

# Temperature-Dependent Functional Response of *Harmonia Axyridis* Pallas (Coleoptera: Coccinellidae) Preying on *Acyrtosiphon Pisum* (Harris) (Hemiptera: Aphididae) Nymphs

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## Research Article

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# Abstract

Functional response models are often used to understand the foraging interactions and determine the suitable biocontrol agents. We determined the functional response of *Harmonia axyridis* to nymph *Acyrtosiphon pisum* at different but constant temperatures (between 15 and 35 °C) and prey densities. Logistic regression and Roger's random predator models were employed to determine the type and parameters of functional response. *Harmonia axyridis* larvae and adults exhibited Type II functional responses to different densities of *A. pisum*. Warming increased both the predation activity and host aphid control mortality. The 4<sup>th</sup> instar and female *H. axyridis* consumed the most aphids. Warming contributed markedly in accelerating the predator action. For fourth instar larvae and female *H. axyridis* adult, the successful attack rates were  $0.234 \pm 0.014 \text{ h}^{-1}$  and  $0.247 \pm 0.015 \text{ h}^{-1}$ ; the handling times were  $0.132 \pm 0.005 \text{ h}$  and  $0.156 \pm 0.004 \text{ h}$ ; and the estimated maximum predation rates were  $181.28 \pm 14.54$  and  $153.85 \pm 4.06$ , respectively. These findings accentuate the high performance of 4<sup>th</sup> instar and female *H. axyridis* and the role of temperature in their efficiency. Further studies exploring intraguild predation and mutual interference will be required to conclude the biocontrol potential of *H. axyridis* to *A. pisum*.

## Introduction

The concept of integrated pest management strategies based on biocontrol agents has received increasing prominence worldwide<sup>1</sup>. It has been implemented with tremendous success to both under fields and greenhouses<sup>2-4</sup>, in the particular context of reducing the large-scale use of pesticides<sup>5-8</sup>. While the adoption of biological control is desirable, the successful implementation depends upon the comprehensive understanding of predator-prey interactions, owing to their fundamental role towards ecosystem functionality and food web stability<sup>9</sup>. Several methods can be applied to quantifying these interactions<sup>10,11</sup>, functional response<sup>12</sup>, numerical response<sup>13</sup>, kill rate<sup>14</sup>, and consumption rate<sup>15</sup> are typically employed when foraging interactions have to formalize<sup>16</sup>. Functional response describes how predation rate changes with resource density<sup>17</sup>. Three types of functional responses can be present, with consumption rate being linear up to a constant plateau (Type I), hyperbolic (Type II), or sigmoid (Type III)<sup>18</sup>, depending upon the parameter of functional response, i.e., the enemy attack rate, and prey handling time. The attack rate defines the steepness of the increase in predation with the increase of prey density, and handling time helps rate the satiation threshold<sup>19</sup>. Many sources of variation are known to modulate outcomes of these functional response parameters<sup>20,21</sup>.

Temperature is a chief driver of biological systems through the temperature-dependent nature of biological rates (e.g., metabolic rates)<sup>24</sup>. Effects of temperature on biological rates are likely to be realized up to species level thus expected to influence the population growth rates and carrying capacities<sup>25</sup> as well as ecosystem functions. Temperature can regulate the prey-predator interactions by altering their responses to metamorphosis and population dynamics<sup>22</sup>. Warming is shown to increase the food intake

of predators<sup>23</sup>. In order to consume large prey biomass, a predator should become quickly adept to search/handle its prey, so that it may spend time on consumption events rather than on prey searching/handling attempts, therefore a change of predatory behavior or functional response may be expected under warming<sup>26,27</sup>. For instance, the handling time decreased, consumption rate increased, and the type of functional response changed from Type II to Type III for *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) preying on *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) when the temperature increased from 18 °C to 27 °C<sup>28</sup>. Similarly, functional response for *Euborellia annulipes* (Dermaptera: Anisolabididae) preying on larvae *Plutella xylostella* (Lepidoptera: Plutellidae) changed from Type III at low temperature (i.e., 18 °C) to Type II at higher temperature (i.e., 25 °C and 32 °C)<sup>29</sup>. A changing functional response with warming may destabilize some predator-prey interactions, it may favor prey depletion by increasing predator action<sup>29</sup>. Therefore, a large body of literature is directed towards understanding the consequence of warming on food webs and stability<sup>30,31</sup>.

The pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae), originally a Palearctic species<sup>32</sup>, has now become a pest of global concern for pulse and legume producers<sup>33</sup>. It has a broad host range, infesting grass pea (*Lathyrus sativus* L.), faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), and lupin (*Lupinus albus* L.) (Fabales: Fabaceae)<sup>34</sup>. The aphid inflicts injury either directly, i.e., by removing sap from succulent phloem tissues or via injecting phytotoxic saliva<sup>35</sup>, or indirectly, i.e., by vectoring multiple plant viruses (e.g., the cucumber mosaic virus, the pea enation mosaic virus, the bean leaf roll virus, and the beet yellow virus)<sup>36,37</sup> or by producing honeydew, subsequently inviting sooty-mould that disturbs plant photosynthetic and respiratory functions<sup>38</sup>. Its prolonged infestation could lead to plant stunting, deformation, and discoloration, ultimately reducing crop yields by 35.7%<sup>39</sup>. The broad host range, complex life cycle, and quick adaptiveness to new environments make it difficult to control this aphid. Moreover, this aphid has been reported for the development of insecticide resistance<sup>40</sup>.

Many aphidophagous ladybird beetles (Coleoptera: Coccinellidae) are known to be exploited for conservative or augmentative biocontrol programs of several economically important aphids in diverse crops, outdoor and in greenhouses, allowing aphid suppression to well below economically damaging levels<sup>41</sup>. *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is a generalist predator, geographically wide-spread<sup>42</sup>, and extensively employed as a biocontrol agent of soft-bodied insects, including aphids in a diversity of crops<sup>43,44</sup>. Various biological aspects of *H. axyridis* of importance for its predatory potential (e.g. phenological characteristics, life table parameters, and functional response (generally with a Type II response), have been investigated with respect to the temperature and other concerning factors<sup>45-49</sup> to many crop pests<sup>50-52</sup>, however, information on its predatory behavior and functional response to *A. pisum* almost lacking<sup>53</sup>.

Here, we report the functional response of *H. axyridis* to *A. pisum* under various thermal conditions. We expect that, based on its close association with predator growth and development, the temperature change will accordingly modify consumption. Further, we aim to assess whether thermal conditions and aphid density affect the functional response of larvae and adult *H. axyridis*.

## Results

The mortalities of host aphid in the controls were different between temperature ( $F_{4, 160} = 78.64$ ;  $P < 0.001$ ; Fig. 1a) and aphid density ( $F_{7, 160} = 116.75$ ;  $P < 0.001$ ; Fig. 1b), each increased with increasing the temperature and host aphid density. The density-wise assessment showed similar behavior for increasing trajectories of host mortalities with warming and increasing aphid density ( $F_{28, 160} = 10.23$ ;  $P < 0.001$ ; Fig. 1c).

*Harmonia axyridis* adults (female:  $30.21 \pm 5.12$  aphids/day; male:  $23.45 \pm 3.71$  aphids/day) and 4th instar ( $32.25 \pm 5.27$  aphids/day) consumed significantly more aphids than preceded instars (Wald  $X^2 = 539.39$ ;  $df = 5$ ;  $P < 0.001$ ; Fig. 2b), with an exponentially increasing consumption with respect to increasing temperature (Wald  $X^2 = 104.86$ ;  $df = 4$ ;  $P < 0.001$ ; Fig. 2a) and aphid density (Wald  $X^2 = 1759.52$ ;  $df = 7$ ;  $P < 0.001$ ; Fig. 2c), peaking at 30°C (i.e.,  $24.41 \pm 6.11$  aphids/day) and 35°C (i.e.,  $27.03 \pm 6.43$  aphids/day), at aphid densities of 128 and 160 aphids/petri dish arena. Three way (temperature  $\times$  stage  $\times$  density: Wald  $X^2 = 15.07$ ;  $df = 140$ ;  $P = 1$ ) and two way (temperature  $\times$  stage: Wald  $X^2 = 27.78$ ;  $df = 20$ ;  $P = 0.115$ ; temperature  $\times$  density: Wald  $X^2 = 20.24$ ;  $df = 28$ ;  $P = 0.855$ ) interactions were non-significant, meaning consumption outcomes for temperature (Fig. 3) and growth stage (Fig. 4) remained unchanged, when evaluated at various aphid densities, separately.

