A future food boom rescues the negative effects of cumulative early-life adversity on lifespan in a small mammal

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SIGNIFICANCE

Harsh early-life conditions influence health and survival during adulthood, but the factors that can ameliorate these effects remain ambiguous. Here, we show that the consequences of early-life adversity on longevity in squirrels are rescued by a high-quality environment later in life if such benefits are had by the entire population. Providing individuals with supplemental food increased lifespan, but did not modify the relationship between early-life adversity and lifespan. However, squirrels that experienced a population-wide future food boom lived longer than squirrels that did not despite early-life adversity. Our findings suggest that individual resilience to early-life

adversity may hinge on population-level patterns of competition and energetic constraint, and thus on the quality of the future environment beyond the individual.

ABSTRACT

Challenging or adverse early-life conditions, even when transient, can have long-lasting effects on individual phenotypes and reduce lifespan across species. If these effects can be mitigated, even in part, by a high quality later-life environment, then differences in future resource access may explain variation in vulnerability and resilience to early-life adversity. Using 32 years of data on 886 wild North American red squirrels, we test the hypothesis that the negative effects of early-life adversity on lifespan can be mitigated by later-life food abundance. We first define early-life adversities as factors that significantly reduce the likelihood of juvenile survival, and find that they had cumulative negative effects on lifespan. We then show that although experimental supplementation with additional food increases individual lifespan, it did not change the consequences of early-life adversity on longevity. A naturally-occurring future food boom experienced in the second year of life, however, did eliminate the longevity costs of a harsh early-life environment. Together, our results demonstrate that adverse conditions experienced early in life reduce lifespan in red squirrels and thus may influence patterns of natural selection beyond juvenile viability. That these effects can be mitigated by a high-quality future environment suggests a non-deterministic role for early-life conditions on later-life phenotypes, and highlights the importance of evaluating the impact of early-life conditions in the context of an animal's entire life course.

INTRODUCTION

In humans, the early-life environment exhibits such profound predictive power over later-life phenotype that the first 1,000 days of life are widely recognized as foundational for determining future health, quality of life, and even human capital (1). Adverse conditions in early life can alter brain development (2), dysregulate the immune and endocrine systems (3, 4), and ultimately increase morbidity and mortality in adulthood (5). Among nonhuman animals, early-life adversities can exhibit similar far-reaching

effects. Challenging ecological conditions during early life are linked to increased adult parasite load in rabbits (6), inflammation in birds (7), poor reproductive performance in hyenas (8), and most consistently, reduced lifespan across species (9–11). From an ecological perspective, a shortened lifespan can result from harsh weather, food scarcity, or increased competition and predation, which can independently or collectively cause physiological changes that reduce longevity (e.g., telomere attrition, (12–14)).

Beyond ecological challenges, an adverse maternal environment can also reduce lifespan (15). Juvenile animals can struggle to access maternal resources due to poor maternal condition, mistimed parturition, or increased rates of sibling competition (16–19). Such challenges may reduce lifespan as a result of life history trade-offs that deprioritize developmental systems that promote longevity (18, 20), or, as hypothesized in humans, by inducing adaptive accelerated reproductive development at the expense of longevity ((21–23), but see (10)).

The negative effects of early-life adversity on lifespan are not uni-dimensional, however, and individuals can be exposed to many forms of early-life adversity simultaneously. Research has therefore begun to investigate the cumulative influence of multiple early-life adversities and their varied combinations. These studies reveal that early-life challenges can combine to collectively reduce longevity (8, 24), fueling an interest in understanding how animals inhabiting heterogeneous environments may cope with clusters of adversity during development. Elucidating these patterns can also provide insight into how animals may respond to the multidimensional environmental shifts caused by human-induced rapid environmental change (HIREC), which can generate distinct but co-occuring sources of adversity (25).

Moreover, some aspects of the future environment may be capable of buffering against long-term costs of adversity. In highly social animals such as nonhuman primates, the quality of an individual's social environment predicts longevity (26), and strong social bonds and high social status during adulthood can ameliorate the negative effects of

early-life adversity on survival (27). In solitary animals, the amelioration of early-life adversity may instead depend on an alternate component of the future environment. For example, in resource pulse ecosystems where animals experience pronounced variation in the temporal availability of resources, individuals can be born into food-scarce environments but subsequently experience a future food boom (28). While this type of mismatch between the developmental and adult environment has been invoked to explain the onset of metabolic disease in humans (29, 30), in wild populations, a resource-rich future environment may free animals from developmental or physiological constraints created by adverse early-life conditions (18, 31). Thus, if the consequences of a challenging early environment can be modified by later-life resource access, future life experience may explain variation in susceptibility to early-life adversity.

Here, we test the hypothesis that the relationship between early-life adversity on lifespan can be modified by future food in a population of wild North American red squirrels (*Tamiasciurus hudsonicus*) inhabiting a resource pulse ecosystem. The masting cycles of white spruce (*Picea glauca)* trees, red squirrels' preferred food source, result in food booms (mast years) and busts (non-mast years) that dramatically impact the availability of spruce cones for squirrels to hoard ahead of winter(32). First, we identify sources of early-life adversity by determining which environmental challenges experienced in the first year of life reduce the probability of juvenile survival (Table 1A). We then examine cumulative effects of early-life adversity on longevity, and test whether these effects can be ameliorated by later-life resource richness using two measures of future food availability (Table 1B). We predict that a resource-rich future will offset, at least in part, the negative effects of early life adversity on lifespan in red squirrels.

Table 1. A) Hypothesized sources of early-life adversity in juvenile squirrels experienced during their first year of life and B) potential resource-related buffers against early-life adversity. References supporting these hypotheses in this study population are provided where available.

