

1 **Husbandry and Maintenance of *Carausius morosus* Laboratory Populations**

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18

19 **Abstract**

20 *Carausius morosus*, the Indian stick insect, is a slender twig-like insect endemic to India.  
21 Though widely introduced through captivity around the world and commonly used in  
22 laboratories or kept as a household pet, standardized animal husbandry laboratory  
23 protocols are lacking. Here we report detailed laboratory culture conditions for *C.*  
24 *morosus*. We maintain stocks at 23 °C, 70% relative humidity, and a 12:12 hour light-dark  
25 photoperiod. This culture has been successfully sustained under these conditions for over  
26 two years, with standardized protocols in place for dietary and cage setup conditions. We  
27 also report methods for egg and hatchling care to support ongoing experiments with *C.*  
28 *morosus*. These standardized methods improve reproducibility and accessibility, enabling  
29 the broader use of *C. morosus* as a laboratory model system for developmental,  
30 behavioral, and physiological studies.

31

32 **Summary**

33 This paper outlines detailed protocols for maintaining a *Carausius morosus* laboratory  
34 colony, including key procedures for animal husbandry, egg and hatchling care, and an  
35 overview of the species lifespan and biological characteristics.

36

37 **Keywords:** Phasmatodea, stick insect, embryogenesis, fecundity, culture methods,  
38 parthenogenesis

39

## 40 **Introduction**

41           As interest grows in alternative non-mammalian model systems, insects offer an  
42 opportunity to explore a wide range of biological diversity while also providing lower-cost  
43 research models, simpler care requirements, and fewer regulatory barriers than  
44 mammalian models <sup>1</sup>. Emerging insect model systems make it possible to investigate  
45 unique biological phenomena, including for example parthenogenesis in stick insects like  
46 *Carausius morosus* <sup>2</sup>. While the fruit fly *Drosophila melanogaster* has shaped our  
47 understanding of developmental and genetic processes since the early 20th century <sup>3</sup>,  
48 other insect model systems such as *C. morosus* present a unique opportunity not only for  
49 developmental biology, but also for investigating motor control, locomotion, and  
50 neurohormone-regulated systems <sup>4</sup>. Additionally, within the field of evolutionary  
51 developmental biology, hemimetabolous (directly developing) insects remain  
52 underrepresented, with the holometabolous (indirectly developing, or undergoing  
53 metamorphosis) *D. melanogaster* serving as the most well-studied model insect. Studying  
54 directly developing insect species like *C. morosus*, which undergoes several nymphal  
55 instar stages before reaching sexual maturity <sup>5</sup>, allows us to fill key phylogenetic gaps in  
56 evolutionary developmental biology research.

57           *C. morosus* are easy to rear in captivity, making them well-suited for both  
58 laboratory research and teaching environments. Their ability to reproduce  
59 parthenogenetically <sup>6</sup> eliminates the need for complex breeding setups, reducing  
60 maintenance demands and simplifying population management. Although successful  
61 rearing benefits from controlled environmental conditions, its husbandry remains  
62 straightforward and cost-effective compared to many other laboratory models. *C.*

63 *morosus* feeds readily on common plant material such as ivy (*Hedera helix*), bramble  
64 (*Rubus fruticosus*), or rose leaves (*Rosa* spp.).

65           Standardized rearing protocols for *C. morosus* are critical for producing consistent  
66 and reproducible results in research studies. To our knowledge, no in-depth protocols for  
67 rearing *C. morosus* in the laboratory have been published to date. In our laboratory, we  
68 have successfully established a comprehensive set of standardized protocols for *C.*  
69 *morosus* laboratory cultures including egg, hatchling, and reproductively mature adult  
70 rearing. With these methods, we aim to make this insect more accessible to the broader  
71 biological research community.

72

73

74 **Protocol**

75

76 **Method 1 - Environmental Rearing Conditions**

77

78 1. The laboratory culture should be maintained in a climate-controlled room or  
79 incubator with environmental settings as follows:

- 80 ● 23 °C
- 81 ● Relative humidity 70 %
- 82 ● Light-Dark photoperiod of 12:12 hours

83

84 **Method 2 - Establishment and Maintenance of Mesh Enclosures**

85

86 **NOTE:** Mesh insect enclosures with fabric mesh ventilation on three sides are  
87 recommended for housing *C. morosus*. An enclosure measuring 24 × 24 × 36 inches is  
88 suitable for maintaining up to 50 adults or, when establishing a new colony, up to 500  
89 hatchlings.

