

Supplementary Information

Effects of ageing on timing of hibernation and reproduction

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S1) Results path analysis (R-package piecewise SEM)

Below we provide the R-Code used to perform and retrieve results from a path analysis using R-package piecewiseSEM¹ computed from a list of models fitted using functions lme R-package nlme² and glmer R-package lme4³. Path analyses were calculated separately for males and females.

Abbreviations used:

hibstart.doy = onset of hibernation (day of year), hibdur.days = hibernation duration, hibend.doy = termination of hibernation (day of year), repro_active = females: giving birth yes/no; males: testis > 20mm for at least three weeks yes/no, log_age = age in years on a logarithmic scale, age.death = longevity (years), bm_before = body mass prior to hibernation, a.name = animal ID, diet = feeding condition (see main text).

Males:

```
modlist=list(
  lme(hibstart.doy~repro_active+age.death+log_age+bm_before,random=~1|year/diet/a.name,na.action
=na.omit,data=datm),
  lme(hibdur.days~repro_active+hibend.doy+hibstart.doy+age.death+log_age+bm_before,random=~1|yea
r/diet/a.name,na.action=na.omit,data=datm),
  lme(hibend.doy~log_age+age.death+repro_active+bm_before,random=~1|year/diet/a.name,na.action=n
a.omit,data=datm),
  glmer(repro_active~log_age+age.death+(1|a.name)+(1|diet),family=binomial,data=datm)
)

> sem.fit(modlist,datm, .progressBar = FALSE,conditional=T)

$missing.paths

missing.path          estimate std.error df crit.value p.value
1 repro_active~bm_before+age.death+log_age
                    -0.0034    0.0076  NA    -0.4432  0.6576

2 hibend.doy~hibstart.doy+age.death+log_age+bm_before+repro_active
                    0.0682    0.0497 119     1.3710  0.1729

$Fisher.C
  fisher.c df p.value
1      4.35  4  0.361

$AIC
  AIC  AICc  K  n
1 72.35 94.593 34 142

> sem.model.fits(modlist)

  Class  Family  Link  n  Marginal  Conditional
1    lme gaussian identity 142 0.1750271  0.9990470 (model hibstart)
2    lme gaussian identity 142 0.9945171  1.0000000 (model hibdur.days)
3    lme gaussian identity 142 0.1755294  0.9419713 (model hibend)
4 glmerMod binomial  logit 142 0.1905036  0.5721905 (model repro)

> coef.table = sem.coefs(modlist, datm)
```

```
> coef.table
```

	response	predictor	estimate	std.error	p.value	
1	hibstart.doy	repro_activeyes	32.935382406	7.732444811	0.0000	***
2	hibstart.doy	age.death	-3.038854601	1.407956948	0.0329	*
3	hibstart.doy	log_age	-30.724454509	14.842936147	0.0406	*
4	hibstart.doy	bm_before	-0.104131171	0.099344493	0.2967	
5	hibdur.days	hibstart.doy	-1.007209540	0.008064035	0.0000	***
6	hibdur.days	hibend.doy	1.008592786	0.013907149	0.0000	***
7	hibdur.days	age.death	-0.262526195	0.155445177	0.0939	
8	hibdur.days	repro_activeyes	-0.575418966	0.754109989	0.4470	
9	hibdur.days	log_age	0.212868851	1.416235559	0.8808	
10	hibdur.days	bm_before	0.000171043	0.009773119	0.9861	
11	hibend.doy	log_age	-35.935478585	9.008333035	0.0001	***
12	hibend.doy	bm_before	0.082843564	0.062218935	0.1856	
13	hibend.doy	repro_activeyes	-3.963374964	4.643869738	0.3951	
14	hibend.doy	age.death	0.262313456	0.916064744	0.7751	
15	repro_active	log_age	3.042688245	0.945166071	0.0013	**
16	repro_active	age.death	0.179133364	0.108114756	0.0975	

```
> sem.coefs(modlist,datm,standardize = "scale")
```

