Manipulation of the social and spatial organization of the vector of Chagas disease, *Triatoma infestans*, by the parasite *Trypanosoma cruzi*

Short title: Behavioral manipulation of Chagas disease vector by the parasite

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Abstract

Insects of Triatominae subfamily are vectors of the parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease. Some species of Triatominae, like *Triatoma infestans* in the Southern Cone of South America, live in the human neighborhood, aggregating in wall or roof cracks during the day and going out to feed on animal or human blood at night. Except for their feeding specialization, these insects share this cycle of activities where social interaction alternate with mainly individual foraging activity with many other arthropods species. The understanding of mechanisms leading to group cohesion is essential for understanding the spatial organization of the insects and the parasite dispersion. Experiments with adults of *T. infestans* were conducted to study how sex and infection by *T. cruzi* affect their aggregation and geotaxis behavior. Thanks to video recordings, lasting 150 min for each experiment, the position of the insects was recorded. We show that the insects presented a high negative geotaxis and aggregative behavior. We demonstrate that males have a higher negative geotaxis and a higher aggregation level than females and that the infection by *T. cruzi* strengthens both behaviors. The aggregation level and the negative geotaxis were stronger in infected insects than in non-infected ones, the difference between sexes being maintained. The question of the influence of the geotaxis on the bug social behavior is also asked, as all the hitherto published works about aggregation were done in horizontal devices. The importance of these results is discussed in term of parasitic manipulation, dispersion of the vector and strategy of its monitoring.

Author summary

Chagas disease, one of the major neglected diseases in Latin America, is caused by a parasite transmitted by feces of hematophagous insects which live in or near the human dwellings, resting in wall cracks during the day, and searching for food at night. Spatial organization of these insects is not known. Thus, we conducted experiments to analyze aggregation (the act of gathering) and geotaxis (move according to gravity) in adult insects, and to study the influence of the parasite on these two behaviors. We demonstrate that males exhibit a higher aggregation and a stronger negative geotaxis

(moving upwards) than females and that the infection strengthens both behaviors in both sexes. These results suggesting a non-homogeneous distribution of individuals on dwellings' walls are epidemiologically important for the control and the monitoring of insects. They also open a new line of research to study how these behaviors are influenced by factors like the heterogeneities of the environment (presence of shelters), or the variability between individuals (different individual stages together, and presence of infection).

Keywords:
Aggregation, gregariousness, Triatominae, individual behavior, geotaxis, sex differences, parasitic manipulation, Chagas disease, *Triatoma infestans, Trypanosoma cruzi*, South America, Bolivia

Introduction

Chagas disease is one of the most important neglected tropical diseases with 6-7 million people who are estimated to be infected, and 20% of the population who are at risk [1–6]. This vector-borne disease is caused by the parasite *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) and is transmitted by contact with infected feces/ urine of hematophagous insects of the Triatominae subfamily (Hemiptera: Reduviidae). Currently, 149 extant species have been described worldwide, and all of them are considered as potential vectors [7]. Most of them live in sylvatic habitats, and only a dozen of species are regarded as vectors of major epidemiological importance due to their capacity to live in the surrounding of the human dwellings where they find stable shelters and food abundance (8, 9,10). *Triatoma infestans*, the subject of our study, is the main vector in the Southern Cone of South America [9]. Except for their feeding specialization, the domiciliary species share similar lifestyle and cycle of activities with many gregarious arthropods including other anthropic species like cockroaches [8,10–13]. During the daytime, they assembled in dark and sheltered places such as cracks in the walls or roof, or behind objects hanging on walls. At night, they leave their shelter to actively seek a host upon which to feed and then, they come back to a resting place to digest. The digestive phase can last from some days to several weeks according to the blood meal size, the individual and the environmental conditions [8,14].

The control strategy for Chagas disease relies mainly on the control of the domestic vectors through chemical control [1]. Face to the increase of the insecticide resistance exhibited by these insects, and to the reinvasion of dwellings by residual or sylvatic population of triatomines [15–18]; it is necessary to study the behaviors leading to a better understanding of their distribution and their dispersion. In this perspective, aggregation and geotaxis are key behaviors; knowing them better and understanding how the parasite dispersion may influence them is fundamental. Indeed, aggregation is a widespread behavior [19–23] that results from a response of individuals to environmental heterogeneity, and from social interactions involving attractions between individuals [24–30]. The social interactions maintain the group cohesion and the associated adaptive values of group living

(help in the reproduction [31,32], defense [23,33], and feeding [32,34]). In triatomines, protection against predation is usually evoked as the main benefice of clustering, but surviving might also be enhanced thanks to protection against hydric loss, and to a higher probability of coprophagy, symbiont exchange, and of sex encounters, as it was showed for other insects [35–39]. Aggregation in triatomines was studied with a focus towards the substances that mediate it, and on the factors that modulate the aggregative response [40–48]. All these works analyzed nymphal instars behavior response; in adults very few is known except that they can aggregate around feces [47]. Geotaxis, also called gravitaxis, is a crucial behavior involved in insect orientation [49]. Animals can exhibit locomotion that is gravitationally directed vertically down or up (positive or negative geotaxis, respectively). Almost nothing is known about triatomine geotaxis, except that *T. infestans* tend to be more concentrated in the upper half of the walls in houses or chicken houses [36,50]. Moreover, to our knowledge, no studies were conducted to analyze the synergy or conflict between gregariousness and geotaxis of triatomines.

It is well-known that parasites can modify physiological, behavioral, and/or morphological traits of their hosts to increase their fitness, even if it is at the cost of the host fitness [51]. This usually means that infected hosts will behave in ways that facilitate the transmission of the parasite [52,53]. Literature about the effects and a possible manipulation of triatomines behavior by *T. cruzi* is relatively sparse, covering only six species: *Mepraia spinolai, Panstrongylus megistus, Rhodnius prolixus, Triatoma brasiliensis, Triatoma dimidiata* and *T. infestans*. Authors have been especially interested in the parasite's effects on four groups of the host's behavior: life-history trait, feeding, defecation, and dispersion/ locomotion. It seems that *T. cruzi* increases the development time and biting rate, and decreases the longevity and defecation time in *M. spinolai* [54,55], but no change was observed in *P. megistus* [56], *R. prolixus* [57], *T. dimidiata* [58], *T. infestans* [59], and almost no change in *T. brasiliensis* [60]. Reproduction was decreased by *T. cruzi* in *T. brasiliensis* [60]. The dispersion was higher in infected females of *T. dimidiata* than in non-infected females; no effect was found in males [61]. The locomotory activity of *R. prolixus* was decreased by infection: the total number of movements was 20% less than that observed in non-infected insects [62]. The time to find a

host for an infected *M. spinolai* was almost twice as fast as for a non-infected insect [55]. In conclusion, modification of the triatomine traits seems to be species-dependent, age-dependent, sex-dependent, and even environment/ physiology-dependent.

In this work, experiments were conducted to study aggregation and geotaxis in adults of *T. infestans* and to analyze the effect of the infection by *T. cruzi*. Our hypotheses, based on the literature, were that these two behaviors –gregariousness and geotaxis - are strongly intertwined and are increased in infected individuals. We show that both sexes exhibit a high clustering, and a high negative geotaxis, males demonstrating a higher response than females. Interestingly, the infection by *T. cruzi* strengthens both behaviors significantly in both sexes.

Methods

T. infestans specimens were collected in dwellings from Yacuiba Municipality, Department of Tarija, in the southern region of Bolivia. The infection rate of the insects was determined by analyzing drops of feces under a light microscope. Bugs were then separated into two groups infected/ non-infected. Insects were reared at 26±1°C, 60±15% RH, 12:12 night:dark cycle, in plastic pots containing a folded piece of paper. They were fed on hens once every two weeks. Four conditions were then studied: non-infected males, non-infected females, infected males and infected females (abbreviated as NegMal, NegFem, PosMal, and PosFem in Figures and Tables). Sixteen experiments were carried out with non-infected males and females respectively, and twelve and nine experiments with infected males and females respectively. Due to experimental problems, five experiments in both sexes using non-infected insects included some infected insects, with a proportion of the infected population being less than 20%. These experiments were kept and analyzed in the same way.

Setup and Methods

147 A glass aquarium was used (50 x 20 x 50 cm) to avoid escaping of *T. infestans* which is unable
148 to climb on glass walls. As Triatominae prefer to rest on a vertical surface, the insects were allowed to
149 climb on one of the vertical surfaces of this aquarium (50 x 50 cm) thanks to a paper sheet (43 x
150 44 cm) [36,50]. The glass setup was washed, and the paper changed at the end of each experiment. It
151 was illuminated by a centered 60W light bulb, placed at 50 cm behind the wall covered by the paper
152 sheet. The paper guaranteed a homogeneous illumination of the setup. A video camera placed in front
153 of the setup recorded the bug activity for 150 min. A 1 m high polystyrene wall surrounded the setup
154 to isolate it. Experiments were conducted in a quiet and dark room to avoid any disturbance.
155
156 At the beginning of the experiment, 10 bugs (8-10 days of starvation) were dropped on the
157 bottom of the setup. They explored their environment rapidly and climbed on the wall. From the
158 recordings, a snapshot was extracted at 1 min, 5 min and then every 5 min up to 150 min (31 snapshots
159 in total). A processing program allowed us to record the spatial position of the thorax of each bug on
160 each snapshot. With these spatial coordinates, the inter-individual distances were computed. As the
161 length of an adult bug is on average 2.5 cm, we fixed the maximum distance of aggregation between
162 two individuals to 4 cm due to a tactile (legs or antennae) or visual perception.
163

164 **Indexes and statistics**

165 Several indexes of position and aggregation were calculated using processing programs: 1) the
166 number of individuals on the paper sheet; 2) the number of aggregated individuals; 3) the number and
167 the size of the clusters; 4) the spatial stability of the individual (% of individuals that were found at
168 time t+1 in a circle of 10mm in radius centered on the coordinate of the insect at time t); 5) the spatial
169 stability of the biggest cluster (study of the distance between the centroid of the biggest cluster at time
170 t+1 and the centroid of the biggest cluster at time t). Finally, the individual position of the insects in
171 the setup at the end of each experiment was analyzed, recording the vertical orientation of the bugs
172 (position 0: head towards the top), to put forward a privileged position. A vertical orientation was
173 defined as inside an angle of ±30° to the vertical, head oriented towards the top or the bottom. Outside
174 this range, the insect is not considered in a vertical position anymore. The structure of the clusters was

also compared between infected males and infected females, conditions where bigger clusters emerged. Each cluster was considered as an undirected network where each node was an individual. Links between nodes were established when the distance between them was less or equal to 4 cm (the threshold for considering aggregation). The cluster K-density (ratio of the number of edges divided by the number of possible edges) was compared for clusters with a size greater than three individuals.

The comparisons between conditions were made thanks to Anderson-Darling k-sample test [63]. In case of obtaining a $p<0.05$, an Anderson-Darling all-pairs comparison test was performed. These statistics were calculated thanks to the functions adKSampleTest() and adAllPairsTest() of the *PMCMRplus* package of R [64,65]. Circular statistics were carried out with Oriana 4.02 (Kovach Computing services). Uniformity of data was tested using Rao's test, mean comparisons using V-test, and distribution comparisons using Mardia-Watson-Wheeler pairwise test. The structure of the clusters was analyzed thanks to *igraph* package in R [66]. The linear regression was done thanks to lm() function, and the comparison of the slopes of regression thanks to *lsmeans* package of R, using Least-squared means (predicted marginal means) [67].

