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2 **Biological Aspects and Predation of *Pygidicrana***  
3 ***v-nigrum* against the Mediterranean Fly *Ceratitis***  
4 ***capitata***

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19 **ABSTRACT**

Fruit flies are pests of great agricultural concern, as they cause serious damage to the global fruit production. However, there are potential entomophagous organisms that can reduce the population of pest species such as *Ceratitis capitata*. Among the voracious and entomophagous Dermaptera predators, the species *Pygidicrana v-nigrum* displays a strong predatory potential to improve the agricultural handling by assisting the reduction of agrochemical use. This study aims to evaluate the biological development and quantification of *P. v-nigrum* consumption and predation of *C. capitata* during the fruit fly's immature stages. Larvae from the 3<sup>rd</sup> instar and pupae of the Mediterranean fruit fly were used, where biological parameters were analyzed, including the duration and nymphal viability, adult insect size (length), sex ratio, survival of adults and egg production, and the ethology of predation behavior. It was found that the *P. v-nigrum* nymphs from the 1<sup>st</sup> to 3<sup>rd</sup> instar did not feed on the pupal stage *C. capitata*. When ingesting the *C. capitata* larvae, the Dermaptera reached the end of the nymph period, on average, after 228 days. The lowest nymphal viability of *P. v-nigrum* was 85.0% and occurred in its 1<sup>st</sup> instar when fed with larvae. The food provided did not influence the size of this regardless of sex; however, predation on *C. capitata* larvae resulted in a higher proportion of females. Furthermore, the survival of the female *P. v-nigrum* was longer than the male, regardless of the food consumed. There were a high number of deposited eggs from *P. v-nigrum* when feeding on pupae. The predatory consumption of *P. v-nigrum* increased when fed with *C. capitata* larvae and pupae,

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regardless of the nymphal or adult phases. It can be concluded, from the results, that the biological development of the *P. v-nigrum* is not affected when fed with the larval and pupal stages of *C. capitata*.

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21 *Keywords: Fruticulture, Dermapterous, biological control*

## 22 **1. INTRODUCTION**

23 The Dermaptera are terrestrial insects with nocturnal habits and about 1800 species are  
24 distributed in the tropical and subtropical regions [1], including several species with  
25 predatory habits. Furthermore, studies have investigated the behavior and the biological  
26 development of the genera *Euborellia* and *Doru*, with the predation of numerous agricultural  
27 pests such as Coleoptera, Lepidoptera, and Hemiptera [2, 3, 4, 5, 6, 7], and Lepidoptera [8,  
28 9], in the egg stages and young forms, respectively. These are commonly known in Brazil as  
29 “tesourinhas” (“earwig”), because they have two tweezer-like structures at the end of the  
30 thorax.

31 Moreover, the tephritids are considered the main pests of global fruticulture, and the direct  
32 damage from these pests has affect production, including costs related to monitoring and  
33 control, or eradication; while indirect damages are caused by the restriction imposed by  
34 certain importing countries [10]. The pests *Ceratitis* spp. and *Anastrepha* spp. are of major  
35 importance for agricultural research. Among the species, we highlight *Ceratitis capitata*  
36 Wiedemann (Diptera: Tephritidae), commonly known as the Mediterranean fruit fly, found in  
37 Brazil.

38 *Pygidicrana v-nigrum* Audinet-Serville, is one of the most prominent species of the family  
39 Pygidicranidae, whose dermapterous insects seek shelter in jackfruit and banana trees. A  
40 previous study fed this dermapterous insect with eggs from *Ephestia kuehniella* Zeller  
41 (Lepidoptera: Pyralidae) and found that the average nymphal period for these *P. v-nigrum*  
42 were 237 days, with nine instars, showing improved development [11]. Thus, it is necessary  
43 to study the biology and ethology of this dermapterous species [12] and its effect on

44 important pests such as *C. capitata*. The knowledge of the biological aspects of this  
45 dermapterous insect regarding its feeding is essential due to the influence on its biological  
46 cycle, as it is present in different environments, it also plays an important role in the  
47 predation of arthropod pests. The following research aimed to analyze the development of  
48 biological characteristics and the ability of *P. v-nigrum* predation when fed with immature  
49 stage *C. capitata*.

