



Hyperaspis polita Weise functional response: effects of predator stages, prey species, and previous feeding experiences

Sadegh Nakhai Madih¹ · Leila Ramezani¹ · Sara Zarghami² · Nooshin Zandi-Sohani¹

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Abstract

At the moment, *Hyperaspis polita* Weise is a key predator of mealybugs in southern Iran. In this study, the efficiency of this predator on *Phenacoccus solenopsis* Tinsley, a serious pest, and *Planococcus citri* Risso as a factitious prey in predator mass rearing, was examined. The functional response experiment was performed for 4th instar larvae, adult male and female of *H. polita* at five prey densities (2, 4, 8, 12, 16, and 32 adult female stage) on these two preys. The shape of functional response curves well fitted the type II for 4th instar larvae, adult male and female of *H. polita* on both preys, whenever reared on one prey, while being exposed to the other prey (previous feeding experiences). Comparisons of attack rates and handling time for 4th instar larvae and adult female did not show any difference for various preys as well as previous feeding experiences. For the adult male, when reared on *P. solenopsis*, the handling time was significantly longer, while it was shorter on *P. citri* and again the same prey was given to it. The predation was highest for the 4th instar larva followed by adult females and adult male on all treatments. In addition, the maximum predation rate was shown when these predators were reared on *P. solenopsis* and were exposed to *P. citri*. The results of this study indicated that *H. polita* can be an effective biological control agent for mealybugs. It also emphasizes the importance of previous feeding experiences in release program protocols for *H. polita*.

Keywords Biological control · Chinese hibiscus · Iran · Mealybugs

Introduction

Mealybugs are one of the severe agricultural pests in tropical and subtropical areas of the world due to their wide range of host plants, severe damages, high reproduction capacities, long lifespans, and frequent overlapping of generation (Franco et al. 2009). Among the mealybug species reported in Iran, *Phenacoccus solenopsis* Tinsley and *Planococcus citri* Risso (*Hemiptera*: Pseudococcidae) are two economically important pests (Mogghadm 2013; Mossadegh et al. 2015). *Phenacoccus solenopsis* (cotton mealybug) is assumed to be native to North America and it is a new exotic pest which was reported by Moghadam and Bagheri (2010) for the first time in south and southwest of Iran (Tinesly 1898; Hodgson et al. 2008; Mossadegh et al.

2015). Although *P. solenopsis* is frequently reported as a pest in cotton fields, it can attack more than 219 plant species belonging to 70 families including various agricultural crops, weeds, as well as ornamental and medicinal plants (Abbas et al. 2010; Ariff et al. 2012; Fand and Suroshe 2015; Fallahzadeh et al. 2015; Mossadegh et al. 2015). In Iran, it is a major pest of ornamental plants especially Chinese hibiscus shrubs, *Hibiscus rosa-sinenensis* L. in urban green space (Mossadegh et al. 2015).

The citrus mealybug, *P. citri* is native to China, and has also been observed in America, Europe, and the Pacific as a pest of citrus and ornamental plants (Bodenheimer 1951; Ben-Dov 1994; CABI/EPPO 1999). Further, *P. citri* is an important vector of plant viruses (Bertin et al. 2016). Currently in Iran, *P. citri* is a serious pest in north citrus orchards and on ornamental plants in greenhouses (MafiPashakolaei 1997; Asadeh and Mossadegh 1991). Nevertheless, considering the high reproduction and compatibility of this species under laboratory condition, it is used as a suitable prey for mass rearing of natural enemies especially coccinellids for controlling mealybugs in the field (Zarghami et al. 2014; Nakhai et al. 2017).

✉ Leila Ramezani
danaus_lp@yahoo.com

¹ Department of Plant Protection, Faculty of Agriculture, Agricultural Science and Natural Resources, University of Khuzestan, Khuzestan, Iran

² Date Palm and Tropical Fruits Research Center, Ahvaz, Iran

In the warm region of Khuzestan province, southwest of Iran, *Hyperaspis polita* Weise (*Coleoptera: Coccinellidae*), is the most abundant and an effective indigenous predator of mealybug species on ornamental plants and citrus orchards (Allizadeh et al. 2013; Mssadegh et al. 2015; Seyfollahi et al. 2016; Nakhaei et al. 2017). Further, according to earlier reports it is one of the most important predators of mealybugs in Lebanon, the eastern coasts of the Mediterranean, Turkey, Pakistan, and dry regions of South Western Asia (Yazdani 1990; Ramindo and van Harten 2000; Ramindo et al. 2006). Various biological aspects of *H. polita* and its potentials have been studied by Seyfollahi et al. (2016), Nakhaei et al. (2016-2017), and Farhadi et al. (2018). All researchers emphasized its importance and suggested it as a capable predator for mass producing and releasing against mealybugs. However, more studies are required to develop this predator within a successful biological control program.