90

- 91 1. Select a mesh enclosure of appropriate size. Ensure the container is clean and  
92 free of debris before introducing the animals.
- 93 2. For establishing a new colony using mature adults, place 25-50 adult females into  
94 a clean 24 × 24 × 36-inch mesh enclosure.
- 95 3. For the establishment of a new colony using hatchlings, add no more than 500  
96 hatchlings into a clean 24 × 24 × 36-inch mesh enclosure.

97

98 4. **Feeding and Maintenance:**

- 99 - 4.1 - Provide fresh foliage such as ivy, bramble, or rose once per week. Ensure all  
100 plant material is pesticide-free.
- 101 - 4.2 - Trim stems to fit vessels (small jars or bottles) filled with tap water to maintain  
102 leaf hydration.
- 103 - 4.3 - Place the prepared foliage inside the enclosure, making sure leaves are  
104 elevated and accessible to the insects. Carefully close the enclosure after  
105 placement.
- 106 - 4.4 - During weekly cage cleanings, remove wilted or dried foliage and any  
107 accumulated excrement from the bottom of the enclosure.

108

109 **NOTE:** Maintaining a clean environment is essential for colony health and well-being.

110

- 111 - 4.5 - Lightly mist each enclosure daily with 1-2 sprays of tap water from a spray  
112 bottle to ensure proper hydration.

113

114 **Method 3 - Collection and Maintenance of *C. morosus* Eggs**

115

- 116 1. Open an enclosure containing adult females.
- 117 2. Using a gloved hand, gently sweep the substrates on the bottom of the cage, which
- 118 contain both eggs and fecal matter, towards the front opening.
- 119 3. Carefully transfer the material using a gloved hand to a 15 cm petri dish for egg
- 120 retrieval.

121

122 **4. Egg Retrieval**

- 123 - 4.1 - Using blunt plastic forceps, carefully pick up each egg and place it into a clean
- 124 6 cm petri dish.

125

126 **NOTE:** Mature eggs are protected by a hardened exochorion and are dark brown,

127 resembling plant seeds (Figure1).

128

- 129 - 4.2 - Label the dish with the date and time of collection for tracking purposes.
- 130 - 4.3 - A 6cm petri dish is sufficient for storing up to 200 eggs. Keep the lid off during
- 131 incubation to allow ventilation.
- 132 - 4.4 - Approximately one week before the expected hatching window (74-80 days
- 133 after egg laying; data not shown), replace the Petri dish cover.
- 134 - 4.5 - Begin daily monitoring of egg containers before the expected hatching date
- 135 to observe emerging hatchlings.

136

137 **NOTE:** Under the laboratory conditions described in **Method 1**, approximately 60% of

138 eggs are expected to hatch between 74 and 80 days after being laid (data not shown)

139

140 **Method 4 - Collection and Maintenance of *Carausius morosus* Hatchlings**

141

- 142 1. Gather 6 cm petri dishes with newly emerged hatchlings.
- 143 2. Using blunt plastic forceps, gently transfer no more than 500 hatchlings into a clean
- 144 24 × 24 × 36-inch mesh enclosure.
- 145 3. Provide fresh foliage as described in **Method 2. 4.1 - 4.3.**
- 146 4. Label new colony enclosures with the following information:
- 147 ● Egg batch collection date(s)
- 148 ● Date of hatching or range of hatching dates
- 149 ● Number of hatchlings added

150

151 **NOTE:** This tracks enclosure age and predicts the start of reproductive maturity.

152

153 5. Lightly mist each enclosure daily with 1–2 sprays of tap water from an 8oz spray  
154 bottle to ensure proper hydration.

155

156 **NOTE:** Hatchlings may remain in the same enclosure throughout development until  
157 adulthood.

158

159 **NOTE:** Under the laboratory conditions described in **Method 1**, approximately 20% of  
160 hatchlings survive to adulthood in 4-5 months (data not shown).

161

162 6. Remove any dead or damaged hatchlings during weekly cage cleaning to prevent  
163 contamination.

164

### 165 **Method 5 - Life Expectancy**

166

167 1. Post-hatching, individuals undergo five to six molts before reaching reproductive  
168 maturity, which under the laboratory conditions described in **Method 1**, happens  
169 around 200 days after hatching (data not shown).

170 2. Total generation time from egg laying to sexual maturity is approximately 9.5  
171 months (data not shown).

