Non-senescent species are not immortal: stress and decline in two planaria species

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Conflict of Interest Statement

The authors have no conflict of interest to declare.

Author Contributions

RSG conceived the idea; JAD, RSG, and AA designed the methodology; JAD and PH collected and digitized data; JAD analysed data; JAD led the writing of the manuscript with assistance from RSG. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability

The data in this paper are published as supporting information in the open access version deposited in bioRxiv.org (DOI: 10.1101/2023.01.12.523873; Deere et al. 2022) (data are in multiple formats; *e.g.*, .pdf, .csv, .cpproj).

ABSTRACT

1. Potential immortality is observed in several species (*e.g.*, prickly pear cactus, hydra, flatworms) and is indicative of their negligible or even negative senescence rates. Unlike in senescent species, which experience reduced individual performance with age due to physiological degradation, species with negligible or negative senescence display mortality rates that remain constant or decline with age, respectively. These rates vary across taxa and are correlated with life history traits. Yet, the extent to which variable resource availability, a key driver of variation in life history traits, impacts species that show negligible or negative senescence is currently unknown.
2. Here, we examine whether and how variation in the quantity, quality, and feeding interval of resources impact population structure, population performance, and life history trait trade-offs in two long-lived planaria that do not senesce: *Schmidtea mediterranea* and *Dugesia tahitiensis*. In a full factorial design, different combinations of resource quantity (reduced intake, standard intake and high intake) and quality (high and low quality) were provided in two different feeding intervals (7-day and 14-day intervals) for 19 weeks.
3. We show that variability in resource availability, via decreases in quantity, quality, and frequency of resources, does not diminish population viability in either species but does result in suboptimal conditions of stress in *S. mediterranea*.
4. The high population viability we report can be attributed to two different mechanisms: increased reproduction or increased investment into maintenance at the expense of reproduction. Moreover, which mechanism was responsible for said high population viability was context-dependent and modulated by the specific life history strategy of the two planaria species.

5. We show that suboptimal conditions can cause stress responses that have significant impacts on non-senescent species. The context-dependent response we observe suggests that species that do not senesce but are subject to suboptimal conditions of stress may ultimately exhibit declines in performance and ultimately die. A clearer understanding of the impact of suboptimal conditions of resource availability on non-senescent species is needed to determine the extent of stress experienced and ultimately whether a species can truly be immortal.

Keywords: immortality, diet, life history trait, planaria, population structure, senescence, trade-off, vitality.

INTRODUCTION

A hydra (a potentially immortal metazoan (Martínez, 1998)) walks out of a bar… and is run over by a bus. Nothing lasts forever, including potential immortality. How age impacts individual performance and fitness has received much attention (Baudisch, 2011; Baudisch & Stott, 2019; Caswell & Salguero-Gómez, 2013; Kirkwood, 1977; Medvedev, 1990; J. W. Vaupel, 2010). The reduction of individual performance with age due to physiological degradation, senescence (Finch, 1994; Jones et al., 2008), was initially thought to be universal, although recent evidence shows that not all species reduce individual performance with age (review by Flatt & Partridge, 2018; (Caswell & Salguero-Gómez, 2013; Jones et al., 2008, 2014; Roper et al., 2021; but see Nelson & Masel, 2017). Indeed, some species like the ocean quahog (*Arctica islandica*) (Abele et al., 2009), sugar cone pine (*Pinus lamertiana*) or guppy (*Poecilia reticulata*) (Roper et al., 2021) have mortality rates that remain constant with age, while other species display mortality rates that decline with age (Roper et al., 2021; Vaupel et al., 2004). The former phenomenon is known as negligible senescence (Finch, 1994, 2009; Vaupel et al., 2004) and the latter as negative senescence (Jones et al., 2014; Vaupel et al., 2004). Negative senescence has been reported in hydra (Martínez, 1998), prickly pear cactus (*Opuntia rastrera*) (Roper et al., 2021), or flatworms (planaria, Phylum Platyhelminthes) (Sahu et al., 2017). Crucially, regardless whether a species is senescent, negligibly senescent, or negatively senescent, its rates of senescence typically correlate with other life history traits (*e.g.*, generation time; Healy et al., 2019; Salguero-Gómez et al., 2016), with the magnitude of actuarial (= survival) senescence largely coupled by the speed of the life history (Jones 2008).

The underlying correlation between life history traits and rates of senescence then raises the question of how drivers of variation in life history traits ultimately impact senescence. Resource availability is a key driver of individual development and population performance (Ozgul et al., 2009; Smallegange, 2011; Stearns, 1992). Yet, studies on how resource availability impacts the life

97 cycle of an organism have largely focused on specific life transitions (*e.g.,* age at maturity (Auer,
98 2010; Smallegange, 2011; Stearns, 1992) or features of the life cycle (*e.g.,* reproduction (Rutstein et
99 al., 2005; Williams, 1996); survival (Ozgul et al., 2009)). Survival, development, and reproduction
100 are key demographic processes in the life cycle of any species. Indeed, these demographic
101 processes define life history traits (*e.g.*, age at maturity, longevity, *etc.*) that, in turn, produce life
102 history strategies that shape individual fitness and dynamics of a population (Roff 1992; Stearns
103 1992; Capdevila & Salguero-Gómez 2019). Life history theory predicts a trade-off in resource
104 allocation between maintenance (survival and development) and reproduction of individuals (Roff,
105 1992; Stearns, 1992). However, the impact of resource availability on these demographic processes,
106 and therefore on life history traits, is not straightforward. The impacts of variability in resources on
107 demographic process can be non-linear (*e.g.*, indirect winter climate on summer foraging
108 conditions; Mysterud et al., 2001) but can also interact with other factors (*e.g.*, anthropogenic land
109 use change) resulting in demographic impacts being exacerbated or buffered (Maron et al., 2015).

110 Given the nuanced impact that resources have on life history traits, a key question in ecology
111 and evolution is to what extent negligibly and negatively senescent species are impacted by
112 variable resource availability. Here, we contribute to filling this gap of knowledge by examining the
113 impact of variability of resources in two long-lived invertebrate species, the planarians *Schmidtea*
114 *mediterranea* (Benazzi, Baguna, Ballister, Puccinelli & Del Papa, 1975) and *Dugesia tahitiensis*
115 (Gourbault, 1977). Obligate asexual planaria do not show physiological senescence (Sahu et al.,
116 2017). In these animals, stem cells also function as germ line, and so many of the well-known
117 somatic mechanisms of senescence (Finkel & Holbrook, 2000; Lemaître et al., 2024; Payne &
118 Chinnery, 2015) do not apply to obligate asexual planarian stem cells (Sahu et al., 2017). However,
119 individuals of planarians can still suffer suboptimal conditions of stress, shrink, and even die (*e.g.*,
120 Ofoegbu et al., 2016). These features provide the perfect opportunity to assess the role of variable

121 resource availability in non-senescent species. Specifically, we focus on the impacts of variable
122 resource availability on planaria population size and population structure, with the rationale for
123 said focus detailed below.

124 Variation in vital rates (*e.g.*, age-specific survival) dictate population level processes such as
125 population growth rate and extinction risk (Hernández-Yáñez et al., 2022; Lande & Orzack, 1988).
126 Where survival does not decline with age, an indication of negligible or negative senescence,
127 population level processes may differ from populations where survival does decline with age
128 (actuarial senescence). For example, in organisms where survival declines with age, the growth rate
129 of their populations is typically reduced compared to populations of organisms with negligible
130 senescence (Edelfeldt et al., 2019). Crucially, variation in survival trajectories that are caused by age
131 effects may also be driven by environmental factors (Edelfeldt et al., 2019; Roach et al., 2009).
132 Therefore, changes at the population level (*e.g.,* size) due to variable resources can be an indicator
133 of changes in survival trajectories of individuals and potentially "senescence" status. Population-
134 level assessments of the impact of variable resources on survival trajectories are a well-established
135 approach in organisms such as yeast (*Saccharomyces cerevisiae*, Jiang et al., 2002). In yeast, lifespan
136 is assessed by the number of times cells within a multicellular colony produces daughter cells
137 through budding (Jiang et al., 2002). Such an approach is then also amenable to assess the impacts
138 of variable resources on mean life expectancy of a population of other species whereby clonal
139 reproduction is a key life history trait (*e.g.*, planaria, Malinowski et al., 2017). Furthermore, focusing
140 on the population level provides the opportunity to assess how variable resources shape population
141 structure. Indeed, population structure can play a key role in the dynamics of a population through
142 the changes of the ratio of large/old individuals to smaller/younger individuals (Benton & Grant,
143 1999; Caswell, 2010).