Results

Negative geotaxis: spatial distribution and total population

The bugs quickly climbed on the wall and stayed there, showing a high negative geotaxis; and after an exploratory phase, the insects began to cluster and rest (Fig 1). After 10 min, more than 80% of the individuals were on the wall (90% after 25 min) for the four conditions; and this proportion remained constant until the end of the experiment where no statistical difference was detected between the four conditions (Fig 2). The bugs were mostly located in the upper half of the setup; the median vertical position reached a plateau value (stationary state) after 15 min with a value greater than 35 cm for the four conditions (Fig 3). Their vertical distributions revealed a statistical difference between sexes, e.g., males were located higher than females, and also between non-infected and infected

individuals, e.g., infected males were higher than non-infected males (Fig 4). These trends were also found inside the top strip of 4 cm (40-44 cm), a zone corresponding to 10% of the total area of the setup and where 56% (80%) of the non-infected (infected) males and 33% (44%) of the non-infected (infected) females were located (Fig 5).

Fig 1. Examples of insect's positions at 150 min in the setup for the four conditions

Fig 2. Median fraction of bugs on the wall. Number of experiments: 16, 16, 9, 12 for NegFem (pink), NegMal (cyan), PosFem (red) and PosMal (blue) respectively. Inserted figure: boxplot distribution of bugs on the wall at 150 min. Anderson-Darling test at 150 min between the 4 conditions: TkN = -0.14, $p = 0.47$

Fig 3. Evolution of the median vertical position (quantiles 25%-75%) of the non-infected and infected insects on the wall during the experimental time. Median position at 150 min: NegFem (pink): 36.7 cm, PosFem (red): 39.0 cm, NegMal (cyan): 40.9 cm, PosMal (blue): 41.8 cm

Fig 4. Boxplot distribution of the vertical position of the individuals at 150 min. Number of observations: non-infected females: 145; non-infected males: 151; infected females: 90; infected males: 117. Anderson-Darling k-sample test between the four conditions for all individuals: TkN = 24.4, $p < 0.001$. Results of Anderson-Darling all-pairs comparison tests are shown at the top of the figure (conditions with different letters correspond to conditions statistically different at $p < 0.001$).

Fig 5. Boxplot distribution of the median fraction of individuals at the top of the setup (40-44cm) at 150 min. Number of observations: 16, 16, 9, 12 for NegFem, NegMal, PosFem, and PosMal respectively. Anderson-Darling k-sample test between the four conditions for all individuals: TkN = 8.3, $p < 0.001$. Results of Anderson-Darling all-pairs comparison tests are shown at the top of the figure (conditions with different letters correspond to conditions statistically different at $p < 0.03$)

At the end of the experiment, more than 80% of the individuals had a vertical orientation from which around 70-80% had the head turned towards the top of the setup (± 30°). The four conditions showed that individuals are not uniformly distributed (Rao's test < 0.01), but rather centered on 0 (V-tests < 0.001, Fig 6). When the distributions of individual orientations were compared, no difference appeared between sexes. Interestingly, the infection affected the orientation of the males which demonstrated a higher proportion of insects with the head towards the bottom when infected (Fig 6).

Fig 6. Vertical orientation of insects for the four conditions. Wedge's angles of 60°, frequencies are shown as radius of the wedge. 0° represents the head to the top. The dark line shows the mean direction and its length, and the standard deviation of the distribution. Number of observations: NegFem: 145, NegMal: 154, PosFem: 90 and PosMal: 117. Rao's tests gave $p < 0.01$ for the four conditions. V-tests (testing the null hypothesis of uniformity against non-uniform distribution with a mean of 0) gave $p < 0.001$ for the four conditions. Mardia-Watson-Wheeler pairwise tests between the four conditions: NegFem/ NegMal: $p = 0.271$, NegFem/ PosFem: $p = 0.156$, NegFem/ PosMal: $p = 0.001$, NegMal/ PosFem: $p = 0.015$, NegMal/ PosMal: $p = 0.003$, PosFem/ PosMal: $p = 0.049$

To summarize, without being infected, both sexes showed a high negative geotaxis that was higher for males than for females (Fig 4). Most of the insects of both sexes were oriented the head toward the top (Fig 6). The infection by *T. cruzi* strengthened this bug's geotaxis, especially for males.

Global Clustering

The median aggregated bug fraction increased up to reach a plateau around 30 min, gathering around 40% and 60% of non-infected and infected females respectively, and 70% and 90% of non-infected and infected males respectively (Fig 7). A statistical difference was observed at 150 min (end of the experiment) between sexes (males showed a higher aggregated fraction than females), and between infected conditions (infected bugs with a higher aggregated level than non-infected ones) (Figs 7 and 8). Insects in all conditions tended to gather in one or two clusters. The biggest cluster

assembled 40% (70%) of the aggregated non-infected (infected) population in males, and 30% (40%) of the aggregated non-infected (infected) population in females (Fig 7). No difference was observed between sexes ($p = 0.047$ and $p = 0.22$ for non-infected and infected bugs), neither between infection conditions (Fig 7). When the structure of the clusters are compared between infected sexes, clusters of infected males looked more compact, with a significantly smaller distance between aggregated individuals, and they also looked denser, with a higher K-density (Fig 9 and 10).

Fig 7. Dynamics of aggregation for the four conditions: NegFem (pink), NegMal (cyan), PosFem (red) and PosMal (blue). A: median fraction of aggregated individuals; B: median size of the biggest cluster; C: spatial stability of the biggest cluster. Anderson-Darling k-sample test at 150 min between the four conditions: (A) TkN = 7.4, $p < 0.001$, (B) TkN = 5.1, $p = 0.001$, and (C) TkN = -0.4, $p = 0.573$. Results of Anderson-Darling all-pairs comparison test are shown with different letters corresponding to conditions statistically different at $p < 0.05$: (A) NegFem: a, NegMal: b, PosFem: b, PosMal: c; (B) NegFem: a, NegMal:b, PosFem: ab, PosMal: b

Fig 8. Relative frequency of experiments according to the aggregated fraction of individuals for the four conditions

Fig 9. Structure of the clusters in infected conditions. Boxplot of the size of edges (or links) between aggregated bugs (left) and K-density of the clusters of size > 3 individuals (right). Anderson-Darling k-sample test for size of the edges: TkN = 9.1, $p < 0.001$ (72 and 255 observations for PosFem and PosMal respectively); and for the K-density: TkN = 4.7, $p = 0.004$ (17 and 21 observations for PosFem and PosMal respectively)

Fig 10. Example of clusters of 10 bugs in infected conditions. Nodes represent the insect positions on the wall. Links between nodes are present only when the distance is less or equal to 4 cm (the aggregative distance)

At the end of the experiments, individuals were very stable in space: the median fraction of individuals that moved less than 1 cm was greater than 60% for the four conditions, and no difference between sexes and infection condition was observed (Fig 11). The biggest cluster also showed strong spatial stability, with no statistical difference between the four conditions (Fig 7).

Fig 11. Boxplot of stable individuals (move < 10 mm between two snapshots) for the four conditions. Anderson-Darling k-sample test between the four conditions: TkN = 2.9, p = 0.01. Results of Anderson-Darling all-pairs comparison tests are shown at the top of the figure (conditions with different letters correspond to conditions statistically different at $p < 0.005$)

In order to verify that the fraction of aggregated individuals was not directly due to the method of calculating this fraction and the increase of the bugs density at the top of the setup, 20,000 repetitions of groups of N simulations were performed ($N = 16$ for NegFem and NegMal, $N = 9$ for PosFem, and $N = 12$ for MalPos). For each simulation, 10 points were vertically distributed following the experimental vertical distribution of the bugs at 150 min (Fig 12), and homogeneously horizontally distributed. For each group of simulations, the mean fraction of aggregated individuals was calculated for each repetition. The mean aggregated fractions found in the simulation were 0.26, 0.42, 0.36, and 0.72 for NegFem, NegMal, PosFem and PosMal respectively, revealing that an increase of the geotaxis leads to a rise in the observed aggregation level. However, the probability of observing a mean aggregated fraction higher or equal to the corresponding experimental one was $p < 0.0001$ for all the conditions, demonstrating that the observed phenomenon involves an active aggregation due to the inter-attraction between individuals.

Fig 12. Vertical distribution of insects on the wall for the four conditions. Fraction of bugs by horizontal strips of 5 mm, from 0 to 440 mm, corresponding to the height of the setup for non-infected (females: pink, males: cyan) and infected bugs (females: red, males: blue)

Fig 13 shows that the fraction of aggregated individuals in a strip of 0.5cm was proportional to the fraction of the population settled in this strip. The slope of the regression line was the lowest for the NegFem condition, and the highest for the PosMal condition, being intermediate and similar for the two other conditions. The slopes of the linear regression were compared computing a model including the interaction between the total number of bugs and the conditions: a significant interaction was found ($F_{3, 348} = 30.04$, $p < 0.001$), giving a slope equal to 0.61 for NegFem, 0.85 for NegMal, 0.84 for PosFem, and 0.92 for PosMal. The comparison of the slopes between the four conditions showed that NegFem's slope was lower than the three other conditions' slopes ($p < 0.001$), MalPos' slope was higher than the three other conditions' slopes ($p < 0.02$), and MalNeg's slope was not different from PosFem's slope ($p = 0.98$). These results demonstrate that, for the same density, the aggregation was higher for males than for females and for infected insects than for non-infected ones.

Fig 13. Fraction of aggregated individuals according to the population present in a horizontal strip of 5mm high for the four conditions. Linear regressions: NegFem (pink): $y = 0.6096\ x - 0.0019$ (SE of slope: 0.0294), $R^2 = 0.83$, $p < 0.001$; NegMal (cyan): $y = 0.8492\ x - 0.0015$ (SE of slope: 0.0169), $R^2 = 0.97$, $p < 0.001$; PosFem (red): $y = 0.8403\ x - 0.0017$ (SE of slope: 0.0268), $R^2 = 0.92$, $p < 0.001$; PosMal (blue): $y = 0.9168\ x - 0.0001$ (SE of slope: 0.0075), $R^2 = 0.99$, $p < 0.001$

In conclusion, we observed an active aggregation whose level was significantly greater for males than for females, and for infected than for non-infected insects. Clusters of infected bugs looked denser and more compact in males than in females, reinforcing this conclusion.

