



Predicting adult lifespan and lifetime reproductive success from early-life reproductive events

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Abstract

Fitness is a product of survival and fecundity, which arise from a host of individual traits that vary among individuals. Estimating fitness is central to many research programs in ecology and evolution. Yet, because researchers are rarely able to track individuals through a complete lifetime, they typically rely on proxies of reproductive success. Because it can be readily measured, the number of offspring produced at first reproduction has been used as a proxy for fitness in some studies, including some on the marine copepod *Tigriopus californicus*. However, to date, no validation of the accuracy of this metric has been completed. We tracked the lifetime reproductive success of *T. californicus* mothers to test whether the number of offspring in a mother's first clutch is a reliable indicator of lifetime reproductive success. We used a repeated measures design to quantify variation in reproductive metrics among individual mothers and within each mother across her lifetime. We found positive associations between first clutch size, adult lifespan, and lifetime reproductive success. We detected negative effects on clutch size, offspring body size and offspring survival due to aging and the increase in reproductive bouts over time. Finally, we observed a trade-off between clutch size and offspring body size. These results indicate that first clutch size is a good predictor of lifetime reproductive success and may serve as a suitable fitness proxy in *T. californicus* copepods. The observed decline in female reproductive traits over time may underscore an emphasis on early reproduction due to the variable conditions this species endures in the wild or, alternatively, may result from resource limitations imparted on females that must mate only once per lifetime.

Introduction

Many studies in ecology and evolution require researchers to estimate fitness. However, even with comprehensive data on survival and reproduction, estimating fitness of an individual is challenging (Arnold 1983; Lande and Arnold 1983; Barker 2009). In many study systems, researchers

have incomplete information on the survival and fecundity of individuals, so they are forced to rely on proxies that are hypothesized to co-vary with lifetime reproductive success. In studies where proxies are used, researchers assume that specific events in a life history sequence reliably predict overall reproductive success. This assumption is based on the prediction that natural selection promotes life history strategies that maximize reproductive success across life history stages (Reznick et al. 2002). The proxies that authors choose to approximate lifetime reproductive success are variable; some are single measures, while others require repeated measurement of traits over time (van de Pol and Wright 2009). As a consequence, a recurring theme in life history research involves exploring relationships among singly or repeatedly measured proxies for reproductive success in order to quantify trait variation among individuals or over an individual's lifetime (Hamel et al. 2009; van de Pol and Wright 2009; Careau and Wilson 2017b).

Zooplanktonic copepods have been proposed as models for studying variation in life history traits and relationships among reproductive variables because of their relatively

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short generation times and suitability to lab environments (Allan 1976). Similarly, the benthic marine copepod *Tigriopus californicus* has been the focus of studies that measured life history traits to better understand speciation (Edmands and Harrison 2003; Burton et al. 2013) and physiological responses to stress (O'Brien et al. 1988; Dybdahl 1995; Heine et al. 2019). *T. californicus* copepods are ideal for quantitative experiments (Edmands and Harrison 2003), because this species has a relatively short lifespan (Vittor 1971), females engage in multiple bouts of reproduction (Burton 1985), and both sexes are amenable to direct experimental manipulation (Powers et al. 2019). These characteristics enable researchers to record simultaneously repeated measures of life history traits on multiple individuals.

T. californicus has a variable average lifespan from as low as 50 days to as high as 125 days depending on environmental temperatures (Vittor 1971; Foley et al. 2019). This relatively short lifespan allows for observing life history traits of multiple generations within a reasonable experimental time period. Although males of this species are free to mate multiple times, females mate only once (Burton 1985). Female *T. californicus* receive a spermatophore from a single mate that is the exclusive source of sperm (Blades-Eckelbrager 1991; Titelman et al. 2007). From this single transfer of sperm, females can produce, carry, and hatch more than a dozen clutches of eggs (Egloff 1966; Burton 1985). A range of traits linked to reproduction and survival including body size, clutch size, number of clutches, lifespan, offspring survival, and development time have been measured in *Tigriopus* copepods either at discrete life history stages or ideally, over the entire lifetime of reproducing females (Vittor 1971; McAllen and Brennan 2009; Han et al. 2016). In some studies, first clutch size has been used as a proxy of lifetime reproductive success, because total lifetime reproduction could not be measured either due to time constraints or the need to perform terminal measures on study animals (Barreto and Burton 2013; Heine et al. 2019). First clutch size has been used as a fitness proxy, alongside oxidative damage, to measure the effects of mitonuclear incompatibilities in *T. californicus* hybrids (Barreto and Burton 2013). Similarly, first clutch size was used to approximate reproductive success in *T. californicus* females exposed to hormetic effects of UV-radiation (Heine et al. 2019). This metric has also been employed in vertebrate studies (Slagsvold 1982; Dhondt et al. 1992). In collared flycatchers, first clutch size has been experimentally manipulated to learn how maternal characteristics affect future offspring (Schluter and Gustafsson 1993). First clutch size was also used as a proxy for fecundity in *Parus* tits to observe the simultaneous effects of population density and habitat heterogeneity (Dhondt et al. 1992). Slagsvold (1982) consider variation in first clutch size in their models to estimate avian reproductive investment in the face of nest predation, habitat selection and seasonal

variation. The modeling revealed that variation in clutch size is not easily explained by a single source of selective pressure (Slagsvold 1982). However, to our knowledge, whether first clutch size is positively related to other measures of reproductive success has not been explicitly described.

Here, we tracked reproductive life history traits over the adult lifespan of *T. californicus* females from sexual maturity until death. We measured several life history traits for each reproductive event in a mother's lifetime using both repeated measures and single measures, depending on the trait. A primary goal of this study was to determine how first clutch size is related to lifetime reproductive success by measuring many lifetime reproductive traits. We analyzed relationships between these traits and first clutch size produced by *T. californicus* mothers using generalized linear models. Next, we analyzed the effect of aging and increasing reproductive bouts among and within female copepods to assess variation in life history traits over time. Finally, we analyzed several life history traits to identify potential tradeoffs among reproductive traits.

Materials and methods

Copepod culture

We have maintained large outbred populations of *Tigriopus californicus* copepods in continuous cultures in 35 psu artificial seawater at room temperature in a temperature controlled lab room (set to 21 °C; range 20–22 °C) on a 12:12 h light:dark cycle and fed live microalgae (*Isochrysis galbana* and *Tetraselmis* sp.). All cultures used in this study came from a stock culture of admixed *T. californicus* copepods from multiple populations. Therefore, individuals in this study do not represent any single-sourced population.

