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Differential evolution of asexual and sexual females in a benign culture environment

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Abstract

Here we report one of the first investigations of evolvability of lifespan and reproduction in metazoans, examining both extrinsic and intrinsic factors. We tested effects on senescence of an environmental variable (simulated lake hydroperiod, the length of time an aquatic habitat is inundated), female reproductive physiology (asexual females that reproduce by ameiosis, versus sexual females reproducing by meiosis), and time in a benign culture environment (minimal, if any, external mortality factors). To do this we established chemostat cultures of the rotifer *Brachionus plicatilis* s.s., and maintained the cultures for 385 d. Hydroperiod alone or in interaction with the effects of time in the benign environment (season) or reproductive physiology had no significant effect on the net reproductive rate, generation time, or rate of aging. Yet combining animals from both ephemeral and permanent hydroperiods revealed a 26% increase in asexual female lifespan across seasons (23% decrease in the rate of aging) and a 56% increase in asexual fecundity, suggesting that maintenance in benign laboratory conditions leads to slower aging. The relative stasis of traits for sexual females implies an impact of reproductive physiology on evolvability. In addition we found a positive correlation between fecundity and lifespan, suggesting an absence of trade-offs in life history traits in the benign laboratory environment.

Keywords

Fecundity; experimental evolution; hydroperiod; life history trade-offs; lifespan; rotifer; temporary waters

Introduction

In aquatic habitats, external mortality factors may alter organisms' life history through direct influences on their physiology, or over time may lead to evolutionary changes. In environments with a short hydroperiod (short duration of the wet phase), organisms that rely on the presence of water for completion of part or the entirety of their life cycle exhibit a range of adaptations to their ephemeral habitat. Many taxa show rapid progression through the life cycle (Schröder *et al.*, 2007; Suhling *et al.*, 2005; Valdesalici & Cellerino, 2003;

Wissinger *et al.*, 2003). Accelerated maturation may hasten attainment of a developmental stage that can persist during a dry spell, or is adapted to terrestrial life (Wellborn *et al.*, 1996).

In addition to the risk of drying, hydroperiod gradients are associated with other stressors. Environmental variables such as conductivity (salinity) and temperature may fluctuate in association with hydroperiod, impacting and at times imposing costs on fitness and life history traits (Campillo *et al.*, 2011; Viayeh *et al.*, 2010; Waterkeyn *et al.*, 2008). Short hydroperiods and high salinities also are associated with reduced species richness (Waterkeyn *et al.*, 2008; Wellborn *et al.*, 1996). On the other hand, the inability of many large-bodied predators to persist in temporary waters can lead to lower predation pressure as habitat permanence decreases (Wellborn *et al.*, 1996). Taxa in habitats with low risk of external mortality factors such as predation (e.g., zoos, laboratories, and other captive populations) often exhibit increased lifespan or a decrease in the rate of aging (Monaghan *et al.*, 2008; Ricklefs, 2008; Ricklefs & Scheuerlein, 2001; Williams, 1957).

Another layer of complexity added to the environmental milieu of habitat permanence and extrinsic mortality factors consists of physiological trade-offs and intrinsic constraints on life history evolution. For instance, it is often hypothesized that reproduction imposes a cost to longevity (Kirkwood & Rose, 1991). Further evidence of physiological effects on lifespan is seen in gender differences. In many species males and females differ in their lifespan, and a recent report of seed beetles suggests gender may affect evolution of longevity (Fox *et al.*, 2011). Complicating matters is the fact that trade-offs may be observed in studies of individuals' current physiology, but not in the evolutionary trajectories of traits in populations (Flatt, 2011). Here we report on an experimental evolution approach (Colegrave & Collins, 2008) to directly test the evolutionary response of lifespan and fecundity to hydroperiod in an environment with little (if any) extrinsic mortality factors, and discuss results in terms of the potential for evolution, i.e., evolvability as described by (Pigliucci, 2008).