	response	predictor	estimate	std.error	p.value	
1	hibstart.doy	repro_activeyes	0.7758052709	0.182139239	0.0000	***
2	hibstart.doy	age.death	-0.1826258057	0.084612973	0.0329	*
3	hibstart.doy	log_age	-0.2076733158	0.100326436	0.0406	*
4	hibstart.doy	bm_before	-0.0769855499	0.073448294	0.2967	
5	hibdur.days	hibstart.doy	-0.9561310266	0.007655084	0.0000	***
6	hibdur.days	hibend.doy	0.5261709102	0.007255195	0.0000	***
7	hibdur.days	age.death	-0.0149766956	0.008867896	0.0939	
8	hibdur.days	repro_activeyes	-0.0128667558	0.016862407	0.4470	
9	hibdur.days	log_age	0.0013658595	0.009087186	0.8808	
10	hibdur.days	bm_before	0.0001200439	0.006859114	0.9861	
11	hibend.doy	log_age	-0.4419838220	0.110796841	0.0001	***
12	hibend.doy	bm_before	0.1114506430	0.083704034	0.1856	
13	hibend.doy	repro_activeyes	-0.1698787534	0.199046222	0.3951	
14	hibend.doy	age.death	0.0286848754	0.100174816	0.7751	
15	repro_active	log_age	0.8731091300	0.271217194	0.0013	**
16	repro_active	age.death	0.4570170833	0.275831629	0.0975	

```
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

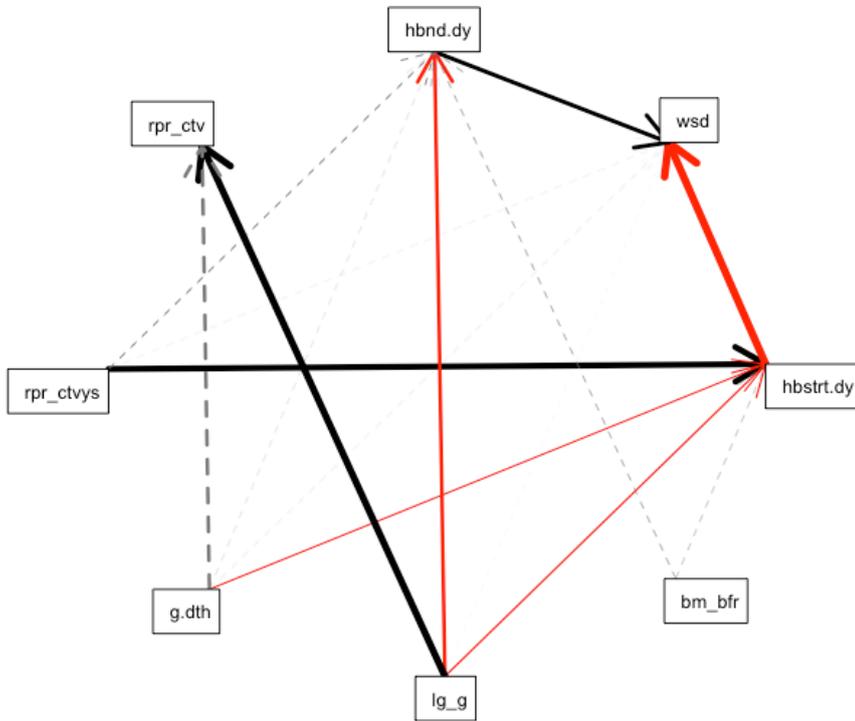


Fig. S1.1: Results plot of the path analysis for male edible dormice R-package piecewiseSEM¹. Arrows represent unidirectional relationships among variables. Red lines indicate negative effects, black line positive effects. Solid lines indicate significant relationships, dashed lines non-significant relationships. The thickness of the line represents the strength of the effect, i.e., it is proportional to the absolute value of the scaled coefficient (see coef.table, sem.coefs for males ¹).

Females:

```
modlist=list(  
lme(hibstart.doy~repro_active+age.death+log_age+bm_before,random=~1|year/diet/a.name,na.action  
=na.omit,data=datf),  
lme(hibdur.days~repro_active+hibend.doy+hibstart.doy+age.death+log_age+bm_before,random=~1|yea  
r/diet/a.name,na.action=na.omit,data=datf),  
lme(hibend.doy~log_age+age.death+repro_active+bm_before,random=~1|year/diet/a.name,na.action=n  
a.omit,data=datf),  
glmer(repro_active~log_age+age.death+(1|a.name)+(1|diet),family=binomial,data=datf)  
)
```

```
> sem.fit(modlist,datf, .progressBar = FALSE,conditional=T)
```

```
$missing.paths
```

```
missing.path          estimate std.error df crit.value p.value  
1 repro_active ~ bm_before + age.death + log_age  
          -0.0024      0.0115  NA  -0.2087  0.8347  
2 hibend.doy ~ hibstart.doy + age.death + log_age + bm_before + repro_active  
          0.0754      0.0716  84   1.0540  0.2949
```

```
$Fisher.C
```

```
fisher.c df p.value  
1      2.8  4  0.591
```

```
$AIC
```

```
AIC  AICc  K  n  
1 70.8 104.321 34 106
```

```
> sem.model.fits(modlist)
```

```
Class Family Link n Marginal Conditional  
1 lme gaussian identity 106 0.06575485 0.9990519 (model hibstart)  
2 lme gaussian identity 106 0.88721109 0.9999031 (model hibdur.days)  
3 lme gaussian identity 106 0.23722244 0.9375330 (model hibend)  
4 glmerMod binomial logit 106 0.21011177 0.4109037 (model repro)
```

