Transgenerational effects on development following microplastic exposure in *Drosophila melanogaster*

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Background. Plastic pollution affects all ecosystems, and detrimental effects to animals have been reported in a growing number of studies. However, there is a paucity of evidence for effects on terrestrial animals in comparison to those in the marine realm.

Methods. We used the fly *Drosophila melanogaster* to study the effects that exposure to plastics may have on life history traits and immune response. We reared flies in four conditions: In media containing 1% virgin polyethylene, with no chemical additives; in media supplemented with 1% or 4% polyvinyl chloride, known to have a high content of added chemicals; and control flies in non-supplemented media. Plastic particle size ranged from 23-500 μm. We studied fly survival to viral infection, the length of the larval and pupal stage, sex ratios, fertility and the size of the resultant adult flies. We then performed crossings of F1 flies in non-supplemented media and looked at the life history traits of the F2.

Results. Flies treated with plastics in the food media showed changes in fertility and sex ratio, but showed no differences in developmental times, adult size or the capacity to fight infections in comparison with controls. However, the offspring of treated flies reared in non-supplemented food had shorter life cycles, and those coming from both polyvinyl chloride treatments were smaller than those offspring of controls.

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17 **Abstract**

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21 **Methods.** We used the fly *Drosophila melanogaster* to study the effects that exposure to
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24 with 1% or 4% polyvinyl chloride, known to have a high content of added chemicals; and control
25 flies in non-supplemented media. Plastic particle size ranged from 23-500 μm. We studied fly
26 survival to viral infection, the length of the larval and pupal stage, sex ratios, fertility and the
27 size of the resultant adult flies. We then performed crossings of F1 flies in non-supplemented
28 media and looked at the life history traits of the F2.

29 **Results.** Flies treated with plastics in the food media showed changes in fertility and sex ratio,
30 but showed no differences in developmental times, adult size or the capacity to fight infections
31 in comparison with controls. However, the offspring of treated flies reared in non-
32 supplemented food had shorter life cycles, and those coming from both polyvinyl chloride
33 treatments were smaller than those offspring of controls.

34 **Introduction**

35 Plastics are found ubiquitously contaminating aquatic and terrestrial systems, but we still know
36 little of their effects on health. Microplastics (plastic particles less than 5 mm, either
37 manufactured, created by weathering of larger plastic fragments or fibers from clothing items

38 (Andrady, 2011; Browne et al., 2011)), represent the overwhelming majority of the total plastic
39 in the sea (Cozar et al., 2014; Eriksen et al., 2014). However, microplastics are also being
40 recovered from freshwater settings (reviewed in (Eerkes-Medrano, Thompson & Aldridge,
41 2015)), from lake systems (Driedger et al., 2015) to mountain catchments (Allen et al., 2019), as
42 well as in terrestrial environments (Rillig, 2012; de Souza Machado et al., 2018).

43 Although lagging behind the work on marine organisms (Bergmann, Gutow & Klages, 2015;
44 Martínez-Gómez et al., 2017b; Rendell-Bhatti et al., 2020), there is growing evidence for impacts
45 on terrestrial animals. Several studies found birds to have ingested microplastics, either directly
46 or via trophic transfer (Holland, Mallory & Shutler, 2016; Zhao, Zhu & Li, 2016), and earthworm
47 growth rate was reduced and mortality increased when exposed to <150µm polyethylene (PE)
48 microplastic particles (Huerta Lwanga et al., 2016). While not affecting its life cycle, the moth
49 *Bombyx mori* shows behavioural alterations when treated with polystyrene (PS) nanoplastics
50 (Parenti et al., 2020). Aquatic mosquito larvae have been found to be able to eat 2 µm PS beads
51 and transfer them to the adult terrestrial life stage (Al-Jaibachi, Cuthbert & Callaghan, 2018).
52 Other diptera have been exposed to dietary plastics. The life traits of the midge *Chironomus*
53 *riparius* were affected by 20-100 µm mixed-polymer plastic microparticles, in particular larval
54 body mass increased and there were changes in the shapes of larval mouth parts and female
55 wings in treated animals (Stanković et al., 2020). In the same organism, on the contrary, another
56 study found that 32-500 µm PE particle exposure reduced larval size, as well as delaying
57 development (Silva et al., 2019). In the soil arthropod *Folsomia candida*, ingestion of 1-63 µm PE
58 microplastic beads is linked to behavioural changes, with reduced velocity and distance of
59 movement (Kim & An, 2020). Movement was also impaired by 0.1-1 µm PS microbead ingestion
60 in *Drosophila melanogaster* (Zhang et al., 2020).