This report documents the evolution and evolvability of senescence and fecundity in rotifers (Fig. 1), basal metazoans with a long history of use in aging studies (Enesco, 1993; Snell & Stelzer, 2005; Wallace & Snell, 2010). Rapid turnover (typical lifespans up to a few weeks) (Wallace & Smith, 2009; Wallace & Snell, 2010) allows studies of evolutionary dynamics for these zooplankton in a tractable timescale. To assess factors involved in evolution of lifespan and reproduction we established six flow-through cultures (chemostats) of *Brachionus plicatilis* s.s. rotifers. Brachionids are cyclical parthenogens that reproduce as asexual (amictic) females until induced to produce sexual (mictic) daughters (Wallace & Smith, 2009; Wallace & Snell, 2010; Wallace *et al.*, 2006). Asexual females only undergo ameiotic parthenogenesis and only produce daughters. Yet sexual females only undergo meiosis and either produce haploid males, or if fertilized at a young age, produce diploid diapausing embryos (and no males). Asexual and sexual females are diploid and can share the same genotype, because one asexual female can undergo parthenogenesis to produce both sexual and asexual daughters (Fig. 1). We tested effects on longevity and fecundity of (1) hydroperiod; (2) time in a benign culture environment (laboratory setting with minimal,

if any, extrinsic mortality factors); and (3) female reproductive physiology (identity as a sexually- or asexually-reproducing female).

Methods

Cultures and hydroperiod treatments

Chemostat culture treatment and maintenance is described in (Smith & Snell, 2012), which investigated evolution of sex and dormancy in response to the hydroperiod treatment. In brief, *Brachionus plicatilis* s.s. Müller, 1786 monogonont rotifers were isolated from water samples from the brackish pond Poza Sur, Spain and maintained as separate, asexually propagated lineages (Carmona *et al.*, 2009). Subcultures of 15 of these lineages provided by colleagues Dimas-Flores and García-Roger were used to initiate six replicate chemostat populations, each inoculated with 10 asexual clonemates per lineage (N=150 animals per chemostat). Cultures were maintained for 385 d in Celllift™ bioreactors (Ventrex), each containing 570 mL medium: 1 million cells mL⁻¹ of the alga *Tetraselmis suecica* in 15 ppt artificial seawater (ASW, Instant Ocean® sea salts). Flow rate was 150 mL d⁻¹, and chambers were kept at 22°C with continuous lighting. Location of chambers was randomized, and re-randomized every 9 weeks.

The six chemostats were randomly assigned to ephemeral or permanent hydroperiod treatments. The 385 d experiment was divided into six growth seasons; at the end of each 9-week growth season ephemeral chambers were reset to mimic desiccation-refilling of ephemeral pools. For a reset, the chamber's contents were emptied, and it was re-inoculated with hatchlings of diapausing embryos (~800) produced in that chemostat in the season immediately prior to the reset. In ephemeral cultures population growth occurred asexually during the season, but induction of sex and production of diapausing embryos during the season was required to survive each reset (N=5). Concomitant to ephemeral resets, every 9th week permanent cultures were cleaned to remove biofilm on the chamber walls and settled debris (including diapausing embryos, which sink and remain dormant until hatching is induced). Cleaned permanent chambers were refilled with their active populations and old medium, minus debris and diapausing embryos. Thus permanent cultures propagated asexually; sexual reproduction did occur, but did not contribute to population growth due to our removal of diapausing embryos (the product of sex). We cannot rule out the potential for a minimal level of hatching prior to removal of diapausing embryos, but typical requirements for diapause prior to hatching (Wallace & Snell, 2010) preclude the opportunity for substantial frequencies of sexual embryo hatchlings in permanent cultures.