172

### 173 **Method 6 – Termination of Insects and Disposal of Enclosure Waste**

174

175 When *C. morosus* are no longer needed for experiments, have died, or eggs are not  
176 required:

177 1. Remove insects and any waste materials from the enclosures. Place all  
178 contaminated materials into an autoclavable waste receptacle.

179 1. Store the waste in a -20°C freezer for at least 72 hours.

180 2. After freezing, transfer the autoclavable waste bag to a biohazard bin for proper  
181 disposal.

182

183

## 184 **Representative Results**

185 We collected data documenting successful laboratory rearing of *C. morosus* from  
186 eggs to reproductively mature adults between September 2024 and November 2025. We  
187 established a laboratory culture stock during this period from eggs following husbandry  
188 steps in **Method 4.1- 4.6** of this protocol in a climate-controlled room with a temperature  
189 of 23 °C, 70% relative humidity, and a 12:12 light-dark photoperiod. Over this 14 month  
190 period, we established, maintained and monitored five cages for adult mortality, eggs laid,  
191 timing of egg hatching, and hatchling survival to adulthood.

192 Data shown in Figures 2 and 3 present observations from the five monitored cages  
193 from setup to adulthood of hatchlings, including the eggs laid and adult mortality for each  
194 cage. After the founder individuals in each cage reached sexual maturity and began laying  
195 eggs, we recorded the number of adults present in each cage to establish the starting  
196 population at the onset of oviposition. Numbers of adults in each cage over time for each  
197 cage is shown in **Figure 2** and Table 1. We interpret the steady decline in survival as  
198 age-related mortality.

199 We monitored egg production in parallel in the same five cages monitored for adult  
200 survival (Figures 2, 3). Daily egg counts per cage were highest during the first 30-50 days  
201 of egg-laying, and decreased gradually over time. Although total egg production varied  
202 among cages (Table 2), this general pattern was consistent across all five replicate cages.  
203 We monitored egg hatching dynamics over a 50-day period for 105 egg batches (Figure  
204 1d), where each egg batch is a group of eggs oviposited in the same cage within a 24-  
205 hour period. We found that once the first egg of a batch had hatched, 90% of eggs from  
206 that batch hatched within 12 days of the emergence of the first hatchling (Figure 1d),

207 independent of batch size (Figure 1b). Using the term “hatch completion” to describe the  
208 proportion of eggs in a batch that have hatched by a given time point following the  
209 emergence of the first hatchling from a batch, we note that the distribution of egg batches  
210 by the day of 90% hatch completion indicates that most batches reached this threshold  
211 between hatching days 10 and 14 (Figure 1c) after emergence of the first hatchling.

212

## 213 **Discussion**

214 The husbandry methods detailed here offer a straightforward and reproducible framework  
215 for maintaining robust laboratory populations of *C. morosus*. Although *C. morosus* has  
216 long been kept in laboratories or as domestic pets, laboratory research husbandry  
217 protocols have varied across studies <sup>6, e.g. 7–10</sup>. Ensuring consistent environmental  
218 conditions and feeding practices is crucial for making *C. morosus* a reliable laboratory  
219 model organism for developmental, physiological, and evolutionary research. Our method  
220 supports long-term colony maintenance without signs of disease, crowding-related stress,  
221 or reproductive decline. Over more than two years of continuous culture, we observed no  
222 obvious pathogen-related mass deaths, and egg and hatchling production remained  
223 stable across generations.

224

### 225 *Developmental Timing in C. morosus Relative to Other Model Insects*

226 Compared to holometabolous insects such as the fruit fly *Drosophila melanogaster*, *C.*  
227 *morosus* exhibits slower developmental rates and a more prolonged juvenile phase.  
228 Whereas *D. melanogaster* completes embryogenesis in ~24 hours and reaches  
229 reproductive maturity within 10-12 days at 25°C <sup>11</sup>, under our rearing conditions *C.*

230 *morosus* requires 74-80 days for embryogenesis and an additional ~200 days to reach  
231 reproductive maturity at 23°C (data not shown). This extended timeline reflects  
232 fundamental physiological and evolutionary differences between hemimetabolous and  
233 holometabolous insects <sup>12</sup>, and is repeatedly found across the Phasmatodea <sup>13-15</sup>. While  
234 other hemimetabolous insects such as the cricket *Gryllus bimaculatus* complete  
235 embryogenesis and reach sexual maturity in a shorter timeframe (ten days and six weeks  
236 respectively at 29°C <sup>16</sup>), the slower development of *C. morosus* provides a unique  
237 opportunity for reproducible research and detailed, longitudinal studies of embryogenesis  
238 and juvenile development.

