1   **A new automated chilled adult release system for the aerial distribution of sterile male tsetse**

2   **flies**

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25

Abstract

Background

Tsetse flies transmit trypanosomes that cause the debilitating diseases human African trypanosomosis (HAT) or sleeping sickness in humans and animal African trypanosomosis (AAT) or nagana in livestock. The riverine tsetse species *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) inhabits riparian forests along river systems in West Africa. The Government of Senegal has embarked on a project to eliminate a population of this tsetse species from the Niayes area with the objective to manage AAT in this area. The project is implemented following an area-wide integrated pest management approach with an SIT component. The SIT can only be successful when the sterile males that are released in the field are of high biological quality, i.e. have the same dispersal capacity, survival and competitiveness as their wild counterparts. To date, sterile tsetse males have been released by air using biodegradable cardboard cartons that were manually dropped from a fixed-wing aircraft or gyrocopter. The cardboard boxes are however expensive, and the system is rather cumbersome to implement.

Methods

A new prototype of an automated chilled adult release system (Bruno Spreader Innovation, (BSI™)) for tsetse flies was tested for its accuracy (in counting numbers of sterile males as loaded into the machine), release rate consistency and impact on quality of the released males. The impact of the release process was evaluated on several performance indicators of the irradiated male flies such as flight propensity, survival, mating competitiveness, premating and mating duration, and insemination rate of mated females.

Results

The BSI™ release system counted with a consistent accuracy and released homogenously tsetse flies at the lowest motor speed (0.6 rpm). In addition, the chilling conditions (6 ± 1 ℃) and the release process (passing of flies through the machine) had no significant negative impact on the males' flight

51   propensity. No significant differences were observed between the control males (no irradiation and no

52   exposure to the release process), irradiated males (no exposure to the release process) and irradiated

53   males exposed to the release process with respect to mating competitiveness, premating period and

54   mating duration. Only survival of irradiated males that were exposed to the release process was

55   reduced (~2 folds), irrespective of whether the males were held with or without feeding.

56   **Conclusion**

57   Although the release process had a negative effect on survival of the flies, the data of the experiments

58   indicate that the BSI machine holds promise for use in tsetse SIT programmes. The results of this

59   study will now need to be confirmed under operational field conditions in West Africa.

Introduction

Tsetse flies (*Glossina* spp.; Diptera: Glossinidae) are hematophagous insects and are the cyclical vectors of two debilitating diseases in sub-Saharan Africa, i.e. human African trypanosomosis (HAT) or sleeping sickness in humans and animal African trypanosomosis (AAT) or nagana in livestock [1,2]. Nagana and sleeping sickness have been major obstacles to rural development and a severe constraint for the development of more efficient and sustainable agricultural production systems in sub-Saharan Africa [3]. AAT limits the exploitation of fertile agricultural land in ~10 million km^2 in this part of Africa and, therefore, tsetse flies are rightly considered one of the root causes of poverty and hunger [4,5]. In West Africa, tsetse flies of the *palpalis* group, i.e. *Glossina palpalis palpalis* (Robineau-Desvoidy), *Glossina palpalis gambiensis* Vanderplank and *Glossina tachinoides* Westwood, are the most important cyclical vectors of these two diseases [6].

Due to the lack of effective vaccines and inexpensive drugs for HAT, and the development of resistance of the AAT parasites against available trypanocidal drugs [7], tsetse control remains a key component for the integrated sustainable management of both diseases [6]. Currently, there are four acceptable control tactics for the integrated management of tsetse vectors, i.e. (i) the live-bait technique (dip, spray, or pour on application of residual insecticides on livestock) [8], (ii) insecticide-impregnated targets/traps [9], (iii) the sequential aerosol technique (SAT) [10], and (iv) the sterile insect technique (SIT) [11–13]. In most cases, sustainable management of tsetse fly populations can only be achieved if the control tactics are implemented following the principles of area-wide integrated pest management (AW-IPM) [14]. For example, IPM applied against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area [15].

Already in the 1970's, an attempt was made to eradicate the *G. p. gambiensis* population from the Niayes region in Senegal, mainly using insecticide-based control tactics. However, area-wide principles were not adhered to and the project failed to create a sustainable tsetse-free zone, leading to re-colonization of the Niayes from relict pockets that had not been treated [16–18].

86   In 2005, the Government of Senegal embarked on a campaign to eradicate a population of *G. p.*

87   *gambiensis* [16] from a 1,000-km^2 area of the Niayes, located in the vicinity of the capital Dakar. The

88   programme has been implemented under the auspices of the Pan African Tsetse and Trypanosomosis

89   Eradication Campaign (PATTEC), a political initiative started in 2001 that calls for intensified efforts

90   to reduce the tsetse and trypanosomosis problem [19]. The Direction des Services Vétérinaires (DSV)

91   of the Ministry of Livestock (Ministère de l'Elevage et des Productions animales) implemented the

92   eradication campaign with support from the Institut Sénégalais de Recherches Agricoles (ISRA) of

93   the Ministry of Agriculture (Ministère de l'Agriculture et de l'Equipement rural). The programme

94   received financial and technical support from the Food and Agriculture Organization of the United

95   Nations (FAO), the International Atomic Energy Agency (IAEA), the Centre de Coopération

96   Internationale en Recherche Agronomique pour le Développement (CIRAD), and the USA State

97   Department under the Peaceful Uses Initiative (PUI). In this AW-IPM programme, conventional

98   suppression methods (insecticide-impregnated traps/targets/nets and insecticide pour-on on livestock,

99   insecticide ground and aerial spray, bush clearing) were integrated with the release of sterile male

100 flies [20].

101 The sterile insect technique (SIT) is a species-specific, safe, efficient, environment-friendly autocidal

102 control tactic to manage populations of insect pests and disease vectors [21–24]. The SIT requires

103 mass-rearing of the target insect, sterilization of the males using ionizing radiation and sequential

104 area-wide releases of a large numbers of the sterile males into the target area. The sterile male flies

105 compete with wild male flies for mating with the wild female population, interrupting their

106 reproductive potential and ultimately resulting in population reduction or elimination [25,26]. The

107 released sterile males, therefore, need to have adequate mobility to find and mate with virgin wild

108 females, and this is vital to successfully implement the SIT component of AW-IPM programmes

109 [25,27,28]. Therefore, routine quality control procedures are crucial to identify weaknesses in fly

110 production and handling procedures that result in low quality of the sterile males, as this may lead to

111 potential programme failure [14,29].

112   The aerial release of sterile male tsetse flies was pioneered in the programme that eradicated a

113   population of *Glossina austeni* from Unguja Island of Zanzibar in the 1990's [13]. The sterile males

114   were packaged in bio-degradable cartons that were manually dropped from fixed-wing aircrafts [30].

115   A similar approach was used during the initial years in the Senegal project, but the boxes were

116   dropped from gyrocopters [20]. In view of the high cost of the bio-degradable cartons and the lack of

117   storage space in the gyrocopters (requiring frequent landings to reload), efforts were made to develop

118   chilled-adult release systems similar to those developed for the release of sterile fruit flies [31,32].

119   However, the release systems for these pests have a very high throughput to obtain release densities of

120   2,500 to 200,000 fruit flies/km^2/week [33], but for tsetse flies the challenge was to develop a machine

121   that could disperse the sterile insects at very low release rates to obtain densities of 15 – 80

122   flies/km^2/week [33]. The aerial release of sterile insects has many advantages as compared with

123   ground releases i.e. it is fast, reaches areas that are inaccessible for ground release and provides a

124   uniform distribution of sterile insects over the target areas. However, it is expensive and in some

125   larger programmes, it represents around 40% of the annual operating budget of the sterile fly

126   emergence and release centres [34]. Therefore, attempts to reduce the cost of the aerial release process

127   are desirable either by reducing the frequency of flights through increasing the quality of the sterile

128   males [30,31] or by using automated chilled-adult release systems that avoid the cost of bio-

129   degradable carton boxes [30,31]. Currently, the majority of SIT programmes release chilled adult

130   insects into the targeted areas [34,35] using small fixed-wing air craft, helicopters or gyrocopters

131   [30,34,36,37]. The use of smaller airplanes or gyrocopters is one way to reduce the aerial release cost,

132   e.g. the use of a gyrocopter in Senegal was the cheapest way at a cost of € 320 per flying hour during

133   the period 2010-2020 [2]. Similarly, replacing the paper boxes and reducing the number of flights

134   using an automated chilled-adult release system will not only reduce the implementation cost but also

135   increase the efficiency of the program through improved sterile male fly distribution [30,33].

136   The use of automated devices to release chilled male tsetse flies was earlier (in 2012 for mexicana 1

137   and 2015 for Mexicana 2) attempted in Senegal using a Mubarqui Smart Release Machine (MSRM2),

138   adapted from the one used to release fruit flies (MSRM1) [37]. Initially, the system gave promising

139   results demonstrating its potential suitability for use in tsetse SIT programmes and provided

140   acceptable distribution when the males were maintained at 9-12°C. However, its operational use

141   revealed its limitation in terms of inadequate consistency of the release rates, which was probably

142   caused by vibrations of the gyrocopter that interfered with the vibrator of the MSRM2 that was used

143   as a fly ejection mechanism [37]. This resulted in an average recapture rate that was lower than the

144   one obtained when sterile male *G. austeni* were released at ambient temperature (25-29°C) using

145   paper cartons in the Zanzibar programme [30]. The disappointing recapture rates of the released male

146   flies prompted the programme to discontinue the use of the MSRM2. A major drawback was that the

147   impact of the release process on the sterile male tsetse quality, including their ability to fly and to

148   survive after passing through the MSRM2, was not well tested before its operational use in Senegal

149   [37]. In this study, a new automated chilled adult BSI prototype (Bruno Spreader Innovation (BSI™),

150   the Aerial Works Company (AEWO), St-Jean le Vieux, France) was tested for the release of sterile

151   male *G. p. gambiensis* flies under laboratory conditions. The release system contains a cylinder

152   rotating against a brush as an ejection mechanism [38] that enables the release of a low number of

153   sterile insects per unit area and time. First, the machine was calibrated, and the consistency of the

154   release rate determined. Thereafter, the impact of the release process on sterile male performance was

155   assessed in terms of flight propensity, mating competitiveness (in walk-in field cages), premating

156   period and mating duration, insemination potential and survival.

157

Material and Methods

159   **Tsetse Flies**

160   All experiments were carried out with flies from a *G. p. gambiensis* colony that was established in

161   2009 at the FAO/IAEA Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria [39,40] from

162   pupae received from a colony maintained at the Centre International de Recherche-Développement

163   sur l'Elevage en zone Subhumide (CIRDES), Bobo Dioulasso, Burkina Faso. The original colony was

164   established in 1972 at Maison-Alfort, France from field pupae collected in Guinguette, Burkina Faso

165   and then transferred to CIRDES in 1975.

