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Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies

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In semelparous populations, dormant germ banks (e.g. seeds) have been proposed as important in maintaining genotypes that are adaptive at different times in fluctuating environments. Such hidden storage of genetic diversity need not be exclusive to dormant banks. Genotype diversity may be preserved in many iteroparous animals through sperm-storage mechanisms in females. This allows males to reproduce posthumously and increase the effective sizes of seemingly female-biased populations. Although long-term sperm storage has been demonstrated in many organisms, the understanding of its importance in the wild is very poor. We here show the prevalence of male posthumous reproduction in wild Trinidadian guppies, through the combination of mark–recapture and pedigree analyses of a multigenerational individual-based dataset. A significant proportion of the reproductive population consisted of dead males, who could conceive up to 10 months after death (the maximum allowed by the length of the dataset), which is more than twice the estimated generation time. Demographic analysis shows that the fecundity of dead males can play an important role in population growth and selection.

1. Introduction

Many organisms have been shown to store sperm inside the female reproductive tract [1]. Some examples include social insects [2], crabs [3], salamanders [4], turtles [5], lizards [6,7], bats [8] and fish [9,10]. Laboratory experiments, where mated females were left without males for extended periods, have shown that in several species sperm can survive stored in females for over a year [11]. However, since many sperm-storing species show last-male preference in siring a brood [12], it is unclear whether sperm storage can contribute significantly to posthumous reproduction in natural populations. An exception to this is seen in a study on side-blotched lizards *Uta stansburiana*, where certain male phenotypes are specialized for sperm competition and posthumous reproduction [7]. However, that study only spanned a single reproductive season and did not test for sperm survival across seasons. It remains to be seen whether posthumous reproduction can occur across breeding events, and consequently have an effect on the maintenance of genetic diversity and longer-term adaptation.

In this study, we assess the dynamics and demographic importance of posthumous reproduction in a fish species that exhibits some of the fastest rates of evolution known to date [13]: the Trinidadian guppy *Poecilia reticulata*. Guppies possess small receptacles in the ovarian cavity capable of storing sperm [14], and nourish it with extracellular ovarian sugars [15]. It has been long known that females are able to produce offspring in the laboratory well after the removal of males [16]. Although several studies emphasize the importance of guppy

sperm storage for sperm competition, cryptic female choice and polyandry [17,18], its role in the long-term dynamics of posthumous reproduction in the wild is completely unknown. Posthumous reproduction can be expected in this species both because females can store sperm and because in nature they have substantially longer lifespans than mature males [19,20].

Our aim is to assess whether, in nature, female guppies can use stored sperm beyond the lifetime of a male. To address the prevalence, demographic importance and selective potential of long-term sperm storage in nature, we analysed data from a longitudinal study of individually marked and pedigreed guppies in the wild. Specifically, we use two analyses: (i) an inferential hidden state–space mark–recapture model to estimate the prevalence and duration of posthumous male reproduction in the population; and (ii) population modelling to evaluate its importance for fitness and population growth.

2. Material and methods

(a) Study population

We used data from a recent guppy introduction experiment into the Lower LaLaja, a tributary of the Guanapo River, in the Northern Mountain Range of Trinidad. On 16 March 2008, we introduced 76 individually marked guppies (38 males and 38 females). The introduction site is a reach 100 m long bounded by two waterfalls. It has an average benthic width of 1 m, and consists of a series of pools connected by riffles. The reach did not contain guppies before introduction and is separated from natural populations by the downstream waterfall. The introduced guppies were collected as juveniles (standard length less than 8 mm) and reared in the laboratory under common garden conditions, keeping sexes separate. Two weeks before introduction, we mated the mature guppies by grouping them in tanks containing five males and five females. We introduced the 38 mated females together with 38 new laboratory males with which they had not mated, thus increasing the opportunity for further mate choice in the streams.

Before introduction, we uniquely marked all individuals by injecting a combination of subcutaneous colour marks of visible implant elastomer (NorthWest Marine Technology) in two of eight possible body positions. We photographed all individuals to measure standard length, and took three to six scales for pedigree reconstruction. Scales were dried and preserved in 0.5 ml O-ring sealed micro-tubes until DNA extraction.

(b) Individual-based data

Every month, all guppies larger than 13 mm were collected and brought back into the laboratory for identification. All new recruits were uniquely marked once they reached 14 mm, as done for the founding females and males. Sexual maturity of males was determined under a dissecting scope by noting whether the gonopodium hood extended beyond the tip of the gonopodium. Standard length was measured from photographs taken of each fish upon its initial capture and each successive recapture. The mean size of mature males in our sample was 17.2 mm, ranging from 14.61 to 19.75. For each photograph, the fish was anaesthetized in a neutrally buffered solution of tricaine methanesulfonate (MS-222), and straightened over a plate next to a ruler. Its picture was then taken with a digital camera mounted on a tripod above and lit with two full-spectrum fluorescent light bulbs. Guppies were tracked from March 2008 to March 2009.