Data collection

T. californicus copepods have generation times that are typically 21–28 days (Powlik 1996). They develop through six naupliar instars and five juvenile copepodid stages, molting at each transition, before reaching maturity (Fig. S1). Mature male copepods clasp and guard juvenile females (i.e., virgin) until the female's final molt into the adult stage, at which point copulation occurs immediately (Vittor 1971; Burton 1985; Powlik 1996). To track female lifetime reproduction, we isolated clasped male–female pairs in a single well of a 24-well plate. We monitored the clasped pair daily until the male released the female, at which point we removed the male and recorded the date as the start of the female's adult lifespan. Here, we note that the measure of adult lifespan of female *T. californicus* does not account for variation in the development time of the female naupliar and juvenile life

stages. This variation could have affected variation in female reproductive traits and should be noted as a potential limitation to conclusions regarding effects on female lifespan. However, Vittor (1971) reported that of all life history traits measured, development time had the least amount of variation across external conditions.

We recorded reproduction metrics (Table S1) from September to December 2017. Assays were started at the same time and monitored together until the end of the experiment. Females were monitored daily for the growth of eggs. Egg gestation was quantified as the difference between egg hatching date and the day eggs were first extruded. Once the eggs hatched, we moved the female to the adjacent well of the 24-well plate and again monitored her daily for subsequent clutches. This process was repeated until the mother's death, at which point the date was recorded as the end of her adult lifespan.

For every clutch that a mother produced, we recorded how many offspring (nauplii) hatched, the clutch number for that mother, and the adult age (in days since reaching maturity) of the mother. Nauplii were monitored in the well in which they hatched. After 14 days, we recorded survival and the ratio of nauplii to juvenile copepodids as a measure of development rate. From here forward, we refer to these two measures as clutch "survival ratio" and "development ratio", respectively. Finally, we measured body size of 2–3 nauplii from each clutch by photographing them under a dissecting microscope on a glass, calibrated measurement slide (Fig. S1). We multiplied their length and width to obtain the approximate body area of each nauplius (mm^2 , converted to μm^2). Photos were taken in triplicate and measurements from each photo were averaged into a single value for each offspring.

Repeated measures of life history traits can reveal important intra-individual variation in trait expression that may be missed when only comparing single measures (Bland and Altman 1995a, b; Hamel et al. 2009; Roche et al. 2016; Careau and Wilson 2017a). Moreover, repeated measures on individuals can be useful for testing for tradeoffs between reproductive traits and aging (Cam et al. 2002), and repeated measures can reveal predicted relationships among traits (Stearns 1989) that single observations at the population level may mask. However, repeated measures of the same individuals violate the assumption of independent samples and must be accounted for in statistical model (van de Pol and Wright 2009; Bakdash and Marusich 2017). Thus, we incorporated mother ID as a random effect in our analyses.

The traits recorded at the level of each clutch (clutch size, clutch number, mother age at hatch, survival ratio, development ratio) and of individual nauplii (body size) represent repeated measures of life history traits within a single mother. We also recorded summary statistics for every mother: the total number of clutches, first clutch size,

average clutch size, largest clutch size, total offspring, average egg gestation time (in days), average clutch survival ratio, average clutch development ratio, average nauplii body size, and total lifespan. Because each of these variables represents a single measure, statistical analyses of these data only represent variation among copepod mothers.

Data analyses

We performed three sets of statistical analyses using linear modeling. All models were run in R v 3.6.1 (The R Core Team 2019); random effects models were fit using the *lme4* package v 1.1.21 (Bates et al. 2015) and the *MCMCglmm* package (Hadfield 2010). In the first set of analyses, we assessed whether the size of a mother's first clutch was positively related to other measures of her reproductive success (Table S1; Fig. S2). Using linear models, we tested for an effect of increasing first clutch size on the following variables: total offspring, average clutch size, largest clutch size, total number of clutches, average offspring survival ratio, average offspring development ratio, average offspring size, average egg gestation time, and mother adult lifespan.

When testing for a significant relationship between first clutch size and total offspring, it is important to consider that the number of offspring in the first clutch contributes to the total offspring count. Therefore, to validate the relationship between first clutch size and total offspring, we ran a simulation in R using the 'rpois' function to generate first clutch and total offspring variables using the means from the observed data under the assumption that the only relationship between the two was due to first clutch size being a part of the offspring total. This represented a null model, (fit with the same negative binomial parameters as the actual model) for which we simulated 1000 effect size estimates between first clutch size and total offspring. We compared and plotted the effect size from the original data against the distribution of 1000 null model effect sizes and performed a significance test (using a cutoff of $\alpha = 0.05$) for the probability that the observed effect size fell within the distribution of the null model effect sizes. If the observed effect size from the original data fell outside the range of values from the null model, this would indicate that the observed effect was not simply due to the addition of the first clutch into the total number of offspring. Figure S2 shows the final distribution of simulated effect sizes plotted with the observed effect. See the R script in the supplement for a step-by-step walk-through of the comparison of the effect size from the original data with that from null model. Briefly, we first defined our simulation function using the means from the observed first clutch data (denoted FCF in script), total clutch data (*F* in script), and the difference between the two (Diff in script; this represented all offspring in the total not attributed to

the first clutch). We then randomly generated a new set of first clutch size values (FCF_{sim}) of the same length as the original data set using the observed mean. Similarly, we generated a new set of random difference values ($Diff_{sim}$) using the mean of the difference between the observed first clutch and total offspring data described above. We added the simulated first clutch sizes and differences together ($FCF_{sim} + Diff_{sim}$) to get a new set of total offspring values (F_{sim}). These new values represent a randomly generated offspring total in which the only relationship between first clutch size and total offspring is due to the first clutch size being added directly to the total (i.e., the relationship is only mathematical and not driven by any biological relationship). We then ran a linear model testing the relationship between the randomly generated first clutch data and the randomly generated total ($F_{sim} - FCF_{sim}$) and extracted the effect size. We repeated this simulation process 1000 times and plotted the distribution of effect sizes against the observed effect size from the observed data model (Fig. S2). We also tested for a significant difference between the simulated effect sizes and the observed effect size using a likelihood ratio test.