Logistic regression between the initial aphid densities offered to larvae and adult *H. axyridis* and the proportion of aphid consumed ( $N_a/N_o$ ) showed all significantly negative values of the linear coefficients  $P_1$ ; exhibiting a Type II functional response across all growth stages and temperatures tested (Table 1). The declining consumption with increasing aphid densities (Fig. 5) also confirmed a Type II functional response. The monotonically declining proportion of consumption with increased aphid densities, led to further confirmation of Type II functional responses (Fig. 6). Clearly, a more linear trend for the proportion of prey eaten with increasing aphid density was noted for 4th instar (Fig. 6d) and female *H. axyridis* (Fig. 6f) at higher (i.e., 30 and 35°C) than lower temperatures.

Table 1

Results of the logistic regression analysis of the proportion of nymphs of *Acyrtosiphon pisum* predated by all stages of *Harmonia axyridis* relative to the initial number of nymphs provided.

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
15°C	1st instar	Intercept	$-7.39 \times 10^{-01}$	$2.26 \times 10^{-01}$	-3.26	0.001
		Linear	$-6.96 \times 10^{-02}$	$1.37 \times 10^{-02}$	-5.05	$4.36 \times 10^{-07}$
		Quadratic	$6.449 \times 10^{-04}$	$1.962 \times 10^{-04}$	3.286	$1.01 \times 10^{-03}$
		Cubic	$-2.040 \times 10^{-06}$	$7.740 \times 10^{-07}$	-2.635	$8.40 \times 10^{-03}$
	2nd instar	Intercept	$-3.67 \times 10^{-02}$	$2.03 \times 10^{-01}$	-0.18	0.85
		Linear	$-8.96 \times 10^{-02}$	$1.25 \times 10^{-02}$	-7.15	$8.15 \times 10^{-13}$
		Quadratic	$8.635 \times 10^{-04}$	$1.771 \times 10^{-04}$	4.876	$1.08 \times 10^{-06}$
		Cubic	$-2.73 \times 10^{-06}$	$6.95 \times 10^{-07}$	-3.941	$8.12 \times 10^{-05}$
	3rd instar	Intercept	1.58	$1.93 \times 10^{-01}$	8.19	$2.42 \times 10^{-16}$
		Linear	$-1.06 \times 10^{-01}$	$9.99 \times 10^{-03}$	-10.67	$< 2 \times 10^{-16}$
		Quadratic	$1.03 \times 10^{-03}$	$1.30 \times 10^{-04}$	7.887	$3.09 \times 10^{-15}$
		Cubic	$-3.24 \times 10^{-06}$	$4.89 \times 10^{-07}$	-6.624	$3.50 \times 10^{-11}$
4th instar	Intercept	3.35	$2.62 \times 10^{-01}$	12.76	$< 2 \times 10^{-16}$	
	Linear	$-1.18 \times 10^{-01}$	$1.08 \times 10^{-02}$	-10.93	$< 2 \times 10^{-16}$	

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
		Quadratic	$1.08 \times 10^{-03}$	$1.26 \times 10^{-04}$	8.580	$< 2 \times 10^{-16}$
		Cubic	$-3.30 \times 10^{-06}$	$4.43 \times 10^{-07}$	-7.462	$8.52 \times 10^{-14}$
	Male	Intercept	2.30	$2.13 \times 10^{-01}$	10.77	$< 2 \times 10^{-16}$
		Linear	$-9.59 \times 10^{-02}$	$9.68 \times 10^{-03}$	-9.91	$< 2 \times 10^{-16}$
		Quadratic	$8.61 \times 10^{-04}$	$1.20 \times 10^{-04}$	7.176	$7.20 \times 10^{-13}$
		Cubic	$-2.62 \times 10^{-06}$	$4.35 \times 10^{-07}$	-6.012	$1.84 \times 10^{-09}$
	Female	Intercept	2.47	$2.19 \times 10^{-01}$	11.27	$< 2 \times 10^{-16}$
		Linear	$-9.75 \times 10^{-02}$	$9.60 \times 10^{-03}$	-10.15	$< 2 \times 10^{-16}$
		Quadratic	$9.13 \times 10^{-04}$	$1.16 \times 10^{-04}$	7.877	$3.36 \times 10^{-15}$
		Cubic	$-2.81 \times 10^{-06}$	$4.13 \times 10^{-07}$	-6.808	$9.90 \times 10^{-12}$
20°C	1st instar	Intercept	$-8.50 \times 10^{-02}$	$1.95 \times 10^{-01}$	-0.43	0.66
		Linear	$-7.39 \times 10^{-02}$	$1.12 \times 10^{-02}$	-6.55	$5.42 \times 10^{-11}$
		Quadratic	$7.037 \times 10^{-04}$	$1.561 \times 10^{-04}$	4.509	$6.52 \times 10^{-06}$
		Cubic	$-2.237 \times 10^{-06}$	$6.045 \times 10^{-07}$	-3.700	$2.16 \times 10^{-04}$
	2nd instar	Intercept	$2.97 \times 10^{-02}$	$1.92 \times 10^{-01}$	0.155	0.87

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
		Linear	$-7.59 \times 10^{-02}$	$1.09 \times 10^{-02}$	-6.92	$4.32 \times 10^{-12}$
		Quadratic	$7.33 \times 10^{-04}$	$1.50 \times 10^{-04}$	4.871	$1.11 \times 10^{-06}$
		Cubic	$-2.33 \times 10^{-06}$	$5.79 \times 10^{-07}$	-4.031	$5.56 \times 10^{-05}$
	3rd instar	Intercept	2.01	$2.03 \times 10^{-01}$	9.92	$< 2 \times 10^{-16}$
		Linear	$-9.10 \times 10^{-02}$	$9.43 \times 10^{-03}$	-9.64	$< 2 \times 10^{-16}$
		Quadratic	$8.24 \times 10^{-04}$	$1.18 \times 10^{-04}$	6.959	$3.43 \times 10^{-12}$
		Cubic	$-2.53 \times 10^{-06}$	$4.33 \times 10^{-07}$	-5.838	$5.29 \times 10^{-09}$
	4th instar	Intercept	3.82	$3.08 \times 10^{-01}$	12.41	$< 2 \times 10^{-16}$
		Linear	$-1.09 \times 10^{-01}$	$1.18 \times 10^{-02}$	-9.25	$< 2 \times 10^{-16}$
		Quadratic	$9.71 \times 10^{-04}$	$1.32 \times 10^{-04}$	7.337	$2.19 \times 10^{-13}$
		Cubic	$-2.90 \times 10^{-06}$	$4.48 \times 10^{-07}$	-6.471	$9.74 \times 10^{-11}$
	Male	Intercept	2.85	$2.36 \times 10^{-01}$	12.04	$< 2 \times 10^{-16}$
		Linear	$-1.06 \times 10^{-01}$	$1.00 \times 10^{-02}$	-10.51	$< 2 \times 10^{-16}$
		Quadratic	$9.86 \times 10^{-04}$	$1.19 \times 10^{-04}$	8.232	$< 2 \times 10^{-16}$
		Cubic	$-3.02 \times 10^{-06}$	$4.22 \times 10^{-07}$	-7.152	$8.54 \times 10^{-13}$