DNA was isolated from scales by binding DNA to silica in the presence of high-molarity sodium iodide [21]. Data for 11 microsatellite loci were collected using LI-COR 4200 DNA analysers (LI-COR Biosciences; electronic supplementary material, table A1). Polymerase chain reactions (PCRs) were performed in 5 or 10 µl volumes using the following reagents: 1× Thermopol Buffer (New England Biolabs), 200–400 ρg DNA, 50 µM each dNTP (NEB), 0.25–0.5 U *Taq* DNA polymerase (NEB) and 0.2 µM each primer. Forward primers were 5′-labelled with IR700 or IR800 dye to allow fluorescent imaging. PCR cycling parameters included 95°C 5 min initial denature, followed by 30 cycles of 94°C 30 s; primer-specific Ta (see the electronic supplementary material, table A1) 30 s; 72°C 60 s; followed by a 5 min soak at 72°C.

Parentage was assigned using the full likelihood method implemented in COLONY v. 2 [22] using all marked individuals, dead or alive, as candidate parents. For 567 offspring genotyped at a minimum of five loci (mean number of loci scored per fish = 10.54), 532 (94%) had their mother and 512 (90%) their father assigned with greater than or equal to 0.9 probability. We confirmed that, while known dead males produced offspring, no known dead female did.

(c) Inference of demographic parameters

Because these data are derived from a monthly mark–recapture census, the age of death is not known with certainty. The absence of an individual in any one census could be explained either by its having died or that it was alive but not seen. Because the study site is separated from habitat downstream by a substantial waterfall, we assume that fish cannot migrate back upstream and emigrants are treated as if they had died (i.e. we estimate apparent survival). Since we only have parentage information for individuals over 14 mm, we can only conservatively estimate the number of conceptions occurring after the sire's death. We assumed that individuals captured at a size smaller than 18 mm (males) or 20 mm (females) were born two months before first capture and conceived three months before first capture. Individuals over these sizes were assumed to be conceived four months before. These criteria are conservative, following known patterns of gestation (25 days) and growth [23], and were confirmed by the fact that the number of recruits was correlated with the number of pregnant females two month prior, as assessed by the peak in abdominal distension shown on their pictures (Torres-Mejía *et al*. 2011, unpublished data).

In order to incorporate the uncertainty of the apparent survival of individuals, we inferred the probability of male lifetime and posthumous reproduction using hidden state–space mark–recapture models [24] applied to the capture and pedigree data. Hidden state–space models are composed of two layers: a process model that defines the probabilities of survival and reproduction, and an observation model that defines the likelihood of observing the data given the process model. For example, in the case of mark–recapture models, the observation of individuals is defined by both the probability of survival (a process model parameter) and the probability of capture (an observation model parameter).

The process model (figure 1) is structured into five different states: juvenile (J), mature and alive before first reproduction (M), adult and alive after first reproduction (R), dead but reproductively active (S), and dead and no longer siring any offspring (D). The transition matrix between these states is

$$\Psi = \begin{pmatrix} \varphi_J(1-\mu) & 0 & 0 & 0 & 0 \\ \varphi_J\mu(1-\rho) & \varphi_M(1-\rho) & 0 & 0 & 0 \\ \varphi_J\mu\rho & \varphi_M\rho & \varphi_R & 0 & 0 \\ 0 & \rho(1-\varphi_M)\sigma & (1-\varphi_R)\sigma & \varphi_S & 0 \\ 1-\varphi_J & 1-\varphi_M & (1-\varphi_R)(1-\sigma) & 1-\varphi_S & 1 \end{pmatrix}.$$

We denote the survival probabilities of the three live stages φ_J, φ_M and φ_R, whereas φ_S denotes the probability that a dead yet reproductively active individual remains so (as opposed to no longer being reproductive). The probability of maturation is denoted by μ, and the probability of a mature individual

