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RESEARCH ARTICLE

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Comparing ageing and the effects of diet supplementation in wild vs. captive antler flies, *Protopiophila litigata*

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Abstract

1. Few studies have simultaneously compared ageing within genetically similar populations in both laboratory and natural environments. Such comparisons are important for interpreting laboratory studies, because factors such as diet could affect ageing in environment-dependent ways.
2. Using a natural population of antler flies (*Protopiophila litigata*), we conducted separate factorial experiments in 2012 and 2013 that compared age-specific male survival and mating success in laboratory cages versus a natural field environment while supplementing their diets with protein or sugar.
3. We found consistent and substantial increases in both survival and mating rates in the laboratory compared to the field, but remarkably, despite these large differences actuarial ageing was only higher in the laboratory than in the field in 2012 and similar in the two environments in 2013. In both years, there was no difference between environments in reproductive ageing.
4. We found that males fed protein had a higher mortality rate than males fed sugar (strong and low support in 2012 and 2013, respectively).
5. In contrast, diet did not strongly impact average mating rates, actuarial ageing or reproductive ageing in either experiment.
6. Our results provide the first evidence that the negative effect of protein on life span reported in many laboratory studies can also occur in wild populations, although perhaps less consistently. They also highlight how laboratory environments can influence life-history traits and suggest caution when extrapolating from the laboratory to the field.

KEYWORDS
actuarial senescence, demographic ageing, diet variation, laboratory–field comparisons, life span, macronutrients, mating rate, reproductive senescence

1 | INTRODUCTION

Evolutionary studies often rest on the fundamental assumption that responses in the laboratory are qualitatively similar to those that would be seen in the field (Briga & Verhulst, 2015; Matos, Rego, Levy, Teotónio, & Rose, 2000; Partridge & Gems, 2007; Sgrò & Partridge, 2001). This assumption can be problematic for life-history traits such as ageing, a highly plastic trait that could be strongly affected by artificial laboratory environments. Ageing evolves because natural selection is generally weaker at later ages than at early ages (Hamilton, 1966; Kirkwood, 1977; Kirkwood & Rose, 1991). This allows the accumulation of late-acting deleterious mutations and favours alleles

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that increase early-life performance even at the cost of late-life survival and reproduction (Medawar, 1952; Williams, 1957). Ageing is a central topic of evolutionary research because it can have important evolutionary and demographic consequences (Charlesworth, 1994; Finch, Pike, & Witten, 1990). In addition, ageing can increase inbreeding depression and alter genetic variance with age (Charlesworth & Hughes, 1996; Escobar, Jarne, Charmantier, & David, 2008). Much of our knowledge of ageing comes from laboratory experiments, so it is important to understand to what extent inferences from laboratory studies can be generalized to natural populations (Kawasaki, Brassil, Brooks, & Bonduriansky, 2008; Reichard, 2016).

Ageing (senescence) is the age-related decline in an individual's physiological function that results in increased mortality risk (frailty) and decreased reproductive rate, although the physiological changes that underlie ageing are often unknown (Medvedev, 1990). Changes in frailty are typically estimated at the population level as the rate of increase in mean mortality rate with age ("actuarial ageing", Ricklefs, 2008) and the decline in reproductive output ("reproductive ageing"), although the latter can also be measured at the individual level. Here, in-line with other evolutionary studies (Charlesworth, 1994; Finch et al., 1990), we focus on these population-level, statistical measures of both actuarial and reproductive ageing and refer to them collectively as "ageing".

Several factors complicate our understanding of ageing in laboratory versus field settings. First, there are substantial taxonomic and methodological biases in studies of ageing. Most information on ageing in natural populations comes from long-lived vertebrates (Nussey, Froy, Lemaitre, Gaillard, & Austad, 2013), while empirical research in the laboratory is dominated by short-lived model invertebrates (Gems & Partridge, 2013; Kirkwood & Austad, 2000). As such, we know much less about ageing in short-lived wild organisms, particularly insects (although see Bonduriansky & Brassil, 2002; Carroll & Sherratt, 2017; Kawasaki et al., 2008; Ryan, Ben-Horin, & Johnson, 2015; Sherratt et al., 2010; Zajitschek, Brassil, & Bonduriansky, 2009). Furthermore, the considerable difference in life histories between the model organisms used in laboratory versus field research complicates extrapolation, and manipulative studies of factors that influence ageing in the field are rare (Nussey et al., 2013; Roach & Carey, 2014).

Second, populations studied in the laboratory are often genetically different from their counterparts in the field due to genetic drift or adaptation to laboratory conditions (Matos & Avelar, 2001), potentially resulting in differences in ageing (Kenyon, 2005; Kirkwood & Austad, 2000). Third, ageing is highly plastic. Evidence for environment-dependent ageing comes from manipulations of social environment (Adler & Bonduriansky, 2011), reproductive investment (e.g. Tatar, Carey, & Vaupel, 1993) and nutrient composition of juvenile (Hooper, Spagopoulou, Wylde, Maklakov, & Bonduriansky, 2017; Runagall-McNaull, Bonduriansky, & Crean, 2015) and adult (e.g. Gems & Partridge, 2013; Lee, Hwang, Artan, Jeong, & Lee, 2015) diets. These studies suggest that differences between environments could substantially alter patterns and mechanisms of ageing (Briga & Verhulst, 2015; Van Voorhies, Fuchs, & Thomas, 2005). Genotypes can also vary in their response to the environment or other factors such as diet (i.e. genotype × environment interactions; Liao, Rikke, Johnson, Diaz, & Nelson, 2010; Vieira et al., 2000).

A few studies have compared actuarial ageing under laboratory versus natural conditions. These typically show that mean life span is extended, and actuarial ageing is slowed, in captive environments (*insects:* Kawasaki et al., 2008; Ryan et al., 2015; *vertebrates*: Bronikowski et al., 2002; Hämäläinen et al., 2014; Ricklefs, 2000; Tidière et al., 2016; *plants*, Roach, 2001; but see Molleman, Zwaan, Brakefield, & Carey, 2007 for an exception). Comparisons of captive versus wild populations of ruminants reveal that captivity can influence ageing and implicate dietary differences as a factor underlying observed patterns (Lemaître, Gaillard, Lackey, Clauss, & Müller, 2013; Müller, Gaillard, Bingaman Lackey, Hatt, & Clauss, 2010). By contrast, to our knowledge, only one review has compared the decline in mating rate with age (i.e. reproductive ageing) between the laboratory and field: Atsalis and Videan (2009) concluded that reproduction declined earlier and faster in captive than in wild chimpanzees. With the exception of Kawasaki et al. (2008), all of these studies compared genetically distinct cohorts in the laboratory and field, meaning that differences in ageing cannot be unequivocally attributed to environmental effects. Because very few studies have investigated ageing and other life-history traits simultaneously and experimentally in genetically similar populations in the laboratory and field, the extent to which patterns of ageing in the laboratory are representative of those in nature is unclear. This limits our understanding of the mechanisms, environment-dependence and fitness consequences of both longevity and ageing.

The lack of experimental field studies is especially problematic for research on the effects of diet on ageing. Dietary nutrients are well known to affect life span and ageing (Gems & Partridge, 2013), and the ratio of macronutrients (protein:carbohydrate) is suggested as a key factor shaping mortality and reproductive patterns in animals (Moatt et al., 2019; Simpson, Le Couteur, & Raubenheimer, 2015). The effects of diet on ageing could interact with other environmental parameters that differ between the laboratory and field (Lemaître et al., 2013; Müller et al., 2010). For example, diet can affect condition and influence behaviour (Lihoreau et al., 2015), including sexual signalling (Hunt et al., 2004; Maklakov et al., 2009) and mating (Blay & Yuval, 1997). If behavioural differences alter the risk of environmentally driven mortality, or "wear-and-tear" in an environment-dependent way, then diet may affect life span and ageing differently in laboratory and field environments. Additionally, dietary nutrients (especially protein) can influence immune responses, wound healing and thermoregulation (Adler & Bonduriansky, 2014). While protein consumption typically accelerates ageing and shortens life span in captive insects (Fanson, Weldon, Perez-Staples, & Simpson, 2009; Ja et al., 2009; Lee et al., 2008; Maklakov et al., 2008), the role of protein in immunity and wound healing could negate or reverse this effect in natural environments (Adler & Bonduriansky, 2014).

The antler fly (*Protopiophila litigata*) uses discarded cervid antlers as substrate for mating, egg-laying and larval feeding. Median

life span in the wild is six days (Bonduriansky & Brassil, 2002), and adult males exhibit a remarkable site fidelity, typically returning to the same antler daily to compete for mates near oviposition sites on the antler surface (Bonduriansky & Brassil, 2005). This site fidelity makes it possible to acquire high-quality longitudinal data and conduct experiments in the field. Previous research provided the first compelling evidence of senescence in a wild insect (Bonduriansky & Brassil, 2002). Antler flies therefore offer unique opportunities to compare life-history patterns in natural versus captive populations.

We carried out the first direct laboratory versus field comparison of both actuarial and reproductive ageing in genetically similar captive and wild cohorts. We also manipulated diet in both environments to compare its effects between the laboratory and field. Since diet cannot be controlled in wild animals, we instead supplemented both captive and wild flies with carbohydrates (sugar) or protein (yeast). Mating rate and presence/absence (survival) were recorded simultaneously in males subjected to these diet treatments both on moose antlers stationed in a natural field environment and in nearby laboratory population cages. Because life-history traits such as life span, reproductive rate and ageing rate are highly plastic, and there are many ways to supplement diet, we carried out this study over two consecutive field seasons using two separate experiments in which diet treatments were applied differently. Our goals were to compare ageing between the laboratory and field and to gain insight into whether diet effects on ageing varied between these environments. As such, consistent findings from both experiments would suggest more robust effects of environment and/or diet, while contrasting patterns between experiments would indicate heterogeneity of effects, the cause(s) of which would require further investigation.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Discarded moose antlers were collected near the Wildlife Research Station in Algonquin Provincial Park, Ontario, Canada, in May 2012 and relocated to forested areas at the Station prior to larval emergence. Experiments were performed in the spring and summer of 2012 (8 June – 23 July) and 2013 (10 June – 12 July). In both years, males were captured when they first appeared on an antler. To control for seasonality effects, multiple cohorts were collected over 28 (2012) and 30 (2013) days. Larval development time in antler flies in their natural environment is unknown, but time to emergence ranges between 33 and 65 days under laboratory conditions depending on the food quality. Antler flies pupate in the soil near their natal antler and adults typically return to this antler to breed, so these males were likely newly emerged adults (Bonduriansky & Brooks, 1998). These "focal" males were marked with individual codes on the thoracic notum using enamel paint and photographed from above without anaesthesia (Bonduriansky & Brooks, 1997), then randomly assigned to a supplemental diet treatment and environment (laboratory or field). We used two different diet supplementation approaches, one applied during each of the two consecutive field seasons. There are many ways to supplement diet, and we do not know which of these may be the most ecologically relevant, but consistent effects across methods would provide a more robust inference. In addition, preliminary analysis of the data from the first year (2012) suggested weak effects of diet, so we wanted to know whether a longer exposure might produce a stronger response. We recognize that the use of somewhat different diet treatments complicates interpretation of differences in results across years (see Discussion).

In 2012, we used a factorial design involving two diets (plus a water-only control) and two environments (field vs. laboratory). On the day of first capture, males were held individually for 1 hr in a glass vial (95 mm x 22 mm) that contained ad libitum carbohydrates (granulated cane sugar in water), protein (deactivated dried Torula yeast in water) or just water. After this diet application, focal males were either released near an antler outdoors (field) or placed into a population cage indoors (laboratory). Males were re-treated for one hour every third day in the laboratory and every third day (or at the first opportunity thereafter) after being recaptured in the field, and subsequently were immediately released at the antler or cage from which they were taken. In 2013, we used an alternative factorial design involving the same two environments (field vs. laboratory), two of the same diets (carbohydrate vs. protein), but with the diets provided in a single two days of exposure after initial capture. Water was provided ad libitum in both sugar and protein diet treatments as in 2012, but there was no water-only control (as there were tighter logistical and time constraints).

2.2 | General environmental conditions

In the field, antlers were situated on 0.8 m tall wooden stands located 15–100 m apart at the edge of the forest surrounding the research station (in 2012) or in small, shaded clearings within the forest (2013). Average surface area (±SE) of an antler was 678.4 ± 81.5 cm^2. Antlers were not enclosed or manipulated in any other way, so marked males released at antlers (and unmarked flies of both sexes) could move without constraint, had unrestricted access to their natural diet (when not in a diet treatment), and experienced a natural range of weather conditions, predators (e.g. spiders and predatory insects), parasites (e.g. mites) and fluctuations in numbers and sex ratios.

In the laboratory, focal males were housed in one of several acrylic cages (3581.6 cm^3) along with wild-collected females. Each cage contained ~20 flies at an approximate M:F sex ratio of 2:1, with a similar number of males from each diet treatment. To hold density and sex ratio approximately constant, dead flies were replaced with new individually marked focal males, generically marked non-focal males (when additional focal males were unavailable) or wild-collected females as necessary. Caged flies were protected from predators, interspecific competitors, shielded from wind and rain, but experienced near-natural fluctuations in temperature, humidity and light as cages were housed in an un-insulated cabin. Both years, focal males in the laboratory had continual access to cane sugar, water and protein (via the oviposition substrate). This particular laboratory environment is one

of many that could be used and that the choice of laboratory environment, along with seasonal variation in abiotic conditions, may affect the presence and magnitude of laboratory–field differences.

Each cage or antler was checked six (occasionally 4–5) times per day every two hours from 0900 to 1900. At each observation, we recorded the presence and mating status of focal males, as well as the sex ratio and total number of flies on the antler or in the cage. Mating status was determined by searching for mating pairs following Bonduriansky and Brooks (1998).

In 2012 (2013), we introduced males onto, and subsequently monitored, a total of six (two) antlers in the field and 11 (12) laboratory cages. In 2012, we excluded males from our analysis that were not seen after their initial release at antlers (to exclude handling-induced mortality; Bonduriansky & Brassil, 2005). There was no mortality during the first diet application in 2012, whereas in 2013 there was ~18.9% and 0% mortality for protein versus sugar males, respectively. However, males were randomly assigned to laboratory versus field after the application of the diet treatment, so mortality during diet application could not affect laboratory/field differences in either year, nor could it affect differences among diets in 2012. Nonetheless, differential mortality during diet application may have reduced our power to detect diet effects in 2013 however (see Results and Discussion).

