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Memory and obesity affect the population dynamics of asexual freshwater planarians

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Abstract

Asexual reproduction in multicellular organisms is a complex biophysical process that is not yet well understood quantitatively. Here, we report a detailed population study for the asexual freshwater planarian *Schmidtea mediterranea*, which can reproduce via transverse fission due to a large stem cell contingent. Our long-term observations of isolated non-interacting planarian populations reveal that the characteristic fission waiting time distributions for head and tail fragments differ significantly from each other. The stochastic fission dynamics of tail fragments exhibits non-negligible memory effects, implying that an accurate mathematical description of future data should be based on non-Markovian tree models. By comparing the effective growth of non-interacting planarian populations with those of self-interacting populations, we are able to quantify the influence of interactions between flatworms and physical conditions on the population growth. A surprising result is the non-monotonic relationship between effective population growth rate and nutrient supply: planarians exhibit a tendency to become 'obese' if the feeding frequency exceeds a critical level, resulting in a decreased reproduction activity. This suggests that these flatworms, which possess many genes homologous to those of humans, could become a new model system for studying dietary effects on reproduction and regeneration in multicellular organisms.

1. Introduction

Unicellular organisms, such as bacteria or yeast, typically replicate through budding or binary fission [1–3]. In contrast, most multicellular organisms reproduce exclusively sexually. Notable exceptions are the freshwater polyp *Hydra* [4–6], the starlet sea anemone *Nematostella vectensis* [7, 8] and planarians [9–14], which may undergo both sexual and asexual reproduction. Planarians are the most complex organisms exhibiting this amazing capability. They can completely regenerate all body structures, including a central nervous system, from a minuscule body part. The regeneration process in planarians is mediated by a large number of stem cells (neoblasts), that comprise ∼25–30% of all cells [13–18] and are homogeneously distributed throughout their body, except in the pharynx (eating tube) and the region anterior to the photoreceptors which are stem cell free. The neoblasts also provide the basis for asexual reproduction by transverse fission: planarians may spontaneously divide into two pieces ('head' and 'tail'), see figure 1(*a*), and rebuild the missing body parts within about a week.

The population behavior of unicellular microorganisms has been thoroughly studied both experimentally [1–3, 19–22] and theoretically [23–27] over the past decades. In contrast, comparatively little is known about the collective fission dynamics of asexually reproducing higher organisms such as planarians. A possible explanation might be that multicellular organisms experience substantially longer reproduction cycles

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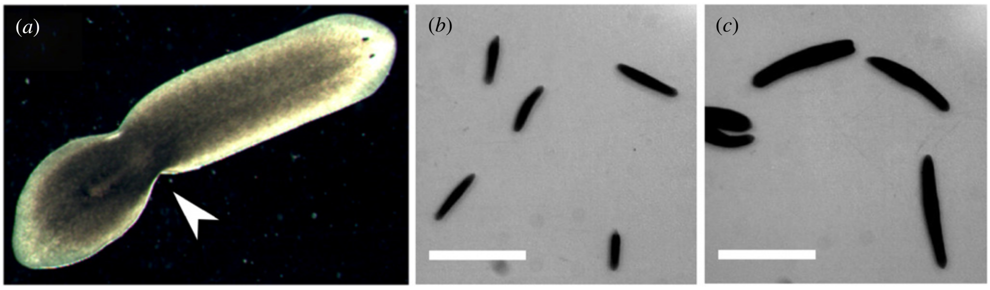

Figure 1. *S. mediterranea* at the onset of fission: (*a*) arrow indicates prospective fission site. Worms grow substantially bigger at higher feeding frequency f (scale bars correspond to 1 cm): (*b*) feeding frequency $f = 0.5$/week and (*c*) $f = 3$/week.

than bacteria or yeast, which usually duplicate within ∼10–20 min [1–3], are easier to cultivate in the lab, and require considerably less lab space and human resources.

Here, we report the first long-term experimental study (∼1 year) of the asexual freshwater planarian *Schmidtea mediterranea*. Our investigations are, among others, motivated by the fact that a detailed understanding of the statistical properties of planarian ensembles is essential for the correct interpretation of stem cell and regeneration experiments on individual organisms. Seminal early studies in the 1960s and 1970s [10, 11, 28–30] focussed primarily on self-interacting populations of various freshwater planarian species and were limited in sample size and duration of observation, making statistical analysis difficult. To overcome such limitations, we performed large-scale experiments with thousands of individuals, allowing for a quantitative comparison of the long-term dynamics (∼1 year) of non-interacting and self-interacting populations, the influence of environmental conditions on growth dynamics, and yielding new insights into the fission statistics of individual planarians.