Bioassays and Analysis

In the latter half of the 1st and the final two (5th and 6th) growth seasons, we conducted life history bioassays to assess females' total lifespan and fecundity. Bioassays are described in (Smith & Snell, 2012), but here we performed more extensive analyses of lifespan and reproduction, including factors independent of hydroperiod. In brief, asexual ovigerous females (P generation) were isolated from chemostat outflow and placed individually in 1 mL medium (5×10⁵ cells mL⁻¹ *T. suecica* in 15 ppt ASW) in the well of a 24 well plate. To establish a cohort of the F1 generation, the next day one neonate female progeny from each

P generation female was transferred individually to a second plate in 1 mL medium; the mother (P generation) was discarded. Every day, cohort F1 females were transferred to fresh medium and their progeny (F2) were counted and discarded. For asexual females, combining ephemeral chemostats we followed 59, 66, and 71 F1 females in the 1st, 5th, and 6th season, respectively (16 N 24 per chemostat per season); for permanent cultures we monitored 53, 71, and 70 females in the 1st, 5th, and 6th season, respectively (17 N 24 per chemostat per season). For unfertilized sexual females, combining ephemeral chemostats we followed 31, 42, and 65 F1 females in the 1st, 5th, and 6th season, respectively (8 N 24 per chemostat per season); for permanent cultures we assayed 41, 29, and 22 females in the 1st, 5th, and 6th season, respectively (3 N 22 per chemostat per season). Inability to distinguish cohort females as asexual (yielding female progeny) or sexual (with male progeny) until they reproduced led to use of unequal numbers of asexual and sexual F1 females. During bioassays, location of test females from the six chemostats was randomized within and among 24 well plates.

Statistical analyses were performed in IBM SPSS v.18 at $\alpha=0.05$. We performed repeated measures linear mixed models analyzed with restricted maximum likelihood (REML) to test for significant factors influencing fecundity and lifespan. Square root transformations of lifespan and fecundity were made to improve normality. Model selection was performed with Bayesian Information Criteria (BIC). Comparison of linear models of total fecundity and lifespan with a square root transformation, to models of non-transformed data with linear, gamma, Poisson with log-link, or negative binomial distribution with log-link, showed a better fit of the square root transformed linear model as seen via a lower BIC by >2300. We compared models with or without a random intercept and found a lower BIC in absence of the random intercept. Final selected (reported) models consisted of square root transformed data and a Scaled Identity covariance structure, with chemostat as the subject ID and a random effect from the repeated measure of season (time). The intercept, hydroperiod treatment, season, and mictic (Table 1) were input as fixed effects with season as a covariate, including all two- and three-way interactions between hydroperiod, season, and mictic status (Table 1). For lifespan, in addition to total lifespan we tested effects on (square root transformed) pre-reproductive, reproductive, and post-reproductive lifespan. The reproductive phase was distinguished by presence of live progeny in the medium (not merely unhatched eggs). Lifespan-fecundity relationships were assessed with Pearson correlation analysis on the square root transformed data.

A multivariate general linear model (GLM) was used to test effects of hydroperiod, mictic status, and the covariate season, as well as interactions among the terms, on population summary statistics. These comprised cohort generation time, T ; net fecundity, Ro ; and the rate of aging, ω . Generation time and net fecundity were computed with PopTools (<http://www.poptools.org>). The rate of aging was calculated using the Weibull aging function as $\omega = a^{1/(1+\beta)}$ (Ricklefs, 2008); this is an alternative to the Gompertz model and mortality rate doubling time, and is less sensitive to initial mortality rates (Ricklefs & Scheuerlein, 2002). To compute ω , we first used SPSS for nonlinear regression to estimate the values of a and β

from the formula $l_x = \exp(-m_o x - \frac{ax^{\beta+1}}{\beta+1})$ as in (Ricklefs, 1998), where l_x represents the

proportion of females surviving to age x , and m_0 is the initial mortality rate. Initial values were input as $m_0 = 0$, $\alpha = 0.0001$, and $\beta = 3$; see (Ricklefs, 1998) for comparison of parameters in mammals and birds.

