

1 **Mosquitoes asses interspecific competition by larvae-released semiochemicals**  
2 **during oviposition habitat selection**

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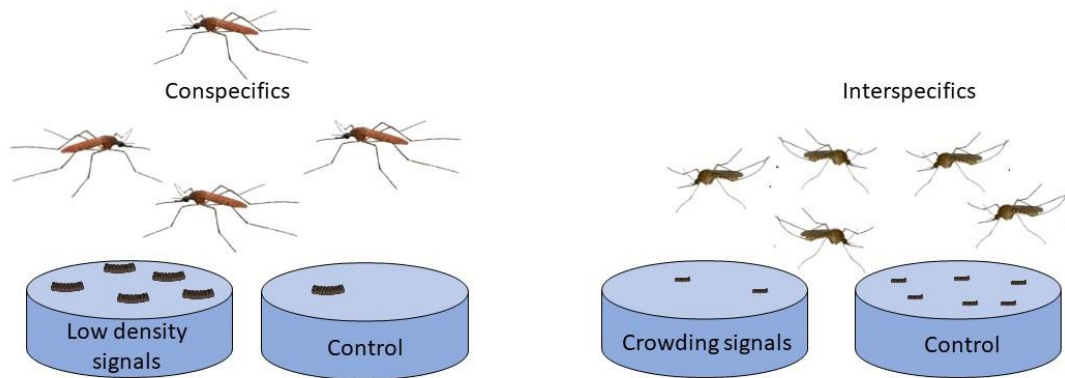
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12 **Abstract:**

13 Numerous species of animals alter their behavior in response to increasing  
14 competition. To do so, they must possess the ability to detect the presence and density  
15 of interspecific competitors. We studied the role of semiochemicals released by  
16 increasing densities of larval *Culiseta longiareolata* Macquart on female oviposition  
17 habitat selection in two sets of field mesocosms. Similarly to *C. longiareolata* larvae,  
18 subordinate *Culex laticinctus* Edwards are periphyton grazers who dwell in rain-filled  
19 pools in the Mediterranean region. We show that *C. laticinctus* females oviposited  
20 significantly less in mesocosm pools that were treated with crowding signals  
21 originating from *C. longiareolata* larvae. In a second field experiment, we placed a  
22 similar number of larvae directly inside the 50 L mesocosms. These low-density  
23 mesocosms did not affect *C. laticinctus* oviposition but were attractive to conspecific  
24 oviposition. These results increase our understanding of the female's ability to detect  
25 species-specific signals indicating increased larval competition.

26 Introduction

27 Interspecies competition, is a reciprocally negative interaction between populations of  
28 two or more species (Rockwood 2015, Begon et al. 2006). This fundamental  
29 interaction between species that share a similar niche is considered one of the most  
30 important factors in the shaping of the ecological community (Begon et al. 2006,  
31 Morin 2011), as well as an important driver of evolutionary speciation (Hutchinson  
32 1959). The high cost of competition is associated with several alterations in animal  
33 behavior. In the presence of a competing species, individuals alter their foraging  
34 activity (Begon et al. 2006, Abrams 2010, Dhondt 2012), as well as produce

35 aggressive interference and shifts in mating success (Dhondt 2012, Grether et al.  
36 2017). Habitat selection is another mechanism affected by competition, and some  
37 animal species shift their activity, in space (to a less desirable habitat) or in time (to a  
38 different time of day or a different season) to reduce competitive interactions  
39 (Abramsky et al. 1990). These behavior alterations happen in response to increased  
40 densities of both populations and thus require the ability to identify competitor  
41 presence as well as an estimation of conspecifics (Rosenzweig 1981). Although  
42 several studies demonstrated the ability of competitors to do just that (Morris et al.  
43 2000, Abramsky et al. 1990, Sandlin 2000), the mechanism of identification is usually  
44 overlooked.

45 Chemical signals or semiochemicals, are an important source of information for  
46 numerous animal species. This is especially true in aquatic systems since odorants  
47 tend to travel better in water compared to auditory and visual signals (Brönmark and  
48 Hansson 2012). Aquatic species alter their behavior in response to semiochemicals  
49 indicating a food source, predator presence, or conspecific density (von Elert 2012) .  
50 With few exceptions e.g. (Wu et al. 2019), studies on behavior alteration as a result  
51 of semiochemicals associated with interspecific competitors looked at mere  
52 recognition and overlooked the density-dependent effects.