Variable	Definition	References

A) Early-life adversities		
Large litter size	relatively large number of siblings born within the same litter (increased sibling competition)	(33)
Litter size mismatched to environmental conditions	litter size is mismatched to future food abundance (e.g.,a large litter is produced in a non-mast year when future food is low, a small litter is produced in a mast year when future food is high)	(34, 35)
Later parturition date	date of birth is relatively late in the breeding season, resulting in increased conspecific competition for territories	(36, 37)
Slow postnatal growth rate	relatively low offspring growth rate during period of maternal dependence calculated as change in body mass from ~0 d to 25 d of age	(35, 37, 38)
Non-mast year	a year in which a white spruce mast does not occur and new food production is scarce	(39)
Elevated squirrel density	relatively high number of squirrels living on study area (increased conspecific competition)	(34, 38, 40, 41)
One year following peak in lynx-hare cycle	a year characterized by a crash in hare density and expected prey-switching by lynx to red squirrels	(42))
Elevated mustelid density	relatively high number of nest predators	(40, 43)
Extremes in winter temperature	relatively warm or cold average winter temperatures	(40)
B) Potential buffers against early-life adversity		
Mast year	encountering a spruce mast event, which results in the production of a superabundance of food, during the second year of life	(39)
Experimental food supplementation	access to a supplemental feeding station at the center of a squirrel's territory	(44)

RESULTS

Independent effects of early-life conditions on juvenile survival and lifespan

Six of the 9 potential sources of early-life adversity were negatively associated with juvenile overwinter survival (Figure 1, Table S1A). Juveniles exhibited poorer overwinter survival if they were born the year following the peak of the lynx-hare cycle (e.g., when there is a crash in the snowshoe hare population; $\beta = -0.76$, $z = -2.28$, $P = 0.022$), when conspecific densities were elevated ($\beta = -0.51$, $z = -4.25$, $P = 0.000$), or when new food was scarce (i.e., a non-mast year, $\beta = -1.29$, $z = -4.79$, $P = 0.000$). Survival was also less likely if juveniles grew slowly during the early postnatal period of maternal dependence (i.e., first 25 days; $\beta = 0.27$, $z = 4.91$, $P = 0.000$), were born later in the breeding season ($\beta = -0.25$, $z = -3.5$, $P = 0.001$; particularly if conspecific density was also high, $\beta = -0.16$, $z = -2.11$, $P = 0.035$), were born into a large litter ($\beta = -0.15$, $z = -2.32$, $P = 0.020$) or a litter whose size was mismatched to the environment (e.g., a large litter in a non-mast year, or a small litter in a mast year; $\beta = 0.35$, $z = 2.98$, $P = 0.003$). There was no effect of mean overwinter temperature, or mustelid density on juvenile survival. Males were less likely to survive their first winter than females ($\beta = -0.76$, $z = -7.83$, $P = 0.000$, Figure 1A, Table S1A), and had shorter lifespans overall ($\beta = -0.14$, $z = -3.18$, $P = 0.001$, Figure 1B, Table S1B).

Only one of the abovementioned factors defined as early-life adversities exhibited continued, independent effects on total lifespan. Squirrels that were born later in the breeding season lived shorter lives than those born earlier ($\beta = –0.07$, $z = -2.68$, $P = 0.007$; Figure 1B, Table S1B).

Cumulative effects of early-life adversity on lifespan

Early-life adversity may exhibit divergent effects on longevity and fitness given that natural selection operates on the latter but not necessarily on the former, so we tested whether lifespan predicted fitness in our population. We found that lifespan positively predicted lifetime reproductive success (number of offspring successfully recruited into the breeding population) in both sexes, such that squirrels that lived longer produced

more recruits over their lifetimes than those who died earlier (β = 2.15, z = 14.61, *P* = 0.000; Figure S1, Table S2).

Most juveniles experienced more than one early-life adversity during the first year of life, and although only parturition date exhibited continued independent effects on total lifespan, the sum total of all early-life adversities experienced cumulatively predicted lifespan (β = -0.08, z = -2.88, *P* = 0.004, Figure 2, Table S3). Squirrels experiencing one adversity lived, on average, 3.1 total years while squirrels experiencing 5 and 6 adversities lived only 2.4 and 2 years, respectively (Figure 2, Table S3).

A high quality future environment mitigates the consequences of early-life adversity

Among squirrels living on the experimental study area, supplementation with a bucket of peanut butter at the center of their territory increased lifespan (β = 0.28, z = 3.18 *P* = 0.001; Figure 3, Table S4). However, we found little evidence that food supplementation changed the relationship between early-life adversity and lifespan (β = 0.04, z = 0.42, *P* = 0.68). By contrast, experiencing a population-wide food boom in the second year of life eliminated the negative effects of early-life adversity on lifespan (β = 0.14, z = 2.01, *P* = 0.04; Figure 4, Table S5).

DISCUSSION

A shortened lifespan is a commonly documented consequence of early-life adversity, reflecting an enduring connection between the early-life environment and end-of-life outcomes. Here, we show that, in line with prior work in humans and other mammals, early-life adversities combine to cumulatively reduce lifespan in North American red squirrels. However, the quality of the future environment modified this relationship. Future food supplementation increased lifespan for those directly receiving additional food, but did not influence the relationship between early-life adversity and lifespan. However, squirrels that experienced a population-wide food boom in their second year of life did not suffer a shortened lifespan as a result of harsh conditions in their first year

of life. Our findings suggest a non-deterministic role of early-life adversity on later-life phenotype, whereby a high quality future environment can buffer individuals against the longevity costs associated with challenging early-life conditions.

Overwinter survival is a key life history stage for juvenile red squirrels as it determines their recruitment into the breeding population the following spring (40, 45). We found survival to be lowest among squirrels that were born later in the breeding season, grew slowly during the postnatal period, or were born into large litters. These patterns reflect potential challenges related to competition and curtailed maternal investment within the developmental environment. Later-born squirrels may be less likely to encounter vacant territories to occupy as many territories will have already been occupied by earlier-born squirrels. Territory acquisition is critical for overwinter survival (40), thus later-born squirrels face increased conspecific competition for territories and in turn, higher rates of early mortality (33). Similarly, sibling competition for maternal resources is largest in large litters. Such competition manifests as a quantity/quality trade-off in which pups born into larger litters exhibit slower growth (46), which reduces a squirrel's ability to compete for its own natal territory as well as territories adjacent to it (33).

Beyond the maternal environment, juvenile mortality was predicted by harsh ecological conditions, including predation risk by Canada lynx, which exhibit prey-switching from snowshoe hares to red squirrels in years after hare populations crash (42, 47). Squirrels born in the year following a hare crash are therefore at the highest risk of predation and more likely to suffer direct predation events by lynx. In addition, elevated squirrel densities (which increase conspecific competition for both food and territories) and food scarcity were additional ecological sources of early-life mortality. In line with prior work, juveniles born in non-mast years when new food is scarce were less likely to survive (34), particularly when maternal reproductive effort was mismatched to the environment such that sibling and conspecific competition was high when food was low (e.g., a large litter in a low food (non-mast) year; (35)).