166   The colony at the IPCL has been maintained using an *in vitro* feeding system with thawed bovine

167   blood (Svaman spol, s.r.o., Myjava, Slovak Republic). The blood was kept frozen at -20°C and

168   irradiated with 1 kGy in a commercial 220 PBq ^{60}Co wet storage panoramic shuffle irradiator. The

169   flies were offered a blood meal three times a week and maintained under a 12L:12D light regime

170   cycle. Pupae were incubated at $24.1 \pm 0.1°C$ and $78.8 \pm 3.7\%$ R.H. for four weeks and adults emerged

171   under the same temperature and humidity conditions. These conditions will henceforth be referred to

172   as standard laboratory rearing conditions.

173   **Radiation**

174   The tsetse puparia were irradiated in air at the IPCL, Seibersdorf, Austria using a Gammacell® 220

175   (MDS Nordion Ltd., Ottawa, Canada) ^{60}Co irradiator. The dose rate was measured by alanine

176   dosimetry as 2.144 Gy·sec^{-1} on 2015-03-03 with an expanded uncertainty $(k = 2)$ of 3.2%. The

177   radiation field was mapped using Gafchromic HD-V2 film and the dose uniformity ratio in the

178   volume used for the experiments was < 1.1. The irradiated group was placed in a petri dish at the

179   centre of a polycarbonate jar (2200 mL) and sandwiched between two phase change packs that kept

180   the temperature below 10°C during irradiation with 120 Gy. Untreated puparia or flies were used as

181   control (0 Gy) and handled in the same manner.

182   **BSI™ automated release device**

183   The BSI™ automated chilled adult release system (hereafter referred to as "BSI™", **Figure 1,**

184   **Supplementary file 1**) and the associated software (BSI Navigator version 1.9.8) installed on a tablet

185   computer (Samsung Galaxy Tab S2), were tested (flight simulations) at the IPCL for its functionality

186   (accuracy and release rate consistency) and impact (chilling and potential physical damage) on the

187   sterile adult males. The BSI™ has a weight of 20 kg and consists of a funnel surrounded by a cooling

188   unit, into which the flies are loaded and held until discharged into cavities on a rotating cylinder that

189   acts as an ejection mechanism, resulting in the release of flies [38]. An optical sensor monitors the

190   number of males released. The machine was switched on at least one hour before the loading of the

191   flies to obtain a stable temperature of $6 \pm 1^{\circ}C$.

192   **Calibration of the BSI™**

193   The accuracy of the BSI™, i.e. its ability to count the numbers of flies released was assessed at speeds

194   ranging from 0.6 to 6 rpm. After selecting a specific speed, each of three batches of a known number

195   (480, 1000 or 2000) of 5-day old sterile male *G. p gambiensis* were immobilized by exposure to a

196   gentle flow of cold air (4°C) in a chiller and loaded into the BSI™ and released. As the chiller and the

197   BSI™ were located in different rooms, the chilled flies were transferred from the chiller to the BSI™

198   on a netted circular container placed on a plastic box filled with ice and covered with aluminium foil

199   to keep the flies immobilized. The released flies were collected on ice to keep them in an

200   anaesthetized state for repeated use in calibration at each rotation speed. Each of the three batches was

201   replicated three times for each of the ten rotation speeds. The flies were discarded after the

202   calibration. The BSI™ software counted and automatically recorded the number of sterile male flies

203   passing through the machine as detected by an optical sensor. This figure was later compared with the

204   hand counts of the flies loaded into the machine to calculate the error. The software automatically

205   generated graphs with trend lines of error rate for the ten selected rotation speeds used in each

206   replicate within the 3 batches of flies. This enabled the selection of the replicate with the best linear

207   curve (with the least errors at each speed), which was later used for the simulated flight releases.

208   **Consistency of the release rate**

209   During calibration the best accuracy was observed at a rotation speed of 0.6 rpm and was therefore

210   used, to assess the consistency of the release rate of the BSI™, i.e. the number of males released per

211   minute. A total of 2000 male flies were exposed to the release process at a rotation speed of 0.6 rpm

212   for three replicates. The BSI™ was switched on one hour before the test and the software set to the

213   following parameters for release of the flies: i) IPCL polygon where the machine was physically

214   located, ii) manual control of the release iii) the numbers of insects available to pass through the

215   machine, iv) a rotation speed of 0.6 rpm and v) a vibration value of the maximum power of the

216   shaker. The duration of the release process was recorded using a digital timer. Male flies that passed

217   through the BSI™ were collected in a container placed on ice. The collection containers were changed

218   every five minutes and the males counted manually. These manual counts were used for comparison

219   with the number of males recorded by the BSI™. A total of seven containers of released flies were

220   collected for each replicate. The flies were discarded after the test.

Impact of the release process on sterile male performance

Preparation of the tsetse flies

223   After emergence of the female *G. p. gambiensis* flies, the remaining male pupae were collected and

224   divided into two groups. Both groups were chilled at 10^0C for one hour before one group of pupae

225   was irradiated. Thereafter, both groups of pupae were covered with sieved, sterilized sand mixed with

226   0.025% fluorescent powder by mass following the procedure used in the operational program in

227   Senegal [41]. Different colours were used for the irradiated and untreated groups. The pupae were

228   placed in emergence cages and the emerged marked males were maintained under standard colony

229   conditions. Virgin female flies were collected from the colony three days prior to the mating

230   competitiveness test. For this study, five different treatment groups of males were used: irradiated

231   males exposed to the release process that were held in the machine for 5 minutes (5m), 60 minutes

232   (60m) and 120 minutes (120m) before passing through the machine, irradiated males that were not

233   exposed to the release process (Irrd) and males from the colony that were neither irradiated nor

234   exposed to the release process (Control). All male flies were offered two blood meals on the 1st and 3rd

235   day after emergence, and 24 hours after the last blood meal, 5-day old males of the treatment groups

236   were immobilised at 4°C for 18 and 36 minutes to allow sorting and counting. A total of 500 male

237   flies were placed into the BSI™ to assess their flight propensity and mating competitiveness and

238   survival after passing them through the machine after 5, 60 and 120 minutes compared with irradiated

239   only and control.

240   *Flight Propensity*

241   The flight propensity of the 5-day old males of the treatment groups was assessed under standard

242   rearing conditions. Flight tests were carried out following the modified FAO/IAEA/USDA protocol

243   [42] in netted cages (45 × 45 × 45 cm) containing a black painted PMMA flight tube (89 mm

244   diameter, 3 mm thick wall, 100 mm high). Light could only enter the tube from the top and the walls

245   were coated with unscented talcum powder to prevent the flies from crawling out of the tube [40]. For

246   each test, an average of 140 (49-410) chilled males were put in a plastic Petri dish (90 mm diameter)

247   with the base covered by black porous paper and the flight tube placed on top. The number of male

248   flies that escaped from the tube (called "flyers") and those that remained in the tubes (called "non-

249   flyers") were recorded after two hours [43]. The cages were placed in a room with fluorescent lights

250   giving an intensity of 500 lux at the flight tubes to attract the emerged flies. Each treatment was

251   replicated eight times. Samples of 30 and 120 male flies that escaped the tube were collected for each

252   of the treatment groups and the control group, respectively, for use in the mating competitiveness test

253   seven days post emergence. The remainder of the flies were discarded.

254   *Mating competitiveness and insemination rate*

255   Mating competitiveness of the male flies was assessed in walk-in field cages containing a potted tree

256   to simulate a natural environment. The netted cylindrical field cages [44] (2.9 m diameter and 2.0 m

257   high) [45] were located inside a greenhouse with temperature and humidity control and natural light

258   that could be supplemented with artificial light from cold white fluorescent tubes. The temperature in

259   the greenhouse ranged from 24°C to 31°C and the relative humidity from 41 to 56% during the

260   observation periods. Light intensity varied from 236 to 5000 lux depending on the position in the cage

261   with areas under the PVC supporting frame and tree leaves recording lower light intensity.

262   Temperature and humidity were recorded from 08:45 h am to 11:30 h am.

263   All mating competitiveness tests were carried out between 9.00 h am and 11.00 h am as described in

264   previous experiments [46]. The male flies of the five treatment groups that were flyers from the flight

265   propensity test, were released in the field cage to compete for mating opportunities with untreated

266   colony males for mating with virgin females. The competitiveness test of each treatment group was

267   replicated eight times. During the test in each replication, four field cages representing the four

268   treatments were located in the centre of the greenhouse and used simultaneously for observations of

269   mating activity, with each of two observers managing two cages.

270   In each field cage 30 three-day old virgin females were released ten minutes before two groups of 7-

271   day old flies (30 males from the control and 30 males from one of the four treatments, as previously

272   described) to compete for mating opportunities at an initial ratio of 2 males:1 female, during the 2-

273   hour observation. The male fly treatments were randomly allocated to different cages each day such

274   that at the end of the experiment each treatment was observed in the same cage at least twice **(Table**

275   **2)**. When the male had successfully engaged the female *in copula*, the mating pair was collected in a

276   tube with netting at both ends (4 cm diameter x 6 cm height) and after the couples disengaged, the

277   males were separated from the females. Each tube was numbered to identify the individual male and

278   female treatment. The period between the release of the male flies in the field cage until copulation

279   was recorded as the pre-mating time. The difference in time between the initiation of successful

280   copulation and separation was recorded as the mating duration. After the end of the mating, males and

281   females were separated to identify the male treatment and the females dissected to estimate

282   spermathecal fill. The fluorescent dyes to discriminate male fly treatments were differentiated using a

283   USB digital microscope (AM4113FVT2, Dino-Lite Europe, Almere, The Netherlands) with UV-light,

284   connected to a PC for display. The female flies were dissected in physiological saline solution under a

285   binocular microscope and the insemination rate and spermathecal fill were assessed subjectively at

286   x100 magnification using a Carl Zeiss compound microscope connected to a PC for display [23]. The

287   spermathecal fill was scored to the nearest quarter for each spermatheca separately as empty (0),

288   partially-full (0.25, 0.50 or 0.75) or full (1.0) and the quantity of sperm transferred was then computed

289   as the sum of the two spermathecal scores. The number of females that mated as a proportion of the

290   total females in each replicate is an indication of the tendency of the flies to mate (proportion mating,

291   PM). The relative mating index (RMI) was defined as the number of mating pairs accounted for by

292   the treatment category as a proportion of the total number of mating pairs. These indices represent the

293   competitiveness of treated males relative to the colony control males [46].

Survival

295   To determine the impact of the release process on the longevity of irradiated males, a survival test was

296   carried out with one group of flies kept under starvation and a second group kept under standard blood

297   feeding conditions. Five-day old males (n = 30) from the treatment and control groups were placed in

298   adult holding cages (diameter 110 mm x height 50 mm) and maintained under standard tsetse rearing

299   conditions. Two groups of flies originating from two different batches of flies (from different weeks)

300   were used for the treatments, resulting in two biological/true replicates. Within each group, treatments

301   were also divided into two replicates resulting in two technical replicates. This totalled 4 replicates (2

302   biological and 2 technical replicates). Male mortality was recorded daily under starvation conditions

303   and three times per week for the standard feeding conditions.