53 Ovipositing mosquito females (Diptera: Culicidae) are excellent models for the study  
54 of semiochemical effects on habitat selection. Mosquitoes are characterized by a  
55 complex life cycle where adults are free to range the landscape but the immature are  
56 confined to the aquatic habitat where they hatched. Female mosquitoes provide little  
57 parental care beyond the selection of an appropriate oviposition site, making  
58 oviposition a critical factor in larval survival. Ovipositing females are attracted to  
59 several bacteria-released semiochemicals that are associated with nutrients for future

60 larvae (Takken 1999, Bentley and Day 1989). Females also use semiochemicals to  
61 detect predators and avoid oviposition in sites where predation risk is high (Bentley  
62 and Day 1989, Angelon and Petranka 2002) . In addition, gravid females can quantify  
63 predators (Silberbush and Blaustein 2011), and conspecific larvae (Wasserberg et al.  
64 2014), rather than simply be aware of their presence. In this study, we examined the  
65 ability of ovipositing mosquito females to detect and respond to the presence and  
66 density of semiochemicals originated by larvae of a dominant competitor species.

67 *Study species:*

68 *Culiseta longiareolata* Macquart is a highly abundant species throughout the  
69 Mediterranean region (Becker et al. 2010). The females typically oviposit in small,  
70 often temporary, rain-filled bodies of water and are often the earliest colonizers of  
71 these habitats following rain (Van Pletzen and Van der Linde 1981, Blaustein and  
72 Margalit 1996, Ward and Blaustein 1994). Because these rain-filled pools are both  
73 ephemeral and limited in number, they are a valuable resource to amphibians and  
74 aquatic insects. *Culex laticinctus* Edwards is a mosquito species whose larvae are  
75 often associated with *C. longiareolata* breeding sites (Margalit and Tahori 1974,  
76 Becker et al. 2010). The larvae of this species are considerably smaller in comparison  
77 to *C. longiareolata* larvae (Figure 1). *Culiseta longiareolata* larvae are considered  
78 herbivorous and feed mainly on periphyton algae and bacteria (Van Pletzen 1981).  
79 Nevertheless, fourth instar *C. longiareolata* larvae are considered as highly aggressive  
80 competitors of other freshwater species such as *Bufo viridis* tadpoles (Blaustein and  
81 Margalit 1994) and other mosquito larvae (Tsurim et al. 2013). This aggressive  
82 behavior towards other aquatic dwellers may result in the death of larvae of other  
83 mosquito species (Al-Saadi and Mohsen 1988, Shaalan 2012) as well as vertebrates  
84 such as *Bufo viridis* tadpoles (Blaustein and Margalit 1994).

85 The purpose of this study was to examine the effects of chemical signals produced by  
86 high densities of the dominant competitor *C. longiareolata* larvae on ovipositing  
87 mosquito females. The study was conducted with field mesocosms that mimic rain-  
88 filled pools that are the natural larval habitats of both species. We hypothesized that  
89 water with high *C. longiareolata* larval density will contain crowding signals. These  
90 signals, indicating high competition will be associated with larval habitat of poor  
91 quality. Ovipositing females are therefore hypothesized to avoid these habitats. In a  
92 second experiment, we examined the effects of the actual larvae who were not  
93 subjected to crowding. According to the Ideal Free Distribution theory, conspecifics  
94 should always prefer habitats with lower density (Fretwell and Lucas 1969).  
95 Subordinate competitor species are likewise hypothesized to avoid competition and  
96 reduce oviposition in habitats containing competitors.

97

## 98 **Methods:**

### 99 *Field experiments:*

100 Field experiments were conducted at the Oranim college campus botanical gardens  
101 (Tivon-Israel) between May and June 2023, a period that is peak activity for  
102 ovipositing mosquitoes in that area. We monitored mosquito oviposition in black  
103 plastic pools sized 66.04×50.8×15.24 cm. Pools were organized in a randomized  
104 block design, containing eight blocks of three treatments (n=24) randomly distributed  
105 within each block. Pools within a block were placed ~1m apart and blocks were  
106 spaced ~10 m from each other. Pools were filled with ~50L tap water and  
107 supplemented with 10g of rodent chow to enhance oviposition. We collected  
108 mosquito egg rafts daily from the water surface of each pool. The collected egg rafts

109 were hatched and the larvae were raised to the 4<sup>th</sup> instar and identified to species using  
110 (Becker et al. 2010).