A detailed analysis of fission waiting times in non-interacting populations reveals that the regeneration of tail fragments exhibits noticeable memory effects. Our experiments further demonstrate that standard growth models [31] that are commonly used to describe asexual unicellular populations, seem to capture certain features of self-interacting planarian populations quite well (e.g. density, temperature dependence), while they fail on other aspects (waiting time distributions in non-interacting populations, nutrition effects). Surprisingly, we find that planarians become ‘obese’[4] if the food supply exceeds a critical threshold, see figures 1(*b*) and (*c*), resulting in decreased reproduction. This suggests that, in addition to their importance in stem cell research [13, 14, 32], planarians could become a valuable model system for studying dietary influences on reproduction and regeneration.

2. Materials and methods

In all our experiments, we used the asexual strain of *S. mediterranea*. Worms were kept in planaria-water [33], fed organic beef liver (at fixed feeding frequency f), and stored in the dark, except for feeding and cleaning. During transverse fission, *S. mediterranea* divides asymmetrically resulting in a large head piece with pharynx and a smaller tail, see figure 1(*a*). Generally, the fission pieces go through a regeneration phase (∼1 week) before the next division may occur, but occasionally, head pieces divide before they have fully regenerated a new tail. In the case of tails, the body rescales, since initially they cannot eat and thus have to use old tissue as energy source for regeneration. Regeneration is followed by a growth period whose duration depends on the initial size of the fission piece. Planarians seemingly have to exceed a minimum size before undergoing fission again; small worms show no fission activity. To study the effects of worm–worm interactions, experiments were carried out for both non-interacting (*N*) and self-interacting (*S*) populations.

N-experiments: each worm was kept in its own petri dish (100 mm diameter × 20 mm height) filled with 25 mL of planarian water at constant temperature $T = 20$ °C, fed once a week ($f = 1$/week), and cleaned twice per week. After each fission, head and tail pieces were separated into new petri dishes and labeled with a code reflecting their entire history. Fission events were counted three times per week, implying a time resolution of $\leqslant 3$ days.

S-experiments: a seed population of N_i worms was placed in a container with a specific volume (V) at a given temperature (T). Off-spring were not separated after fission. For experiments involving varying feeding frequency (f), each box was cleaned three times a week, set by the highest feeding frequency of $f = 3$/week. Otherwise, boxes were cleaned twice a week. For all sets, sufficient food per worm was provided during feeding so that competition for food is not a limiting factor. Planarians were manually counted once per week. To prevent bacterial infection, 50 μL of gentamicin was added as necessary. For the various experiments, several boxes were kept under different, but constant physical conditions (V, T, f).

Planarian mass and areas were measured using two sets of 40 worms from populations kept at different feeding frequencies $f = x$/week, $x = 3, 2, 1, 0.5$. The mass was measured by placing each set on a piece of filter paper, removing excess water and weighing them on a Mettler Toledo lab balance. Then pictures of each individual worm were taken with a LEICA MZ16FA stereo microscope (Leica Microsystems, Wetzlar, Germany) using a Basler A601f CCD

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[4] Throughout, the term ‘obese’ is used to describe a substantial gain in body volume.

camera (Basler AG, Ahrensburg, Germany) and Basler BCAM Viewer (Basler AG, Ahrensburg, Germany) and their area was calculated using ImageJ software (US National Institutes of Health, Bethesda, MD, USA).

3. Results and discussion

3.1. Fission waiting time distributions and memory effects

We first determine the unperturbed fission waiting time distributions (FWTDs) for heads and tails for non-interacting planarian populations. Since head and tail fragments are separated after the fission event in these experiments, each planarian lives in its own microenvironment and there is no competition for space or food. Each experiment begins with a seed worm; the day of its first fission is defined as $t = 0$. If all off-spring survive then there are 2^g worms per fission generation g. In general, however, generations $g \gg 1$ are incomplete due to long waiting times and/or death of individuals. By monitoring the individual fission events over several generations, we obtain empirical waiting time histograms for each worm family. The results are summarized in figure 2. The diagrams in figures 2(*a*) and (*b*) illustrate how different fission generations contribute over an observation time of $t \sim 300$ days to the joint FWTDs shown in figure 2(*c*).