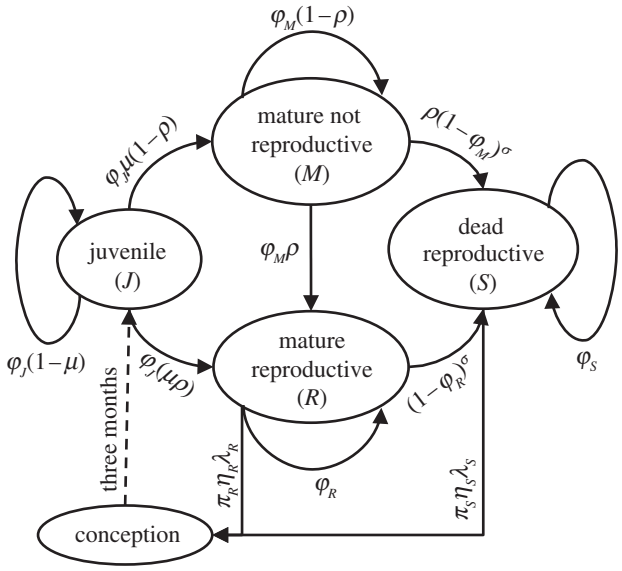

Figure 1. Schematic of the life cycle transitions of modelled male guppy populations (process model). Transitions are monthly with the exception of the transition from conception to juvenile of markable size, which is assumed deterministic and occurs in three months. See §2 for details and table 1 for parameter definitions.

becoming reproductively active is ρ. In order to transition from an alive to a dead yet reproductive state, an individual must die with probability $1 - \varphi$ and its sperm remain stored in some female(s) with probability σ. In other words, σ is the probability of remaining reproductively active given that they died. The model transitions are defined such that maturation and reproductive activity occurs before death. This is necessary in order to accommodate the census structure, where an individual can be captured as a juvenile in one census, but mature, reproduce and die before the next census.

Reproduction is modelled as stage- and month-dependent. Since data on reproduction often have an excess of zeros (corresponding to events with no reproduction), we model reproduction as a zero-augmented Poisson process [25]. Such a process, also known as a 'hurdle' process, is determined by a mixed distribution where reproduction occurs with binomial probability π and the number of offspring is conditioned on reproduction, and follows a zero-truncated Poisson distribution with mean λ. The frequency of zeroes is thus determined by a binomial probability $(1 - \pi)$ that can exceed the expectation of the Poisson process. Reproduction can occur in the two reproductive states R (alive) and S (dead). Consequently, the expected stage- and month-specific numbers of conceived offspring reaching markable size is

$$R_{J,t} = R_{M,t} = 0,$$
$$R_{R,t} = \pi_{R,t}\lambda_{R,t}$$
$$\text{and} \quad R_{S,t} = \pi_{S,t}\lambda_{S,t}.$$

Both sons and daughters are counted in the estimation of λ, and an additional stage- and month-specific parameter η describes the proportion of males. Note that all reproduction refers to individuals that reach 14 mm, yet is defined as occurring at conception, in order to discern whether the individual is sired before or after the father's death. In other words, recruitments are backdated by three months before their first capture at greater than 14 mm. Consequently, the model inherently incorporates in parameter π and λ the probability of recruitments successfully reaching markable size. Although this does not inform on the brood size at birth, it does incorporate all offspring that reach maturity, and is thus an appropriate measure of fitness.

The observation model simply consists of the probability of observing an individual given that the process model predicts it to be alive (i.e. observable). This allows us to account for imperfect detection of individuals. The model specifies that live individuals (states J, M and R) be detected with probabilities p_t that depend on the month (i.e. census).

Finally, we constructed a female-only model to fit to the female data in order to compare survival rates of both sexes. Because female maturity cannot be assessed externally and females do not reproduce posthumously, the female model only included two stages: pre-reproductive J and reproductive R.

We fitted the models to the capture–recapture and parentage data using a Bayesian framework. Priors for all binomial parameters were defined as uniform distributions between 0 and 1, while those for expected offspring λ are taken as a uniform distribution between 0 and 100. We ran three independent Markov chain Monte Carlo (MCMC) chains of 100 000 iterations with an added burn-in period of 50 000. Samples were thinned every 100 iterations. Convergence was assessed using the Gelman–Rubin statistic [26]. Model fitting was implemented in JAGS v. 3.2 through R package R2jags [27]. We set the MCMC to track the estimated number of individuals in each stage category each month, the proportion of individuals sired by dead males, as well as the mean reproductive output with respect to the age (if alive) or time of death (if dead) of the sire. Age was counted starting at first reproduction. Note that these parameters sampled from the MCMC chain represent statistical estimates that incorporate both the observed data and the uncertainty on the missing data given the estimated observation parameters (e.g. recapture probability).