239

#### 240 *Colony Size, Survival Rates, and Adult Reproduction*

241 Proper colony setup and careful management of population density were key to keeping  
242 the stocks healthy. Maintaining a maximum of approximately 500 hatchlings or 50 adults  
243 per 24 × 24 × 36-inch enclosure ensured stable conditions, a healthy colony and  
244 reproducible developmental observations.

245 Comparing average daily mortality with the number of founding adults in a cage  
246 (Figure 4a; Table 1) revealed no predictable relationship between these two parameters.  
247 Similarly, there was not a strong predictive relationship between total number of founding  
248 adults and total egg production (Figure 4b; Tables 1, 2). Instead, cages with lower  
249 average daily adult mortality showed a mild tendency to have higher average daily egg  
250 output (Figure 4c). However, differences among cages suggest that egg production varied  
251 per adult (Tables 1, 2), which could mean that factors beyond adult survival contribute to

252 fecundity patterns, and that individual females may also vary in how many eggs they lay  
253 each day.

254 Egg production in all monitored cages generally declined over the course of the  
255 experiment (Figure 3). Most cages had the highest daily egg-laying rates early in the  
256 reproductive period (first 50 days), after which production gradually tapered off (Figure  
257 3). This pattern is consistent with our observation that individuals typically reach sexual  
258 maturity and begin laying eggs around 135 days post-hatching, and mortality starts  
259 approximately 90 days later (~225 days post-hatching; data not shown).

260

#### 261 *Timing and Dynamics of Hatching*

262 Across 105 monitored egg batches, most hatching occurred within a consistent 12-day  
263 period following the onset of emergence (Figure 1c), regardless of the number of eggs in  
264 a batch (Figure 1d). Similarly, there was no correlation between batch size and the timing  
265 of 90% hatch completion (Figure 1b). This predictable hatching period is a useful feature  
266 of *C. morosus* colony management because it allows hatchlings to be grouped into  
267 cohorts of similar age, improving developmental staging and reducing experimental  
268 variability. Knowing when bulk hatching will occur also allows for better planning of  
269 enclosure turnover and resource allocation, including the timely preparation of  
270 enclosures, foliage, and tracking materials, an especially important consideration for  
271 large-scale or long-term studies.

272

#### 273 *Egg Handling and Substrate Considerations*

274 Our egg-handling method involves collecting eggs directly from the enclosure floor and  
275 incubating them in ventilated Petri dishes. This approach maintains high viability while  
276 making eggs easily observable, unlike cricket systems that collect eggs in substrates such  
277 as coconut fiber or cotton wicks <sup>17</sup>. The hard exochorion of *C. morosus* eggs provides  
278 protection during handling and against desiccation, making it possible to incubate eggs  
279 safely in Petri dishes without additional substrate, while maintaining high hatch rates.  
280 Taken together, these standardized procedures provide a structured approach for  
281 maintaining healthy *C. morosus* populations for reproducible developmental, behavioral,  
282 genetic, or physiological research. Consistent environmental controls (23 °C, 70% RH,  
283 12:12 LD), regular enclosure maintenance, and predictable developmental timelines  
284 make *C. morosus* an accessible and low-cost model system. Importantly, unlike some  
285 cricket systems where crowding, cannibalism, or pathogen load can rapidly destabilize  
286 colonies <sup>18</sup>, *C. morosus* are easy to maintain, making them well suited for large-scale  
287 experiments and studies spanning multiple generations. As *C. morosus* becomes more  
288 widely adopted as a hemimetabolous model species, use of standardized methods should  
289 promote reproducibility and comparability across studies in multiple areas of insect  
290 biology.