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
	Female	Intercept	2.68	$2.36 \times 10^{-01}$	11.39	$< 2 \times 10^{-16}$
		Linear	$-8.48 \times 10^{-02}$	$9.81 \times 10^{-03}$	-8.64	$< 2 \times 10^{-16}$
		Quadratic	$7.68 \times 10^{-04}$	$1.15 \times 10^{-04}$	6.661	$2.72 \times 10^{-11}$
		Cubic	$-2.35 \times 10^{-06}$	$4.04 \times 10^{-07}$	-5.827	$5.65 \times 10^{-09}$
25°C	1st instar	Intercept	$4.89 \times 10^{-01}$	$1.87 \times 10^{-01}$	2.61	0.009
		Linear	$-8.64 \times 10^{-02}$	$1.06 \times 10^{-02}$	-8.12	$4.54 \times 10^{-16}$
		Quadratic	$8.267 \times 10^{-04}$	$1.463 \times 10^{-04}$	5.652	$1.59 \times 10^{-08}$
		Cubic	$-2.619 \times 10^{-06}$	$5.645 \times 10^{-07}$	-4.639	$3.51 \times 10^{-06}$
	2nd instar	Intercept	$4.75 \times 10^{-01}$	$1.78 \times 10^{-01}$	2.65	0.007
		Linear	$-6.27 \times 10^{-02}$	$9.40 \times 10^{-03}$	-6.67	$2.48 \times 10^{-11}$
		Quadratic	$5.60 \times 10^{-04}$	$1.26 \times 10^{-04}$	4.435	$9.22 \times 10^{-06}$
		Cubic	$-1.75 \times 10^{-06}$	$4.81 \times 10^{-07}$	-3.641	$2.71 \times 10^{-04}$
	3rd instar	Intercept	2.73	$2.31 \times 10^{-01}$	11.79	$< 2 \times 10^{-16}$
		Linear	$-1.04 \times 10^{-01}$	$1.00 \times 10^{-02}$	-10.38	$< 2 \times 10^{-16}$
		Quadratic	$9.53 \times 10^{-04}$	$1.21 \times 10^{-04}$	7.859	$3.86 \times 10^{-15}$

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	<i>Pr(z)</i>
		Cubic	$-2.92 \times 10^{-06}$	$4.32 \times 10^{-07}$	-6.749	$1.49 \times 10^{-11}$
	4th instar	Intercept	4.12	$4.02 \times 10^{-01}$	10.25	< 2e-16
		Linear	$-7.67 \times 10^{-02}$	$1.47 \times 10^{-02}$	-5.21	$1.89 \times 10^{-07}$
		Quadratic	$5.94 \times 10^{-04}$	$1.58 \times 10^{-04}$	3.755	$1.73 \times 10^{-04}$
		Cubic	$-1.71 \times 10^{-06}$	$5.18 \times 10^{-07}$	-3.312	$9.26 \times 10^{-04}$
	Male	Intercept	4.03	$3.24 \times 10^{-01}$	12.41	< $2 \times 10^{-16}$
		Linear	$-1.13 \times 10^{-01}$	$1.23 \times 10^{-02}$	-9.22	< $2 \times 10^{-16}$
		Quadratic	$9.95 \times 10^{-04}$	$1.36 \times 10^{-04}$	7.307	$2.73 \times 10^{-13}$
		Cubic	$-2.94 \times 10^{-06}$	$4.58 \times 10^{-07}$	-6.429	$1.29 \times 10^{-10}$
	Female	Intercept	4.95	$4.22 \times 10^{-01}$	11.73	< $2 \times 10^{-16}$
		Linear	$-1.28 \times 10^{-01}$	$1.50 \times 10^{-02}$	-8.52	< $2 \times 10^{-16}$
		Quadratic	$1.15 \times 10^{-03}$	$1.59 \times 10^{-04}$	7.259	$3.89 \times 10^{-13}$
		Cubic	$-3.48 \times 10^{-06}$	$5.18 \times 10^{-07}$	-6.718	$1.84 \times 10^{-11}$
30°C	1st instar	Intercept	1.22	$1.90 \times 10^{-01}$	6.46	$1.04 \times 10^{-10}$
		Linear	$-1.00 \times 10^{-01}$	$1.03 \times 10^{-02}$	-9.75	< $2 \times 10^{-16}$

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
		Quadratic	$9.454 \times 10^{-04}$	$1.394 \times 10^{-04}$	6.781	$1.19 \times 10^{-11}$
		Cubic	$-2.942 \times 10^{-06}$	$5.334 \times 10^{-07}$	-5.515	$3.48 \times 10^{-08}$
	2nd instar	Intercept	1.98	$2.02 \times 10^{-01}$	9.82	$< 2 \times 10^{-16}$
		Linear	$-1.05 \times 10^{-01}$	$9.75 \times 10^{-03}$	-10.77	$< 2 \times 10^{-16}$
		Quadratic	$9.99 \times 10^{-04}$	$1.24 \times 10^{-04}$	8.060	$7.61 \times 10^{-16}$
		Cubic	$-3.12 \times 10^{-06}$	$4.56 \times 10^{-07}$	-6.833	$8.29 \times 10^{-12}$
	3rd instar	Intercept	4.40	$3.25 \times 10^{-01}$	13.52	$< 2 \times 10^{-16}$
		Linear	$-1.46 \times 10^{-01}$	$1.26 \times 10^{-02}$	-11.58	$< 2 \times 10^{-16}$
		Quadratic	$1.34 \times 10^{-03}$	$1.41 \times 10^{-04}$	9.465	$< 2 \times 10^{-16}$
		Cubic	$-4.06 \times 10^{-06}$	$4.82 \times 10^{-07}$	-8.420	$< 2 \times 10^{-16}$
	4th instar	Intercept	4.97	$7.80 \times 10^{-01}$	6.37	$1.87 \times 10^{-10}$
		Linear	$-3.00 \times 10^{-02}$	$2.86 \times 10^{-02}$	-1.04	0.29
		Quadratic	6.043e-05	3.021e-04	0.200	0.841
		Cubic	-2.243e-07	9.595e-07	-0.234	0.815
	Male	Intercept	7.65	$6.89 \times 10^{-01}$	11.09	$< 2 \times 10^{-16}$
		Linear	$-2.10 \times 10^{-01}$	$2.25 \times 10^{-02}$	-9.32	$< 2 \times 10^{-16}$

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
		Quadratic	$1.87 \times 10^{-03}$	$2.22 \times 10^{-04}$	8.433	$< 2 \times 10^{-16}$
		Cubic	$-5.47 \times 10^{-06}$	$6.86 \times 10^{-07}$	-7.986	$1.4 \times 10^{-15}$
	Female	Intercept	4.96	$7.32 \times 10^{-01}$	6.77	$1.25 \times 10^{-11}$
		Linear	$-3.59 \times 10^{-02}$	$2.60 \times 10^{-02}$	-1.38	0.16
		Quadratic	$2.75 \times 10^{-05}$	$2.69 \times 10^{-04}$	0.102	0.919
		Cubic	$1.38 \times 10^{-07}$	$8.49 \times 10^{-07}$	0.163	0.871
35°C	1st instar	Intercept	1.42	$1.89 \times 10^{-01}$	7.50	$6.00 \times 10^{-14}$
		Linear	$-9.36 \times 10^{-02}$	$9.71 \times 10^{-03}$	-9.63	$< 2 \times 10^{-16}$
		Quadratic	$8.732 \times 10^{-04}$	$1.280 \times 10^{-04}$	6.823	$8.92 \times 10^{-12}$
		Cubic	$-2.731 \times 10^{-06}$	$4.828 \times 10^{-07}$	-5.658	$1.54 \times 10^{-08}$
	2nd instar	Intercept	2.57	$2.22 \times 10^{-01}$	11.57	$< 2 \times 10^{-16}$
		Linear	$-1.15 \times 10^{-01}$	$1.01 \times 10^{-02}$	-11.37	$< 2 \times 10^{-16}$
		Quadratic	$1.089 \times 10^{-03}$	$1.25 \times 10^{-04}$	8.706	$< 2 \times 10^{-16}$
		Cubic	$-3.37 \times 10^{-06}$	$4.52 \times 10^{-07}$	-7.458	$8.77 \times 10^{-14}$
	3rd instar	Intercept	4.00	$3.12 \times 10^{-01}$	12.81	$< 2 \times 10^{-16}$

