4. Relationship between the frequency of interference behaviour and frequency of stationary behaviour (A), feeding behaviour (B), grooming behaviour (C), walking behaviour (D) and other minor behaviour (E).

3.2.1 Flow diagram

The behavioural sequences of both dual and individual female *H. axyridis* exhibit distinctive patterns (Fig. 6(A,B)). *Harmonia axyridis* actively responded to the presence of aphids by exhibiting attacking, consuming and grooming behaviours that slowed the pace of movement and reduced the potential searching area, which likely increased the probability of host encounter. Moreover, a repeated cycle of walking, consuming, grooming and stationary behaviour was observed for individual and paired *Harmonia axyridis* when aphid densities were above 10 and 30, respectively (Fig. 6(A,B)).

3.3 Functional responses

Significant negative linear terms were detected from the logistic regressions observed for both individual and paired *H. axyridis*. Therefore, the functional responses for both individual and paired *H. axyridis* preying on *M. persicae* over a 1-h period were of the type II random predator equation (Fig. 7(A–C)). In addition, the attack rates and handling times of the functional response models were all significant (Table 2). The observed and estimated functional responses for pairs of *H. axyridis* overlapped across all prey densities. As expected, the overall prey consumption for paired *H. axyridis* was almost double that of individual *H. axyridis*