Head and tail fragments possess distinctly different FWTDs: the random waiting time T_H, elapsing before a head fragment undergoes fission, is approximately exponentially distributed with the probability density function (PDF)

$$p_H(t) = \frac{\mathrm{e}^{-t/\theta_H}}{\theta_H}. \qquad (1)$$

From our data we estimate the empirical mean $\theta_H = 16 \pm 3$ days, corresponding to the solid (red) line in figure 2(*c*).

By contrast, the FWTD of the tail fragments is more complex and cannot be described by a one-parameter distribution. In order to understand the weakly multi-modal structure of the tail waiting time PDF in figure 2(*c*), it is helpful to distinguish tails that descend from heads (TfHs) and from tails (TfTs). Surprisingly, we find that TfHs and TfTs have significantly different FWTDs, see the inset of figure 2(*c*). This implies that memory effects play an important role in the fission statistics of tails. Can we understand this memory effect from a biological perspective? A possible explanation is the existence of an anterior–posterior gradient in neoblast mitotic activity and composition [34] as well as a possible metabolic gradient [35]. These findings suggest that TfT pieces face extra challenges compared to TfHs: TfTs need to regenerate from the less active body part and, moreover, must rebuild more complex structures, such as the central nervous system, the photoreceptors and a large variety of tissue types, twice in a row. Future data on long-term observations of tail lines over many generations will allow us to test whether these tails restore their activity levels over time or not since the latter would lead to a successive increase of FWTs over the course of the observation time. The implications of such a slow-down in reproductive behavior are severe: it would suggest that pure tail lines experience a gradual dilution of their overall stem cell population or of a specific subpopulation, which is required for tail regeneration and survival. A comparative analysis of pure tail lines at different generations using molecular markers for stem cell specific proteins [34, 36] will allow us to address this question in the future. In this context, it will also be interesting to study the role and distribution of Gap junction proteins [37], which are plasma membrane channels important for cell–cell communication and have recently been shown to be involved in stem cell regulation in *S. mediterranea* [34, 38].