```
> coef.table = sem.coefs(modlist, datf)
```

```
> coef.table  
response predictor estimate std.error p.value  
1 hibstart.doy repro_activeyes 15.66018241 7.87237108 0.0499 *  
2 hibstart.doy log_age -16.17922505 14.53748679 0.2689  
3 hibstart.doy age.death -1.57683432 1.43441286 0.2747  
4 hibstart.doy bm_before 0.09285748 0.11511078 0.4221  
5 hibdur.days hibstart.doy -0.93165812 0.03823779 0.0000 ***  
6 hibdur.days hibend.doy 0.92803146 0.05314982 0.0000 ***  
7 hibdur.days bm_before 0.09772778 0.04498413 0.0327 *  
8 hibdur.days age.death -0.75925928 0.55768740 0.1771  
9 hibdur.days repro_activeyes -1.67043213 3.09172169 0.5904  
10 hibdur.days log_age 1.31788831 6.03808858 0.8278  
11 hibend.doy log_age -45.04480313 10.45508755 0.0000 ***  
12 hibend.doy bm_before 0.16172194 0.08296344 0.0546  
13 hibend.doy age.death -0.60513335 1.04100755 0.5626  
14 hibend.doy repro_activeyes 1.87171858 5.69266025 0.7431  
15 repro_active log_age 4.40659077 1.61300025 0.0063 **  
16 repro_active age.death -0.14063443 0.18182462 0.4392
```

