Measuring the host-seeking ability of *Aedes aegypti* destined for field release

Running head: Measuring *Aedes aegypti* host-seeking ability

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Abstract

Host-seeking is an essential process in mosquito reproduction. Field releases of modified mosquitoes for population transformation rely on successful host-seeking by female mosquitoes, but host-seeking ability is rarely tested in a realistic context. We tested the host-seeking ability of female *Aedes aegypti* mosquitoes using a semi-field system. Females with different *Wolbachia* infection types (*w*Mel-, *w*AlbB-infected and uninfected) or from different origins (laboratory and field) were released at one end of a semi-field cage and recaptured as they landed on human experimenters fifteen meters away. Mosquitoes from each population were then identified with molecular tools or through marking with a consistent weight of fluorescent powder. *Wolbachia*-infected and uninfected populations had similar average durations to landing and overall recapture proportions, as did laboratory and field-sourced *A. aegypti.* These results suggest that the host-seeking ability of mosquitoes is not negatively affected by *Wolbachia* infection or long-term laboratory maintenance. This method provides an approach to study the host-seeking ability of mosquitoes across a long distance which will be useful when evaluating strains of mosquitoes that are planned for releases into the field to suppress arbovirus transmission. An adjustment of this method may also be useful in sterile insect release programs because male host-seeking and swarming around female feeding sites can also be investigated.

Introduction

The management of arboviral diseases has become increasingly important to global health in recent decades.[1] The occurrence of arboviral diseases such as dengue, Zika, Japanese encephalitis and West Nile fever is increasing, especially in tropical and subtropical areas. [2,3,4] These viruses require blood-feeding mosquitoes to complete their life cycle,[5] with mosquitoes from the genera of *Culex* and *Aedes* being particularly important.[6] An effective way to control arbovirus transmission is to suppress the vector mosquito populations. Pesticides are widely used for this purpose but this can lead to the evolution of physiological resistance, alongside other undesirable effects associated with pesticide use.[7, 8] The sterile insect technique (SIT),[9] incompatible insect technique (IIT),[10] and the release of insects carrying a dominant lethal gene (RIDL)[11] are promising non-insecticidal alternatives, where wild-type females that mate with the released "modified" males have few viable offspring, decreasing the population size.

An alternative approach aims to decrease the ability of mosquitoes to transmit viruses by introducing endosymbiotic *Wolbachia* bacteria.[12, 13] *Wolbachia* are transmitted maternally and can invade natural populations through cytoplasmic incompatibility and any beneficial effects on host reproduction.[14, 15] When introduced into mosquitoes from other insects, some *Wolbachia* strains reduce their capacity to transmit viruses.[12, 16] *Aedes aegypti* infected with the *w*Mel *Wolbachia* strain have been introduced into field populations, with the first releases taking place in Cairns, Australia in 2011.[17] In locations in Australia where *Wolbachia* have established there have been no confirmed locally-transmitted cases of dengue occurring within the release areas.[18, 19]

Population replacement and suppression strategies ideally should be preceded by investigations to assess their potential for success, address safety concerns,[20] and perform community engagement.[18, 21] When using *Wolbachia* to block arbovirus transmission, fitness costs imposed on their hosts such as adult life-shortening,[22] reduced quiescent egg viability,[23] and reduced starvation resistance of larvae[24] must be considered. Such effects mean that *Wolbachia* must exceed a threshold frequency in order to spread in natural populations.[17, 25, 26] SIT, IIT and RIDL programs are simpler in that the only concern is male fitness, but still require the released males to have a high competitiveness to ensure successful mating with wild females.[27] Populations reared in the laboratory can adapt to the artificial conditions which may reduce field performance.[28, 29] For instance, laboratory maintenance can lead to the loss of pesticide resistance,[30] greatly reducing fitness in release areas with heavy pesticide use.[21]

Fitness assays are usually carried out in the laboratory to detect fitness costs, but during releases mosquitoes must locate hosts or mates under variable environmental conditions. Performance under laboratory conditions often does not translate to performance in the field.[31, 32, 33] Males from the transgenic OX3604C strain of *A. aegypti* successfully suppressed laboratory populations[34] but were much less effective under semi-field conditions due to a strong mating disadvantage.[35] For *Wolbachia* releases, density-dependent effects,[25] loss of cytoplasmic incompatibility,[36] and incomplete maternal transmission[37] may account for the slower-than-expected spatial spread of infections in natural populations[37, 38] or even failed establishment[39] despite success under more controlled conditions.[12]

Successful host-seeking is key to population replacement programs since female mosquitoes require blood for reproduction. Females locate a potential blood source by tracking exhaled CO_2 over tens of meters, then approach and land on the host by detecting thermal plumes, host odors, moisture and visual contrast.[40, 41, 42] *Wolbachia* infections do not affect the attraction of *A. aegypti* to human odors in the laboratory,[43] but successful host-seeking in the field will depend on the detection of olfactory cues from a long distance, visual and temperature signals from a shorter distance and flight ability.

In this paper, we tested the host-seeking ability of female *A. aegypti* using a semi-field cage in North Queensland, Australia[44] to simulate an outdoor setting. Females were released at one end of the semi-field cage and then recaptured by two experimenters seated at the other end. This method allows for a direct comparison of host-seeking ability between different mosquito strains in a common environment. To test the method we compared mosquitoes with the *w*Mel and *w*AlbB *Wolbachia* strains, which are now being released into the field in disease control programs,[17] (Nazni et al., unpublished data) against uninfected counterparts. To evaluate whether laboratory adaptation could affect host-seeking as demonstrated in laboratory experiments previously,[45] we also compared a laboratory population to a population collected recently from the field.