Results

Cohort life history assays in the 1st and final two growth seasons (5th, 6th) revealed changes in lifespan over time, with significant contributions from the main effect of season and the interaction of season with reproductive physiology (mictic status, i.e., identity as an asexual female producing daughters or a sexual female producing males) (Table 1, Fig. 2). Mean asexual lifespan in days (± 1 SE) increased 26% over six seasons: season 1, 8.4 (± 0.3); season 5, 11.0 (± 0.2); season 6, 10.5 (± 0.3). However for sexual females, mean lifespan in days (± 1 SE) changed little: season 1, 8.1 (± 0.3); season 5, 8.5 (± 0.4); season 6, 8.4 (± 0.3). Hydroperiod only had a significant impact on the pre-reproductive period, and there only in interaction (hydroperiod*mictic status, $P=0.034$; hydroperiod*mictic status*season, $P=0.040$). For asexual females, there was a decrease from the first to final season in the pre-reproductive lifespan in both hydroperiods from a mean in days (± 1 SE) of 1.64 (± 0.14) to 0.94 (± 0.03) in the ephemeral hydroperiod, and from 1.57 (± 0.15) to 1.00 (± 0.00) in the permanent hydroperiod. For sexual females there was a much smaller change, but with a slight increase in the ephemeral hydroperiod from 0.94 (± 0.04) to 1.00 (± 0.02), and a slight decrease in the permanent hydroperiod from 1.10 (± 0.05) to 1.00 (± 0.05). Most notable changes were the decrease in pre-reproductive lifespan, and increase in total, reproductive, and post-reproductive lifespan of asexual females (Fig. 3). The greater change in asexual versus sexual females, and impact of seasons (time in the benign culture environment), are seen in the significant effects of the mictic status*season interaction on total lifespan and each lifespan phase ($P<0.001$, each).

Similarly, mictic status ($P=0.019$), season ($P<0.001$), and their interaction ($P<0.001$) had significant impacts on total lifetime fecundity. Asexual fecundity increased by 56% across seasons, from a mean (± 1 SE) of 11.8 progeny (± 0.5) in the 1st season to 18.4 (± 0.3) in the 6th season. Sexual females showed little change in numbers of offspring, from a mean (± 1 SE) of 12.7 (± 0.6) in the 1st season, to a mean of 13.0 (± 0.5) in the 6th season. Hydroperiod did not influence fecundity, either as a main effect or in interactions, with the term coming closest to a significant contribution being the interaction of hydroperiod*season. Averaged across asexual and sexual females, in ephemeral cultures mean lifetime fecundity (± 1 SE) increased from 11.6 (± 0.5) in the 1st season to 16.4 (± 0.4 SE) in the 6th season, and for permanent cultures mean fecundity increased from 12.6 (± 0.6) in the 1st season to 16.0 (± 0.5) in the 6th season.

We found a positive correlation between total lifespan and fecundity, pooling (square-root transformed) data for seasons 1, 5, and 6 (Fig. 4). Positive correlations were seen for data from asexual and sexual females combined (Pearson's $r=0.493$, $P<0.001$, $df=618$), and remained when a partial correlation was run controlling for the effects of season (Pearson's partial $r=0.456$, $P<0.001$, $df=617$). The positive relationship also was seen for asexual females without (Pearson's $r=0.535$, $P<0.001$, $df=388$) or with controlling for the impact of season (Pearson's partial $r=0.448$, $P<0.001$, $df=387$). Although the correlation coefficient

was slightly smaller, positive correlations were found for sexual females as well, without (Pearson's $r=0.328$, $P<0.001$, $df=228$) or with controlling for the impact of season (Pearson's partial $r=0.326$, $P<0.001$, $df=227$).