One of the key aspects for understanding the predator-prey interactions is examining the functional response as it often correlates with biocontrol efficacy, and therefore can determine the success or failure of the predator in biological control programs (Murdoch and Oaten 1975; Pervez and Omkar 2005). Functional response is defined as the number of preys eaten per predator as a function of prey density (Solomon 1949; Holling 1966). Typically, the functional response is conveniently classified into three general types named I, II, and III, which are described as linear, non-linear with saturation, and sigmoid patterns, respectively (Holling 1959). However, there are several other possible forms such as type IV (i.e. dome shaped) (Luck 1985), and type V (i.e. negative exponential) (Sabelis 1992), as well as functional response with predator interference (Hassell 1978; Omkar and Pervez 2004). Two factors are important in determining the functional response type, handling time and attack rate rate (Holling 1965; Hassell 1978). The coefficient of the attack rate estimates the proportion of predation to prey density, and depends on the prey encounter rate by the predator, the probability of the prey to be attacked when encountered, and the probability that an attack will result in capture capture (Thompson 1975; Bailey 1986). The other factor is the handling time that helps estimate the satiation threshold and reflects the cumulative effect of time taken for capturing, killing, subduing, and digesting prey (Veeravel and Baskaran 1997; Pervez and Omkar 2005).

The purpose of the present study was to determine the efficacy of 4th instar larvae and that of both female and male adults of *H. polita* on two invasive mealybug species in Iran. Initially, the functional response of these three efficient stages of *H. polita* on the adult *P. solenopsis* and *P. citri* was studied and then the effects of previous feeding experience were tested by changing the prey rearing.

Material and methods

Prey and predator cultures

Phenacoccus solenopsis was collected from *Hibiscus rosa-sinensis* L. shrubs, in Mollasani (48°30' E, 32°20' N), Khuzestan Province, southwestern Iran in the spring of 2016. Individuals of *P. citri* were supplied from an insectarium culture originating from a citrus orchard in northern Iran. Cultures of both mealybug species were mass-reared on potato sprouts (*Solanum tuberosum* L.) in boxes (24×16×10 cm) tightly covered with a fine mesh net. *H. polita* adults were collected from *H. rosa-sinensis* infested with *P. solenopsis* in the above-mentioned region and reared on sprouted potatoes infested with *P. solenopsis* plus *P. citri* for two generations before being used in experiments. The stock colonies of all insects were maintained in an incubator at 30±1°C, 65±5% RH, and 14L:10D photoperiod.

Functional response assessments

For this experiment, 4th instar larvae (< 12 h olds), adult male and female *H. polita* (5-day-old) were reared separately on either *P. solenopsis* or *P. citri*. The aim of this test was to determine the functional response of predator stages mentioned on its preys, and whether these behavioral characteristic would differ with different preys offered or with previous feeding experience. In the first half of the experiment, the functional response of three stages of *H. polita* reared on *P. solenopsis* was determined to different densities of *P. solenopsis* adult female and three stages of *H. polita* reared on *P. citri* to different densities of *P. citri* adult female separately. Before the experiment, Individuals of *H. polita* were kept without food for 12 h in a micro tube (1.5 ml) in order to adjust their hunger level. Thereafter, each predator was introduced into a plastic container (9×7×3 cm) with different densities of *P. citri* adult female or *P. solenopsis* as prey on potato discs as host plant. Each container had a 20 mm diameter hole in the middle of the lid, which was covered with a piece of fine muslin to provide ventilation. The densities of prey evaluated were 2, 4, 8, 12, 16, and 32. After 24 h, each predator was removed and the number of preys consumed was recorded. Each treatment (prey densities) was repeated 18 times. In the second half of this experiment, the functional response of three stages of *H. polita* reared on *P. citri* to different densities of *P. solenopsis* adult female and reared on *P. solenopsis* to different densities of *P. citri* adult female was evaluated in exactly the same way as described previously. In this way, we evaluated the effects of the previous feeding experience by changing the prey from rearing to the experiment.

Statistical analysis

The data on predation rates of each predator when supplied with different prey with each prey offered alone were analyzed by a three-way ANOVA with three different predator stages, four levels of prey [*P. citri*- *P. citri* (CC), *P. citri*-*P. solenopsis*(CS), *P. solenopsis*- *P. solenopsis* (SS), *P. solenopsis*- *P. citri* (SC)], six different densities as the factors, and interactions between the treatments. Next, multiple comparisons were performed with the Tukey’s post hoc test. The statistical analyses were conducted in SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

