Decades of field data reveal that turtles senesce in the wild

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Abstract
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Keywords
aging, lifespan, painted turtle, reproduction, senescence

Disciplines
Ecology and Evolutionary Biology | Evolution | Population Biology | Terrestrial and Aquatic Ecology | Zoology

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Decades of field data reveal that turtles senesce in the wild

Short title: Turtles senesce in the wild

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Keywords: Aging | lifespan | painted turtle | reproduction | selection | senescence

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Abstract

Lifespan and aging rates vary considerably across taxa, thus understanding the factors that lead to this variation is a primary goal in biology and has ramifications for understanding constraints and flexibility in human aging. Theory predicts that senescence – declining reproduction and increasing mortality with advancing age – evolves when selection against harmful mutations is weaker at old ages relative to young ages, or when selection favors pleiotropic alleles with beneficial effects early in life despite late-life costs. Yet in many long-lived ectotherms, selection is expected to remain strong at old ages because reproductive output typically increases with age, which may lead to the evolution of slow or even negligible senescence. We show that, contrary to current thinking, both reproduction and survival decline with adult age in the painted turtle, *Chrysemys picta*, based on data spanning >20 years from a wild population. Older females, despite relatively high reproductive output, produced eggs with reduced hatching success. Additionally, age-specific mark-recapture analyses revealed increasing mortality with advancing adult age. These findings of reproductive and mortality senescence challenge the contention that chelonians do not age, and more generally provide new evidence of reduced fitness at old ages in non-mammalian species that exhibit long chronological lifespans.
Significance

Turtles are icons of longevity, popularly characterized as lacking aging and remaining robust as they get older. Indeterminate growth and a positive relationship between body size and fecundity suggest that a greater proportion of reproductive output could come from older, rather than younger, individuals. However, studies of turtle populations are typically too short to empirically test these assertions. We tracked >1000 painted turtles for >20 years in a population in northwest Illinois, USA. Contrary to traditional thought, successful reproduction and survival declined as the turtles aged. Consistent with life-history theory, the observed senescence in reproduction and survival in this population may be due to relatively high extrinsic mortality as a result of human disruption.

Author Contributions

FJJ and DAW designed and conducted the research; DAW, DAWM and AMB analyzed the data; and all authors wrote the paper.
Introduction

Why do some organisms show little to no signs of aging as they get older, whereas others exhibit substantial physiological deterioration and reproductive senescence with advancing age (1, 2)? This question has motivated many studies of aging in the wild, ranging from reviews of demographic aging (3, 4) to compilations of mechanistic studies (e.g., (5) and references therein). Senescence should evolve when selection is weaker on deleterious traits expressed at an old age relative to those expressed at a young age (6). Indeed, mutations with senescent effects can persist due to a trade-off between beneficial effects early in life and pleiotropic detrimental effects late in life (7, 8). Importantly, the persistence of age-specific deleterious mutations will therefore be influenced by levels and sources of extrinsic mortality (e.g., predation, resource scarcity, infectious disease) and the particular ages at which extrinsic sources cause death (9, 10). For example, the onset of physiological or reproductive deterioration is expected to occur relatively early in populations exposed to high levels of extrinsic mortality of adults. Accordingly, such organisms are expected to evolve rapid development/growth, high reproductive effort at young ages, and a shortened lifespan. For species or populations that experience low extrinsic mortality rates, physiological and reproductive function are expected to decline more slowly with advancing age, or with a delay in the onset of senescent decline (7). Moreover, such age-specific selection dynamics can result in negligible (11) and even negative senescence (12).

To clarify the role of natural selection in shaping both reproductive and general senescence and lifespans, an understanding of not only mortality, but also reproductive parameters and their fitness consequences, across adult ages is required. For instance, although declining fertility in old age indicates reproductive senescence (13), how the resultant age-related
changes in reproductive output translate to lifetime fitness is largely unknown. Indeed, even constant reproductive output with age may not necessarily translate into increasing Darwinian fitness with advancing age; reproduction would actually need to increase if selection against deleterious mutations is to remain strong across the lifespan (28). Intuitively, one would expect that relatively low reproductive output (due to declining fertility) would result in reduced fitness, but such an effect may not hold if the quality of offspring (and hence the likelihood that offspring contribute to future generations) differs as individuals age. That is, the relative number of progeny produced by old versus young females may not necessarily reflect age differences in reproductive fitness if there is age-related variation in offspring quality and survival. Finally, whether a post-reproductive life stage exists in any given species and how such a post-reproductive stage affects an individual’s lifetime reproductive fitness are pressing questions in evolutionary biology (14, 15).