Data analysis

305   The consistency of release rate data was analyzed using a linear mixed-effects model fitted by

306   maximum likelihood. The flight propensity, the relative mating index and the spermatheca values data

307   were analyzed using a generalized linear mixed-effects model fitted by maximum likelihood (Laplace

308   approximation) with a "binomial (logit)" family. Treatments were used as fixed effects and replicates

309   were used as random effects. The premating and mating duration data were analyzed using linear

310   regression models. The number of matings achieved was tested for equality of performance between

311   treated and control males using the RMI and the Log likelyhood ratio test for comparison of means

312   [47]. Spermathecal fill categories were analyzed using a Kruskall-Wallis test [47,48]. The survival of

313   flies of different treatments was analyzed using Kaplan-Meier survival curves [49]. Survival curves

314   were compared using the cox.surv model where the treatment was used as explanatory variable and

315   the survival as the response variable. The data were statistically analyzed and graphs created in Excel

316   and R version 3.6.2. [50] using RStudio Desktop version 1.2.5033 [51] with the packages; ggplot2

317   [52], nlme [53], lme4 [54], survival [55], coxme [56] and MASS [57].

Results

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Calibration of the BSI™

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320 The calibration results were used to assess the accuracy of the machine by counting the loaded

321 number of flies for comparison with the number of flies released as counted and recorded by the

322 machine. The software automatically generated tables and graphs of the machine counts and

323 calculated the error rates for the three batches (480, 1000, 2000 flies) and their replicates. The results

324 showed average error rates above 45% of the numbers counted by the machine compared with the

325 hand-loaded number of flies into the machine, and this error rate increased with increasing motor

326 speed (F = 494, df = 1, 88, $P < 0.001$) (**Figure 2A**). The lowest error rates (47.4% ± 1.5, 47.9 ± 0.7;

327 and 45.9 ± 0.4 for loads of 480, 1000 and 2000 flies, respectively) were observed at the lowest motor

328 speed of 0.6 rpm. The error rate was not affected by the number of flies loaded into the machine (P =

329 0.245) (**Table 1**; **Figure 2B**).

Consistency of the release rate of the BSI™

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331 The release rates of the sterile flies over time as shown by the manual hand-counts, the counts

332 recorded by the optical sensor and estimated counts (after correction by the software using the error

333 rate estimated as explained above) are presented in **Figure 3A**. The actual release rate as given by the

334 manual counts did not vary significantly with time (F = 3.5849, df = 1, 20, P = 0.0736) after an

335 approximately one-minute initial delay **(Figure 3B, Supplementary file 2)**. There were no significant

336 differences in release rates between the three replicates of the 2000 flies used ($P > 0.05$). The manual

337 and estimated counts were strongly correlated (t = 67.348, df = 1, 22, $P > 0.001$) showing that the

338 machine was able to correct any errors in counts during the release period, in an accurate and

339 consistent manner (**Figure 3C**).

340   **Sterile male *G. p. gambiensis* performance after exposure to the release process with the BSI**

341   *a)* **Male flight propensity**

342   The flight propensity of the male flies was more than 60% for all replicates and mating activity was

343   observed in all cages for all treatments (**Table 2**). Untreated colony males had an average flight

344   propensity of 88.3%, which was significantly higher than that of the treatment males (z = 5.290, *P* <

345   0.001). However, the flight propensity of males that were only irradiated (Irrd) and of irradiated males

346   that were exposed to the release process for different durations (5, 60 and 120 minutes) was similar

347   (**Figure 4**, **Table 2**).

348   *b)* **Mating competitiveness**

349   When flies were released in the walk-in field cages, they generally landed on the supporting frame of

350   the cage, its side walls and roof. The flies would clean themselves and occasionally fly short

351   distances. The irradiated only (Irrd) males or the males that were irradiated and exposed to the release

352   process at different durations (5m, 60m and 120m) competed successfully with the untreated colony

353   males under our experimental field cage conditions. There were no significant differences in the

354   relative mating index values between irradiated only males (Irrd) and the treatment males (**Table 2,**

355   **Figure 5**). The relative mating indices showed that the untreated colony male flies do not

356   outcompeted the male flies that were irradiated but not exposed to the automated chilled release

357   process or the male flies that were loaded into the machine except 120m (G= 4.591. df=1, *P* = 0.032)

358   (**Supplementary file 3**).

359   **c) Pre-mating period and mating duration**

360   Mating pairs were formed soon after the males were released in the field cages. There were no

361   significant differences in the pre-mating period between the only irradiated males (Irrd) and the

362   irradiated males exposed to the release process at different durations (5m, 60m and 120m). The

363   premating period also did not differ between the different treatments (**Figure 6a**). Similarly, there

364   were no significant differences in the mating duration between only irradiated males and the treatment

365   males (5m, 60m and 120m) (**Figure 6b**, **Table 2**).

366

367   d**) Insemination rate**

368   Females mated with males of the different treatments had similar insemination rates (**Figure 7A**). In

369   general, 55-69% of the dissected mated females showed spermatheca that were completely filled with

370   sperm regardless of the treatment to which the males were exposed, while the rest of the dissected

371   mated females had a similar distribution of empty and partially filled spermatheca (13-26%) **Figure**

372   **7B**).

373

374   **e) Male survival**

375   The results from the Kaplan-Meier analysis indicate that under starvation conditions, most of the

376   males died within two weeks but they survived relatively longer (50% of males survive > 20 days

377   regardless treatments) when receiving a normal feeding regime of three blood meals per week (**Figure**

378   **8**). Survival of males chilled for 120 minutes before release and starved lived significantly shorter

379   than only irradiated males ($z = 2.954$, $P = 0.00313$). However, no significant difference in male

380   survival was observed between irradiated only males (Irrd) and those exposed to the release process

381   for 5 or 60 minutes (**Figure 8A**). Under feeding conditions, males that were only irradiated (Irrd) died

382   significantly faster than males of the 5 min treatment group ($z = 2.90$, $P = 0.00369$). However, no

383   significant differences were observed between the irradiated only treatment (Irrd) and the 60m and

384   120m treatment groups (**Figure 8B**).

Discussion

386   The main objective of this laboratory study was to evaluate a new prototype of an automated chilled

387   adult release device (BSI™) to release sterile male tsetse flies from the air, firstly to assess its

388   functionality and suitability for the release of sterile tsetse flies at low density (i.e. 10 males /km^2) and

389   secondly, to assess the impact of the release process under chilled conditions on the quality of the

390 sterile males. Generally, during flight simulations in this study the BSI™ device released flies at a

391 consistent release rate (60±9.58 males/min for the speed of 0.6 rpm), demonstrating its functional

392 ability to homogenously release sterile tsetse flies in target areas (an important aspect of sterile insect

393 release devices) [31]. The accuracy and consistency may be attributed to its release mechanism that

394 consists of a rotating cylinder which rotates at a constant speed (0.6 rpm), allowing a loading of

395 similar number of flies per unit time into the cylinder. This mechanism is different from the ones used

396 in earlier release devices, i.e. a conveyor belt or a vibratory feeder used in the MSRM1 and MSRM2,

397 respectively [37]. Although, it is noted that the MSRM2 fulfilled the requirement of the tsetse

398 eradication program in Senegal for very low release rates (~50 flies/km^2) and was routinely used for

399 some time to replace the release cartons, the machine was still not able to achieve the lowest rate of

400 10 flies/km^2 without manipulation from the handlers and has been discontinued from use as previously

401 mentioned [37].

402 The BSI™ is controlled by Bluetooth® from a tablet computer that includes a completely automated

403 guidance and navigation system, providing the pilot of the release aircraft with the polygon areas and

404 the necessary data on the wild flies' distribution and density on the ground. The control system is also

405 able to display the release rates and the physical conditions in the machine such as temperature,

406 relative humidity, speed and vibration power of the shaker. The automated navigation system gives an

407 advantage in accuracy and homogeneity over earlier release systems such as the use of carton boxes

408 that was prone to human error [37]. The observed accuracy and consistent release rates can also be

409 attributed to the absence of clumping of flies in the machine during release regardless of the number

410 of flies loaded into the device (max. 2000 flies). Clumping was prevented by maintaining the flies in a

411 free-flowing granular state at a temperature of 6 ±1°C, an improvement as compared with the

412 previously used MSRM2 machine where clumping was a serious problem [37]. The BSI™ has several

413 advantages compared with the MSRM2 machine in terms of weight (20 kg versus 64 kg), power (2-

414 3A -12V versus 100 A), and it is less bulky. However, like the MSRM2 device, it can be easily

415 installed in a gyrocopter **(Figure 1b)**. These advantages of the BSI™ device in terms of its efficient

416 release mechanism, its automated navigation system, its low weight and power requirements and the

417 absence of clumping of the flies, will make it an attractive option for operational use in field release

418 programs. It can be operated solely by the pilot, and hence eliminates the necessity of a release

419 coordinator, reducing the costs of tsetse fly aerial release [37].

420 Generally, the results show that the combined effect of chilling and the mechanical abrasion

421 experienced by the flies when passing through the release device did not have a significant negative

422 impact on flight ability, relative mating index, premating and mating duration of the flies as compared

423 to the irradiated flies. In this case, the absence of a negative effect may have been contributed by

424 shorter chilling conditions that did not exceed three hours (including the handling procedure before

425 the longest release period). In addition, the insemination rates in female flies that had mated with

426 males that had been chilled in the machine for at least an hour before being released, were not

427 significantly reduced. This does not corroborate the results of the Mutika et al [43] study that

428 simulated long distance transport of pupae and the release of *G. p. gambiensis* as chilled adults (pupae

429 stored for 5 days at 10°C and sterile males stored up to 30 h at 5.1 ± 0.4°C), i.e. prolonged chilling of

430 adults affected the biological quality of the flies, and the study recommended that the duration of

431 chilling should be minimized. The significant reduction in flight ability regardless of the chilling and

432 release process) of irradiated males as compared with the control males (not irradiated, not passed

433 through the machine indicates a significant negative impact of irradiation on the released males. These

434 results are in agreement with many previous studies that report a dose dependent negative impact of

435 irradiation on male insect quality [58–60]. In addition, these results are in agreement with those of

436 Diallo et al. [61], who found that irradiation combined with chilling conditions increased flight

437 propensity in comparison with irradiation alone. Overall, our study further agrees with that of Mutika

438 et al [44] on the combined effect of irradiation and chilling in that a significantly lower proportion of

439 sterile males stored at low temperatures succeeded in securing mates compared with untreated males.

440 Generally, irradiation only did not affect the survival of starved male flies but the release process at

441 120 min significantly reduced their survival. The lower survival observed in our study is in agreement

442 with Mutika et al. which clearly stated that the longer the adults are kept at the low temperatures, the

443 lower their biological quality [43]. Also, this result agrees with the study of Diallo et al. [61] in which

444   starved sterile males that emerged from chilled pupae showed an average survival of 4-5 days.