Sample sizes are shown in Table S1. We made a total of 16,737 observations in 2012 (N = 432 males, 38.6 ± 26.1 *SD* observations/male) and a total of 10,907 observations in 2013 (N = 219 males; 49.8 ± 38.2 observations/male). The total number of flies per cage in the laboratory was similar to the mean number of flies per antler across all observations (i.e. unmarked females and marked and unmarked males) in 2012 (laboratory: 19.8 ± 2.7 vs. field: 17.9 ± 7.0; Figure S1), but lower than that in 2013 (laboratory: 21.1 ± 3.7 vs. field: 87.1 ± 48.9; Figure S2). The average sex ratio was slightly less male-biased in the laboratory than in the field both in 2012 (0.66 ± 0.02 vs. 0.84 ± 0.03, respectively; Figure S3) and in 2013 (0.67 ± 0.04 vs. 0.73 ± 0.15; Figure S4).

2.3 | Statistical analyses

Because diet treatments differed between years, data were analysed separately by year. For each year/experiment, we tested the effects of environment, diet and their interaction on actuarial and reproductive ageing, as well as on average mortality and mating rates using R v3.4.4 (R Development Core Team, 2013). Including sex ratio and number of flies did not qualitatively change the results of our model selection (Tables S3–S10). A male's probability of being sighted on antlers did not decrease with his age so did not inflate our estimates of ageing in the field (Table S11). Models were ranked according to corrected Akaike's information criterion (AICc; Hurvich & Tsai, 1989), and ΔAICc values for terms of interest were calculated by comparing the best fit models that included versus excluded that term. When the difference in ΔAICc between best competing models was lower than two, we discuss only the simpler model (Burnham & Anderson, 2002).

The effects of environment, diet and their interaction on mean mortality rate were analysed using semi-parametric Cox proportional hazards models (coxme package; Therneau, 2018) and fully parametric survival models (survival package; Therneau, 2015). We included potentially confounding covariates in all models either as fixed effects (body size and emergence date) or as random effects (cage/antler identity). Because body size is a strongly condition-dependent trait in antler flies (Oudin, Bonduriansky, & Rundle, 2015), as in many other insects (Blanckenhorn, 2000; Cotton, Fowler, & Pomiankowski, 2004), for Cox analyses, we included an interaction between log body size and environment to test for potential differences in condition-dependent mortality between the laboratory and field (effect of emergence date on body size was first removed; Supporting Information Appendix S1). Individuals observed alive on the last day of the experiment were censored (n = 1 field individual in 2012 and n = 17 laboratory individuals in 2013). As detection probability is high in this system, we assumed that other wild individuals died on the day following their last observation (Bonduriansky & Brassil, 2002). For each year separately, we first compared five Cox models (null, environment effect only, diet effect only, additive environment and diet effects, environment × diet interaction). This semi-parametric approach allows testing for the effect of each variable on mortality hazard without defining the underlying hazard function, but cannot be used to test for effects on actuarial ageing rates (Appendix S1). The assumption of proportional hazards was verified for all the effects except that of environment in 2012 (Table S4d).

To investigate effects on actuarial ageing rate, we fit different parametric survival distributions for each year and each environment independently. The two-parameter Weibull and the gamma distributions were best supported based on Kolmogorov–Smirnov goodness-of-fit tests and AICc model selection, while the Gompertz distribution was poorly supported (Delignette-Muller, 2015; Appendix S1, Table S2, Figures S5–S8 for details). We used the two-parameter Weibull over the gamma for the parametric analyses because there is an explicit formula for the Weibull (but not gamma) mortality rate. The Weibull parameter, α, quantifies the increase in mortality with age for a sample of individuals (α = 1 indicates no ageing; α > 1 or <1 denote positive and negative ageing, respectively; Appendix S1).

For each year separately, we compared four different models to test the effects of environment, diet and their interaction on α (null, environment effect only, diet effect only and environment × diet interaction; an additive model cannot be fit with the survival package). We controlled for differences in survival independent of ageing by including the interaction between environment and diet on the Weibull scale in all four models (our Cox analyses showed this interaction affected mortality rates in both years; see Results). The 95% confidence interval of each Cox and Weibull parameter was computed with 1,000 simulations using a multivariate normal distribution with the mean equal to the best model estimates and variance–covariance matrix equal to its Hessian matrix (Rode, Charmantier, & Lenormand, 2011). Mortality was high on the day following release, potentially due to handling effects (see Results). This increase was visible in 2012 (Figure 1a) when males were released immediately

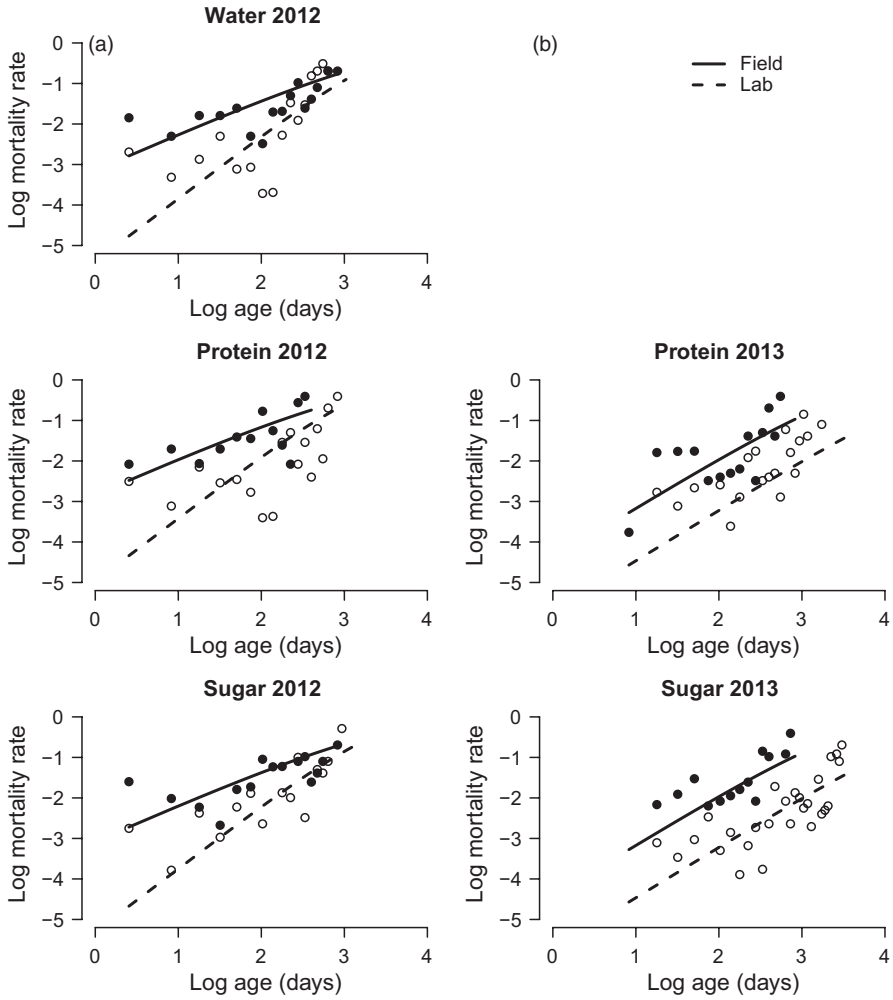

FIGURE 1 Effects of diet and environment on male actuarial ageing in 2012 (a) and 2013 (b) experiments. Observed mortality rate in the field and the laboratory (solid and open circles, respectively) and fitted mortality rate in the field and the laboratory (solid and dashed lines, respectively) increased with age for males fed water, protein or sugar (top, middle and bottom panels, respectively). Diet affected mortality but not its increase with age (see Results)

after marking, but not in 2013 (Figure 1b) when they were kept and fed for two days before being released. To verify that our results in 2012 were not influenced by this early-life mortality, we refit the Weibull models after removing the 56 males that were seen on their day of release but never afterwards.

The effects of environment, diet and their interaction on reproductive ageing were analysed using the LME4 package (Bates, Mächler, Bolker, & Walker, 2014). We modelled the mating success of a male, *p*, as the number of matings observed for that male on a given day divided by the number of observation periods for that day using generalized linear mixed models with a binomial error distribution and a *logit* link function. We quantified reproductive ageing as the change in mating success with age. For each environment separately, we first determined whether reproductive ageing was better described by linear or quadratic age effects (Appendix S1). We included antler/cage, season and observation day as random effects, as well as individual identity to account for repeated measures and potential differences in reproductive performance between individuals (Ericsson, Wallin, Ball, & Broberg, 2001; Reid, Bignal, Bignal, McCracken, & Monaghan, 2003). We then combined the data from the two environments and tested for differences in reproductive ageing between environments and diets by fitting 18 models with different combinations of environment, diet, age and their interactions, separately by year. The 95% confidence interval of each parameter was computed with 1,000 simulations using the bootMer function (Bates et al., 2014). Finally, we also performed additional tests to investigate potential trade-offs between average mating rate and reproductive ageing, to ensure that our results were not affected by differences in sex ratio and total number of flies (i.e. density) between environments, and to confirm that our estimates of reproductive ageing were not biased by disappearance of low-quality individuals ("selective disappearance") (Ivimey-Cook & Moorad, 2018; van de Pol & Verhulst, 2006; see Appendix S1 for details).

3 | RESULTS

3.1 | Average mortality rate

The median life span of laboratory males was nearly twice that of field males in both 2012 (median life span in days, pooling across diets [95% CI], field: 6 [2–13], laboratory: 11 [2–17.6]) and 2013 (field: 8 [4–16.9], lab: 13 [4–27]). These differences were strongly supported (ΔAICc = 4.94 and 14.03 for Cox models without an environment effect in 2012 and 2013, respectively, Table 1). Mortality rates were

more than 60% lower in the laboratory compared to the field in both years (Figure 1, Table S4a). An additive effect of diet was also present in the best fit Cox model in 2012 and in the second best fit model in 2013 (Table 1). Indeed, alternative models with no diet effect or with an interaction between diet and environment were supported in 2013 (ΔAICc = 0 and ΔAICc = 0.52, respectively, Table 1), but not in 2012 (ΔAICc = 4.99 and ΔAICc = 2.77, respectively, Table 1). In 2012, the best Cox model provided evidence that the protein diet increased mortality in both the laboratory and in the field (sugar- or water-fed males had, respectively, a 25% and 30% reduction in mortality rates compared to protein-fed males, Table S3a). In 2013, the additive Cox model suggested the same pattern (sugar-fed males had an 18% reduction in mortality rates compared to protein-fed males, Table S3a), but this effect had low support (ΔAICc = 0.41, Table S3a). In both years, the effect of body size did not differ between environments (Table S3a).

3.2 | Actuarial ageing

Mortality rates increased with age in both years, indicating actuarial ageing (Figure 1, Table 2). The rate with which mortality increased with age was lower in the field than in the laboratory with strong support in 2012 (α_{field} = 1.91, $\alpha_{laboratory}$ = 2.58, Table S6a; ΔAICc = 10.52 for a model without an environment effect on shape) but not in 2013 (α = 2.28; ΔAICc = 0.98 for a model with a higher ageing rate in the field (α_{field} = 2.41) than in the laboratory ($\alpha_{laboratory}$ = 2.14); Table S6a). In 2012, we observed an increased mortality rate on the day following the release of males (Figure 1a; potentially a handling-related effect), but the more rapid senescence observed in the laboratory compared to field remained qualitatively unchanged when males dying on this day were excluded from the analysis (Table S5b). Diet did not affect ageing rates in either year (ΔAICc = 2.58 and 2.17, for a model with a diet effect on the Weibull shape parameter in 2012 and 2013, respectively).

3.3 | Average mating rate and reproductive ageing

Laboratory males had a higher average mating rate compared to field males in both 2012 (average per cent males mating per day, pooling across diets ± *SD*; field: 5.4% ± 5.0%, laboratory: 20.0% ± 9.6%) and 2013 (field: 9.4% ± 1.2%, laboratory: 14.9% ± 8.0%). Support for these differences between environments was strong in both years (Figure 2; ΔAICc = 42.77 and 11.6 for models lacking an environment effect in 2012 and 2013, respectively; Tables S7 and S9). There was weak support for a higher mating rate of males fed sugar or protein compared to males fed water in 2012 (ΔAICc = 1.89 for a model lacking a diet effect; Tables 3 and Table S8a; Figure 2). Mating rate did not differ between sugar- and protein-fed males in 2013 (ΔAICc = 1.45 for a model with a diet effect; Table 3; no water control treatment in 2013). Long-lived males also had a higher mating rate (positive effect of life span in Table S12c), except in the field in 2013 (negative effect of life span in Table S12c).