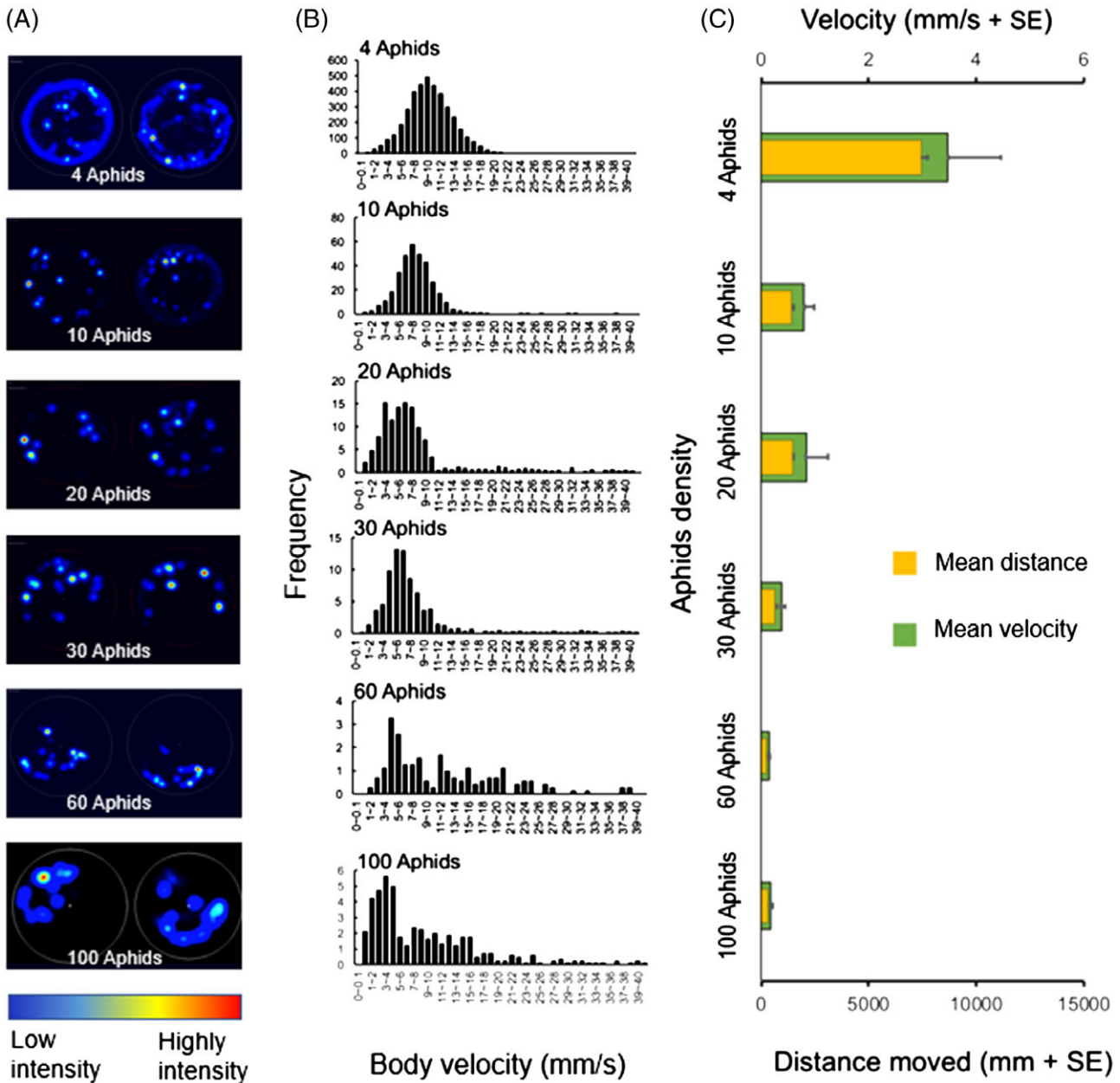


Figure 5. (A) Examples of location intensity heat plots illustrating where *Harmonia axyridis* spent their time during trials with different aphid densities. (B) The frequency of velocity distribution of individual *H. axyridis*. (C) Mean velocity and distance moved of individual female *H. axyridis* at different aphid densities during the 1-h experimental period.

(Fig. 8(D)). The attack rate (a) and handling time (T_h) confirmed this relationship numerically (Table 2). The attack rate was highest in the paired treatment, while the handling times in the individual and paired treatments were not significantly different.

The functional response parameters (a and T_h) at different aphid densities are illustrated in Fig. 8. The rate of successful searches fluctuated as prey density changed for both individual *H. axyridis* (a : $X^2 = 11.326$, $df = 5$, $P = 0.045$) (Fig. 8(A)) and paired *H. axyridis* (a : $X^2 = 16.725$, $df = 5$, $P = 0.005$) (Fig. 8(C)). In comparison, handling time did not change with aphid density for individual *H. axyridis* (T_h : $X^2 = 1.459$, $df = 5$, $P = 0.918$) (Fig. 8(B)) or paired *H. axyridis* (T_h : $X^2 = 1.393$, $df = 5$, $P = 0.925$) (Fig. 8(D)). For individual predators, the calculated attack rate was higher than the estimated attack rate when aphid density was below 60, while the CIs for calculated

and estimated attack rates overlapped when aphid densities were 60 and 100. The estimated T_h was double that calculated. For paired *H. axyridis*, the estimated combined attack rate was similar to the rate calculated using video recording data for aphid densities of 4, 10, 20 and 60, whereas the estimated T_h was slightly higher than that calculated.

4 DISCUSSION

This study demonstrated the ability of automated video tracking to quantify the feeding behaviour of aphid predators feeding on different densities of prey by linking the parameters in the functional response model and actual predator behaviour. As an important ecological process that reflects the relationship