144 Here, we develop a full factorial design where we experimentally manipulate three different

145 dimensions of resource availability (quantity, quality, and feeding interval) to examine how
146 resource variability impacts the population structure, population performance, and life history trait
147 trade-offs of two potentially immortal species, the planarians *S. mediterranea* and *D. tahitiensis*.
148 We test three hypotheses regarding the impacts of these three dimensions of resource availability
149 on population size and structure, and underlying life history trade-offs, as detailed in Table 1.

150

151 **METHODS**

152 **Study system**

153 To examine how resource variability impacts the population structure, population viability, and
154 life history trait trade-offs, we chose planaria as a study system due to their key life history traits.
155 Notably, the majority of planarians are capable of restorative and physiological regeneration due to
156 injury or damaged/sick cells (Elliott & Sánchez Alvarado, 2013). Their ability to replace damaged or
157 sick cells contributes to the typically long-lived life history of planarians (Elliott & Sánchez Alvarado,
158 2013). Indeed, under favourable, constant conditions, planaria are theoretically immortal (Elliott &
159 Sánchez Alvarado, 2013). Planaria are carnivorous and feed on live arthropods, annelids, and
160 molluscs. However, planaria also scavenge on individuals that have recently died (Abel et al., 2022;
161 Vila-Farré & C. Rink, 2018). Prey preference has also been shown in several planaria species (Vila-
162 Farré & C. Rink, 2018). Nonetheless, this preference can vary among localities and when the
163 preferred prey is scarce (Vila-Farré & C. Rink, 2018). Importantly, individual planarians are also able
164 to shrink when faced with unfavourable conditions (González-Estévez, 2009).

165 Here, we study responses to variability in resource availability by *Schmidtea mediterranea* and
166 *Dugesia tahitiensis*, two closely related, freshwater planarian species from the order Tricladida.
167 Both species are free-living and naturally occur in slow running and still water, but are easily reared

168 in the lab (Sousa & Adell, 2018). The two species also differ in size, with *D. tahitiensis* being larger
169 (≈10mm in length) than *S. mediterranea* (≈5mm). *S. mediterranea* can reproduce sexually and
170 asexually, whereas *D. tahitiensis* is an obligate asexual. We used asexual lines of *S. mediterranea* for
171 our experiments to directly compare the two species. Asexual reproduction in planaria occurs via
172 binary fission, which results in two genetically identical clones of smaller size from the fissioning
173 individual (Malinowski et al., 2017). Details of stock culture maintenance are described in the
174 Supporting Information.

175

176 **Experiment**

177 To test our hypotheses regarding how variability in resource availability impacts populations of
178 *S. mediterranea* and *D. tahitiensis* (Table 1), we randomly assigned populations to a full-factorial
179 design for each of the two species. This design included three resource factors: quality, quantity,
180 and feeding interval (Fig. 1, Box 1). Quality of resource had two levels, differing in energy content
181 and relative protein-to-carbohydrate ratio: high quality (high carbohydrate level, *HQ* hereafter) *vs.*
182 low quality (low carbohydrate level, *LQ* hereafter). The high quality diet was organic calf's liver and
183 the low quality diet bloodworm. The protein-to-carbohydrate ratio indicates resource quality, with
184 low carbohydrate-to-protein content corresponding to poorer quality (Lee et al. 2008). The two
185 diets differed in their energy content (109 kcal/100g in the liver *vs.* 14 kcal/100g in the bloodworm)
186 and in their relative protein-to-carbohydrate ratios (1:0.35 in liver *vs.* 1:0.17 in bloodworm).
187 Quantity of resources had three levels, which differed in the amount of calories available in each
188 feeding: standard calorie intake (*SI*: 0.001g/individual, representative of intake to maintain
189 laboratory populations (Jochen, 2018)), restricted calorie intake (*RI*: 0.0005g/ind.), hyper-calorie
190 intake (*HI*: *ad libitum* which was at least 10 fold, approx. 0.01g/individual, that of the SI). Different
191 combinations of resource quantity and quality were provided at two different intervals: once every

192 seven days (*7D*), or every 14 days (*14D*). The total amount of resources provided over a 14-day
193 period was equivalent for both feeding interval treatments (*i.e.*, one 14-day interval equalled two 7-
194 day intervals) (see Fig. 1). As such, across the most extreme diet treatments, there is over a 300-
195 fold difference in calories provided (per individual per unit time). Within our experimental setup,
196 the equivalent to a control would be either the high quality (*HQ*), standard intake (*SI*), seven days
197 interval (*7D*) treatment (*i.e.*, *HQ-SI-7D*) or the low quality (*LQ*), standard intake (*SI*), 14 days interval
198 (*14D*) treatment (*i.e.*, *LQ-SI-14D*). Here, we determine resources provided at an interval of every
199 seven days (*7D*) of a high quality non-restricted (standard intake for the organism) diet as our
200 control (i.e., *HQ-SI-7D*).

201 To evaluate interactive effects of resource availability on population structure, population
202 viability, and life history trait trade-offs, we replicated all treatments in the full factorial (n = 12
203 levels: 2 qualities: *HQ vs. LQ*; 3 quantities: *RI*, *SI* & *HI*; 2 intervals: *7D vs. 14D*) five times for each
204 species. A replicate consisted of a petri dish (25mm deep × 150mm Ø) containing 10 clonal
205 individuals each, henceforth referred to as a population (total n = 600 ind./spp.). For each
206 population, individuals were randomly isolated from one of the three stock cultures and placed in
207 the population 7-8 days before the start of the experiment. Importantly, as individuals of both
208 species are clonal, and stock cultures were initiated with individuals from the same cohort,
209 individuals within each population were of the same chronological age. During this time, individuals
210 were not fed to ensure individuals across all treatments were in a similar starved state at the start
211 of the experiment. Treatment populations were randomised into three blocks containing 20
212 populations per species per block. For each species, all experimental blocks lasted 19 weeks (133
213 days). Blocks were staggered in three different start dates due to the logistical constraint of
214 sampling all 60 populations on the same day, as such staggered blocks allowed a more logistically
215 feasible sampling of 20 populations per day. Block 1 of the experiment started on the 4th February
216 2020, and block 2 on the 30th April 2020. In block 3, the start date for *S. mediterranea* and *D.*

217 *tahitiensis* differed due to low numbers available for *S. mediterranea* from the stock populations. As
218 such, block 3 for *D. tahitiensis* started on the 1st May 2020 and for *S. mediterranea* on the 19th June
219 2020. Part way through the experiment, due to the COVID-19 pandemic, the location of the
220 experiment was changed within 2 hours, while retaining the same environmental conditions, see
221 Fig S1 in the Supporting Information.

222 Within each population, every week, population extinction was determined, and asexual
223 reproduction (clonal reproduction) estimated by counting all living and dead individuals. The weekly
224 determination of population extinction and estimation of reproduction was done to determine
225 changes in population size. The expectation is that, in H1 and H3 (Table 1), risk of population
226 extinction will decrease due to increased reproduction, while in H2 (Table 1) extinction risk will
227 decrease due to investment in maintenance. Additionally, size (area in mm^2) of each individual
228 flatworm within each population was measured weekly to determine growth/shrinkage. Size
229 measurements were calculated from photographs taken of each population (details of image
230 digitization can be found in the Supporting Information). Our expectations here are that for H1,
231 individual size will be normally distributed as the average resource intake among individuals will not
232 be impacted due to the high quantity of resources available. For H2, environments will be
233 dominated by larger individuals due to competitive effects; and for H3, reduced feeding interval of
234 resources will result in greater rate of size oscillation as more frequent resources does not restrict
235 fissioning events to larger individuals (Nentwig & Schauble, 1974).