445   Similarly, under feeding conditions, the males released after a short duration within the machine (5

446   min) died significantly faster than the irradiated (Irrd) and non-released males (control). Surprisingly,

447   males released after a longer time in the machine survived better, which might be due to a reduced

448   metabolic rate due to the chilling that could have stimulated cellular repair mechanisms of the somatic

449   damage [61].

450   Despite the encouraging results, the BSI™ has limitations that ideally need to be improved. First,

451   there were errors in fly counts that were related to more than one fly at a time being loaded into the

452   cavity of the rotating cylinder. This can somehow be corrected as the BSI™ software allows a factor

453   to be included to correct errors in counts (as displayed on the navigation page during the flight

454   simulations). This error might be related to the cavity size and tests should be conducted to assess the

455   effect of different (reduced) sizes of the cavity to receive only one fly at a time. However, even after

456   the correction of errors the software estimated lower maximum counts (ca. 1598 (**Figure 3A**)),

457   compared with the 2000 flies loaded into the machine. This estimation may be improved by

458   implementing the statistical model used in this study (**Figure 3B**) to give more accurate predictions of

459   the real counts. Additionally, handling (counting, sorting) of flies before the release process could

460   have contributed to the overall performance of the flies because of the increased length of time under

461   low temperatures. In future, with new technologies that are currently under research [62,63], male and

462   female pupae can be separated on day 23-24 post larviposition, hence eliminating the counting and

463   sex sorting steps before loading into the BSI™ as done in this study as well as the transport under

464   chilling which was not part of this study. In addition, the counting could also be eliminated by using

465   weight or volume to estimate the number of flies, especially where large numbers of flies will need to

466   be loaded into the machine during SIT programs.

467   The sterile males released in Senegal are produced in tsetse mass-rearing facilities located in other

468   countries (i.e, The Slovak Academy of Sciences, Slovakia, the IPCL, Seibersdorf, Austria and the

469   Insectary of Bobo Dioulasso (IBD) and CIRDES, Bobo Dioulasso, Burkina Faso) and require

470   transport under chilled conditions (10°C) to prevent emergence during shipment [29,41,61,64].

471   Although in this study the male pupae were chilled at 10°C for 1-2 hours before irradiation, this

472   treatment did not accurately mimic what occurs in the operational release program in Senegal, i.e. the

473   pupae in the study were not packaged or shipped and the chilling time of the adult flies at 10°C was

474   relatively short. Further studies are needed to assess the combined effect of chilling, packaging and

475   shipment with the release process using the BSI™. In addition, the distribution and recapture of

476   released males under field condition remains to be assessed.

477   In conclusion, in an effort to expand the repertoire of machines available for tsetse release in the field

478   and improve previously tested release mechanisms, our results show that the BSI™ has great potential

479   that merits consideration for use in the current SIT release program in Senegal.

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Author Contributions

490   Conceived and designed the experiments: AMMA AGP MJBV JB JBr. Performed the experiments:

491   CKM GNM. Analyzed the data: AMMA AGP JB GNM. Contributed reagents/materials/analysis

492   tools: CKM AMMA AGP MJBV JB. Wrote the paper: CKM GNM AMMA AGP MJBV JB EB JB

493   MTS BS MVO.

Conflicts of interest statement

495   Jimmy Bruno designed the release system (Bruno Spreader Innovation, (BSI™) and works for his

496   own enterprise Jimmy Bruno, AEWO (aerial works).

497   **Availability of data and materials**

498   Materials described in the manuscript, including all relevant raw data, are available in this link

499   https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/AQIW8P .

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681

682   **Figure Legends**

683   **Figure 1.** The BSI™ a: images showing (i) top view of the open BSI™ showing the storage funnel

684   where tsetse flies are held at 6°C, (ii) 3-dimention technical drawing showing different parts on the

685   BSI release machine. b: a gyrocopter carrying the BSI release machine in field operation.

686   **Figure 2:** Effect of motor speed and initial fly number on the error rate of the BSI™. a: Impact of

687   motor speed (rpm) on the error rate during counting of the sterile males released by the BSI™. b:

688   Impact of initial number of flies on the error rate during the counting of sterile males released by the

689   BSI™ at the lowest speed (0.6 rpm). The graphs represent the minimum, first quartile, median, third

690   quartile and maximum for each treatment. Values indicated by the same lower-case letter do not differ

691   significantly at the 5% level.

692   **Figure 3:** Consistency of release rate of BSI™ a: Cumulative count of flies (manual, actual counts

693   and estimated counts by the machine) over time at the lowest speed (0.6 rpm). Comparison of the

694   recorded release rate against the actual rate (by hand count) b: The release consistency of flies per

695   minute, c; Prediction of manual counts from estimated counts using a linear model.

696   **Figure 4:** Impact of the release process through the BSI™ on male flight propensity. The graph

697   represents the minimum, first quartile, median, third quartile and maximum for each treatment. Values

698   indicated by the same lower-case letter do not differ significantly at the 5% level.

699   **Figure 5:** Impact of the release process through the BSI™ on male relative mating index (RMI). This

700   graph represents the minimum, first quartile, median, third quartile and maximum for each treatment.

701   Values indicated by the same lower-case letter do not differ significantly at the 5% level.

702   **Figure 6:** Impact of the release process through the BSI™ on male premating period (a) and mating

703   duration (b). The graph represents the minimum, first quartile, median, third quartile and maximum

704   for each treatment.

705   **Figure 7**. Impact of the release process through the BSI™ on the insemination rate of mated females

706   a: Mean spermatheca value, b: distribution of spermatheca fill.

707   **Figure 8:** Impact of the release process through the BSI™ on *Glossina palpalis gambiensis* male

708   survival under starvation (A) and feeding (B) conditions.

709

710 **Table 1**: Averages (±SE) counts of calibration of the BSi release machine using batches of 480, 1000 and 2000 *G. palpalis gambiensis* males

Speed (rpm)	0.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6.0
counts ±SE (480)	252.7 ± 7.2	224.7 ± 4.4	213.7 ± 4.3	218.7 ± 6.1	199 ± 8.5	190 ± 3.1	186.7 ± 5.8	190.7 ± 5.8	175.7 ± 8.8	159.7 ± 2.9
% error	47.4 ± 1.5	53.2 ± 0.9	55.5 ± 0.9	54.4 ± 1.2	58.5 ± 1.9	60.4 ± 0.8	61.1 ± 1.2	60.3 ± 1.3	63.4 ± 1.9	66.7 ± 0.8
counts ±SE (1000)	520.7 ± 6.6	476.3 ± 4.3	474.3 ± 19.6	428.3 ± 6.4	398.7 ± 10.8	380.3 ± 12.7	353.7 ± 13.0	348.3 ± 9.0	337.7 ± 9.1	319 ± 7.0
% error	47.9 ± 0.7	52.4 ± 0.2	52.6 ± 2.2	57.2 ± 0.7	60.1 ± 1.1	62 ± 1.2	64.6 ± 1.1	65.2 ± 0.6	66.2 ± 0.2	68.1 ± 0.7
counts ±SE (2000)	1082.7 ± 7.2	1009 ± 28.3	941 ± 30.0	871.7 ± 22.7	847.3 ± 3.2	823 ± 38.8	749.3 ± 11.9	755.7 ± 5.8	752.7 ± 16.9	695.7 ± 8.7
% error	45.9 ± 0.4	49.6 ± 1.4	53 ± 1.5	56.4 ± 1.1	57.6 ± 0.2	58.9 ± 1.9	62.5 ± 0.6	62.2 ± 0.3	62.4 ± 0.8	65.2 ± 0.4

711 *Each combination of density and speed was technically replicated three times.

712

713 **Table 2**. The mean (±sd) response variables for the flight propensity test and mating
714 competitiveness observations. 5m, 60m, and 120m: irradiated males held chilled for 5 min, 60
715 min and 120 min, respectively before passing through the BSI release machine, Irrd: irradiated
716 males but not passed through the BSI release machine and Control: non-irradiated males from
717 the colony.

	5m	60m	120m	Irrd	Control
Flight propensity (%)	77.8±8.61	75.7±7.2	73.9±12.1	77.2±6.3	88.3±7.3*
Relative mating Index	0.46±0.14	0.46±0.11	0.41±0.10	0.39±0.16	0.56. ±0.14
Premating time (minutes)	45.00±39.00	33.00±30.00	37.00±35.00	45.00±39.00	40.00±34.00
Mating duration (minutes)	81.00±31.00	71.00±30.00	72.00±27.00	70.00±28.00	80.00±30.00*
Spermathecal Fill	1.53(±0.78)	1.36(±0.80)	1.35(±0.84)	1.53(±0.79)	1.54(±0.73)