61 Despite this growing body of literature demonstrating negative effects, the specific effects of
62 plastic exposure on health outcomes remain almost completely unknown (Campanale et al.,
63 2020; Hirt & Body-Malapel, 2020). Exposure to different types and sizes of plastics has been
64 known to modulate the immune system as shown by altered gene expression (producing stress
65 and immune related proteins) in aquatic animals such as molluscs (*Mytilus galloprovincialis*
66 exposed to either 3 µm PS or 1-50 µm PE (Capolupo et al., 2018; Détrée & Gallardo-Escárate,
67 2018) and fish (*Danio rerio* exposed to 700 nm PS beads (Veneman et al., 2017) and *Sparus aurata*
68 exposed to 40-150 µm polyvinyl chloride (PVC) (Espinosa, Cuesta & Esteban, 2017)). 130µm PVC
69 particles increased phagocytic activity of immune cells in the annelid *Arenicola marina* (Wright
70 et al., 2013)), and PS nano and microparticles increased immune cell counts the crustacean
71 *Daphnia magna* (Sadler, Brunner & Plaistow, 2019; Aljaibachi et al., 2020). Moreover, immune
72 inflammatory response was triggered in molluscs (80 µm PE particles) and fish (0.5 and 50 µm PS
73 beads) (von Moos, Burkhardt-Holm & Koehler, 2012; Jin et al., 2018). Other immune reactions
74 have been detected after 250-1000 µm PE particle ingestion in earthworms (*Eisenia Andrei*)
75 (Rodriguez-Seijo et al., 2017) and in human cell lines exposed to 0.5-100 µm PS beads (Hwang et
76 al., 2020).

77 How these physiological responses translate to the immune capacity of the animals is not known,
78 but they have been regarded as potential interference with resistance to disease (Greven et al.,
79 2016). The objective of this study was to test the effect of microplastic contamination in the

80   immune response to viral infection using the amenable model *D. melanogaster*, as well as
81   observing their effect in the life cycle of the flies which was not previously known.

82   **Materials and Methods**

83   Plastic particles.

84   Polyethylene (PE) particles were obtained by grinding commercial PE nurdles (Merck, catalogue
85   number 428043). Polyvinyl Chloride (PVC) particles were obtained by grinding commercial
86   white PVC prime virgin plasticised pellets kindly provided by Northern Polymers and Plastics
87   Ltd. (UK). PE nurdles are virgin (additive-free) particles, while PVC are plasticised pellets with
88   potentially high levels of hazardous chemical additives such as phthalates (Navarro et al., 2010)
89   and polyaromatic hydrocarbons (Rendell-Bhatti et al., 2020). Both pellets were ground using a
90   SPEX Sample Prep Freezer/Mill 6870 cryogrinder using 4 cycles of two minute runs at 15 beats
91   per second with a cooling time of 2 minutes between cycles. Both types of nurdles had similar
92   mechanical properties in grinding. Fragments were sieved through 500, 250, 125 and 23 μm
93   meshes, and a mixture was assembled (10% of 250-500 μm particles, 80% of 125-250 μm and
94   10% 23-125 μm). Microplastic particles were added to *Drosophila melanogaster* cornmeal
95   media at either 1% PE, 1% PVC or 4% PVC (weight/volume, w/v) in the F1 only. Controls were
96   reared in non-supplemented cornmeal media, as were all flies in the F2. Cornmeal media recipe
97   and nutritional information is provided in the supplementary materials (Supplementary table
98   1).

99   F1 matings

100   *D. melanogaster* mating cages were set up with apple juice 3% agar plates where females laid
101   enough eggs to obtain the desired large amount of synchronous embryos. All embryos were
102   pooled together and suspended in water. 10 μl embryo squirts were added to each vial,
103   alternating conditions so one vial of each condition was prepared each time, for a total of 30
104   vials per treatment. Embryo squirts allow a consistently similar number of embryos to be added
105   in each vial. Flies were left to develop at 25°C, 12h:12h day night cycle, 70% humidity.

106   F1 life history measurements

107   30 tubes for each control, 1%PVC and 1%PE treatments were used to study pupation and
108   eclosion times. For each tube, time of pupation of every larva and time of eclosion of every
109   adult was recorded. Offspring of every treatment were pooled to determine the ratio of males
110   and females. For experimental set-up reasons, as we could not obtain 30 tubes for this
111   condition, 4% PVC was not used to study developmental times or sex ratio. 4% PVC was
112   however used for viral experiments and size measurements, as well as to set up matings to
113   study fertility and F2 life history.