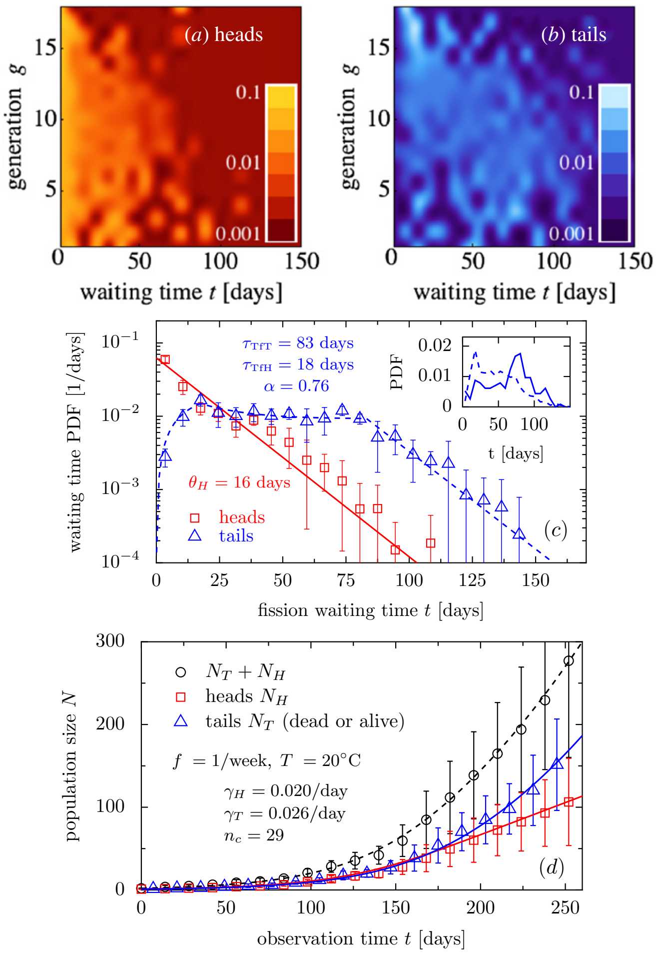

Figure 2. Non-interacting populations. Data summary: five families, total number of fissions for heads $n_H = 1623$ and tails $n_T = 680$, death cases $d_H = 2$, $d_T = 148$. (*a*), (*b*) Density plots of the waiting time PDFs per fission generation based on combined raw data. Generations $g \gg 1$ are generally incomplete due to long waiting times and/or death of individuals. (*c*) FWTDs obtained by averaging over five families (□, △); bin width = 7 days. Lines represent the theoretical models $p_{H/T}(t)$ from (1) and (3) with α denoting the relative fraction of TfHs in the data sample and Θ_H the mean waiting time of heads. Inset shows the experimentally measured FWTDs for TfHs (dashed) and TfTs. (d) Mean population growth. Symbols indicate an average over five families. Lines represent the solution of (6) with fit parameters $(\gamma_H, \gamma_T, n_c)$. Error bars correspond to sample standard deviations.

To obtain a simple quantitative model for the observed FWTDs, we may decompose the total waiting time of either tail type as

$$T_T^\sigma = T_R^\sigma + T_H, \qquad \sigma \in \{\mathrm{TfT}, \mathrm{TfH}\}, \qquad (2)$$

where T_R^σ is the recovery time required to 'build' a new head and T_H is the exponentially distributed waiting time of heads. As a minimal assumption, we take T_R^σ to be uniformly distributed on the interval $[0, \tau_\sigma]$. The joint tail waiting time PDF can then be written as

$$p_T(t) = \alpha\ p_{\mathrm{TfH}}(t) + (1-\alpha)\ p_{\mathrm{TfT}}(t), \tag{3}$$

where α is the relative fraction of TfHs in the data sample and

$$p_\sigma(t) = \frac{\mathrm{e}^{-t/\theta_H}}{\tau_\sigma}[\mathrm{e}^{\tau_\sigma/\theta_H} + (\mathrm{e}^{t/\theta_H} - \mathrm{e}^{\tau_\sigma/\theta_H})\Theta(\tau_\sigma - t) - 1]. \tag{4}$$

$\Theta(z)$ denotes the Heaviside step-function, i.e. $\Theta(z) := 0, z < 0$ and $\Theta(z) = 1, z \geqslant 0$. Using the value θ_H from above, we can estimate the parameter triple $(\alpha, \tau_{\mathrm{TfH}}, \tau_{\mathrm{TfT}})$ from our data and obtain the dashed (blue) curve in figure 2(*c*), which agrees very well with the experimentally measured waiting time distribution of the tails (△).

The observation that the fission statistics of the tail fragments exhibits non-negligible memory effects implies that more detailed mathematical models for the stochastic time evolution of non-interacting planarian families should go beyond the typically considered class of Markovian tree-models [39–43]. We hope that future data will allow us to constrain non-Markovian branching models. In fact, our present data suggest that even the fission statistics of head fragments is very weakly non-Markovian. However, at least in the first approximation, these effects appear negligible for understanding the main features of the family trees.

3.2. *Population dynamics of isolated planarians*

The data from the *N*-experiments can be used to reconstruct the growth of a non-interacting planaria population. For each family, the total number N of worms at time t can be written as a sum of N_H heads and N_T tails, $N(t) = N_H(t) + N_T(t)$. Generally, the number of heads can only increase if a tail undergoes fission and vice versa. According to our data, death cases of heads are extremely rare (less than 0.5%) and, therefore, can be neglected. By contrast, a non-negligible number $D_T(t)$ of tails have died before time t and cannot contribute to fission. Defining N_T as the total number of tails (dead or alive), a simple model for the dynamics of the head/tail-subpopulations is given by the rate equations

$$\frac{\mathrm{d}}{\mathrm{d}t}N_T = \gamma_H N_H, \qquad \frac{\mathrm{d}}{\mathrm{d}t}N_H = \gamma_T(N_T - D_T), \tag{5}$$

with $\gamma_{T,H} \geqslant 0$ denoting the effective fission rates. The model equations (5) can be closed either by specifying a differential equation for D_T or by postulating a functional dependence between $N_{T,H}$ and D_T. For simplicity, we opt for the latter approach. Experimental observations show that, initially, both head and tail populations grow exponentially, whereas at larger times the fraction of heads increases only linearly, see figure 2(*d*). This asymptotic behavior in the head data suggests a phenomenological ansatz for D_T such that the growth model (5) reduces to

$$\frac{\mathrm{d}}{\mathrm{d}t}N_T = \gamma_H N_H, \qquad \frac{\mathrm{d}}{\mathrm{d}t}N_H = \gamma_T n_c \tanh(N_T/n_c), \tag{6}$$

where n_c is a threshold parameter. At large times, when $N_T(t) \gg n_c$, equations (6) imply

$$N_H \simeq \gamma_T n_c t, \qquad N_T \simeq \gamma_H \gamma_T n_c t^2/2. \tag{7}$$