(d) Demographic analysis

We used the estimated process model parameters to define monthly population transition matrices of males [28]. This allowed us to evaluate the demographic consequences of reproduction after death. The model was male-only and therefore omits the production of females. To account for estimation uncertainty, we constructed 1000 transition matrices with each of the 1000 MCMC samples of parameters estimated during the data-model fit. The male-only transition matrix was defined as follows:

$$T_t = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & \pi_{R,t}\eta_{R,t}\lambda_{R,t} & \pi_{S,t}\eta_{S,t}\lambda_{S,t} \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & \varphi_{J,t}(1-\mu_t) & 0 & 0 & 0 \\ 0 & 0 & 0 & \varphi_{J,t}\mu_t(1-\rho_t) & \varphi_{M,t}(1-\rho_t) & 0 & 0 \\ 0 & 0 & 0 & \varphi_{J,t}\mu_t\rho_t & \varphi_{M,t}\rho_t & \varphi_{R,t} & 0 \\ 0 & 0 & 0 & 0 & \rho_t(1-\varphi_{M,t})\sigma_t & (1-\varphi_{R,t})\sigma_t & \varphi_{S,t} \end{pmatrix}.$$

In order to make the population model demographically consistent, it is necessary to include three new transitions with 100 per cent probability, representing the three months from conception to markable size. Note that this does not mean that we assume zero mortality of juveniles, but because conception is defined based on production of two-month-old fish (plus a month of gestation), this aspect of the model must follow.

From these matrices, we calculated the sensitivities and elasticities of population growth to the different stage transitions ($s_{i,j}$ and $e_{i,j}$, respectively). Sensitivities describe the change in population growth rate expected from a small absolute change in a population parameter, whereas elasticities describe the expected change due to a relative change in the parameter. They quantify the absolute and relative importance of life-history transitions to population growth and fitness [28]. To calculate these, we used Tuljapurkar's method for variable environments [22]. Because we were interested in the effect of life-history parameters on the realized demography, we applied the method to the precise sequence of monthly transition matrices rather than a random sample of them. Elasticities and sensitivities for the first three states are shown pooled as an indicator of the importance of the 'black box' of demographic processes occurring before fish are marked.

Table 1. Parameter estimates and standard errors drawn from posterior distributions of the male and female model fits.

		Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
male model											
φ_J	juvenile survival	0.53 ± 0.13	0.48 ± 0.29	0.75 ± 0.19	0.56 ± 0.28	0.78 ± 0.12	0.58 ± 0.15	0.76 ± 0.11	0.93 ± 0.05	0.89 ± 0.07	0.94 ± 0.05
φ_{MM}	non-reproductive adult survival	0.21 ± 0.07	0.70 ± 0.14	0.66 ± 0.18	0.90 ± 0.07	0.57 ± 0.09	0.73 ± 0.10	0.61 ± 0.11	0.81 ± 0.08	0.79 ± 0.08	0.64 ± 0.11
φ_R	reproductive adult survival	0.53 ± 0.11	0.82 ± 0.09	0.74 ± 0.09	0.91 ± 0.06	0.68 ± 0.08	0.85 ± 0.07	0.69 ± 0.19	0.82 ± 0.08	0.68 ± 0.09	0.94 ± 0.05
φ_S	reproductive survival of dead males	0.51 ± 0.29	0.69 ± 0.20	0.74 ± 0.18	0.79 ± 0.15	0.66 ± 0.20	0.76 ± 0.07	0.52 ± 0.14	0.74 ± 0.18	0.58 ± 0.24	0.50 ± 0.29
μ	probability of maturation	0.88 ± 0.10	0.50 ± 0.29	0.65 ± 0.24	0.49 ± 0.29	0.88 ± 0.11	0.83 ± 0.14	0.90 ± 0.09	0.92 ± 0.05	0.60 ± 0.10	0.75 ± 0.07
ρ	probability of becoming reproductive if mature	0.33 ± 0.03	0.43 ± 0.15	0.29 ± 0.16	0.34 ± 0.10	0.21 ± 0.07	0.22 ± 0.08	0.25 ± 0.08	0.12 ± 0.05	0.22 ± 0.05	0.02 ± 0.02
σ	probability of remaining reproductive after death	0.56 ± 0.21	0.37 ± 0.27	0.80 ± 0.16	0.53 ± 0.28	0.86 ± 0.11	0.59 ± 0.26	0.59 ± 0.26	0.57 ± 0.25	0.50 ± 0.28	0.49 ± 0.28
π_R	probability of reproducing if reproductive alive	0.96 ± 0.04	0.82 ± 0.09	0.74 ± 0.09	0.61 ± 0.09	0.72 ± 0.07	0.57 ± 0.08	0.66 ± 0.08	0.50 ± 0.09	0.38 ± 0.08	0.54 ± 0.08
π_S	probability of reproducing if reproductive dead	0	0.21 ± 0.14	0.16 ± 0.12	0.21 ± 0.11	0.36 ± 0.15	0.35 ± 0.10	0.29 ± 0.11	0.40 ± 0.14	0.29 ± 0.12	0.48 ± 0.21
λ_R	average number of offspring if reproductive alive	2.71 ± 0.37	5.25 ± 0.64	1.82 ± 0.38	1.74 ± 0.36	2.41 ± 1.67	2.85 ± 0.42	2.26 ± 0.37	1.46 ± 0.31	1.73 ± 0.41	1.55 ± 0.30
λ_S	average number of offspring if reproductive dead	0	n.a.	n.a.	2.12 ± 1.04	1.67 ± 0.59	1.44 ± 0.37	1.64 ± 0.50	1.29 ± 0.27	1.77 ± 0.60	1.67 ± 0.50
η_R	proportion of males in broods of live males	0.42 ± 0.06	0.49 ± 0.06	0.42 ± 0.08	0.46 ± 0.08	0.38 ± 0.05	0.48 ± 0.06	0.55 ± 0.07	0.30 ± 0.08	0.46 ± 0.10	0.44 ± 0.08
η_S	proportion of males in broods of dead males	0.50 ± 0.29	0.76 ± 0.21	0.63 ± 0.26	0.39 ± 0.21	0.32 ± 0.17	0.41 ± 0.14	0.32 ± 0.14	0.52 ± 0.15	0.63 ± 0.16	0.42 ± 0.13
p	capture probability if alive	1	0.82 ± 0.08	0.95 ± 0.05	0.95 ± 0.05	0.90 ± 0.06	0.86 ± 0.05	0.88 ± 0.05	0.81 ± 0.06	0.82 ± 0.06	0.85 ± 0.05
female model											
φ_J	pre-reproductive survival	0.19 ± 0.09	0.71 ± 0.21	0.72 ± 0.17	0.96 ± 0.03	0.81 ± 0.08	0.79 ± 0.11	0.84 ± 0.11	0.66 ± 0.10	0.67 ± 0.13	0.78 ± 0.11
φ_{RM}	reproductive adult survival	0.90 ± 0.06	0.95 ± 0.05	0.92 ± 0.05	0.92 ± 0.04	0.94 ± 0.04	0.95 ± 0.03	0.98 ± 0.02	0.95 ± 0.02	0.94 ± 0.03	0.90 ± 0.05
ρ	probability of becoming reproductive	0.43 ± 0.05	0.30 ± 0.22	0.42 ± 0.24	0.37 ± 0.17	0.29 ± 0.19	0.58 ± 0.26	0.45 ± 0.26	0.78 ± 0.19	0.25 ± 0.21	0.58 ± 0.27
π_R	probability of reproducing if reproductive	0.93 ± 0.06	0.85 ± 0.08	0.68 ± 0.09	0.50 ± 0.08	0.57 ± 0.08	0.44 ± 0.07	0.33 ± 0.05	0.18 ± 0.03	0.11 ± 0.03	0.16 ± 0.03
λ_R	average number of offspring if reproduced	3.16 ± 0.42	4.53 ± 0.52	1.75 ± 0.36	1.53 ± 0.30	2.15 ± 0.28	1.66 ± 0.25	1.76 ± 0.28	1.2 ± 0.17	1.25 ± 0.21	1.69 ± 0.29
p	capture probability if alive	1	0.73 ± 0.09	0.74 ± 0.09	0.75 ± 0.08	0.93 ± 0.03	0.77 ± 0.04	0.78 ± 0.04	0.87 ± 0.03	0.86 ± 0.03	0.80 ± 0.03

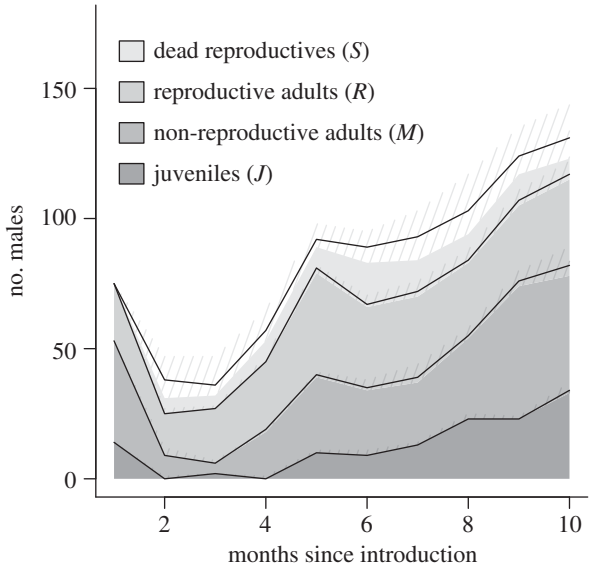

Figure 2. Monthly estimates of the number of males in each of the four states. Solid lines, mean estimate; hatched areas, 95% credible intervals.