Similar to correlations between socioeconomic status, educational attainment, and health in humans, environmental covariance can generate clusters of adversity in which multiple ecological challenges co-occur (48). Though only parturition date exhibited continued, independent effects on lifespan, all sources of early-life adversities predicted lifespan when considered cumulatively, illustrating the need to consider that early-life challenges alone may not explain variation in lifespan until they compound with other simultaneous challenges. Most squirrels experienced at least 1 source of early-life adversity, and the consequences of harsh early-life conditions for lifespan increased with increasing numbers of early-life adversities. This pattern echoes previous work in both humans and nonhuman animals indicating that clusters of early life adversity may be particularly prevalent in populations inhabiting highly fluctuating environments and/or environments in which multiple sources of early-life adversity are expected to coincide (8, 24).

Human research has long endeavored to explain how the biological embedding of early-life adversity leads to variation in individual health and longevity (49, 50). What remains largely unknown are what, if any, factors can buffer against such embedding. Moreover, if the negative effects of harsh early environments are non-deterministic such that they can be mitigated by other factors like a high quality later-life environment, then consideration of an individual's entire life course is essential to explaining variation in susceptibility to early-life adversity. We found that squirrels that received a supplemental food bucket on their territories lived longer than those that did not, but the relationship between early-life adversity and longevity was unaffected by food supplementation. By contrast, squirrels that experienced a population-wide food boom (i.e., "mast year") in their second year of life did not suffer reduced lifespans as a result of early-life adversity. Although they occur episodically, the boom of food produced in mast years serves as a catapult for Darwinian fitness in red squirrels, increasing both annual and lifetime reproductive success (34, 35). Our results suggest that mast events can also alleviate the longevity costs of harsh early-life conditions, potentially by relieving energetic constraints at the population level and thus increasing environmental quality and reducing food competition for the entire population of squirrels.

These results extend our current understanding of the role of early life effects on later-life phenotype by uncovering one dimension of the future environment, population-wide resource availability, that can buffer against the negative effects of early-life adversity on lifespan. Although food booms and their effects may be unique to resource pulse ecosystems, they reflect dramatic increases in environmental quality that benefit entire populations, and serve as natural experiments that mimic large-scale environmental perturbations (28). Beyond enhancing Darwinian fitness, we show that population-wide food booms alter expected relationships between early-life environments and later-life phenotype, and possibly patterns of senescence, in ways that providing individuals with supplemental food cannot. Inter-individual variation in vulnerability and resilience to early-life adversity may therefore hinge on changes in larger-scale patterns of competition and constraint in which individual benefits to longevity and fitness hinge on benefits conferred to the entire population.

MATERIALS AND METHODS

Study system

We have studied North American red squirrels (*Tamiasciurus hudsonicus*) in the southwestern Yukon, Canada (61°N, 138°W) since 1989 (32). Detailed information about the study system and field methods can be found elsewhere (32, 45). Briefly, we followed squirrels from birth until death on two separate ~40 hectare study areas (Kloo or “KL” and Sulphur or “SU”) as well as an experimental study area (Agnes or “AG”), identifying individual squirrels using uniquely labeled metal ear tags placed shortly after birth while still in their natal nest or at first capture during regular live-trapping. We censused the entire population in May and August or September of each year. Because red squirrels are highly territorial and trappable, our detection probability does not differ from 1 and a lack of detection during a census is indicative of death (51). This enables us to confidently estimate lifespan (median = 3.5 y, maximum = 9 y; (45)).

Life-history and fitness data

We determined female reproductive status via abdominal palpation for fetus development and by monitoring individual mass gain during regular live-trapping. Within

a few days of birth, we located each nest using radio-telemetry, counted, ear clipped (for unique marking within each litter and tissue sample), and weighed each pup (to the nearest tenth of a gram). About 25 days later, we reweighed each pup and affixed a set of permanent metal ear tags. Because growth is linear during this period of development (37), we calculated pup postnatal growth rate as the mass gain per day. To calculate lifetime reproductive success, we summed the number of recruits produced by each squirrel over their lifetime. We determined the sire of each pup produced by analyzing tissue samples, assigning loci with GENEMAPPER software 3.5 (Applied Biosystems), and assigning paternity with CERVUS v.3.0 with 99% confidence (52, 53). Details on microsatellite loci isolation and paternity assignment can be found in prior studies (54–56). We considered a pup as recruited into the breeding population if they survived to 200 days old (37, 57, 58).

Temperature data

We used daily temperature records from the Haines Junction weather station, which is located ~35 km SE from our study area (Climate ID 2100630, 60.77°N, 137.57°W), to calculate yearly mean overwinter temperatures from the months of October to the following March. Prior studies in our population using data from this weather station demonstrate that mean overwinter temperatures capture thermoregulatory costs of temperature extremes and impact juvenile survival and litter failure (40, 43).

Predator data

We used data on two predators of juvenile red squirrels from our study area, Canada lynx (*Lynx canadensis)* and mustelids (short-tailed weasel *Musela erminea,* least weasel *M. nivalis,* and marten *Martes americana*), from the Kluane Boreal Forest Ecosystem Project (1987-1996) and the Community Ecological Monitoring Program (1996-present). Lynx and mustelid densities were calculated as the average snow track count per 100 kilometer transect (59). We also calculated the density of snowshoe hares (*Lepus americanus)*, on which Canada lynx specialize, using mark-recapture (59) because lynx prey-switch to red squirrels following crashes in hare population densities (47). Following prior studies (42), we binned the hare-lynx cycle into 4 categories based on the location in the cycle: peak hare density (when both hare and lynx density are

high), 1-year post hare peak (when hare density crashes, but lynx density remains high), 2-years post peak (when lynx density crashes), and any other year in the cycle. Low juvenile recruitment suggests red squirrel predation risk from lynx is highest 1-year post hare peak (42).

Measures of food availability

Food boom. Each year, we counted the number of visible cones on one side of the top 3 meters of a consistent subset of trees (between 159-254 trees) on each study area (60). We then log (+1) transformed counts and calculated the mean to represent an annual index (61). We then defined years with a superabundance of cones as mast years, which occur once every 3-7 years (34).

Experimental food supplementation. From 2005-2017 except for years following the 2010 and 2014 masts, we experimentally supplemented a subset of squirrels living on a separate study area (AG) by hanging a bucket containing 1 kg of peanut butter between two trees at the center of the supplemented squirrel's territory. We replenished peanut butter approximately every 6 weeks from October to May and replenished peanut butter to any lactating females through the summer months. One kg of peanut butter is approximately equal to the resting metabolic needs of an individual for 70 days (62, 63).