In the second set of analyses, we modeled the effect of aging and increasing reproductive bouts over time on the following set of variables: clutch size, offspring size per clutch, clutch survival ratio, clutch development ratio, and egg gestation time per clutch. Since these traits were recorded multiple times over a mother's lifetime, we modeled their relationships using a generalized linear mixed-effects model (negative binomial or Poisson for count data, logistic for proportions). We also included mother ID as a random effect to capture among-individual variation in quality in measured traits and to control for variation due to any traits that we did not measure. Thus, effect sizes from the model incorporated both variation among and within individual females over time. We separately partitioned trait covariation into covariation among and within copepod mothers (Table S2) using a bivariate model fit using *MCMCglmm* following the recommendations of Carreau and Wilson (2017a, b) and the methods of Roche et al. (2016) (see R script in supplement for details on prior specification and model parameters).

In the third set of analyses, we quantified relationships between clutch-related variables, while controlling the effect of mother age, to look for potential trade-offs between life history traits (Tables S3, S4). In these analyses, we used a generalized linear mixed effects model for all response variables except offspring body size, which was continuous with normally distributed residuals. To model the effect of body size on survival and development, we scaled body size (μm^2) to reduce model eigenvalues and improve model resolution. This was necessary whether we modeled body size in μm^2 or in mm^2 . As in the second set of analyses, we quantified

among and within individual trait covariation with a bivariate model fit using *MCMCglmm*.

For all analyses, we report model results as an effect size estimate (β). This is written as a regression coefficient (slope) for linear models and an odds ratio converted to a percent increase/decrease for generalized linear models (denoted by % after the β value). We assessed statistical significance using confidence intervals. Confidence intervals that include a value of 0 are equivalent to a p value of >0.05 . We report covariances between traits with the results on the effect of aging on life history traits (Table S2) and the results on the relationships among clutch-level traits (Table S4). All figures were built in R using 'ggpubr' (Kassambara 2019).

Results

We tracked lifetime reproductive success and life history metrics of 23 females that produced 178 clutches during the study period. From these clutches, we recorded body size measurements on 227 offspring. On average, females lived 45 days past their final molts (ranging between 17 and 74 days). In total, females produced 4400 offspring, 1462 of which survived (33%); of those that survived, 1120 reached their juvenile molt at the time of the 2-week checkpoint (77%).

Effect of first clutch size on other life history traits among mothers

We found that first clutch size is significantly and positively associated with other life history traits (Figs. 1, S2 and S3; Table 1). We found that as first clutch size increased, mother adult lifespan increased as well ($\beta = 2.79\%$; 95% CI 0.28–5.37) (Table 1; Fig. 1). We also found that mothers that had larger first clutches also had more total offspring ($\beta = 4.05\%$; 95% CI 0.56–7.68) and each of their clutches tended to be larger on average ($\beta = 0.22$; 95% CI 0.01–0.42) (Table 1; Fig. 1).

When we simulated the relationship between first clutch size and total reproductive output under a null model (wherein the only relationship between the two variables was due to the addition of first clutch size into the total number of offspring), we found that the observed effect from the original data shown above ($\beta = 4.05\%$) was significantly different ($p < 0.001$) than the distribution of effects under the null model (range = approx. -0.01 to 0.03%) (Fig. S2). This indicated that the observed effect estimated from the original data was not simply due to the addition of the first clutch into the total offspring count.

We found that first clutch size was an unreliable predictor of other life history and reproductive measures even though several modeled relationships fell just short of statistical

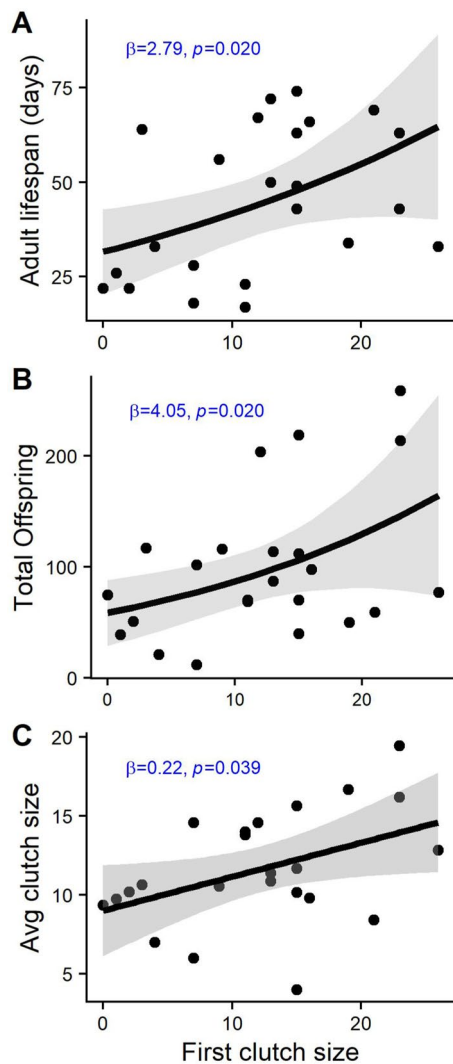


Fig. 1 The relationship between first clutch size of *Tigriopus californicus* females and adult lifespan (a), total offspring (b), and average clutch size (c). Small circles represent individual samples. Model-predicted regression coefficients and 95% confidence intervals are shown by the line and shaded area

significance (Table 1; Fig. S3). These include a mother's largest clutch size, total number of clutches, average clutch survival ratio, average clutch development ratio, average offspring body size, and average egg gestation time.

Effect of aging and increasing reproductive bouts on clutch-level traits

Most of the traits analyzed using the *MCMCglmm* model showed greater intra-individual covariance than inter-individual covariance (Table S2). The majority of the *T. californicus* mothers showed similar variation in life history traits over their lifetime (Figs. 2, S4). In some cases, trait covariance among mothers was positive, while trait covariance within mothers was negative (Table S2). This observation is not uncommon in life history data (van Noordwijk and de Jong 1986). The covariance among and within individuals combines (along with unmeasured variation and error) to give the effect sizes (β) described below.

We observed that for every additional day a mother aged, there was a decrease in clutch size ($\beta = -1.85\%$; 95% CI -2.70 to -1.01) and offspring body size ($\beta = -36.64 \mu\text{m}^2$; 95% CI -61.65 to $-9.46 \mu\text{m}^2$) (Fig. 2; Table 2). For every additional day added to a mother's age, there was an increase in egg gestation time ($\beta = 0.78\%$; 95% CI 0.27 to 1.28) (Fig. 2; Table 2). While we did not find that aging had a significant effect on clutch survival ratio and development ratio, these traits did decrease overall as mothers aged (Fig. S4; Table 2).