```
> sem.coefs(modlist,datf,standardize = "scale")
```

```
response predictor estimate std.error p.value  
1 hibstart.doy repro_activeyes 0.463890414 0.23319807 0.0499 *  
2 hibstart.doy log_age -0.134288033 0.12066162 0.2689  
3 hibstart.doy age.death -0.120570783 0.10968085 0.2747  
4 hibstart.doy bm_before 0.074727542 0.09263629 0.4221  
5 hibdur.days hibstart.doy -0.825852141 0.03389524 0.0000 ***  
6 hibdur.days hibend.doy 0.599988662 0.03436230 0.0000 ***  
7 hibdur.days bm_before 0.069715687 0.03209005 0.0327 *
```

8	hibdur.days	age.death	-0.051462817	0.03780013	0.1771
9	hibdur.days	repro_activeyes	-0.043862615	0.08118309	0.5904
10	hibdur.days	log_age	0.009696395	0.04442479	0.8278
11	hibend.doy	log_age	-0.512613387	0.11897971	0.0000 ***
12	hibend.doy	bm_before	0.178443027	0.09154136	0.0546
13	hibend.doy	age.death	-0.063441469	0.10913801	0.5626
14	hibend.doy	repro_activeyes	0.076019572	0.23120655	0.7431
15	repro_active	log_age	1.234694188	0.45195671	0.0063 **
16	repro_active	age.death	-0.363006480	0.46934478	0.4393

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

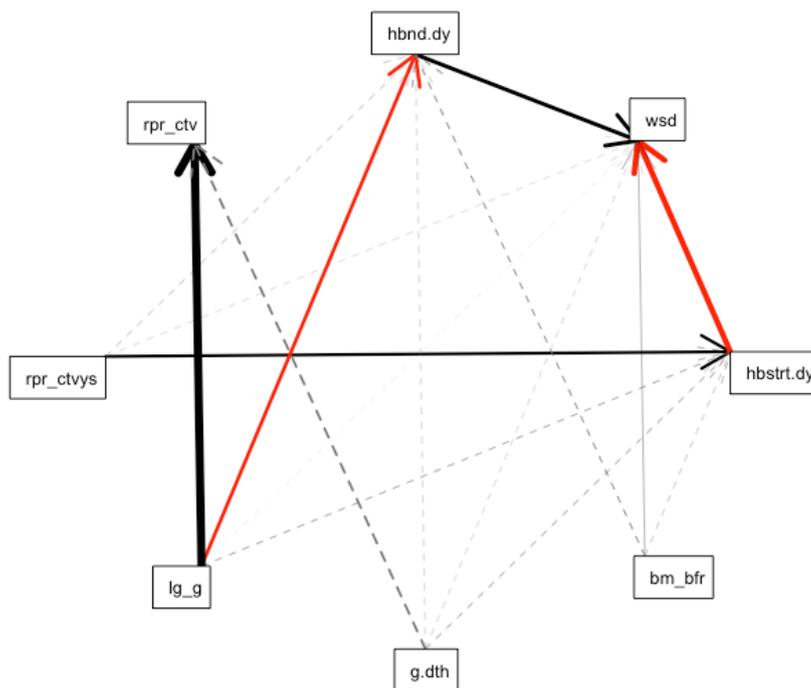