After accounting for differences in average mating rate between environment and across diets, mating rate decreased quadratically

TABLE 1 Results of the AICc model selection for Cox proportional hazards survival models. All models included an interaction between environment and residual log body size and emergence date as fixed effects and antler/cage identity as a random effect

Year	Model	Df	Log likelihood	AICc	ΔAICc
2012	Environment + diet	7	−2139.09	4292.44	–
	Environment × diet	9	−2138.39	4295.20	2.77
	Diet only	6	−2142.56	4297.32	4.88
	Environment only	5	−2143.64	4297.42	4.99
	Null model	4	−2147.23	4302.55	10.12
2013	Environment only	5	−879.86	1770.00	–
	Environment + diet	6	−879.01	1770.41	0.41
	Environment × diet	7	−878.00	1770.52	0.52
	Null model	4	−888.03	1784.24	14.24
	Diet only	5	−887.45	1785.17	15.17

TABLE 2 Results of AICc model selection for two-parameter Weibull survival models. The second parameter of all Weibull models included the interaction between environment and diet, residual log body size and standardized emergence date as fixed effects and antler/cage identity as a random effect

	Shape parameter	Df	Log likelihood	AICc	ΔAICc
2012	Environment	11	−1145.54	2313.70	0.00
	Environment × diet	15	−1142.56	2316.28	2.58
	Diet	12	−1149.74	2324.22	10.52
	Intercept	10	−1152.51	2325.54	11.84
2013	–	8	−632.64	1281.96	0.00
	Environment	9	−632.04	1282.94	0.98
	Diet	9	−632.63	1284.13	2.17
	Environment × diet	11	−631.45	1286.17	4.22

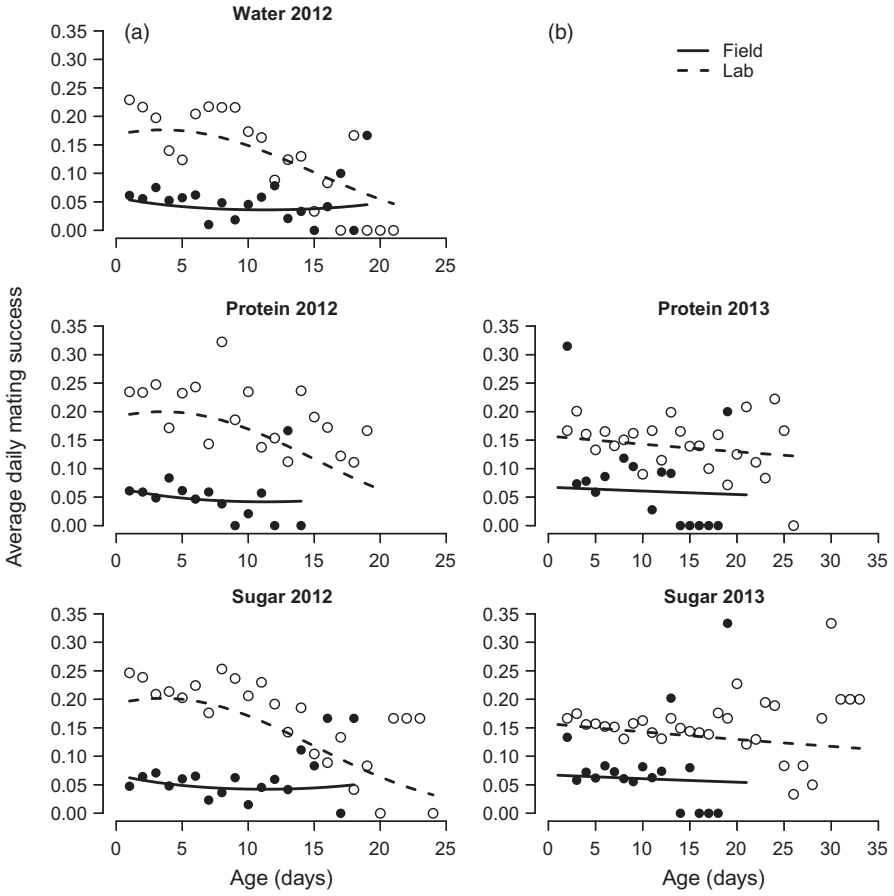

FIGURE 2 Effects of diet and environment on male reproductive ageing (i.e. average daily mating success) in 2012 (a) and 2013 (b) experiments. Observed mating rate in the field and in the laboratory (solid and open circles, respectively) and fitted mating rate in the field and in the laboratory (solid and dashed lines, respectively) decreased with age for males fed water, protein or sugar (top, middle and bottom panels, respectively)

with age with strong support in 2012 (ΔAICc = 19.69 for model lacking an age effect; Table S7) and linearly with age with low support in 2013 (ΔAICc = 0.02 for model lacking an age effect; Table 3). Hence, evidence for reproductive ageing was strong in 2012, but weak in 2013 (Figure 2). Accounting for selective disappearance did not change the estimated age effects from the best fit model (Table S12c). Differences in reproductive ageing between environments were weakly supported in 2012 (ΔAICc = 0.55 for a model without an environment × age interaction; Table 3) and unsupported in 2013 (ΔAICc = 2.02 for a model with an environment × age interaction; Table 3, Figure 2). In both years, diet did not affect reproductive ageing and did not interact with environment (Table 3). Finally, males with a higher-than-average mating rate did not exhibit more rapid reproductive senescence (ΔAICc > 1.78 for models that included a correlation between the random intercept and the random age slope of each male, Table S12a).

4 | DISCUSSION

We observed a large difference in median life span between environments (83.3% and 62.5% higher in the laboratory than in the field in 2012 and 2013, respectively) which resulted from mortality rates that were approximately 60% lower in the laboratory than in the field in both years. This is consistent with many (Bronikowski et al., 2002; Carroll & Sherratt, 2017; Hämäläinen et al., 2014; Kawasaki et al., 2008; Ricklefs, 2000; Roach, 2001), but not all (Molleman et al., 2007; Müller et al., 2010) previous studies. Lower mortality in laboratory compared to field environments probably resulted from a more reliable food supply in the laboratory, lower foraging costs (Piper & Partridge, 2007), and from protection from predators, parasites, competitor species and inclement weather. Importantly, the higher mortality that we observed in the field was not due to any single antler (Figure S6), to lower re-sighting rates for older males (see Methods), to age-dependent migration to non-monitored antlers (Table S11), or to mortality during diet application (environments were assigned after diet application). Elevated mortality on the day of release was likely due to handling-induced effects, but could alternatively be the result of frail, low-quality individuals that tend to die early in life.

Differences between environments in actuarial ageing were more complex and varied between years. Males showed faster actuarial ageing in the laboratory relative to the field in 2012. This more rapid actuarial ageing resulted in instantaneous mortality rates converging between environments for old individuals (Figure 1). However, in 2013 (where sample size and therefore statistical power were lower), we observed a weakly supported trend in the opposite direction, with faster actuarial ageing in the field than in the laboratory (Figure 1). Longer life span in both years in the laboratory was therefore not due to a slower rate of ageing in this environment, but

TABLE 3 Results of the AICc model selection for reproductive ageing. Mating rate declined (on a logit scale) quadratically with age in 2012 (strongly supported) and linearly with age in 2013 (weakly supported). Models with ΔAICc > 10 are presented in Tables S7 and S9. All models included residual log body size and emergence date as covariates and male identity, antler/cage identity, emergence date and the interaction between observation, environment and day as random effects

Year	Effects on senescence	Effects on average mating rate	Df	Log likelihood	AICc	ΔAICc
2012	Environment × (age + age^2)	Environment + Diet	14	−2819.96	5668.07	–
	Environment × (age + age^2)	Environment × Diet	16	−2818.08	5668.35	0.28
	Age + age^2	Environment + Diet	12	−2822.26	5668.62	0.55
	Age + age^2	Environment × Diet	14	−2820.34	5668.82	0.75
	Environment × (age + age^2)	Environment	12	−2822.93	5669.96	1.89
	Age + age^2	Environment	10	−2825.04	5670.15	2.08
	Environment × (age + age^2) + diet × (age + age^2)	Environment + Diet	18	−2817.95	5672.14	4.07
	Environment × (age + age^2) + diet × (age + age^2)	Environment × Diet	20	−2816.46	5673.22	5.15
	Diet × (age + age^2)	Environment	16	−2820.80	5673.78	5.71
	Diet × (age + age^2)	Environment × Diet	18	−2819.24	5674.73	6.65
2013	Age	Environment	9	−1652.01	3322.13	–
	–	Environment	8	−1653.04	3322.16	0.02
	–	Environment + Diet	9	−1652.74	3323.58	1.45
	Age	Environment + Diet	10	−1651.79	3323.70	1.57
	Environment × age	Environment	10	−1652.01	3324.16	2.02
	Age	Environment × Diet	11	−1651.12	3324.40	2.27
	–	Environment × Diet	10	−1652.19	3324.51	2.37
	Diet × age	Environment + Diet	11	−1651.34	3324.83	2.70
	Environment × age	Environment + Diet	11	−1651.79	3325.73	3.60
	Diet × age	Environment × Diet	12	−1650.84	3325.87	3.74
	Environment × age	Environment × Diet	12	−1651.12	3326.43	4.30
	Environment × age + diet × age	Environment × Diet	12	−1651.34	3326.86	4.73
	Environment × age +diet × age	Environment × Diet	13	−1650.84	3327.90	5.77

rather resulted from a lower baseline mortality in the laboratory (i.e. lower intercepts in Figure 1). Very few comparisons of ageing rates in wild versus captive insects have been carried out, but patterns vary for those that have. Carroll and Sherratt (2017) found faster actuarial ageing in captive compared to wild butterflies, whereas a study on neriid flies found faster actuarial ageing in wild compared to captive males (Kawasaki et al., 2008). Rapid actuarial ageing in the wild can result from an increase in age-dependent environmentally driven mortality (Kawasaki et al., 2008; Roach, 2001), but this process cannot explain the more rapid ageing that we observed in the laboratory in 2012. Our results could arise from the selective disappearance of low-quality individuals in the field (e.g. due to condition-dependent susceptibility to predation). If low-quality individuals have higher mortality rates on average, this could explain the more rapid actuarial ageing we observed in the laboratory compared to the field. However, contrary to this expectation, average mortality rates in each environment were affected similarly by log body size (used as a proxy for individual quality). However, body size may be a poor indicator of individual condition (Wilder, Raubenheimer, & Simpson, 2015), most notably in the field, and further studies using other proxies for condition (e.g. carbohydrate or fat content; (Rode & Morrow, 2009) are needed to test whether condition-dependent environmentally driven mortality results in slower ageing at the population level (Chen & Maklakov, 2012).

In both years, our diet manipulation affected the average mortality rate but not the actuarial ageing rate (i.e. changes in mortality with age). Overall, males fed sugar or water had ~18%–30% lower average mortality rate than those fed protein. Protein:carbohydrate ratio plays an important role in shaping life span and reproduction (Grandison, Piper, & Partridge, 2009), and, consistent with our results, a negative effect of protein consumption on longevity has been observed in multiple laboratory studies of insects (Adler, Cassidy, Fricke, & Bonduriansky, 2013; Fanson et al., 2009; Ja et al., 2009; K. Lee et al., 2008; Maklakov et al., 2008). Our study provides the first evidence that protein consumption can increase mortality rate not only under benign laboratory conditions but also under natural conditions, and thus suggests that this well-known effect is ecologically relevant. Increased protein has also been shown to enhance sexual

signalling (Hunt et al., 2004) and mating success (Blay & Yuval, 1997; Taylor & Yuval, 1999) in males of some species, which might explain the negative effect of protein consumption on male mortality in our study. However, support for an effect of diet in the field in the 2012 experiment but not in the 2013 experiment suggests that this effect is less consistent under natural conditions than in the laboratory. The effect of diet on average mortality rate is likely to depend both on the age of individuals upon exposure and the duration of this exposure (e.g. Stroustrup et al., 2016), so different results between experiments could reflect differences in our feeding protocol. It is also possible that a negative effect of protein consumption was weaker in 2013 because of substantial mortality of protein-fed males during the two-day diet application, which may have disproportionately eliminated individuals that were most susceptible to protein's harmful effects prior to release.

Average mating rate was higher in the laboratory compared to the field in both years (a difference of ~14.5% and 5.5% in 2012 and 2013, respectively). In nature, males can only mate when females arrive at an antler and operational sex ratios on antlers are typically strongly male-biased (Bonduriansky & Brooks, 1999). However, differences in mating rate between environments were still strongly supported when differences in sex ratio and fly numbers were accounted for statistically. Antler fly females visit antlers less frequently than males and can fly away to escape male harassment (Bonduriansky & Brooks, 1999), so the constant availability of females in laboratory cages may have contributed to this elevated mating rate in the laboratory. Preventing females from escaping male sexual attention is likely a common feature of laboratory environments and may give males more control over mating, increasing the opportunity for sexual conflict (Yun, Chen, Singh, Agrawal, & Rundle, 2017). This may have important consequences for inferences concerning sexual conflict, and an elevated mating rate in the laboratory may affect other life-history traits. Males with higher mating rates also tended to have longer life spans (except in the field in 2013), suggesting that any trade-off between reproduction and survival may be masked by variation in individual condition.

Consistent with a previous study of antler flies (Bonduriansky & Brassil, 2005), we detected reproductive ageing in both years (although support was weak in 2013 where sample size was smaller). Importantly, selective disappearance of low condition/short-lived males (van de Pol & Verhulst, 2006) did not bias our estimates of reproductive senescence. In addition, evidence for differences in reproductive ageing between environments was weak in 2012 and absent in 2013, suggesting that the substantially higher mating rate in the laboratory did not cause accelerated reproductive ageing, perhaps as a result of greater access to resources. Consistent with this, within environments males with higher mating rates did not have faster declines in mating rate. This apparent absence of a trade-off between mating rate and reproductive ageing both between and within environments contrasts with the only other study that compared patterns of reproductive ageing in captive versus wild chimpanzees (Atsalis & Videan, 2009). In our case, diet also did not affect reproductive ageing in either year, although there was some evidence that males fed sugar or protein mated more frequently than males provided with water only in 2012. Additional data are needed to determine the consistency and generality of these results.

Several of our results differed between our 2012 and 2013 experiments. These differences could be at least partially due to inter-annual variation in environment conditions. However, dietary treatments, sample sizes and the precise location of antlers also changed, potentially contributing to variation in results. Additionally, in 2013 there was elevated mortality on the protein diet during the treatment period before the flies were released. This might affect our ageing estimates, but because flies were split between laboratory and field after application of the diet treatments, it should not impact our analysis of differences between laboratory and field environments. Quantifying ageing in the wild is logistically demanding, particularly in small insects. Our experiment was not designed to quantify inter-annual variation, so between-year differences should be regarded as tentative. Conversely, consistent patterns across our 2012 and 2013 experiments (e.g. higher mating rate and greater longevity in the laboratory vs. field, and actuarial ageing in both environments) can be regarded as especially robust, given that they were maintained despite these differences. Density and sex ratio varied throughout the life of each male and between years, but our results remained qualitatively unchanged when controlling for such variation both between and within environments (Appendix S1). Going forward, multi-season/year longitudinal studies will be important to quantify year-to-year variation and gain insight into the environmental variables that may contribute to it.

In summary, ours is the first study to investigate actuarial and reproductive ageing simultaneously in genetically similar wild and captive cohorts. Our data provide strong evidence that survival and reproduction can differ dramatically between laboratory and natural environments, and more tentative evidence that ageing rate can also differ between environments. We found that laboratory males lived much longer and mated much more often than wild males. We also found that males in the laboratory showed faster actuarial ageing than males in the field (2012; a pattern that contrasts with results of most previous studies), but this was not observed in 2013. Furthermore, we detected an overall higher average mortality rate of males fed protein compared to those fed sugar or water in both years (but with low support in 2013), providing the first evidence that the negative effect of protein on life span reported in many laboratory studies can also occur in wild populations, although perhaps less consistently.