Temperatures	Growth Stages	Parameters	Estimates	S.E.	Z-Value	Pr(z)
		Linear	$-1.21 \times 10^{-01}$	$1.21 \times 10^{-02}$	-10.03	$< 2 \times 10^{-16}$
		Quadratic	$1.05 \times 10^{-03}$	$1.36 \times 10^{-04}$	7.714	$1.22 \times 10^{-14}$
		Cubic	$-3.11 \times 10^{-06}$	$4.66 \times 10^{-07}$	-6.672	$2.52 \times 10^{-11}$
	4th instar	Intercept	6.90	1.07	6.44	$1.13 \times 10^{-10}$
		Linear	$-1.22 \times 10^{-01}$	$3.59 \times 10^{-02}$	-3.40	0.0006
		Quadratic	$1.14 \times 10^{-03}$	$3.59 \times 10^{-04}$	3.173	$1.50 \times 10^{-04}$
		Cubic	$-3.75 \times 10^{-06}$	$1.11 \times 10^{-06}$	-3.385	$7.13 \times 10^{-04}$
	Male	Intercept	9.20	1.15	7.94	$1.94 \times 10^{-15}$
		Linear	$-2.25 \times 10^{-01}$	$3.61 \times 10^{-02}$	-6.25	$4.08 \times 10^{-10}$
		Quadratic	$1.93 \times 10^{-03}$	$3.41 \times 10^{-04}$	5.660	$1.51 \times 10^{-08}$
		Cubic	$-5.50 \times 10^{-06}$	$1.01 \times 10^{-06}$	-5.437	$5.43 \times 10^{-08}$
	Female	Intercept	5.13	$8.52 \times 10^{-01}$	6.02	$1.72 \times 10^{-09}$
		Linear	$-2.91 \times 10^{-02}$	$3.14 \times 10^{-02}$	-0.92	0.35
		Quadratic	$6.93 \times 10^{-05}$	$3.31 \times 10^{-04}$	0.209	0.834
		Cubic	$-3.27 \times 10^{-07}$	$1.05 \times 10^{-06}$	-0.311	0.756

Estimates of functional response parameters, determined through Rogers random predator model, revealed that the *H. axyridis* exhibited the highest attack rate (Fig. 7a), the shortest handling time (Fig. 7b), and the maximum predation (Fig. 7c) at higher temperatures, and typically at later growth stages. The outcomes of all three parameters were generally low at low thermal conditions (15 and 20°C) but started to improve with warming, with the best results at 30 and 35°C. The outcomes of all three parameters were much low for 1st, 2nd, and 3rd instar when compared with the 4th instar and adult *H. axyridis*. Female *H. axyridis* performed much better than male *H. axyridis*, especially in terms of handling time and maximum predation rate (Fig. 7). The attack rate of 4th instar (i.e.,  $0.234 \pm 0.014 \text{ h}^{-1}$ ) was greatest at 30°C, whereas of adult male (i.e.,  $0.180 \pm 0.01 \text{ h}^{-1}$ ) and female (i.e.,  $0.247 \pm 0.015 \text{ h}^{-1}$ ) was at 35°C. The handling time of 4th instar (i.e.,  $0.132 \pm 0.005 \text{ h}$ ) and adult female (i.e.,  $0.156 \pm 0.004 \text{ h}$ ) was shortest at 35°C. The maximum predation rate for 4th instar (i.e.,  $181.28 \pm 14.54$ ), adult male (i.e.,  $91.90 \pm 21.48$ ) and female (i.e.,  $153.85 \pm 4.06$ ) was obtained at 35°C.

## Discussion

Exploring the temperature influence on predator-prey interactions<sup>16</sup> can be highly insightful towards conservative or augmentative biocontrol programs<sup>54</sup>. As insects are ectotherms, their development and biology are responsive towards temperature<sup>55</sup>, so are their behavioral trophic interactions<sup>16,56</sup>. Theories concerning temperature change and its influence on ecosystem functions, including foraging interactions<sup>57</sup>, are gaining increasing prominence since the past decade<sup>30,31</sup>. The current research is the first report exploring functional response of *H. axyridis* to *A. pisum* at different growth stages and temperatures. The temperature ranges we have tested are existent across a range of temperate or sub-tropical regions. We determined increasing host aphid mortality with warming, meaning warming can lead towards prey depletion. A low-temperature threshold (i.e., 20 °C) is reported the best for aphid growth and development, whereas, higher temperatures > 30 °C become unfavorable for aphid fertility and development, and discourage its population buildup<sup>93</sup>. We showed a Type II functional response by all stages of *H. axyridis*, within the tested host aphid density ranges and thermal conditions, with maximum predation by the 4th instar and female *H. axyridis* between 25 and 35°C. Warming is shown to accelerate insect metamorphosis. Accelerated growth/development enhances metabolic rate and energy gain requirements<sup>50,58</sup>, which the predator meets by consuming large meals<sup>59</sup>, explaining our results of heightened predation under warming. Heightened predation under warming (between 14 and 35 °C), is already known for *H. axyridis* preying on aphids *C. juglandicola* or *P. juglandis*<sup>46</sup>, or eggs of *Spodoptera litura* F. (Lepidoptera: Noctuidae)<sup>50</sup>, and for other coccinellids, *Adalia bipunctata* L., *Hippodamia convergens* Guérin-Ménéville and *Coccinella septempunctata* L., preying on aphid *Myzus persicae* (Sulzer)<sup>26,60</sup>.

Functional response models are employed to quantifying the consumption relationship between a predator and its resource<sup>12</sup>, and a typical model illustrating these relations is the Type II, explaining a curvilinear increase in predation with respect to increasing prey density, changing to asymptotic at high

prey densities, and thenceforth hanging around constant by reason of satiation<sup>12</sup>. A Type II response is often a characteristic of those predators that provide efficient control at smaller resource<sup>61</sup>, though often associated with unstable predator-prey population dynamics<sup>17,62,63</sup>, owing to the decreasing risk of predation with resource abundance. This means a negative density-dependent mortality, which can unstable population dynamics<sup>64</sup>. Many sources of variations are shown to have regulatory roles towards functional response, including prey/predator biology, switching, preference, host distribution, patch allocation time of predator, and intra/interspecific competitions<sup>65-67</sup>.

In our findings, despite its positive role towards consumption, no change in *H. axyridis* functional response type (i.e., Type II) was observed towards thermal changes. A changing functional response type with thermal changes has been reported for many predators<sup>29</sup>, but rarely for coccinellids. *Harmonia axyridis* when preying on immature *S. litura*<sup>50</sup>, or when preying on *C. juglandicola* or *P. juglandis* with respect to the temperature<sup>46</sup>, *Rhopalosiphum padi* L. and *Sitobion avenae* F. to the fertilizer treatments<sup>68</sup>, *Lipaphis erysimi* K. (Hemiptera: Aphididae), *Cacopsylla chinensis* (Yang & Li) (Hemiptera: Psyllidae) and *Danaus plexippus* L. (Lepidoptera: Nymphalidae) to prey identity<sup>51,69,70</sup>, *A. gossypii*, *Myzus persicae* S., *M. nicotianae* S., *Aphis glycines* Matsumura (Hemiptera: Aphididae) and *Diaphorina citri* (Hemiptera: Psyllidae) to growth stage<sup>71-73</sup> have showed frequently a Type II functional response<sup>65</sup>. However, the change of functional response type with respect to prey distribution<sup>74</sup> or prey quality (either pesticide treated or untreated) have been shown for *Harmonia axyridis*<sup>48,49</sup>, suggesting complex nature of predator-prey interactions, and, emphasizing the need for further assessments of current findings with regard to general factors like pesticides<sup>75</sup>. Insecticides from synthetics and biopesticides groups are commonly applied worldwide<sup>76-78</sup> and shown to have profound effects, both positive or negative, on behavioral or physiological responses of predators<sup>45,79</sup>.

The attack rate and handling time are important parameters, describing functional response magnitude. The attack rate (also called the space clearance rate or attack efficiency) describes the ability a predator possesses to catch its prey in a given time frame, and handling time describes the time lost from searching per host consumed<sup>80</sup>. A high attack rate means that the predator is adept at quickly removing hosts from the volumes or areas it is searching, and low handling time means how quickly a predator traps, hunts, and digests prey<sup>80</sup>. In our findings, the parameter estimates showed greater variations across predator growth stages, with frequently better estimates at later growth stages and higher temperatures. Maximum daily predation rates were temperature-dependent, especially their increase, and the handling time of *H. axyridis* presented an exponential decrease for all growth stages at the lowest thermal conditions, meaning predators will spend less investment on foraging, convert that to resting, for example, and decrease predation event<sup>81,82</sup>. Conversely, the greater number of prey consumed owing to the warming implies a decrease in handling time with a resultant increase in time to approach other prey at alleviated temperature<sup>83</sup>. The increased metabolic rates under warming were associated with greater energy demands, which should expectedly maximize food intake and foraging activity. Determining these

ge confirmed poor response by the first three instars as

reported earlier<sup>72,84</sup>, whereas better by final instar and adult *H. axyridis* (especially female when compared with male)<sup>85,86</sup> suggesting *H. axyridis* better biocontrol efficiency at later stages, plausibly owing to better searching efficiencies and agilities. The 4th instar requires large meals to attain the required weight for pupation<sup>87</sup> and adult predators have to prepare for reproduction<sup>69</sup> and other functions related to egg maturation or fertilization<sup>88</sup>.