718 *: Treatment with significant difference from other treatments

i

ii

Refrigerated air

Temperature sensor

Fridge

Tank

Vibration motor

Tank support

Presence cells

Spacer

Stepper motor

Brush

Wheel rotating

Relative humidity and temperature sensor

Ambiant air

Compting cell
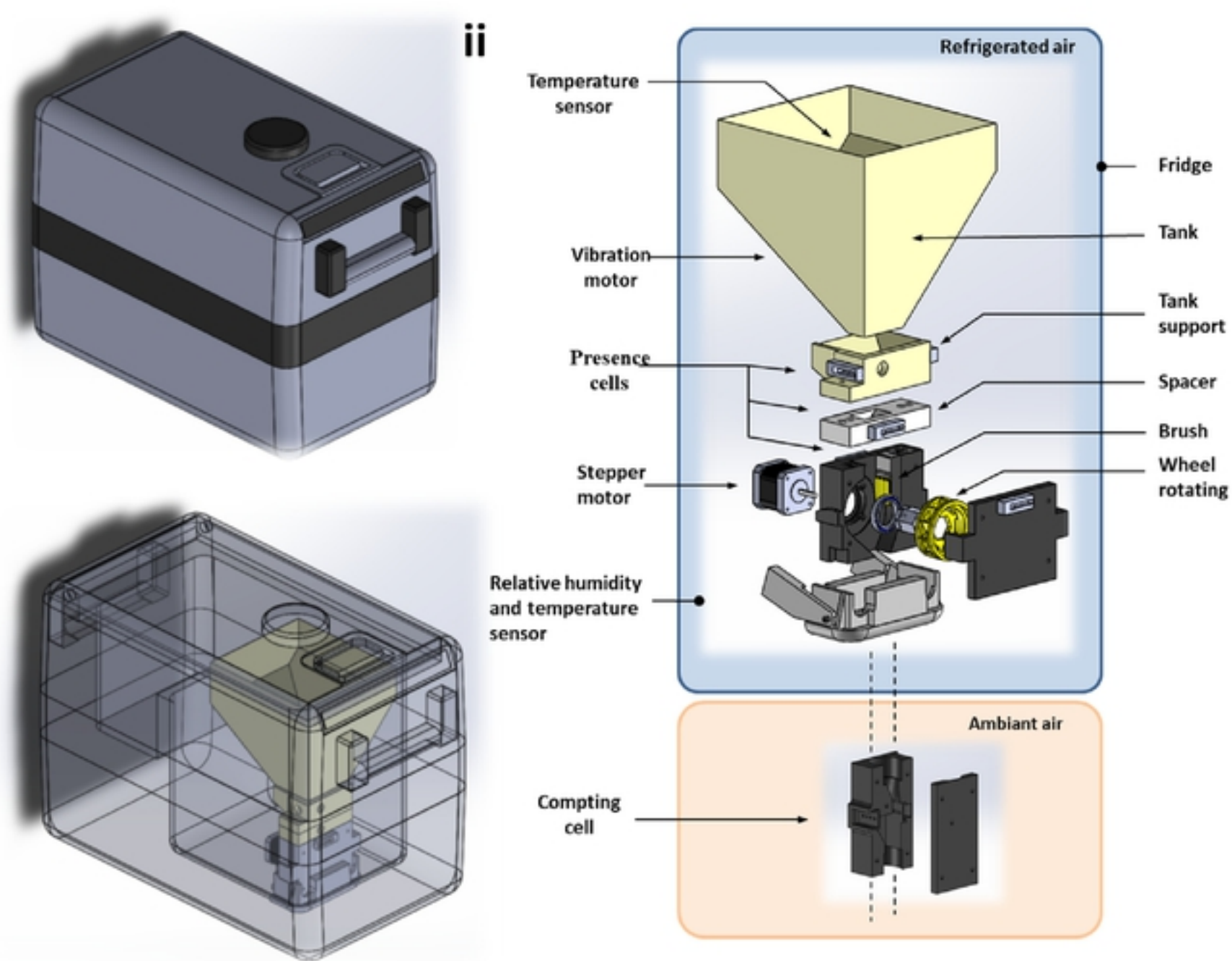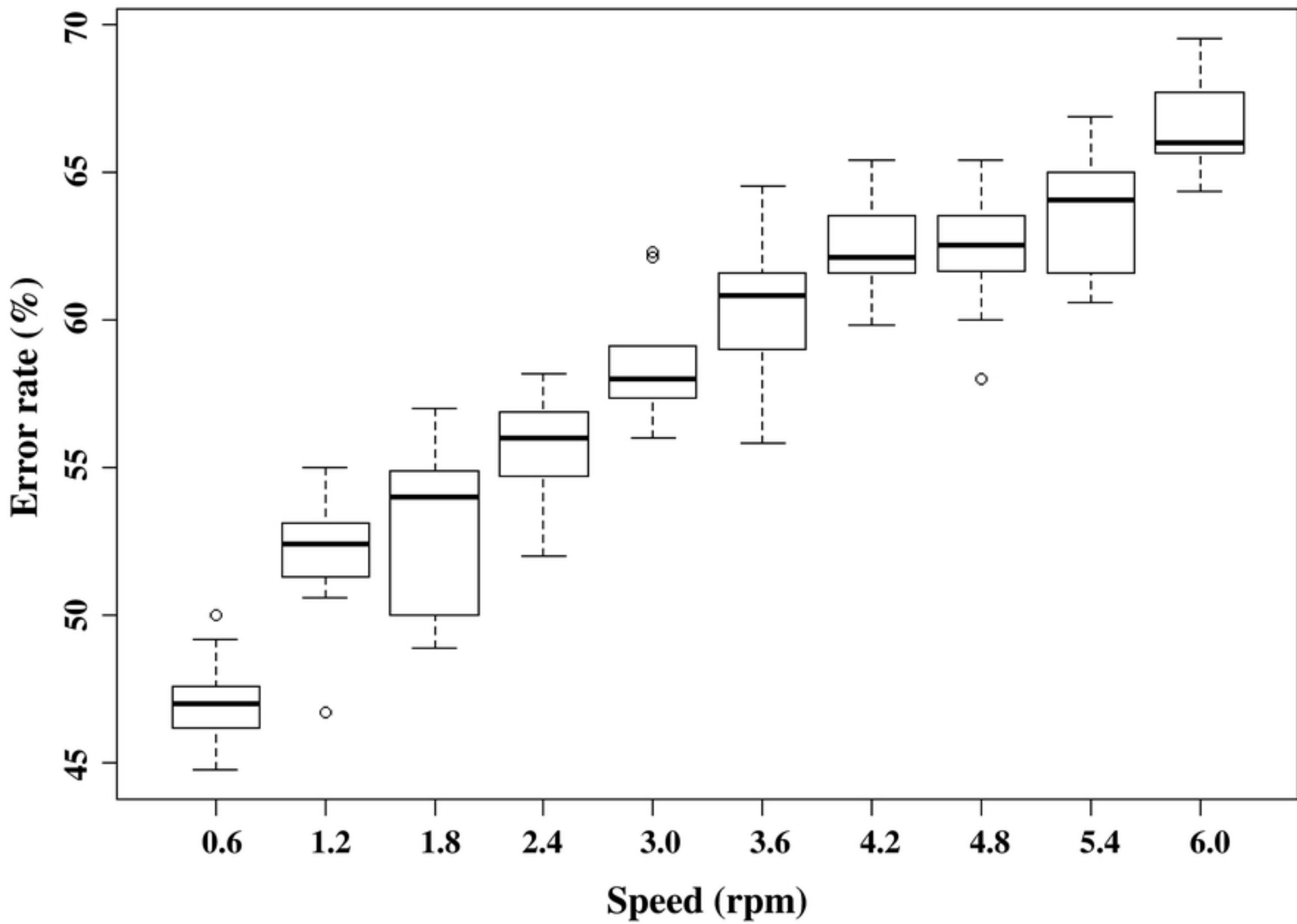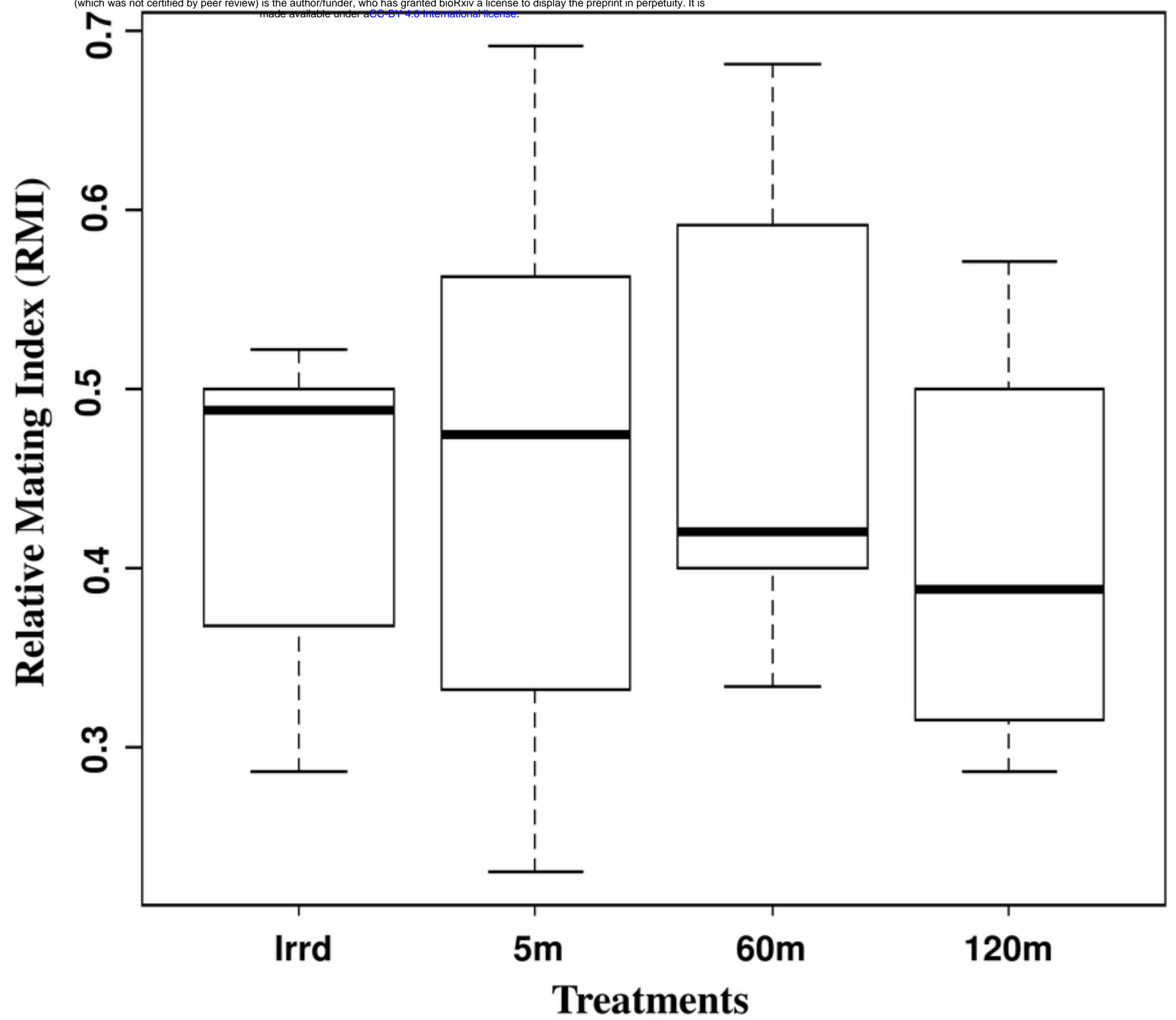
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Figure 1b