236

237 **Size distribution analysis**

238 To test impact of variation in resource availability on individual body size, we used the size
239 spectrum of populations as a method of quantifying the distribution of body size (Edwards et al.,
240 2017). Where populations are increasing in size, with increases in small individuals, this implies

increased reproductive output. To do so, we calculated the size spectra slope values (*b*) for each population, of both species, across treatments following Carvalho et al. (2021). Briefly, *b* describes the relative abundance of a population in relation to body sizes, thus providing a good proxy to quantify the distribution of body size within a population. A steeper (more negative) *b* indicates fewer large-bodied and/or more small-bodied individuals, which is an indication of a right-skewed size population structure. Values of *b* can vary substantially with estimates ranging from -6.75 to -0.40 been reported (Arranz et al., 2021; Carvalho et al., 2021). To estimate *b*, for each species separately, we used the abundance density function (Carvalho et al., 2021):

$$N(x) = nx^{b}\left(\frac{b+1}{x_{max}^{b+1} - x_{min}^{b+1}}\right) \quad (1)$$

where n is the number of individuals, x the area of each individual (mm^2), x_{max} and x_{min} are the maximum and minimum area of individuals that were digitized. To calculate the maximum and minimum values, all populations for each treatment were pooled and maximum and minimum values calculated separately for each treatment for each week.

For each week of the experiment, *b* values were calculated using the area measurements of each individual within a given population. The *b* value for each of the five populations of each treatment was calculated. As such, here *b* relates to how the populations responds to the varying quality, quantity, and feeding interval of resources provided. Next, we applied a Bayesian framework to determine changes in the mean *b* values using the "bmrs" package (Bürkner, 2017) in R version 4.0.3 (R Core Team, 2020). Given that the estimated *b* values are based on size values, there is the potential that the *b* values may show a right-skewed distribution due to the ability of planaria to shrink. Thus, we initially compared models with a Gamma distribution to that of a Gaussian distribution to identify the model with the best fit. We identified that in the case of both

265 *S. mediterranea* and *D. tahitiensis* a model with a gaussian distribution had the best fit (Supporting
266 Information, Table S2). The final model used to model the response variable *b*, had a Gaussian
267 distribution and adaptive priors with treatment (categorical with 12 levels), week (19 weeks) and
268 block (3 blocks) as fixed variables and population (60 populations) as a random effect. Presented
269 are the median estimate and 50% and 95% compatibility intervals of the posterior probability.
270 Compatibility intervals can be considered equivalent to confidence intervals in a frequentist
271 approach (Gelman & Greenland, 2019). See Supporting Information for model details and Table S3
272 & S4 for final priors of each model.

273

274 **Population size analysis**

275 To determine the impact of variation in resource availability on population size, we calculated
276 changes in weekly population size for each species. We applied a Bayesian framework to determine
277 changes in population size using the same "bmrs" package as before. Population size was modelled
278 with a Poisson and Gaussian distribution to determine best fit. In the case of *S. mediterranea* the
279 model with the best fit was identified as one with a Gaussian distribution (Supporting Information,
280 Table S5) and adaptive priors, with treatment (categorical with 12 levels), week (19 weeks) and
281 block (3 blocks) as fixed variables, population as a random effect (60 populations) and population
282 size the response variable. In the case of *D. tahitiensis*, the model with the best fit had a Poisson
283 distribution with a log link function (Supporting Information, Table S5) using adaptive priors, with
284 treatment (categorical with 12 levels), week (19 weeks) and block (3 blocks) as fixed variables,
285 population as a random effect (60 populations) and population size the response variable. As
286 before, median estimated values are presented with 50% and 95% compatibility intervals. See
287 Supporting Information for model details and Table S6 & S7 for final priors of each model.

288 Crucially by analysing changes in population size and population structure (as identified by the
289 size distribution analysis), we can provide insights in potential reproductive output given our
290 knowledge of the biology and life cycle of both species (Carter et al., 2015; González-Estévez, 2009;
291 Malinowski et al., 2017; Nentwig & Schauble, 1974). For example, a population that is increasing in
292 its size with increases in the number of small individuals implies increased reproductive output. In a
293 population where individuals are shrinking, fissioning (reproduction) is not occurring and
294 populations are not increasing. As such there will not be overlap with the size distributions of small
295 individuals due to reproductive output and small individuals as a result of shrinking.

296

297 **RESULTS**

298 **Body size distributions**

299 Over time, size spectra slope values (*b*) for *S. mediterranea* became more negative for most
300 treatments (Fig. 2), implying a more right-skewed population. The consistent decline in *b* values
301 indicates a change in the size structure of the populations with an increase in smaller individuals
302 and fewer larger individuals. Overall, 14-day interval treatments had more negative *b* values than 7-
303 day interval treatments (Fig. S3). In the high-quality treatments, when compared to *HQ-SI-7D* (our
304 control), only the 14-day feeding interval high intake (*HQ-HI-14D*) and 14-day feeding interval
305 reduced intake (*HQ-RI-14D*) treatment had smaller *b* values and as such more right-skewed size
306 structure, with the *HQ-RI-14D* treatment fulfilling the size prediction of H2 (Table 1). This outcome
307 suggests that in high quality treatments populations changed in size structure with an increase in
308 the number of small individuals and a decrease in the number of larger individuals over time (Fig.
309 2). When changes in structure were found, this change in structure was more prominent in
310 treatments where feeding interval was 14 days. However, in the case of the low-quality treatments,

311 only the 7-day feeding interval reduced intake (*LQ-RI-7D*) treatment did not show a change in size
312 structure that differed from that of *HQ-SI-7D* (Fig. S3). All other low quality treatments showed a
313 greater change in size structure (*i.e.*, more negative *b* values) with more right-skewed populations
314 over time (Fig. 2). In the case of *LQ-HI-7D*, the size prediction of H1 was not fulfilled as size was
315 predicted to be normally distributed.

316 For *D. tahitiensis*, size spectra slope values (*b*) varied less in the 7-day feeding interval
317 treatments than the 14-day feeding interval treatments (Fig. 3). When compared to *S.*
318 *mediterranea*, *D. tahitiensis* populations were less right-skewed. When assessing the outcomes in
319 terms of the size predictions of H1 and H2 for *D. tahitiensis*, treatment *LQ-HI-7D* fulfilled the
320 predictions of H1 as body size was normally distributed (Table 1). However, for treatment *HQ-RI-*
321 *14D* the size predictions of H2, a right-skewed population, was not fulfilled (Table 1). In both species
322 experimental block 1 differed from block 2 and block 3 in several treatments (Fig. S3 & S4).
323 However, this pattern was not consistent between treatments and species (see Fig. 2 and 3, light
324 blue points). Still, even with the block differences, the trends of all blocks were the same
325 (increasing, decreasing, or stable). Overall, there was no consistent trend in how *b* values across
326 treatments differed to that of *HQ-SI-7D*. As such, treatment effect does not predict variation in size
327 structure over time.

328

329 **Population trends**

330 Overall, *D. tahitiensis* populations had higher numbers than populations of *S. mediterranea* at
331 the end of the experiment (Fig. 4 & 5). Population size of *S. mediterranea* varied between
332 treatments that differed in resource quantity (*HI*: high intake, *SI*: standard intake, *RI*: reduced
333 intake) and interval of feeding (*7D*: every seven days, *14D*: every 14 days) but was consistent across

334 resource quality (*HQ*: high quality, *LQ*: low quality) (Fig. 4A & B). The exception being the high
335 intake resource quantity treatments with a 7-day feeding interval (*HI-7D*). In this case, the *HQ-HI-7D*
336 treatment, where the resource was of a high quality, the population declined compared to the *LQ-*
337 *HI-7D* treatment, where the population increased over time (Fig. 4A & B, Fig. S5). All populations
338 where resource was provided every 14 days showed a decrease in population size over time
339 compared to populations when feeding interval was every seven days, where counts either stayed
340 constant (*HQ-RI-7D*) or increased over time (*HQ-SI-7D, LQ-HI-7D , LQ-SI-7D*) (Fig. 4A & B Fig. S5).
341 The exception here was the *HQ-HI-7D* treatment, where population size declined over time. The
342 decline in *HQ-HI-7D* does not fulfil the prediction of H3 where the expectation was an increase in
343 reproduction and a larger population size. However, the decrease in population size of treatment
344 *HQ-RI-14D* supports the population size prediction of H2. As such, the outcome of *HQ-RI-14D* fulfils
345 the full prediction of H2 that population size will decrease and body size will be right-skewed.