At the population level, a multivariate GLM of generation time (T), net reproductive rate (R_0), and the rate of aging (ω) revealed no effect of hydroperiod as a main effect or in interactions. However, the main effects of mictic status ($P<0.001$) and season ($P<0.001$) as well as their interaction ($P=0.014$) all had a significant value for Wilk's λ in multivariate tests of these terms. These were followed by between-subject tests of each response term. Both asexual and sexual females showed (slight) decreases in T , with a longer generation time in each season for asexual females but no difference in the trajectories across seasons of either female type (Fig. 5). However, season ($P=0.001$) and the season*mictic status interaction ($P=0.014$) significantly impacted both R_0 and ω . For asexual females, R_0 increased and ω decreased across seasons, while these traits remained relatively unchanged for sexual females (Fig. 6, Table 2).

Discussion

Our study represents one of the first to experimentally investigate extrinsic environmental factors affecting evolution of the metazoan lifespan along with intrinsic evolvability determinants. Hydroperiod (aquatic habitat permanence) had little effect on lifespan, although there was a significant (if slight) impact of the hydroperiod*season*mictic status interaction on the pre-reproductive lifespan. For asexual females, there was a decrease from the first to final season in the pre-reproductive lifespan in both hydroperiods, whereas pre-reproductive lifespan for sexual females showed a slight increase in the ephemeral hydroperiod, and a slight decrease in the permanent culture. The change in sexual females seems contrary to the expectation of shorter lifespans in ephemeral compared to permanent systems, but the change was so small to warrant further testing to verify its significance (Suppl. Table 1). However, time in a benign culture environment and reproductive physiology clearly were associated with rapid evolution of life history traits. We observed a 26% increase in lifespan and a 56% increase in fecundity by asexual female rotifers over the 385 d (84 generations) in laboratory culture. Absence of a substantial change in sexual females' lifespan illustrates a role of female reproductive status on evolvability (broadly defined as the potential for evolutionary change, but for further description see (Pigliucci, 2008)). In the paragraphs that follow we discuss the relation of hydroperiod, benign culture environments, and reproductive status to life history evolution. We also address the positive correlation between lifespan and fecundity.

Inhabitants of temporary waters such as zooplankton and juvenile phases of aquatic insects often show faster development rates than their permanent water counterparts (De Block *et al.*, 2008; Dudycha & Tessier, 1999; Jocque *et al.*, 2010; Wissinger *et al.*, 2003). This acceleration may hasten the transition to a stress-tolerant dormant stage, metamorphosis from an aquatic juvenile to a terrestrial adult, and/or the time of first reproduction (De Block *et al.*, 2008; Vanschoenwinkel *et al.*, 2010; Waterkeyn *et al.*, 2008; Wellborn *et al.*, 1996). Potentially the long (9 weeks) hydroperiod relative to the rotifer lifespan (~1–3 weeks) did not impose strong enough selection to cause more pronounced evolution of faster, shorter

life histories in the chemostats. *Hexarthra* sp. rotifers in the Chihuahuan Desert show faster development rates than most rotifer species, but the extremely ephemeral rock pools they inhabit may fill and dry within days (Schröder *et al.*, 2007). Although the importance of the relative scale of hydroperiod duration to the inhabitants' lifespan may appear obvious, hydroperiod classification schemes often are based on abiotic features such as length of inundation, e.g., (Brock *et al.*, 2003; Serrano & Fahd, 2005). Scaling duration of inundation or drying frequency to the study organisms' lifespan may provide a more meaningful classification scheme for hydroperiod, emphasizing time periods more or less likely to impose selection pressure.