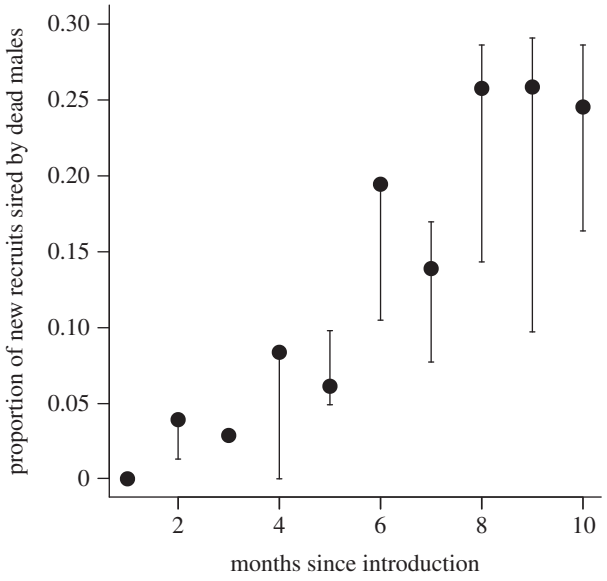

Figure 3. Estimated proportion of offspring sired by dead males and 95% credible intervals.

We estimated male and female life expectancies at first reproduction using the geometric mean of the monthly reproductive survival rates $G(\varphi_R)$ and calculating $G(\varphi_R)/(1 - G(\varphi_R))$.

3. Results

The data consist of 278 male individuals captured an average of 2.56 times each. The probability of capturing an individual if alive was high (see estimates in table 1): only in 18 per cent of the cases where an individual was known to be alive (due to later reappearance in the census) was it not caught. There were 164 reproductive events, of which 47 (28.1%) were conceived after the last time the male was seen, and 25 of them (15.2%) at least two months after. Post-mortem conceptions represent 73 out of 540 (13.5%) births.

(a) Demographic parameter inference

The estimated monthly parameters and standard errors for the male and female state-space models are summarized in table 1. The first two months are characterized by low male and female survival probably due to introduction stress. After that, male survival and recruitment shows a seasonal pattern where high male mortality corresponds with the typically wettest months (July–November, with a small dry season in September or October). The survival of reproductive females, however, remains relatively stable throughout the year (table 1).

Monitoring of individuals in the MCMC samples showed that 54.6 ± 2.6 per cent (error indicates s.d.) of the 278 males in the dataset reproduced at least once, of which 50.8 ± 4.9 per cent did so only while alive, 33.6 ± 4.1 per cent did so also posthumously and 15.6 ± 2.8 per cent only reproduced posthumously. Only 21.1 ± 6.7 per cent of the individuals that reproduced posthumously were founder individuals, whose sperm-storage patterns could have been affected by the initial introduction mortality. Figure 2 shows the estimated numbers and proportions of each stage class through time. The figure reveals seasonality typical of the system, with wet seasons associated with population declines. Note that the total population as defined in our demographic model is larger than the number of live individuals because it includes those individuals estimated dead but still reproductively active. The population starts with no dead reproductives because the population was initiated with fish that were collected as juveniles, reared in single-sex groups, then mated to other members of the population before being released. It is only after release that some founders died and it became possible to identify offspring as being sired by dead fathers. Throughout the months, there is an increase in the proportion of dead individuals that remain reproductively active, reaching a maximum of up to 19.6 ± 6.7 per cent of the total population and 31.4 ± 0.09 per cent of the reproductive population in month 6 (August 2008, during the peak of the wet season). On the last month (January 2009), 15.4 ± 5.6 per cent of the total population ($29.4 \pm 8.9\%$ of the reproductive population) was represented by the stored sperm of dead males. Note that in periods where the population of live individuals declines, the total population remains stable thanks to the increase in the number of dead reproductive males (i.e. male genotypes stored as sperm in female bodies). The monthly proportion of new recruits conceived by dead males grows from 0 to 23.7 ± 3.7 per cent in 10 months (figure 3).