Material and Methods

Mosquito strains and maintenance

120 *A. aegypti* mosquitoes in this study were reared at 26-28°C in a controlled temperature
121 room at James Cook University, Cairns, using methods described previously.[46] We
122 performed two sets of experiments to compare the effects of *Wolbachia* infection and
123 laboratory maintenance on host-seeking ability respectively. To test for the effects of
124 *Wolbachia* infection, we used uninfected, *w*Mel-infected and *w*AlbB-infected *A. aegypti*
125 with a similar genetic background. Populations infected with *w*Mel and *w*AlbB were derived
126 from lines transinfected previously.[12, 47] The *w*Mel population was collected from Cairns,
127 Australia in May 2013 from regions that had been invaded two years earlier[17, 48] while the
128 *w*AlbB population was crossed to an Australian background and maintained in the
129 laboratory.[49] The uninfected population was established from *A. aegypti* (*Wolbachia*-
130 uninfected) eggs collected in Cairns, Queensland, Australia in November 2015.[50] Females
131 from all *Wolbachia*-infected lines were backcrossed for three generations to the uninfected
132 males to ensure a similar genetic background before the experiments.[23] To test for the
133 effects of laboratory maintenance we compared the host-seeking ability of laboratory and
134 field populations. The laboratory population was identical to the uninfected population
135 described above and had been maintained in the laboratory for 27 generations. The field
136 population of *A. aegypti* (*Wolbachia*-uninfected) was collected in September 2018 from the
137 same location as the laboratory population and was a mix of the first and second laboratory
138 generations at the time of experiments.
139
140 For each release, the compared colonies were hatched synchronously, provided with
141 TetraMin® fish food tablets (Tetra, Melle, Germany) *ad libitum* and the larval density was
142 controlled to 150 in 1 L water to ensure matched eclosion. After pupation, approximately 80
143 pupae were selected with a mix of 80% females and 20% males and left to emerge as adults

144 in one cage (BugDorm-4M1515 Insect Rearing Cage). Each cage was provided with a cup of
145 10% sucrose and water and left for at least 4 d to ensure that females had matured and
146 mated, but not blood fed. One day before the release, sugar cups were removed with only
147 water cups remaining to starve the females, since sugar feeding may affect host-seeking
148 behavior.[51, 52] The released females were 5 d old in both the *Wolbachia* infection
149 comparison and the laboratory maintenance comparison.
150
151 **Release-recapture method**
152
153 We used a semi-field system (17.5 × 8.4 m) at James Cook University, Cairns, Australia
154 containing soil, vegetation, a "Queenslander" house structure (Qld) and a ventilation system
155 to match outside ambient temperatures to simulate natural conditions (Figure 1).[44]
156 Mosquitoes were released near the door side from a box with a mesh lid while two
157 experimenters were seated within the Qld structure to attract mosquitoes from the other
158 end (Figure 1). Two temperature loggers (Thermochron; 1-Wire, iButton.com, Dallas
159 Semiconductors, Sunnyvale, CA, USA) were placed near the release point and two were
160 placed under the Qld structure to monitor temperatures during experiments
161 (Supplementary Table 1).
162
163
164 **Figure 1.** Interior of the semi-field cage. (a) View of the door from inside the Qld. (b) View of
165 the Qld from the door. (c) Schematic diagram of the cage showing the release point and the
166 location of two experimenters.
167

168

169 Females from all populations in the comparison were aspirated into a single release box
170 (Supplementary Figure 1) and placed in the semi-field cage to acclimate for at least 30
171 minutes before experiments commenced. For the *Wolbachia* infection comparison, 50
172 uninfected, 50 *w*Mel-infected and 50 *w*AlbB-infected females were released into the box.
173 For the laboratory maintenance comparison, 50 laboratory and 50 field source females were
174 released. Females that were damaged during handling were replaced.

175

176 Two experimenters wore bug net mesh hats, long-sleeved shirts and shorts, exposing only
177 their lower legs to restrict the area where mosquitoes could land. The same two
178 experimenters undertook all experiments. Experimenters sat on the floor within the Qld
179 structure, 1 m apart (Figure 1) with an electronic timer, mechanical aspirators (Model
180 2809C, BioQuip Products, Inc, Rancho Dominguez, CA, USA) and 15 collection vials nearby.
181 The experiment commenced by pulling the fishing line to remove the mesh lid from the box
182 to release the mosquitoes (Supplementary Figure 1), after which the timer was immediately
183 started. Females landing on exposed skin were collected with mechanical aspirators as they
184 landed. Collection vials were replaced with empty vials at 3-minute intervals until 42
185 minutes had elapsed. After 42 minutes, both experimenters moved to the opposite end of
186 the cage to capture mosquitoes that did not land during the experiment. Collections
187 occurred until no more mosquitoes were detected after a thorough search of the semi-field
188 cage. Between experiments, two Biogents Sentinel (BGS) traps (Biogents AG, Regensburg,
189 Germany) were placed inside the semi-field cage to assist in the capture of any remaining
190 mosquitoes. At least one hour before each experiment commenced, the experimenters

191 searched the semi-field cage and used an electric mosquito swatter to kill any mosquitoes
192 found.
193
194
195 *Wolbachia infection comparison*
196
197 The host-seeking experiment was repeated seven times with 50 uninfected, 50 *w*Mel-
198 infected and 50 *w*AlbB-infected females. Females collected from each replicate and time
199 interval were stored in absolute ethanol at 4°C for wing length measurements, DNA
200 extraction and *Wolbachia* screening. One replicate was discarded from other analyses due
201 to the loss of samples during wing dissection.
202
203 Field-collected *A. aegypti* are smaller and more variable in size than laboratory-reared *A.*
204 *aegypti*.[53] Since host-seeking females collected from the field in a previous experiment
205 tended to be larger than non-host-seeking females,[54] we tested whether host-seeking speed
206 and successful host seeking within 42 minutes was affected by size. We measured the wing
207 length of females from two experimental replicates to obtain an indication of their body
208 size.[55] Intact wings were dissected from individual females and fixed under a 10 mm circular
209 coverslip (Menzel-Gläser, Braunschweig, Germany) using Hoyer's solution[56] for further
210 observation and measurement with an NIS Elements BR imaging microscope (Nikon
211 Instruments, Japan).[24]
212
213 DNA extraction and *Wolbachia* screening were conducted according to the methods of Lee,
214 et al. [57] DNA from whole mosquitoes was extracted using 200 µL of 5% Chelex 100 Resin