Figure 2a
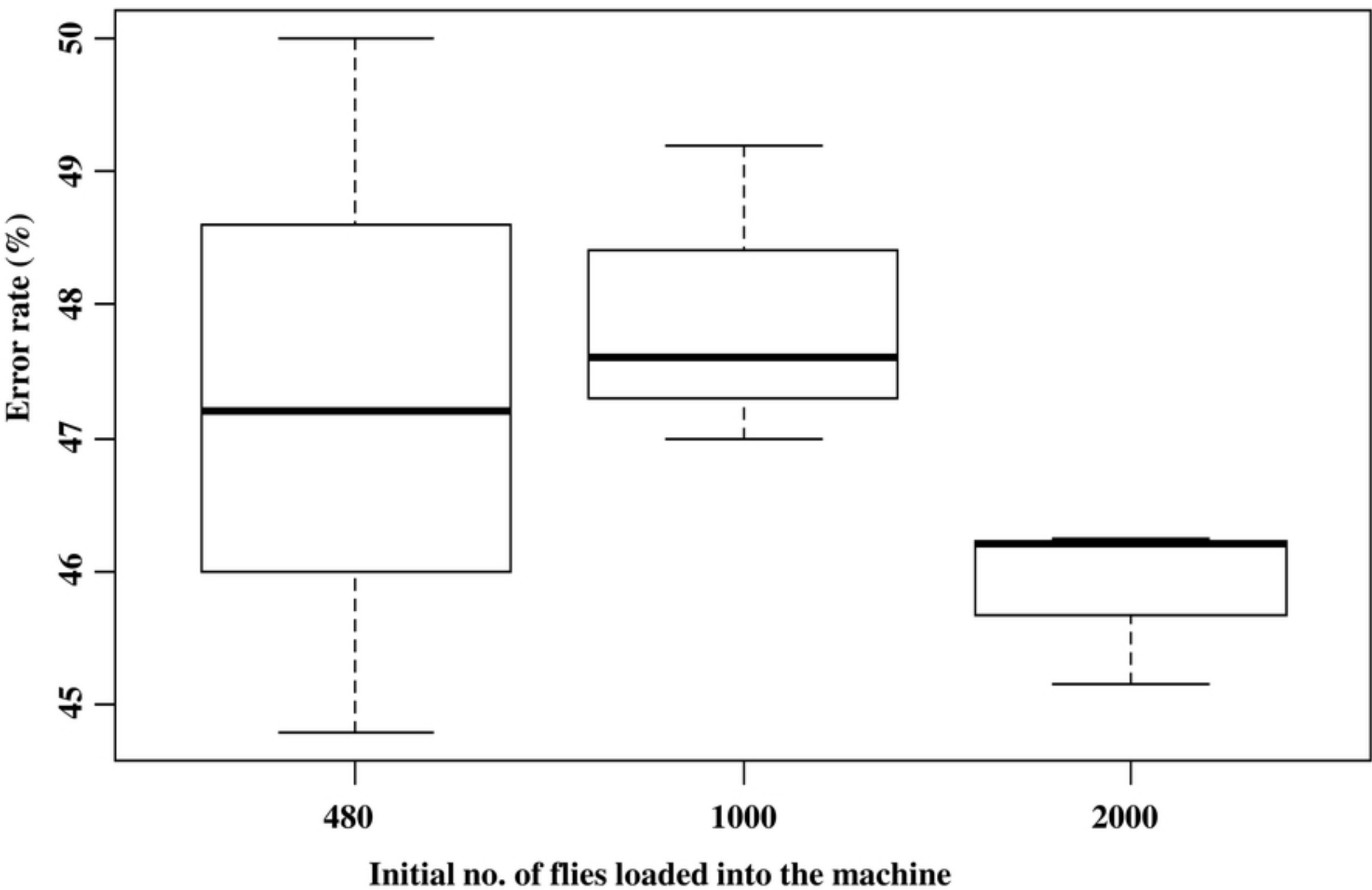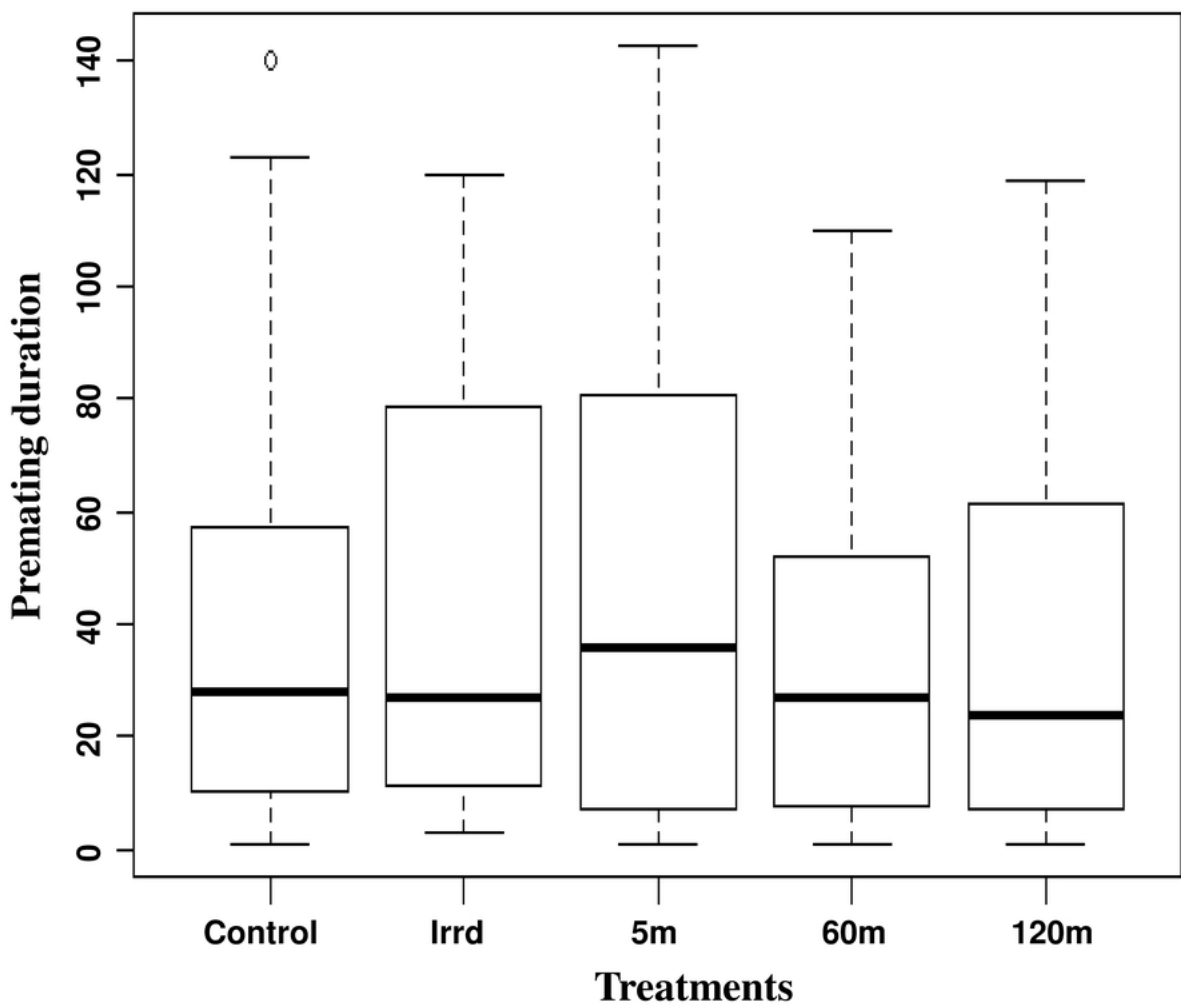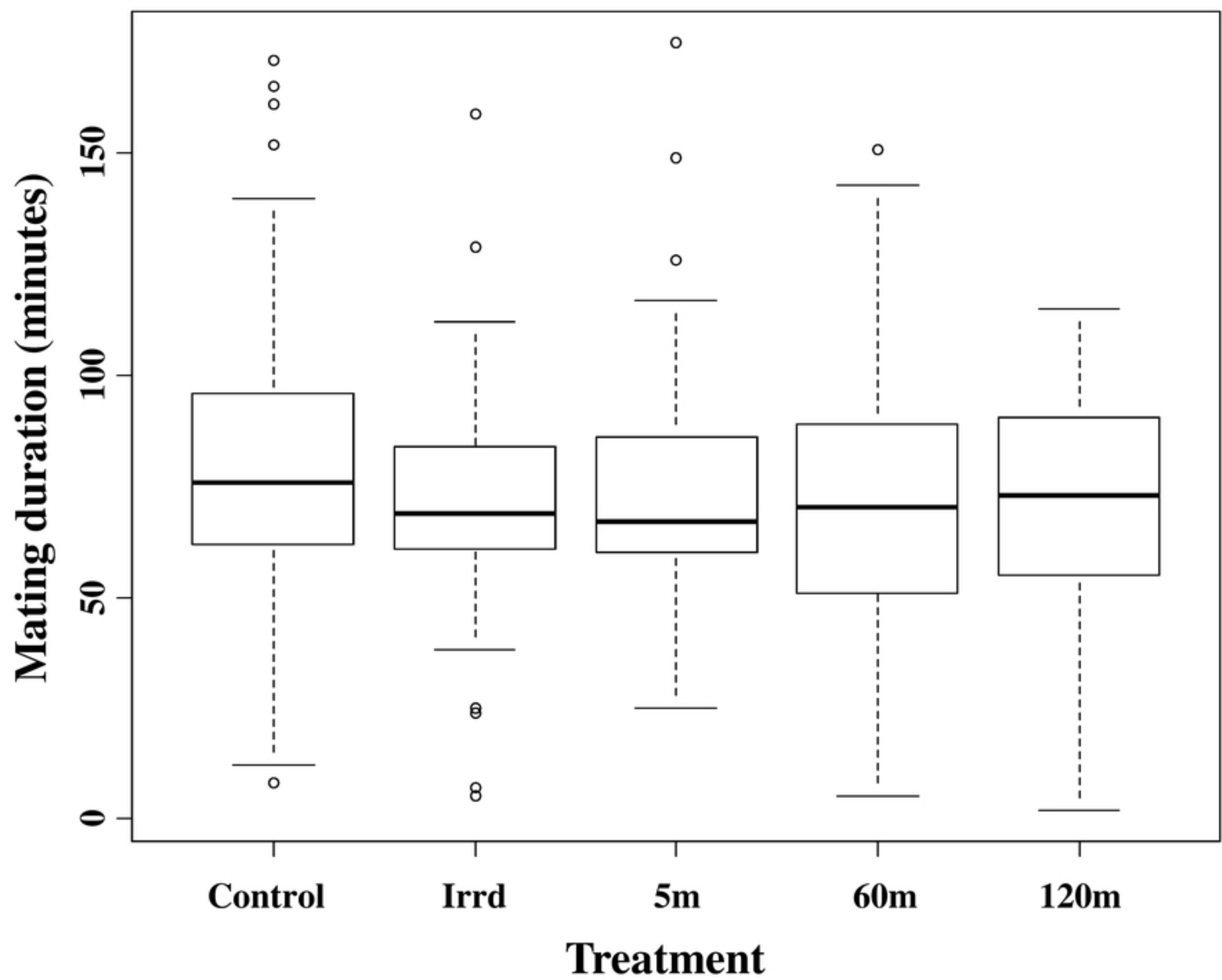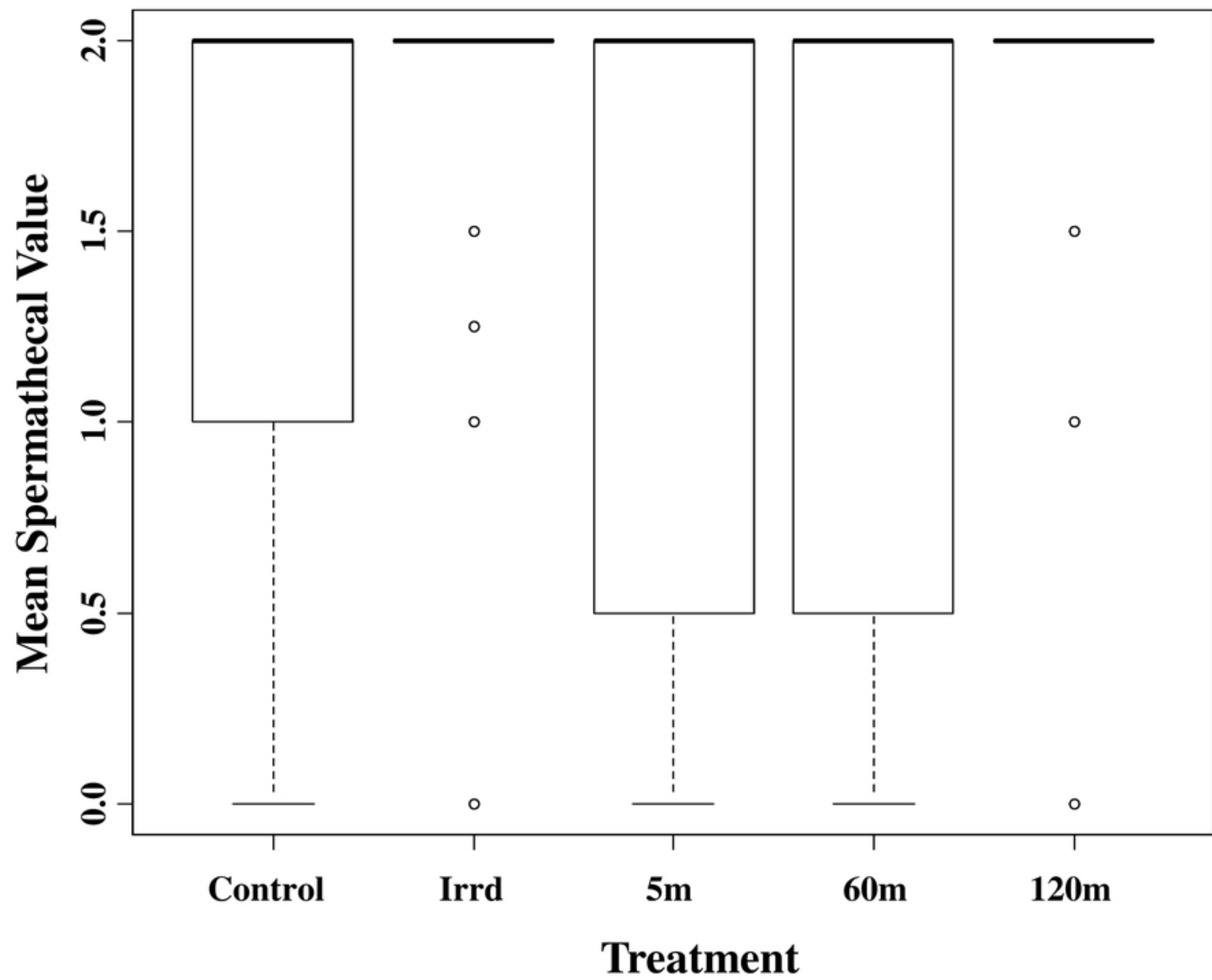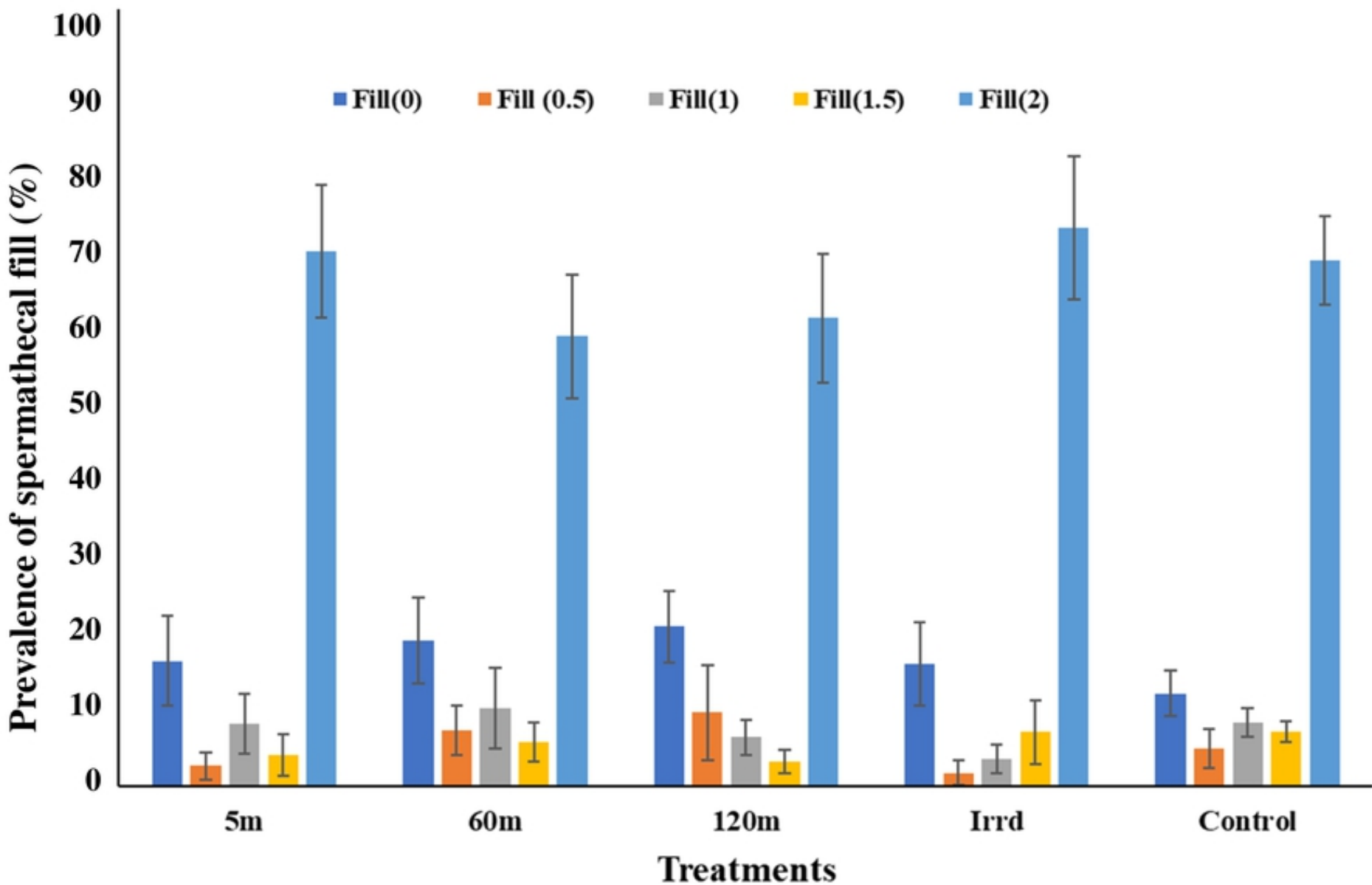