111 *Crowding signals experiment:* We produced water-containing signals of highly  
112 crowded *C. longiareolata* larvae by placing 200 4<sup>th</sup> instar larvae in 400 ml plastic  
113 cups for 24 hours. This density of 500 larvae/L is considered high but not an untypical  
114 density for this species (Blaustein and Margalit 1996, Blaustein and Margalit 1994). A  
115 second treatment with medium crowding signals included 20 larvae in 400 ml (50  
116 larvae/L), and a third set included control cups with no larvae. Larvae were fed with  
117 ~0.05 grams of finely grounded fish flakes (42.2% crude protein) that were added to  
118 each cup. We filtered the water daily and replaced the dead and pupated larvae.  
119 Conditioned water (without the larvae) was added to the experimental pools each day  
120 at sunset. All pools were emptied and refilled every 5 days.

121 *Low-density larvae experiment:* We placed 2 densities of living *C. longiareolata*  
122 larvae directly in the field mesocosms. The higher density included 200 4<sup>th</sup> instar  
123 larvae, roughly the amount larvae originated from a single egg raft (Van Pletzen and  
124 Van der Linde 1981, Al-Jaran and Katbeh-Bader 2001). The lower concentration  
125 treatment consisted of 20 larvae per pool, and the third pool was a control pool  
126 without larvae. Pupated larvae were replaced daily and all pools were emptied and  
127 refilled every 5 days.

128

129 *Statistical analysis:*

130 We used the total number of all egg rafts, per pool, collected for each mosquito  
131 species across all dates as a dependent variable. We used square root transformations  
132 of these values with an addition of 0.5 to all values, to homogenize among-treatment

133 variance (Yamamura 1999). The homogeneity of variance was then tested using  
134 Levene's test. We conducted separate univariate ANOVAs for each mosquito species  
135 in each of the experiments using "Block" and "Treatment" as fixed factors. Treatment  
136 means were compared using Fisher's protected LSD when the main effect of  
137 treatment had  $p < 0.1$  using  $\alpha = 0.05$  for individual LSD comparisons. All analyses  
138 used SPSS statistics for Windows version 24 (IBM 2016).

139

## 140 **Results**

141 The first field experiment ran for 15 days (May 3<sup>rd</sup>-May 18<sup>th</sup> 2023) and the second for  
142 25 days (May 22<sup>nd</sup>- June 16<sup>th</sup> 2023). During these periods, we collected a total of 335  
143 and 340 egg rafts from both setups respectively. One of the blocks contained an  
144 especially low number of egg rafts during the first period and was removed from the  
145 analysis. All egg rafts collected in both field experiments belonged to one of three  
146 mosquito species. *Culiseta longiareolata* (Macquart), *Culex laticinctus* (Edwards) and  
147 *Culex pipiens* (Linnaeus). The egg rafts of these three species appeared in similar  
148 amounts and consisted of 34.3%, 34%, 31.6%, 25.6%, 40.9%, and 33.5%  
149 respectively.

150 In the first field experiment with signals of crowded *C. longiareolata*, the three  
151 species showed significantly different responses to the signals. *Culiseta longiareolata*  
152 egg raft distribution was not affected by the treatments ( $F_{2,12} = 0.1$ ;  $p = 0.9$ ). By  
153 contrast, *C. laticinctus* oviposition was significantly affected by the different  
154 treatments ( $F_{2,12} = 5.25$ ;  $p = 0.02$ ) with significantly fewer egg rafts oviposited in pools  
155 containing signals of either high or medium-crowded larvae (Figure 2). The

156 oviposition distribution pattern of *C. pipiens* was not affected by the treatments ( $F_{2,12}$   
157  $=0.39$ ;  $p=0.69$ ).