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AUTHORS' CONTRIBUTIONS

B.S.M. designed the project, collected data, assisted in data analysis, and wrote the manuscript. N.O.R. performed data analysis, generated figures and wrote the manuscript. R.B. assisted in project design, data collection and wrote the manuscript. H.D.R. assisted in project design, data collection and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Survival and mating rate data and scripts for statistical analyses are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.406jj88 (Mautz et al. 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Appendix S1 *Additional information on methods, materials and statistical analyses including supporting tables S1- S12 and supporting figures S1 – S11*

Experimental design

In 2012, males were captured off antlers by gentle aspiration and stored in groups of approximately 20 in glass vials (95 mm x 22 mm) prior to marking and subsequent application of the experimental treatments. Males were marked before application of the diet treatment throughout the 2012 season. In 2013, males were collected individually by placing a small glass scintillation vial over the fly on the antler and were stored in these vials prior to marking or treatment. At the start of the field season males were marked and then placed in a diet treatment. Part way through the 2013 season, this was changed with males receiving the diet treatment first and then subsequently being marked before release.

Marking was performed following Bonduriansky and Brooks (1997). In brief, flies were individually placed in a cylindrical tube that had one end covered with clear plastic wrap. A plunger inserted through the other end was then used to gently push the fly against the plastic wrap with their ventral (2012) or dorsal (2013) side up. This unit was then placed under a stereomicroscope (Omano SZMN Series stereomicroscope and Stereo Zoom Binocular model SZ501101) and a ruler with 0.5-mm markings was placed on top of the unit next to the fly. The fly was photographed using a Nikon CoolPix S630 camera by placing the camera lens flush with one ocular eyepiece. The fly was then transferred to a similar tube with a fine-gauged mesh screen on the top. The same plunger was then used to restrain the male such that his scutum and scutellum were exposed between the wires. Males were then placed back under the microscopes and marked with a unique identifying code using different colours of Testors® enamel paint applied with a 2-4 hair paintbrush. In both years, some males did not survive or were lost during the marking/handling process and were therefore not included in the data set.

Carbohydrate and protein diets were created by soaking a small piece of cotton in a watery paste of natural granulated cane sugar (Sweet Source©, Canada) or a similar watery paste made from powdered, deactivated torula yeast (Lake States® Type B, Lallemand Inc, QC, Canada McCormick, Canada). The water treatment consisted solely of a water-soaked piece of the same cotton. The cotton pads were lightly squeezed to remove excess water before being placed in a glass vial (95 mm x 22 mm).

Statistical Analysis

We used different proxies for body size in 2012 (length from tip of head to end of the abdomen) and in 2013 (thorax length), as data collected after 2012 showed that the two measures were strongly correlated in males and the latter was easier to collect (Oudin, Bonduriansky, & Rundle, 2015). As average male body size covaries negatively with emergence date, we used the residuals from a regression of log body size on the day of emergence (Bonduriansky & Brassil 2005). Emergence date was scaled to a mean of zero and standard deviation of one prior to analysis (Schielzeth, 2010).

Actuarial ageing rate

We first fit Cox proportional hazards mixed models using the following formula:

$$\boldsymbol{h_t = h_{0t} \times e^{Xb+Zu}} \qquad (1),$$

where $\boldsymbol{h_t}$ is a vector of the hazard function at age *t*, $\boldsymbol{h_{0t}}$ is an unspecified baseline hazard at age *t*, $\boldsymbol{b}$ is a vector of fixed effects, $\boldsymbol{u}$ is a vector of random antler/cage identity effects, and $\boldsymbol{X}$ and $\boldsymbol{Z}$ are incidence matrices relating the observations to the fixed and random effects respectively. Fixed effects in $\boldsymbol{b}$ comprised the overall mean, two factors (an environment factor with two levels, field vs. lab, and a diet factor with three levels in 2012, protein, sugar, and water, and with two levels in 2013, protein vs. sugar) and two covariates (residual log body size and standardized emergence date). The random

effects in $\boldsymbol{u}$ were assumed to follow a normal distribution with zero mean vector and variance σ^2 ($\boldsymbol{u} \sim N(0, \sigma^2 \boldsymbol{I_n})$), where $\boldsymbol{I_n}$ represents the identity matrix of dimension equal to the number of cages/antlers. For each year separately, we compared five different models for hazards rates (null, environment only, diet only, environment and diet additive effects, environment × diet interaction models), including potentially confounding variables in all five models either as fixed effects (residual log body size and standardized emergence date) or as random effects (cage/antler identity). The exponentiated coefficient of a predictor variable represents the ratio of the mortality rate of one class of individuals for this variable relative to the mortality rate of a reference class. For example, exp(estimate) = 0.66 for sugar fed males in the lab in 2013 represents a 34% reduction (1 - 0.66 = 0.34) in mortality rate of sugar-fed males in the lab compared to those fed protein in the lab and a 75% reduction (1 – (0.66 × 0.38) = 0.75) in mortality rate of sugar-fed males in the lab compared to those fed protein in the field. To check the assumption of proportional hazards, we refit the model with ΔAICc = 0 using the coxph function and tested the independence between the scaled Schoenfeld residuals and age at death using the *coxzph* function in the survival package and checked visually the martingale and deviance residuals (Therneau, 2015). The assumption of proportional hazards was verified for all the effects but the effect of environment in 2012 (Table S4d).

For parametric survival analyses, we performed model selection in two steps. First, we compared the goodness of fit of different survival distributions (exponential, two-parameter Weibull, three-parameter Weibull, log-logistic, lognormal, Gompertz, and gamma) using the *flexsurv* (Jackson, 2016) and the *fitdistplus* (Delignette-Muller, 2015) packages. The different distributions correspond to different hazard functions with constant, increasing, decreasing, U and inverted-U shapes. In addition, the hazard function of the three-parameter Weibull includes a baseline mortality rate corresponding to age-independent mortality (Ricklefs, 1998). We evaluated the goodness-of-fit of each distribution to the data of each environment × diet combination using Kolmogorov-Smirnov tests (Table S2) and by

visually comparing the empirical and fitted cumulative distribution functions (Fig. S5, S6) using the *fitdistplus* package (Delignette-Muller, 2015) and by plotting the fitted cumulative hazard of each model against the non-parametric Kaplan-Meier estimates (Figures S7, S8) using the *flexsurv* package (Jackson, 2016). For each year and each environment separately, we also determined the distribution that best fit the data using AICc model selection. For each distribution, we fitted the following saturated model:

Scale = diet + residual log body size + emergence date + antler/cage identity.

Shape = diet

Baseline mortality = intercept

Antler/cage identity was included a fixed effect in each model as the *flexsurv* package does not allow for random effects. Note, that the shape parameter does not exist for the exponential distribution and that the baseline mortality parameter only exists in the three-parameter Weibull model. The two-parameter Weibull and the gamma distribution provided the best fit to the data (Table S2, Fig. S5-S8). Both gamma and two-parameter Weibull models fit the left tail of the distribution similarly, but they differed in their fit of the right tail and center of the distribution (Weibull being worse for the former, but better for the latter; Fig. S5 and S7). We chose to use the two-parameter Weibull (defined by its scale, λ, and shape, α, parameters) over the gamma for the parametric analyses as the latter cannot be fit with the *survival* package. In addition, and in contrast to the gamma distribution, there is an explicit formula for the two-parameter Weibull hazard function, defined as $h(t) = \alpha \frac{t^{\alpha-1}}{\lambda^{\alpha}}$, where the shape parameter, α, quantifies the increase in mortality rate of a population of a given age, t, ($\alpha = 1$ indicates constant mortality with age; $\alpha > 1$ or < 1 denote increasing or decreasing mortality with age respectively), and the scale parameter, λ, represents the 0.63-quantile of the survival distribution (i.e. the time at which ~63% of the individuals are dead; Crawley, 1993). For example, the mortality rate

increases linearly with age, *t*, when α ~2. Ageing measures associated with lifespan or maximum lifespan do not quantify actuarial senescence accurately (Baudisch, 2011; Moorad, Promislow, Flesness, & Miller, 2012) and several alternative measures of senescence have been proposed to describe the increase in mortality rate with age (e.g. Ricklefs & Scheuerlein 2002; Baudisch, 2011; Moorad, Promislow, Flesness, & Miller, 2012). We used the shape parameter of the Weibull distribution to investigate the effect of environment, diet and their interaction on ageing rates. The Weibull hazard rate increases linearly with age t on a log-log scale ($\log(\mathrm{h}(t) = \log(\frac{\alpha}{\lambda^{\alpha}}) + (\alpha - 1)\log(t)$), where both α and λ determine the intercept, $\log(\frac{\alpha}{\lambda^{\alpha}})$. Hence, the shape parameter is directly related to the derivate of the log hazard rate at age *t*, $\frac{dLog(h(t))}{dt} = \frac{\alpha-1}{t}$ (Ricklefs and Scherlein 2002), to the "relative rate of ageing" ($\frac{\frac{dh(t)}{dt}}{h(t)} = \frac{\alpha-1}{t}$, Kraus et al. 2013), and to the dispersion of the age as measured with Gini coefficient ($G = 1 - 0.5^{\frac{1}{\alpha}}$; Archer et al. 2018; Gigliarano et al. 2017; Gini 1912).

As the survival package does not support additive effects on Weibull shape, we performed model selection by comparing four different models for α (null, environment effect only, diet effect only, and environment × diet interaction). We controlled for differences in survival independent of ageing by including the interaction between environment and diet, residual log size, emergence date and antler/cage identity as a random effect on the scale parameter, λ in all four models tested. Goodness of fit was assessed by comparing the observed (i.e. proportion of individuals dying over 2-day intervals) and fitted mortality rates for different age classes using log-log plots (Fig. 1). The later was computed based on the Weibull survival function (proportion of individuals alive at time *t*), $S(t) = e^{-\left(\frac{t}{\lambda}\right)^{\alpha}}$, as: $\frac{S(t)-S(t+2)}{S(t)}$ (Crawley, 1993).

For both Cox proportional hazards and the two parameter Weibull analyses, we conducted additional analyses to investigate if differences between the lab and the field where due to differences in sex ratio/number of flies. We included the interaction between average daily number of flies and environment and between the average daily sex ratio and environment in all the models tested. The best model(s) when the number of flies and sex ratio when not included also had $\Delta AICc < 2$ when controlling for these effects in both Cox proportional hazards and Weibull models (Tables S3 and S5), but the estimates were different (Tables S4 and S6). Including sex ratio and total number of flies as covariates did not reduce the R^2 of the environment effect (e.g. $R^2 = 0.016$ vs. $R^2 = 0.019$ for Cox models that do or do not consider sex ratio and total number of flies in 2012 and $R^2 = 0.074$ vs. $R^2 = 0.051$ for Cox models that do or do not consider sex ratio and total number of flies in 2013, Table S4c). This indicates that sex ratio and total number of flies do not account for differences in mortality rates observed between environments.

Trade-offs between mating rate in early vs. late-life

We only considered variation in mating rate intercept between males using male identity as a random effect in our main analysis. We tested if males with a higher mating rate early in life had a sharper decline in mating rate using random slope models (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We compared our best models in 2012 or 2013 respectively that included a male identity random intercept with two models. The first model included a random effect of male identity on both the intercept and the age slope, whereas the second model included these two random effects and a correlation between them (Zuur et al., 2009). Models were compared using AICc (see main text). In both 2012 and 2013, the model with a random intercept for each male and no random slope had the lowest AICc ($\Delta AICc > 1.78$ for the other models, Table S12a).

Efffect of selective disappearance on the estimate of reproductive ageing

We performed additional analyses to investigate whether our estimates of reproductive ageing where biased by selective disappearance due to removal of low-quality individuals from the population (Nussey, Coulson, Festa-Bianchet, & Gaillard, 2008; Nussey, Froy, Lemaitre, Gaillard, & Austad, 2013; van de Pol & Verhulst, 2006). We compared three models. The first model corresponded to the model with lowest AICc in 2012 or 2013 from our main analyses (Tables S7c and S9c). The second model included individual lifespan as a covariate, whereas the third model included an interaction between environment and lifespan (to test for difference in selective disappearance between environments, Table S12b). Estimates for reproductive ageing (i.e. age effects) from the model with the lowest AICc (Table S12c) did not differ from those of the best model in our main analyses (Table S8a and S10a). Hence, our estimates of reproductive ageing were not biased by selective disappearance.

Test of potential confounding factors in our actuarial and reproductive ageing analyses

Our primary aim is to test for differences in survival and mating success between the field environment and a lab environment similar to the one typically used in senescence studies in insect. As differences in the average sex ratio or in the average number of flies daily present on each antler or in each cage represent inherent differences between the two environments, we did not control for these variables in the main analyses. Here we further investigate whether the effect of environment on survival or mating rate was due to differences in sex ratio or in total number of flies between environments. If this were the case, we would expect that the best model including an effect of environment would no longer be supported when accounting for sex ratio and total number of flies. To determine this, we included interactions between environment and standardized sex ratio and between environment and total number of flies in all models tested. The models with the lowest AICc in the main analyses were

consistently the best models in these supplementary analyses (Tables S3-S9). We also investigated whether the effect of environment on mortality rate and mating rate was partly due to differences in sex ratio and total number of flies (density) between environments. For example, the lower mating rate observed in the field could be due to a lower sex ratio in this environment. Contrary to the environment factor, sex ratio and total number of flies vary both between and within environments. If these variables underlie some of the differences between environments, we would expect the explanatory power of the environment effect (i.e. its coefficient of determination, R^2) to be lower in models that do account for these covariates compared to models that do not. We therefore tested for a decrease in the R^2 of the environment effect in models that accounted for these variables. For each year separately, we fit the model lowest AICc with and without an effect of environment and computed likelihood-based coefficients of determination (R^2, Ives, 2017) by hand for Cox analyses and using the rr2 package (Ives & Li, 2018) for mating rate analyses. We then refit two similar models that controlled for the effects of sex ratio and total number of flies to determine whether the R^2 of the environment effect decreased when accounting for sex ratio and total number of flies. The model with lowest AICc remained unchanged when accounting for sex ratio and total number of flies (Tables S3, S5, S7, S9) and the R^2 of the environment effect did not decrease in this best model (Tables S4, S8 and S10). This indicates that sex ratio and total number of flies do not account for differences in mating rates observed between environments.