Here we analyze 24 years of individual-based birth, death, and reproductive data gathered for more than 1000 marked individuals since 1988 from a population of painted turtles (*Chrysemys picta*) inhabiting the backwaters of the Mississippi River in the state of Illinois, USA (16). We quantify age-related changes in mortality and reproduction from capture-mark-recapture and reproductive / nesting behavior data of known-age individuals. We assessed hatching success of eggs each September (17, 18), which we use as a measure of fitness. With these unique long-term data we ask: (i) how does reproductive output and reproductive behavior change with advancing age? (ii) how does fitness change with advancing age? and (iii) how does mortality change with advancing age? We then interpret our findings in the context of evolutionary theories for rates of senescence.
Results

Age-specific reproduction. Survival and physical data were collected on 2234 nests constructed by 600 painted turtles with known identities and reproductive ages (Table S1). Although reproductive and nest microhabitat variables generally increased with age, only egg mass and nest vegetation cover exhibited a statistically significant increase (Fig. 1). Importantly, however, our analyses detected a significant interactive effect of age x plastron length on egg mass ($F_{14,891}=6.3, P<0.001$) such that the generally positive relationship between female body size and egg size waned with older ages: the slopes declined and the y-intercepts increased with advancing age (Fig. 2). These findings imply that investment into egg size is similar between relatively small and large late-age females, but large late-age females invest more than smaller females of all other age-classes (sensu (19)). Thus, the relatively large eggs produced by old females resulted in larger hatchlings than those produced by young females, irrespective of maternal body size (see Fig. 1b).

Age-specific fitness. Females that produced large eggs and frequent clutches yielded more successfully-hatched offspring than individuals that produced small eggs and infrequent clutches ($P$-values<0.05, Table 1). Fitness (number of eggs hatched per year) declined in relatively old turtles despite a moderate age-related increase in total egg output (number of eggs produced each year; Fig. 3). Moreover, fitness was influenced by the interactions of total egg output and the number of clutches produced at each reproductive age (Fig. 3). Specifically, fitness declined with age, particularly for individuals with relatively high seasonal egg output (i.e., defined as either total egg output or clutch frequency per season); in animals with overall low egg output, an age-related decline in fitness was not evident. Because depredated nests were excluded from these
analyses, extrinsic mortality of eggs due to nest predation did not drive this age-related variation in hatching success (patterns were generally similar when depredated nests were included in the analyses, see Figures S2 – S4).

**Age-specific mortality.** Mortality analysis that included encounter histories (capture-mark-recapture) for 1031 females from 1993-2012 revealed measurable mortality senescence – i.e., accelerating mortality probability with advancing age (Fig. 4). The Gompertz-function \( u_x = Ae^{bx} \) where \( u_x \) is the age-specific mortality hazard, \( A \) is the initial mortality rate at first nesting - i.e., the first year of the adult stage, and \( b \) is the rate of increasing mortality) had the lowest DIC value among those models considered (see Table 2). Estimated parameters were \( A \) (initial mortality rate / year) = 0.102 (95% credible interval: 0.090 to 0.116) and \( b = 0.050 \) (0.034 to 0.067). This result corresponds to a doubling of mortality every 13.8 years (10.3 to 20.2). Two additional curves are included in Fig. 4 to provide comparison to mammalian-type curves for baboons \( (u_x = 0.028e^{0.123x}, (20)) \) and domestic dogs \( (u_x = 0.02e^{0.23x}, (21)). \)

**Discussion**

Survival senescence has generally been thought to be negligible or absent in various reptiles ((22-26) but see (3)). This is so because traits that either protect against predators (such as external ribcages and venom, reviewed in (27)) or that increase reproductive success with advancing age (such as indeterminate growth and fecundity) may overcome the declining power of natural selection with advancing age that characterizes natural populations (10, 28). In the specific case of *C. picta*, which has both adult-protective morphology and indeterminate growth and fecundity, lifetime fitness would increase if individuals maintain successful and increasing
reproduction through adulthood. Such steady or even increasing reproduction with advancing age is particularly likely for species that exhibit low rates of extrinsic mortality at old ages, indeterminate growth, or a positive relationship between body size and fecundity (e.g., (25)) (i.e., many reptiles, including turtles (26)). Although our aim was to understand whether and how indeterminate growth and fecundity may offset declining late-life selection, our 24-year data set on *C. picta* revealed instead a significant decline in both reproductive success and adult survival for females. Moreover, when we tested among competing models for mortality acceleration, some of which would allow deceleration later in life, the Gompertz model had the most support (Table 2), suggesting increasing mortality acceleration over the entire adult lifespan (Fig. 4).