158 In the second field experiment with live larvae in the pools, *C. longiareolata*  
159 oviposition showed a dramatic response to the presence of larvae ( $F_{2,14}=10.64$ ;  
160  $p=0.002$ ), with many more egg rafts found in pools added with 200 larvae per pool  
161 when compared to pools added with 20 larvae per pool or control pools (Figure 3a).  
162 The egg raft distribution of both *C. laticinctus* and *C. pipiens* was not significantly  
163 affected by the presence of *C. longiareolata* larvae ( $F_{2,12}=5.25$ ;  $p=0.87$  and  $F_{2,12}$   
164  $=0.39$ ;  $p=0.69$  respectively, Figure 3b-c).

165

## 166 **Discussion:**

167 This study focused on the role of semiochemicals indicating increasing competition in  
168 habitat selection. We hypothesized that water containing highly crowded *C.*  
169 *longiareolata* larvae (20 and 200 larvae in 400 ml.) will contain chemical signals.  
170 These signals would be associated with highly crowded larval habitats, and avoided  
171 by ovipositing females. *Culiseta longiareolata* females did not respond to these  
172 conspecific crowding signals (Figure 2a). Furthermore, a similar number of  
173 conspecific larvae that were not crowded were attractive to gravid females (figure 3a).  
174 Conspecific density does not necessarily cause an immediate decrease in habitat  
175 suitability. A low number of conspecifics may be favorable to colonizers over an  
176 empty habitat (Fretwell and Lucas 1969). This trend was shown for ovipositing  
177 mosquitoes in response to increasing conspecific larvae (Wasserberg et al. 2014) and  
178 eggs (Williams et al. 2008). It is suggested that a habitat with low conspecific density



179 may indicate site persistence, potential mates, and overall appropriate conditions  
180 without increased competition.

181 By contrast to conspecifics, the presence of interspecific-dominant competitors  
182 reduces habitat quality even at low densities (Forsman et al. 2008, Eccard and Ylönen  
183 2002, Abramsky et al. 1990). Mosquito larvae are often confined to the oviposition  
184 site until metamorphosis. The presence of interspecific larvae from a dominant  
185 competing species at this site will often result in reduced survival (Livdahl and Willey  
186 1991). Even if survival to metamorphosis is not significantly reduced, interspecific  
187 competition results in other factors associated with a decline in population size as  
188 reduced adult body size, longevity, or changes in time to metamorphosis (Silberbush  
189 et al. 2014). In our case, competition can be completely avoided by placing the larvae  
190 in a competition-free habitat during oviposition. Our results show that females of the  
191 subordinate *C. laticinctus* preferred to oviposit in pools that lacked condition water  
192 that previously contained crowded larvae of the dominant *C. longiareolata* larvae  
193 (Figure 2b). Similar numbers of larvae placed in a higher volume of water did not  
194 trigger a significant response (Figure 3b). these results strongly indicate that  
195 ovipositing females responded to chemical signals from crowded larvae, indicating a  
196 habitat with high competition.

197 The distribution of *C. pipiens* egg rafts was not significantly affected by the *C.*  
198 *longiareolata* cues (Figure 2c) or larvae (Figure 3c). The global distribution of *C.*  
199 *laticinctus* generally overlaps with that of *C. longiareolata* (Becker et al. 2010). The  
200 larvae of these two species often co-occur in recently filled freshwater pools (Kiflawi  
201 et al. 2003, Becker et al. 2010, Margalit and Tahori 1974). *Culex pipiens* on the other  
202 hand, are characterized by global distribution and by their ability to inhabit a very

203 wide variety of water sources (Becker et al. 2010). As such, this species may be less  
204 sensitive to competition and cannot therefore detect and respond to them.

205

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211 of interest.