## 50 **2. MATERIAL AND METHODS**

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52 The research was carried out in the Laboratory of Entomology (LEN), Campus II of the  
53 Federal University of Paraíba (UFPB), Areia, Paraíba State, Brazil. The experiments were  
54 performed at  $25 \pm 2$  °C,  $70.0 \pm 10.0\%$  relative humidity (RH), and a 12 h photophase.  
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### 56 **2.1 Rearing of *Pygidicrana v-nigrum* and the Mediterranean fruit fly *Ceratitis*** 57 *capitata*

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59 The nymphs and adults were kept in transparent plastic containers (6.0×8.0 cm) with  
60 moistened absorbent paper and fed on an artificial diet consisting of the following  
61 ingredients: milk powder (130 g), beer yeast (220 g), wheat bran (260 g), and nipagin (40 g),  
62 and an initial ration of chicken meat (350 g). The eggs were laid and fixed anywhere in the  
63 container by the female, who protects them until hatching into nymphs. The feed and  
64 absorbent paper were both exchanged weekly. Alcohol (70%) was applied to the lid of the  
65 container to inhibit the emergence of microorganisms.  
66

67 The Mediterranean fruit flies were grown in the Laboratory of Entomology in the conditions  
68 already stated above. Their larvae were fed an artificial diet composed of beer yeast (120 g),  
69 raw carrot (600 g), and nipagin (5 g). The adults were kept in cages (50 × 50 × 60 cm) and  
70 fed daily with a solution of 10.0% honey in distilled water, provided in cotton placed on the  
71 cage during the adult stage.

## 73 **2.2 Biological Development of Earwig *Pygidicrana v-nigrum* fed on *Ceratitis*** 74 *capitata*

75 The bioassays were organized by a completely randomized design (CRD) with two food  
76 treatments with 20 *P. v-nigrum* nymphs for each treatment and one individual nymph per  
77 replicate. The food (prey) used was 3<sup>rd</sup> instar larvae and pupae of *C. capitata* (<24 h old),  
78 which were unviable at low temperatures, leading to the death of *P. v-nigrum*. These were  
79 supplied in enough quantity for the development of the earwigs, as defined in the preliminary  
80 tests. To evaluate the biological characteristics, the following parameters were assessed:  
81 nymphal duration and viability, adult insect size (length), sex ratio, adult survival, and egg  
82 production per posture.

## 83 **2.3 Predation Capacity of Earwig on *Ceratitis capitata***

84 We used 190 specimens of earwig, 110 of which were fed on 3<sup>rd</sup> instar larvae and the  
85 remaining 80 were fed on pupae of *C. capitata*. The nymphs and adults of the predator were  
86 individualized in Petri dishes (9.0 × 1.5 cm) and fed with 3<sup>rd</sup> instar larvae or pupae of *C.*  
87 *capitata*. The food was supplied in a quantity higher than that consumed by the predator  
88 daily at each instar or stage, so that the number of 3<sup>rd</sup> instar larvae and pupae consumed  
89 could be counted and the predation capacity per day of consumption could be determined.  
90 This number of 3<sup>rd</sup> instar larvae and supplied pupae was observed daily in preliminary trials.

## 91 **2.4 Statistical Analysis**

92 The experiments were carried out using a CRD. For the research into the biological aspects  
93 of the predator, the food consisted of larvae or pupae of the Mediterranean fruit fly, with 20  
94 replicates for each food treatment. The sex ratio was calculated by dividing the number of  
95 females with the total number of individuals (females + males) according to [13]; the adult  
96 survival probability was analyzed using a non-parametric test and estimated using the

97 Kaplan-Meier survival test (Log-Rank test), using the MedCalc<sup>®</sup> software; and the means of  
 98 the analysis of variance of the other characteristics were compared by the F-test at the 5.0%  
 99 probability level. Data were analyzed by the Assistat 7.7 program [14]. The predation  
 100 capacity research involved the use of the 3<sup>rd</sup> instar larvae or pupae of the Mediterranean fruit  
 101 fly, with 15 repetitions for each different food treatment. The predator's consumption was  
 102 measured using regression analysis.