346 In *D. tahitiensis*, populations either increased or remained stable across all treatments (Fig. 5A
347 & B). When comparing *HQ* and *LQ* treatments, *HQ* treatments with feeding intervals of seven days,
348 regardless of resource quantity, had a higher population size than *LQ* treatments with 7-day feeding
349 intervals, except for *HQ-HI-7D* and *LQ-HI-7D*, which had similar counts. When resource was
350 provided every 14 days, there was no clear trend; the *HQ-HI-14D* treatment had lower counts than
351 the *LQ-HI-14D* treatment, *HQ-SI-14D* had higher counts than *LQ-SI-14D* and *HQ-RI-14D* had similar
352 counts to *LQ-RI-14D* (Fig. 5A & B, Fig. S6). In the case of *HQ-RI-14D* population size increased which
353 did not fulfil the population predictions of H2. For treatments *LQ-HI-*7D and *HQ-HI-7D* population
354 size increased, thus fulfilling the population predictions of H1 and H3 respectively. For both species,
355 experimental block 1 (light blue lines, Fig. 4 & 5) had population growth rates lower than block 2
356 and block 3. However, for all treatments, the population response (increasing, decreasing, or stable
357 counts) of block 1 did not differ from that of block 2 or block 3 (Fig. 4 & Fig. 5, Fig. S5 & S6).

358

359 **DISCUSSION**

360 Compared to senescent species (Lemanski et al., 2020; Pardo et al., 2013), a comprehensive
361 understanding of the impact of variable resource availability on species that show negligible or
362 negative senescence is lacking (but see Schaible et al., 2011). Here, we contribute to this gap of
363 knowledge by incorporating variation in resource availability in two non-senescent species: the
364 flatworms *S. mediterranea* and *D. tahitiensis* (Sahu et al., 2017). Our findings show how, when
365 multiple factors of resource availability interact (*i.e.*, quality, quantity and frequency), these non-
366 senescent species show population-level responses expected from suboptimal conditions of stress.
367 Specifically, in *S. mediterranea*, we observe suboptimal conditions of stress through population
368 decline and individual shrinkage when resources are made available are every 14 days, regardless of
369 the resource quality or quantity. However, conditions of stress in *Dugesia tahitiensis* are less
370 frequent and occur only when resources are reduced, regardless of resource intervals, with only
371 individual-level shrinkage being noted as evidence of stress, since we observe no population
372 decline.

373 The way these factors of resource availability impacts individuals within populations is context
374 dependent in our experiment. The high population viability (only two of the 20 populations went
375 extinct in our experiment, both were *S. mediterranea*) and variable response in reproduction (as
376 proxied by population size and structure) across treatments is also likely driven by the specific life
377 history strategy of our species. Generally, planarians can restore their own damaged cells (Elliott &
378 Sánchez Alvarado, 2013). However, restoring damaged cells is costly and requires resources (Akashi
379 & Gojobori, 2002). Naturally, if most resources are used for maintenance, fewer resources will be
380 available for reproduction. The high population viability we report here in response to the variable
381 resource availability is indicative of the fundamental trade-off between survival and reproduction

382 under variable environmental conditions (Stearns, 1992). Here, theory predicts that, in long lived
383 species such as our planarians (Elliott & Sánchez Alvarado, 2013), resources will be primarily
384 invested in maintenance of individuals and not reproduction (Erikstad et al., 1998; Sæther et al.,
385 2013). As highlighted earlier, reproduction in planaria occurs via binary fission, and so individuals in
386 our experimental setup are clonal and thus of the same age. As such, any changes in the structure
387 and size of a population can be thought of as a reflection of the survival-reproduction trade-off. A
388 less right-skewed population, where there are fewer smaller individuals, is indicative of reduced
389 reproduction as new recruits are smaller. In these populations of reduced reproduction, individuals
390 invest in maintenance that results in reduced/no growth with little change in body size. In general,
391 for *S. mediterranea*, such a survival-reproduction trade-off can be observed across the 7-day
392 feeding treatment, but not when the feeding interval was every 14 days, where population size
393 decreased, indicating reduced reproduction and reduced survival of individuals. For *D. tahitiensis*,
394 only populations of low quantity and with a feeding interval every 14 days show a survival-
395 reproduction trade-off of high survival and reduced reproduction. Such context-dependant trade-
396 offs have also been shown in *Hydra magnipapillata*, another non-senescent species (Schaible et al.,
397 2011). There, the authors found a clear maintenance-reproduction trade-off when resources and
398 temperature were constant, whereby investment into reproduction resulted in reduced survival.
399 However, under fluctuating environmental conditions, the authors reported no clear maintenance-
400 reproduction trade-off. This context-dependent survival-reproduction trade-off then begs the
401 question, where there is an apparent lack of trade-off between survival and reproduction (see
402 Metcalf 2016), do we see a trade-off with body size?

403 The size structure of our populations varied across treatments, but not in a way that was
404 consistent with our hypotheses (Table 1). For *S. mediterranea*, populations become more right-
405 skewed across treatments, with two exceptions in the 7-day feeding interval treatment. The right-

406 skewed populations describe an increase in the number of smaller individuals and decline of larger
407 individuals in the populations (Carvalho et al., 2021). Such changes in population structure can
408 occur when there is increased reproduction, as more recruits of a smaller size enter the population.
409 *S. mediterranea* reproduce asexually through fission, where larger individuals fission into two, with
410 the posterior product (new recruits) generally smaller than the anterior fission product (now a
411 smaller individual too) (Peter et al., 2001). Thus, fissioning results in fewer larger individuals and an
412 increase in the number of smaller individuals. However, the right-skewed size structure due to
413 reproductive events will only be reflected in populations that are increasing in number as in these
414 populations no mortality of individuals was observed. Where *S. mediterranea* populations show a
415 decline or remain stable in size, the change in structure is not driven by decreased reproduction.
416 Instead, the life history of planaria seems to be driving the response. A key trait in the life history of
417 planaria is the ability to shrink when resources become scarce (Mangel et al., 2016; Rink, 2013).

418 The ability to shrink allows individuals of some species to survive resource-scarce periods of
419 time until conditions improve (Ebert, 1967; Marinovic & Mangel, 1999; Wikelski & Thom, 2000). In
420 planaria, when individual shrinkage occurs, cell numbers reduce through autophagy during which
421 energy resources are supplied to stem/regenerative (neoblasts) cells (González-Estévez, 2009;
422 Romero & Baguñà, 1991). This process ensures that maintenance levels can be met and as
423 individuals attempt to maintain homeostasis, fissioning (asexual reproduction) does not occur.
424 Furthermore, in *S. mediterranea* and in sister species of *D. tahitiensis* (*i.e.*, *D. japonica* and *D.*
425 *dorotocephala*) the probability of fissioning declines as individuals get smaller, with a minimum size
426 where fissioning can occur (Carter et al., 2015). In the case of sexually reproducing planaria,
427 reproductive organs are often consumed during shrinkage (Saló, 2006), thus preventing further
428 reproduction. Shrinkage is a strategy found in several taxa and been suggested to mitigate the
429 impact of fluctuating or variable environments on individual survival (Ebert, 1967; Marinovic &

430 Mangel, 1999; Salguero-Gómez & Casper, 2010, 2011). Indeed, shrinkage maintains relatively high
431 levels of survival during periods of resource scarcity compared to strategies where shrinkage does
432 not or cannot physically occur. As such, the high levels of survival ensure population viability. For
433 instance, in the Galápagos marine iguanas (*Amblyrhynchus cristatus*), individuals shrink up to 20%
434 of their body length when nutritional resources are low following El Niño (Wikelski & Thom, 2000);
435 those individuals that shrink more have a higher rate of survival, suggesting the adaptive value of
436 shrinkage. When nutritional resources improve during La Niña events, body length then increases
437 again, as is the case with planarians when they receive increased nutritional resources after periods
438 of low nutrient availability (González-Estévez, 2009; Thommen et al., 2019).