Though employed commonly to understand the predator-prey interactions related to food consumption and food web stability<sup>89,90</sup>, the functional response models are usually applied under controlled conditions and do not thoroughly represent the field circumstances, hence fail to count for complex and diverse ecological interactions. For example, small-scale setups (such as Petri dish) may not mimic the actual field conditions<sup>91</sup>, and a predator with abundant prey resource, as under controlled conditions, will never going to experience the challenges of emigration or cannibalism<sup>92</sup>, which could occur under poor resource availability. Despite these limitations, the functional response models allow us to understand complex foraging interactions and forecast ecosystem stability. We determined the strong efficiency of *H. axyridis* towards *A. pisum* control, but the predation activity depended upon the growth stage and thermal conditions imposed. The 4th instar and female *H. axyridis* emerged as the best performing biocontrol candidates, with best efficiencies under warming conditions. Another striking effect of warming was determined on prey mortality that increased with warming. This implies that warming may lead towards aphid depletion and consequently trigger intraguild predation or other antagonistic interactions among predator populations<sup>94</sup>. This allows us to suggest that *H. axyridis* can be used at low temperatures (between 20 and 25 °C), but the risk of intraguild predation can be expected at higher temperatures. Provision with alternative prey resources could be a feasible way of supporting this generalist predator without changing functional response type; however, further research is needed to synthesize careful conclusions. Thus, we recommend further studies to evaluate current findings with the inclusion of the other contributable factors, such as intraguild predation, alternative prey resource, mutual interference, pesticides, etc., so that the actual predation behaviour of *H. axyridis* to *A. pisum* may be approached and an efficient biological control may be developed and implemented.

## Methods

Cucumber (*Cucumis sativa* L., cv. Negin; Cucurbitales: Cucurbitaceae) and broad bean (*Vicia faba* L.; Fabales: Fabaceae) seedlings were grown from seeds purchased from Caoxian County, Shandong, China. The seeds were sown in 30 × 25 cm diameter regular pots filled with (3:1) soil: manure. The seedlings were maintained under greenhouse conditions of 12–26°C, 45–55 % RH, and 16:8 h (Light: Dark) photoperiod, and subsequently used for rearing and conducting functional response assays. The plant materials used were obtained with prior permission, and the present study is in compliance with relevant guidelines and legislation.

For establishing *A. pisum* culture, the initial populations of aphid collected from unsprayed alfalfa fields were subsequently brought to the laboratory and reared on broad bean plants inside net cages (20 × 10 ×

30 cm height). The stock culture of *H. axyridis* was developed from a pre-established laboratory colony, already available in the same laboratory. The predator was reared on *A. pisum* infested bean plants (7–8 leaves) inside net cages (60 × 42 × 30 cm height) for three consecutive generations at laboratory conditions of  $24 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 16:8 h (Light: Dark) photoperiod. Bean plants were checked daily for predator eggs. Egg batches when found were carefully removed, placed on tissue paper in Petri dishes (9 cm), and transferred to a computer-operated growth chamber, maintained at settings of  $25 \pm 1^\circ\text{C}$ ,  $65 \pm 5\%$  RH and 16:8 h (Light: Dark) photoperiod. The post-emergence larvae were separated and reared in Petri dishes containing aphid as their diet, refreshed daily. The whole culture was maintained at the Department of Plant Protection, Huazhong Agricultural University, China.

The experimental arena consisted of clear Petri dishes (9 cm diameter), with a micromesh screen over the top for ventilation and bottom covered with clean cucumber leaf disk. The desiccation of cucumber leaf disc was prevented by adding 1% agar solution<sup>95</sup>. The assays were performed with *H. axyridis* larvae (i.e., 1st instar, 2nd instar, 3rd instar, 4th instar) and adults (male, female) at constant temperatures (i.e., 15, 20, 25, 30, 35°C). The homogeneity of predator age was maintained within each tested growth stage. The first instar larvae were separated one by one shortly after hatching to avoid sibling cannibalism. Hatchlings were reared in Petri dishes (9 cm diameter) until maturity on 4th instar nymphs (100–150 aphids/day). Female *H. axyridis* included mated individuals<sup>72</sup>. First instar larvae were starved for about 6h, whereas subsequent instars/stages were starved for 24 h to standardize hunger level, according to Islam, et al.<sup>50</sup>. The moist cotton roll offered humidity to all predators during starvation. The use of 4th instar aphid was ensured throughout the experiments as a way to prevent predator preference switch according to prey size<sup>96</sup>. Using a fine camel hairbrush, the aphids at different densities (i.e., 2, 4, 8, 16, 32, 64, 128, and 160 aphids) were transferred in Petri dishes, allowed for 30 minutes to uniformly spread and settle over the substrate, and thereafter were transferred to a computerized growth chamber at various fixed temperatures (i.e., 15, 20, 25, 30, 35°C),  $70 \pm 5\%$  RH and 16:8h (Light: Dark) photoperiod. The whole experiment was replicated 10 times for each prey density, growth stage, and temperature. The numbers of aphid consumed were recorded every 24th h. Control replicates were kept free from *H. axyridis* to account for natural mortality and correct *A. pisum* consumption by the predator as a function of natural mortality. Predation mortality data were corrected for control mortality by applying Abbott's correction<sup>97</sup>.

The control mortality data were analyzed between temperatures, aphid densities, and their interaction, by using Univariate Analysis of Variance (ANOVA) in SPSS (version 21), fitting the above three variables as fixed factors against the dependent variable (i.e., host mortality). Significant ( $P < 0.05$ ) effects were further compared by using Tukey's Honestly Significant Difference (HSD) multiple comparisons Test. Prior to analysis, the mortality data were tested for normality and homogeneity of error variance (i.e., homoscedasticity) by using Shapiro-Wilk and Levene tests, and  $Y = \sqrt{x + 1}$  transformed to improve compliance with these assumptions. All means and standard errors in text and figures are calculated with untransformed data.

Aphid consumption by *H. axyridis* for temperature, growth stage, density, and their two-way and three-way interactions were analyzed by using Generalized Linear Models (GLM) in SPSS (version 21). Kolmogorov-Smirnov test confirmed non-normal distributions of data ( $P > 0.05$ ), and due to over-dispersion, the data were fitted with negative binomial distribution and a log link function, and factors and interaction effects were analyzed by using the Wald Chi-Square test for a confidence level (CI) of 95%. If needed, the multiple follow up tests were run to analyze the temperature and growth stage effects, separately, at each aphid density, and the significance for each test was adjusted by following Bonferroni correction to avoid Type 1 error.

Analysis of the functional response was done in two different phases<sup>17</sup>: first phase involved the determination of type and estimation of the parameters of the functional response curve. It is compulsory to find the type of functional response for calculating the functional response parameters using a proper model. The type was determined by applying logistic regression of the proportion of prey eaten as a function of initial prey density offered. A polynomial logistic regression equation assuming a binomial distribution of data to define the type of functional response<sup>17</sup> (Eq. 1) was fitted as under:

$$\frac{N_a}{N_o} = \frac{\exp(P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}{1 + \exp(P_o + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)} \quad (1)$$

Where  $N_a$  and  $N_o$  indicate the number of prey consumed and the initial prey density offered, respectively, and  $\frac{N_a}{N_o}$  is the proportion of prey consumed. The  $P_o$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the regression parameters representing intercept or constant, linear, quadratic, and cubic coefficients, respectively. The coefficients were calculated by using the maximum likelihood method. The values of the linear and quadratic coefficients indicate the nature of functional response either it is Type II or Type III. When the value of a linear parameter is negative, the functional response is Type II, and if it is positive with a negative quadratic coefficient, then response is of Type III. The Type II response shows that the proportion of prey consumption decreases as the prey density increases, and a Type III response represents that the proportion of prey consumed increases until an inflection point and then decreases<sup>17</sup>. Once the functional response type was determined, the second phase started where functional response parameters were determined. For which, data were fitted to Rogers' type II random predator equation, with the help of non-linear least square regression, and determined and analyzed the parameters of functional response. As the prey was not changed or replaced during the entire experiment, the random predator equation was determined to be more appropriate for such a dataset<sup>98</sup>. The attack rate ( $a$ ) and handling time ( $T_h$ ) were calculated by using the random predator model as under: (Eq. 2).

$$N_a = N_o [1 - \exp(a(T_h N_a - T))] \quad (2)$$

Where,  $a$  is the attack rate,  $T_h$  is the handling time,  $T$  is time available for predator during the experiment. Here, " $g/m$ " function was used to fit the logistic regression, and the parameters (attack rate  $a$  and handling time  $T_h$  of functional response) were estimated by using *FRAIR* (Functional Response Analysis in

R, version 4.0.0)<sup>99</sup> in the R statistical environment<sup>100</sup>. The maximum predation rate is the ratio between  $T/Th^{101}$  and estimates the maximum amount of prey that a predator can consume in a given time frame.