Statistical analysis

We conducted all analyses in R version 4.0.2. We used the package *lme4* to conduct generalized linear mixed models (GLMM) and the package *visreg* to visualize partial regressions. We controlled for study area (fixed effect), litter number (fixed effect), litter identity (random effect), and year (random effect) in all models. Some models also contained mother identity as an additional random effect if the model would converge with this additional structure.

We defined early-life factors as early-life adversities if they significantly reduced the likelihood of juvenile survival using a set of 9 putative early-life adversities assembled based on prior work in our study population (Table 1). To do this, we constructed a model to test which of these factors experienced during a squirrel's birth year were

related to survival over a squirrel's first winter to the following May (i.e., spring census) using a binary error distribution (survived yes/no). We used pup growth rate, litter size (number of pups), parturition date (day-of-year), mast year (yes/no), year in the hare-lynx cycle (hare peak, 1-year post peak, 2-years post peak, other), squirrel population density, mustelid density, and mean winter temperature (see rationale for these predictions in Table 1). We also included interactions of litter size x mast, parturition date x squirrel density, and pup growth rate x squirrel density as predictors as the effects of these factors on juvenile survival may be dependent on other co-occurring variables (e.g., being born late in the year may only have a negative effect if conspecific competition that year is high, etc.).

We next confirmed that lifespan was a fitness-relevant trait by testing whether the total number of recruits produced during a squirrel's life (response) was related to lifespan (# of years), sex, and their interaction as predictors using a GLMM with Poisson distribution. To determine if early-life adversities exerted continued, independent effects on lifespan beyond the juvenile period, we ran an identical model to the one described above except we used lifespan (i.e. longevity conditional upon survival to 200 days) as the dependent variable rather than juvenile survival. We then tested whether early-life adversities exhibited cumulative effects on lifespan. To do this, we summed the total number of early-life adversities (1 = exposed, 0 = not exposed) that each squirrel experienced. For continuous variables, we binned data into the lower or upper half of the distribution, determined which bin reduced juvenile survival, and assigned this bin as an adversity (e.g., if the upper half of the distribution of conspecific density reduced juvenile survival, then squirrels experiencing densities in that upper bin were considered to be exposed to adversity and thus assigned "1"). We then ran another GLMM (Poisson) examining the relationship between the cumulative number of early life adversities (fixed effect) and lifespan (response). We confirmed this effect was linear by additionally testing for quadratic ($z = -1$, $p = 0.921$) and cubic ($z = 0.5$, $p = 0.603$) terms and fitting splines with general additive models.

Finally, we tested whether future resources (a future food boom and experimental supplementation with peanut butter) ameliorated the cost of early-life adversities on lifespan (i.e., # of early-life adversities x resource). First, we tested whether experimental supplementation with *ad libitum* peanut butter ameliorated costs of early-life adversity. We restricted this analysis to cohorts born between 2004-2015 to focus on cohorts that had the potential to live at least 4 years before monitoring ended on the experimental grid in 2019. Squirrels (N = 263 individuals, 10 cohorts) either received a bucket of peanut butter on their territory or did not (binary variable). Then, we ran a model in which we added an interaction of early-life adversity with whether an individual encountered a spruce mast during its second year of life.

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FIGURE LEGENDS

Fig 1. Harsh conditions in the first year of life independently predict poor overwinter survival and reduced lifespan. (A) Six of the 9 potential early-life adversities were associated with a reduced likelihood of juvenile overwinter survival (i.e., survival past the first 200 d). **(B)** Parturition date was the only early-life adversity to demonstrate a continued effect on lifespan for those individuals that survived their first winter. Forest plots depict results of generalized linear mixed-effects model testing which early-life factors predict juvenile overwinter survival (N = 3,699 squirrels) and lifespan (N = 886 squirrels). Purple bars denote factors that significantly ($P > 0.05$)

negatively correlate with survival; green bars denote factors that significantly positively correlate with survival; gray bars denote non-significant factors.

Fig 2. Early-life adversities cumulatively reduce longevity. Although only two early-life factors significantly independently associated with lifespan, squirrels that experienced multiple adversities identified as reducing juvenile overwinter survival (Figure 1) exhibited shorter lifespans. Partial residual plot depicts the relationship between the number of early-life adversities experienced and lifespan (N = 886 squirrels).

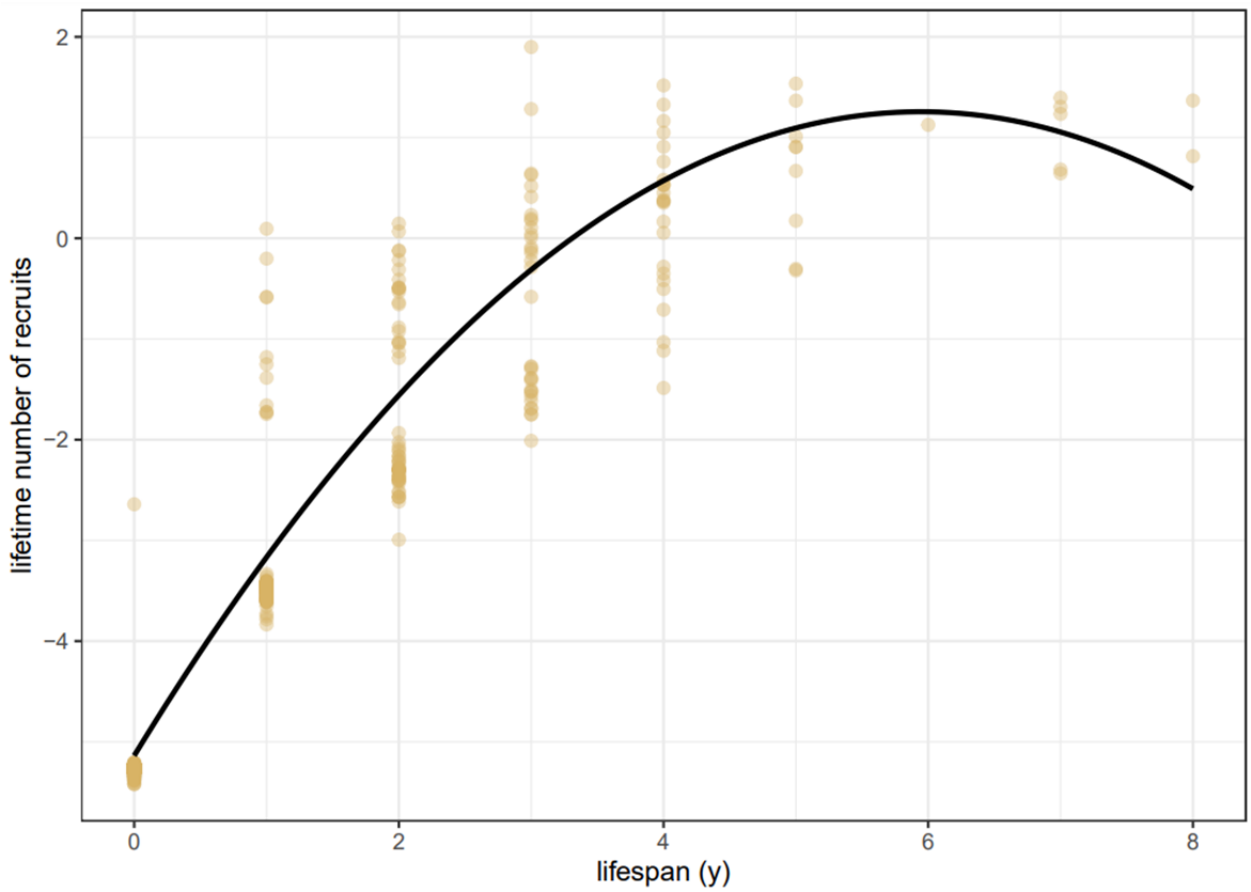
Figure 3. Experimental food supplementation extends lifespan but does not change the relationship between early-life adversity and lifespan. Scatterplot depicts partial residuals from a generalized linear-mixed effects model testing whether providing individual squirrels with *ad libitum* peanut butter at the center of their territories could ameliorate the negative effects of early-life adversity on lifespan (N = 263 squirrels).