Our results differ from those for another *C. picta* population, which exhibits both overall low mortality and no differences in survivorship of old- versus middle-aged females (22). However, the survivorship curves of (22) are consistent with gently increasing mortality with advancing age in that population (estimating mortality as \(-\text{Ln}(l_x)\)). Thus, estimating the rate and form of mortality senescence by computing instantaneous mortality rates (e.g., Bronikowski & Flatt 2010) reveals that our study and perhaps a previous long-term study of *C. picta* reveal small but measurable mortality increases with advancing age. Our study population differs from this other population in that ours has experienced a relatively long history of human-driven mortality. The extent to which this human-induced mortality is in any way age-dependent is unknown. Specific human-induced mortality derives primarily from injuries to nesting females (e.g., when crossing roads) and injuries to adults of both sexes in the water column (e.g., by boating activity). Were these to manifest in an age-dependent manner across the lifespan, the age-specific selection gradients could be altered. In contrast, if the increased mortality affects all adult ages equally, we expect acceleration of the entire life history, as the population age-
structure would become skewed towards young ages. A comparison of the high mortality population (this study) and a lower mortality population (22) supports this contention. Specifically, we detected rapid early growth rates (Fig. S5), increased reproductive effort at young ages, early maturation, and reduced lifespan of turtles in our population (29). Thus, these two painted turtle populations fit strikingly well with predictions from life-history theory based on the force and sensitive ages of mortality (28, 30) (Fig. S4). Overall then, these studies support the notion that the life-history strategy is a set of correlated traits that respond predictably to natural selection.

Interestingly, in contrast to reproduction and survival, measures related to body size and behavior did not exhibit senescence (Figs. 1, S5). This result accords with evolutionary senescence theory in that such traits (i.e., growth and learning, respectively) could buffer selective forces that accelerate death and infertility rates (9, 31). At the same time, hatching success and adult mortality – traits that are substantially influenced by physiological processes (26) – exhibited senescence. Long-term research that not only assesses shifts in survival and reproductive parameters with age, but also quantifies age-related changes in physiology and offspring fitness may reveal that senescent patterns in long-lived reptiles are more common than what traditional paradigms suggest (32, 33). In addition, long-term studies that explore trade-offs between early- and late-life performance will provide crucial information for better understanding these declines in reproduction or survival as individuals age. For example, greater resource allocation to early-life growth or reproduction is expected to come at a cost to late-life performance that can lead to age-related declines in reproduction and survival (i.e., disposable soma theory of aging: (34)). These patterns have been observed in many vertebrates (35),
including reptiles (36, 37), and could contribute to the reproductive and survival senescence observed here in *C. picta*.

Determining the universality of reproductive aging has received great recent interest both in terms of documenting its existence and calculating whether reproductive aging occurs at similar (vs. faster) rates than general senescence (13). Analyses have taken the form of either measuring the rate of declining fertility and reproductive success with advancing age (38, 39) or measuring the distribution of age of last successful reproduction and comparing that to the distribution of death (15). Our analysis is similar to the former of these approaches, and we indeed document reproductive decline with advancing age, although not in measures traditionally used (such as rates of conception), but rather by calculating egg hatching success. Similarly, Congdon et al. (23) reported greater embryo mortality due to arrested development in eggs produced by the oldest female age class in Blanding’s turtles. Other long-lived species tend to exhibit reproductive senescence at rates that are faster than somatic aging, and that ends at an earlier age than death, which yields a post-reproductive lifespan (primates including humans: (15), killer whales: (40), but see elephants: (41, 42)). These mammalian species may exhibit reproductive senescence due to a shelf-life of their primary oocytes, but this is unlikely the case in *C. picta* because reptiles are not oocyte-limited (43). One firm conclusion is that reproductive senescence in turtles and other ectothermic amniotes cannot be attributable to adaptive explanations invoked in the mammalian literature (e.g., the “grandmother hypothesis,” which largely rests on the role of parental care to increase one’s own fitness or the fitness of relatives (44)).