The relationship between the predation rate and initial density of available prey was investigated by fitting functional response curves for each predator’s stages in two steps (Juliano 2001). In the first step, the type (shape) of the functional response was described by determining how well the data fitted the types I, II or III functional responses, using a polynomial logistic regression of the proportion of prey consumed (N_a/N_0) as follows:

$$\frac{N_a}{N_0} = \frac{\exp (P_0+P_1N_{0+}+P_2N_0^2+P_3N_0^3)}{1 + \exp (P_0+P_1N_{0+}+P_2N_0^2+P_3N_0^3)} \tag{1}$$

where, N_a is the number of prey consumed, N_0 denotes the initial prey density, and the parameters P_0 , P_1 , P_2 , and P_3 are the constant, linear, quadratic, and cubic parameters respectively, related to the slope of the curve. The above parameters were estimated using the CATMOD procedure in SAS software (Juliano 2001; SAS Institute Inc 2003). The data sets for all predator stages of *H. polita* were fitted individually to Eq. 1, and the types of functional response were determined by examining the signs of P_1 and P_2 . If P_1 was positive and P_2 was negative, a type III functional response was evident. However, if P_1 was negative the functional response was a type II (Juliano 2001).

In the second step, after determining the type of functional response, a nonlinear least squares regression (PROC NLIN, SAS Institute Inc 2003) was used to estimate the type II response parameters (T_h , and a) using Rogers’ random predator Eq. (2), which is the most appropriate type II functional response in situations with prey depletion (Rogers 1972):

$$N_a = N_0 \{ 1 - \exp [a (T_h N_0 - T)] \} \tag{2}$$

where, T represents the total time that predator and prey are exposed to each other (24 h), a is the attack rate (hours), and T_h (hours⁻¹) denotes the handling time in hours. The Parameters’ estimates were compared by inspecting asymptotic 95% confidence intervals.

The maximum predation rate (T/T_h), which represents the maximum number of preys that can be consumed by an

individual during 24 h, was calculated using the estimated T_h . The value of the coefficient of determination was estimates as follows:

$$r^2 = 1 - \text{residual sum of squares/corrected total sum of squares.}$$

Results

The number of preys consumed (when each prey species was given alone) was significantly affected by predator’s stages ($F_{2,1332} = 170.289$, $P < 0.0001$), prey density ($F_{5,1332} = 223.296$, $P < 0.0001$) and prey species ($F_{3,1332} = 4.859$, $P = 0.002$), for predators or previous feeding experience of predators. Further, a significant interaction ($P < 0.05$) was found between predators and prey for supplying ($F_{6,1332} = 2.380$, $P = 0.027$), as well as predators and prey density ($F_{10,1332} = 20.182$, $P < 0.0001$). However, the interaction between the number of preys eaten by predators and ultimate prey \times prey density and predators \times ultimate prey \times prey density was not significantly different ($F_{30,1332} = 0.181$, $P = 1.0$) (Table 1).

The effect of the density of both prey species (*P. solenopsis* or *P. citri*) on the 4th instar larvae as well as adult male and female *H. polita* reared on either *P. solenopsis* or *P. citri* indicated that the number of preys eaten by predators increased with growing the prey density (Fig. 1).

The polynomial logistic regression analysis of the proportion of both preys eaten by predators *H. polita* reared on either *P. solenopsis* or *P. citri* revealed a type II functional response (Table 2) in all bioassays. The linear coefficient P_1 was negative for these stages, i.e. the proportion of prey consumed declined steadily with an increase in the initial number of preys offered, suggesting a type II functional response (Fig. 1).

The lowest attack rate by the 4th instar larvae corresponded to *P. citri*, while the attack to *P. solenopsis* showed the highest rate (Table 3). However, this parameter of prey

Table 1 A three-way ANOVA with three different predator stages, two supplying prey, the six different densities as factors, and interactions among the treatments

P	F	Mean squares	df	Source
.000	170.289	179.249	2, 1332	P*
.002	4.859	5.115	3, 1332	H
.000	223.296	235.045	5, 1332	D
.027	2.380	2.505	6, 1332	P×H
.000	20.181	21.243	10, 1332	P×D
.989	0.352	0.371	15, 1332	H×D
1.000	0.181	0.190	30, 1332	P×H×D

*P, Predators; H, Preys; D, densities of prey

Fig. 1 Functional responses 4th instar larvae, adult female and male of *H. polita* to different density of *P. solenopsis* (S) and *P. citri* (C). Symbols are observed data and lines were predicted by predicted by by logistic regression models