Figure 4. A future food boom eliminates the negative effects of early-life adversity on lifespan. Squirrels that experienced multiple adversities but encountered a spruce mast event during their second year of life did not suffer shortened lifespans despite multiple adversities. Scatterplot depicts partial residuals from a generalized linear mixed-effects model testing whether encountering a food boom (mast year) in the second year of life (yes/no) modified the relationship between cumulative early-life adversities and longevity (N = 886 squirrels).

SUPPLEMENTARY MATERIALS

Figures

Figure S1. Lifespan positively predicts lifetime reproductive success in Yukon red squirrels. Among both male and female squirrels, longer lives were associated with more offspring successfully recruited into the breeding population over the lifetime (lifetime reproductive success; N = 1,197 squirrels). Scatterplot depicts partial residuals from a generalized linear mixed-effects model.



Tables

Table S1. Harsh early-life conditions independently predict reduced juvenile survival, with limited independent effects on lifespan. A) Juvenile squirrels were more likely to die overwinter (i.e., the first 200 days of life) if they were male, born into large litters, born later in the breeding season, grew slowly during the first 25 days postnatal of life, it was a low food (i.e., non-mast) year, squirrel densities were high, or it was the year following a crash in the snowshoe hare population (more likely to experience prey-switching by lynx to squirrels). Squirrels born into large litters in non-mast years (litter size x mast interaction) or late in the breeding season in high density years (squirrel density x parturition date) also exhibited a smaller likelihood of overwinter survival. B) Only sex and parturition date were independently associated with total lifespan, such that squirrels born male or later in the breeding season lived shorter lives. There was a trend toward shorter lifespans for squirrels born in high squirrel density years or in the year following a hare crash. Analysis of lifespan was restricted to squirrels that survived through their first winter (i.e., to adulthood). Results reflect output from generalized linear mixed-effects models. Continuous predictors were centered to a mean of zero and expressed in standard deviations.

	(A) Juvenile survival (N = 3,736)				**(B) Adult lifespan (N = 913)**			
Independent terms	**Estimate**	**SE**	**Z**	**P**	**Estimate**	**SE**	**Z**	**P**
intercept	**-1.30**	**0.16**	**-8.13**	**0.000**	**1.03**	**0.06**	**17.46**	**0.000**
grid (SU)	0.05	0.11	0.43	0.670	-0.03	0.05	-0.62	0.533
sex (male)	**-0.76**	**0.10**	**-7.83**	**0.000**	**-0.14**	**0.04**	**-3.18**	**0.001**
litter size	**-0.15**	**0.07**	**-2.32**	**0.020**	0.01	0.03	0.17	0.865
parturition date	**-0.26**	**0.06**	**-4.50**	**0.000**	**-0.07**	**0.02**	**-2.68**	**0.007**
growth rate	**0.27**	**0.06**	**4.91**	**0.000**	0.02	0.02	0.74	0.458
mast (no)	**-1.29**	**0.27**	**-4.79**	**0.000**	0.11	0.09	1.20	0.231
squirrel density	**-0.51**	**0.12**	**-4.25**	**0.000**	-0.07	0.04	-1.81	0.071
lynx-hare cycle (peak)	0.22	0.32	0.70	0.482	-0.13	0.12	-1.12	0.262
lynx-hare cycle (1-yr post peak)	**-0.76**	**0.33**	**-2.28**	**0.022**	-0.27	0.14	-1.91	0.056
lynx-hare cycle (2-yrs post peak)	-0.33	0.33	-0.99	0.321	-0.07	0.12	-0.55	0.585

mustelid density	0.06	0.11	0.58	0.559	0.03	0.04	0.72	0.473
mean winter temperature	-0.03	0.11	-0.25	0.800	-0.05	0.04	-1.33	0.183
litter size x mast (yes)	**0.35**	**0.12**	**2.98**	**0.003**	-0.02	0.05	-0.42	0.675
parturition date x squirrel density	**-0.16**	**0.08**	**-2.11**	**0.035**	0.00	0.03	0.05	0.961
growth rate x squirrel density	-0.09	0.07	-1.43	0.153	0.02	0.02	0.67	0.503
random effects			**variance**	**std. dev.**			**variance**	**std. dev.**
litter identity			0.76	0.87			0.01	0.12
maternal identity			0.00	0.00			0.02	0.14
cohort			0.21	0.46			0.02	0.14

Table S2. Lifespan is positively correlated with lifetime reproductive success in red squirrels. Squirrels that lived longer lives also successfully recruited more pups into the breeding population (lifetime reproductive success). Results reflect output from a generalized linear mixed-effects model. A non-significant interaction between sex and lifespan was removed to construct the final model below.