A critical question is whether senescent changes in mortality (as in primates) or in reproduction drive declines in fitness. In reptiles, which are often characterized as indeterminate
growers, few studies document either mortality senescence or the decline in some physiological trait with advancing adult age (3). Because many species with indeterminate growth increase fecundity with increasing age (due in part to increasing body size), the question becomes at what level of increased fecundity will selection cease declining with advancing age? Although we demonstrate survival senescence in our population of *C. picta*, the age-specific increase in mortality is considerably less rapid than that reported for mammalian taxa (e.g., Fig. 4), which is consistent with the view that rates of senescence in these long-lived reptiles are relatively slow. These divergent patterns may be explained by very different rates of extrinsic mortality in turtles (or their ancestors) compared to mammals. Indeed, due to their protective morphology (shell) and lack of senescence in immune function (45), turtles likely experience considerably less late-age mortality due to extrinsic factors than do mammals. This comparison between turtles and mammals fits with theoretical expectations for patterns of survival senescence in these taxa, but our results challenge the traditional view that long-lived ectotherms, such as turtles, do not exhibit declining reproductive function with advancing age. Indeed, declines in fitness with age will be exacerbated by a combination of increased mortality rate and decreased reproductive success, as we demonstrate in these long-lived ectotherms.

**Methods**
Since 1988, nesting patterns of painted turtles (*Chrysemys picta*) have been continually monitored at the Thomson Causeway Recreation Area in northwestern Illinois (Carroll County, 41°57’N, 90°7’W) (10-12). Unmarked females exhibiting 5-7 annuli on the pectoral scutes were classified as primiparous (i.e., a reproductive age of one, chronological age 5-7 years old). Thus all future recaptures of these animals were at a known age (19). Individuals that could not be
assigned ages confidently were excluded from analyses of reproduction unless the turtles were recaptured across a 15+ year time span, in which case data collected from captures \( \geq 15 \) years after initial capture were included in the oldest age class (i.e., chronological age \( \geq 20 \) years old). During each nesting season (mid-May to early July), the study area was monitored intensively for nesting turtles. All females at the nesting grounds were individually marked and their eggs were weighed and counted (typically within 4 hours of oviposition). All eggs were returned to their nests and allowed to incubate naturally, and nests were checked for predation almost daily until the end of June of each year. Microhabitat data (shade cover and distance from the water) were also collected for each nest to assess age-related changes in nesting behavior. Nesting behaviors are important because maternal choice of nest microhabitat differs among age classes (46) and can influence egg survival (17). These data are available through Dryad (details in Acknowledgments).

**Age-specific reproduction.** Analyses of age-specific fitness included 600 individuals with known ages captured from 1997-2010. Of these individuals, 65% were observed nesting more than once, and most were first-time nesters (Table S1). Thus, our comparisons across ages were largely based on longitudinally sampled individuals. Linear mixed models were used to evaluate age-specific variation in reproductive variables (i.e., egg mass, clutch size, clutch frequency, total egg output per female per season, hatchling mass) and nest microhabitat variables (i.e., nest shade cover, distance from nest site to nearest water). In cases where females nested 2 or 3 times per season, within-season mean female values were used for egg mass, clutch size, nest shade cover, and nest distance to water. Each dependent variable was standardized to a mean of zero and unit variance within each year prior to analysis. Reproductive age (1-15 years) was defined
as a fixed main effect and plastron length was a covariate. The interaction term of plastron length x reproductive age was removed from models when it was not significant, which occurred in all cases except in the analysis of egg mass. Maternal identity was included as a random effect to account for repeated measurements on females across ages. Linear and second-order polynomial regressions were employed to detect age-specific trends in reproductive and nest microhabitat variables.