439 The impact of variable resources on body size due to shrinkage observed in *S. mediterranea*
440 populations is unlikely occurring in populations of *D. tahitiensis*. Fewer *D. tahitiensis* treatments
441 showcased populations becoming more right-skewed (*i.e.*, more smaller individuals) over the
442 course of the experiment. There, where populations become more right-skewed, populations do
443 not show a decline in population size and, unlike the case with *S. mediterranea*, changes in
444 population structure are likely due to fissioning. Populations of *D. tahitiensis* that have a less right-
445 skewed size structure increased in size during the experiment. Within these population, individuals
446 are likely experiencing increased competition for resources. Increased resource competition among
447 individuals results in contest competition, where an individual interferes with other individuals to
448 obtain resources (Nicholson, 1954). In competitive environments, smaller individuals are typically
449 outcompeted by larger individuals within the population as larger individuals tend to be more
450 dominant (Petersson & Järvi, 2000; Stewart & Tabak, 2011). In our study, contest competition
451 results in differences in average resource intake among individuals, with larger planarian individuals
452 able to consume more resources per feeding event. As such, competition between two individuals
453 of planarian naturally results in the larger of the two growing more. The competitive exclusion of

454 smaller individuals thereby increases the number of larger individuals in the population, resulting in
455 less right-skewed populations. Here, competition for resources resulted in mortality of smaller
456 individuals and faster growth in the surviving, larger individuals. Evidence of smaller individuals
457 being outcompeted by larger individuals has been observed in several species, including other
458 planaria (Reynoldson, 1964; Reynoldson & Sefton, 1972). For example, in a 20 month study of a lake
459 population of *Planaria torva*, Reynoldson and Sefton (1972) found changes in the size distribution
460 of individuals in the population that were attributed to intraspecific competition for resources.
461 Similarly, in the brown trout (*Salmo trutta*), Petersson & Järvi (2000) observed that sea-ranch trout,
462 which is 13% larger than its wild-type counterpart, had higher dominance than the latter.

463 The high population viability reported here highlights the ability of planaria to mediate impacts
464 of variation in resource availability. However, as mentioned before, the mechanisms that allow
465 shrinkage and regeneration of individuals do not preclude individuals from suffering suboptimal
466 conditions of stress and ultimately dying. Said resource-availability driven stress is observed in the
467 few *S. mediterranea* populations that went extinct and those populations declining in numbers.
468 Why such extinction events and declining numbers only occurred in populations of *S. mediterranea*
469 and not *D. tahitiensis* can be attributed to differences in their ability to regenerate. Regeneration is
470 not uncommon within the Metazoa, with almost every phylum having a species with this ability
471 (Alvarado, 2000). Furthermore, regeneration can be classified into two categories highlighting the
472 variability of the phenomenon: 1) morphallaxis, the regeneration in the absence of active cell
473 proliferation (*e.g.*, *Hydra*) and 2) epimorphosis, the regeneration with cell proliferation *(e.g.*, limb
474 and tail regeneration in vertebrates) (Alvarado, 2000; Morgan, 1901). In the case of planaria, both
475 forms of regeneration are utilised but are context dependent; morphallaxis to restore scale and
476 proportion (*e.g.*, when shrinking occurs) and epimorphosis for the formation of a new organ (*e.g.*,
477 due to a fission event) (Pellettieri, 2019).

478 The lack of local extinction and declining numbers in the examined populations of *D.*
479 *tahitiensis*, compared to *S. mediterranea*, is likely due to the former species being able to
480 implement epimorphosis and repair damaged cells more efficiently than the latter (Peter et al.,
481 2001). Indeed, *Dugesia tahitiensis* has one of the highest regenerative and fissioninig capacities and
482 the most neoblasts (adult stem cells found in Platyhelminthes and Acoela) within the Tricladida
483 taxon (Baguñá & Romero, 1981; Peter et al., 2001). High neoblast density in the cellular tissue
484 between the body wall and organs is a prerequisite for efficient regeneration after fission and,
485 when compared to *S. mediterranea*, *D. tahitiensis* have higher cell counts (Peter et al., 2001).
486 Moreover, neoblast self-renewal activity can be impacted by resource availability with reduced
487 resource levels resulting in lowered self-renewal activity (Mangel et al., 2016). Reduced neoblast
488 self-renewal activity will then enhance the negative impacts experienced by *S. mediterranea*. The
489 variation in self-renewal activity brought about by variation in resource availability will ultimately
490 impact survival trajectories of individuals. As such, the fittest individuals are more likely to survive
491 to extreme ages that increases the population's average survival, a pattern that has also been
492 described in other animals (Vaupel & Yashin, 1985). This age-by-environment interaction has also
493 been reported in some long-lived plants, *e.g.* in *Plantago lanceolata* (Roach et al., 2009) and
494 *Fumana procumbens* (Edelfeldt et al., 2019), where variation in survival trajectories are modulated
495 by resource availability.

496 No species is truly immortal. The changing environment can play a significant part in the levels
497 of stress and individual faces, regardless of its senescence rates. Here, we contribute to the under-
498 represented empirical demonstration of the impact of variation in resource availability in two
499 species that do not senesce. Our findings support previous work on another non-senescent species
500 (*Hydra magnipapillata*; Schaible et al., 2011), showing that the impacts of fluctuating resources on
501 the trade-off between reproduction and maintenance is context dependent. Importantly, we

502 highlight that two non-senescent species respond differently to variation in resource availability;
503 one species can suffer population extinction and the other population growth, highlighting
504 differential suboptimal conditions of stress under changing environmental conditions. A clearer
505 understanding of how fluctuating environments, particularly in resources availability, impacts
506 species that show the unique trait of negligible or negative senescence will be key to fully
507 understand the role of this life history trait and ultimately whether anything is indeed immortal.

508

509 **References**

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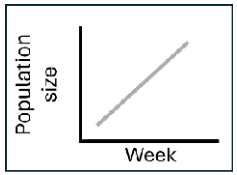
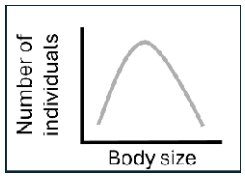
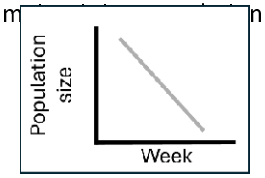
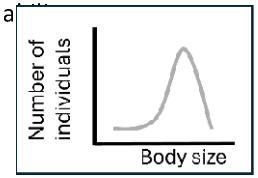
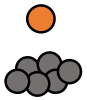
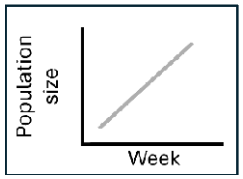
Table 1. Hypotheses of the impact of the variability in three different dimensions of resource availability (quantity, quality, and feeding interval) on population structure, population performance, and life history trait trade-offs in two species of Planaria; *Schmidtea mediterranea* and *Dugesia tahitiensis*. Here we show results for each species, supporting (✓) or rejecting (✕) each hypothesis. High quality (🟠); low quality (🟣); high intake (); reduced intake (⚫); feeding intervals every 14 days (14-Day); feeding intervals every seven days (7-Day).