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### 3. RESULTS AND DISCUSSION

#### 106 Biological Development of *P. v-nigrum* fed with *C. capitata*

107 There were nine instars during the nymphal period of *P. v-nigrum*, although some individuals  
 108 only went through seven or eight stages regardless of the food (Table 1). This behavior was  
 109 related to the adequacy of food, which can result in the lengthening or reduction of the  
 110 number of instars, as the development of insects is affected by biotic and/or abiotic factors. It  
 111 has been found in another study that the species *Tagalina papua* Bormans (Dermoptera:  
 112 Pygidicranidae), belonging to the same family as *P. v-nigrum*, survived six instars [12].

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**Table 1. Average duration (days) and viability (%) of the stages of *Pygidicrana v-nigrum* fed with larvae and pupae of *Ceratitidis capitata***

Duration (days)									
Food	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>
Larvae	21.46	22.40	24.40	27.06a	25.60a	26.40a	34.40a	35.27a	38.50a
Pupae	-	-	-	22.66b	23.20a	28.06a	32.93a	40.33a	31.50a
CV (%)				22.13	21.32	24.85	20.72	28.64	8.72
Viability (%)									
Larvae	85.0	88.2	93.3	100.0	100.0	100.0	100.0	100.0	100.0
Pupae	-	-	-	95.0	94.7	100.0	100.0	100.0	100.0

116 Means followed by the same letter in the column do not differ statistically.

117

118 Table 1 shows that 1<sup>st</sup> to 3<sup>rd</sup> instar nymphs of the predator did not consume pupae, but  
119 instead only consumed the larvae of this tephritid. The lack of consumption by early nymphs  
120 is due to the fragility of their oral apparatus in contrast to the stiffness of the integument of  
121 the pupa, making it impossible to break it down for ingestion. For an insect to feed, several  
122 characteristics of the food should be analyzed, among these are the color, shape, size  
123 (length), temperature, sound, texture, and hardness [15]. In the 4<sup>th</sup> instar there was a  
124 statistically significant difference. Nymphs that consumed pupae had a shorter instar period  
125 (22.66 days on average). This reduction of *P. v-nigrum* instar may have occurred due to  
126 ingestion of the previous food (standard diet), as it provided the necessary nutrients for  
127 proper development.

128

129 The mean nymphal viability of the dermapterous species varied from 85.0% to 100.0% for  
130 those fed with larvae and between 94.7% and 100.0% for those fed with pupae, inferring a  
131 high viability of *P. v-nigrum* regardless of the food consumed. The failure of the nymphs (in  
132 the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> instars) regarding the consumption of pupae. The natural alternative for  
133 this predator would be to search for prey with a soft tegument; in addition, the Dermaptera  
134 order are omnivores are omnivores, so other alternatives are available. The results of this  
135 research confirm that the prey is a suitable nutritional source for *P. v-nigrum* development.

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137 There was no statistically significant difference between the sizes of the predators, in its  
138 adult stage, regardless of whether it was fed with pupae or larvae of the *C. capitata* (Table  
139 2). The females reached a size-range of 3.0 to 4.2 cm and 3.3 to 4.2 cm when fed with  
140 larvae and pupae, respectively; whereas males were in the size-range of 3.2 to 3.9 cm for  
141 both food treatments. The result of *P. v-nigrum* individuals larger than 4.0 cm exceeds what  
142 has already been reported in the literature for the order Dermaptera. Working with species *T.*  
143 *papua*, found a length of 2.9 to 3.6 cm [12]. The *P. v-nigrum* sex ratio, regardless of the food,

144 is within the expected and suitable values for laboratory breeding, with the ratio of one male  
145 per one female (1:1) being enough for reproductive success.

146 **Table 2. Average size (length) and sex ratio of *Pygidicrana v-nigrum* when fed with**  
147 **larvae and pupae of *Ceratitis capitata***

Food	Size		Sex Ratio
	Female (cm)	Male (cm)	
Larvae	3.48a	3.47a	0.60
Pupae	3.73a	3.61a	0.46
CV (%)	9.81	7.21	

148 Means followed by the same letter in the column do not differ statistically from  
149 each other by F test (P = 0.05).