## Declarations

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## Author Contributions

XZ conceived the idea and supervised the laboratory trials. YI and FMS designed the protocol of laboratory experiments. YI performed the experiments, analyzed the data in R software, and wrote the initial draft. FMS and XR analyzed the data in SPSS software, and wrote the final draft. MR, XR, MY, LX and XZ reviewed the manuscripts. All authors discussed and approved the publication of the manuscript.

## Competing Interests

The authors declare there are no competing interests.

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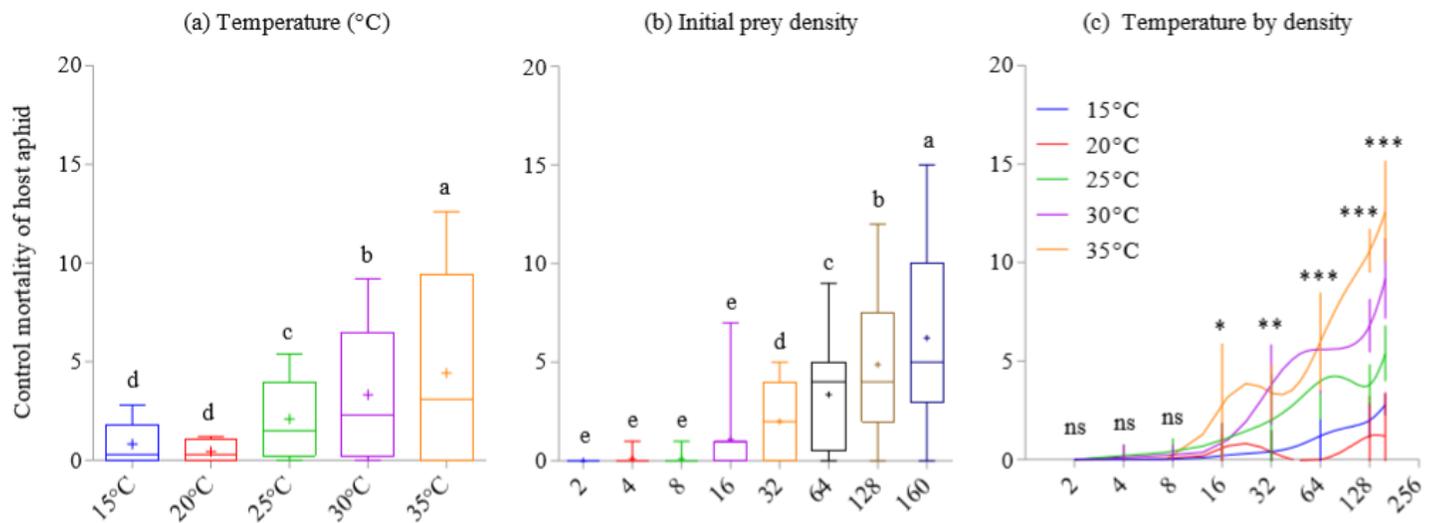
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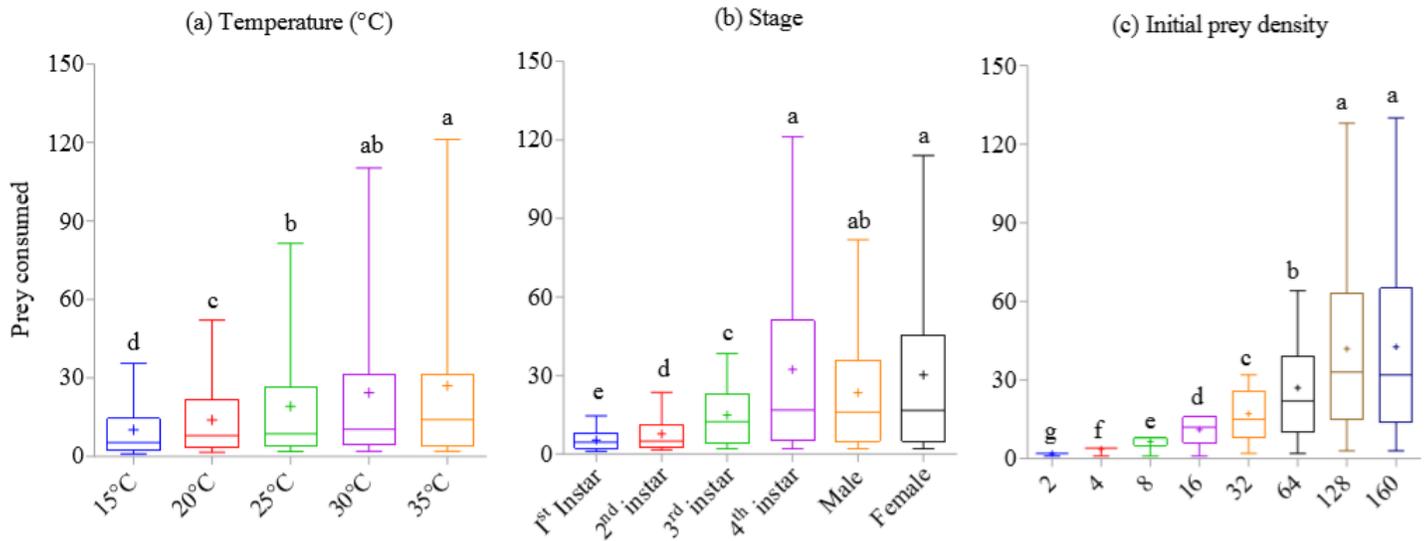
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## Figures