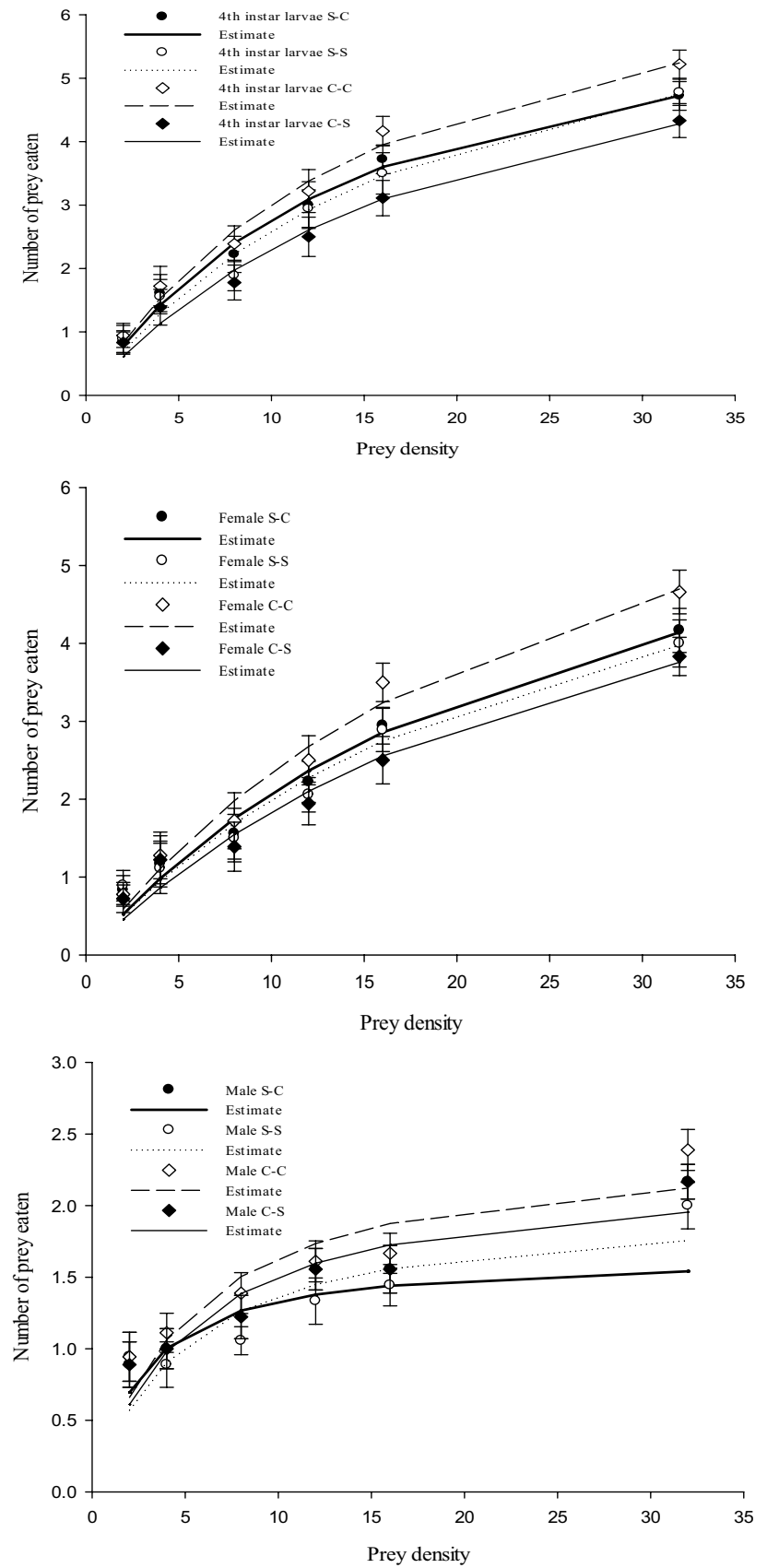


Table 2 Results of logistic regression analysis of the proportion of prey consumed by 4th instar larvae, adult female and adult male of *H. polita* as a function of initial prey density