Our observed increase in actuarial ageing in the field could be due to older males visiting antlers less often compared to young males, rather than older males having a higher mortality rate *per se*. We therefore tested whether the probability of a male being observed on at least one antler during the day, p increased with its age. We used generalized linear mixed models with a binomial error distribution and a logit link function. We compared three models: a model with only linear age effects (age), a model with quadratic age effects (age + age^2) and a null model (no age effect). Residual log body size

and emerge date were included as covariates in all models tested and individual identity was included as a random effect to account for repeated measures. To account for seasonal effects, and for the correlation among mating observations on a given day, we also included emergence date and an interaction between observation day and environment as random effects. The effect of age was not supported in 2012 (ΔAICc = 2.02 for a model with a linear age effect, Table S11a) and was supported in 2013 but with a positive age effect (ΔAICc = 3.82 for the null model, Table S11a). Estimates of the best models for 2012 and 2013 are reported in Table S11b.

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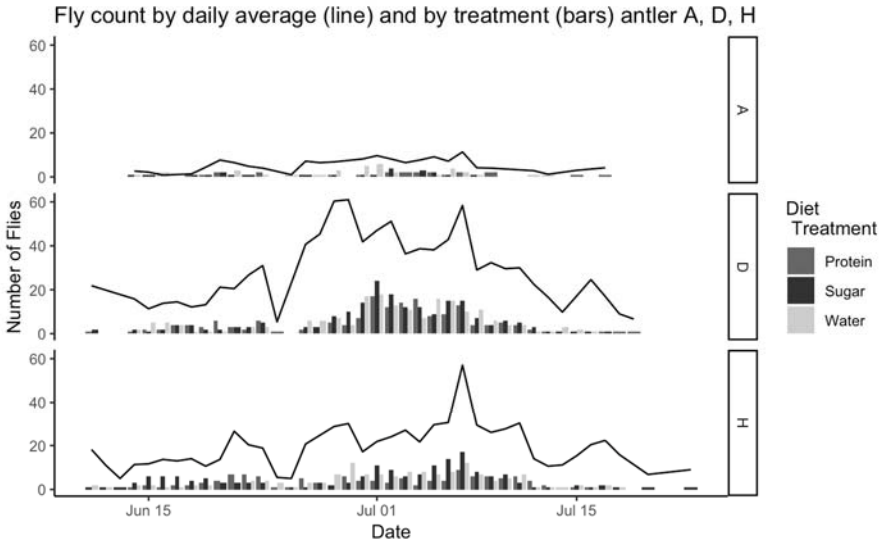
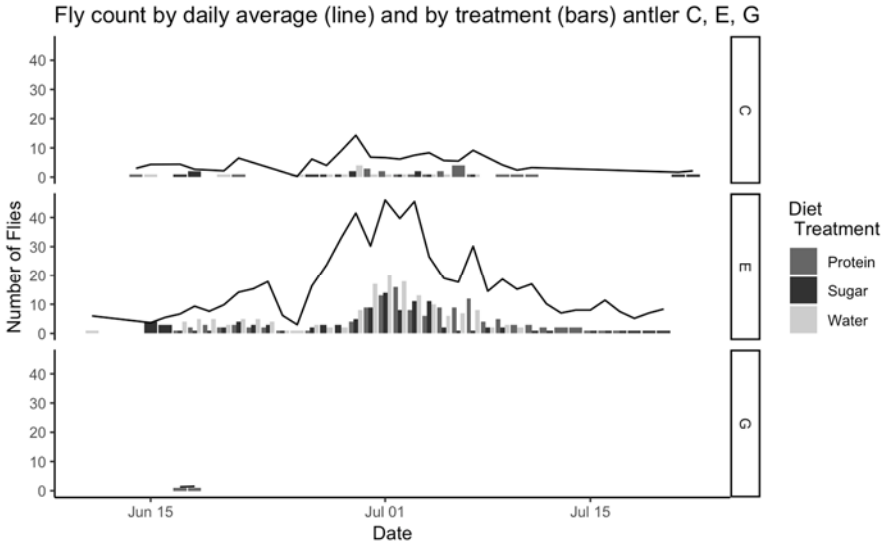
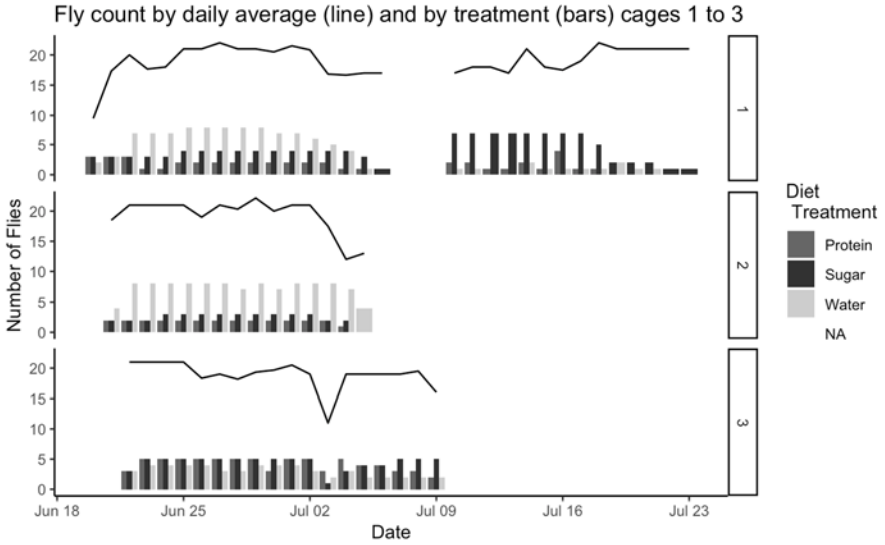

Fly count by daily average (line) and by treatment (bars) antler A, D, H
Number of Flies
A
D
H
Diet Treatment
Protein
Sugar
Water
Jun 15
Jul 01
Jul 15
Date

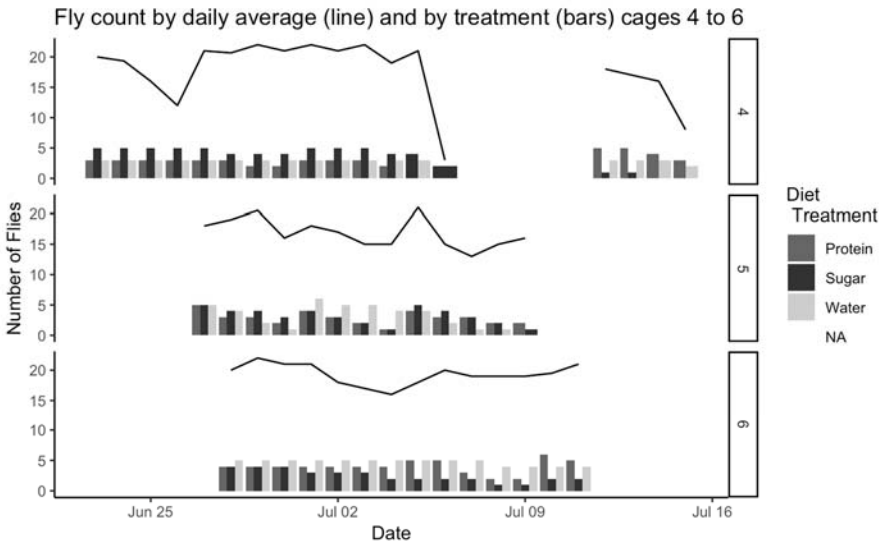
258


Fly count by daily average (line) and by treatment (bars) antler C, E, G
Number of Flies
C
E
G
Diet Treatment
Protein
Sugar
Water
Jun 15
Jul 01
Jul 15
Date

259


Fly count by daily average (line) and by treatment (bars) cages 1 to 3
Number of Flies
1
2
3
Diet Treatment
Protein
Sugar
Water
NA
Jun 18
Jun 25
Jul 02
Jul 09
Jul 16
Jul 23
Date

260



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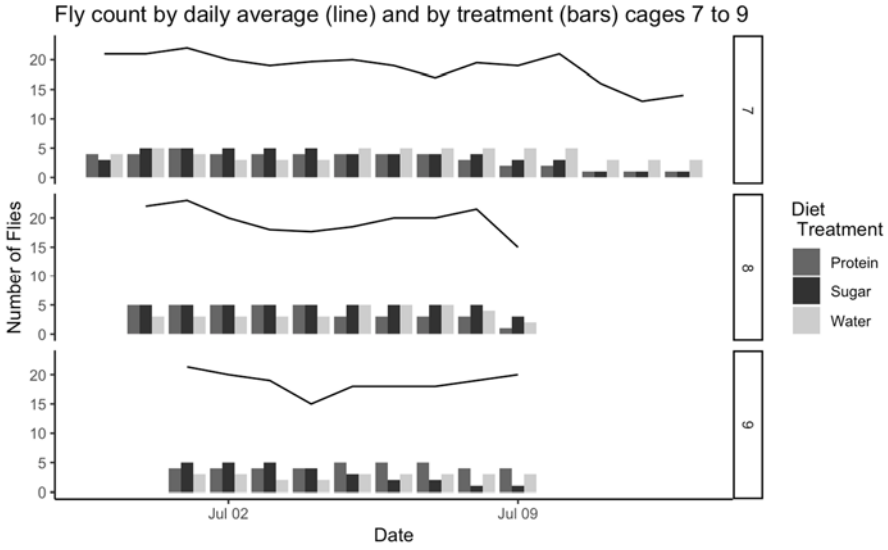

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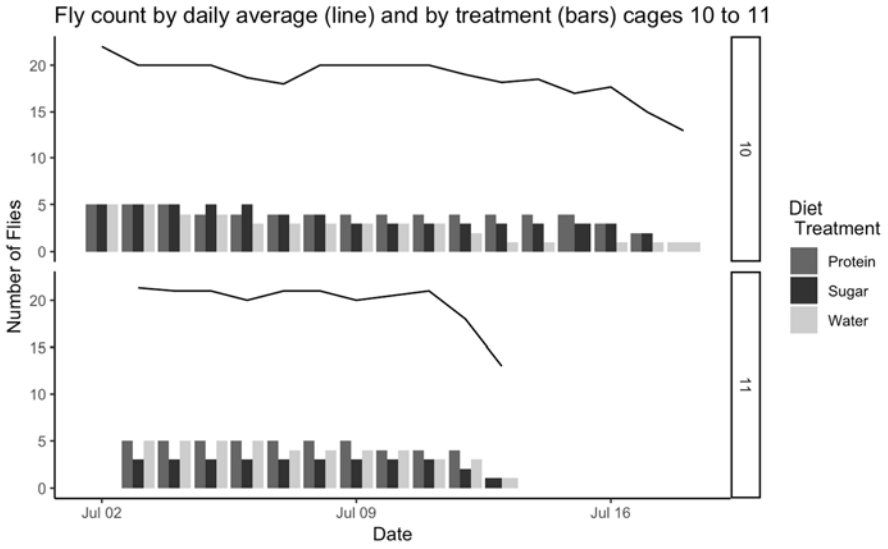

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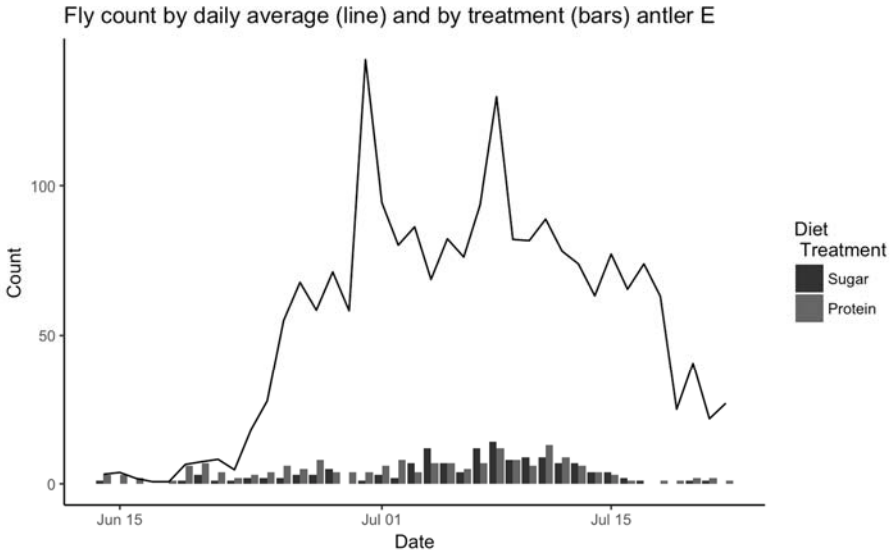
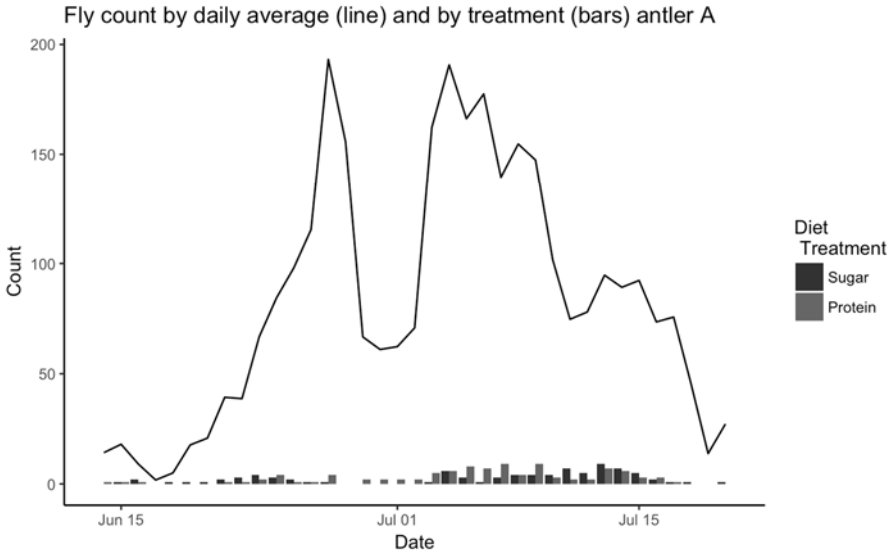
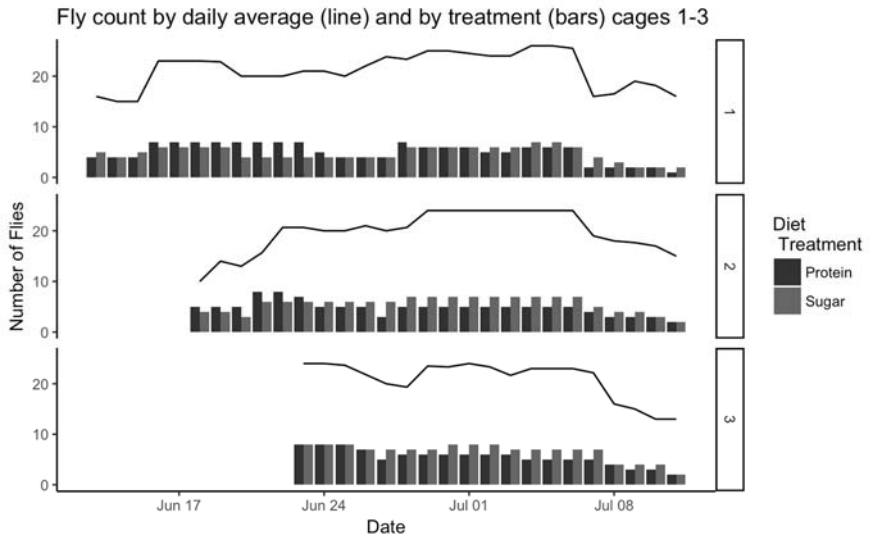
Figure S1. Daily average total number/count of flies (line) and in each treatment (bars) by date for each antler and cage in 2012. Cages 1 and 4 were used twice with a 4- and 5-day interval between uses, respectively (hence the separate sets of bars).