For every additional clutch that a mother produced, there was a decrease in clutch size ($\beta = -5.18\%$; 95% CI -9.04 to -1.23) and offspring body size ($\beta = -131.69$; 95% CI -230.01 to -22.35) (Fig. 2; Table 2). However, there was not a reliable predictive relationship between increasing reproductive bouts and egg gestation time, clutch survival ratio, and clutch development ratio (Fig. S4; Table 2).

Table 1 Results from generalized linear models analyzing the relationship between first clutch size and other reproductive traits

Predictor	Response	Sample size	Effect size	β	Lower CI	Upper CI	p value
1st clutch size	Total offspring	23	Odds ratio	4.05	0.56	7.68	0.020
	Average clutch size	23	Reg. Coef.	0.22	0.01	0.42	0.039
	Largest clutch size	23	Odds ratio	1.67	-0.12	3.49	0.072
	Mother adult lifespan	23	Odds ratio	2.79	0.28	5.37	0.020
	Tot. # of clutches	23	Odds ratio	2.20	-0.38	4.87	0.092
	Survival ratio average	23	Odds ratio	1.71	-9.51	14.81	0.774
	Development ratio avg.	23	Odds ratio	1.84	-9.36	14.94	0.757
	Offspring size average	23	Reg. Coef.	13.25	-68.08	94.58	0.738
	Egg gestation average	23	Reg. Coef.	0.03	-0.01	0.07	0.166

Bold values represent statistically significant results

Fig. 2 The relationship between increasing mother age (in days) and increasing number of reproductive bouts on clutch size (**a**, **b**), offspring size (**c**, **d**), and egg gestation time (**e**, **f**). The black line represents the population mean and the grey lines represent individual mothers

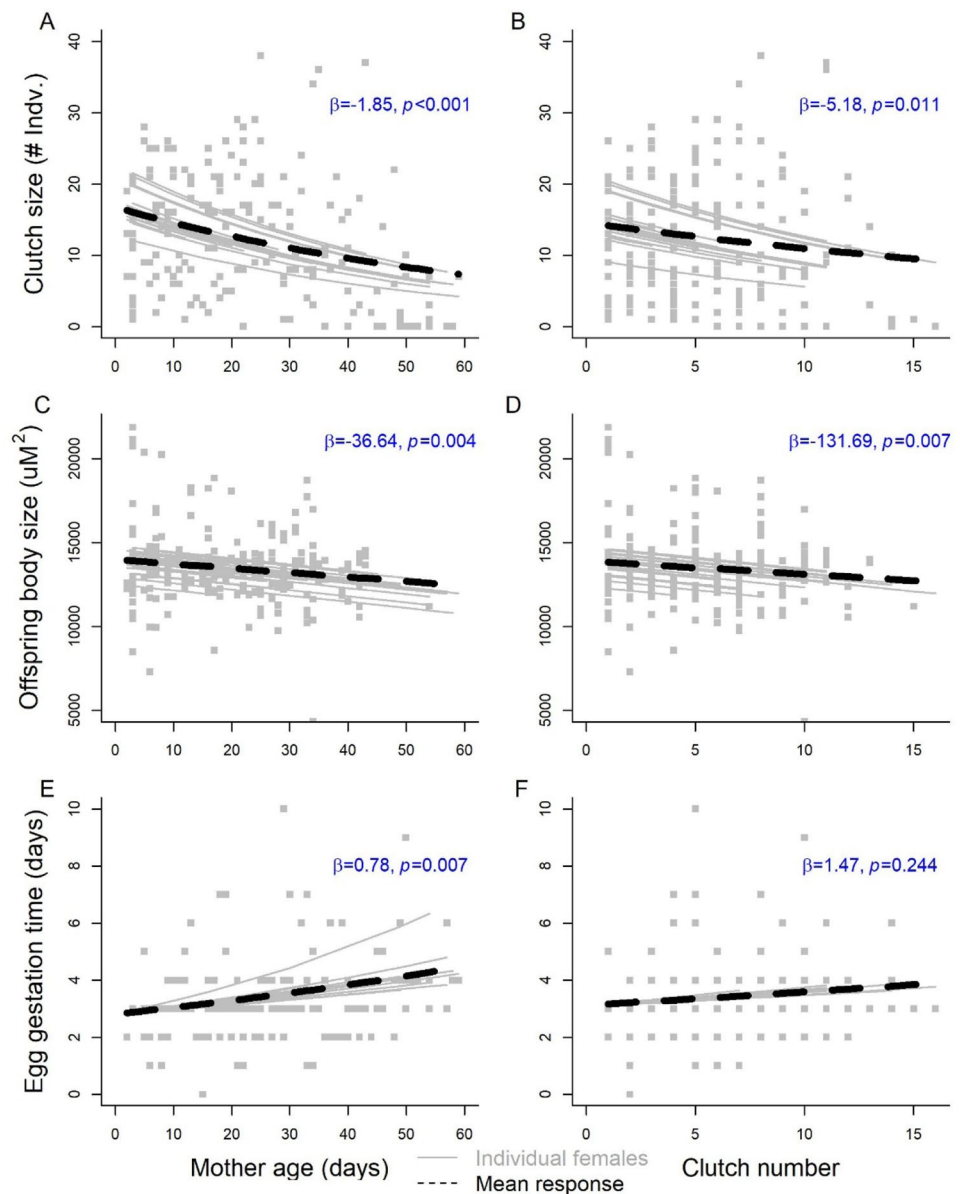


Table 2 Results from generalized linear models analyzing the relationship between mother age, clutch number, and other reproductive traits

Predictor	Response	Sample size	Effect size	β	Lower CI	Upper CI	p value
Mother age	Clutch size	178	Odds ratio	-1.85	-2.70	-1.01	< 0.001
	Offspring size	227	Reg. Coef.	-36.64	-61.65	-9.46	0.004
	Survival ratio	168	Odds ratio	-2.01	-4.35	0.31	0.091
	Dev. ratio	168	Odds ratio	-2.24	-4.63	0.09	0.062
	Egg gest.	178	Odds ratio	0.78	0.20	1.36	0.007
Clutch number	Clutch size	178	Odds ratio	-5.18	-9.04	-1.23	0.011
	Offspring size	227	Reg. Coef.	-131.69	-230.01	-22.35	0.007
	Survival ratio	168	Odds ratio	-3.01	-13.31	8.15	0.586
	Dev. ratio	168	Odds ratio	-5.15	-15.05	5.48	0.331
	Egg gest.	178	Odds ratio	1.47	-0.88	4.03	0.244

Bold values represent statistically significant results

Effects of increasing clutch size, offspring body size and egg gestation time

Most of the relationships between clutch size, offspring body size, egg gestation time and other reproductive traits show greater within-individual covariance than among-individual covariance (Table S4). We observed similar relationships between these variables in most females (Figs. 3, S5, S6). We found a trade-off between the number of offspring in a given clutch and the offspring body size in that clutch; as body size increased, clutch size decreased ($\beta = -9.73\%$; 95% CI -16.63 to -2.24 ; Fig. 3; Table S3). However, we did not find any reliable association between offspring body size and clutch survival ratio or clutch development ratio (Fig. S5; Table S3). Further, we did not find a reliable predictive relationship between clutch size and clutch survival ratio or clutch development ratio (Fig. S5; Table S3).