	Lifetime reproductive success (N = 1,197)			
Independent terms	**Estimate**	**SE**	**Z**	**P**
intercept	**-4.5**	**0.32**	**-14.15**	**0.000**
lifespan	**2.15**	**0.15**	**14.61**	**0.000**
lifespan (quadratic)	**-0.18**	**0.02**	**-10.17**	**0.000**
sex(male)	**-0.63**	**0.21**	**-2.93**	**0.000**
random effects			**variance**	**std. dev.**
litter identity			0.61	0.78
cohort			0.06	0.24

Table S3. **The number of early-life adversities experienced in the first year of life cumulatively predict lifespan**. The more co-occurring, independent early-life adversities a squirrel experienced in their first year of life, the shorter their total lifespan. Results depict output from generalized linear mixed-effects model; number of adversities were scaled to a mean of zero and expressed in standard deviations.

	Lifespan (N = 886)			
Independent terms	**Estimate**	**SE**	**Z**	**P**
intercept	**1.02**	**0.05**	**20.31**	**0.000**
# adversities	**-0.08**	**0.03**	**-2.88**	**0.004**
grid (SU)	-0.04	0.05	-0.94	0.346
sex (male)	**-0.14**	**0.04**	**-3.21**	**0.001**
# adversities * sex (male)	-0.02	0.04	-0.37	0.712
random effects			**variance**	**std. dev.**
litter identity			0.02	0.14
maternal identity			0.02	0.13
cohort			0.03	0.17

Table S4. **Individual supplementation with peanut butter increases lifespan but does not alter the relationship between early-life adversity and longevity.** On a separate experimental study grid, a subset of squirrels received a bucket of peanut butter at the center of their territory. Squirrels that received a bucket on their territory lived longer than those that did not, but the negative relationship between early-life adversities and lifespan remained was not modified by whether a squirrel received a supplemental food bucket. Results depict output from a generalized linear mixed-effects model. Early-life adversities were scaled to a mean of zero and expressed in standard deviations.

	Lifespan (N = 263)			
Independent terms	**Estimate**	**SE**	**Z**	**P**
litter number (second)	-0.04	0.12	-0.36	0.722
sex (male)	-0.09	0.08	-1.11	0.264
# adversities	0.04	0.09	0.51	0.613
bucket (yes)	**0.28**	**0.09**	**3.18**	**0.001**
# adversities x bucket (yes)	0.04	0.09	0.42	0.677
random effects			**variance**	**std. dev.**
cohort			0.02	0.14

Table S5. A food boom experienced in the second year of life rescues the negative effects of early-life adversity on lifespan. Regardless of how many early-life adversities a squirrel experienced in their first year of life, they did not suffer a shortened lifespan if they experienced a food boom (mast year) in their second year of life. Results depict output from a generalized linear mixed-effects model. Number of adversities were scaled to a mean of zero and expressed in standard deviations.

	Lifespan (N = 886)			
Independent terms	**Estimate**	**SE**	**Z**	**P**
intercept	**1.03**	**0.05**	**19.35**	**0.000**
# adversities	**-0.10**	**0.03**	**-3.42**	**0.000**
# adversities x year 2 mast (yes)	**0.14**	**0.07**	**2.01**	**0.044**
year 2 mast (yes)	-0.08	0.11	-0.78	0.437
grid (SU)	-0.04	0.05	-0.79	0.427
sex (male)	**-0.14**	**0.04**	**-3.30**	**0.001**
random effects			**variance**	**std. dev.**
litter identity			0.02	0.14
maternal identity			0.01	0.12
cohort			0.03	0.17