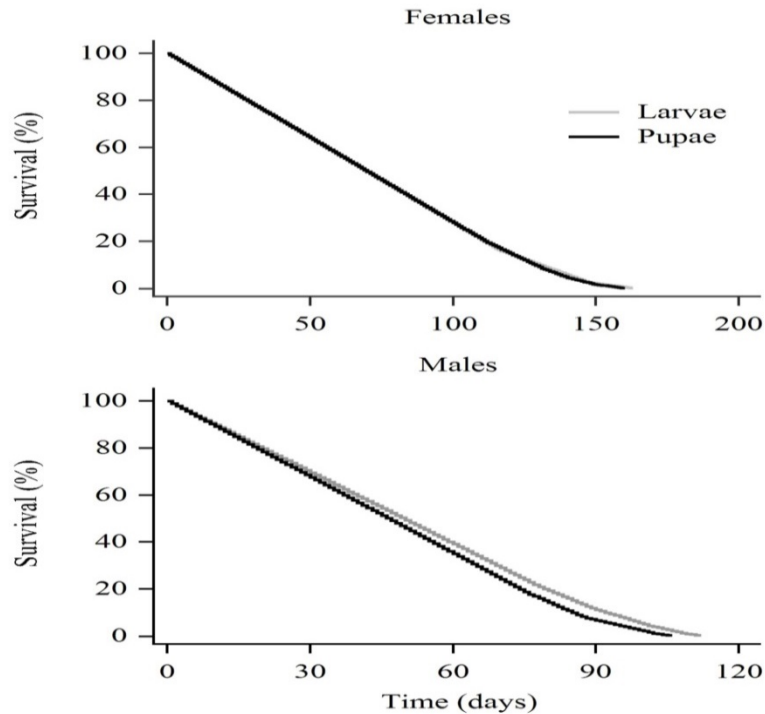
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151 The survival time of the *P. v-nigrum* when feeding on *C. capitata* was longer for adult  
152 females than for male insects (Fig. 1). In female insects, at 50 days, approximately 70.0% of  
153 the individuals were alive; at 80 days, there were only 40.0% of the initial amount; and after  
154 reaching 115 days, only 20.0% of the original adult females were left. At the end of their  
155 longevity, *P. v-nigrum* females averaged 160 and 163 days when consuming *C. capitata*  
156 larvae and pupae, respectively. Regarding male survival, it was found that at 50 days,  
157 approximately 70.0% of the individuals were alive; at 80 days, there was only 40.0% of the  
158 initial amount; and at 115 days, only 20.0% were alive.

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160 The males showed a change in survival behavior at around 60 days between feeding with  
161 pupae and larvae. The latter prolonged the survival, but this variation in survival behavior  
162 was still exceptionally low. A similar longevity found for the *P. v-nigrum* species was also  
163 found in the literature for the species *Doru luteipes* Scudder (Dermaptera: Forficulidae), and  
164 *Euborellia peregrina* Mjöberg, *Euborellia annulipes* (Dermaptera: Anisolabididae), when  
165 consuming insect-pests.

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**Figure 1. Average probability of adult survival for *Pygidicrana v-nigrum* fed with larvae and pupae of *Ceratitidis capitata***

As for the number of deposited eggs for species *P. v-nigrum* fed with larvae and pupae of the Mediterranean fruit fly *C. capitata*, there was statistically significant difference (Table 3). Females fed with larvae produced, on average, 49.25 eggs whereas those that ingested pupae produced, on average, 101.75 eggs. Egg production is related to the accumulation of energy and nutrients and the quantity and quality of the food ingested, which explains the reproductive behavior of the insect. The production of eggs or progeny involves energy and nutrient accumulation, which is also affected by both biotic and abiotic factors [16].



206 **Table 3. Average number of eggs per posture of *Pygidicrana v-nigrum* fed with different**  
 207 **stages of *Ceratitis capitata***

Food	Number of eggs per posture
Larvae	49.25b
Pupae	101.75a
CV (%)	44.23

208 Means significantly differed from each other as determined by the F test (P = 0.05).

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210 The occurrence of a gradual oviposition of *P. v-nigrum* females was observed for days,  
 211 during which time they were fed with pupae between 4 to 11 days, and with larvae, between  
 212 4 to 5 days. There was maternal care of the *P. v-nigrum* female during the oviposition, where  
 213 it licked the eggs and always remained above or beside the egg. It is understood that by  
 214 licking them, the mother releases secretions that simultaneously humidify and also protects  
 215 the eggs from harmful microorganisms [12].