Egg gestation time reliably predicted clutch size, clutch survival ratio, and offspring body size (Fig. S6; Table S3). For every additional day that a mother spent carrying her eggs, we observed a decrease in clutch size ($\beta = -10.57\%$; 95% CI -16.96 to -3.70) and clutch survival ratio ($\beta = -26.63\%$; 95% CI -44.08 to -3.73). For every extra day in egg gestation time, there was an increase in offspring body size ($\beta = 316.59$; 95% CI 127.29 – 500.46). We did not find a significant association between egg gestation time and clutch development ratio (Fig. S6; Table S3).

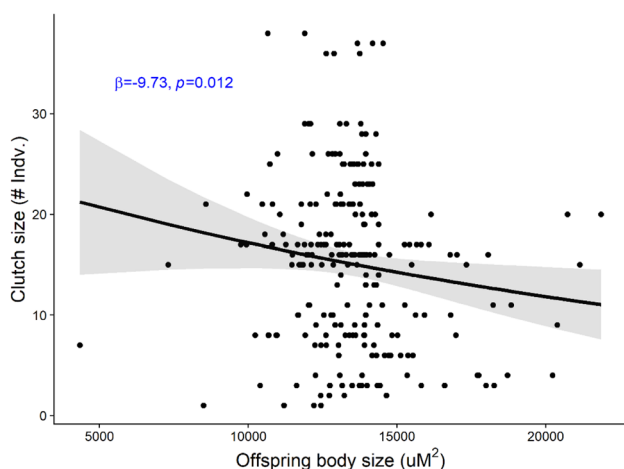


Fig. 3 The significant negative relationship between clutch size and offspring size. The black line represents the population mean and the grey shading represents the 95% confidence interval around the slope

Discussion

Theory predicts that organisms will adopt life history strategies that maximize lifetime reproductive success in a given environment. Nevertheless, in most populations of plants and animals, there is variation among individuals in life history traits (van Noordwijk and de Jong 1986; Le Galliard et al. 2004; Hamel et al. 2009). Understanding the variation in life history traits among individuals in a population is key to understanding potential tradeoffs among investments and payoffs in terms of lifetime reproductive success and establishing the best proxies for total reproductive success. In *T. californicus* copepods, we observed that first clutch size was positively associated with other life history traits considered to be components of reproductive success (Figs. 1, S2, S3) and thus may be a useful, fairly easily measured proxy for fitness. However, we acknowledge that many of our results include effects with large confidence intervals indicating large variation in the data (see Tables 1, 2, and S3 for exact values). Therefore, caution should be taken to interpret these results in the context of both statistical and biological significance. To this end, it is helpful to interpret the relationships between traits based not only on p values and confidence intervals, but the strength of effects (effect sizes are listed in Tables 1, 2, S3 and on Figs. 1, 2, 3 and S3, S4, S5, S6).

A foundational prediction in life history theory is that investment in early reproduction comes at the cost of a decrease in lifespan or in the capacity to invest in later reproduction (Stearns 1989). Contrary to this prediction, however, many studies find positive relationships between investment in early reproduction and investment in subsequent reproductive bouts or survival (van Noordwijk and de Jong 1986; Hamel et al. 2009). The observation that some individuals are able to afford investment in two or more traits predicted to trade-off with one another has been dubbed the “Big Houses, Big Cars” phenomenon (Reznick et al. 2000). We observed that with *T. californicus* copepods, individuals that produced larger first clutches (early investment in reproduction) also lived longer, produced more offspring in total, and produced more offspring per clutch on average. Importantly, our observed effect size of the relationship between first clutch size and total offspring fell well outside the distribution of simulated null model effect sizes, and this suggests an underlying biological connection between first clutch size and total offspring in *T. californicus*. Based on these observations, it appears that, at least in laboratory cultures, because first clutch size positively covaries with other life history traits among *T. californicus* copepods (Table 1), it may be a good proxy for lifetime reproductive success. However, the conclusions from this study pertain to captive animals maintained

with unlimited food, and stable temperature. It is possible that tradeoffs between early reproduction and investment in survival or later reproduction could emerge if copepod mothers were exposed to external stressors (Stearns 1989; Garnier et al. 2016).

We observed a general decrease in reproduction with age in *T. californicus* mothers. This is also observed in other organisms, although the decline usually comes after a period of priming to reach a peak in reproductive success (Clutton-Brock 1984; Sæther 1990; Komdeur 1996; Cam et al. 2002). In the case of *T. californicus* copepods, we observed that older females had smaller clutch size, smaller offspring, and tended to have slightly lower offspring survival, and slower offspring development (Figs. 2, S4; Table 1; although the decreases in survival and development were not statistically significant). Moreover, this decrease in life history traits was fairly uniform across all *T. californicus* mothers in this study (Figs. 3, S5, S6; Table S2).

It is possible that strong selection from external conditions may be responsible for shaping a consistent response to aging among mothers. *T. californicus* inhabits splash pools that present highly variable environments in which populations are prone to rapid extinction (Vittor 1971; Dybdahl 1994). *T. californicus* escape predators by inhabiting pools far above the high tide line (Dethier 1980), but such pools are prone to large variations in salinity. Moreover, many of these splash-pools are partly or fully exposed to the sun, exposing animals to fluctuating temperatures and variable UV-radiation. Fluctuating salinity (Dybdahl 1995), temperature (Tangwancharoen and Burton 2014), and UV-radiation (Heine et al. 2019) can significantly affect harpacticoid copepod reproduction and offspring development. In a highly variable environment, selection should favor short generation times and the early production of many offspring enabling populations to grow rapidly (Reznick et al. 2002; Engen and Sæther 2017). It is possible that *T. californicus* exhibits patterns reminiscent of an *r*-selected species (Vittor 1971), because these copepods are minimally restricted by population growth due to the presence of a continuous food supply from organic run-off into their splash pools (p. 15 of Vittor 1971) and a low risk of juvenile mortality (Tangwancharoen and Burton 2014). A decrease in reproductive success over time may be observed when risk of adult mortality is low and the opportunity to reproduce is great (Godfray et al. 1991). However, it has been suggested that *T. californicus* does not fit traditional definitions of an *r*-selected species (Vittor 1971). Instead, *T. californicus* may exhibit slower declines in reproductive traits, at least near the end of their lives, relative to other iteroparous organisms (Schaffer 1974; Clutton-Brock 1984; Evans et al. 2011) to maintain a strategy of opportunistic breeding (Vittor 1971). This may be due to the need to establish (or re-establish) colonies after displacement or local extinction in splash pools.