(Bio-Rad Laboratories, Hercules, CA) and 3 µL of Proteinase K (20 mg/ mL, Bioline Australia Pty Ltd, Alexandria NSW, Australia). Extractions were diluted by 1/10, pipetted into four positions of a 384-well plate and amplified with mosquito-specific (*mRpS6*) primers, *A. aegypti*-specific (*aRpS6*) primers, *Wolbachia* *w*Mel-specific (*w1*) primers and *Wolbachia* *w*AlbB-specific (*wAlbB*) primers[48, 49, 58, 59] using a LightCycler 480 system (Roche Applied Science, Indianapolis, IN, USA). Robust and similar amplification of *mRpS6* and *aRpS6* (within one cycle) was expected for each individual. Uninfected *A. aegypti* were expected to show no amplification and therefore, no crossing point (Cp) value, with both *w1* and *wAlbB* primers. *A. aegypti* were classified as *w*Mel-infected when they exhibited no amplification with *wAlbB* primers and low Cp values (< 28) and a Tm within the expected range for *w1* primers based on *w*Mel-infected laboratory controls. *w*AlbB-infected *A. aegypti* tested positive for *wAlbB, mRpS6* and *aRpS6* primers but also showed late amplification (Cp > 28) with *w1* primers. Individuals were therefore classified as wAlbB-infected when they exhibited a low Cp value (< 28) with *wAlbB* primers, a Tm within the expected range for *wAlbB* primers and an amplification curve shape consistent with *w*AlbB-infected laboratory control values (Supplementary Figure 2). At least two consistent technical replicates were obtained for each individual.

Laboratory maintenance comparison

In this experiment, laboratory and field populations were marked with different colors of fluorescent powder (DayGlo, Barnes Products Pty Ltd, Moorebank, NSW, Australia) before release since the two populations could not be distinguished by molecular assays. Orange, blue and yellow colors were used and were cycled between replicates. To reduce potential

negative effects of marking, we used a minimal, but visually identifiable amount (Supplementary Figure 3) by weighing powder on a microbalance (Sartorius BP 210 D). One hour before the release, 50 females from each population were aspirated into two separate 70-mL specimen cups containing approximately 0.4 mg of fluorescent powder in different colors. The cups were shaken gently to coat the mosquitoes evenly in powder before placing them in the release box (Supplementary Figure 1). Recaptured females were killed by freezing at -20 °C for 30 minutes and identified under a microscope using a UV flashlight. This experiment was repeated six times.

Data analyses

Data visualization and ANOVA analyses were conducted using R studio with the packages Rmisc,[60] plyr,[61] and ggplot2.[62] Mosquitoes were captured at three-minute intervals and assigned a value based on the median time of each catching interval for average landing time calculations. Mosquitoes caught after 42 minutes were considered as not landing. A two-way ANOVA analyzed differences in average landing time of the landed mosquitoes and the number of females that landed by treating population as a fixed factor and experimental replicate as a random factor. One-way ANOVA was used to compare the wing length of mosquitoes caught at different intervals by treating landing time as a factor. Cumulative landing proportions over time were analyzed with log-rank tests in IBM SPSS Statistics version 25 by combining replicate experiments together.

Results

263

264 *Wolbachia infection comparison*

265

266 We compared the host-seeking ability of uninfected, *w*Mel-infected and *w*AlbB-infected
267 females when released simultaneously in a semi-field cage. On average, more than 30% of
268 the mosquitoes were captured during the first three minutes of the experiment, with
269 approximately 70% landing over the course of 42 minutes (Figure 2A). We compared the
270 cumulative landing proportions of each population when combined across replicates and
271 found no significant differences between *Wolbachia*-infected and uninfected females (log-
272 rank: *w*Mel : uninfected: $\chi^2 = 1.428$, df = 1, P = 0.232; *w*AlbB : uninfected: $\chi^2 = 2.9$, df = 1, P
273 = 0.089, Figure 3A).

274

275 The average time to landing of each population was used as an estimate of host-seeking
276 speed (Figure 2b, 2d). Average time to landing did not differ significantly between
277 uninfected (mean ± SE: 9.5 ± 0.9 minutes), *w*Mel-infected (7.6 ± 0.6 minutes) and *w*AlbB-
278 infected (7.5 ± 0.3 minutes) females (two-way ANOVA: *w*Mel : uninfected: $F_{1,5} = 2.503$, P =
279 0.174; *w*AlbB : uninfected: $F_{1,5} = 6.434$, P = 0.052). There was also no significant effect of
280 replicate on average time to landing in either comparison (*w*Mel : uninfected: $F_{5,5} = 0.617$, P
281 = 0.696; *w*AlbB : uninfected: $F_{5,5} = 2.009$, P = 0.231). We compared the total proportion of
282 females landing as an indicator of overall host-seeking success (Figure 2c, 2e); here there
283 were also no significant differences between populations (*w*Mel : uninfected: $F_{1,5} = 0.282$, P
284 = 0.618, *w*AlbB : uninfected: $F_{1,5} = 2.426$,P = 0.180). There was a significant effect of
285 replicate in the *w*AlbB comparison ($F_{5,5} = 7.505$, P = 0.023) but not in the *w*Mel comparison
286 ($F_{5,5} = 3.482$, P = 0.099).

Figure 2. Host-seeking ability of 5 d old *w*Mel-infected, *w*AlbB-infected and uninfected *A. aegypti* females in a semi-field cage. (a) Cumulative landing proportions of females on human experimenters across all replicates. Lines represent means and error bars represent standard errors. (b-c) Comparisons of average time to landing (b) and proportion landing (c) between uninfected and *w*Mel-infected females, plotted separately for each replicate. (d-e) Comparisons of average time to landing (d) and proportion landing (e) between uninfected and *w*AlbB-infected females, plotted separately for each replicate.