291 **Disclosures:**

292 The authors have no conflicts of interest to declare.

293

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295

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300 their support, including former lab member Dr. Upendra Bhattarai (current affiliation:

301 Brown University) who set up the initial colony.

302

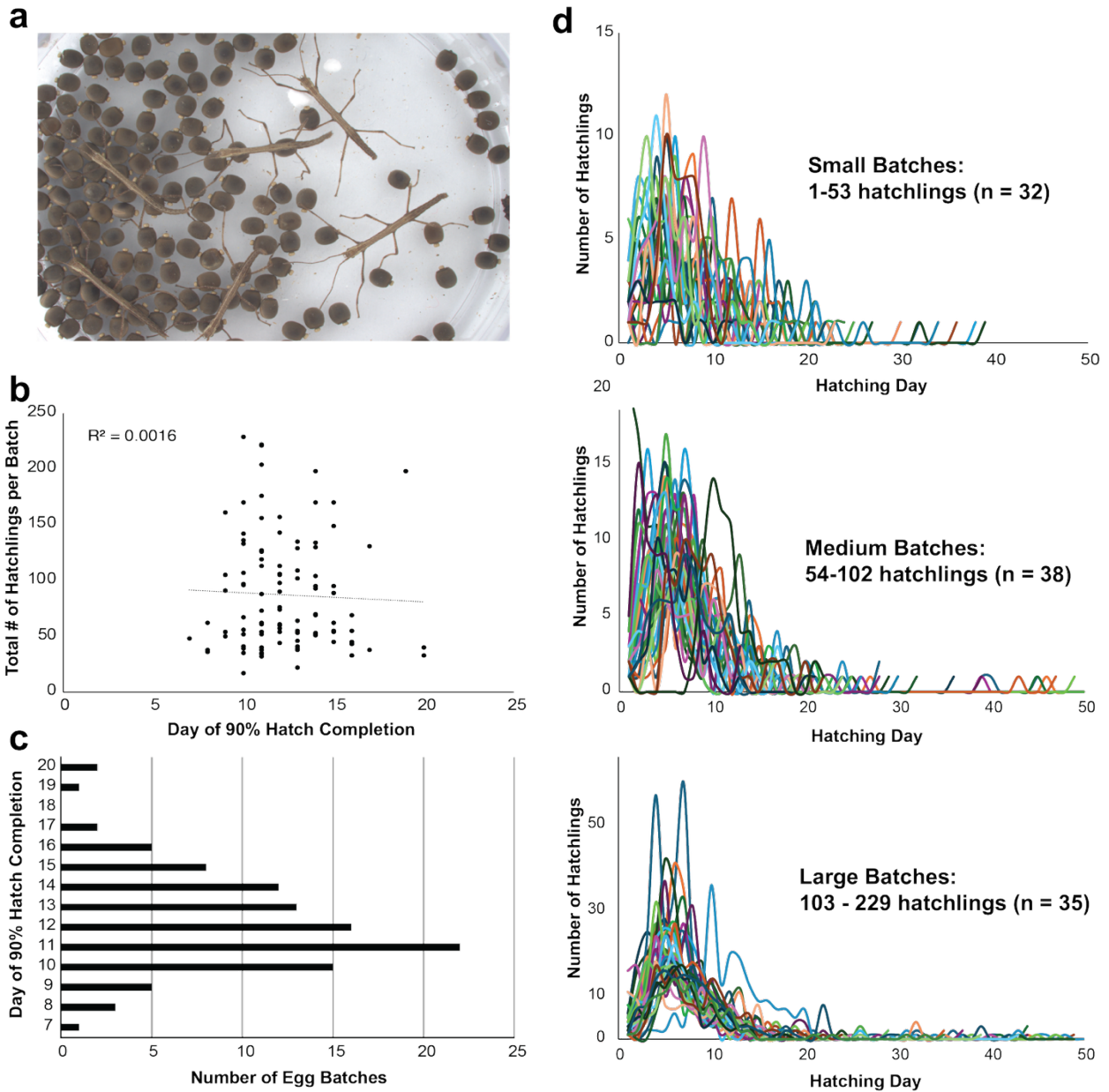
## Table of Materials Used in Article

<b>Material</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
8oz Spray bottle	Amazon	B000H88PCU	For misting mesh enclosures
Autoclavable polypropylene bags, W x H=30.5 x 61 cm (12 x 24)	VWR®	95042-554	For disposal
Disposable Petri dishes 150 x 15 mm	VWR®	25384-326	For egg collection
Disposable Petri dishes 60 x 15 mm	VWR®	25384-092	For egg incubation
Ivy, bramble, rose leaves	N/A	N/A	Collected outside
Mesh insect enclosures	Amazon	B09GS2CH1G	Used to house insects
Tall glass container	N/A	N/A	Used to propagate and hold plant leaves
Tap water	N/A	N/A	For misting
Utoolmart 10pcs anti-static tweezers	Amazon	B07YZ5FB27	Used for collecting and handling eggs and hatchlings