Fig. S1.2: Results plot of the path analysis for female edible dormice R-package piecewiseSEM¹. Arrows represent unidirectional relationships among variables. Red lines indicate negative impacts, black line positive impacts. Solid lines indicate significant relations, dashed lines non-significant relations. The thickness of the line represents the strength of the effect i.e., it is proportional to the absolute value of the scaled coefficient (see coef.table, sem.coefs for females).

S2) Predictions from lm and lme models for effects of age (logarithmic scale) on reproduction and timing of hibernation

Table S2.1: Model prediction from our models included in the path analysis (see above, point S1) for logarithmic effects of age (in years) on the probability to develop tangible testes or give birth to a litter (reproduction), onset of hibernation (hibstart at day of year), hibernation duration (hibdur, number of days), and end of hibernation (hibend at day of year) for males and females. The age is given in years. Model predictions for timing of hibernation show pure age effects as investment in to reproduction was kept constant (at its mean).

Significant effects are indicated by an asterisk (*).

males

age	reproduction*	hibstart*	hibdur	hibend*
1	0.31	248.99	255.65	141.80
2	0.50	239.74	255.71	130.99
3	0.62	234.33	255.75	124.66
4	0.69	230.49	255.78	120.17
5	0.74	227.51	255.80	116.69
6	0.77	225.08	255.82	113.84
7	0.80	223.02	255.83	111.44
8	0.82	221.24	255.84	109.35
9	0.84	219.67	255.85	107.51
10	0.85	218.26	255.86	105.87
11	0.86	216.99	255.87	104.38
12	0.88	215.83	255.88	103.02
13	0.88	214.76	255.89	101.77

females

age	reproduction*	hibstart	hibdur	hibend*
1	0.03	242.99	275.94	166.92
2	0.10	238.12	276.33	153.36
3	0.18	235.27	276.57	145.43
4	0.27	233.25	276.73	139.80
5	0.35	231.68	276.86	135.44
6	0.42	230.40	276.96	131.87
7	0.49	229.32	277.05	128.86
8	0.55	228.38	277.13	126.24
9	0.59	227.55	277.20	123.94
10	0.64	226.81	277.26	121.88
11	0.67	226.14	277.31	120.01
12	0.71	225.53	277.36	118.31
13	0.73	224.97	277.41	116.75

S3) Accuracy of hibernation duration estimates based on nest-box controls

To justify the assumption that the absence in nest-boxes reflected accurately the time spent in hibernation we analysed data from enclosure housed animals implanted with temperature