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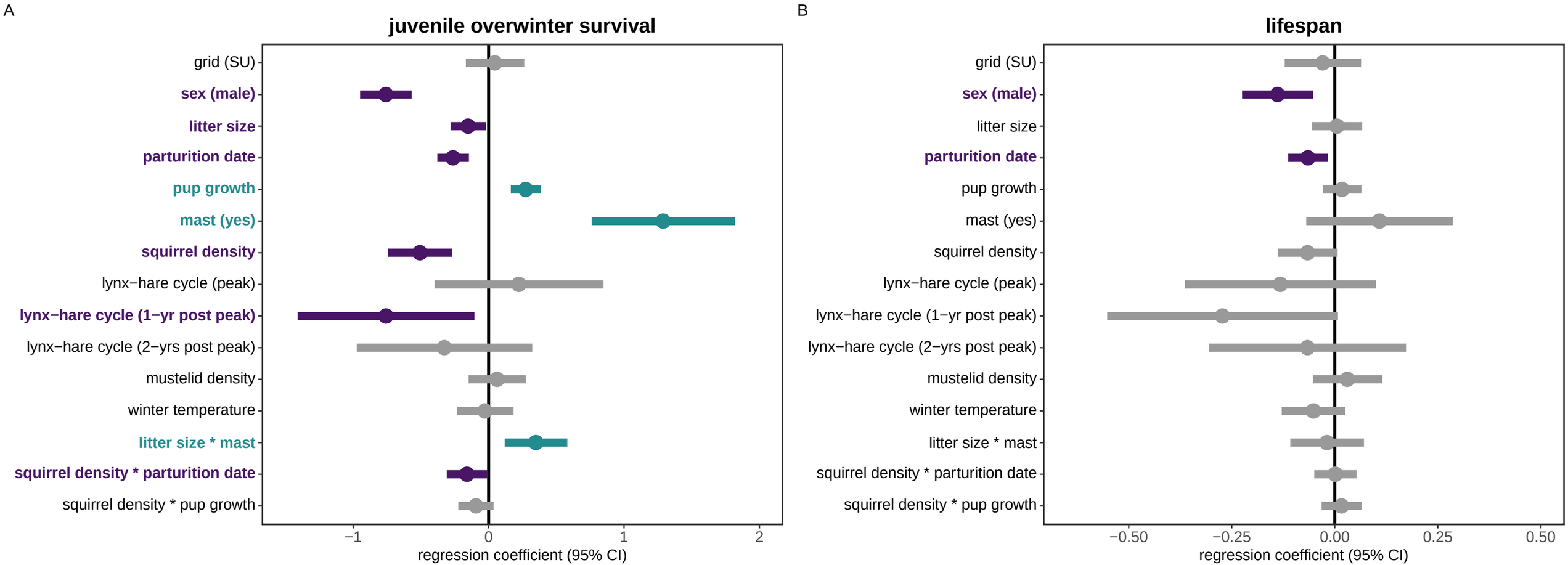
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sex (male)
litter size
parturition date
pup growth
mast (yes)
squirrel density
lynx−hare cycle (peak)
lynx−hare cycle (1−yr post peak)
lynx−hare cycle (2−yrs post peak)
mustelid density
winter temperature
litter size * mast
squirrel density * parturition date
squirrel density * pup growth
−1
0
1
2
regression coefficient (95% CI)
B
lifespan
grid (SU)
sex (male)
litter size
parturition date
pup growth
mast (yes)
squirrel density
lynx−hare cycle (peak)
lynx−hare cycle (1−yr post peak)
lynx−hare cycle (2−yrs post peak)
mustelid density
winter temperature
litter size * mast
squirrel density * parturition date
squirrel density * pup growth