In our experiments, this asymptotic growth regime is reached after approximately 150 days. To obtain estimates for the model parameters $(n_c, \gamma_{T,H})$, we employ a two-step procedure: first, we fit the asymptotic expressions to data with $t > 150$ days. This gives estimates for γ_H and the product $\gamma_T n_c$. In the second step, these values are used to numerically integrate (6) for different choices of the threshold parameter to identify the value n_c that minimizes the sum of residuals for the full N_T raw data set. The resulting curves agree well with the averaged data, see figure 2(*d*). We conclude that the model (6) captures the main features of the population dynamics of non-interacting planarians.

Within our rate model (5), the sub-exponential growth at large times effectively arises due to an increased tail death rate over time. A possible biological cause could be that an increasing number of tails becomes too small to regenerate and/or the inheritance of 'aging factors' such as protein aggregates [44]. We hope that future data will allow us to address this point in detail and to eventually constrain improved branching models [41, 42].

By looking at large populations of non-interacting planarians whose entire history is known, one can also investigate potential differences in the reproductive behavior of individuals. Since all worms are clonal, such differences would have to be caused by epigenetic and/or environmental effects; genetic mutations are unlikely since this would imply an unrealistically high mutation rate. We did in fact observe a significant degree of variability in worm behavior; its in-depth analysis is an exciting avenue for future research, which will require a combination of pattern analysis, RNA sequencing and immunohistological studies.

3.3. *Self-interacting populations under different physical conditions*

The *N*-experiments provide a benchmark for quantifying how worm–worm interactions affect the population growth. At the start of the *S*-experiments, $N_i \leqslant 20$ worms were placed in several identical boxes. When being confronted with a new environment, planarians may go through an adaptation period. To account for this, we define, for each box separately, $t = 0$ as the time when the number of planarians in the box exceeded $N_0 = 20$ for the first time.

Compared with a population of isolated individuals, we expect that interactions such as competition for space, pollution of habitat, mutual infection, or even cannibalism effectively reduce reproduction [11, 45–47]. Adopting a minimal mathematical model, we naively assume that the population dynamics can be described by the standard logistic equation [31] with effective growth rate γ and critical carrying capacity N_c,

$$\frac{\mathrm{d}}{\mathrm{d}t}N = \gamma N\left[1 - \left(\frac{N}{N_c}\right)\right], \tag{8}$$

Table 1. Self-interacting populations. Environmental factors and best fit parameters of the logistic model (8) for an initial population size $N_0 = 20$. B denotes number of boxes under identical conditions and r^2 the goodness of fit measure.

B	V (ml)	T (°C)	f (1/week)	γ (1/day)	N_c	r^2
5	250	20	1	0.032 ± 0.001	548 ± 14	0.94
11	100	20	1	0.023 ± 0.001	192 ± 3	0.94
2	100	23	1	0.029 ± 0.002	139 ± 3	0.97
2	100	15	1	0.020 ± 0.004	43 ± 2	0.94
2	100	20	3	0.022 ± 0.001	99 ± 2	0.99
2	100	20	2	0.039 ± 0.001	122 ± 2	0.99
2	100	20	0.5	0.017 ± 0.002	37 ± 2	0.98

as commonly considered in population studies of unicellular organisms [1–3]. $N(t)$ is identified with the mean population, obtained by averaging over several boxes at identical external conditions. The solution of (8) with the initial condition $N(0) = N_0$ reads [31]

$$N(t) = \frac{N_c N_0 e^{\gamma t}}{N_c + N_0(e^{\gamma t} - 1)}. \tag{9}$$

By fitting the growth law (9) to the raw data, we obtain estimates for the model parameters (γ, N_c) as summarized in table 1.