**Age-specific fitness.** We used generalized linear mixed models to test for linear selection on each reproductive variable and interactions with reproductive age using egg hatching success per female as a measure of fitness (standardized within season, i.e., $\omega_{\text{relative}} = \omega / \omega_{\text{mean}}$ (28)). This measure of fitness (number of successfully-hatched eggs per clutch) strongly correlates with the likelihood of offspring recruiting into future adult age classes (10), and is therefore a meaningful proxy for maternal reproductive success (i.e., fitness). Independent variables (standardized to a mean of zero with unit variance) included plastron length, egg mass, clutch size, clutch frequency, nest shade cover, distance from the nest site to nearest water, and total egg output per female per season, as well as all 2-way interactions with reproductive age. Three-way and higher-order interactions were not included in the model because they were not relevant to our specific questions, and models with higher-level interactions often would not converge, as degrees of freedom were reduced by including these factors. Non-significant interaction terms were sequentially removed from the initial model, and final models were selected based on the lowest AIC score. Again, maternal identity was included as a random effect to account for repeated measurements on females across ages. Parameter estimates for fixed effects were
calculated using restricted maximum likelihood and statistical significance was determined with 
\( F \)-tests.

The selection analyses described above were performed twice. First, to assess non-
predation-related variation in fitness, all depredated nests were excluded from our analyses.
Second, to assess how nest predation impacts the relationship between the reproductive
parameters and fitness (i.e., selection), all depredated nests (depredated nests had fitness=0) were
included in the second set of analyses. Standardizations of all traits (including fitness) were re-
calculated accordingly for the subsets of nests used in each analysis. Results from analyses that
included versus excluded depredated nests were compared qualitatively. Because the potential
fate of eggs in depredated nests (had they been left intact) is unknown, this qualitative
comparison assumes that nest predation is random with respect to the variables measured. A
generalized linear mixed model using reproductive traits (egg mass, clutch size, clutch number)
and nest microhabitat (vegetation cover, nest distance to water) as independent variables and
maternal identity as a random effect showed that this assumption was met (see Table S2).

Additional analyses were performed to confirm and visualize significant effects.
Regression analyses (weighted by sample size due to repeated measures per female) were used to
assess the relationship between fitness and reproductive and nest variables. Second-order
polynomial regressions were used to assess age-specific trends in fitness. Lastly, to visualize
two-way interactions between age and reproductive output on fitness, selection surfaces were
calculated with a first-degree polynomial locally-weighted scatterplot smoothing technique
(LOESS; for total egg output) and a negative exponential smoothing technique (for number of
clutches produced) in Sigma Plot.
**Age-specific mortality.** Analyses of age-specific mortality included 1031 individuals. These individuals included the 600 used in the analyses above, but also included turtles with unknown ages that were captured from 1993-2012. To test for a signature of demographic senescence in mortality, accelerating mortality models were fit to mark-recapture data for both known age and unknown age females using a Bayesian hierarchical analysis (30, 31). DIC was used to choose among alternative models for the survival function fit using the BaSTA package (30) (ver. 1.3) in R (ver. 2.14; R development Core Team 2011) (Table 2). Four survival functions were considered: exponential, Gompertz, logistic, and Weibull functions (31, 32). In the exponential model, survival is constant across ages, while the other three models all allow for decreasing survival as individuals become older. Thus, the alternative models permitted assessment of whether there was evidence for demographic senescence and, if so, what form it took. In addition, models that included an age-independent constant to the survival function (Makeham coefficient) were considered for the Gompertz, logistic, and Weibull models. The approach does not allow for temporal or cohort effects to be incorporated. However, previous work detected no evidence for an overall temporal trend in survival across the study period (50) or for cohort effects on survival related to developmental conditions in the population (37). Finally, alternatives that differed in whether detection was constant or variable among all years were examined.

**Acknowledgments.** We are extremely grateful to the many volunteers, students, and postdocs that have been involved with fieldwork at Turtle Camp over the past decades. We greatly appreciate ongoing support from the U. S. Fish and Wildlife Service and the U. S. Army Corp of Engineers. Thanks to members of the Janzen Lab and T. Schwartz for helpful comments on
earlier drafts of this paper, and J. Sherwood for assistance with ArcGIS software. This research has been approved by the Iowa State University Institutional Animal Care and Use Committee and the Illinois Department of Natural Resources. Reproductive data and encounter histories are available through Dryad: (http://dx.doi.org/accession pending). Funding for this research was provided by the National Science Foundation (grants DEB-9629529, DEB-0089680, DEB-0640932 to FJJ). Additional support was provided by the National Institutes of Health (grant RO1AG049416).