Resource availability	**Prediction**	**Outcome**	
		D. tahitiensis	***S. mediterranea***
H1: High quantity, low quality environments 🟣 ⚫⚫⚫⚫ **7-Day**	Population size will increase, and individual size will be normally distributed. High quantity, low quality environments are richer in protein; these environments typically promote reproduction (Lee, 2015) and, as such, maintain population viability (Bayliss & Choquenot, 2002).  	✓	✕
H2: Low quantity, high quality environments 🟠 ⚫ **14-Day**	Population size will decrease and be dominated by larger individuals skewing the population size structure to the right. Environments with fewer, but higher quality resources, will likely be dominated by larger individuals that outcompete smaller individuals (Szabo, 2002; Ward et al., 2006). In environments with fewer high quality resources, available resources will be preferentially allocated to maintenance because, in organisms with indeterminate growth like planarians, energy allocation is typically prioritised for maintenance over reproduction and development (Jokela & Mutikainen, 1995), thus m[illegible]n via[illegible]  	✕	✓

H3: Environments with more resources of higher quality  **7-Day**	There will be an increase in clonal reproduction and subsequently larger population sizes. Where resource supply is high and constant, populations support higher numbers of individuals, typically decreasing population extinction risk (Bayliss & Choquenot, 2002; Lande et al., 2003) 	✓	✗

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BOX 1 Glossary

HQ-HI-7D: Populations provided with high quality (HQ), high intake (HI) resources at intervals of every seven days.

HQ-HI-14D: Populations provided with high quality (HQ), high intake (HI) resources at intervals of every 14 days.

HQ-SI-7D: Populations provided with high quality (HQ), standard intake (SI) resources at intervals of every seven days.

HQ-SI-14D: Populations provided with high quality (HQ), standard intake (SI) resources at intervals of every 14 days.

HQ-RI-7D: Populations provided with high quality (HQ), reduced intake (RI) resources at intervals of every seven days.

HQ-RI-14D: Populations provided with high quality (HQ), reduced intake (RI) resources at intervals of every 14 days.

LQ-HI-7D: Populations provided with low quality (LQ), high intake (HI) resources at intervals of every seven days.

LQ-HI-14D: Populations provided with low quality (LQ), high intake (HI) resources at intervals of every 14 days.

LQ-SI-7D: Populations provided with low quality (LQ), standard intake (SI) resources at intervals of every seven days.

LQ-SI-14D: Populations provided with low quality (LQ), standard intake (SI) resources at intervals of every 14 days.

LQ-RI-7D: Populations provided with low quality (LQ), reduced intake (RI) resources at intervals of every seven days.

LQ-RI-14D: Populations provided with low quality (LQ), reduced intake (RI) resources at intervals of every 14 days.

Figure legends

Figure 1. Full factorial experimental design to examine how three key dimensions of resource (quality, quantity, and interval of resource availability) shape population size, population structure and life history trait trade-offs of two flatworm species, *Schmidtea mediterranea* and *Dugesia tahitiensis*. Resource quality has two levels (top right panel): high quality (*HQ*; orange dots) and low quality (*LQ*; purple dots). Resource quantity has three levels (middle right panel): restricted intake (*RI*; single grey dot), standard intake (*SI*; three grey dots) and hyper caloric intake (*HI*; six grey dots). Resource interval has two levels (bottom right panel): every seven days (*7D*; black line), every 14 days (*14D*; grey line). Each population was initiated with 10 individuals and replicated five times resulting in a total of 600 individuals and 60 populations per species. Populations were counted and photographed weekly.

Figure 2. Population structure in *Schmidtea mediterranea* populations changes across time in most treatments. Weekly size spectra slope values (*b*) for *S. mediterranea*. A) Calculated size spectra slope (*b*) values (black points) for all populations for high quality (*HQ*,) treatments over 19 weeks. B) Calculated size spectra slope (*b*) values (black points) for all populations for low quality (*LQ*,) treatments over 19 weeks. Blue points indicate estimated *b* values with compatibility intervals (0.95) for each block (block 1 – light blue; block 2 – blue; block 3 – dark blue). Left panels in each graph indicate feeding intervals every 14 days and right panels feeding intervals every seven days. Top panel represent high intake (*HI*,), middles panel standard intake (*SI*,) and bottom panel reduced intake (*RI*,) treatments.

Figure 3. Population structure in *Dugesia tahitiensis* populations has little change in structure across calorie restriction treatments. Weekly size spectra slope values (*b*) for *D. tahitiensis*. A) Calculated size spectra slope (*b*) values (black points) for all populations for high quality (*HQ*,) treatments over 19 weeks. B) Calculated size spectra slope (*b*) values (black points) for all populations for low quality (*LQ*,) treatments over 19 weeks. Blue points indicate estimated *b* values with compatibility intervals ( ) for each block (block 1 – light blue; block 2 – blue; block 3 – dark blue). Left panels in each graph indicate feeding intervals every 14 days and right feeding intervals every seven days. Top panel represent high intake (*HI*,), middles panel standard intake (*SI*,) and bottom panel reduced intake (*RI*,) treatments.

Figure 4. Population size for *Schmidtea mediterranea* varies but with no clear patterns across calorie restriction treatments. Weekly population size of *S. mediterranea* over the 19 weeks for A) high quality (*HQ*,) treatments and B) low quality (*LQ*,) treatments. Black points indicate raw data of weekly population size; blue lines indicate estimated population size for each block (block 1 – light blue; block 2 – blue; block 3 – dark blue). Shaded grey regions indicate compatibility intervals: light grey 0.95, grey 0.8, dark grey 0.5. Left panels indicate feeding intervals every 14 days and right panels feeding intervals every seven days. Top panel represent high intake (*HI*,), middles panel standard intake (*SI*,) and bottom panel reduced intake (*RI*,) treatments.

Figure 5. Population size for *Dugesia tahitiensis* varies but with no clear patterns across calorie restriction treatments. Weekly population size of *D. tahitiensis* over the 19 weeks for A) high quality (*HQ*,) treatments and B) low quality (*LQ*,) treatments. Black points indicate raw data of weekly population size; blue lines indicate estimated population size for each block (block 1 – light blue; block 2 – blue; block 3 – dark blue). Shaded grey regions indicate compatibility intervals: light

817 grey 0.95, grey 0.8, dark grey 0.5. Left panels indicate feeding intervals 14 days and right

818 panels feeding intervals every seven days. Top panel represents high intake (*HI*,), middles

819 panel standard intake (*SI*,) and bottom panel reduced intake (*RI*,) treatments.