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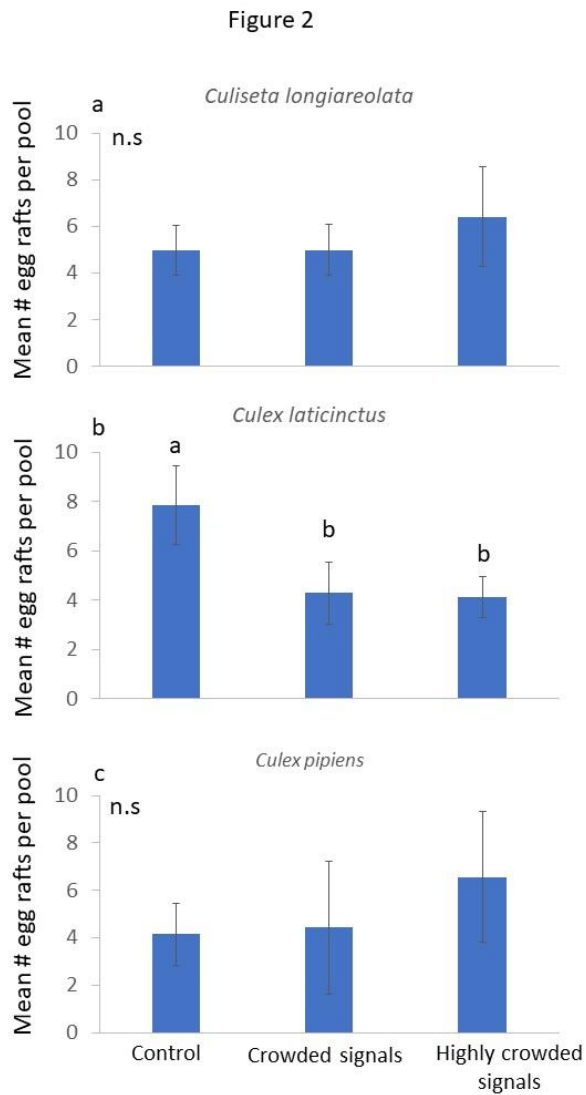
312 Figure 1- Fourth instar larval: *Culiseta longiareolata* (top) and *Culex laticinctus*.



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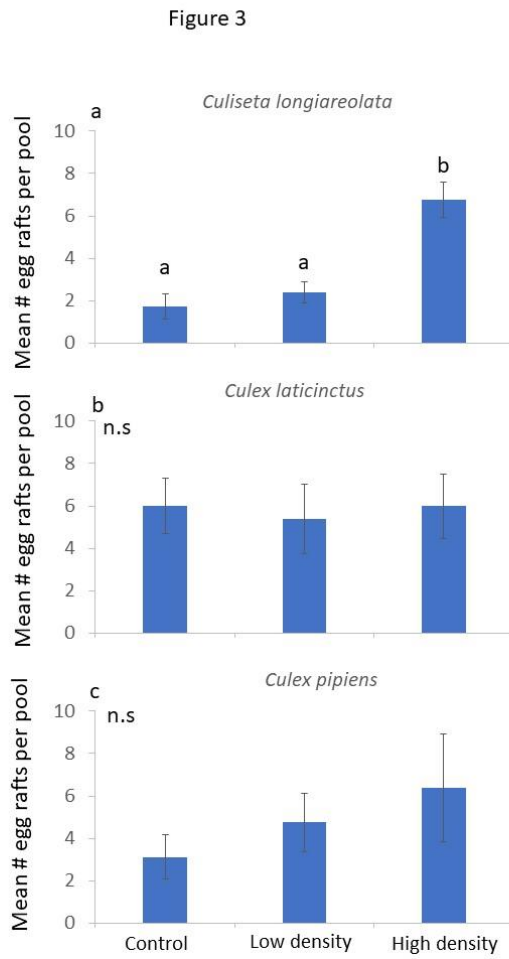
315 Figure 2 – Effect of *Culiseta logiareolata* larvae conditioned water on ovipositing a.  
316 *Culiseta logiareolata*; b. *Culex laticinctus*; and c. *Culex pipiens* oviposition, n=7 per  
317 treatment, error bars stand for  $\pm 1$  SE. Different letters indicate treatments that are  
318 significantly different based on post hoc comparisons. N.s., not significant.



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320

321 Figure 3 – Effect of *Culiseta logiareolata* larvae on ovipositing a. *Culiseta*  
322 *logiareolata*; b. *Culex laticinctus*; and c. *Culex pipiens* oviposition, n=8 per treatment,  
323 error bars stand for  $\pm 1$  SE. Different letters indicate treatments that are significantly  
324 different based on post hoc comparisons. N.s., not significant.



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