loggers (iButtons, (Maxim Dallas, USA,⁴, n = 33 data sets). Hibernation was defined as the period between the first and last occurrence of body temperature < 25°C for at least 24h. We did not observe an influence of sex ($F_{1,30} = 1.34$, $P = 0.2561$) or age ($F_{1,30} = 0.01$, $P = 0.9436$) on the termination of hibernation between the two methods. The onset of hibernation was also not affected by age ($F_{1,30} = 0.83$, $P = 0.3689$) or sex ($F_{1,30} = 0.43$, $P = 0.5181$) between implanted or capture-recapture data sets.

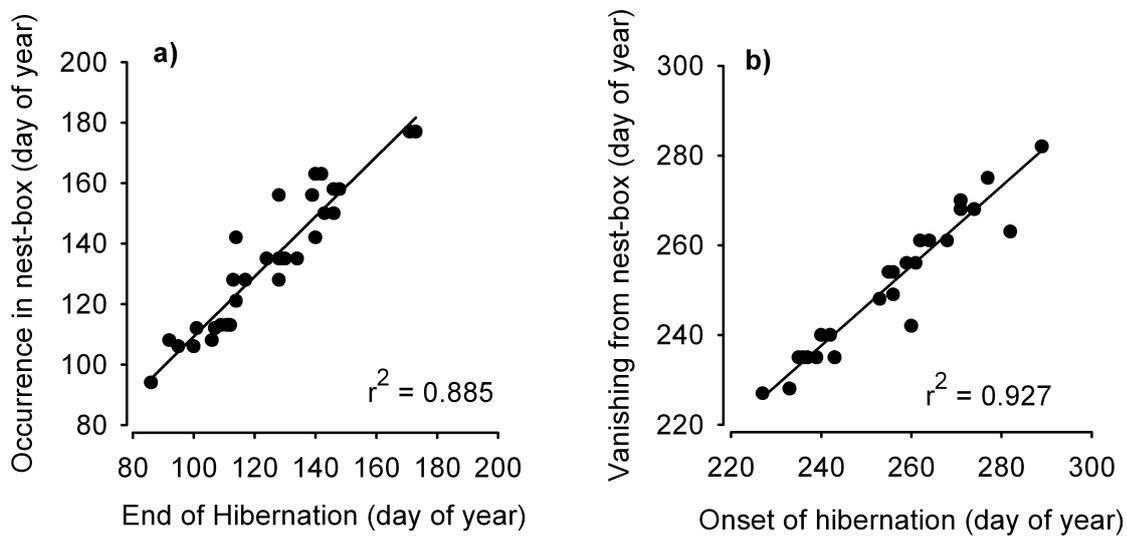


Fig. S3.1: Correlation between (a) occurrence in nest-boxes and termination of hibernation and (b) vanishing from nest-boxes and onset of hibernation.

S4) Relationships between variables of reproduction, hibernation and survival and age on a linear scale

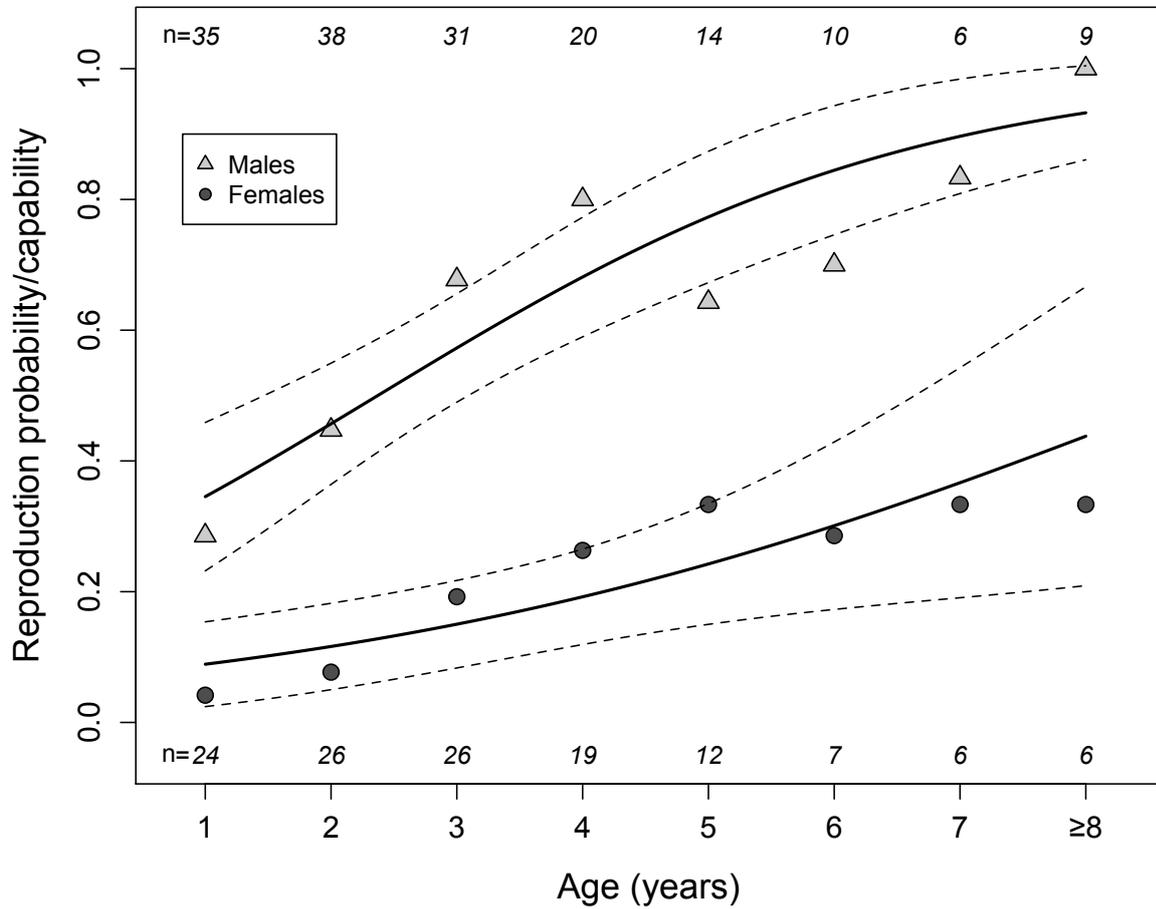


Fig. S4.1: Reproductive probability (females) or capability (males) as a function of age on a linear scale.

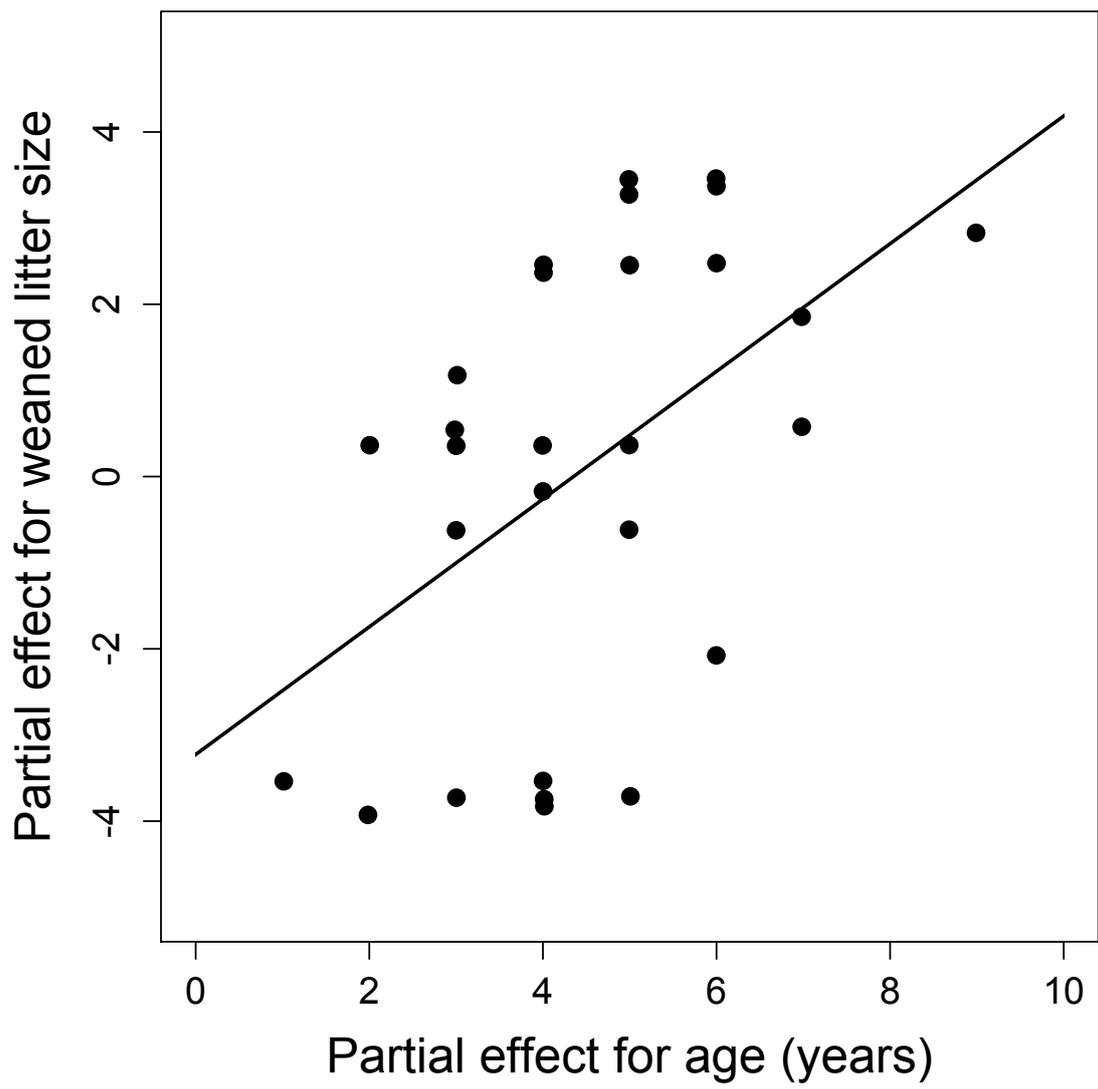


Fig. S4.2: Partial regression plot of linear effects of age on weaned litter size.

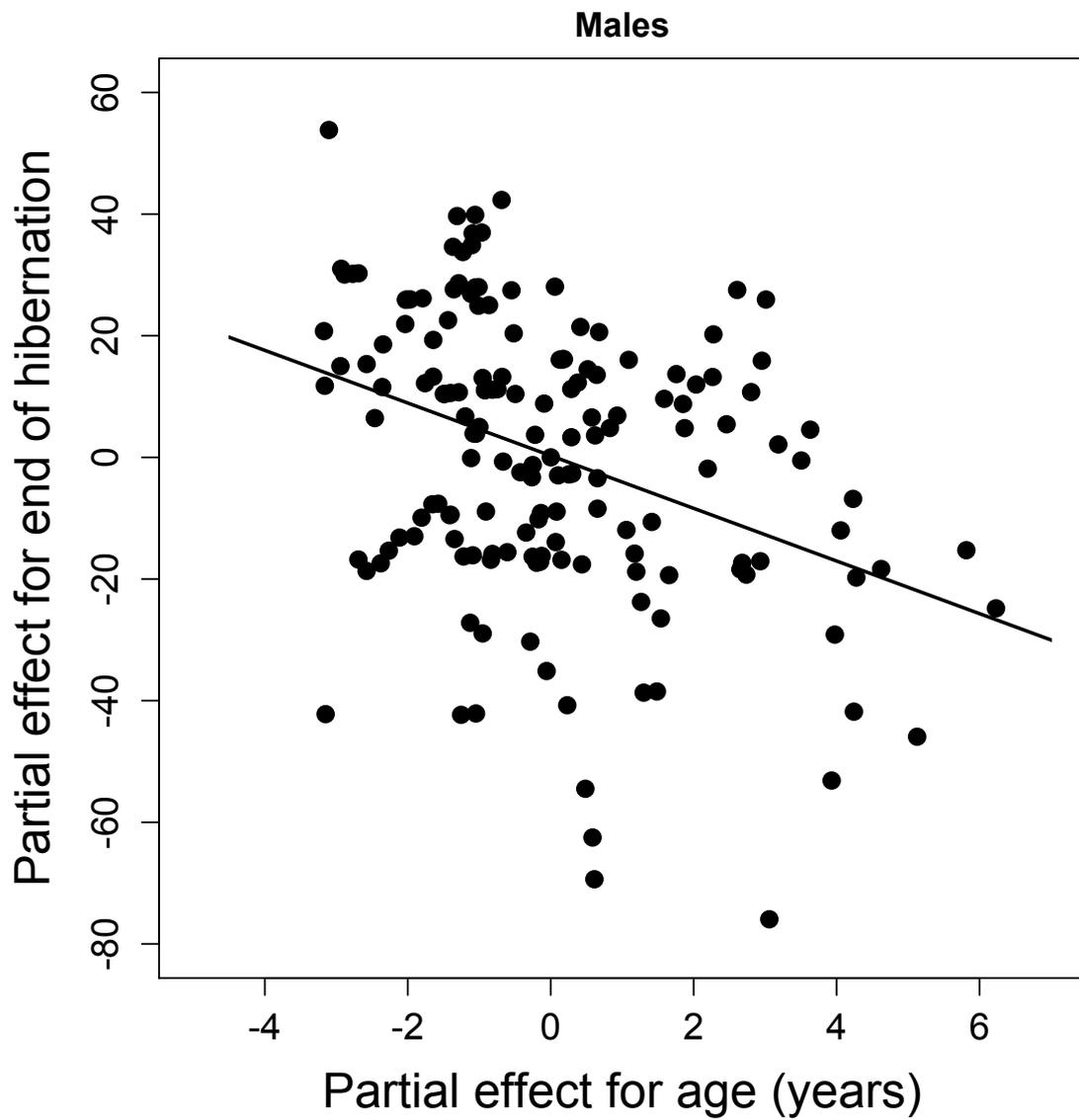