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821 **Figure 1**

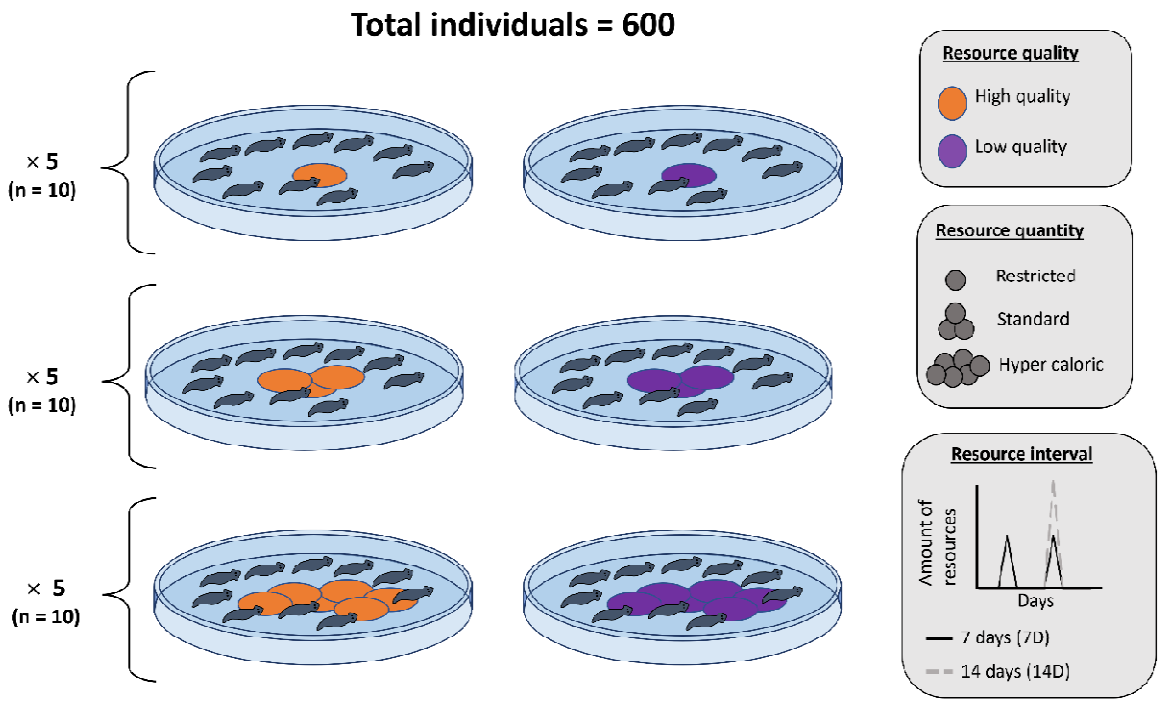

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Figure 2

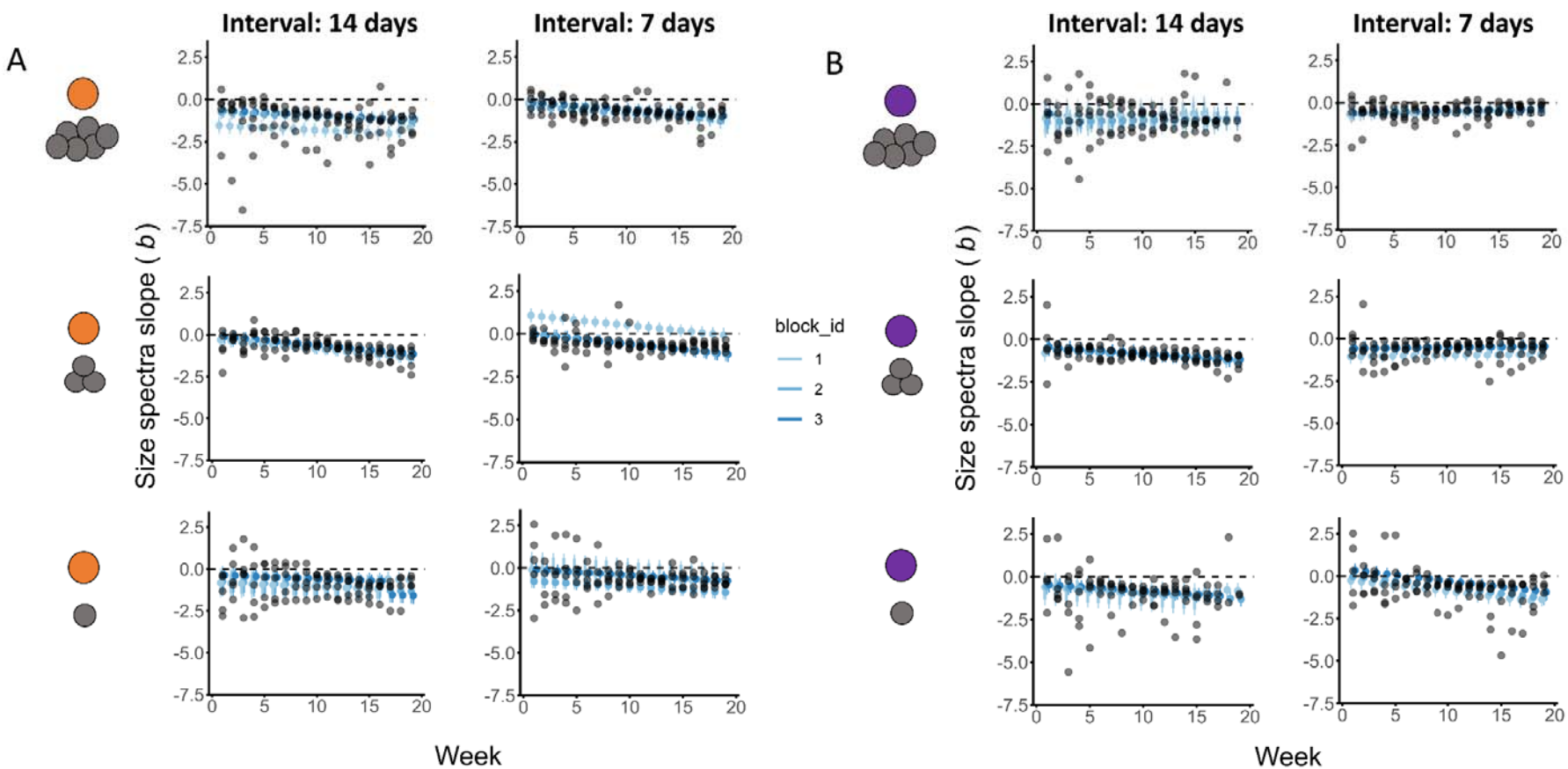
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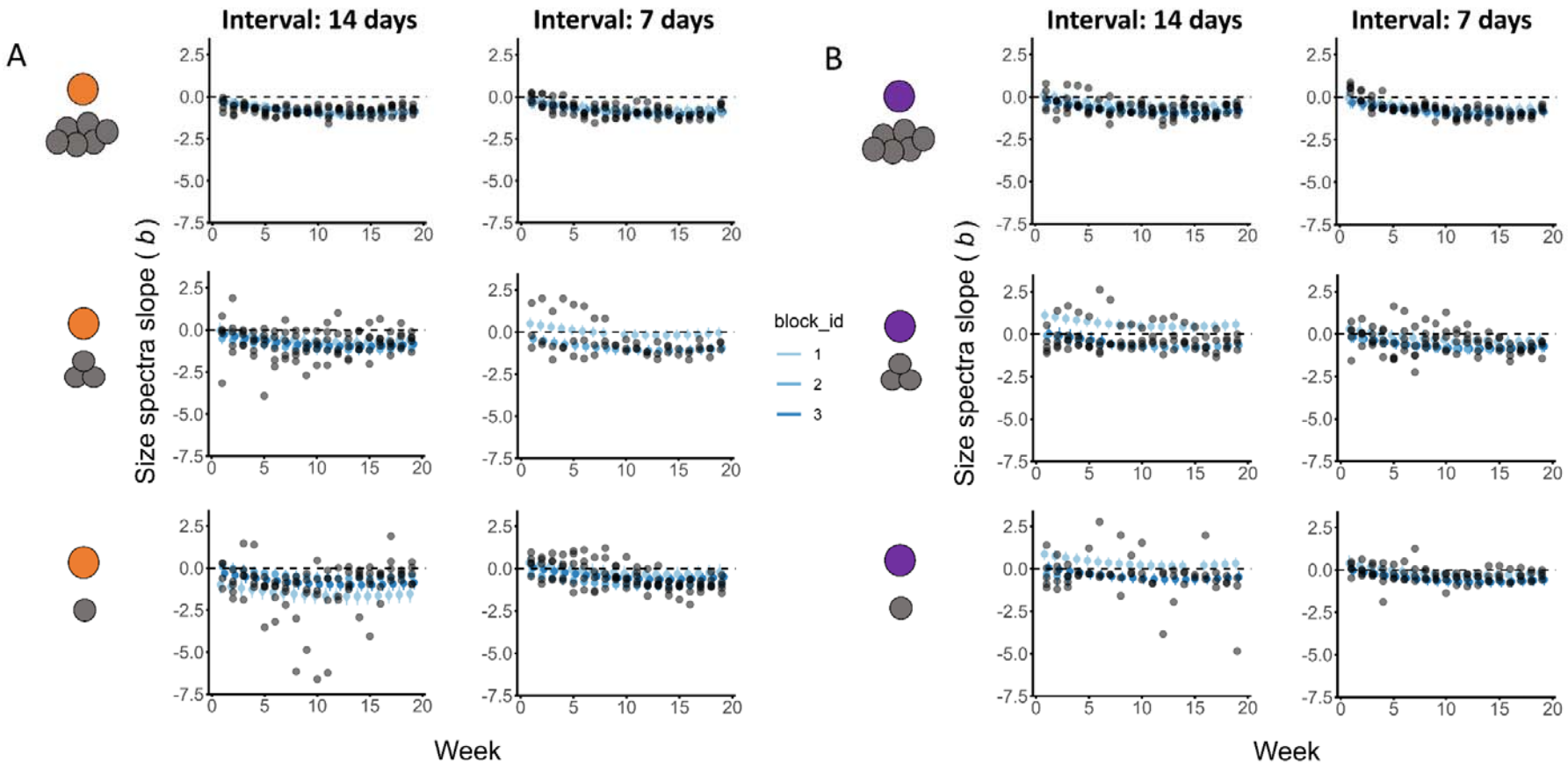
Figure 3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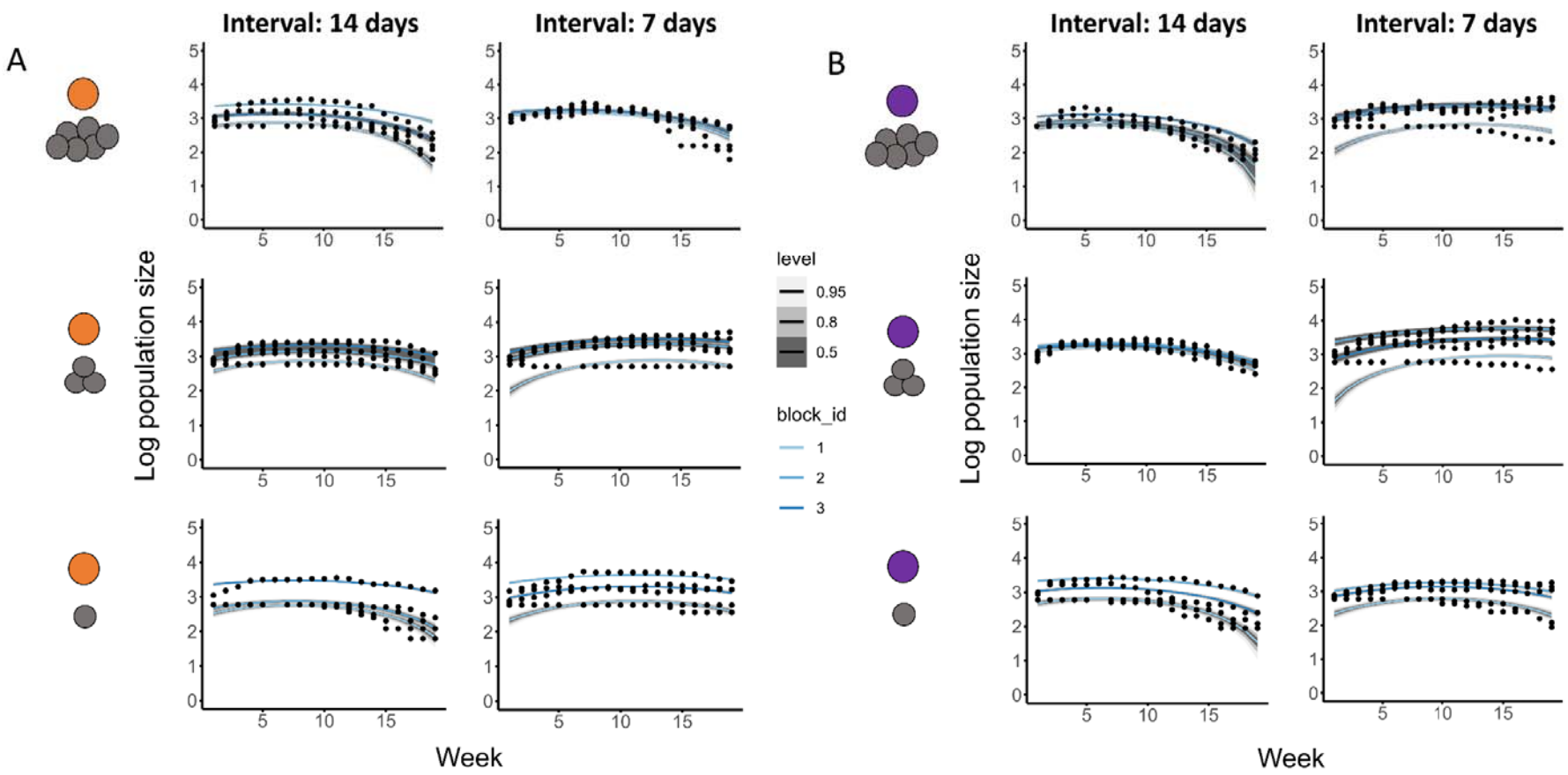
838 **Figure 4**