Fly count by daily average (line) and by treatment (bars) antler E
Count
100
50
0
Jun 15
Jul 01
Jul 15
Date
Diet
Treatment
Sugar
Protein

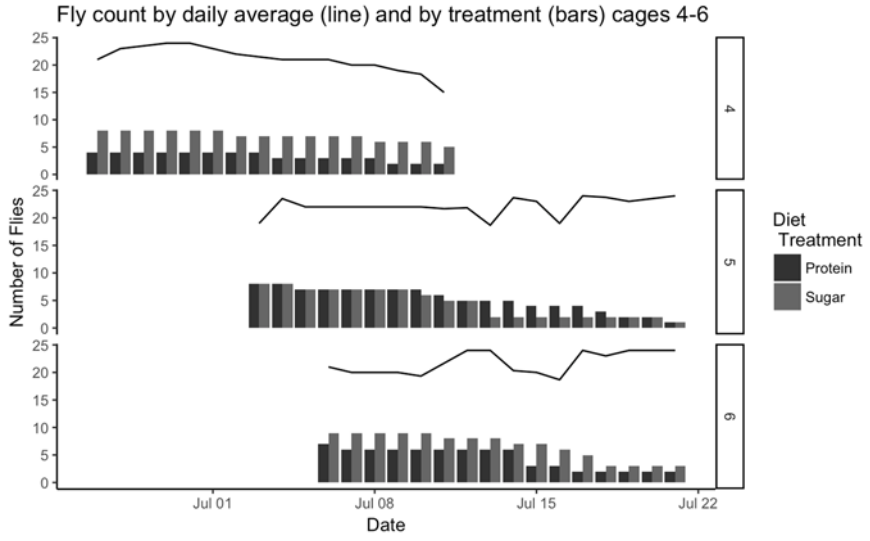
269


Fly count by daily average (line) and by treatment (bars) antler A
Count
200
150
100
50
0
Jun 15
Jul 01
Jul 15
Date
Diet
Treatment
Sugar
Protein

270


Fly count by daily average (line) and by treatment (bars) cages 1-3
Number of Flies
0
10
20
1
2
3
Jun 17
Jun 24
Jul 01
Jul 08
Date
Diet Treatment
Protein
Sugar

271


Fly count by daily average (line) and by treatment (bars) cages 4-6
Number of Flies
0
5
10
15
20
25
4
5
6
Jul 01
Jul 08
Jul 15
Jul 22
Date
Diet Treatment
Protein
Sugar

272

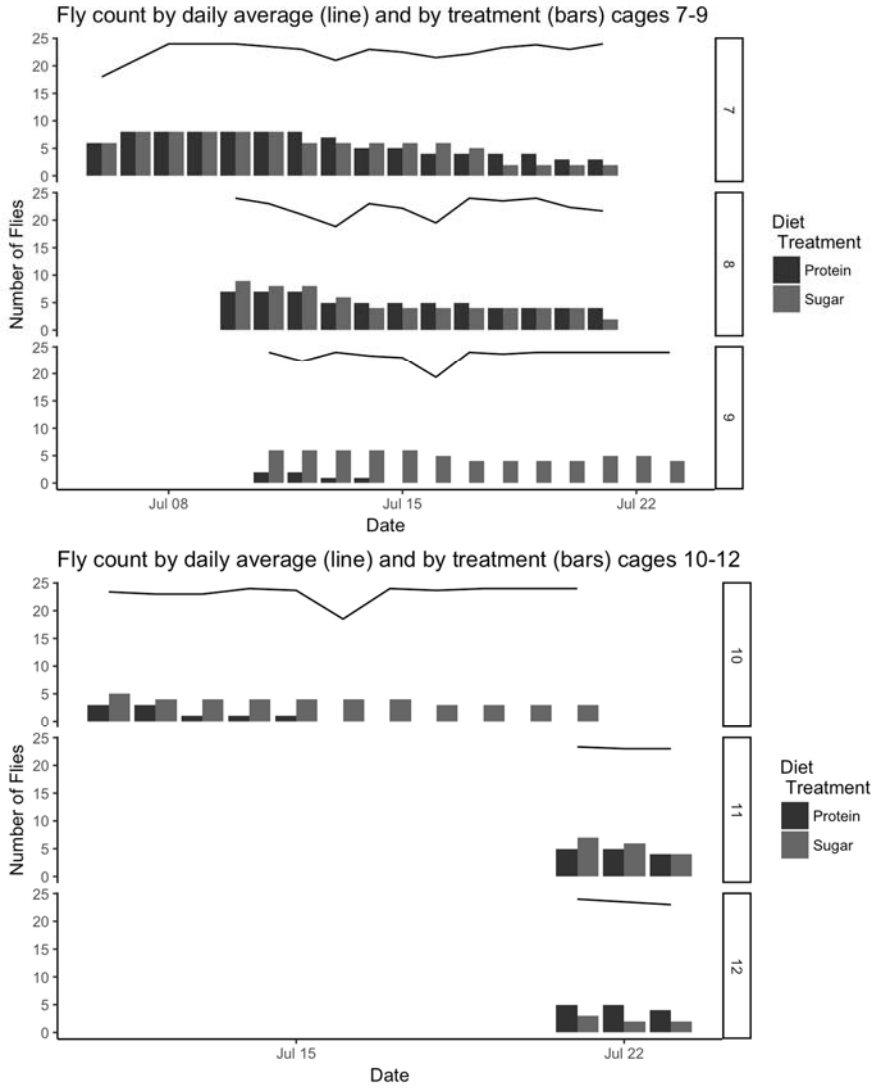
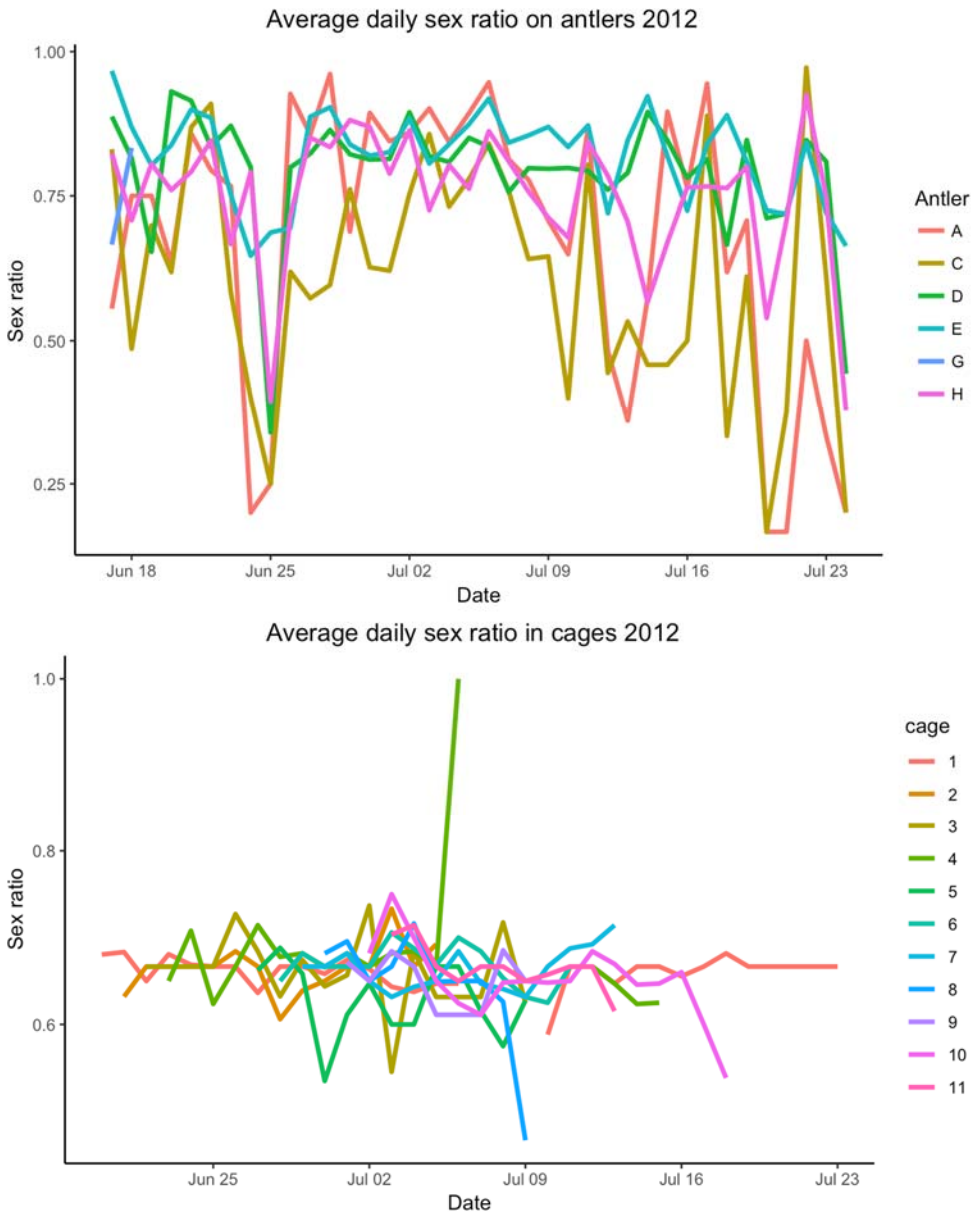


Figure S2. Average total number/count of flies (line) and in each treatment (bars) by date for each antler and cage in 2013.



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284 Figure S3. Average daily sex ratio by antler and cage in 2012.

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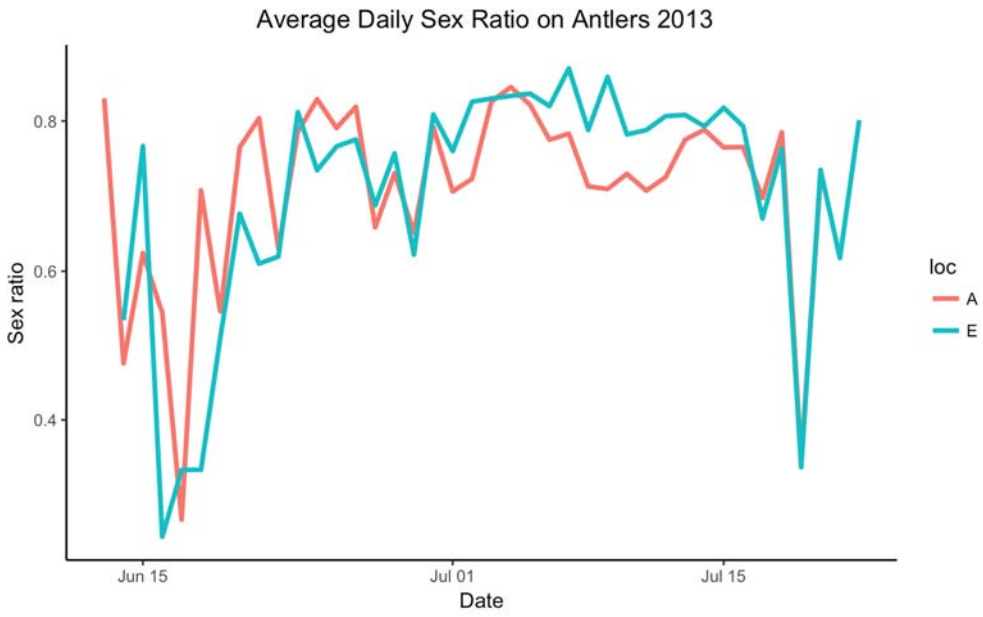