Fig. S4.3: Partial regression plot of linear effects of age on the time of emergence from hibernation in male dormice.

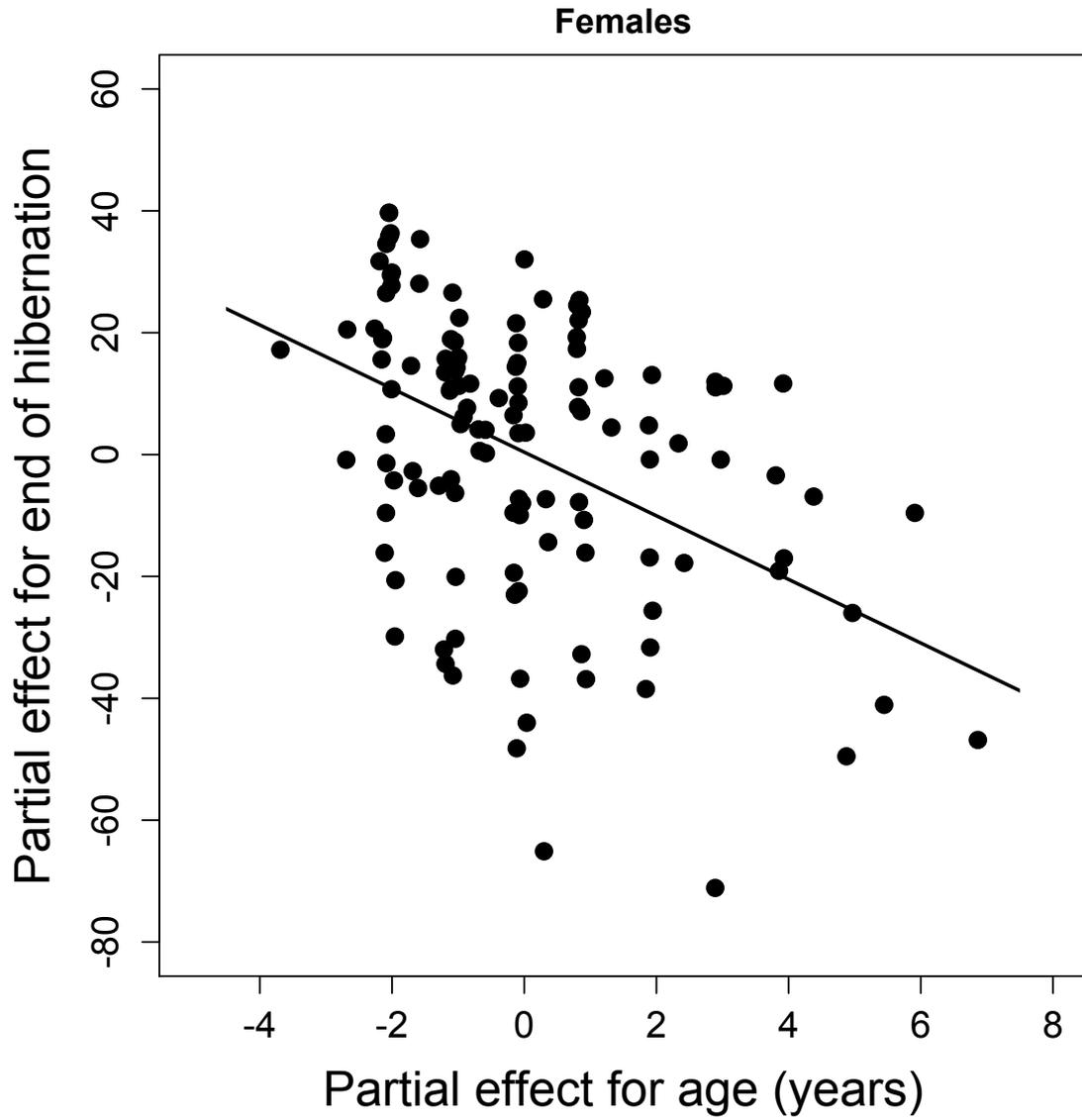


Fig. S4.4: Partial regression plot of linear effects of age on the time of emergence from hibernation in female dormice.

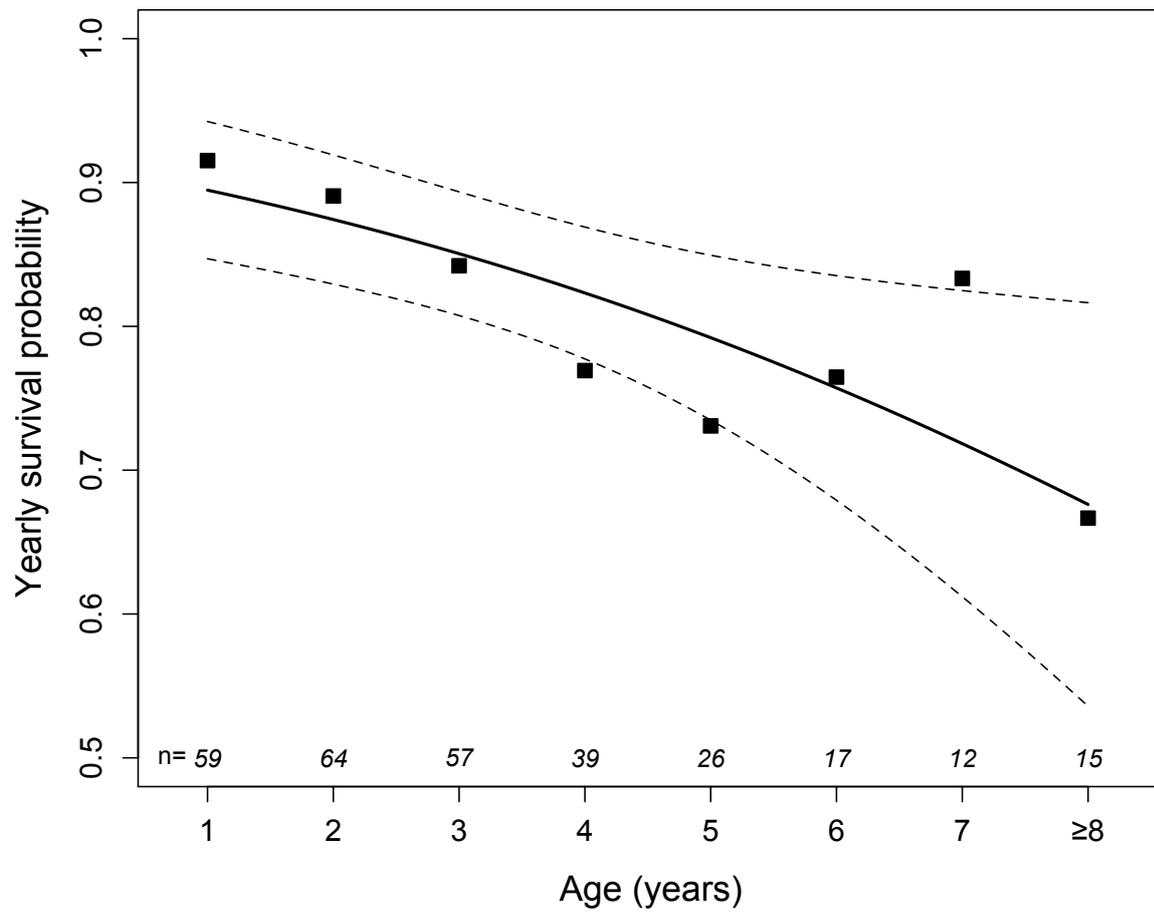


Fig. S4.5: Effects of age (on a linear scale) on yearly survival in dormice kept in outdoor enclosures.

References

- 1 Lefcheck, J. S. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.* **7**, 573-579, doi:10.1111/2041-210X.12512 (2016).
- 2 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & and the R Development Core team. nlme: Linear and Nonlinear Mixed Effects Models (R Package Version 3.1-127) (2016). <<http://CRAN.R-project.org/package=nlme>>.
- 3 Bates, D., Maechler, M., Bolker, B. & Walker, S. *lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-9*, <<http://CRAN.R-project.org/package=lme4>> (2015).
- 4 Bieber, C. & Ruf, T. Summer dormancy in edible dormice (*Glis glis*) without energetic constraints. *Naturwissenschaften* **96**, 165-171, doi:10.1007/s00114-008-0471-z (2009).