Discussion

This work represents the first detailed analysis of aggregation and geotaxis in adult males and females of *T. infestans*, and how both sexes are affected by the presence of *T. cruzi*. As shown before

340 in nymphal instars [43,68], adults exhibited an active aggregation due to the inter-attraction between
341 individuals, illustrating the social character of these insects. We show that a stable aggregation
342 emerges for both sexes, but the fraction of aggregated individuals and the density of clusters are higher
343 for males than for females. This difference between the genders is maintained under infection by *T.*
344 *cruzi*, but the latter reinforces the gregariousness in both sexes.
345
346 Our results are in agreement with previous studies. Indeed, a multi-factorial analysis (using
347 species, development stages and, feces source altogether) of the aggregative response of individuals to
348 feces shows that the aggregation level was lower (but not statistically different) for females than for
349 males [47]. It is well established that clustering or reduction of the inter-individual distances of social
350 and subsocial/ presocial arthropods reduces various stresses and therefore energy consumption
351 [37,69,70]. Different studies show that clustering reduces water loss [37–39,70]. We hypothesize that
352 the clustering of *T. infestans* individuals provides a similar benefit. As their weight is lower than
353 females' one, males could be under higher hydric stress. So they could tend to have a higher gregarious
354 trend. Moreover, it could be more adaptive for females to be less aggregative to disperse their eggs and
355 increase their probability of survival. In our experiments, males and females were supposed to be in
356 similar physiological status due to their comparable period of starvation (8-10 days), but in case of
357 infection, *T. cruzi* and *T. infestans* compete for nutrients, bug individuals showing a reduced resistance
358 to starvation when they are infected [71]. It might be speculated that infected bugs were in a stronger
359 starvation state and therefore were more gregarious to reduce the cost of the different stresses.
360
361 We almost know nothing about the distribution of triatomines inside a dwelling. Even if all the
362 stages are found in the upper part of the walls [36,50], our results suggest that males and females rest
363 in a stratified manner on the walls, males being above of females. Domestic cockroaches have a
364 similar way of life than triatomines, except the feeding habits. *Periplaneta americana*, for instance,
365 shows a preference for vertical areas, and males were vertically positioned above females [72]. Due to
366 the vertical air current, they can detect the female pheromone easily and oriented themselves towards
367 them. In triatomines, the sex pheromone is emitted by the metasternal glands of females, inducing

368 males to move towards the females (positive anemotaxis) [14]. On a wall, due to the difference in
369 temperature between the bottom and the top (up to 6°C [36]), an air current move towards the top of
370 the wall could allow the males to feel at some distance the sexual pheromone released by the females.
371 Most of the individuals showed a vertical orientation with the head towards the top, allowing them to
372 escape quickly from predators generally located below. Infection by *T. cruzi* enhanced the negative
373 geotaxis, especially in males, for which it was also observed a higher proportion of individuals with
374 the head toward the bottom.
375
376 It is interesting to note that a part of the experiments with non-infected insects was in fact
377 composed of a mix of non-infected and infected insects (less than 20%). One or two individuals are
378 not enough to observe a change at the level of the group. The latter suggests that a high proportion of
379 infected insects is required to change the group behavior.
380
381 Behavioral alterations upon infection are called parasitic manipulation when they are adaptive
382 for the parasite, altering phenotypic traits of its host in a way that enhances its probability of
383 transmission. There are some examples where the parasitism affects the geotaxis and the gregarious
384 behavior of the hosts [73–78]. Is there any advantage to *T. cruzi* to strengthen the negative geotaxis
385 and the aggregation behavior in males of *T. infestans*? Two hypotheses can be put forward: 1)
386 increasing the longevity of the vector, far from the predators present on the ground surface; that can
387 result from both a higher negative geotaxis and a higher aggregation level. As previously mentioned,
388 as aggregation could reduce the stress for the *T. infestans*, it could also increase the survival of *T.*
389 *cruzi*; 2) facilitate the host-finding. It could be easier for them to find a food source being higher on a
390 vertical surface. Actually, these insects are known to let themselves fall on the host [79]: the common
391 name of these insects in South of Latin America is "vinchuca" – meaning in Quechua "bug that lets
392 itself fall," i.e., from roof to reach sleeping people to feed on them. [61] have already reported an
393 increase of the dispersion on the field of infected females of *T. dimidiata*. Nymphal instars of *R.*
394 *prolixus* showed, on the contrary, a reduction of their locomotory activity [62].
395

A low height device like the setup used in these experiments allowed us to highlight differences, between sexes, and between infected and non-infected insects. The questions that the results generate showed that little is known about the spatial distribution of the insects in natural conditions and how they behave. Our results predict that in a natural/anthropic environment the percentage of infected insects should increase with the height of the settlement. More experiments have to be conducted to understand the dispersion and aggregation behaviors of *T. infestans*, both in the laboratory and on the field. For example one of our hypotheses concerns the influence of the height of the setup: the higher the setup, the greater would be the spatial segregation between the four categories. The response of the bugs should be modulated according to factors like the development stages and the physiological condition of the insects, the bug density, the numbers of available and suitable shelters, and the infection of the bugs. One important question is the robustness of our results in front of the presence of shelters (small cavities). Similarly, another question is about how mixed groups would distribute (both sexes, and/ or both infected and non-infected bugs).

Our results also open a new vision for controlling/ monitoring triatomines on the field, letting appear a higher risk coming from the upper part of the rooms. More precise studies about bug distribution on the field should help to improve control and monitoring by trapping [80]. Finally, our results lead us to propose simple tests easily feasible in the field, based on the geotaxis and the aggregative behavior of the bugs, to detect infected insects.