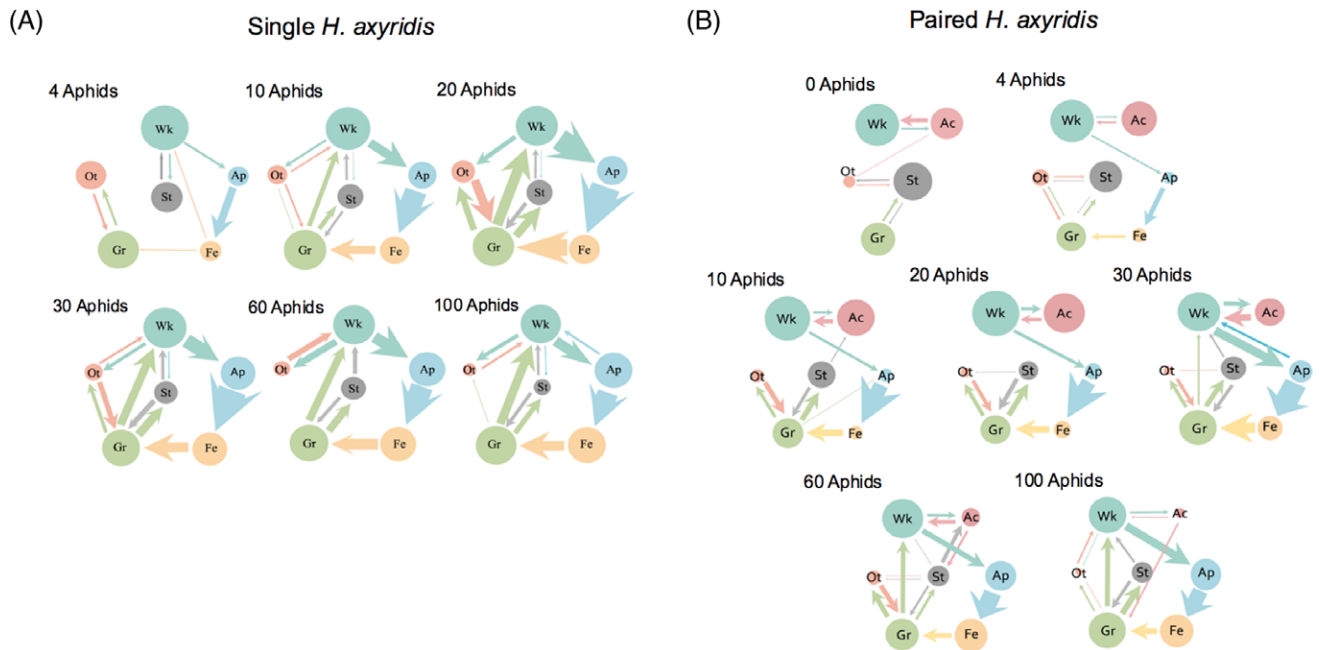


Figure 6. Markov chain models of behavioural transitions of (A) individual *Harmonia axyridis* and (B) paired *H. axyridis* searching leaf disks infested with 0 (paired *H. axyridis* only), 4, 10, 20, 30, 60 or 100 fourth instar aphids. The size of the shapes represents the relative ratio of behaviour frequency to the overall frequency of behavioural events. Line thickness represents the transition probability. The behaviours faeces, struggling and open-wing were pooled in the analysis as 'Other minor'. Connecting lines show the non-random patterns of behaviour that are likely to happen ($P < 0.05$). The widths of connecting lines show the proportion of the standardised residual to residuals of deviations from expected values.

between various predator–prey behaviours and prey densities, the functional response is widely applied to determine the efficiency of biological agents in pest management practices. Our results indicated that the associated behavioural component of prey consumption by an aphidophagous coccinellid was modified by prey density, especially for dual predator trials. However, interference interactions did not affect overall prey consumption under our experiment conditions. In our dual predator trials, *H. axyridis* interacted with each other in various ways from slight contact to direct combat, while no lethal contact was observed (Table 1). Although the percentage duration of interference interactions was extremely low compared to other behaviour, the interference behaviour occurred frequently and could affect the occurrence of other behaviour. Interference behaviour occurred across prey densities, while a negative relationship between the frequency of interference behaviour and prey consumption behaviour was detected. On the other hand, the frequency of interference behaviour was positively linked with all of the other behaviour recorded. This evidence suggests that aggressive behaviour somehow makes *H. axyridis* more active, without actually affecting the overall prey consumption. This result partly supports a previous study, which also demonstrate that the cost of interference behaviour between coccinellid predators under relatively high prey densities could be ignored.³⁵ In addition, for some predators, digestion could reduce the effects of interference. Thus, interference interactions might not reduce prey consumption if they coincide with the period of digestion.³⁶

In this study, prey density did not directly affect the duration of foraging behaviour of individual *H. axyridis*. However, visible 'escaping behaviour' (walking quickly along the edge of the arena) was detected at low aphid density. In comparison, area-restricted foraging was observed at aphid densities from 10 to 100. *Harmonia axyridis* individuals were most mobile at the low aphid densities, which decreased as aphid density increased. This behaviour

pattern was observed in previous studies, which indicated that coccinellid exhibit area-restricted foraging in patches with high prey density.^{26,37,38} In addition, *H. axyridis* spent more time feeding and grooming at high aphid densities. This observation indicated that grooming might be related to prey consumption and digestion. Various studies have indicated that grooming plays a vital role in cleaning sensory organs.³⁹ Therefore, as prey consumption increased, *H. axyridis* spent more time cleaning (grooming) themselves, with feeding frequently being followed by grooming at all aphid densities. Moreover, we found that *H. axyridis* exhibited a cycle of behaviour, including walking, consuming, grooming and remaining stationary, when prey density was above 10 (individual) or 30 (paired). However, the stability of the foraging cycle was influenced by both prey density and conspecific interactions. The Markov chains transition probabilities showed the sequential predictability of *H. axyridis* behaviours, which is similar to previous studies with insects.^{40,41}

Both individual and paired *H. axyridis* exhibited type II functional responses, which was consistent with previous studies.^{26,42–44} Pairs of *H. axyridis* exhibited neutral interactions, indicating that intra-specific interactions might not affect the control efficiency of *H. axyridis*. Although no obvious relationship between the percentage duration of interference interactions and prey density was detected, dual *H. axyridis* were more active than individuals. This result indicated by negative relationship between the duration of stationary behaviour and aphid density when foraging in pairs. In addition, when foraging with conspecifics, the percentage duration of both feeding and grooming behaviour increased with prey density. This was in agreement with a previous study that suggested that interference interactions at high prey densities are weak or absent for aphidophagous ladybirds.^{35,45} The estimates of attack rate calculated from the behavioural data diverged from estimates obtained from the regression analysis. It is possible that the functional response parameters estimated