Nevertheless, the current study reveals that rotifers' lifespan and fecundity evolved in the benign laboratory setting. The chemostat cultures were devoid of many of the extrinsic mortality factors that would occur in natural environments, such as abiotic (e.g., salinity and temperature fluctuations) or biotic stressors (e.g., predation, starvation) (Kuefler *et al.*, 2012; Miracle & Serra, 1989; Schneider & Frost, 1996; Snell, 1986; Wallace & Snell, 2010; Wallace *et al.*, 2006). It has been hypothesized that removal of extrinsic, environmental mortality factors may lead to evolution of longer lifespan (Monaghan *et al.*, 2008; Williams, 1957; Williams *et al.*, 2006). This suggestion, sometimes referred to as Williams' hypothesis (Williams *et al.*, 2006), may explain the increased lifespan that observed in the rotifer cultures. Not only did total lifespan increase, but also the reproductive lifespan and post-reproductive lifespan increased, with an earlier onset of reproduction and increase in fecundity of asexual females. These shifts may suggest an overall increase in fitness.

The concomitant increase in reproduction and lifespan and their positive correlation do not demonstrate the typically negative relationship proposed by two prominent theories of the evolution of aging: the antagonistic pleiotropy and disposable soma (Hughes & Reynolds, 2005; Monaghan *et al.*, 2008). The antagonistic pleiotropy hypothesis focuses on trade-offs in genes affecting longevity and fecundity, while the disposable soma hypothesizes trade-offs in resource allocation to somatic maintenance versus reproduction (Kirkwood & Melov, 2011; Monaghan *et al.*, 2008). To date evidence of trade-offs in reproduction and lifespan is mixed, and can be complex (Anderson *et al.*, 2011; Flatt, 2011; Reznick *et al.*, 2004). In nature resources (e.g., food) could be limiting and lead to trade-offs not seen in captive populations (Ricklefs & Cadena, 2007). If the disposable soma theory applies to our system, one may expect that limiting food or other resources in the chemostats (or wild populations) would result in the expected trade-off of lifespan and fecundity. Indeed, a recent study reported that calorically restricted *B. plicatilis* live longer but have fewer progeny (Kaneko *et al.*, 2011). Yet another study applying chronic caloric restriction to *B. plicatilis* did not find decreased fecundity, and suggests absence of a trade-off in reproduction with somatic maintenance (Gribble & Mark Welch, in press). Yet studies of animals in their natural habitats under exposure to typical environmental stressors may be necessary for some trade-offs to be detected (Van Voorhies *et al.*, 2006).

Beyond suggesting that trade-offs in reproduction and longevity may not always occur (at least in benign environments), our study shows the potential for intrinsic factors influencing evolutionary capacity, with physiological status as an asexual or sexual female substantially influencing the evolvability of *Brachionus plicatilis* s.s. The impact of female reproductive

status on evolution of lifespan and fecundity was related to the physiology of these female types (ability to reproduce asexually or sexually), not to any effects of more or less sexual reproduction in prior generations. The contribution of sex itself to evolvability appeared inconsequential, because there was no difference between ephemeral cultures (which underwent 5 bouts of sex between growth seasons) and permanent cultures (where sex occurred but sexually produced diapausing embryos were removed from the culture before hatching; only asexual reproduction was allowed to contribute to active population growth). The difference between the rotifer female types is that asexual females only produce daughters via ameiotic parthenogenesis, whereas sexual females only produce males via meiosis. (Females were not mated in bioassays; sexual females mated at a young age still would have undergone meiosis, but produced diapausing embryos following fertilization). Initially, mean lifespans for asexual and sexual females were similar, but asexual females increased lifespan and fecundity, whereas these traits changed little for sexual females.