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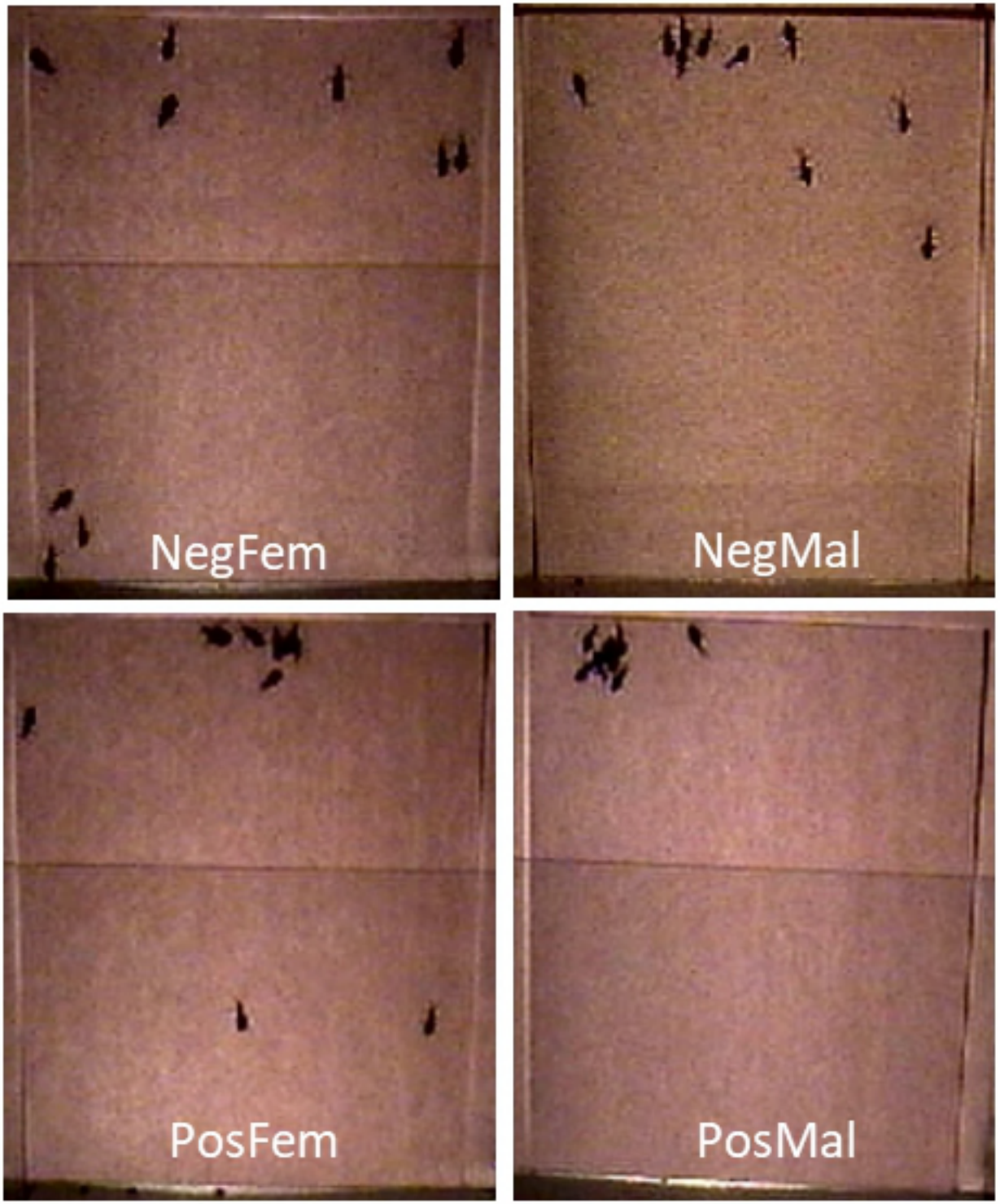
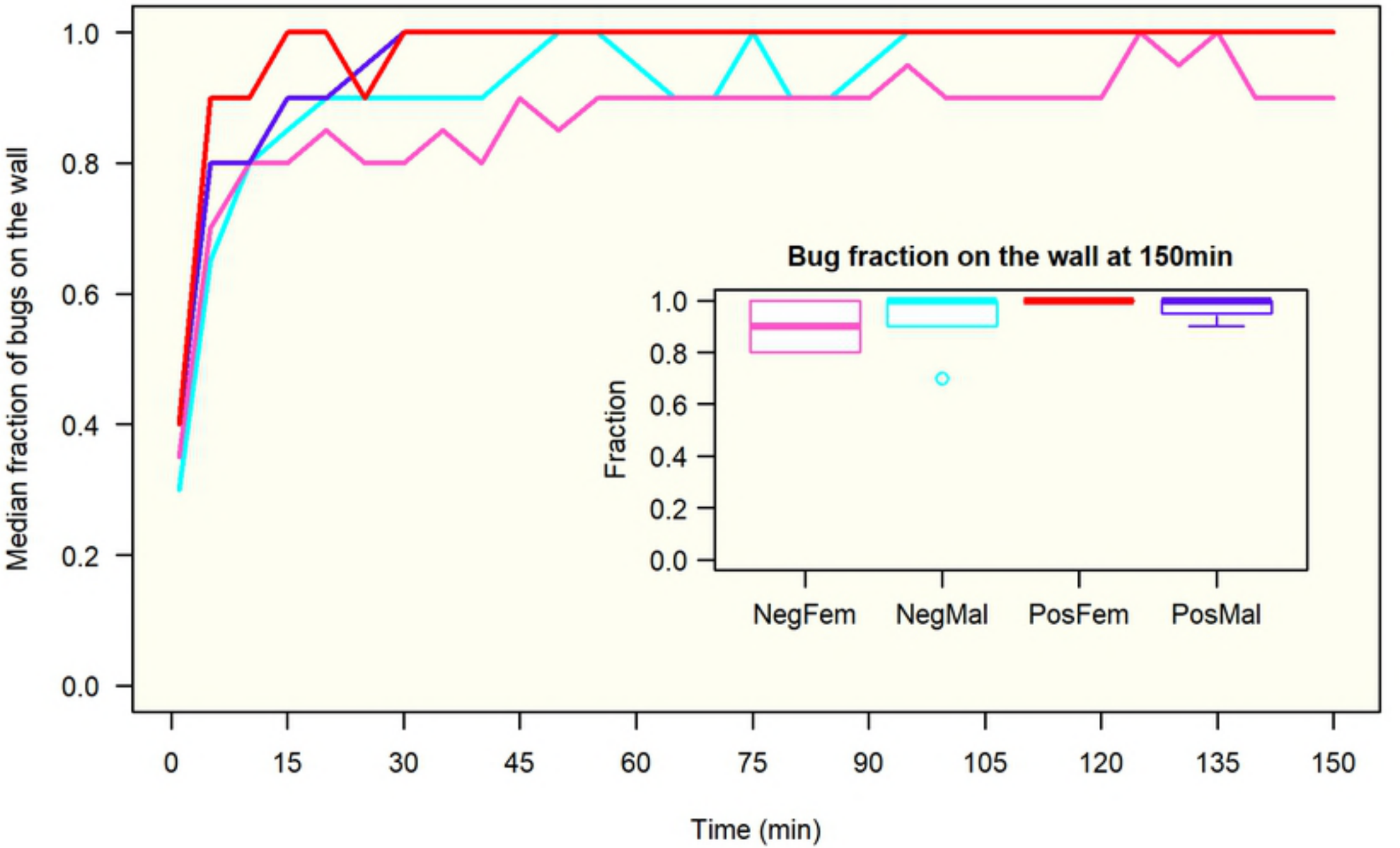