Females from two replicates of the *Wolbachia* infection comparison were measured for wing length (Figure 3). There was no significant effect of wing length on host-seeking speed, measured by capture interval ($F_{14,251} = 0.708$, $P = 0.766$). Females landing within the first three minutes (2.81 ± 0.02 mm, $n = 102$) did not differ in size from females collected after 42 minutes had elapsed (2.80 ± 0.03 mm, $n = 57$), suggesting no difference in size between fast host-seeking females and non-host-seekers ($F_{1,80} = 1.311$, $P = 0.256$).

Figure 3. Wing lengths of female *A. aegypti* collected during two replicates of the *Wolbachia* infection host-seeking experiment. Points represent wing lengths of individual females collected across each 3-minute interval of the experiment. Wing lengths of females captured after 42 minutes had elapsed were also included.

311

312 *Laboratory adaptation comparison*

313

314 In comparisons of laboratory and field *A. aegypti* females, approximately 60% of the
315 released mosquitoes were caught over the duration of the experiments. Cumulative landing
316 proportions did not differ significantly between field and laboratory populations when
317 combined across replicates (log-rank: $\chi^2 = 2.275$, df = 1, P = 0.131, Figure 4a). The average
318 time to landing did not differ significantly between field (mean ± SE: 12.3 ± 1.1 minutes) and
319 laboratory (10.5 ± 1.3 minutes) females (two-way ANOVA: $F_{1,5} = 2.346$, P = 0.186, Figure 4b),
320 with no significant effect of replicate ($F_{5,5} = 2.876$, P = 0.136). Furthermore, the total
321 proportion of females landing did not differ between field and laboratory females ($F_{1,5}$ =
322 0.745, P = 0.428, Figure 4c), with no significant effect of replicate ($F_{5,5} = 4.647$, P = 0.059),
323 suggesting that laboratory maintenance does not affect host-seeking ability.

324

325

326 **Figure 4**. Host-seeking ability of field and laboratory *A. aegypti* females in a semi-field cage.
327 (a) Cumulative landing proportions of females on human experimenters across all replicates.
328 Lines represent means and error bars represent standard errors. (b-c) Comparisons of
329 average time to landing (b) and proportion landing (c) between field and laboratory females,
330 plotted separately for each replicate.

331

332

333

334 **Discussion**

Suppressing the transmission of dengue and other arboviruses by releasing *Wolbachia*-infected mosquitoes is becoming increasingly popular, with releases taking place in at least 12 countries (https://www.worldmosquitoprogram.org/; https://www.nea.gov.sg/corporate-functions/resources/research/wolbachia-aedes-mosquito-suppression-strategy/project-wolbachia-singapore; https://www.imr.gov.my/wolbachia/). For releases to succeed, the strain intended for deployment needs to have comparable fitness to wild-type mosquitoes, which should be tested prior to large-scale field release. The semi-field cage setting is widely used as an intermediate step between laboratory studies and open field releases. [63, 64, 65] Semi-field experiments have been used to test the mating success and invasive ability of *Wolbachia* infections[12, 66, 67] and for evaluating the efficacy of novel mosquito traps and pesticides.[65, 68, 69] But while host-seeking is critical for the success of *Wolbachia* replacement programs, the strains used in field releases including *w*Mel and *w*AlbB have not been evaluated for their effects on host-seeking ability in a realistic way.

We compared the host-seeking ability of female *A. aegypti* with different *Wolbachia* infection types and from laboratory and field origins in a semi-field cage. Our method was similar to the method developed by McMeniman, et al. [70] In their study, the host-seeking ability of wild-type and *Gr3* mutant females lacking a response to CO_2 was compared by releasing mosquitoes in the middle of the cage and leaving them to disperse naturally for 5 hours before the experiment. In our design, female mosquitoes were released simultaneously at a single release point fifteen meters away from the experimenters, thus standardizing the distance over which host-seeking is tested and allowing mosquitoes to

359 combine their flight ability with the detection of olfactory, visual and thermal queues to
360 locate and land on experimenters. This is the first time that a semi-field approach has been
361 used to evaluate the host-seeking ability of mosquitoes with *Wolbachia* strains intended for
362 field deployment.
363
364 We found no significant differences between *A. aegypti* with different *Wolbachia* infection
365 types on host-seeking ability in our experiments. Females with the *w*Mel and *w*AlbB strains
366 should therefore not be at a disadvantage in terms of host-seeking if released into the field.
367 Although a study with a Puerto Rican *A. aegypti* population indicated that laboratory
368 maintenance altered attraction to human odors,[45] no significant differences were found in
369 overall host-seeking between laboratory and field populations in our semi-field
370 experiments. Therefore, our laboratory maintenance protocol[46] should not lead to
371 compromised host-seeking ability in the field, though other factors that can coincide with
372 laboratory maintenance such as inbreeding may reduce fitness.[71] Different rearing
373 procedures, such as the use of membrane feeders, non-human blood or small cages may
374 also affect host-seeking ability if adaptation occurs.
375
376 In the absence of molecular tools, visual marking is needed to distinguish between
377 populations in the same experiment. However, overapplication of powder may affect
378 longevity and behavioral responses, with effects depending on the method and the color
379 used for marking.[72, 73, 74] Although the two sets of experiments were conducted at different
380 times, no significant differences were found between marked and unmarked uninfected
381 laboratory females in terms of average arrival time (One-way ANOVA: $F_{1,10} = 0.388$, P =