References


Figure legends

**Fig. 1.** Age-specific variation in reproductive and nesting parameters in adult painted turtles (*Chrysemys picta*). Graphs show age differences in *(a)* egg mass, *(b)* hatchling mass, *(c)* clutch size, *(d)* clutch frequency, *(e)* total egg output, *(f)* vegetation cover over nest, and *(g)* distance of nest to water. Dependent variables are standardized to a mean of zero and unit variance. Statistical values are results from quadratic regressions. Data represent least-square means (and 1 standard error) calculated from mixed linear models with plastron length as a covariate and maternal identity as a random effect. Reproductive ages 1 to 14 reflect actual ages of 5-7 to 19-21 years old, and reproductive age 15 includes individuals that are ≥ 20 years old.

**Fig. 2.** Age-specific relationships between plastron length and egg mass. Graphs show the age-specific *(a)* slopes and *(b)* y-intercepts from regression analyses of plastron length versus egg mass *(c)*. The negative relationship *(r²=0.53, P=0.002)* between reproductive age and slope coupled with the positive relationship *(r²=0.75, P<0.001)* between age and y-intercept illustrate that late-age females invest less per egg than do young females at relatively large body sizes. At small body sizes, old individuals invest more per egg than do young individuals. Panel *(c)* illustrates the relationships between plastron length and egg mass for young (ages 1-3) and old (ages 13-15) females. The range of body sizes for old individuals was relatively narrow, but young individuals exhibited a large range of sizes that encompassed the size range of old females. Regression lines for all ages are in Figure S1.
Fig. 3. Age-specific variation in relative fitness for adult painted turtles (*Chrysemys picta*).

Reproductive ages 1 to 14 reflect actual ages of 5-7 to 19-21 years old, and reproductive age 15 includes individuals that are $\geq$ 20 years old. *(a)* Change in relative fitness (egg hatching success) with reproductive age (least-square means $\pm$ 1 standard error). *(b)* Interaction between total egg output and reproductive age. Reproductive output is represented as the total number of eggs produced (per female) within a season. *(c)* Interaction between total number of clutches produced per season and age. Statistical results are in Table 1. These graphs are based on analyses that excluded depredated nests.

Fig. 4. Gompertz model fit for age-specific mortality for painted turtles and comparison species (data for baboons (20) and dogs (21); See text for parameter estimates). For turtles, although initial mortality was relatively high, mortality rates increased at a much slower rate than seen in many mammals.
Table 1. Results from two final models of selection on age-specific reproductive traits in painted turtles (*Chrysemys picta*). The first model excluded depredated nests, and thus fitness (dependent variable) represented variation in relative egg hatching success due solely to factors other than nest predation. The second model included depredated nests (relative fitness=zero for depredated nests). Both models included maternal identity as a random effect. Relationships are illustrated in Fig. 3 and Fig. S2-S4.

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<td>Plastron length</td>
<td>0.0694 (0.07)</td>
<td>1, 763</td>
<td>1.1</td>
<td>0.293</td>
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<tr>
<td>Number of clutches produced</td>
<td>0.0500 (0.13)</td>
<td>1, 763</td>
<td>0.2</td>
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<td>Egg mass</td>
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<td>1, 763</td>
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<td>Clutch size</td>
<td>-0.1237 (0.08)</td>
<td>1, 763</td>
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<td>Total egg output per season</td>
<td>0.4019 (0.14)</td>
<td>1, 763</td>
<td>8.1</td>
<td>0.005</td>
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<tr>
<td>Nest vegetation cover</td>
<td>0.1580 (0.05)</td>
<td>1, 763</td>
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<td>Nest distance to water</td>
<td>-0.0097 (0.05)</td>
<td>1, 763</td>
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Table 2. Hierarchical capture-mark recapture models fitted in a Bayesian framework were used to select among alternative survival models to describe mortality patterns for turtles. Functions included those where senescence occurs (gompertz, logistic, and weibull) and the exponential where no senescence occurs. In addition, support for a Makeham parameter and for temporal variation in detection among years was examined. The model with the smallest DIC value had the greatest support.