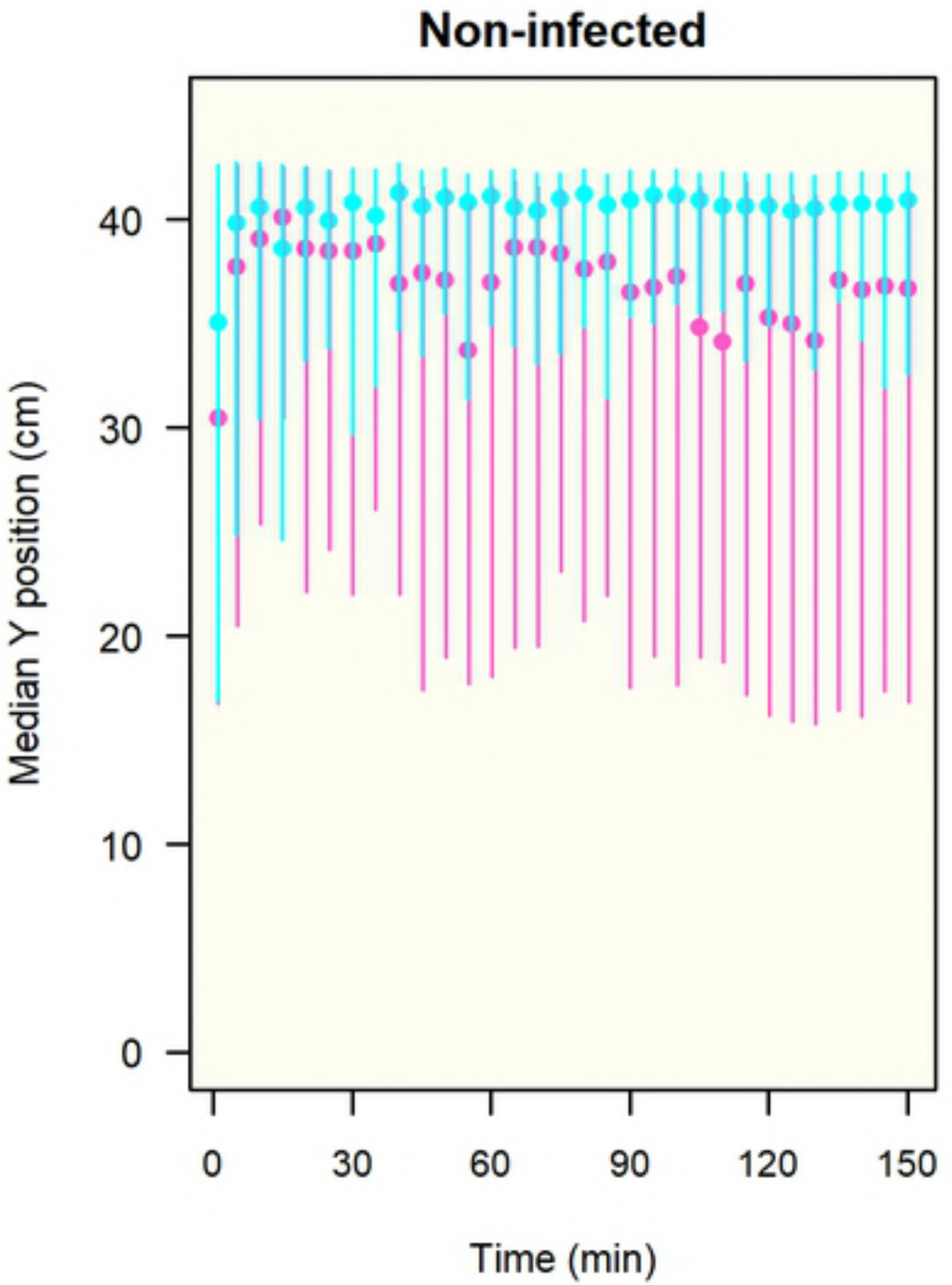
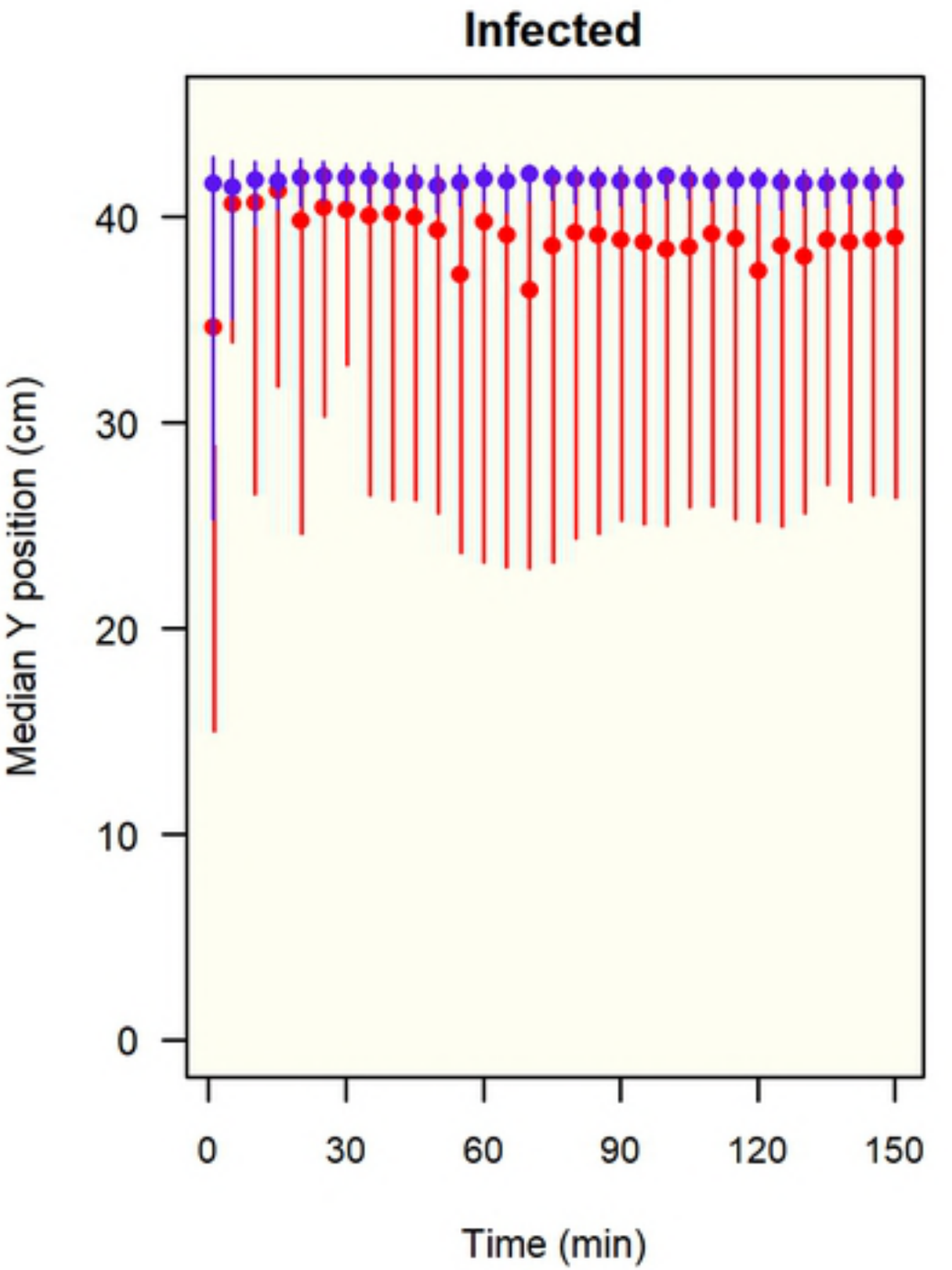
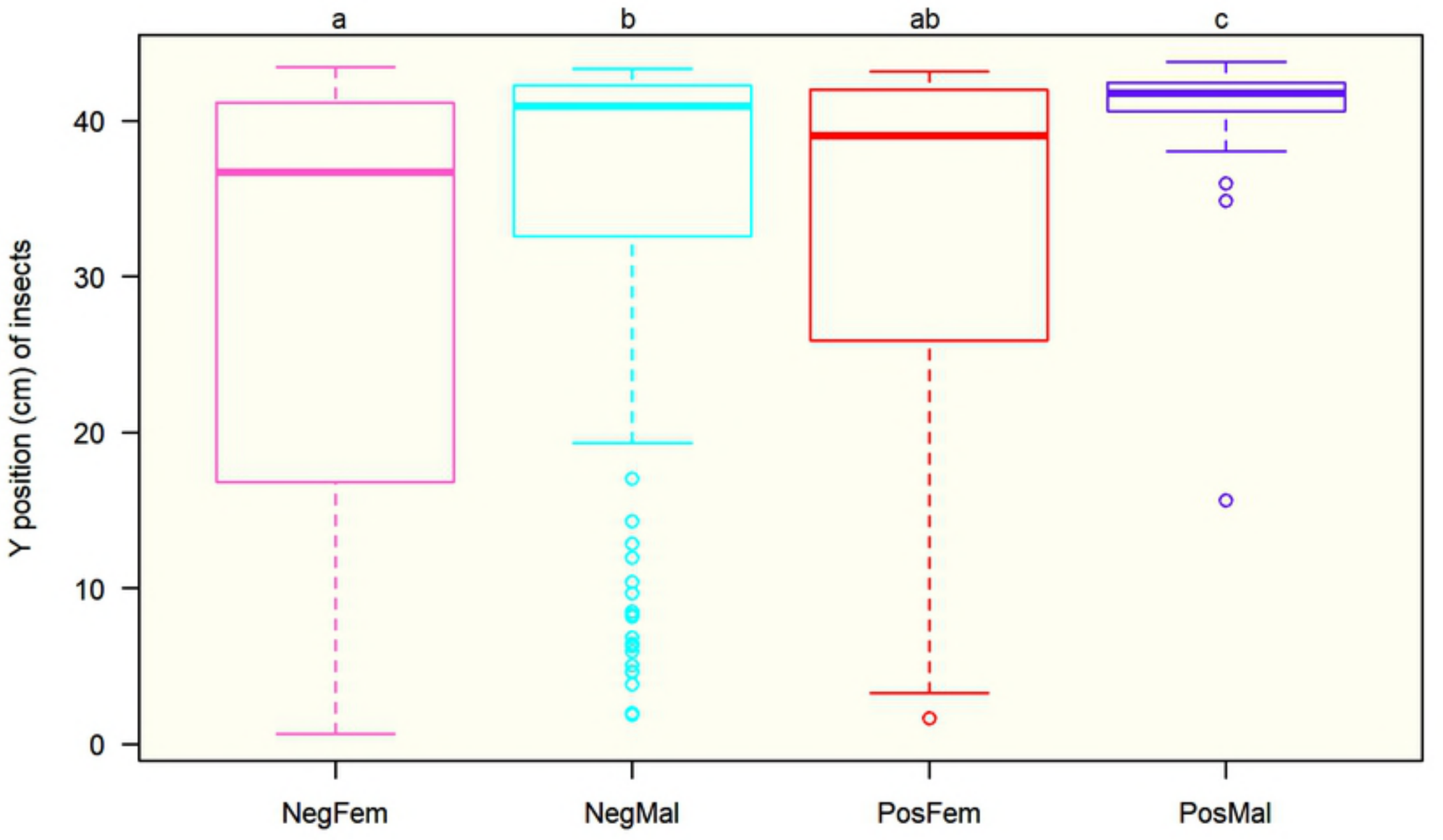
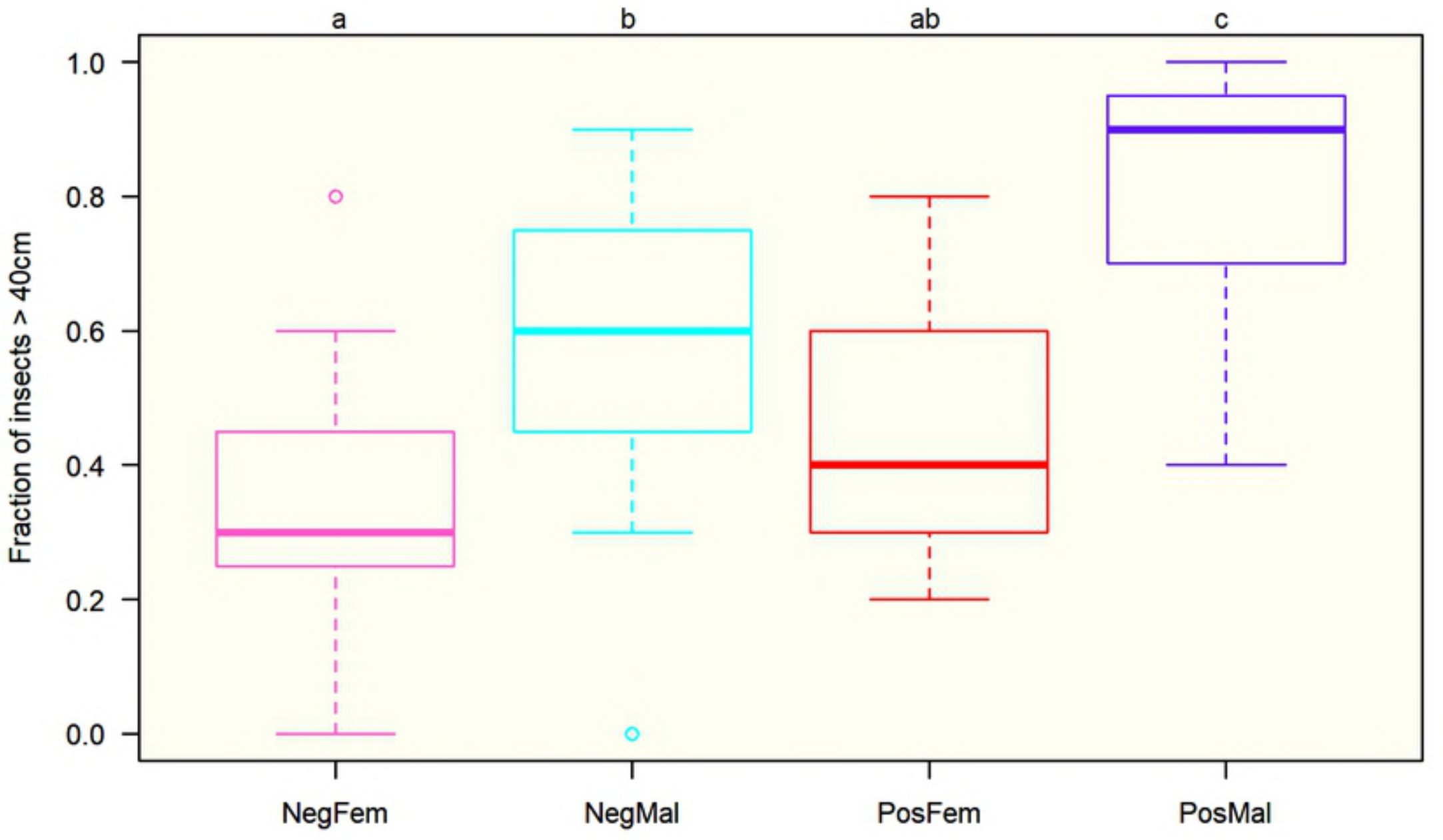