Natural populations of *T. californicus* show cohort-specific and sex-specific responses to environmental stressors such as increased temperature, hypersaline or hyposaline conditions, copper toxicity and pollutant endocrine disruptors (Foley et al. 2019). Current evidence suggests that female *T. californicus* show increased resilience to these many of these stressors compared to males (Foley et al. 2019). Moreover, some wild populations show higher resilience to stressors than others, likely due to the influence of local adaptation. For example, southern *T. californicus* populations show greater resilience against heat stress compared to northern populations that inhabit cooler climates (Foley et al. 2019). Because we did not sample from specific *T. californicus* populations, in favor of using admixed cultures, it is unclear whether the relationships among early reproduction, other life history traits, and aging that we observed would be ubiquitous across geographical space. It is possible that the reproductive patterns we observed also vary with changes to the environment.

In *T. californicus*, we observed a significant negative relationship between the size of offspring upon hatching and the number of offspring per clutch. This agrees with studies in other copepods (Cooney and Gehrs 1980; Guisande et al. 1996) and in other taxa (Christians 2000; Brown 2003). One often-cited explanation for this negative relationship is that there is a physiological constraint on the total mass of offspring that can be developed in a given physical space, such that offspring size trades off with the quantity of offspring (Reiss 1991). However, it is also possible that mothers that are larger can carry larger clutches on average and may be able to allocate more resources to lifetime reproductive success than smaller mothers (i.e., mobilize more nutrient stores more efficiently) (Sakai and Harada 2001). Body size is often used as a key measure of individual quality in higher taxa (Clutton-Brock 1984; Jakob et al. 1996) and in copepods (Timi et al. 2005). When analyzing trade-offs in the parasitic copepod *Lernanthropus cynoscicola*, Timi et al. (2005) found that the negative relationship between offspring size and clutch size disappeared when controlling for variation due to the body size of the mother. Yet, the relationship between body size of the mother and reproductive success in other invertebrates remains equivocal (Bosch and Vicens 2006). In this study, we did not record the body size of mothers. However, by including mother ID as a random effect in the model we did account for broad variation among *T. californicus* females that would have included variation due to body size (and any other unmeasured difference among them). Indeed, not all traits related to individual quality can be measured directly (Wilson and Nussey 2009).

We detected a negative relationship between gestation duration and the resulting hatching success (clutch size, see Heine et al. 2019) and the survival of offspring (Fig. S6). This may be driven by the fact that the longest

gestation times were tied to older mothers. We controlled for mother age in our statistical models examining the relationship between egg gestation and clutch size or offspring survival; in the clutch size model the effect of the mother age covariate was statistically significant ($\beta = -1.58\%$; 95% CI -2.43 to -0.73), but in the offspring survival model the mother age effect was not ($\beta = -1.27\%$; 95% CI -3.67 to 1.19). It may be possible that gestation time is a good indicator of aging in *T. californicus*, because the longer that a mother carried her eggs, the less likely her offspring were to survive and the fewer offspring she produced. In species such as *T. californicus*, mothers carry their eggs until hatching. During this time, mothers deposit nutrients essential to offspring growth and survival (Hirst and López-Urrutia 2006). As *T. californicus* mothers age, they may be less able to allocate resources to eggs in a timely manner, increasing their gestation times and resulting in lower quality clutches.

It is also possible that the increased egg gestation times and decreased reproductive output in older females (Fig. 2) were due to a decline in spermatophore material or in a mother's ability to efficiently utilize proteins and nutrients from the spermatophore. Since *T. californicus* females must use the same male spermatophore to produce all of their offspring (Burton 1985), it is possible that proteins and nutrients in the spermatophore—or the ability to efficiently utilize these proteins and nutrients—lasts only to a certain extent (Iwasa et al. 1984; Godfray et al. 1991). However, more research on the usage and allocation of spermatophore material is needed to determine if this is true (Titelman et al. 2007). Regardless, the evolutionary constraint imparted by the need to produce a lifetime of offspring from a single fertilization event highlights a key difference between females of singly mating species (such as *T. californicus*), and females of other iteroparous species that pool multiple spermatophores to create a large resource pool or that replenish sperm through multiple matings (Arnqvist and Nilsson 2000; Titelman et al. 2007).

We also observed that increased egg gestation times were associated with increased offspring body size (Fig. S6). We would expect that if longer egg gestation times were associated with decreased survival, that offspring body size should be smaller in longer developing eggs as well (i.e., body size and survival are usually positively correlated) (Smith and Fretwell 1974; Warner and Shine 2007). The fact that offspring body size was not significantly correlated with offspring survival could simply be due to the fact that the largest offspring were associated with the longest developing clutches, which in turn were produced by the oldest mothers. We did observe that the relationship between offspring body size and offspring survival was indeed a positive one when controlling for the age of the mother (Table S3).

Conclusion

The results we present in this study may highlight important differences between females of iteroparous species that mate once, like the *T. californicus* copepod, and those that must mate multiple times to replenish reproductive material from new sperm. Female *T. californicus* copepods must use the same spermatophore over an entire lifetime of producing new offspring. Moreover, the life cycle and the evolutionary pressures imparted by the environment on *T. californicus* may provide explanations for the patterns in reproduction over time that we observed. Indeed, the highly variable environment in *T. californicus*-inhabited splash pools, as well as the relatively short life span of the species, may drive an early emphasis on initial reproductive bouts.

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Data availability All data can be found in the online supplementary information and will also be available upon request from the corresponding author.

Compliance with ethical standards

Conflict of interest The authors have no competing interests to declare.

Code availability The code used to perform the analyses in this study is included in the online supplemental material.

Ethical approval Not applicable.