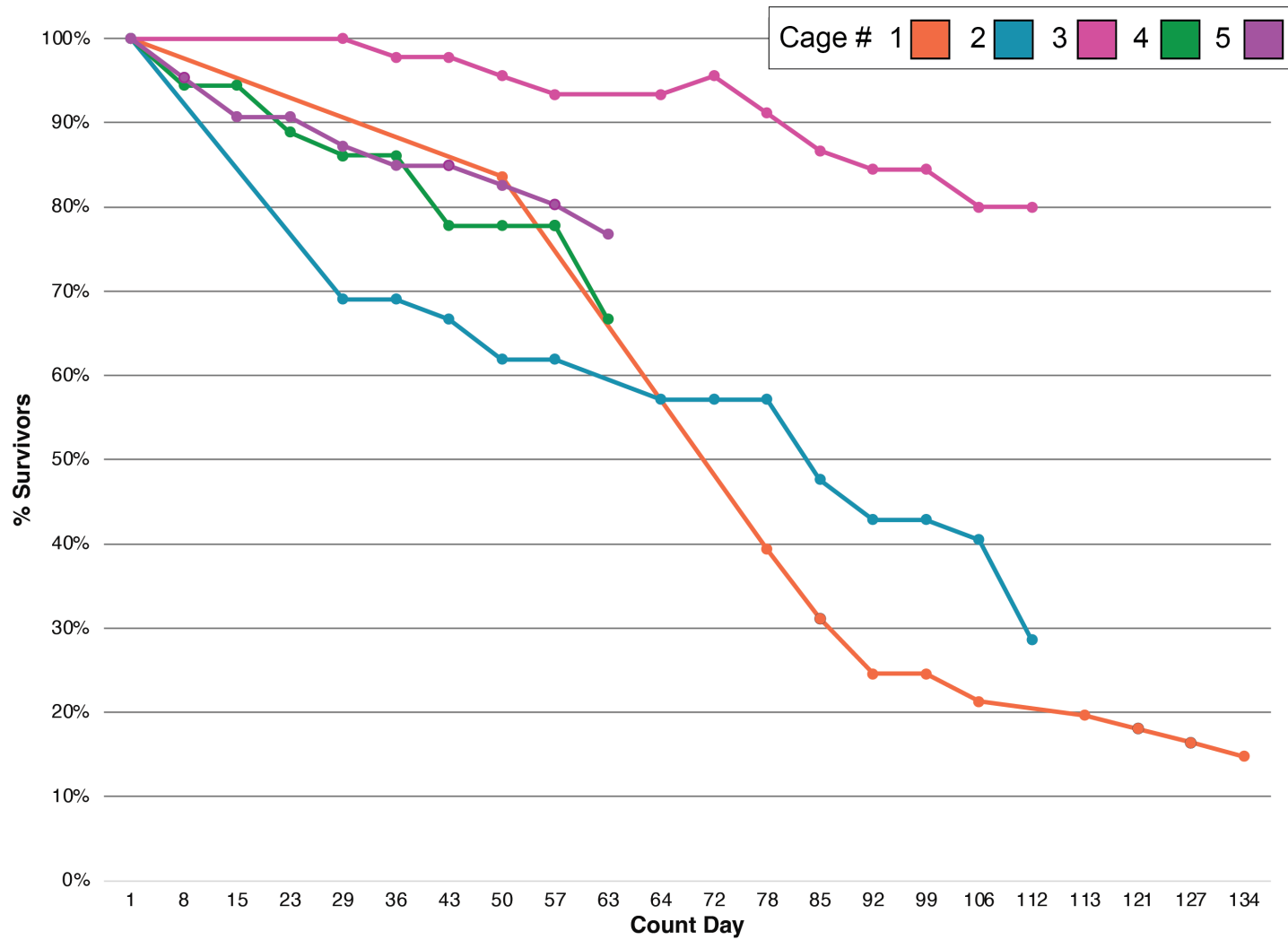
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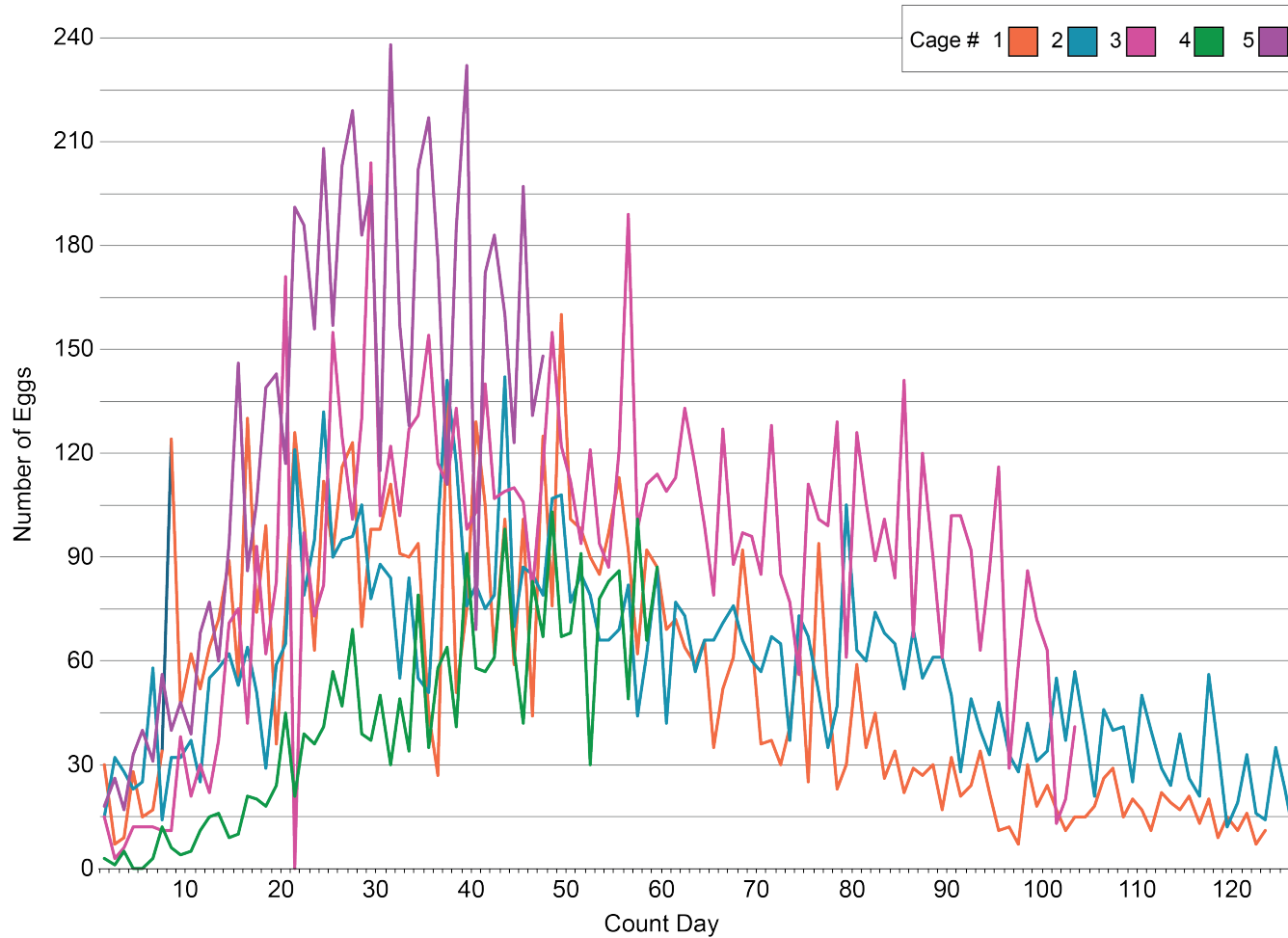
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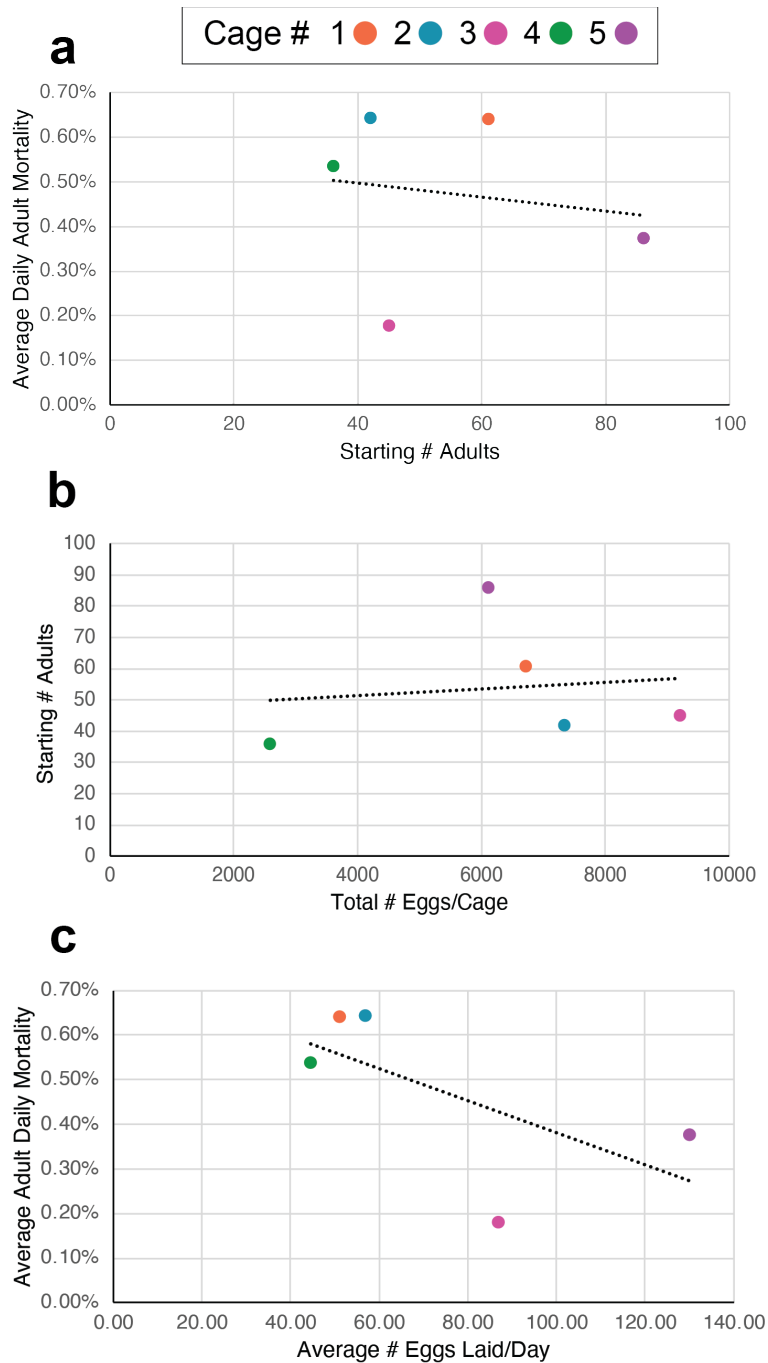
**Figure 1. Egg hatching dynamics.** (a) Representative image of hatchlings emerging in a Petri dish containing eggs. (b) Relationship between total hatchlings per batch and the number of days required to reach 90% hatch completion. (c). Distribution of egg batches reaching 90% hatch across hatching days 7-20. (d) Hatching trajectories over a 50-day collection period for 105 egg batches collected prior to the establishment of the cages monitored for Figures 2 and 3. Batches were grouped by total hatchling number using percentile cutoffs.