The estimated reproductive output (as percentage of the population recruitments sired at any given time) for individuals of different reproductive ages, counted as months since they become reproductive (i.e. entering stage *R*), shows some fluctuation due to a correlation between mean individual age and season (figure 4*a*). Reproductive success of dead males shows a decline with time since estimated death, as expected if last-male precedence is more common and there is a sperm dilution effect as new males mate (figure 4*b*). Nevertheless, males are capable of conceiving viable offspring for at least eight months after death, as has been found in laboratory studies where a single male is allowed to mate [16,29]. This is a conservative estimate of the length of sperm storage, given that this estimate cannot account for fertilizations resulting from sperm storage while the sire is alive. For comparison, we also include the pattern of age-specific reproductive success for females drawn from the female model (figure 4*c*). These graphs can only be interpreted as an ontogenetic trend if the

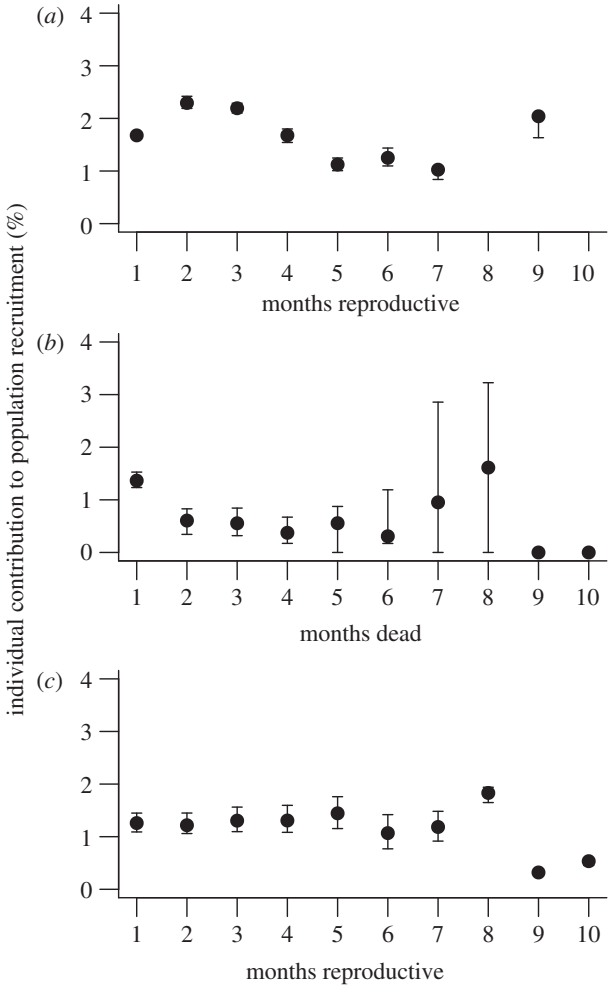

Figure 4. Estimated average reproductive success (percentage of recruits contributed by the individual at any given time) for (*a*) live males in relation to months since they became reproductive, (*b*) dead males in relation to the time since death and (*c*) females in relation to months since they became reproductive. Note that each individual contributes multiple points, tallied for each month that it was estimated present in the population in the (*a*,*c*) live or (*b*) dead reproductive states. Error bars indicate 95% credible intervals of the mean (they do not reflect the variance in the sample, but rather the uncertainty in the mean stemming from the fact that there is uncertainty in the individual's state).

environment had been constant throughout the study. Given that the study incorporates dry as well as wet months, this is not strictly the case, and hence the temporal variations may be reflective of the correlation between population stage structure and season.

(b) Demographic analyses

Excluding the pre-census stages, relative changes in the survival of live reproductive adults show the strongest effect on population growth (elasticity), followed closely by the fecundity of both live and dead reproductive adults, which have similar effects (table 2). Sensitivities are strongest for the fecundity of live males (table 2).

The only way for males to make such a large posthumous contribution to an individual's reproductive success is if some females have much longer lifespans than the males they mated with. Indeed, the life expectancy of females at first reproduction (15.28 ± 3.57 months) is much higher than that of males (3.05 ± 0.44 months).

4. Discussion

We have presented an evaluation of the importance and dynamics of posthumous reproduction in a wild animal population with sperm storage. Our results show that posthumous reproduction is common among male Trinidadian guppies due to the greater longevity of females, who can store sperm. As a consequence, dead males sire a significant proportion of population recruits (figure 3).

Previous laboratory studies on the Trinidadian guppy demonstrated their ability to store sperm for months within the folds of their ovaries when no further males were allowed to mate [16,29]. When laboratory studies allow for multiple males to mate with a female sequentially, last-male precedence seems to be more common, but first-male precedence is also observed at times [30–32], suggesting that the usage of stored sperm is not only controlled by sperm decay, but also a certain degree of cryptic female choice [33]. Given the prevalence of last-sperm precedence, the longevity of stored sperm in a natural scenario was unclear. Our results show that stored sperm does indeed display the lifespan observed in the laboratory, and that despite showing the expected pattern of decay, it plays a significant role in individual fitness and the population dynamics of the species (figures 2–4 and table 2).