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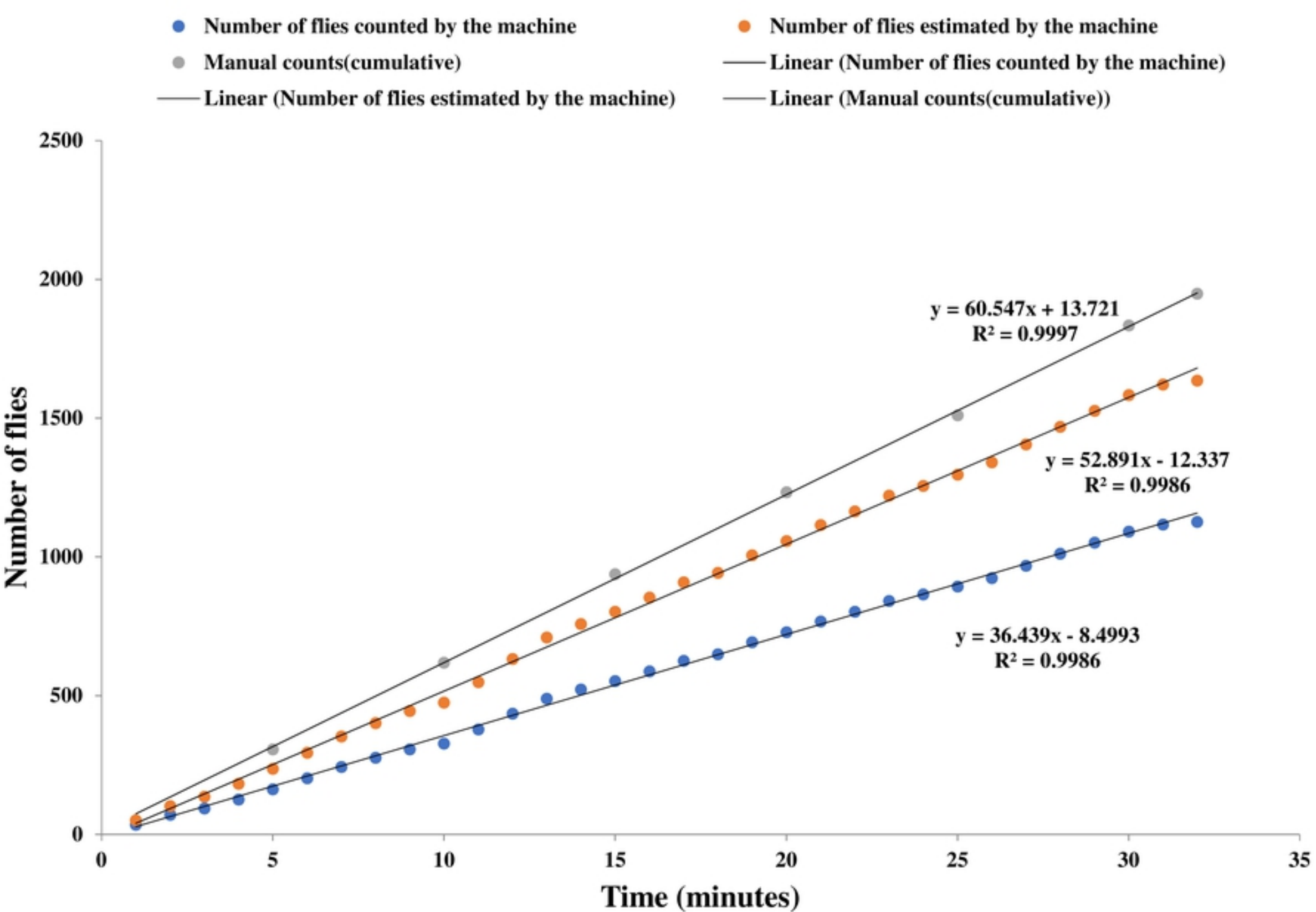
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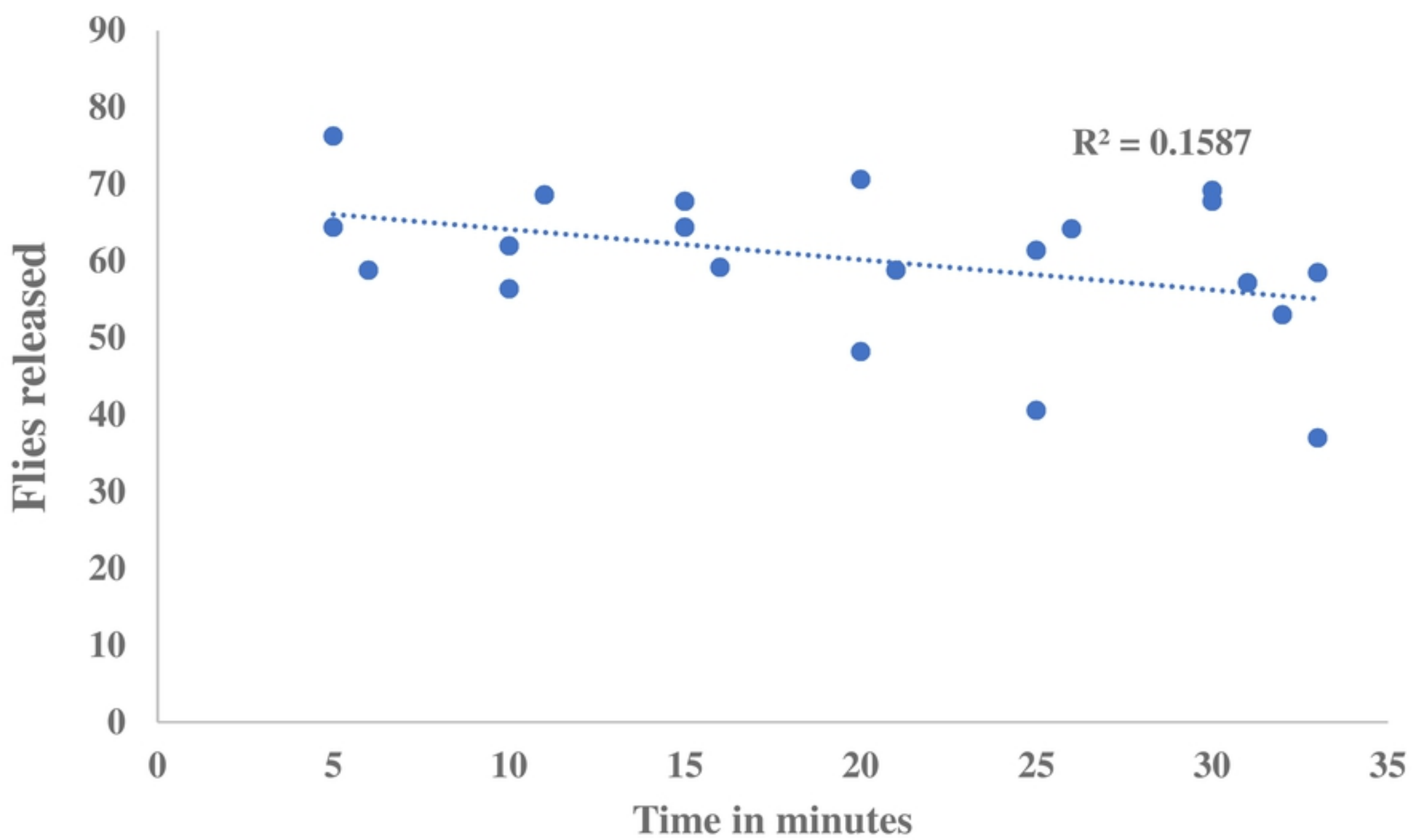
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