216

217 Another observed characteristic was that if disturbances occurred at any point during the  
 218 incubation time, the *P. v-nigrum* might consume all of its eggs [9]. The authors infer that  
 219 behavior possibly occurred due to the handling of cleaning, humidification, and exchange of  
 220 the food in the breeding containers. Furthermore, when working with *D. luteipes*, a decrease  
 221 in viability was observed when the male was left in contact with the female after intercourse,  
 222 which also attributed to the male-caused disturbance which led to the female's consumption  
 223 of her eggs.

#### 224 **Predation of *P. v-nigrum* on *C. capitata***

225 The predator consumption increased over time when fed with the larvae and pupae of *C.*  
 226 *capitata* (Fig. 2). Early instar (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup>) *P. v-nigrum* consumed only larvae, as they  
 227 were not successful with the pupae food. Furthermore, at 35 days the 4<sup>th</sup> and 5<sup>th</sup> instar  
 228 nymphs had consumed more larvae than pupae. The 6<sup>th</sup> instar nymphs consumed more

229 pupae at the end of their stage. The predation of the 7<sup>th</sup> and 9<sup>th</sup> instar *P. v-nigrum* was higher  
230 for larvae, but the difference was not statistically significant. The 8<sup>th</sup> instar nymphs had  
231 similar predatory behavior. Regarding male and female adult consumption, there was higher  
232 larvae consumption in *P. v-nigrum* females than in males. The consumption of larvae by  
233 males was slightly higher than their consumption of pupae, although this was not statistically  
234 significant.

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236 The behavior of this dermapterous species in the present study makes it a possible  
237 potentiator for the consumption of the pupae and 3<sup>rd</sup> instar larvae stages of this global pest.  
238 Its increasing consumption, regardless of the stage of development, shows its voracity in the  
239 constant search to meet its nutritional needs. In addition to this, it consumed more than  
240 necessary, that is, there was accumulation of reserves to aid in its nymphal development,  
241 ecdysis and reproductive processes.

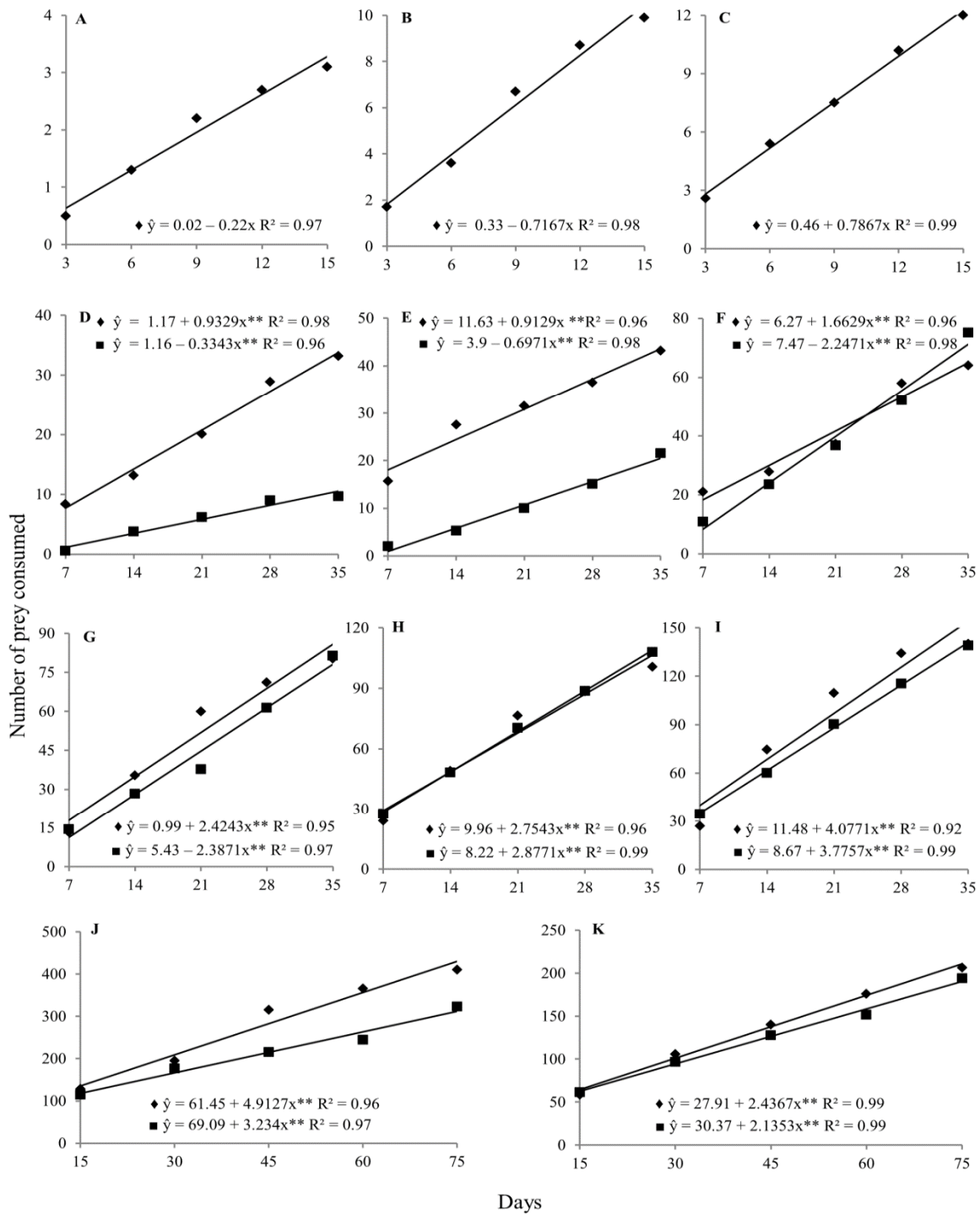
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243 During the experiment, the daily consumption behavior of the predator was inconsistent,  
244 reaching peaks of high daily food consumption interspersed with days where little or no food  
245 was consumed due to its food satiation. The same behavior of consumption was found by  
246 [17], with the species *E. annulipes*, when fed with eggs and caterpillars of the species  
247 *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae).