382 0.547) and proportion landing ($F_{1,10}$ = 0.387, P = 0.548), which suggests that the minimal
383 amount of fluorescent powder used for marking does not affect host-seeking ability.
384
385 We also ran experiments with *w*Mel, *w*AlbB-infected and uninfected *A. aegypti* females that
386 were 20 d old and found no significant differences between populations (Supplementary
387 Figure 4). Although mosquitoes of different ages were not compared in the same
388 experiment, we found that 20 d old females had slower average times to landing (Two-way
389 ANOVA: ages: $F_{1,24}$ = 8.567, P = 0.007, colonies: $F_{2,24}$ = 1.407, P = 0.264) but higher landing
390 proportions (ages: $F_{1,24}$ = 5.802, P = 0.024, colonies: $F_{2,24}$ = 0.461, P = 0.636) compared to 5 d
391 old females by treating mosquito age and colony as fixed factors. This suggests that host-
392 seeking ability may be influenced by mosquito age, but direct comparisons between ages in
393 the same experiment are needed to confirm this finding.
394
395 Many factors can influence mosquito attraction to humans including environmental
396 temperature and humidity, in addition to the CO_2, skin emanations, body heat and moisture
397 of the host.[75, 76, 77] While all mosquitoes in each experiment were reared under the same
398 conditions and were a similar age, we observed substantial differences in average times to
399 landing and landing proportions between replicates (Supplementary Tables 2 and 3). We
400 found no effect of temperature or the time of releases (Supplementary Table 1) according
401 to Spearman's rank correlation (P > 0.05), suggesting that temperature and time of day did
402 not substantially influence host-seeking. We also ran a power analysis using an online
403 calculator (http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-
404 Equality) with a 80% power test using the average times and standard deviations of
405 *Wolbachia*-infected and uninfected colonies. For 20% differences in our studies (7.5 minutes

406 for *w*Mel or *w*AlbB-infected *A. aegypti* while 9.5 minutes for uninfected *A. aegypti*), at least
407 15 replicates are needed to detect an effect, while a difference of 30% could be detected
408 with six replicates.
409
410 In addition to studying the host-seeking ability of females, it may be possible to extend this
411 method to male mosquitos. For SIT, IIT and RIDL approaches, testing the competitiveness of
412 males before the release is essential.[27] A previous semi-field cage study showed that
413 *Wolbachia* infection does not reduce the competitiveness of *A. aegypti* males.[67] However, in
414 nature, adult female densities will not be as high as in semi-field cage tests; males will
415 typically locate and fly around a human host first before detecting female flight tones and
416 initiating courtship behaviour.[78, 79, 80] In a pilot experiment where we released males into the
417 semi-field cage, we found that *A. aegypti* males exhibited a similar host-seeking response to
418 females (Supplementary Figure 5), but this requires further testing.
419
420 In conclusion, we have developed a method to test the host-seeking ability of female *A.*
421 *aegypti* populations under semi-field conditions. While changes in host-seeking behavior
422 due to *Wolbachia* infections and laboratory adaptation are apparent from some laboratory
423 studies, it is important to test host-seeking in a way that reflects natural conditions.
424 Comparisons of host-seeking ability using this approach will be informative when evaluating
425 mosquito strains for field release. This method can also be used to compare other factors
426 such as age and rearing conditions which can help to better understand the host-seeking
427 behavior of female mosquitoes.
428
429 **Acknowledgements**

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Disclosures

The authors declare that no conflicts of interest exist.