'P-value	χ^2 -value	SE	Estimates	Parameters	Predator
0.3529	0.86	0.4634	0.4306	Intercept	4th instar
0.0520	3.77	0.1302	-0.2529	Linear	C-C
0.1471	2.10	0.00979	0.0142	Quadratic	
0.1803	1.80	0.000194	-0.00026	Cubic	
0.6807	0.17	0.4809	0.1979	Intercept	C-S
0.0418	4.14	0.1380	-0.2810	Linear	
0.1313	2.28	0.0105	0.0158	Quadratic	
0.1749	1.84	0.000208	-0.00028	Cubic	
0.5003	0.45	0.4720	0.3181	Intercept	S-S*
0.0390	4.26	0.1342	-0.2770	Linear	
0.1195	2.42	0.0101	0.0158	Quadratic	
0.1554	2.02	0.000201	-0.00029	Cubic	
0.7616	0.09	0.4693	0.1424	Intercept	S-C
0.1275	2.32	0.1324	-0.2018	Linear	
0.3014	1.07	0.00999	0.0103	Quadratic	
0.3544	0.86	0.000198	-0.00018	Cubic	
0.8112	0.06	0.4845	0.1157	Intercept	Adult females
0.0288	4.78	0.1384	-0.3025	Linear	C-C
0.0637	3.44	0.0105	0.0194	Quadratic	
0.0758	3.15	0.000207	-0.00037	Cubic	
0.8636	0.03	0.5008	0.0861	Intercept	C-S
0.0280	4.83	0.1466	-0.3222	Linear	
0.0997	2.71	0.0112	0.0185	Quadratic	
0.1449	2.12	0.000223	-0.00033	Cubic	
0.4278	0.63	0.4918	0.3900	Intercept	S-S*
0.0045	8.06	0.1437	-0.4078	Linear	
0.0184	5.55	0.0110	0.0258	Quadratic	
0.0277	4.84	0.000218	-0.00048	Cubic	
0.6777	0.17	0.4908	0.2040	Intercept	S-C
0.0165	5.75	0.1422	-0.3411	Linear	
0.0527	3.75	0.0108	0.0210	Quadratic	
0.0725	3.23	0.000215	-0.00039	Cubic	
0.4381	0.60	0.5043	0.3910	Intercept	Adult males
0.0162	5.78	0.1521	-0.3657	Linear	C-C
0.1369	2.21	0.0118	0.0176	Quadratic	
0.2362	1.40	0.000238	-0.00028	Cubic	
0.6029	0.27	0.5140	0.2674	Intercept	C-S
0.0172	5.67	0.1559	-0.3714	Linear	
0.1248	2.36	0.0121	0.0186	Quadratic	
0.2071	1.59	0.000244	-0.00031	Cubic	
0.4095	0.68	0.5225	0.4310	Intercept	S-S*
0.0066	7.38	0.1616	-0.4391	Linear	
0.0726	3.22	0.0127	0.0228	Quadratic	
0.1351	2.23	0.000256	-0.00038	Cubic	
0.3077	1.04	0.5223	0.5328	Intercept	S-C
0.0025	9.17	0.1616	-0.4892	Linear	
0.0304	4.69	0.0126	0.0274	Quadratic	
0.0612	3.51	0.000254	-0.00048	Cubic	

*This data is reported by Nakhaei et al. (2016–2017)

by the 4th instar larvae was not tied to the previous feeding experience. The attack rate for the adult female of *H. polita* did not vary significantly when the predator was supplied with different preys (ultimate prey) or reared on different preys (factitious prey). Instead, the highest attack rate for adult male of *H. polita* was determined when reared on *P. solenopsis* with the attack carried out on *P. citri*, while the lowest was achieved when reared on *P. solenopsis* and the attack rate carried out on *P. solenopsis*. However, based on asymptotic 95% confidence intervals, for the values of attack rate for 4th instar larvae, adult females and males of *H. polita*, the observed difference was not statistically significant as there was overlapping between them. Also, the estimated handling times for 4th instar larvae and adult female of *H. polita* were similar in all treatments (Fig. 2). Inversely, for an adult male, the handling time was significantly longer when reared on *P. solenopsis*, while *P. citri* was used as prey, and shorter when reared on *P. citri*, using the same prey. This indicates that the predator responds differently in terms of the handling time and attack rate to the predator stages, prey species, prey for rearing, and previous feeding experiences. The value of the coefficient of determination indicated that Rogers random predator equations (Eq. 2) adequately described the functional responses of all three stages of *H. polita* (see values for R^2 , Table 3).

The maximum predation rate was highest for 4th instar larva followed by adult females and adult male on all treatments. In addition, the maximum predation rate was shown when these predators were reared on *P. citri* and were offered to *P. citri*, while the lowest predation was shown on *P. citri* and were offered to *P. solenopsis* (Tables 1 and 3).

Discussion

Hyperspis polita plays an important role in controlling some economic mealybugs including *Nipaeococcus viridis* Newstead (Khodaman 1993; Farhadi et al. 2018), *Planococcus vovae* (Nasnonov) (Lotfalizadeh and Ahmadi 2000), *Maconellicoccus hirsutus* (Green) (Fallahzadeh and Hesami 2004; Allizadeh et al. 2013), *Planococcus ficus* (Signoret) (Fallahzadeh et al. 2011), and *P. solenopsis* in warm regions of the south and southwest of Iran (Mossadegh et al. 2015; Seyfollahy et al. 2016; Nakhaei et al. 2016-2017). In these regions, mealybugs are controlled with imported coccinellid, *Cryptolaemus montrouzieri* Mulsant. However, the inability of this predator to tolerate the warm summers of these regions has resulted in unsuccessful control of mealybugs in orchards or ornamental plants (Mossadegh et al. 2008). According to many reports proving the good performance of *H. polita*, extensive attention has been paid to using it in controlling mealybugs. According to Seyfollahy et al. (2016), *H. polita* could successfully survive, develop, and