Figure 3(*a*) depicts the data for interacting worms kept under conditions similar to those in the N-experiments for two different box volumes. The solid curves indicate the best fit of the logistic law (9). Good agreement with the data confirms that this simple model provides a useful description of the mean population dynamics. In the limit of a very large box, interactions between the worms become rarer and the carrying capacity is increased. However, compared with the quadratic increase of the non-interacting population at large times, see dashed curve in figure 3(*a*), we note that interactions significantly reduce the asymptotic population growth.

The effect of the water temperature T on the population dynamics is illustrated in figure 3(*b*). The effective growth rate γ increases with T in the considered temperature range, which is likely due to a higher metabolism at higher temperatures. Accordingly, water pollution due to metabolic activity and bacteria growth rises with temperature, causing a decreased carrying capacity N_c. Qualitatively, the growth-density dependence and the existence of an optimal growth temperature agree with data on unicellular organisms [48, 49].

When studying the effect of food supply, however, we obtained an unexpected, surprising result: a non-monotonic dependence between growth rate and feeding frequency f, as evident from figure 3(*c*) and table 1. At low feeding frequencies, the fission rate increases with increased feeding as reported for other asexually reproducing invertebrates, such as *N. vectensis* [7, 8]. However, when fed too frequently, planarian populations grow substantially slower. In fact, we observe that with increasing feeding frequency f worms tend to become 'obese' see figures 1(*b*) and (*c*). The increase in worm size with feeding frequency is quantified in figure 4, which depicts the mean mass and the worm-size (area) distributions for planarian populations at different feeding frequencies. A possible explanation for the decreased fission rate is that animals need to fully release their gut contents before they can fission. This hypothesis is supported by the fact that obese animals that were not fed for several days rapidly resumed fission activity. An additional or alternative cause could be nutrition-induced secondary effects on the molecular level. We were unable to maintain colonies at higher feeding frequencies under the same conditions; worms at $f \geqslant 4$/week died within 2 weeks. Several worms dissolved within a few days, probably because of bacteria infections due