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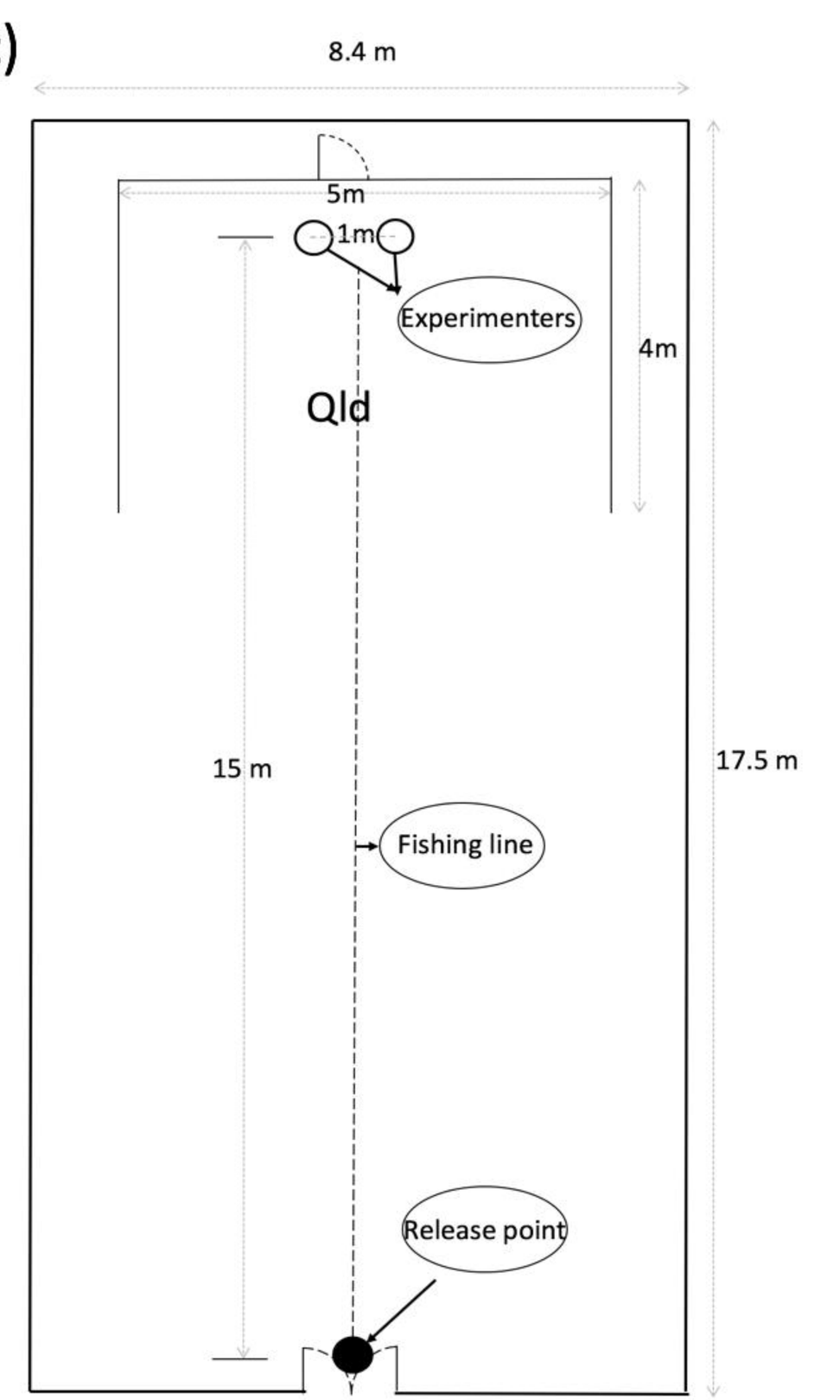
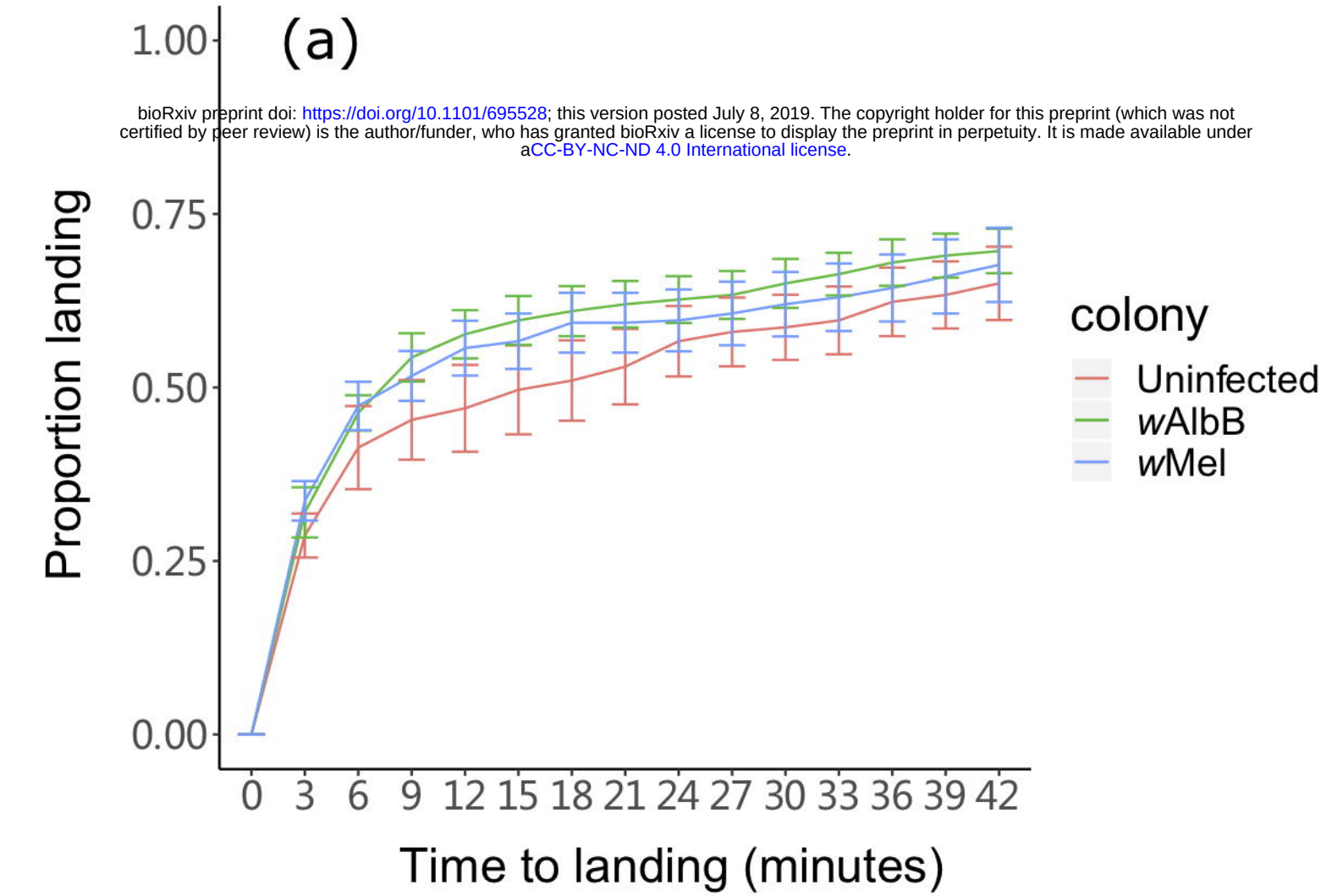
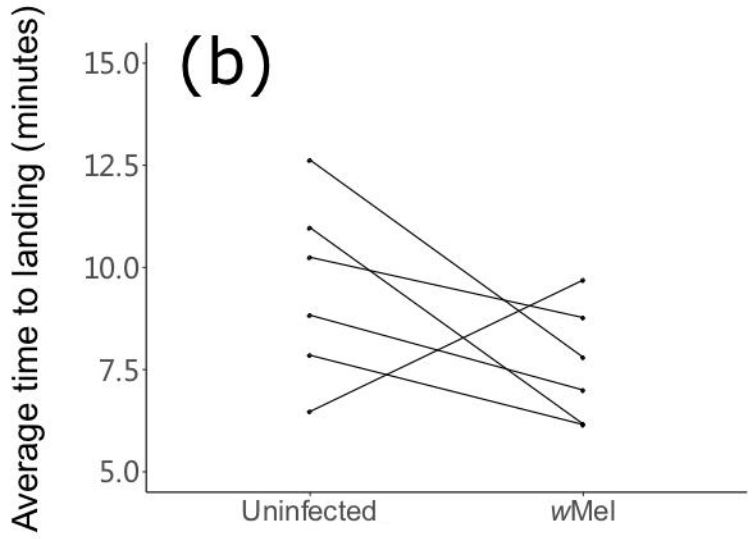
(a)