Table 3 Parameters estimated by the random predator equation as well as R^2 and maximum predation rate (T/T_h) for 4th instar larvae, adult female, and male of *H. polita*

R^2	T/T_h	Handling time (h)			Attack rate (h^{-1})(a)			Predator
		95% CL		Mean \pm S.E	95% CL		4th instar Mean \pm S.E	
0.884	7.4	3.9413	2.5134	3.2274 \pm 0.3601	0.0325	0.0180	0.0253 \pm 0.00366	C-C
0.814	6.7	4.7484	2.4620	3.6052 \pm 0.5766	0.0224	0.0112	0.0168 \pm 0.00283	C-S
0.851	7.2	4.2284	2.4240	3.3262 \pm 0.4551	0.0255	0.0137	0.0196 \pm 0.00298	S-S*
0.855	6.6	4.5174	2.7459	3.6317 \pm 0.4468	0.0310	0.0158	0.0234 \pm 0.00384	S-C
Adult females								
0.814	7.1	4.0399	1.8550	2.9474 \pm 0.5510	0.0206	0.0107	0.0157 \pm 0.00252	C-C
0.719	6.8	5.2049	1.8249	3.5149 \pm 0.8524	0.0160	0.00720	0.0116 \pm 0.00221	C-S
0.786	6.9	4.9474	2.0056	3.4765 \pm 0.7419	0.0178	0.00835	0.0131 \pm 0.00238	S-S*
0.754	7.2	4.6580	2.0155	3.3368 \pm 0.6664	0.0182	0.00902	0.0136 \pm 0.00232	S-C
Adult males								
0.837	2.4	11.5797	8.2042	9.8920 \pm 0.8513	0.0328	0.0130	0.0229 \pm 0.00498	C-C
0.808	2.2	12.7233	8.7499	10.7366 \pm 1.0021	0.0306	0.0112	0.0209 \pm 0.00489	C-S
0.785	2.0	14.5153	9.5098	12.0126 \pm 1.2624	0.0300	0.00906	0.0195 \pm 0.00528	S-S*
0.748	1.7	16.8722	12.1744	14.5233 \pm 1.1848	0.0493	0.0121	0.0307 \pm 0.00940	S-C

*This data is reported by Nakhaei et al. (2016–2017).

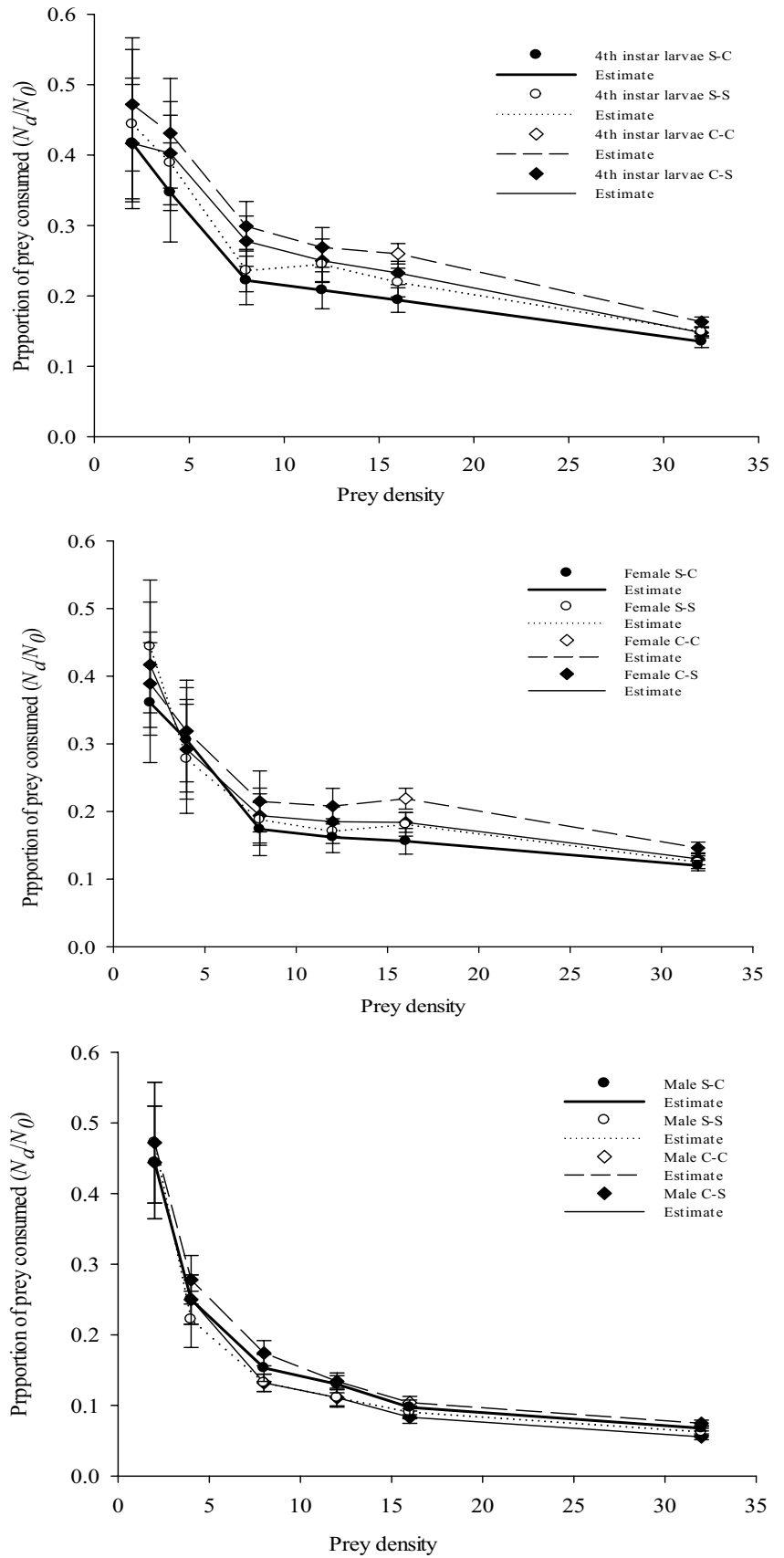
reproduce on *P. solenopsis* in a wide range of temperatures from 25 to 35 °C, with an optimal temperature of 30 °. Nakhai et al. (2017) investigated some biological parameters on *P. solenopsis* and *P. citri* as prey on potato sprout at 30 °C and reported that both of them are suitable for mass production of this predator. Moreover, Nakhai et al. (2016–2017) reported that the 4th instar larvae and the adult females of *H. polita* are effective biological controls agent against *P. solenopsis*. According to Farhadi et al. (2018), the egg *N. viridis* is the most preferred life stage by *H. polita* at 30 °C. All researchers emphasized its importance and suggested it as a capable predator for mass producing and releasing against mealybugs. Thus, more information, such as prey species and previous feeding experience of the searching efficiency of this predator as well as the factors affecting it help us better understand the efficiency of this predator to develop this predator within a successful biological control program.