NegFem
NegMal
PosFem
PosMal


Median fraction of bugs on the wall
1.0
0.8
0.6
0.4
0.2
0.0
0
15
30
45
60
75
90
105
120
135
150
Time (min)
Bug fraction on the wall at 150min
Fraction
1.0
0.8
0.6
0.4
0.2
0.0
NegFem
NegMal
PosFem
PosMal

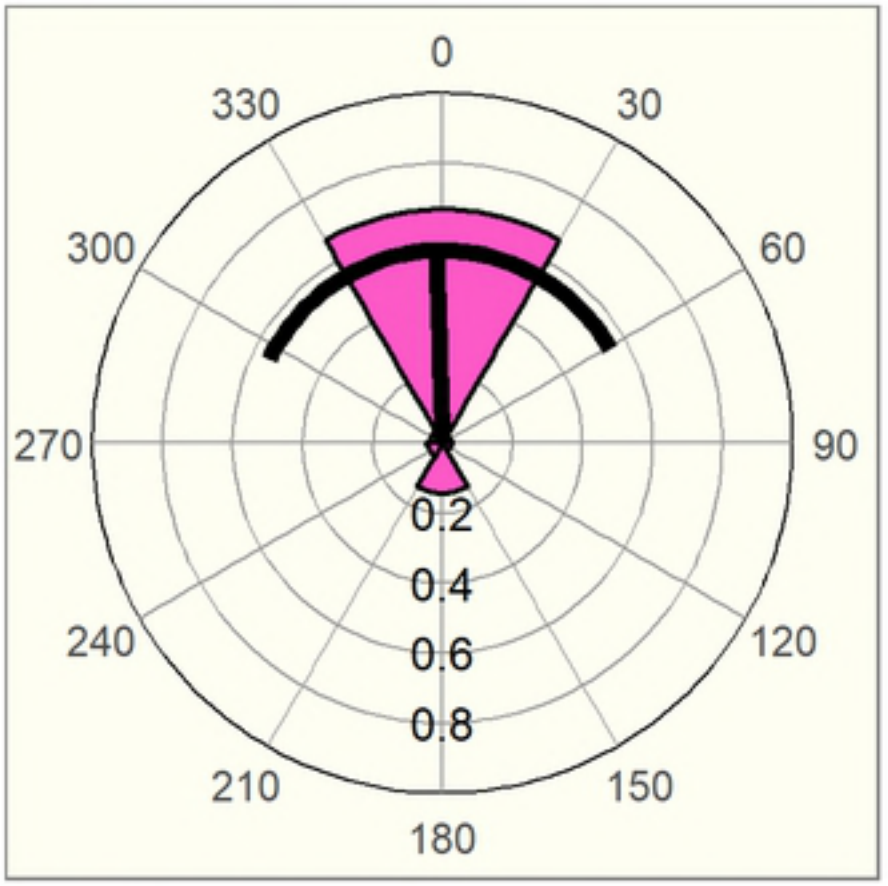

Non-infected
Median Y position (cm)
0
10
20
30
40
0
30
60
90
120
150
Time (min)


Infected
Median Y position (cm)
0
10
20
30
40
0
30
60
90
120
150
Time (min)

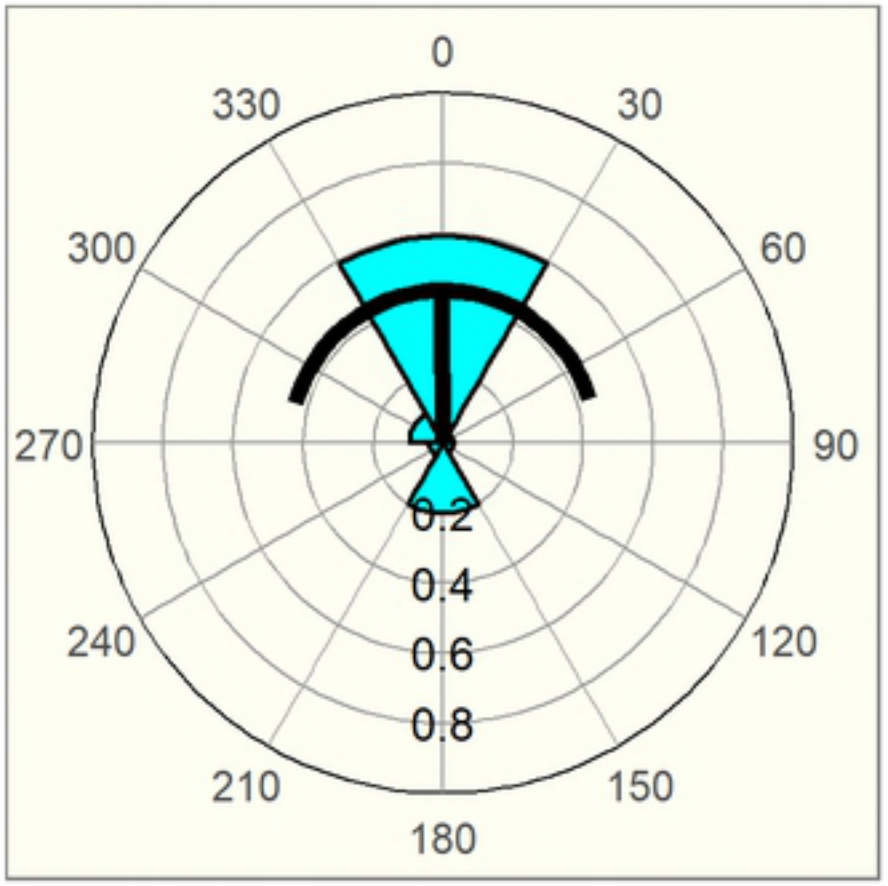

a
b
ab
c
40
30
20
10
0
Y position (cm) of insects
NegFem
NegMal
PosFem
PosMal


a
b
ab
c
1.0
0.8
0.6
0.4
0.2
0.0
Fraction of insects > 40cm
NegFem
NegMal
PosFem
PosMal