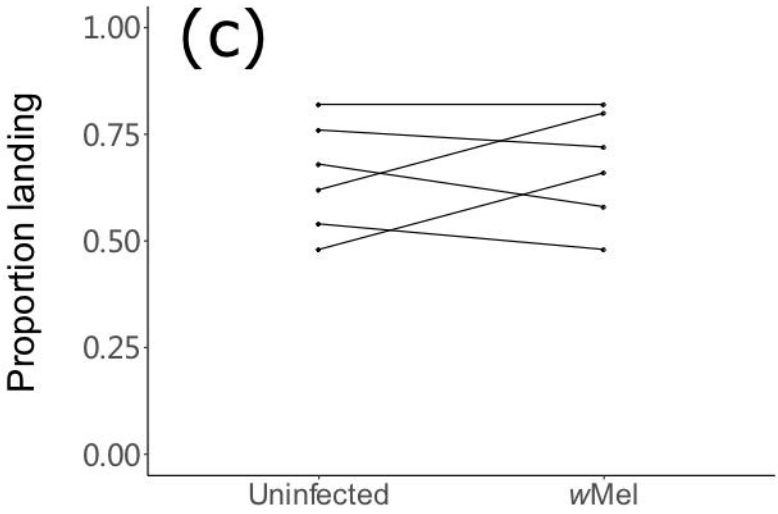
(b)

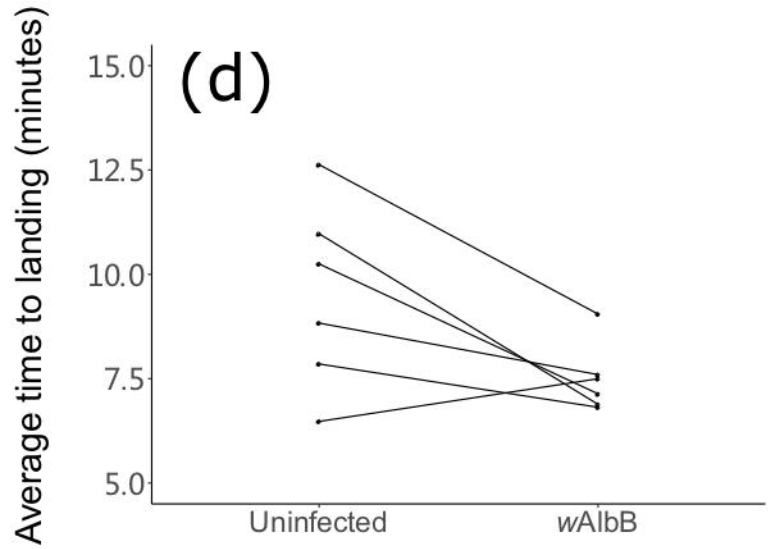
(c)