References

- Allan JD (1976) Life history patterns in zooplankton. *History* 110:165–180
- Arnold SJ (1983) Morphology, performance and fitness. *Integr Comp Biol* 23:347–361. <https://doi.org/10.1093/icb/23.2.347>
- Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav* 60:145–164. <https://doi.org/10.1006/anbe.2000.1446>
- Bakdash JZ, Marusich LR (2017) Repeated measures correlation. *Front Psychol* 8:1–13. <https://doi.org/10.3389/fpsyg.2017.00456>
- Barker JSS (2009) Defining fitness in natural and domesticated populations. In: van der Werf J, Graser HU, Frankham R, Gondro C (eds) *Adaptation and fitness in animal populations*. Springer, Netherlands, Dordrecht, pp 3–14

- Barreto FS, Burton RS (2013) Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. *Proc R Soc B Biol Sci* 280:7. <https://doi.org/10.1098/rspb.2013.1521>
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Blades-Eckelbrager PI (1991) Functional morphology of spermatophores and sperm transfer in calanoid copepods. In: Bauer RT, Martin JW (eds) *Crustacean sexual biology*. Columbia University Press, New York, pp 246–270
- Bland JM, Altman DG (1995a) Calculating correlation coefficients with repeated observations: part 2—correlation between subjects. *BMJ* 310:633. <https://doi.org/10.1136/bmj.310.6980.633>
- Bland JM, Altman DG (1995b) Statistics notes: calculating correlation coefficients with repeated observations: part 1—correlation within subjects. *BMJ* 310:446. <https://doi.org/10.1136/bmj.310.6977.446>
- Bosch J, Vicens N (2006) Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behav Ecol Sociobiol* 60:26–33. <https://doi.org/10.1007/s00265-005-0134-4>
- Brown CA (2003) Offspring size-number trade-offs in scorpions: an empirical test of the van Noordwijk and de Jong model. *Evolution* (N Y) 57:2184–2190. <https://doi.org/10.1111/j.0014-3820.2003.tb00397.x>
- Burton RS (1985) Mating system of the intertidal copepod *Tigriopus californicus*. *Mar Biol* 86:247–252. <https://doi.org/10.1007/BF00397511>
- Burton RS, Pereira RJ, Barreto FS (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annu Rev Ecol Evol Syst* 44:281–302. <https://doi.org/10.1146/annurev-ecolsys-110512-135758>
- Cam E, Link WA, Cooch EG, Monnat JY, Danchin E (2002) Individual covariation in life-history traits: seeing the trees despite the forest. *Am Nat* 159:96–105. <https://doi.org/10.1086/324126>
- Careau V, Wilson RS (2017a) Of uberfleas and krakens: detecting trade-offs using mixed models. *Integr Comp Biol* 57:362–371. <https://doi.org/10.1093/icb/ixc015>
- Careau V, Wilson RS (2017b) Performance trade-offs and ageing in the ‘world’s greatest athletes’. *Proc R Soc B Biol Sci* 284:1–9. <https://doi.org/10.1098/rspb.2017.1048>
- Christians JK (2000) Trade-offs between egg size and number in waterfowl: an interspecific test of the van Noordwijk and de Jong model. *Funct Ecol* 14:497–501. <https://doi.org/10.1046/j.1365-2435.2000.00444.x>
- Clutton-Brock TH (1984) Reproductive effort and terminal investment in iteroparous animals. *Am Nat* 123:212–229. <https://doi.org/10.1086/284198>
- Cooney JD, Gehrs CW (1980) The relationship between egg size and naupliar size in the calanoid copepod *Diaptomus clavipes* Schacht^{1,2}. *Limnol Oceanogr* 25:549–552. <https://doi.org/10.4319/lo.1980.25.3.0549>
- Dethier MN (1980) Tidepools as refuges: predation and the limits of the harpacticoid copepod *Tigriopus californicus* (Baker). *J Exp Mar Biol Ecol* 42:99–111. [https://doi.org/10.1016/0022-0981\(80\)90169-0](https://doi.org/10.1016/0022-0981(80)90169-0)
- Dhondt AA, Kempenaers B, Adriaensen F (1992) Density-dependent clutch size caused by habitat heterogeneity. *J Anim Ecol* 61:643. <https://doi.org/10.2307/5619>
- Dybdahl MF (1994) Extinction, recolonization, and the genetic structure of tidepool copepod populations. *Evol Ecol* 8:113–124. <https://doi.org/10.1007/BF01238245>
- Dybdahl MF (1995) Selection on life-history traits across a wave exposure gradient in the tidepool copepod *Tigriopus californicus* (Baker). *J Exp Mar Biol Ecol* 192:195–210. [https://doi.org/10.1016/0022-0981\(95\)00063-W](https://doi.org/10.1016/0022-0981(95)00063-W)
- Edmands S, Harrison JS (2003) Molecular and quantitative trait variation within and among populations of the intertidal copepod *Tigriopus californicus*. *Evolution* (N Y) 57:2277–2285. <https://doi.org/10.1111/j.0014-3820.2003.tb00239.x>
- Egloff DA (1966) Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod *Tigriopus californicus*. Stanford University, Stanford
- Engen S, Sæther BE (2017) r- and K-selection in fluctuating populations is determined by the evolutionary trade-off between two fitness measures: growth rate and lifetime reproductive success. *Evolution* (N Y) 71:167–173. <https://doi.org/10.1111/evo.13104>
- Evans SR, Gustafsson L, Sheldon BC (2011) Divergent patterns of age-dependence in ornamental and reproductive traits in the collared flycatcher. *Evolution* (N Y) 65:1623–1636. <https://doi.org/10.1111/j.1558-5646.2011.01253.x>
- Foley HB, Sun PY, Ramirez R, So BK, Venkataraman YR, Nixon EN, Davies KJA, Edmands S (2019) Sex-specific stress tolerance, proteolysis, and lifespan in the invertebrate *Tigriopus californicus*. *Exp Gerontol* 119:146–156. <https://doi.org/10.1016/j.exger.2019.02.006>
- Garnier A, Gaillard JM, Gauthier D, Besnard A (2016) What shapes fitness costs of reproduction in long-lived iteroparous species? A case study on the Alpine ibex. *Ecology* 97:205–214. <https://doi.org/10.1890/15-0014.1>
- Godfray H, Partridge L, Harvey P (1991) Clutch size. *Annu Rev Ecol Syst* 22:409–429. <https://doi.org/10.1146/annurev.ecolsys.22.1.409>
- Guisande C, Sánchez J, Maneiro I, Miranda A (1996) Trade-off between offspring number and offspring size in the marine copepod *Euterpina acutifrons* at different food concentrations. *Mar Ecol Prog Ser* 143:37–44. <https://doi.org/10.3354/meps143037>
- Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm {R} package. *J Stat Softw* 33:1–22. <https://doi.org/10.1002/ana.22635>
- Hamel S, Côté SD, Gaillard J, Festa-bianchet M (2009) Individual variation in reproductive costs of reproduction: High-quality females always do better. *J Anim Ecol* 78:143–151. <https://doi.org/10.1111/j.1365-2656.2007.0>
- Han J, Lee M-C, Kim D-H, Lee YH, Park JC, Lee J-S (2016) Effects of trimethoprim on life history parameters, oxidative stress, and the expression of cytochrome P450 genes in the copepod *Tigriopus japonicus*. *Chemosphere* 159:159–165. <https://doi.org/10.1016/j.chemosphere.2016.05.085>
- Heine KB, Powers MJ, Kallenberg C, Tucker VL, Hood WR (2019) Ultraviolet irradiation increases size of the first clutch but decreases longevity in a marine copepod. *Ecol Evol* 9:9759–9767. <https://doi.org/10.1002/ece3.5510>
- Hirst A, López-Urrutia A (2006) Effects of evolution on egg development time. *Mar Ecol Prog Ser* 326:29–35. <https://doi.org/10.3354/meps326029>
- Iwasa Y, Suzuki Y, Matsuda H (1984) Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. *Theor Popul Biol* 26:205–227. [https://doi.org/10.1016/0040-5809\(84\)90030-3](https://doi.org/10.1016/0040-5809(84)90030-3)
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61. <https://doi.org/10.2307/3545585>
- Kassambara A (2019) ggpubr: “ggplot2” based publication ready plots. R package version 0.2.1. <https://cran.r-project.org/package=ggpubr>
- Komdeur J (1996) Influence of age on reproductive performance in the Seychelles warbler. *Behav Ecol* 7:417–425. <https://doi.org/10.1093/beheco/7.4.417>
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* (N Y) 37:1210–1226. <https://doi.org/10.1111/j.1558-5646.1983.tb00236.x>