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251 **Figure 2. Predatory consumption of nymphs in their 1<sup>st</sup> (A), 2<sup>nd</sup> (B), 3<sup>rd</sup> (C), 4<sup>th</sup> (D), 5<sup>th</sup>**  
 252 **(E), 6<sup>th</sup> (F), 7<sup>th</sup> (G), 8<sup>th</sup> (H), 9<sup>th</sup> (I) instars, and of female (J) and male (K) adults of**  
 253 ***Pygidicrana v-nigrum* when fed with the larvae and pupae of *Ceratitis capitata*. (◆)**  
 254 **corresponds to the 3<sup>rd</sup> instar larvae of *C. capitata* and (■) to the pupae food.**

255 *Pygidicrana v-nigrum* consumption of larvae and pupae of the *Ceratitis capitata* throughout  
256 the juvenile stage was similar to the adult stage of the predator *P. v-nigrum* (Fig. 2) with the  
257 exception of the 1<sup>st</sup> to 3<sup>rd</sup> instars, where there was no consumption of pupae (Fig. 2A, B, &  
258 C). There was predominantly more in larvae consumption than pupae consumption, both in  
259 the juvenile and adult stages (Fig. 2). However, there was only a higher consumption of  
260 pupae than larvae in the 6<sup>th</sup> and 8<sup>th</sup> instar (Fig. 2F, & H), but this only occurred in the interval  
261 between 20 and 25 days after their ecdyses. Larger larvae consumption may be of nutritional  
262 benefit to the predator which has the need for increased consumption to meet its  
263 requirements. Physical characteristics, such as hardness, shape, and surface pilosity, in  
264 addition to the allelochemicals and nutritional elements, influence the consumption and  
265 digestion of food [16].

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#### 267 **4. CONCLUSIONS**

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269 The 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar nymphs of the predator *Pygidicrana v-nigrum* did not  
270 consume the pupae of the prey *Ceratitis capitata*. The dermapterous species *P. v-nigrum*  
271 had successfully developed regardless of the growth phase of the supplied *C. capitata*.  
272 Further studies on species *P. v-nigrum* are required to determine its potential as a *C.*  
273 *capitata* regulator and its use in biological control programs.

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#### 276 **COMPETING INTERESTS**

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278 Authors have declared that no competing interests exist.

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