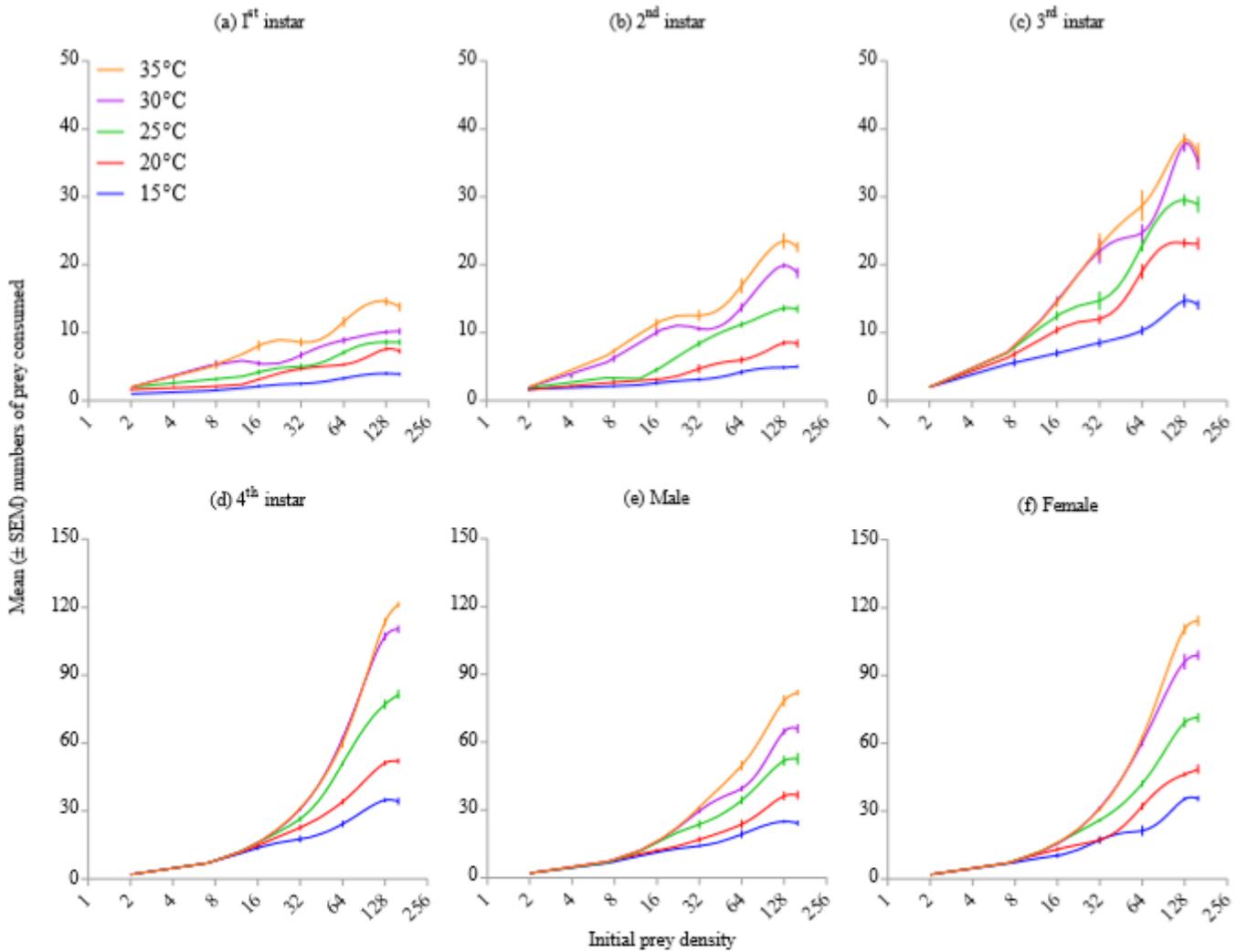
**Figure 1**

Control mortality of host aphid across temperatures and initial prey densities. Box plots showing the range of data (lower and upper quartiles, and extreme values), median, and mean (symbols). Box plots as in panels a, b, labelled with the different letter are showing significant differences among group means ( $P < 0.05$ ; Tukey's HSD test), whereas panel c showing significant difference ( $P < 0.05$ ) based on non-overlapping 95% CI of difference. \*, \*\*, and \*\*\* denote significance at 0.05, 0.01, and 0.001 levels, respectively.



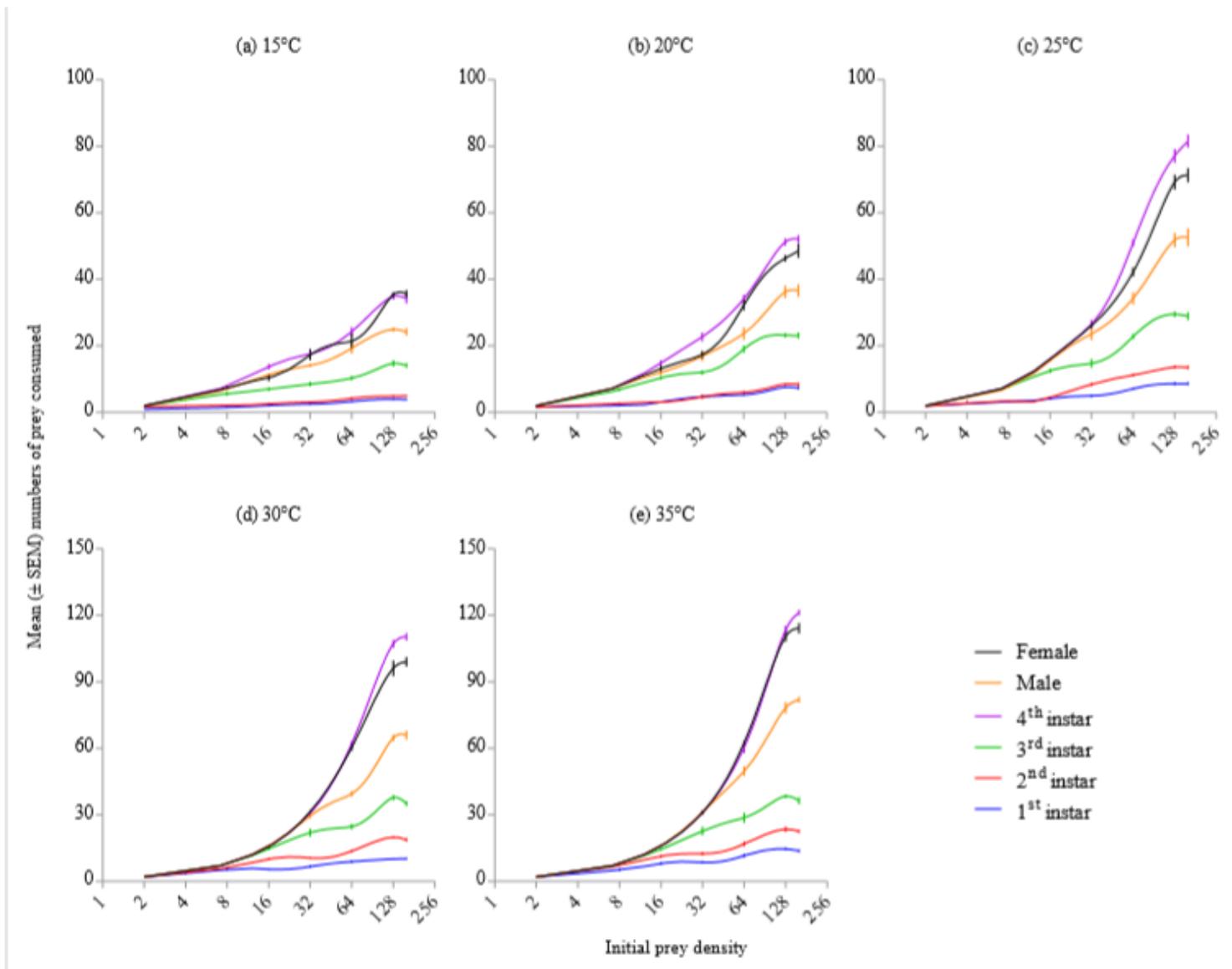
**Figure 2**

Mean number of prey consumed by *Harmonia axyridis* across temperatures, predatory stages and initial prey densities. Box plots showing the range of data (lower and upper quartiles, and extreme values), median, and mean (symbols). Box plots labelled with the different letter are showing significant differences among group means according to Wald chi square test with 95% CI of difference.