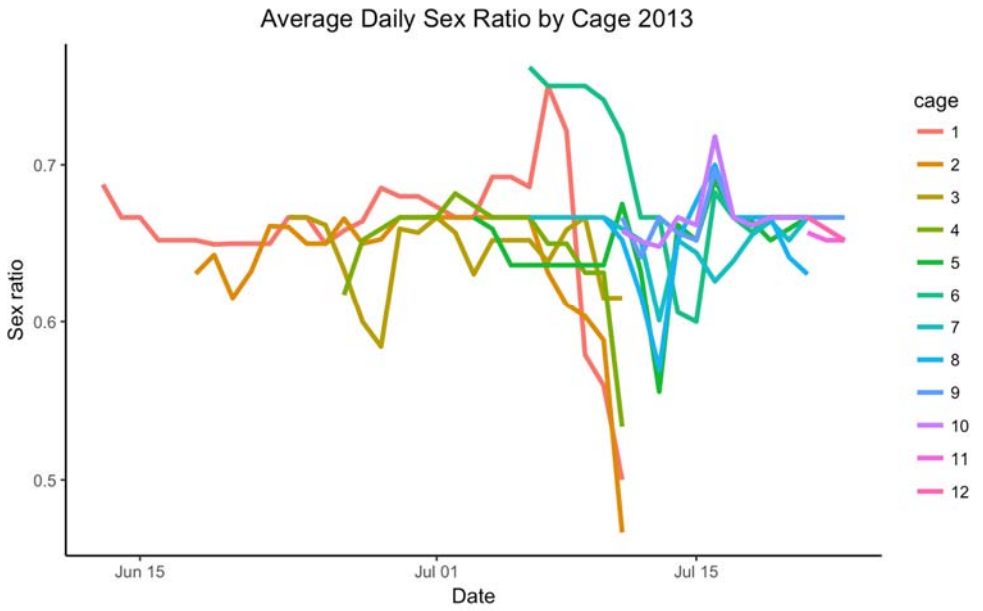
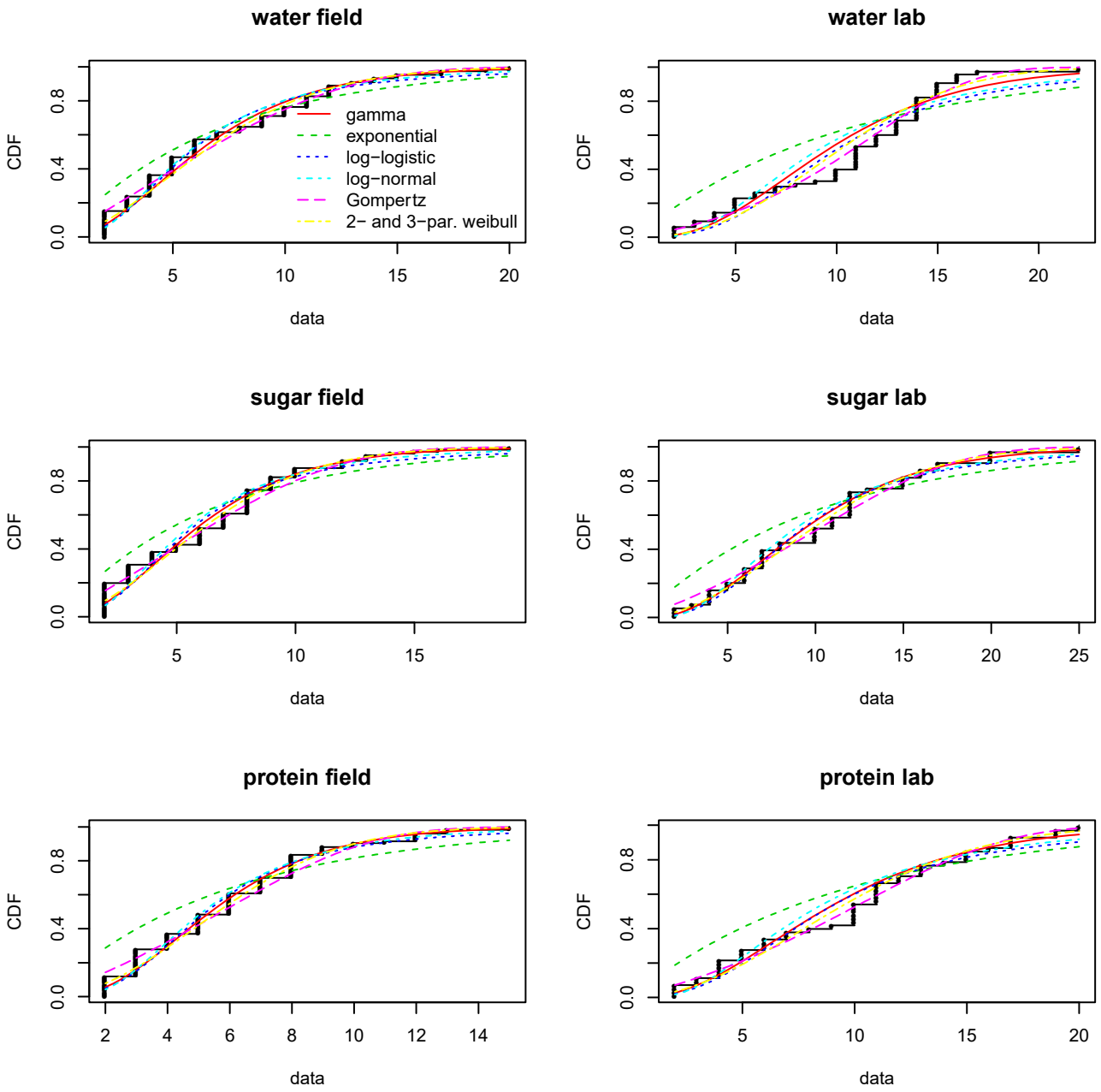


Figure S4. Average daily sex ratio on antlers and in cages in 2013.



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297 Figure S5 Goodness-of-fit comparing the empirical and fitted cumulative distribution function for each

298 survival distribution in 2012.

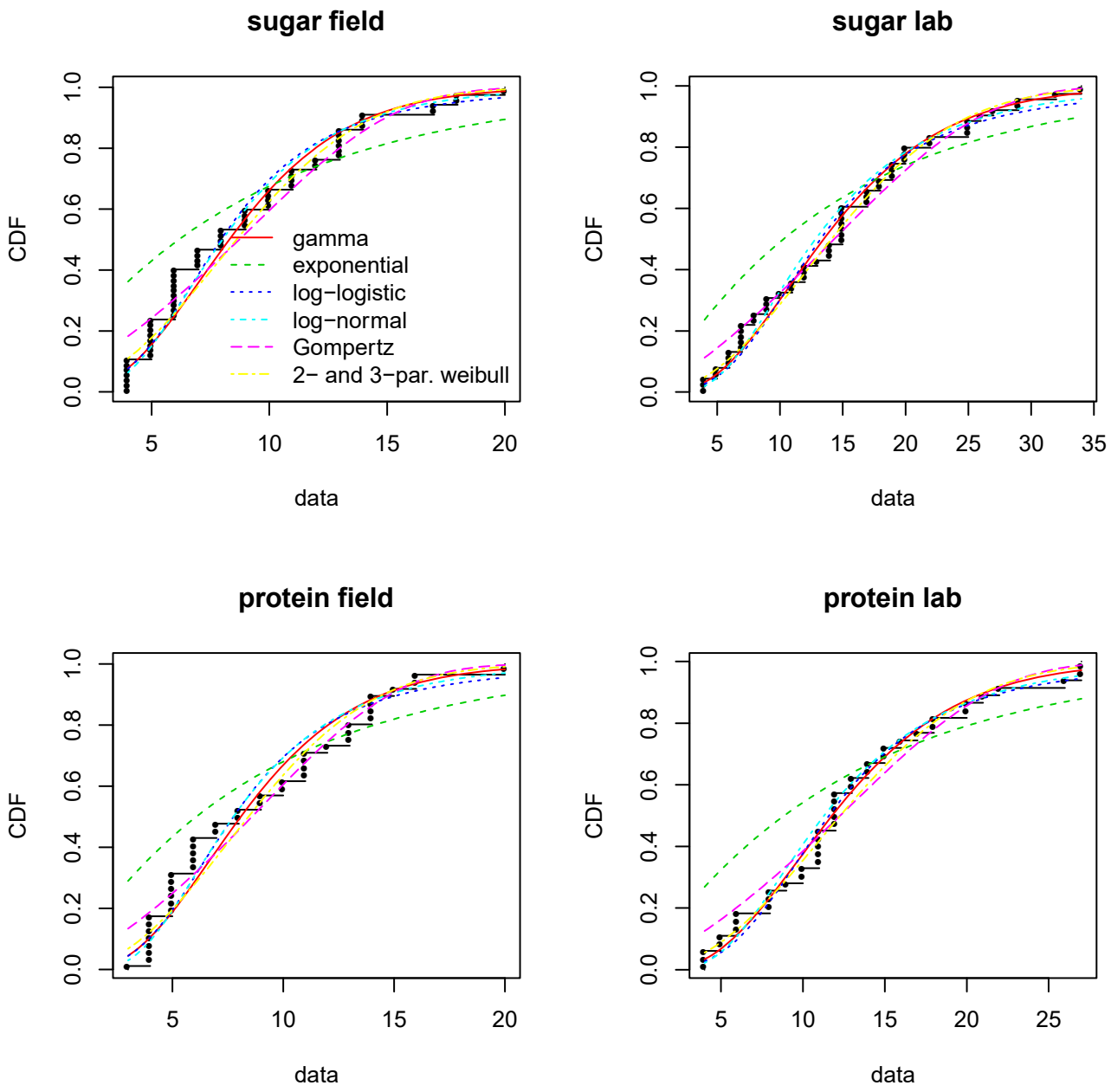


Figure S6 Goodness-of-fit comparing the empirical and fitted cumulative distribution function for each survival distribution in 2013.

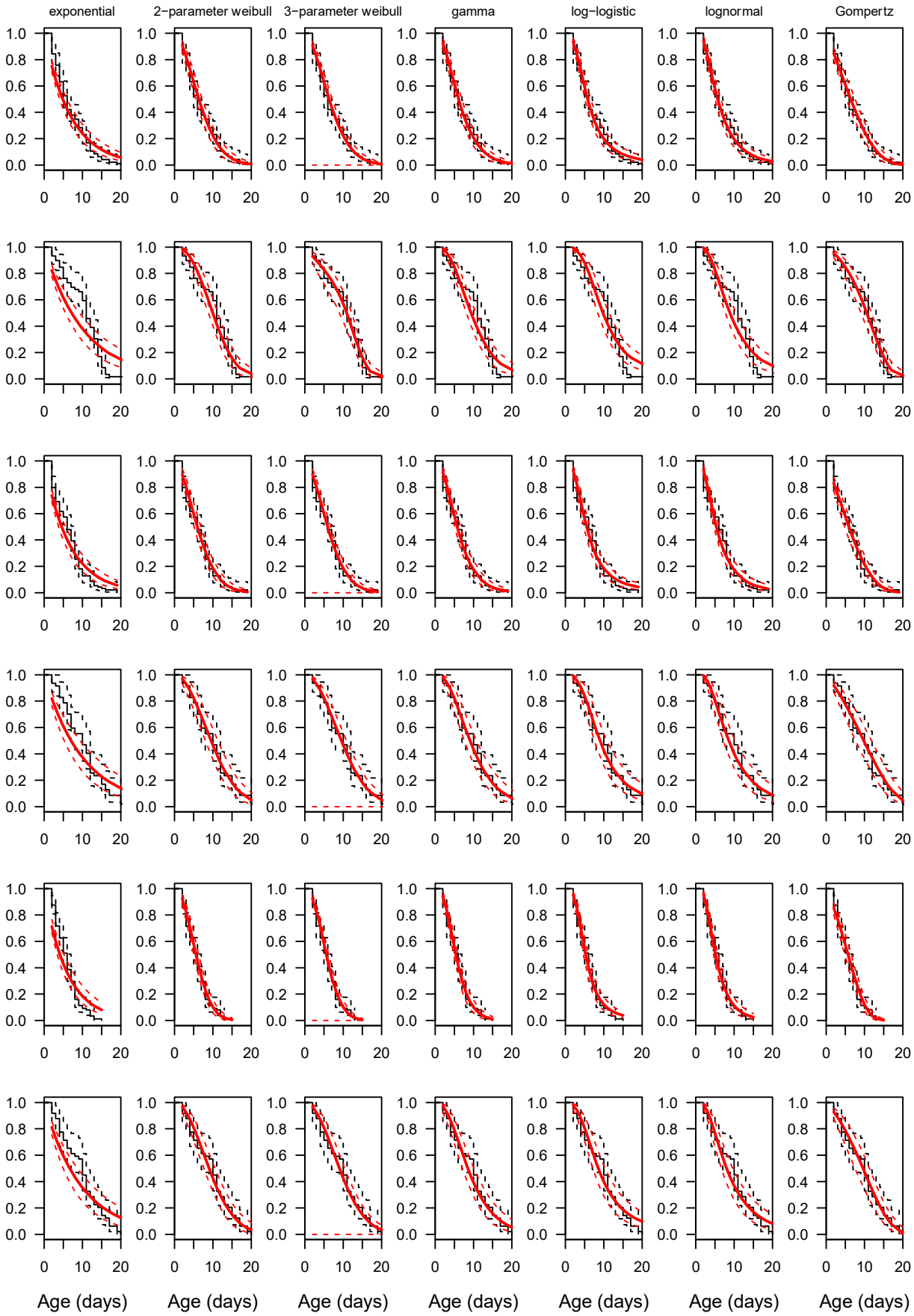

exponential
2−parameter weibull
3−parameter weibull
gamma
log−logistic
lognormal
Gompertz
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
Age (days)

Figure S7. Goodness-of-fit of the survival curve of each of the seven survival distributions for each environment × diet combination in 2012. Red line: fit based on the parametric distribution, black line: fit based on non-parametric Kaplan-Meier estimates. Dashed lines represent 95% confidence intervals. The different rows represent different the diet × environment combinations (from top to bottom: water/field, sugar/field, protein/field, water/lab, sugar/ lab, protein/ lab).