The differential evolution of asexual and sexual females likely do not reflect genotypic differences because a single asexual female can give birth to both asexual and sexual daughters (Fussmann *et al.*, 2007; Wallace *et al.*, 2006), resulting in identical genotypes (Fig. 1). What remains to distinguish asexual and sexual females' reproductive phenotypes and in turn their different evolvability may be epigenetic factors, but further work is needed to examine molecular differences between female types. Asexual females in both of the final two growth seasons lived longer than females in season 1 although culture (chemostat and bioassay) conditions were not changed between seasons. This suggests the increased longevity represents an evolutionary change, as opposed to phenotypic plasticity in varying conditions. The evolutionary mechanism may represent clonal selection from genetic variation present in the founding population. Yet the maximum asexual female lifespan in the 6th growth season was 24% greater than the maximum seen in the initial season (17 d initially, versus 20 d and 21 d in seasons 5 and 6, respectively), suggesting the potential that other factors (e.g., new mutations, ameiotic recombination) may have contributed to the evolutionary changes. Repetition of the experiments reported here, but starting all chemostat populations a single clonal female, is recommended to test the requirement of standing genetic variation for life history evolution. Yet regardless of the evolutionary mechanism, our study complements prior works by showing that beyond gender differences, features underpinning the differences between animals that reproduce via ameiosis or meiosis may influence their life history evolution.

Furthermore, the observed increase in rotifers' post-reproductive lifespan is intriguing and worthy of further study. Post-reproductive individuals should be immune to selection pressures given that they do not contribute to future generations (Kirkwood & Melov, 2011). This may represent an indirect effect, such as decreased extrinsic mortality factors and non-limiting resources (continuous algal food inflow) allowing for longer post-reproductive life. The benign chemostat environment could ameliorate resource trade-offs allowing for high investment in both somatic maintenance and fecundity. Ongoing next-generation sequencing approaches in our laboratory are being used to investigate changes in gene expression associated with the evolution of rotifer aging, and may help elucidate molecular controls of this evolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Brachionus plicatilis pedigree from two parental (P) asexual females through three progeny generations (F1–F3). Gray shading encompasses females with the same genotype (i.e., a P1 female and her asexually produced progeny). A single locus with two alleles is represented in the homozygous (AA or aa) and heterozygous (Aa) state to aid tracking of genotypes. Females are diploid; males are small and haploid. A diapausing embryo is produced when a male fertilizes a young (neonate) sexual female, and will hatch after obligate dormancy to yield an asexual female. Unfertilized sexual females produce males. Stars depict where meiosis occurs to produce progeny; all other reproduction is ameiotic.

Figure 2.

Survivorship curves of asexual (A) and unfertilized sexual (B) females. Curves depict the proportion (l_x) of females surviving to age x for the first and final two 9 week growth seasons.

Figure 3.

Pre-reproductive, reproductive, and post-reproductive lifespan of (A) asexual and (B) unfertilized sexual females.

Figure 4.

Correlation of total lifetime fecundity and lifespan, here shown for asexual females. Trendlines from separate linear regressions of data from seasons 1, 5, and 6 are included to facilitate visualization of lifespan-fecundity relationships. Correlations were positive with or without controlling for effects of season (the latter via partial correlation analysis), for asexual or sexual females analyzed separately or altogether ($P < 0.001$).

Figure 5.

Generation time of asexual and sexual females for the first and final two seasons. Bars represent means (± 1 SE).

Figure 6.

Net reproductive rate of asexual and sexual females for the first and final two seasons. Bars represent means (± 1 SE).

Table 1

Repeated measures linear mixed models analyzed with restricted maximum likelihood analysis of square root transformed lifespan and lifetime fecundity, reporting F statistics and *P* values for fixed effects. In all cases, df (numerator/denominator) were 1/162.

	Fecundity	Total Lifespan	Pre-reproductive lifespan	Reproductive lifespan	Post-reproductive lifespan
Intercept	F 2444.348	4040.440	2054.452	4384.767	417.723
	P <0.001	<0.000	<0.001	<0.001	<0.001
Hydroperiod	F 2.106	0.055	1.321	0.249	0.200
	P 0.147	0.814	0.251	0.618	0.655
Mictic Status	F 5.491	0.015	35.324	29.794	32.248
	P 0.019	0.902	<0.001	<0.001	<0.001
Season	F 56.091	23.565	37.450	17.757	28.827
	P <0.001	<0.001	<0.001	<0.001	<0.001
Hydroperiod * Mictic Status	F 0.232	0.118	4.490	0.331	0.155
	P 0.630	0.731	0.034	0.565	0.694
Hydroperiod * Season	F 2.763	0.075	0.003	0.896	0.055
	P 0.097	0.784	0.960	0.344	0.815
Mictic Status * Season	F 33.746	14.197	23.292	15.885	18.848
	P <0.001	<0.001	<0.001	<0.001	<0.001
Hydroperiod * Mictic Status * Season	F 0.027	0.004	4.239	0.142	0.495
	P 0.871	0.949	0.040	0.707	0.482