Clearly, posthumous reproduction has important fitness consequences for males, as it allows them to expand their reproductive lifespan to equal that of females. This is evidenced by the fact that male fitness (or demographic growth rate) has a high sensitivity to transition probabilities from alive to dead reproductive states, despite it involving the death of the individual (table 2). Moreover, elasticities for live or posthumous reproduction are similar (table 2), implying a similar relative contribution to fitness, and thus similar selection pressures on the live and posthumous reproduction.

A further potential advantage of posthumous reproduction—not accounted for by the elasticity analysis above—is the ability of the sperm to survive conditions that the adult phenotype may not. In our populations, there is a clear seasonal trend in male survival and reproduction (table 1 and figure 2), but the survival of reproductive females shows little or no fluctuation and remains high during the wet season (table 1), allowing the sperm of dead males to survive inside females. This situation is in a way reminiscent of seed banks in plants and dormant egg banks in freshwater plankton, where genetic variation is preserved underground, sheltered from the conditions aboveground [34,35]. Analogously, if female survival is more resilient to environmental change (as suggested by their higher lifespan than males under such seasonal environment), sperm can survive stored in them, preserving the male genotypes that would not have otherwise survived.

From the female perspective, we can think of two adaptive explanations of long-term sperm storage. First, sperm storage buffers the shortage of potential male mates in fluctuating environments. Second, sperm storage may serve as a coin-flipping (or bet-hedging) strategy whereby genetically diverse sperm can accumulate in a female and increase the probability of producing adapted offspring under a variety of environments [36,37]. This mechanism, if persistent through a series of environmental shifts, has been shown to allow the maintenance and increase in the genetic diversity of annual plants with dormant seed banks [38,39]. More generally, it can be argued that the existence of overlapping generations where one of the life stages is shielded from selection can maintain variation

Table 2. Elasticities and sensitivities of population growth to matrix transitions (95% credible intervals in parentheses). Sensitivities to biologically impossible transitions are in italics.

	conception	*J*	*M*	*R*	*S*
elasticities					
conception	0	0	0	0.07 (0.05–0.9)	0.09 (0.04–0.12)
J	0.41 (0.34–0.46)	0.01 (0.00–0.03)	0	0	0
M	0	0.05 (0.04–0.07)	0.05 (0.03–0.08)	0	0
R	0	0.05 (0.04–0.07)	0.04 (0.02–0.05)	0.13 (0.09–0.20)	0
S	0	0	0.01 (0.00–0.02)	0.02 (0.01–0.04)	0.05 (0.02–0.12)
sensitivities					
conception	*1.11 (0.83–1.45)*	*0.33 (0.21–0.49)*	*0.31 (0.20–0.47)*	0.23 (0.18–0.29)	0.10 (0.05–0.18)
J	0.42 (0.28–0.62)	0.09 (0.05–0.14)	*0.09 (0.06–0.14)*	*0.07 (0.05–0.09)*	*0.03 (0.01–0.06)*
M	*0.53 (0.37–0.79)*	0.12 (0.08–0.17)	0.10 (0.06–0.14)	*0.08 (0.06–0.11)*	*0.04 (0.02–0.08)*
R	*1.40 (1.03–2.02)*	0.42 (0.29–0.60)	0.24 (0.17–0.36)	0.18 (0.11–0.27)	*0.10 (0.04–0.30)*
S	*0.99 (0.45–2.86)*	*0.24 (0.11–0.46)*	0.19 (0.10–0.33)	0.14 (0.09–0.25)	0.08 (0.03–0.18)

[40]. The essence of the argument is that, when selection is buffered at certain life stages (e.g. dormant seeds or surviving iteroparous adults), genotypes that are selected against may re-emerge under different environmental conditions. Following this logic, long-term sperm storage by females can also buffer the loss of genetic variation in organisms where females outlive males, enabling males represented in stored sperm to reproduce, even after death.

Our results also have important implications for the measurement of selection in sperm-storing organisms. Standard measures of selection (e.g. selection gradients [41]) need to consider the pool of current phenotypes or genotypes under which selection is acting. Measuring only the phenotypes of the visible fraction of the population (alive) could strongly bias these estimates, particularly if the invisible fraction is not a random sample of the current population. Whether sperm storage can explain the outstanding evolvability of guppies through the maintenance of hidden genetic variation is yet to be determined. We hope that our discovery of the extent and importance of posthumous reproduction in this wild population of guppies encourages studies on the role of sperm storage in the adaptation of populations to changing environments.

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