**Figure 2. Adult mortality dynamics.** Survival trajectories across five replicate *C. morosus* experimental cages over time. Percent survival is plotted over time for individuals in each monitored cage. See summary metrics in Table 1.



**Figure 3. Egg production dynamics.** Number of eggs collected from the same five cages monitored in Figure 2. Cages 1 through 5 were monitored daily for the total of 47, 58, 106, 129 and 132 days, respectively. The plot shows data from the first 126 days of oviposition. See summary metrics in Table 2.



**Figure 4. Relationships between adult numbers, adult mortality, and egg production per cage.** (a) There is no significant relationship ( $R^2 = 0.025$ ) between average adult daily mortality and the number of founding adults in a cage. (b) There is no significant relationship ( $R^2 = 0.017$ ) between total egg production and the number of founding adults in a cage. (c) There is a moderate negative correlation ( $R^2 = 0.411$ ) between average daily egg production and average adult daily mortality. Average adult daily mortality = (% total mortality) / (total number of days monitored)

**Table Legends** [Table Download Link](#)

**Table 1. Adult survival and mortality dynamics across experimental cages.** Adult survival outcomes for five replicate experimental cages. For each cage, we report the date range of cage establishment, the time between cage establishment and initiation of adult counts, the duration of adult monitoring, starting and ending adult population sizes, adult mortality over the monitored period, and average daily mortality.

**Table 2. Egg production across experimental cages.** Egg-laying output of five replicate experimental cages containing mature adults. For each cage, we report the start and end dates of egg collection, the total duration of egg collection, cumulative egg production per cage, and the average number of eggs laid per day.

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