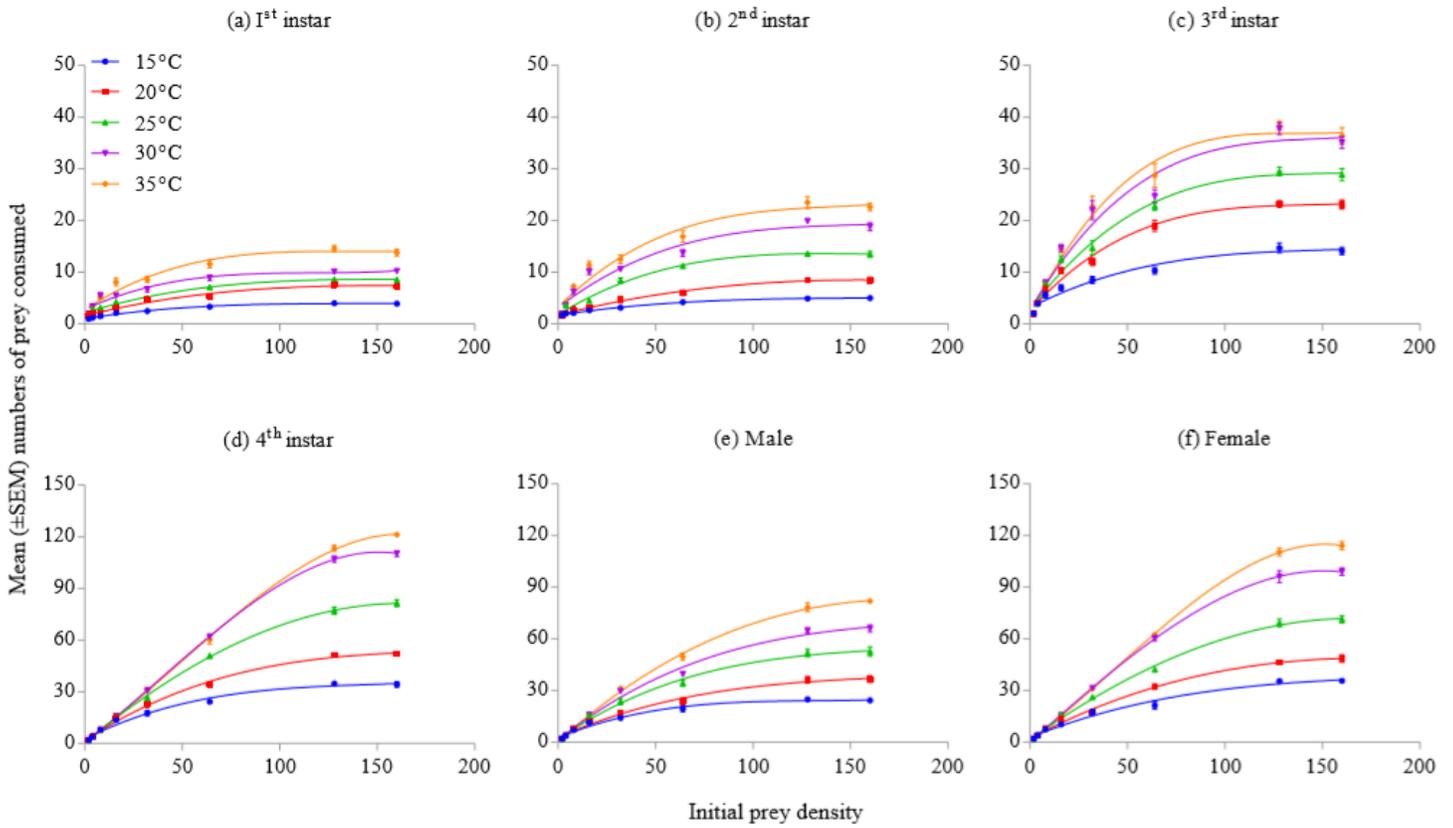
**Figure 3**

Mean ( $\pm$ SEM) numbers of prey consumed by *Harmonia axyridis* across temperatures with respect to the predator growth stages and initial prey densities.



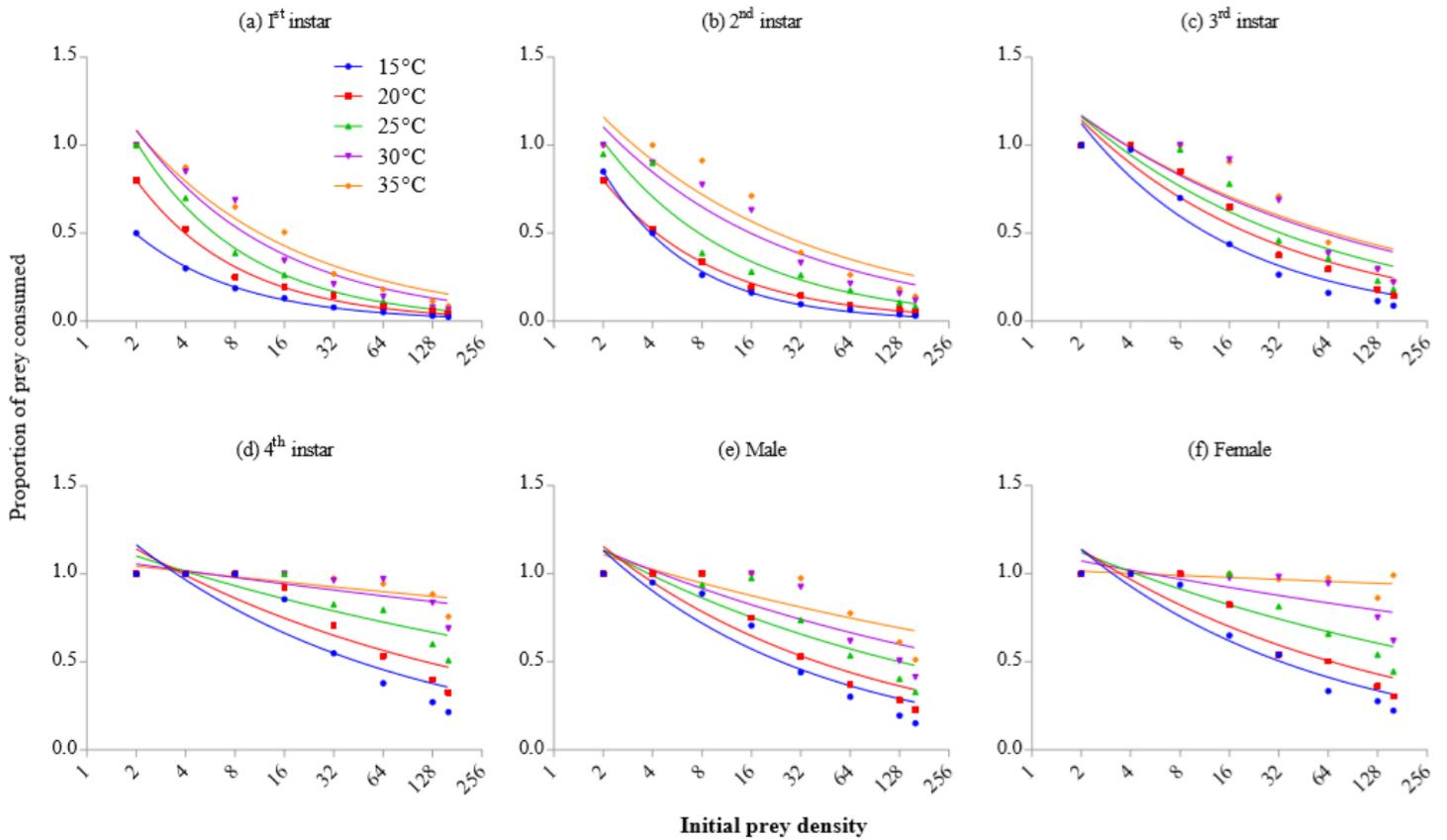
**Figure 4**

Mean ( $\pm$ SEM) numbers of prey consumed by *Harmonia axyridis* across predatory stages with respect to the thermal conditions imposed and initial prey densities.



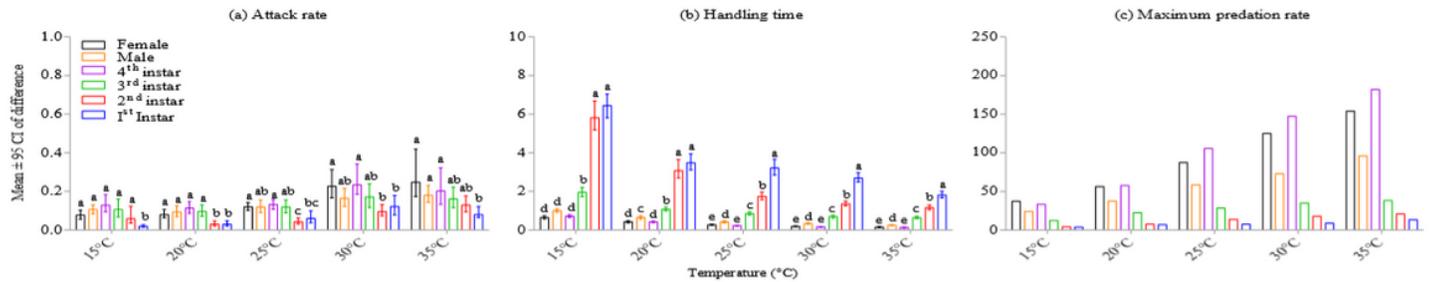
**Figure 5**

Type II functional response curves fitted by Roger's random predator equation of *Harmonia axyridis* across temperatures.



**Figure 6**

Proportion of prey consumed by *Harmonia axyridis* across temperatures and initial prey densities.



**Figure 7**

Functional response parameters of *Harmonia axyridis* across temperatures, resulting from bootstrapped functional response parameters. Bars are estimated means with 95 CI of difference. Different letters above the bars within each temperature are showing significant difference ( $P < 0.05$ ) among various growth stages.