NegFem



NegMal



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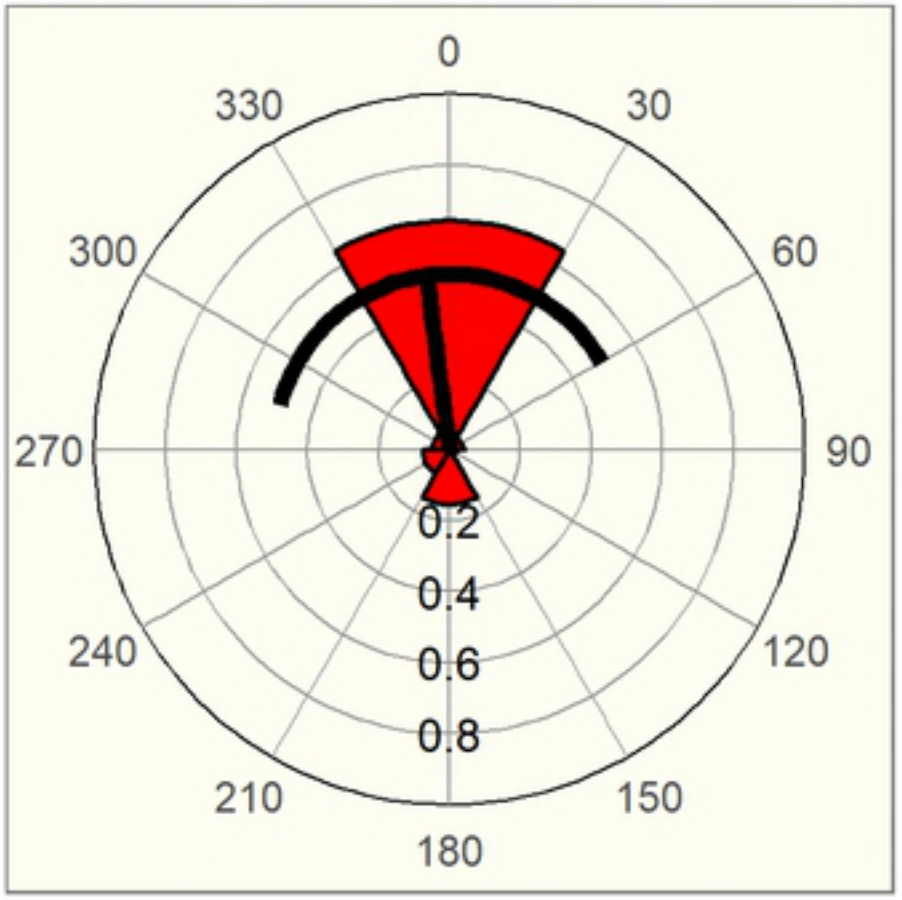
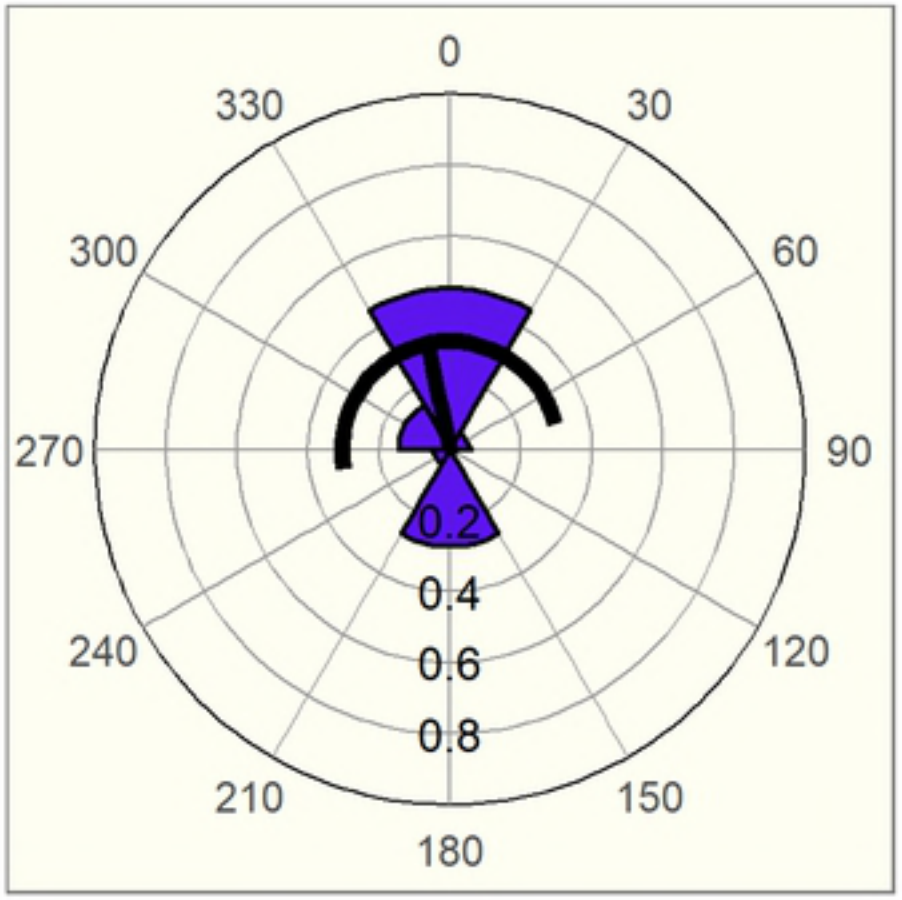
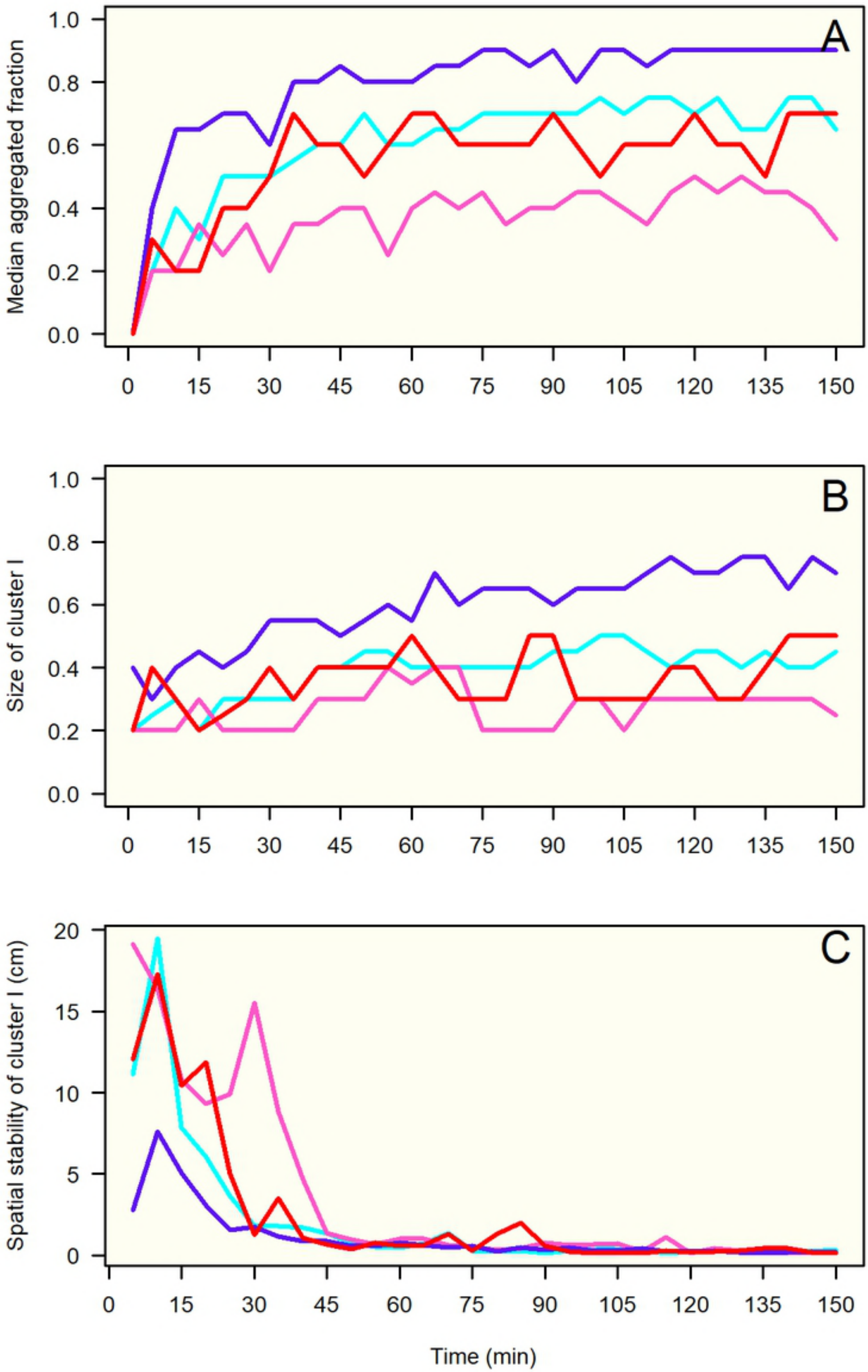
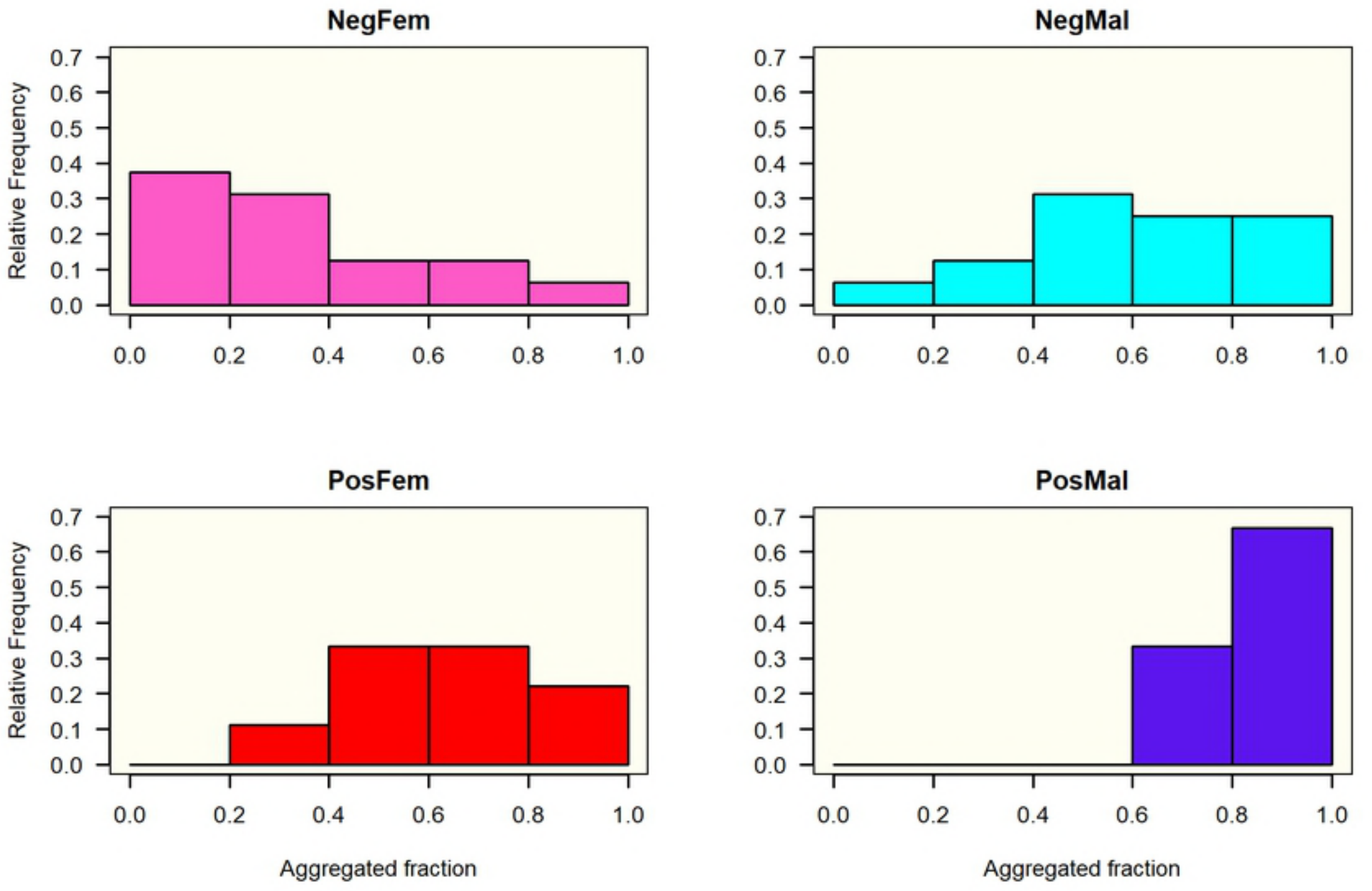
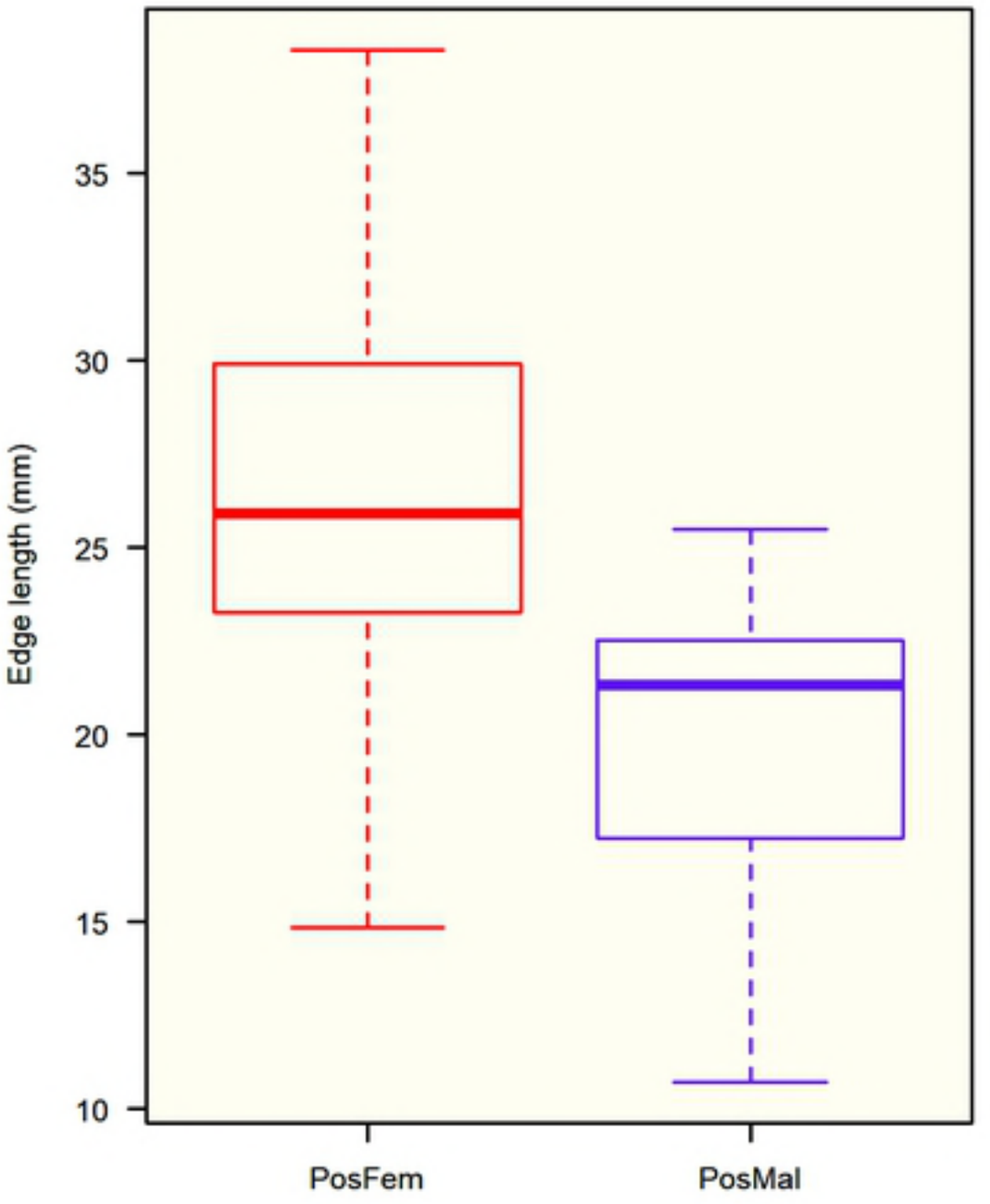