<table>
<thead>
<tr>
<th>Model</th>
<th>Makeham?</th>
<th>temporal?</th>
<th>DIC</th>
<th>ΔDIC</th>
<th>pD</th>
<th>k</th>
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<td>8198.8</td>
<td>833.7</td>
<td>292.8</td>
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<td>100.9</td>
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<td>gompertz no yes</td>
<td>7365.1</td>
<td>0.0</td>
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<td>70.5</td>
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<td>Weibull no no</td>
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<td>*</td>
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<td></td>
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<tr>
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<td>*</td>
<td>*</td>
<td>*</td>
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</tr>
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</table>

* model did not converge
Fig. 1
Fig. 3
Fig. 4
Supplementary Materials:

**Fig. S1.** The relationship between plastron length and egg mass for females of all ages. As age increases, the range of body sizes for moms narrows, but young individuals exhibited a large range of sizes that encompassed the size range of old females.
Fig. S2. Bubble plots for relative fitness versus (a) egg mass ($r^2=0.02$, $P=0.019$) and (b) total egg output ($r^2=0.16$, $P<0.001$) for analyses that excluded depredated nests, and (c) nest vegetation cover ($r^2=0.01$, $P=0.010$) and (d) total egg output ($r^2=0.05$, $P<0.001$) for analyses that included depredated nests. For graphs c and d, six outliers with fitness > 7 were removed for visualization, but were retained in the statistical analyses. Regression analyses were weighted by the sample size for each female, which reflect the relative size of the bubble (e.g., large bubbles contain multiple data points from single females). Additional statistical results for these relationships are in Table 1.
Fig. S3. Age-specific variation in relative fitness for analyses that (a) excluded and (b) included depredated nests. Data represent least-square means (and 1 standard error) calculated from mixed linear models and maternal identity as a random effect. Reproductive ages 1 to 14 reflect actual ages of 5-7 to 19-21 years old, and reproductive age 15 includes individuals that are ≥ 20 years old.
Fig. S4. Selection surfaces for age and reproductive output for adult female painted turtles (*Chrysemys picta*). The top graphs (a, b) illustrate results from analyses that excluded depredated nests, and the bottom graphs (c, d) illustrate results from analyses that included depredated nests. Reproductive output is represented as the total number of eggs produced (per female) within a season (graphs a, c), and number of clutches produced per season (graphs b, d). Statistical results are in Table 1.
Fig. S5. Relationship between reproductive age and plastron length (quadratic regression: $r^2=0.82$, $P<0.001$). For meaningful comparison with other studies, we also quantified this relationship up to reproductive age eight using linear regression (dotted line). Linear regression equation: Plastron length = 1.24*(age) + 149; $r^2=0.97$, $P<0.001$. The slope of the relationship between adult age and plastron length is substantially higher in our study population than that reported in Michigan populations (Slope = 1.2 at our site versus 0.5, 0.55, 0.75 at other sites (47-49), suggesting relatively fast growth rates in our population. The same pattern is evident when plastron length is standardized to a mean of zero and unit variance within years.
Table S1. Age-specific sample sizes of females and nests used in our statistical analyses, and percentage of depredated nests. Data reported for the number females represent the total number of unique nesting females at each age, and the total in the bottom row represents the number of unique females used in this study, rather than the sum total down each column.

<table>
<thead>
<tr>
<th>Reproductive age</th>
<th>Excluding depredated nests</th>
<th>Including depredated nests</th>
<th>% nests predated</th>
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<td></td>
<td>Females</td>
<td>Nests</td>
<td>Females</td>
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<tr>
<td>1</td>
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<td>276</td>
<td>590</td>
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<tr>
<td>2</td>
<td>105</td>
<td>151</td>
<td>231</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>87</td>
<td>168</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>75</td>
<td>166</td>
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<td>5</td>
<td>35</td>
<td>51</td>
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</tr>
<tr>
<td>9</td>
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<td>10</td>
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</tr>
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<td>15</td>
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</tr>
<tr>
<td>Total unique</td>
<td>355</td>
<td>845</td>
<td>600</td>
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Table S2. Effect of reproductive and nest variables on nest predation. Analyses were performed with a generalized linear mixed model including maternal identity as a random variable.

<table>
<thead>
<tr>
<th>Trait</th>
<th>ndf</th>
<th>ddf</th>
<th>F</th>
<th>P-value</th>
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<td>Egg mass</td>
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<td>1876</td>
<td>0.01</td>
<td>0.925</td>
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<td>1</td>
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<td>Nest distance to water</td>
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