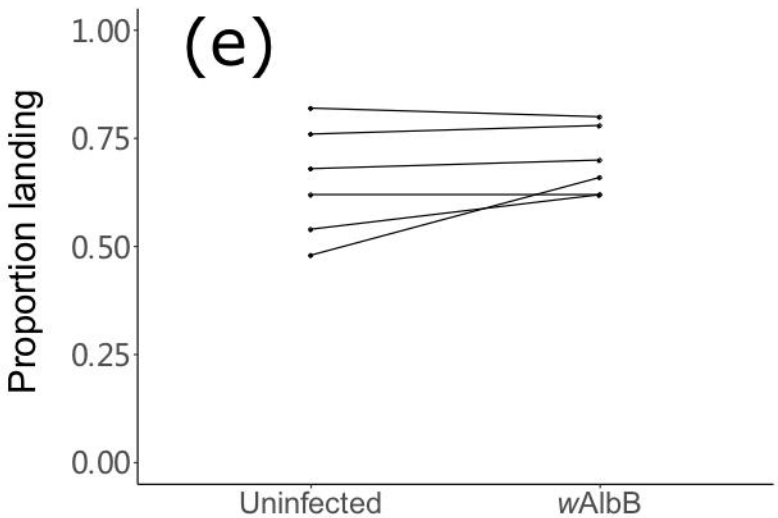


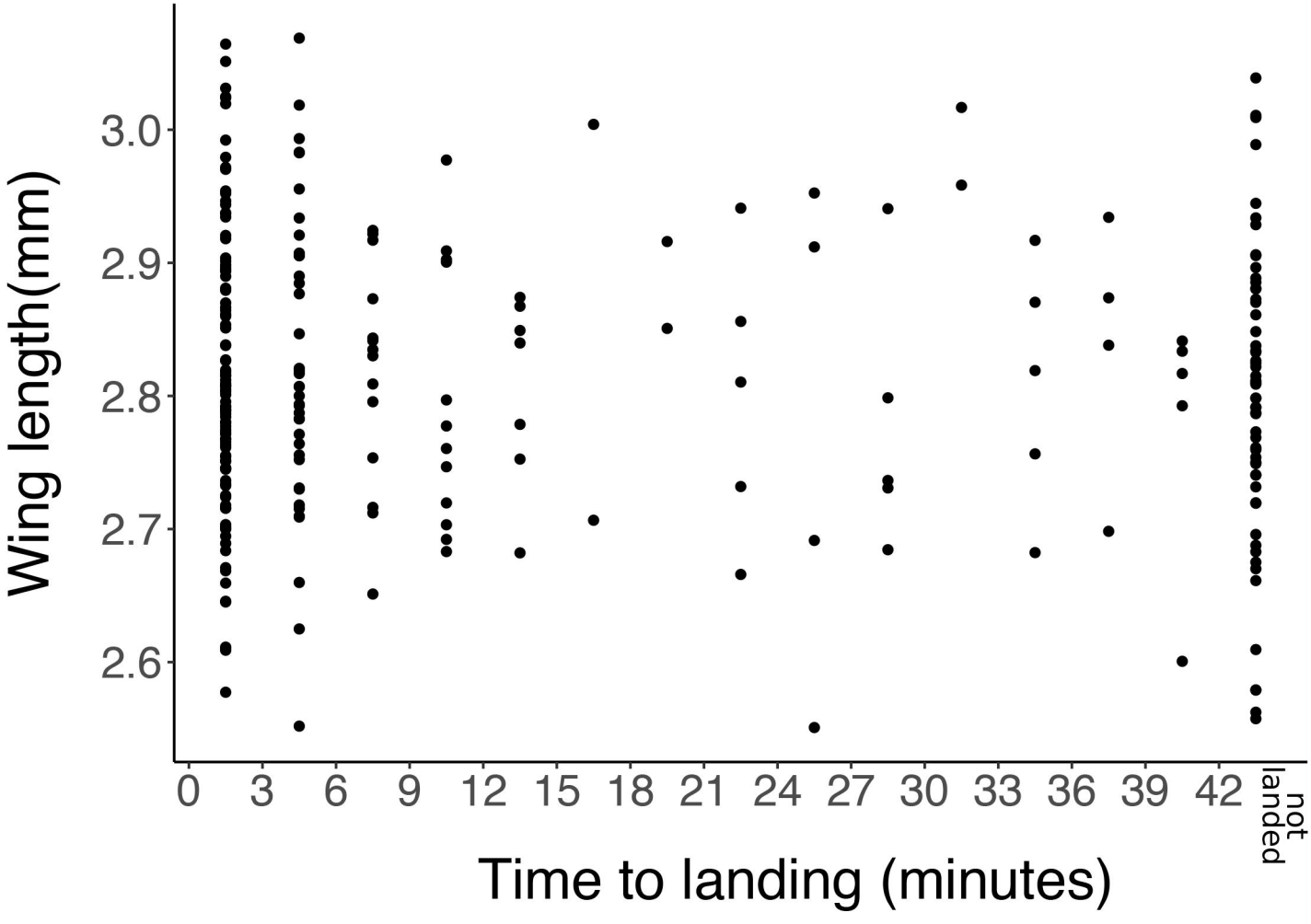











Wing length(mm)
3.0
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2.8
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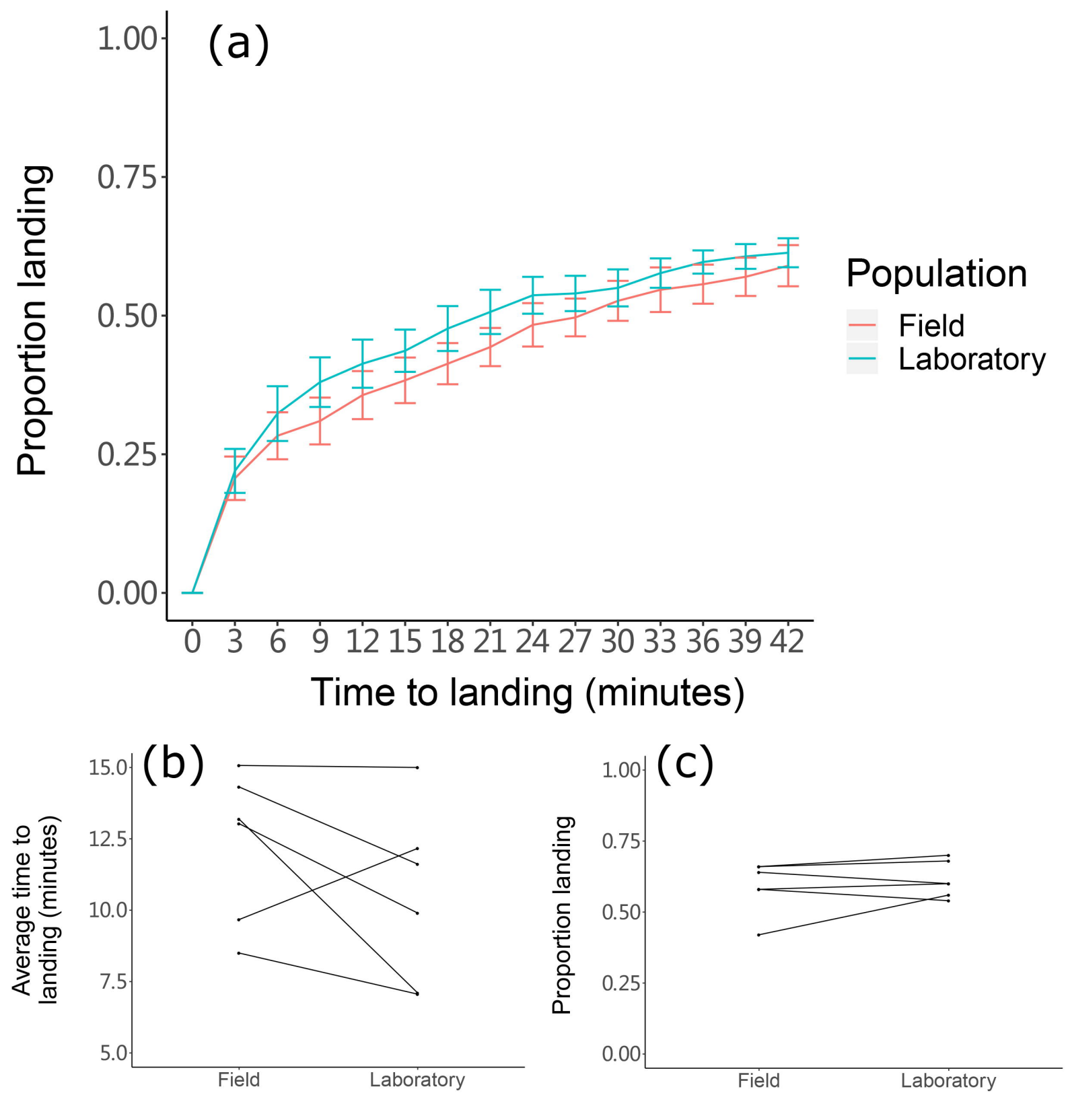
42
not landed
Time to landing (minutes)


(a)
Proportion landing
1.00
0.75
0.50
0.25
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0 3 6 9 12 15 18 21 24 27 30 33 36 39 42
Time to landing (minutes)
Population
Field
Laboratory
(b)
Average time to landing (minutes)
15.0
12.5
10.0
7.5
5.0
Field
Laboratory
(c)
Proportion landing
1.00
0.75
0.50
0.25
0.00
Field
Laboratory