- Le Galliard J-F, Clobert J, Ferrière R (2004) Physical performance and darwinian fitness in lizards. *Nature* 432:502–505. <https://doi.org/10.1038/nature03057>
- McAllen R, Brennan E (2009) The effect of environmental variation on the reproductive development time and output of the high-shore rockpool copepod *Tigriopus brevicornis*. *J Exp Mar Biol Ecol* 368:75–80. <https://doi.org/10.1016/j.jembe.2008.10.013>
- O'Brien P, Feldman H, Grill E, Lewis A (1988) Copper tolerance of the life history stages of the splashpool copepod *Tigriopus californicus* (Copepoda, Harpacticoida). *Mar Ecol Prog Ser* 44:59–64. <https://doi.org/10.3354/meps044059>
- Powers MJ, Hill GE, Weaver RJ (2019) An experimental test of mate choice for red carotenoid coloration in the marine copepod *Tigriopus californicus*. *Ethology* 126:344–352. <https://doi.org/10.1111/eth.12976>
- Powlik JJ (1996) Ecology of *Tigriopus californicus* (Copepoda, Harpacticoida) in Barkley sound. University of British Columbia, Vancouver
- Reiss MJ (1991) The allometry of growth and reproduction. Cambridge University Press, Cambridge
- Reznick D, Nunney L, Tessier A (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol* 15:421–425. [https://doi.org/10.1016/S0169-5347\(00\)01941-8](https://doi.org/10.1016/S0169-5347(00)01941-8)
- Reznick DN, Bryant MJ, Bashey F, Jun N (2002) r- and K-selection revisited: the role of population regulation in life-history evolution. *Evolution* (N Y) 83:1509–1520
- Roche DG, Careau V, Binning SA (2016) Demystifying animal “personality” (or not): Why individual variation matters to experimental biologists. *J Exp Biol* 219:3832–3843. <https://doi.org/10.1242/jeb.146712>
- Sæther BE (1990) Age-specific variation in reproductive performance of birds. *Curr Ornithol* 7:251–283
- Sakai S, Harada Y (2001) Why do large mothers produce large offspring? Theory and a test. *Am Nat* 157:348–359. <https://doi.org/10.1086/319194>
- Schaffer WM (1974) Selection for optimal life histories: the effects of age structure. *Ecology* 55:291–303. <https://doi.org/10.2307/1935217>
- Schluter D, Gustafsson L (1993) Maternal inheritance of condition and clutch size in the collared flycatcher. *Evolution* (N Y) 47:658–667. <https://doi.org/10.1111/j.1558-5646.1993.tb02119.x>
- Slagsvold T (1982) Clutch size variation in passerine birds: the nest predation hypothesis. *Oecologia* 54:159–169. <https://doi.org/10.1007/BF00378388>
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *Am Nat* 108:499–506. <https://doi.org/10.1086/282929>
- Stearns SC (1989) Trade-offs in life-history evolution. *Funct Ecol* 3:259. <https://doi.org/10.2307/2389364>
- Tangwancharoen S, Burton RS (2014) Early life stages are not always the most sensitive: heat stress responses in the copepod *Tigriopus californicus*. *Mar Ecol Prog Ser* 517:75–83. <https://doi.org/10.3354/meps11013>
- The R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Timi JT, Lanfranchi AL, Poulin R (2005) Is there a trade-off between fecundity and egg volume in the parasitic copepod *Lernanthropus cynosicola*? *Parasitol Res* 95:1–4. <https://doi.org/10.1007/s00436-004-1242-1>
- Titelman J, Varpe Ø, Eliassen S, Fiksen Ø (2007) Copepod mating: chance or choice? *J Plankton Res* 29:1023–1030. <https://doi.org/10.1093/plankt/fbm076>
- van de Pol M, Wright J (2009) A simple method for distinguishing within- versus between-subject effects using mixed models. *Anim Behav* 77:753–758. <https://doi.org/10.1016/j.anbehav.2008.11.006>
- van Noordwijk AJ, de Jong G (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *Am Nat* 128:137–142
- Vittor B (1971) Effects of the environment on fitness-related life history characters in *Tigriopus californicus*. PhD Dissertation. <https://doi.org/10.1017/cbo9781107415324.004>
- Warner DA, Shine R (2007) Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* 154:65–73. <https://doi.org/10.1007/s00442-007-0809-9>
- Wilson AJ, Nussey DH (2009) What is individual quality? An evolutionary perspective. *Trends Ecol Evol* 25:207–214. <https://doi.org/10.1016/j.tree.2009.10.002>

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