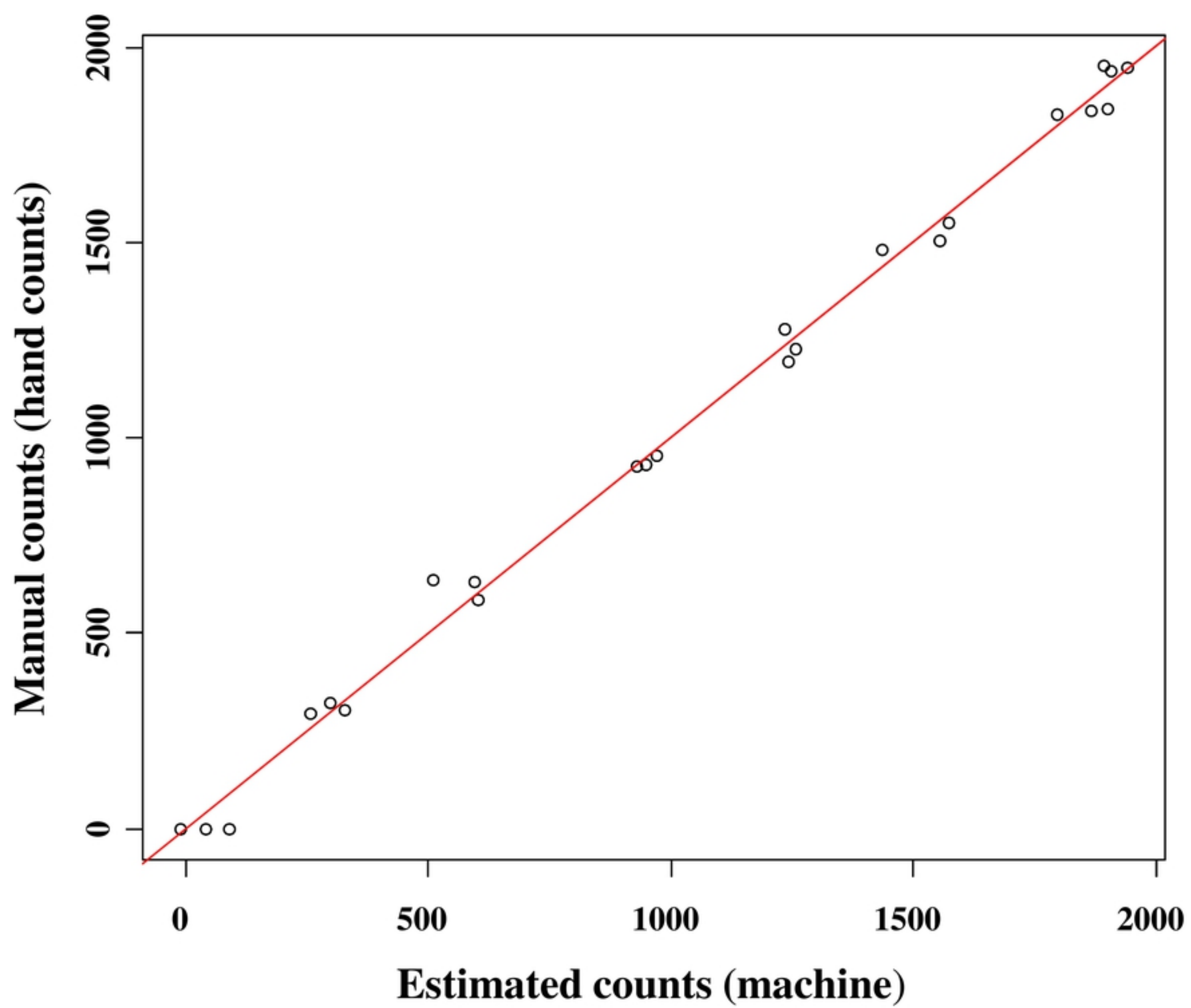
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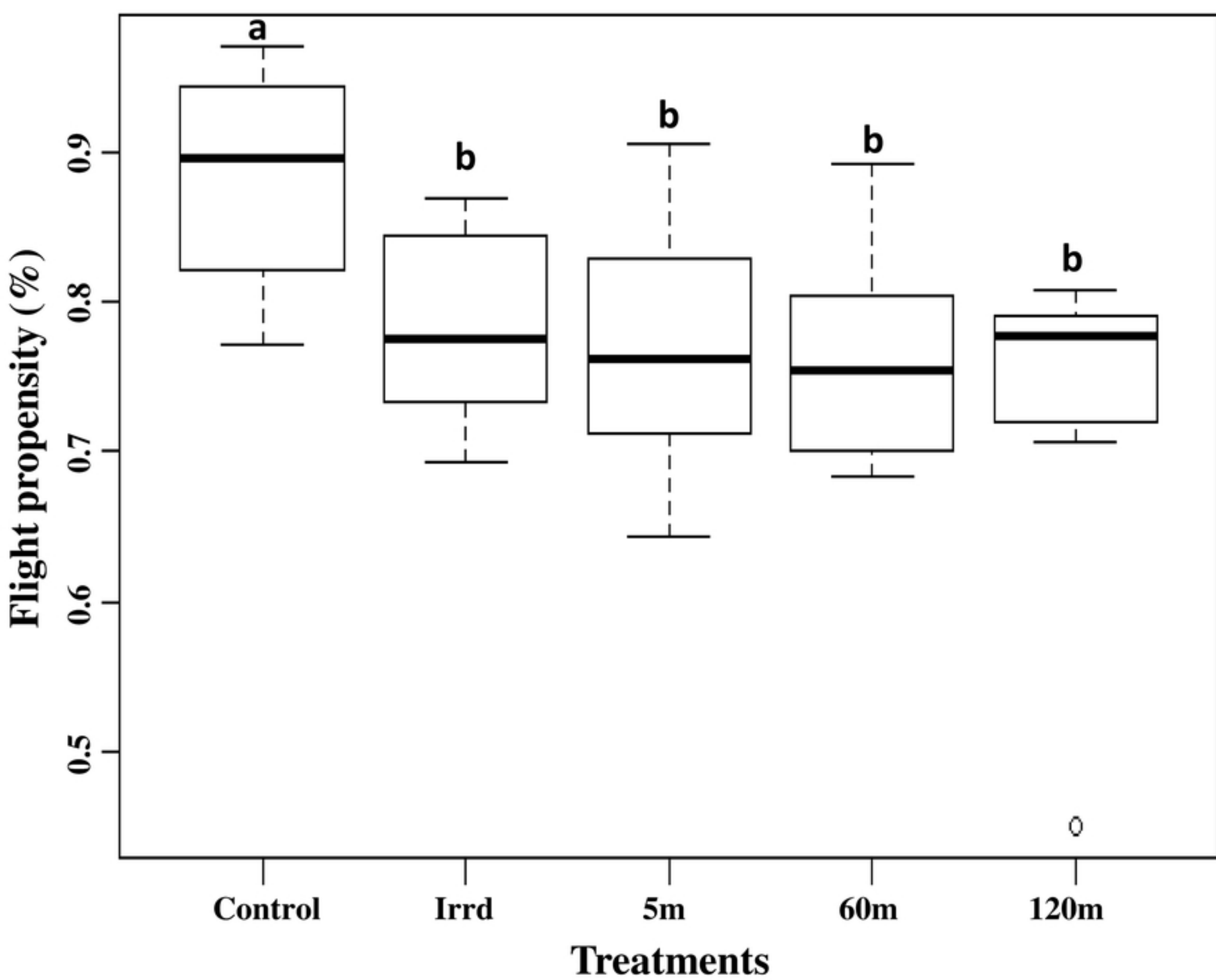
Figure 4

Figure 5

Figure 6a

Figure 6b

Figure 7a

Figure 7b

Figure 8a

Figure 8b