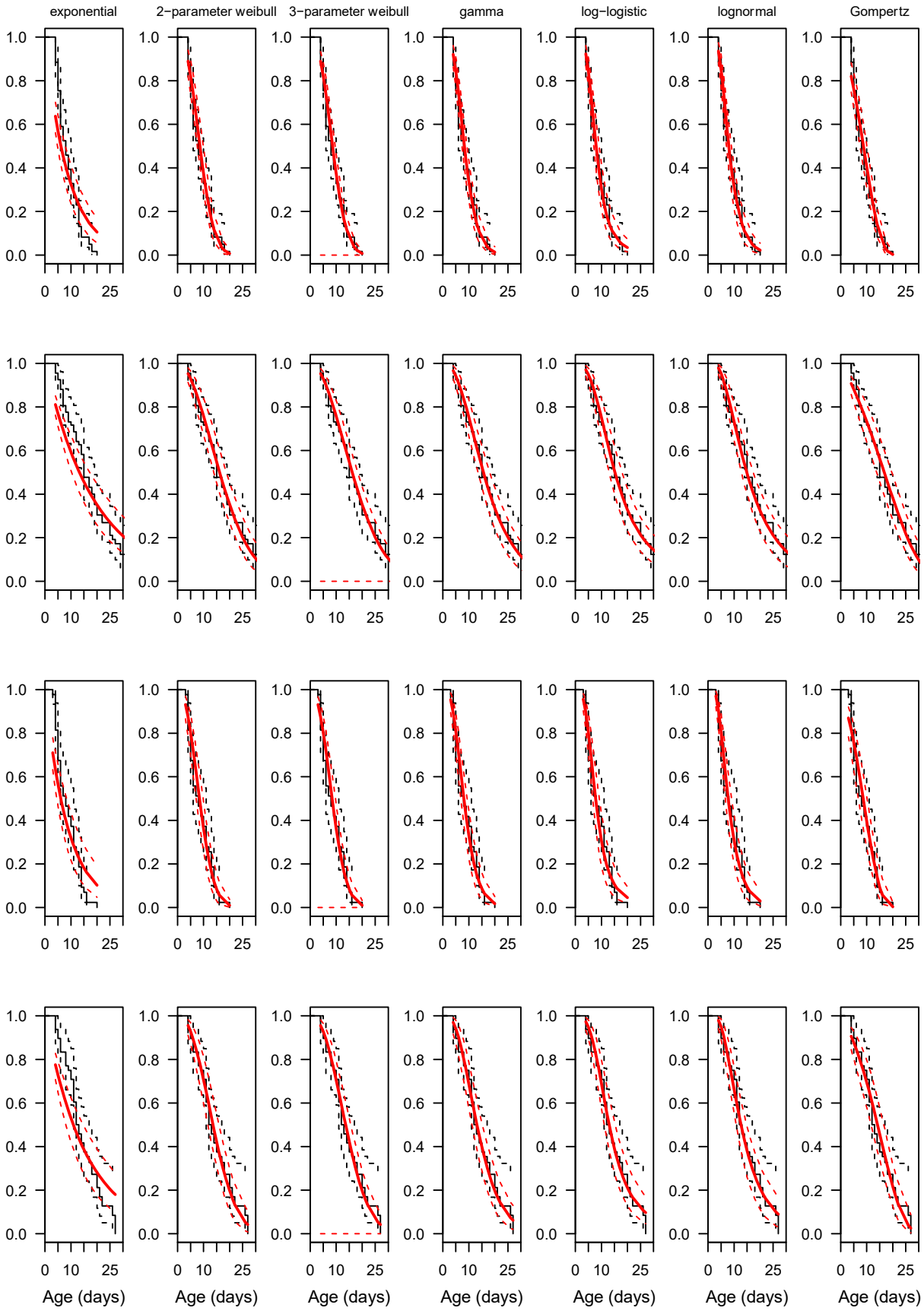

exponential
2−parameter weibull
3−parameter weibull
gamma
log−logistic
lognormal
Gompertz
1.0
0.8
0.6
0.4
0.2
0.0
0
10
25
Age (days)

Figure S8 Goodness-of-fit of the survival curve of each of the seven survival distributions. Red line: fit based on the parametric distribution, black line: fit based on non-parametric Kaplan-Meier estimates. Dashed lines represent 95% confidence intervals. The different rows represent different the diet × environment combinations (from top to bottom: sugar/field, protein/field, sugar/ lab, protein/ lab).

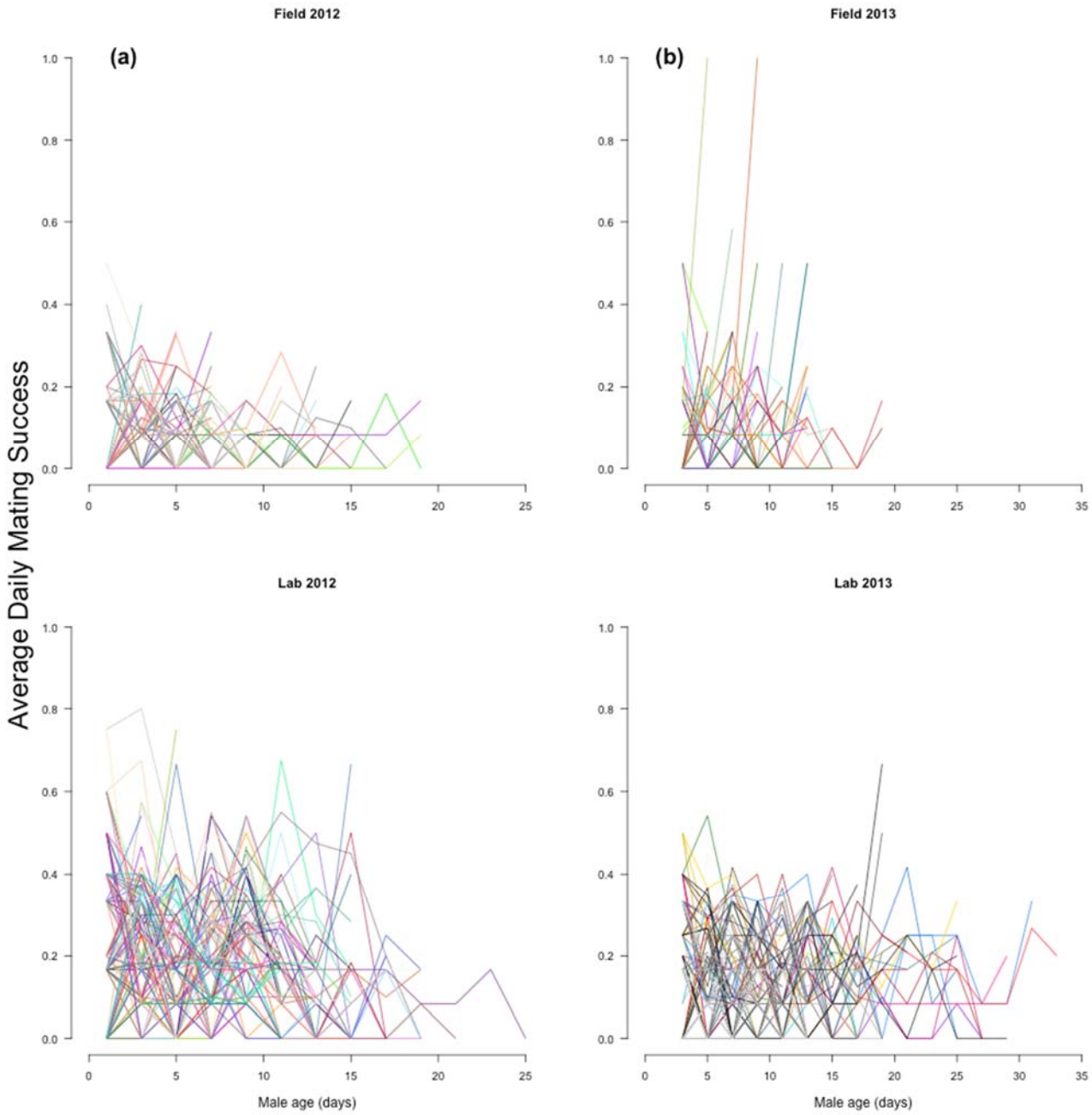


Figure S9 Age-specific male mating success at the individual level. Mating success was computed over two-day intervals and is shown with a unique color for each male.

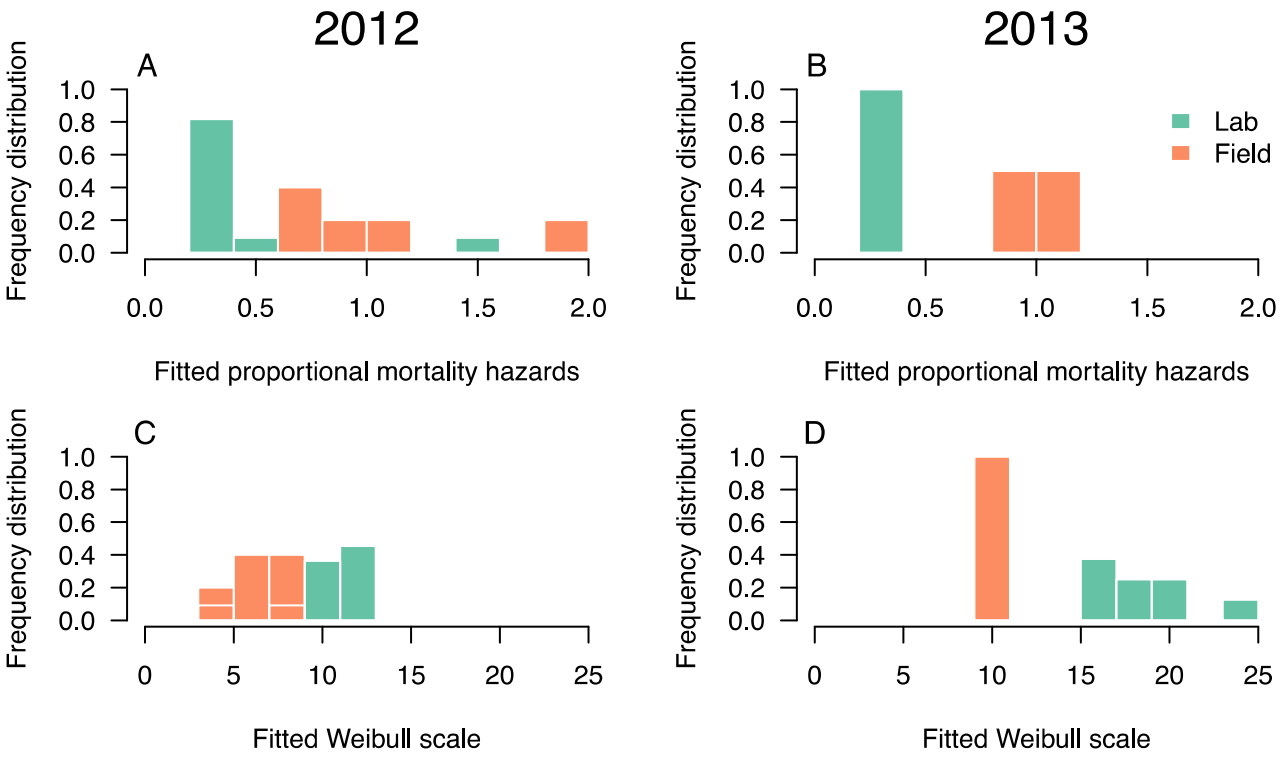
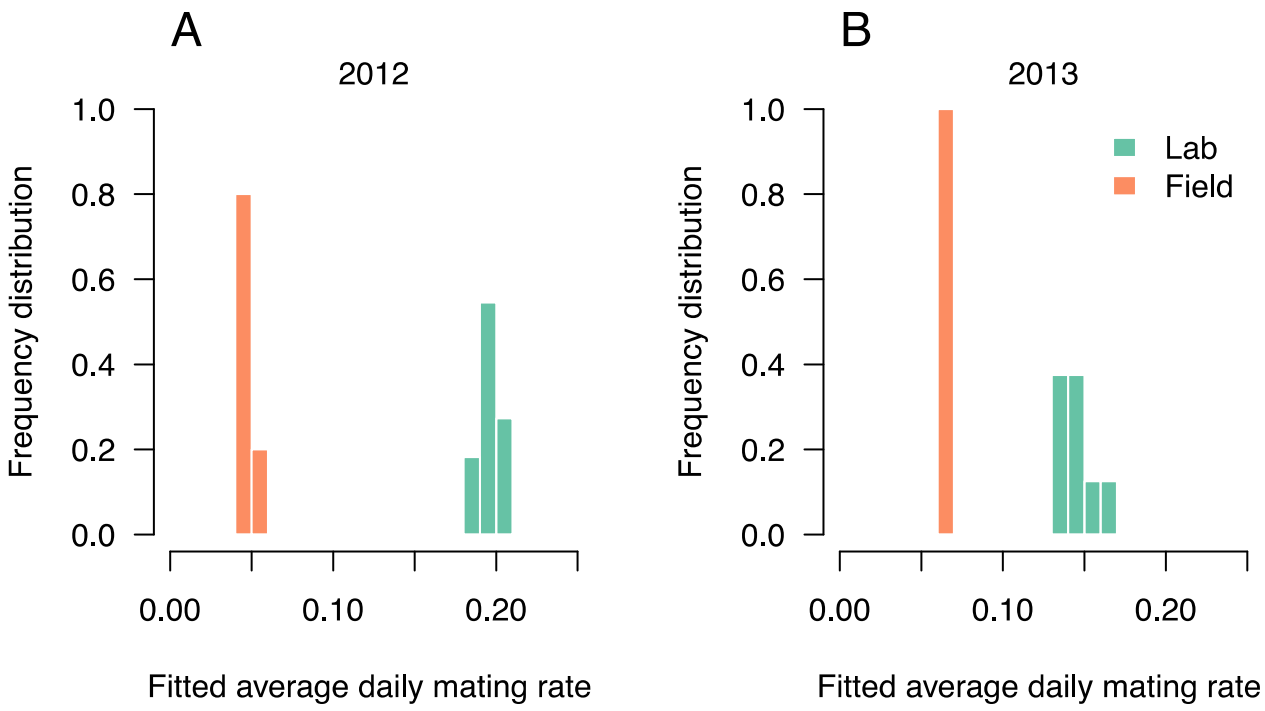


Figure S10 Distribution of fitted proportional mortality hazards in (A) 2012 and in (B) 2013 and fitted Weibull scales across antlers in the field and across cages in the lab in (C) 2012 and in (D) 2013. Fitted values were computed using the estimates and the BLUPs of the model with lowest AICc for a male with a protein diet. For fitted proportional hazards, estimates are represented relative to the average hazard of males in the field environment (i.e. which have a proportional mortality hazard of one).



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334 Figure S11 Distribution of average mating rate across antlers in the field and across cages in the lab in

335 (A) 2012 and in (B) 2013. Fitted average mating rates were computed using the estimates and the

336 BLUPs of the model with lowest AICc for a male of an average age with a protein diet.

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