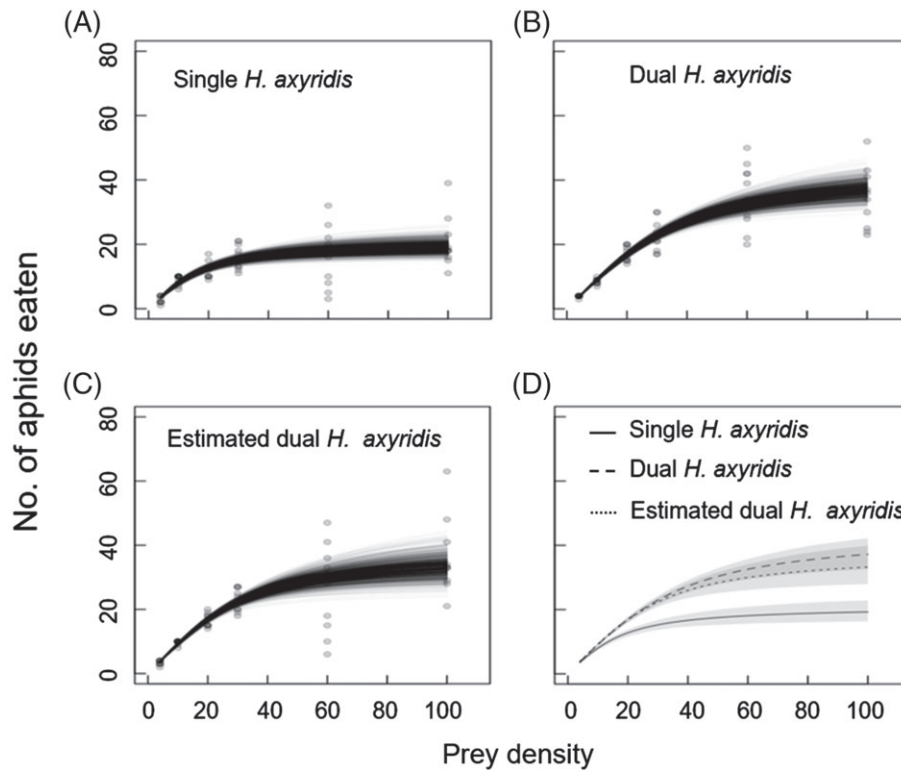


Figure 7. Predatory functional responses of (A) individual, (B) conspecific and (C) estimated predator combinations of *Harmonia axyridis* towards *Myzus persicae*. Circles represent raw data points; lines represent bootstrapped functional responses ($n = 2000$). (D) Individual, paired and estimated functional responses of *H. axyridis*. Lines indicate fitted functional response curves, while shaded areas represent the 95% CIs. Estimated responses were derived from data of individual predators.

Table 2. Linear logistic regressions of the proportion of *Myzus persicae* eaten by individual, paired and estimated dual *Harmonia axyridis*, and functional response parameters for Rogers random predator equation (a and T_h with mean and 95% CI)

Treatments (E: estimated)	Linear coefficient, P	Attack rate a (estimate and 95% CI)	P (Z value)	Handling time T_h (estimate and 95% CI)	P (Z value)
1 <i>H. axyridis</i>	-0.025, <0.001	2.597 (1.56–3.53)	<0.001 (8.995)	0.048 (0.038–0.057)	<0.001 (19.50)
2 <i>H. axyridis</i>	-0.027, <0.001	3.010 (2.31–3.66)	<0.001 (12.806)	0.023 (0.019–0.027)	<0.001 (21.760)
E <i>H. axyridis</i>	-0.027, <0.001	3.358 (2.37–4.24)	<0.001 (11.564)	0.026 (0.020–0.033)	<0.001 (22.245)

from curve-fitting might not have efficiently linked the predator behaviour with different prey densities. In comparison the behavioural data bridged the functional response parameter with real behaviour components, which helped with the interpretation of factors that influence the functional responses. In the observed foraging behaviour, the attack rate was directly related to specific behaviours that occurred when a predator encountered, attacked and consumed prey.^{13,18,46} Although the previous study with predatory mites demonstrated that through maintaining constant prey density during the experiment, functional response parameters obtained from both behaviour observation and model estimation were similar.³⁵ In practice, functional response parameters obtained from regression analysis often differ from those measured directly from behavioural observations.¹ For example, it is difficult to include the actual digestion time in the handling time and therefore digestion time is usually omitted. In addition, the foraging duration T should reflect the actual time spent foraging, including searching and handling prey. However, many studies treat T as the overall time available for foraging, which includes all non-foraging activities.^{8,46} In the present study, although a