The present study indicated that the shape of functional response was not affected by the changes in the developmental stages of the *H. polita*, prey species, or supplying prey/previous feeding experiences. In all cases, *H. polita* showed the type II feeding response on *P. citri* and *P. solenopsis* as prey. In other words, a convex curve, which means an evident increase in the number of consuming preys was observed with increasing in the density of prey until a plateau was reached. Similarly, Nakhaei et al. (2016–2017) reported the same result for 4th larval stage, adult female, and adult male of *H. polita* on *P. solenopsis*. On the other hand, Farhadi et al. (2018) reported the types II and III for 4th larval stage, adult female and adult male of *H. polita* on *N. viridis*.

Most researchers believe that natural enemies exhibiting type III, which show density dependence host/prey mortality positively are supposed to manage the host population (Murdoch and Oaten 1975; Zarghami et al. 2016), on the other hand, many type II responses (i.e. inversely density dependent mortality) have been successfully established and managed host populations. This type of (type II) response is also the most common type among natural enemies, as reported for successful coccinellid in controlling pests, especially mealybugs, such as *Nephus regularis* Sicard, *Scymnus coccivora* Ayyar, *Hyperaspis maindroni* Sicard and *Nephus includens* (Kirsch) on *P. citri* and *P. ficus* (Milonas et al. 2011), *Nephus arcuatus* Kapur on *N. viridis* (Zarghami et al. 2016), *C. montrouzieri* on *P. citri* (Abdollahi Ahi et al. 2012; De Bortoli et al. 2014), *M. hirsutus* (Green) (Torres and Marcono 2015) and *P. solenopsis* (Fand et al. 2010). However, in the field, due to the environmental complexity (Pennings 1990), such as temporal and spatial variability of temperature (Streams 1994), as well as plant characteristics the predation might be different.

The functional responses were evaluated according to their parameters. The attack rate is one of the important components which explain how steeply the functional response curve rises with increasing the prey density. In this research, attack rates were not different among three stages of *H. polita*, feeding on two different types of prey, or previous feeding experience. Among researchers, a variety of responses have been shown. For example, Milonas et al. (2011) presented that the attack rate did not show significant difference between various types of prey (*P. citri* and *P. ficus*) or different stages of *N. includes* (2nd and 4th instar

Fig. 2 Mean (\pm SE) proportion of *P. solenopsis* (S) and *P. citri* (C) consumed by 4th instar larvae, adult female and male of *H. polita*. Symbols are observed data and lines were predicted by logistic regression models



larvae). Also, Zarghami et al. (2016) reported similar results for the different developmental stages of *N. arcuatus* (1st to 4th instar larvae and adult males and females) feeding on *N. viridis* egg. However, Farhadi et al. (2018) reported that the adult male attack rate of *H. polita* was significantly shorter than that of adult female and 4th instar larvae on *N. viridis* eggs. Furthermore, Wu et al. (2018) indicated that the searching rates of female adults of *Harmonia axyridis* (Pallas) on *Melanaphis sacchari* (Zehntner) were highest, followed by those of male adults, fourth, third, second, and first instar larvae. According to a literature review, there is no information about the effect of previous feeding experience on the attack rate of insects.