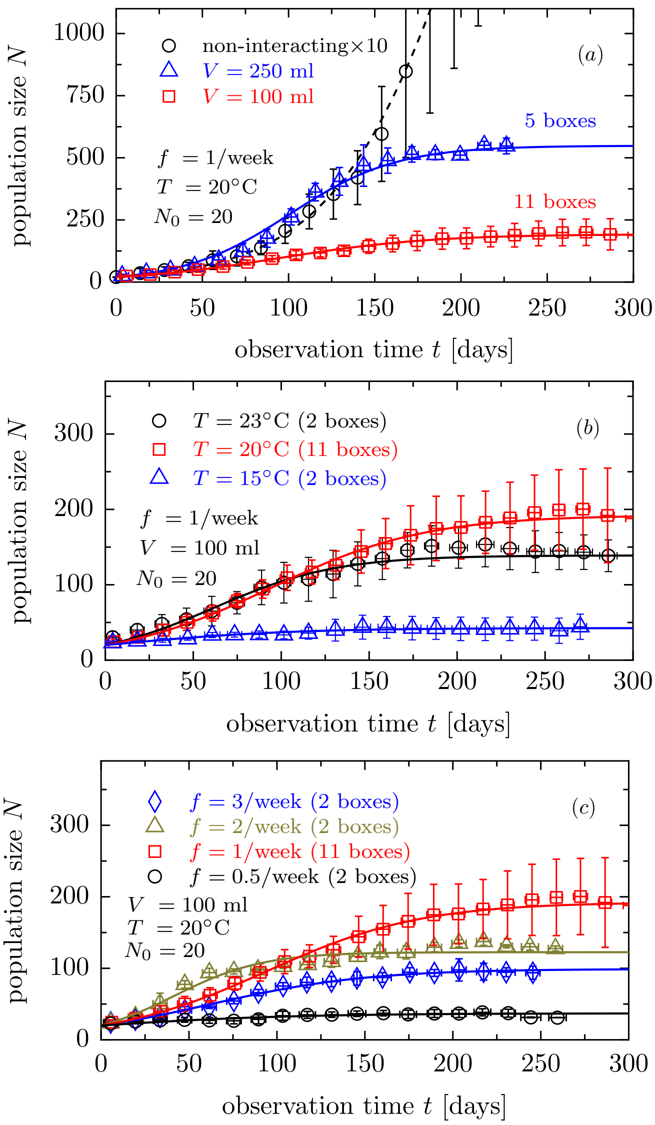

Figure 3. Self-interacting populations under different physical conditions. (*a*) Different volumes V at same T, f: for comparison, we also plot the growth curve of the non-interacting population (dashed/○) from figure 2(*d*), renormalized to $N_0 = 20$. (*b*) Different temperatures T at same V, f. (*c*) Different feeding frequencies f at same V, T. Solid lines represent the best fit of the logistic model (9) to the raw data, using the fixed initial value $N_0 = 20$ and fit parameters from table 1. To obtain an averaged population dynamics (symbols), data were binned in successive time intervals of length $\Delta t = 14$ days; time and worm count coordinates of data points in each bin were averaged with error bars indicating the sample standard deviations.

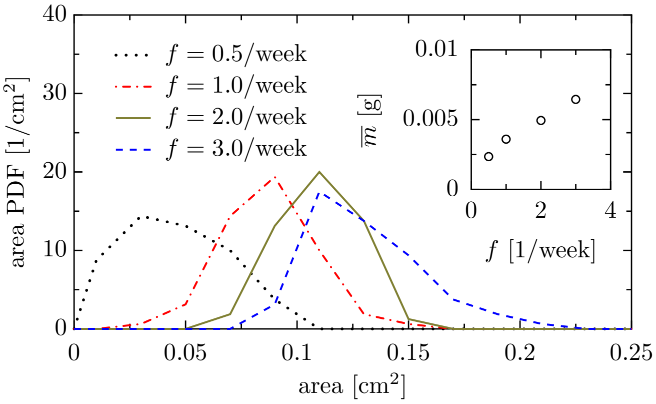

Figure 4. Experimentally determined worm-size (area) distribution at four different feeding frequencies. The inset shows the mean mass per worm. Sample size: 80 worms per feeding frequency.

to increased excrements in the water; other worms feeding on dying siblings led to an accelerated death of the entire population. At high worm densities, similar observations were made for populations that were fed three times per week, but these populations could recover.

4. Conclusions and outlook

We have presented the first detailed population analysis of the asexually reproducing freshwater planarian species *S. mediterranea*. Our long-term study of non-interacting families shows that the fission waiting time of head fragments is approximately exponentially distributed. By contrast, the corresponding tail fragments exhibit a non-exponential waiting time distribution whose multi-modal fine-structure implies that the underlying fission statistics is non-Markovian. To predict the observed distributions from biological and biophysical principles and to understand their evolutionary meaning pose interesting, unsolved questions for future investigations. Their answer will require a deeper understanding of the interplay of stem cell biology and biomechanics during fission and regeneration. While significant progress has been made in the last few years in planarian stem cell biology [13, 14, 17, 18, 36, 50], the study of fission biomechanics has been experimentally challenging. The development of new techniques for fission induction and observation on the microscopic scale will be necessary for a complete understanding of the observed memory effects.

From a more mathematical perspective, our results suggest that the family trees of asexually reproducing planarians may provide an interesting realization of non-Markovian binary trees. A related theoretical problem concerns the question whether the empirically measured waiting time distributions can be described by suitable generalizations of periodically modulated two- and multi-state stochastic models [51], e.g., by interpreting the fission process as a barrier crossing problem [52, 53] from a meta-stable pre-fission state to a more stable post-fission state that becomes gradually unstable due to regeneration and growth of the organism. From a biological point of view, however, it would be interesting to perform a comparative analysis of the population dynamics in asexually reproducing invertebrates and to investigate whether similar memory effects can also be observed in *Hydra* [4–6] and *Nematostella* [7, 8].

Finally, our experiments corroborate that interactions and physical parameters (e.g. temperature, volume) strongly affect the population dynamics of competing individuals [29], which can to a good approximation be described by standard logistic growth models [31]. However, our study also revealed an unexpected non-monotonic relationship between food supply and population growth: *S. mediterranea* become 'obese' if the feeding rate exceeds a critical threshold, leading to a decreased reproduction rate. This remarkable observation deserves further investigation on the molecular level in the future. Since *S. mediterranea* possess many genes homologous to those of humans [14, 32, 54], these organisms could become a useful model to study dietary effects on reproduction and regeneration, in addition to their importance in stem cell research [13, 14, 32]. A particularly interesting question in this context concerns whether or not genotype-by-diet interactions, as recently reported for the fruit fly *Drosophila m.* [55], can also be observed in planarians and other asexual invertebrates.

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