detailed behaviour time budget was obtained, it was still difficult to distinguish between foraging and non-foraging activity. Therefore, it was difficult to categorise behaviours such as grooming and walking as ‘foraging’ or ‘non-foraging’. In addition, motionless behaviours, such as stationary and grooming, may also involve digestion, which might be included in the handling time. Despite this, the calculated handling time of paired *H. axyridis* was similar to that estimated from curve-fitting. This result might have been obtained because the digestion times of paired predators were shorter than those of individual predators at certain prey densities, thus the observed prey consumption time of paired predators was similar to the handling time estimated from curve-fitting.

5 CONCLUSIONS

Overall, using data from a common and simple predator–prey system in the laboratory, this study incorporated behavioural information to observed patterns of predation. This approach provides a useful framework for interpreting specific behaviours of individual natural enemy to population-level processes, and could

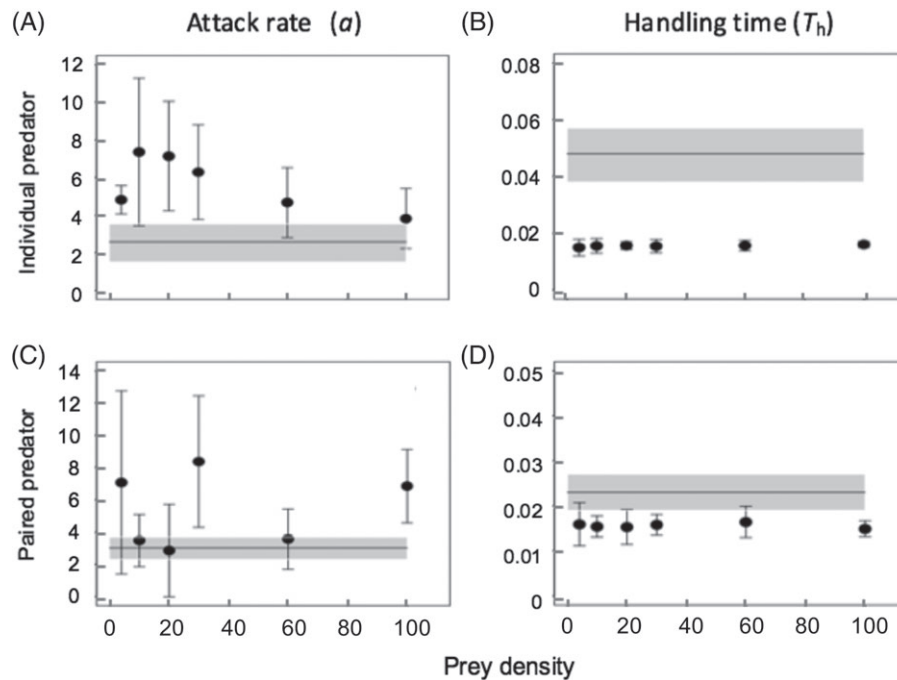


Figure 8. Functional response parameters for attack rate of (A) individual and (C) paired *Harmonia axyridis*, and handling times of (B) individual and (D) paired *H. axyridis*. Circles indicate the mean and error bars represent the CI calculated from video recording data at different prey densities. Lines and shaded areas in (A) and (C) indicate parameters for attack rates and the corresponding CIs for individual and paired predators, respectively. Lines and shaded areas in (B) and (D) represent handling times of individual and paired *H. axyridis*, respectively, calculated from the regression model.

be used as an important complementary method for traditional end-point assays. In addition, our results indicate that automated behaviour tracking of natural enemies might be further developed as a standardized way of selecting and evaluating the potential of a wide range of biological control agents under the influence of various biotic or abiotic factors. In this study, both prey density and conspecific interactions affected the duration and patterns of the foraging behaviour of *H. axyridis*. Although interference interactions between predators occurred across all the prey densities in our confined experimental areas, the outcome of prey consumption was not affected. Therefore, from the applied perspective, our results indicated that intraspecific interactions of predators might not affect their biological control efficiency under certain conditions. Further studies are required to evaluate the behaviour of both predator and prey on natural host plants. More realistic behavioural mechanisms might lead to a more comprehensive understanding of predator–prey interactions, enhancing pest management practices.

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