Estimates of covariance parameters for repeated measures variance (± 1 SE) for the traits are as follows: fecundity variance = 0.517 (± 0.030), Wald Z = 17.493, $P < 0.001$; total lifespan variance = 0.228 (± 0.013), Wald Z = 17.493, $P < 0.001$; pre-reproductive lifespan variance = 0.072 (± 0.004), Wald Z = 17.493, $P < 0.001$; reproductive lifespan variance = 0.101 (± 0.006), Wald Z = 17.493, $P < 0.001$; post-reproductive lifespan variance = 0.584 (± 0.033), Wald Z = 17.493, $P < 0.001$.

Table 2

Rate of aging (ω) of asexual and sexual female rotifers.

Growth season	Rotifer type	m_o	α	β	$\omega(d^{-1})$
1	asexual	1.17E-3 (1.17E-3)	5.61E-3 (2.38E-3)	2.28 (0.31)	0.167 (0.005)
5	asexual	3.95E-3 (3.57E-3)	5.82E-5 (4.80E-5)	4.49 (0.42)	0.120 (0.003)
6	asexual	1.47E-3 (6.95E-4)	6.31E-4 (4.76E-4)	4.09 (0.70)	0.128 (0.004)
1	sexual	0.00 (0.00)	7.58E-3 (4.28E-3)	2.33 (0.39)	0.173 (0.007)
5	sexual	9.34E-3 (6.83E-3)	1.64E-3 (7.57E-4)	2.96 (0.45)	0.155 (0.010)
6	sexual	3.56E-3 (2.70E-3)	3.15E-3 (1.43E-3)	2.99 (0.52)	0.169 (0.007)

Initial inputs for nonlinear regression estimation of parameters were $m_o=0$, $\alpha=1.00E-4$, $\beta=3$; values of ω were computed per chemostat from estimated parameters and averaged across chemostats. Reported values are means for the six chemostats ($\pm$ 1 SE). Parameter estimation and calculation of ω followed (Ricklefs, 1998).

Table 3

General linear model of net reproductive rate (Ro), generation time (T), and the rate of aging (ω), reporting F statistics and P values. For the multivariate model, degrees of freedom (numerator/denominator) were 3/26; for between-subject tests of Ro , T , and ω the $df = 1/28$.

	Multivariate test (Wilk's Λ)			Ro	T	ω
	F					
Intercept	F	2791.145	220.564	5769.606	782.515	
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Hydroperiod	F	0.148	0.216	0.012	0.086	
	P	0.930	0.646	0.915	0.771	
Mictic Status	F	14.563	1.381	46.033	0.005	
	P	<0.001	0.250	<0.001	0.943	
Season	F	11.435	18.619	11.151	15.401	
	P	<0.001	<0.001	0.002	0.001	
Hydroperiod * Mictic Status	F	0.151	0.072	0.399	0.044	
	P	0.928	0.791	0.533	0.836	
Hydroperiod * Season	F	0.206	0.577	0.190	0.032	
	P	0.891	0.454	0.666	0.859	
Mictic Status * Season	F	4.304	10.325	0.005	6.857	
	P	0.014	0.003	0.945	0.014	
Hydroperiod * Mictic Status * Season	F	0.370	0.260	1.080	0.027	
	P	0.775	0.614	0.308	0.871	