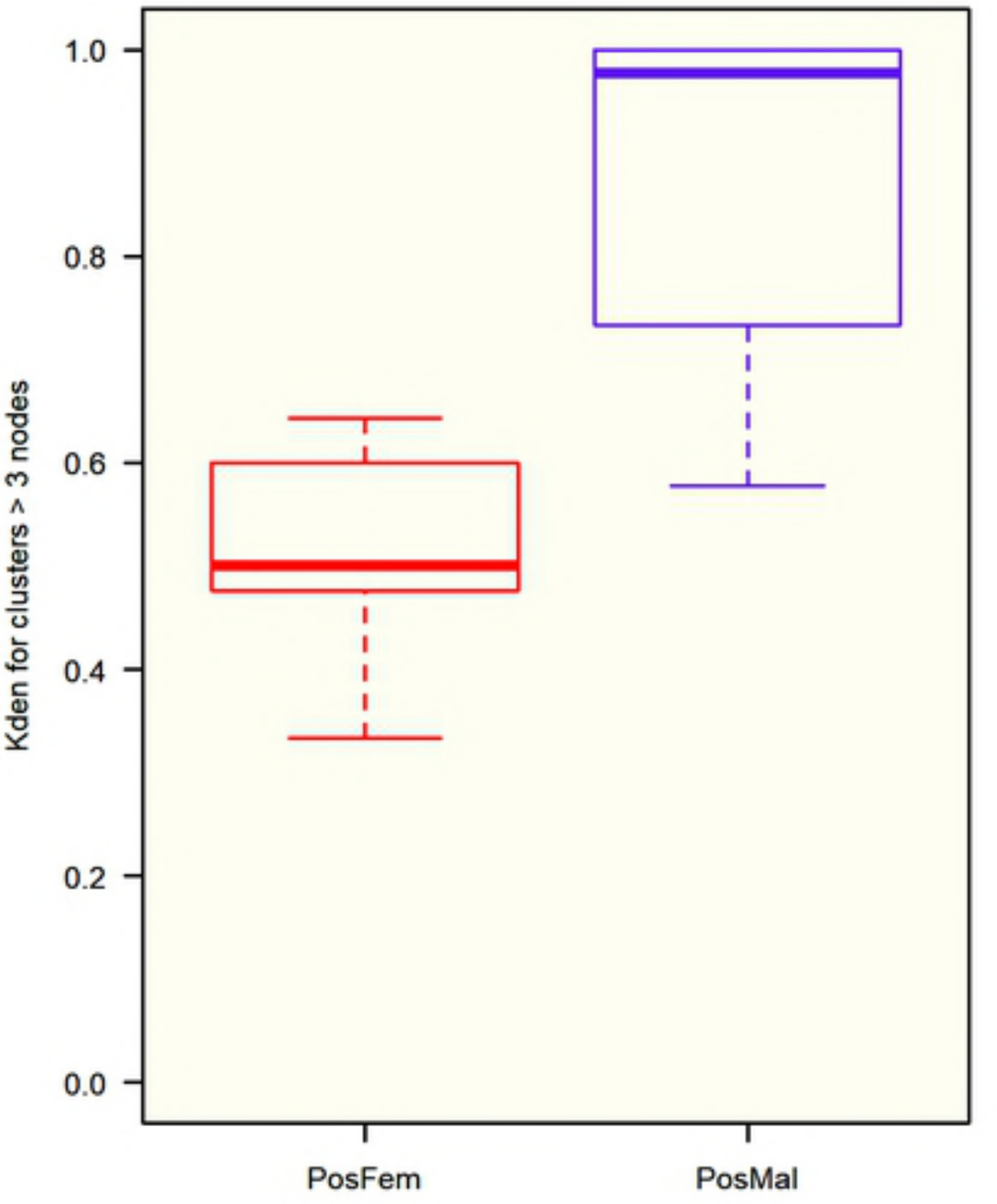
PosMal

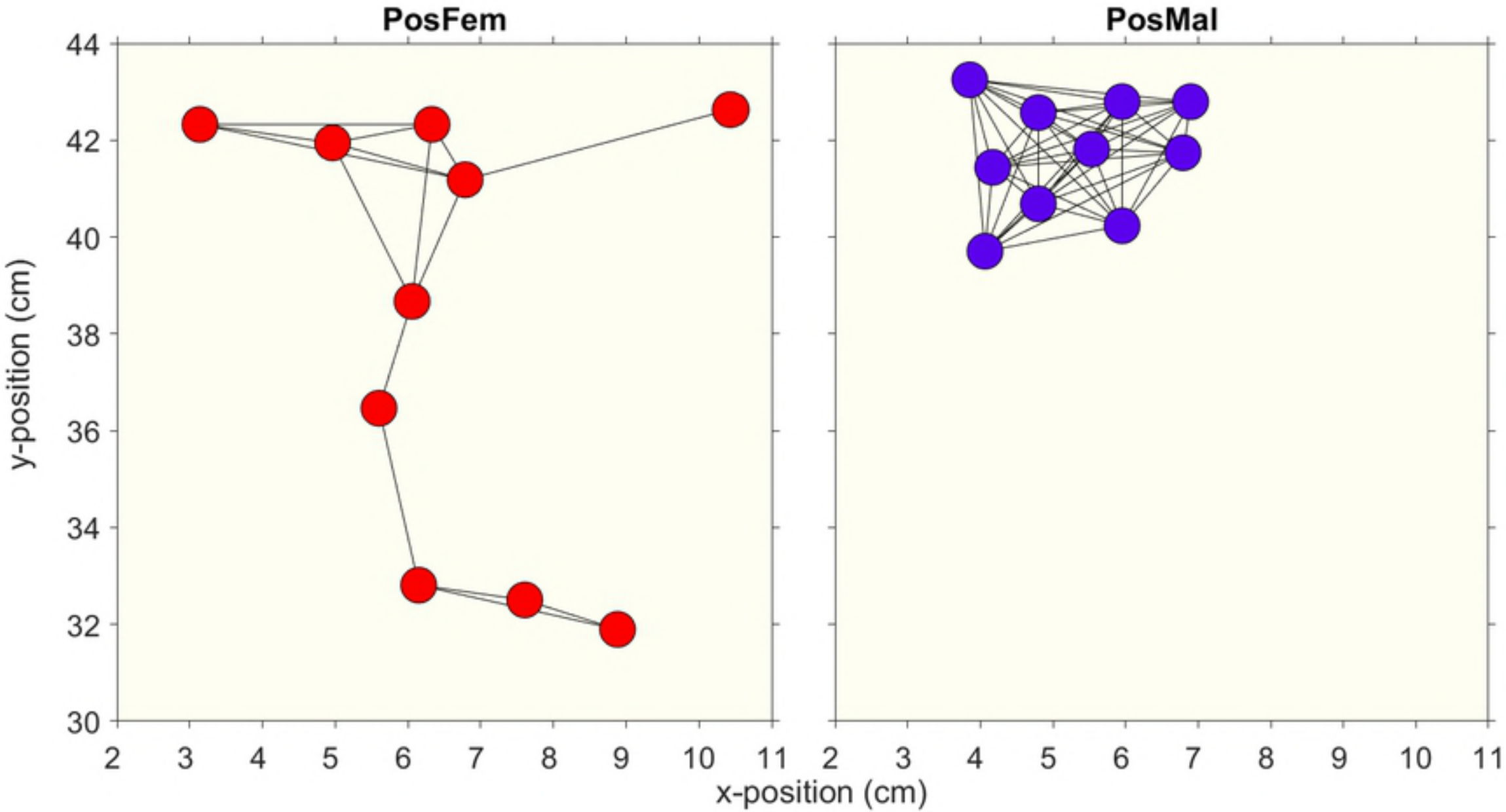


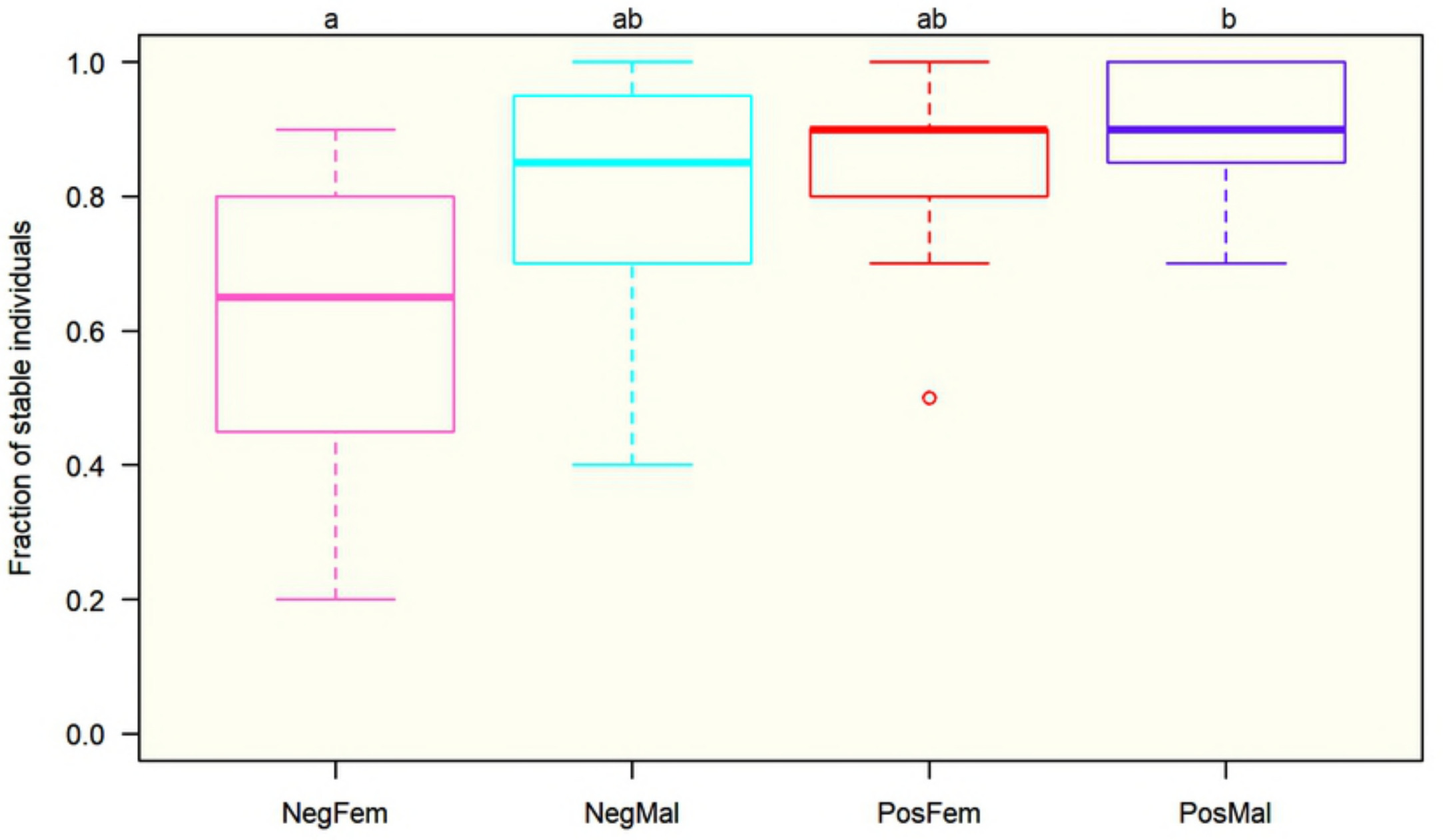

A
Median aggregated fraction
1.0
0.8
0.6
0.4
0.2
0.0
0
15
30
45
60
75
90
105
120
135
150
B
Size of cluster I
1.0
0.8
0.6
0.4
0.2
0.0
0
15
30
45
60
75
90
105
120
135
150
C
Spatial stability of cluster I (cm)
20
15
10
5
0
0
15
30
45
60
75
90
105
120
135
150
Time (min)

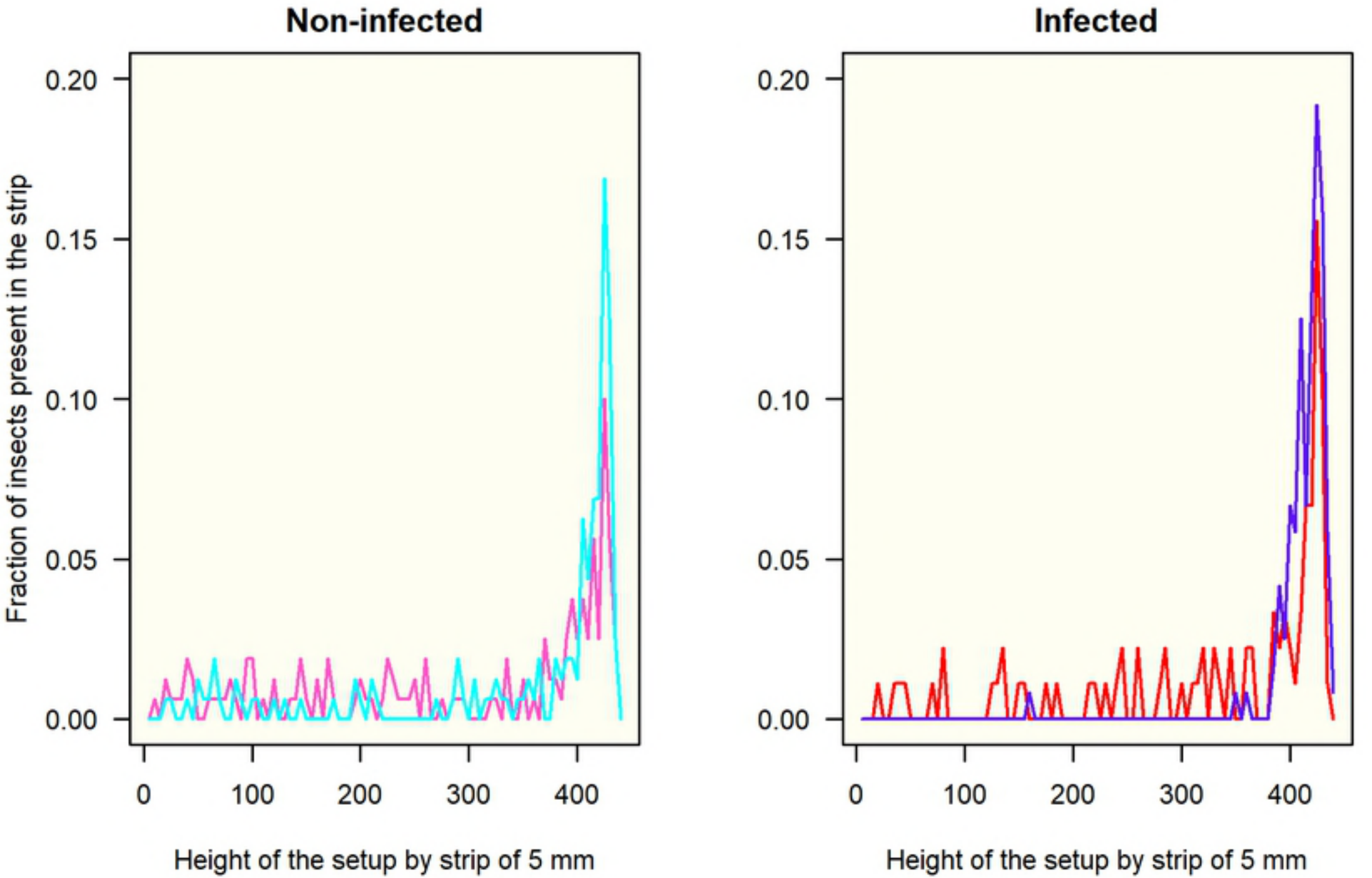

NegFem
NegMal
PosFem
PosMal
Relative Frequency
Aggregated fraction
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.0
0.2
0.4
0.6
0.8
1.0

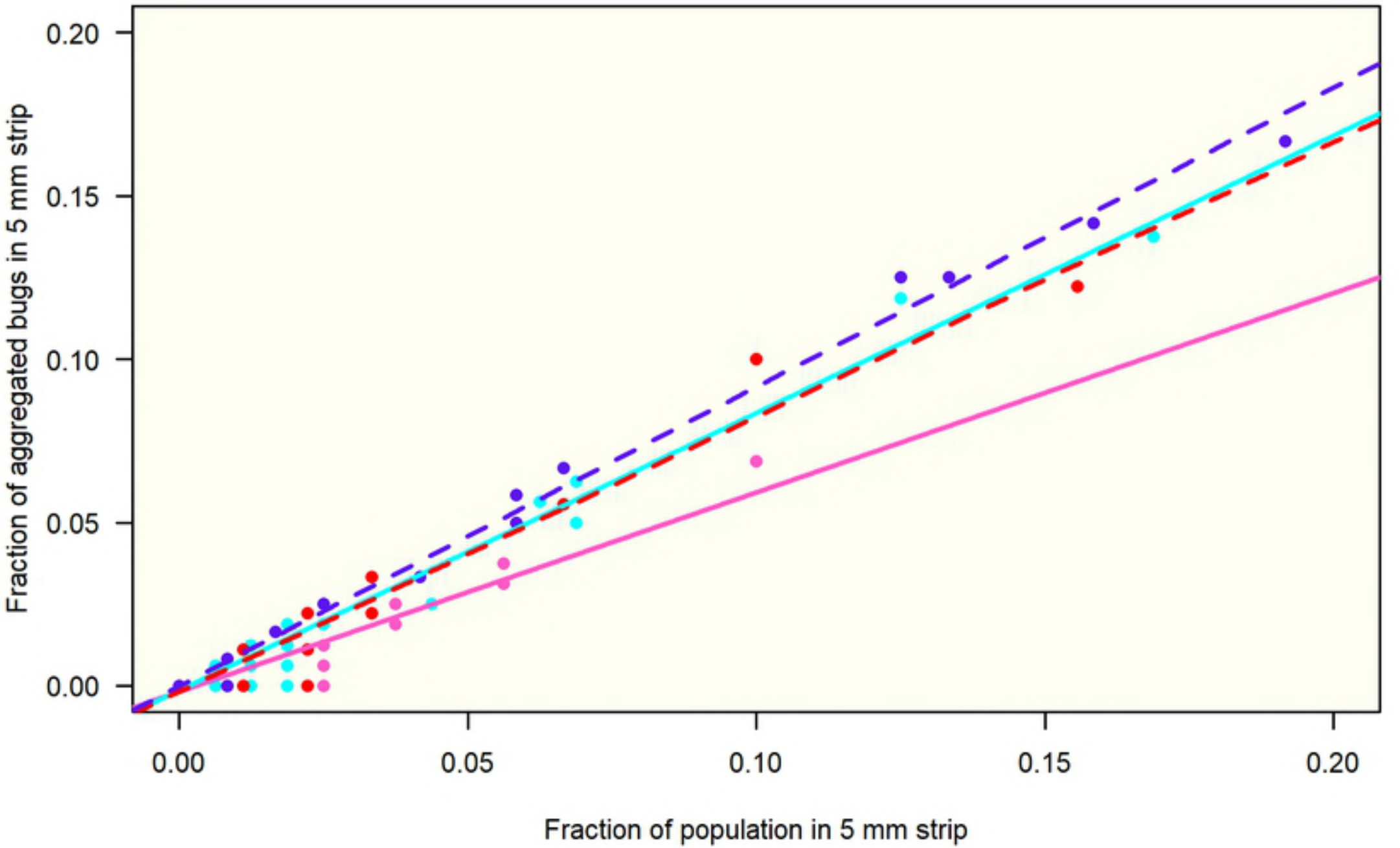

Edge length (mm)
35
30
25
20
15
10
PosFem
PosMal


Kden for clusters > 3 nodes
1.0
0.8
0.6
0.4
0.2
0.0
PosFem
PosMal


PosFem
PosMal
y-position (cm)
x-position (cm)
44
42
40
38
36
34
32
30
2
3
4
5
6
7
8
9
10
11


a
ab
ab
b
1.0
0.8
0.6
0.4
0.2
0.0
Fraction of stable individuals
NegFem
NegMal
PosFem
PosMal


Non-infected
Infected
Fraction of insects present in the strip
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
Height of the setup by strip of 5 mm
Height of the setup by strip of 5 mm


0.20
0.15
0.10
0.05
0.00
Fraction of aggregated bugs in 5 mm strip
0.00
0.05
0.10
0.15
0.20
Fraction of population in 5 mm strip