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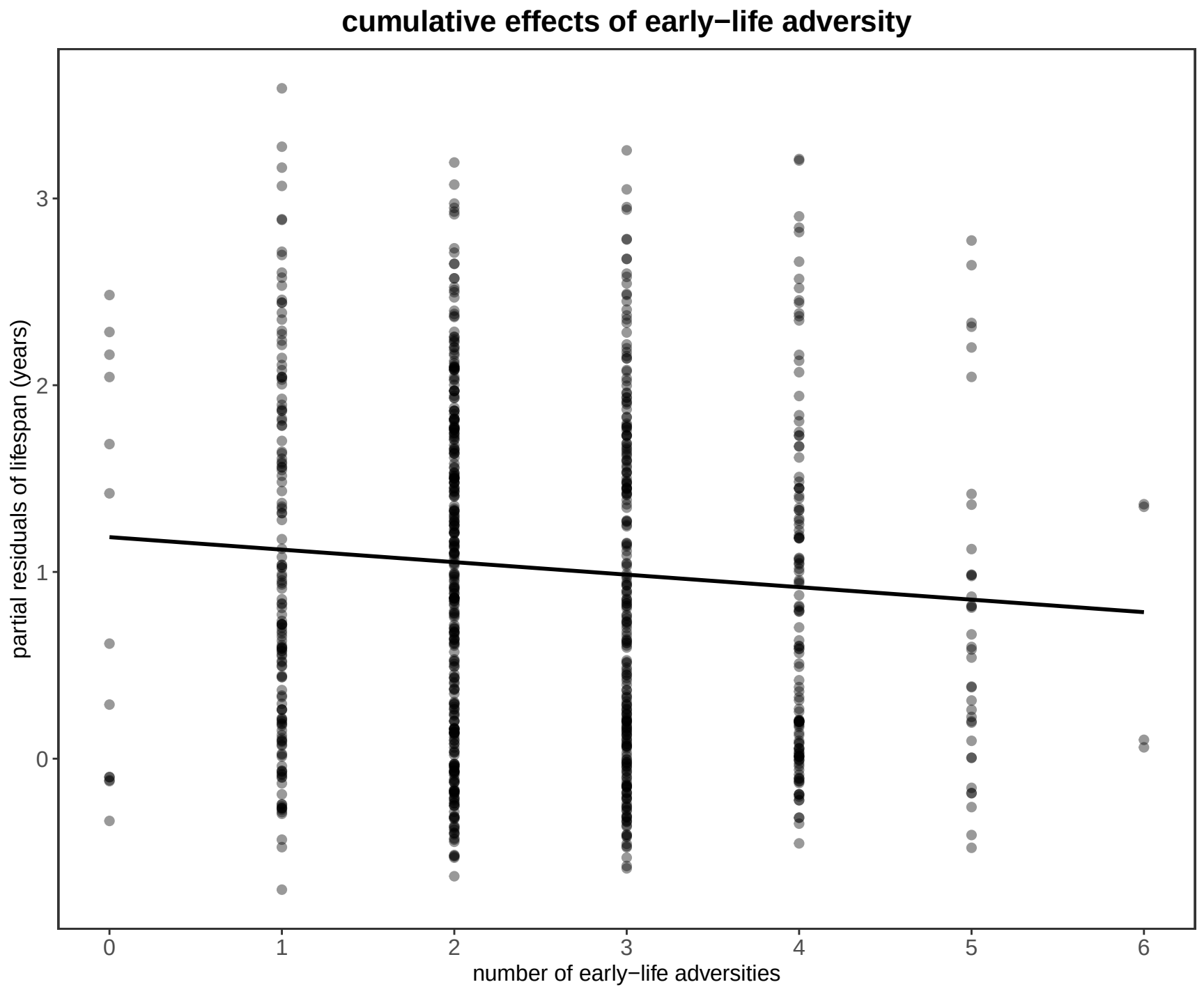
regression coefficient (95% CI)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partial residuals of lifespan (years)
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1
2
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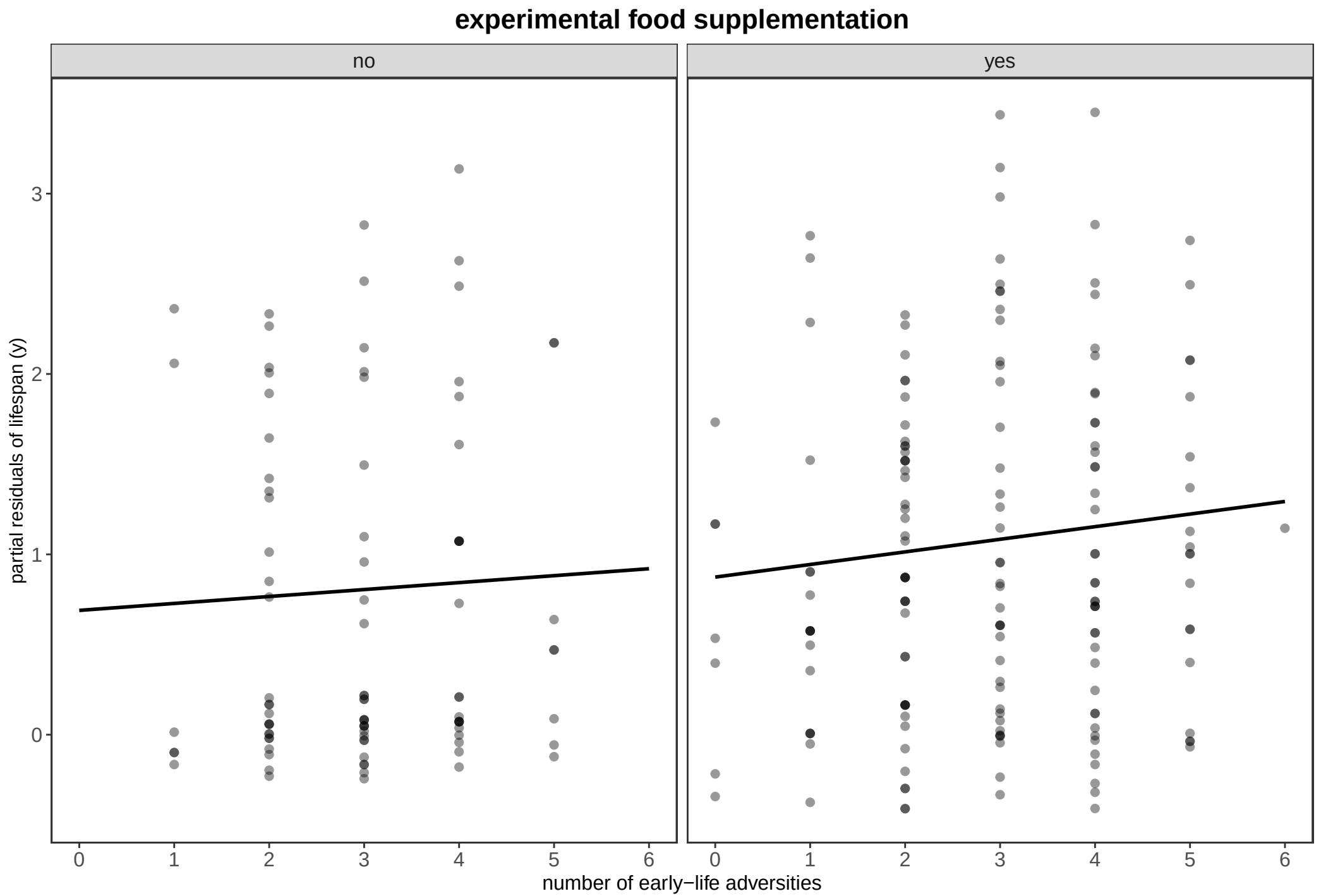
5
6
number of early−life adversities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partial residuals of lifespan (y)
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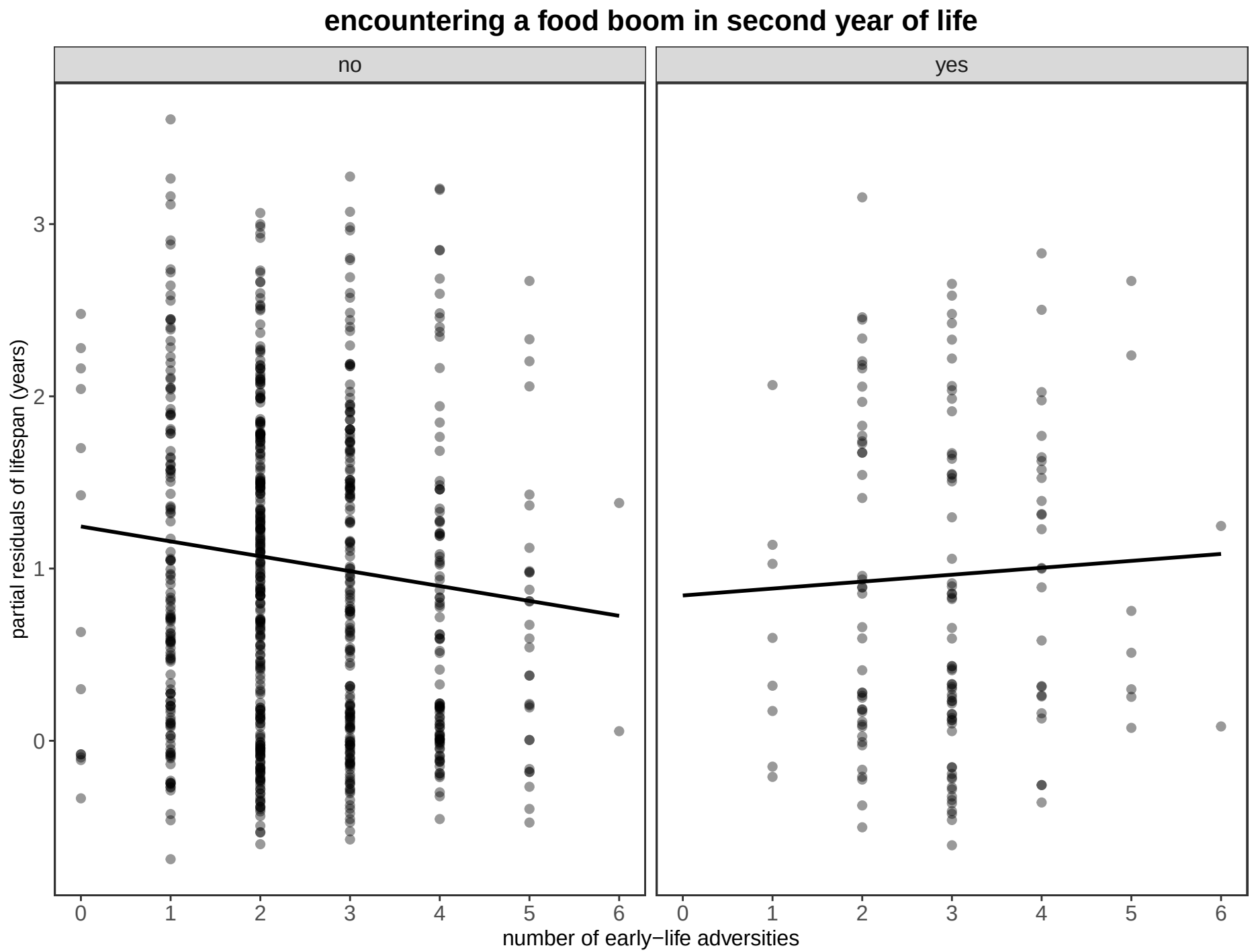
1
0
0
1
2
3
4
5
6
number of early−life adversities


encountering a food boom in second year of life
no
yes
partial residuals of lifespan (years)
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2
1
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6
number of early−life adversities