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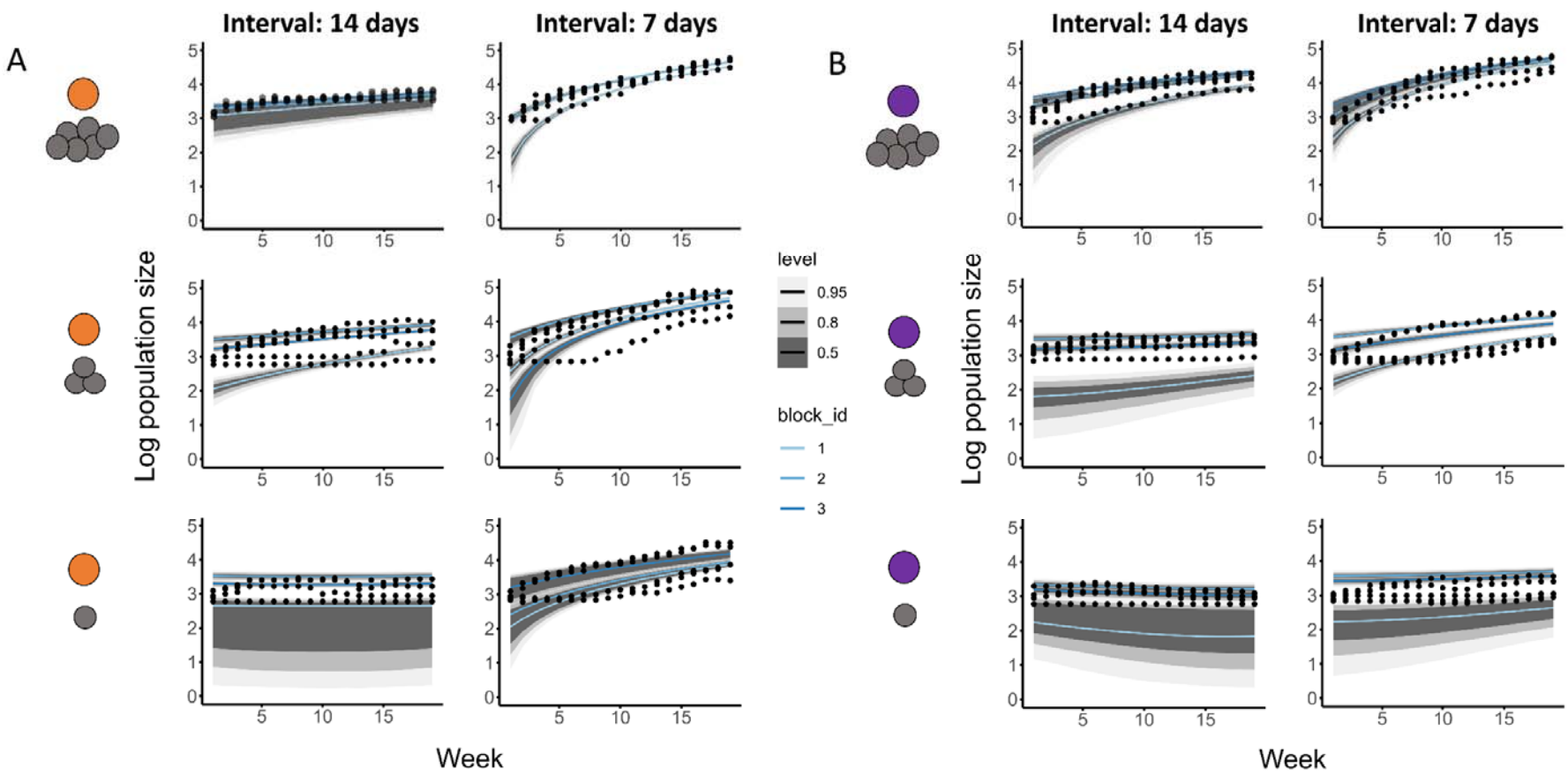
841 **Figure 5**



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