Another important parameter of predator–prey interactions is the handling time. This parameter reflects the cumulative effect of time taken during capturing, killing, subduing, and digesting preys (Veeravel and Baskaran 1997). The significantly shorter time to handle the prey for adult female and 4th instar larvae of *H. polita* and greater for adult male was similar to the results of Nakhaei et al. (2016–2017) and Farhadi et al. (2018). Furthermore, the handling time was high for other predators such as *H. variegata* feeding on *A. fabae* (Farhadi et al. 2010), *N. includens* feeding on *A. gossypii* (Bayoumy 2011), *Propylea quatuordecimpunctata* feeding on *Aphis fabae* (Papanikolaou et al. 2011), *N. arcuatus* feeding on *N. viridis* (Zarghami et al. 2016), and *H. axyridis* on *M. sacchari* were high (Wu et al. 2018). The handling time of the adult female and 4th instar larvae were not affected by prey species and previous feeding experience, while it was affected for the adult male. The adult male spent more time on *P. citri* when reared on *P. solenopsis* and then ate *P. citri*. Milonas et al. (2011) reported that handling time of 4th instar larvae of *N. includens* changed with prey species (T_h on *P. citri* < *P. ficus*), but for 2nd instar larvae, this parameter did not show significant changes with prey species. Unfortunately, no studies have examined the effect of previous experience on the predators.

In the present study, our results showed that 4th instar larvae, adult males and females of *H. polita* are voracious and eat a large amount of prey from low (2 preys) to maximum density (32 preys). The maximum predation rate per day (T/T_h) indicates the number of predators required to suppress those preys in the field affected by predator stages and previous feeding experience. This parameter was more important for 4th instar larva due to their higher requirements for food and energy to grow and attain the critical weight for pupation or to a higher search rate (Bayoumy 2011), which were followed by adult females. The voracity of females was more than 3 times higher than that of adult males. This difference

could be correlated with their larger size and high nutrient requirement for egg production and oviposition (Van den Meiracker and Sabelis 1999).

When compared to other coccidiphagous coccinellid, *H. polita* is more voracious and consumes more *P. solenopsis* and adult *P. citri* as prey. The examples include 4th instar larvae (3.70 adult stage of prey per day) and adult male as well as female of *C. montrouzieri* (8.62) (Kaur and Virk 2011), adult female of *Hyperaspis maindroni* Sicard (0.4), *C. montrouzieri* (0.4), *Nephus regularis* Sicard (0.2), and *Scymnus coccivora* Ayyar feeding on *P. solenopsis* (0.2), 4th instar larvae of *H. maindroni* Sicard (13.6), *C. montrouzieri* (18), *N. regularis* (9.2), and *S. coccivora* feeding on *P. solenopsis* (9.6) (Fand et al. 2010), *N. arcuatus* males feeding on *P. citri* (1.25) *N. viridis* (1.42) and adult females feeding on *P. citri* (3.55) and *N. viridis* (3.44) (Zarghami et al. 2014).

One of the most common methods for rearing natural enemies of mealybugs is using *P. citri* prey in laboratory or insectarium. Research review has indicated that the insect's previous feeding experience of prey can change their future food selection and food preference, modifying subsequent feeding behavior of coccinellids' predator (Houck 1991; Lucas et al. 1997; Zarghami et al. 2014). However, until now no one has paid much attention to the problems that occur for predators eating on prey in the laboratory and then released for controlling other preys not exposed to them so far. Our study showed that previous feeding experience had an influence on predation rate of 4th instar larvae and the male prey, i.e. it ate more prey when reared on ultimate prey, while adult female predation was not affected by previous feeding experiences.

Based on our results, regarding the type II functional response and voracity, the fourth instar larvae and adult female of *H. polita* are good candidates for releasing and controlling *P. solenopsis* or *P. citri* in the field. Meanwhile, the pre-released prey should be considered. This is one of the first steps in demonstrating the potential success of *H. polita* as a biological control agent against mealybug pests in Iran. Note that before regarding it as an appropriate agent, other information such numerical response, foraging behavior, and population dynamics of target pest -*H. polita*- in the field must be investigated to reach a logical conclusion.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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