114   F2 matings

115   To be able to assess fertility of the exposed flies, 3-day old female and male flies for each
116   treatment obtained from the life cycle experiment were crossed at a ratio one female to two
117   males per vial, in 20 vials. Food supplied was cornmeal media with no addition of plastic
118   particles, and rearing conditions were 25°C, 12h:12h day night cycle, 70% humidity. After 30
119   hours, males were removed. Females were changed to a new vial every three days, up to day

120   15, when the female was discarded from the last tube. This way, five tubes with offspring were
121   obtained for each female, unless she died before the end of this period.

122   F2 life history measurements

123   For each vial, we recorded time of first pupation and of first adult eclosion. Each tube was kept
124   for 8 days after the first adult emerged to allow eclosion of all pupae. At day 8, all adult flies
125   from that tube were counted, discerning between males and females. Adult size, total number
126   of flies obtained per female, as well as ratio of male and female, and total number of flies
127   obtained per treatment were investigated.

128   Size

129   Adult size was approximated by wing length (Sharma, Tregenza & Hosken, 2011) for F1 and F2
130   flies. Adults were kept in ethanol until both wings of 30 males for each treatment were
131   dissected, flat mounted on a slide and measured under a Leica DM IL LED microscope using the
132   LAS X software to undertake measurements.

133   Infection survival

134   Virus infection survival experiments were performed on 60 F1 males of the same age for each
135   treatment. Due to logistical constraints, only the F1 was subject to infection. Viral treatments
136   included non-injected males (control), control injected males (sterile challenge with Ringer's
137   solution) and virus injected males (viral challenge) for each media treatment (control, PE 1%,
138   PVC 1% and PVC 4%). Males were kept in groups of ten. Two-day old flies were anesthetized on
139   CO_2 and inoculated using a 0.0125 mm diameter stainless steel needle that was bent to a right
140   angle *ca.* 0.25mm from the end (Fine Science Tools, CA, USA). The bent tip of the needle was
141   dipped into the *Drosophila* C virus (DCV) strain B6A (TCID50 = $6.32×10^9$) which is derived from
142   an isolate collected from *D. melanogaster* in Charolles, France (Jousset et al., 1972). At the dose
143   used, 100% of the flies are successfully infected (Longdon et al., 2013). Flies were inoculated
144   through the pleural suture on the thorax (Longdon et al., 2018). We selected this route of
145   infection as oral inoculation has been shown to lead to stochastic infection outcomes in *D.*
146   *melanogaster.* However, once the virus passes through the gut barrier, both oral and pin-
147   pricked infections follow a similar course, with both resulting in the same tissues becoming
148   infected with DCV (Cherry & Perrimon, 2004; Arnold, Johnson & White, 2013; Ferreira et al.,
149   2014; Chtarbanova et al., 2014). Infection of DCV by inoculation is highly pathogenic in adult D.
150   melanogaster, causing increased mortality, metabolic and behavioural changes and nutritional
151   stress in the intestine, producing similar pathologies to starvation (Christian, 1987; Arnold,
152   Johnson & White, 2013; Chtarbanova et al., 2014; Vale & Jardine, 2017). Survival was recorded
153   daily until all infected animals had died.

154   Statistical analysis

155   To determine whether the different media treatments caused a significant effect on adult size
156   and larval and pupal stages duration we applied a Kruskal-Wallis analysis of the variance
157   because data normality and homoscedasticity were not met. The multiple comparison Dunn's
158   test was employed to determine the cases with significant differences (P < 0.05). All tests were
159   performed using the R software, version 3.5.1 (R Core Team, 2019).

160   To assess whether plastic exposure affected female fertility in the F1, we applied a generalized
161   linear model with zero-inflated negative binomial distribution (log link function), using total
162   adult offspring as response variable and plastic treatment as fixed effect. We then used a Tukey
163   test to perform multiple pairwise comparisons between treatments.

164   To evaluate how a rearing environment containing plastics influenced the ability of the resulting
165   adults to resist an immune challenge with a viral pathogen (DCV), we used a parametric survival
166   analysis (the *survreg* function in R (Therneau, 1999)). The maximal model included, the
167   response variable of the number of days that each individual survived after the viral challenge,
168   control sham infection or non-challenged was administered, any individuals who survived
169   beyond 22 days were censored and recorded as such. The fixed effects included the plastic
170   treatment, the viral treatment and the replicate. The Weibull distribution was used for analysis
171   as it showed the best fit with minimum error deviance. There was no effect of replicate in the
172   maximal model ($\chi2 = 1361.14$, df = 1361, p = 0.94), and was removed from further analysis.

173   **Results**

174   Virus resistance

175   In order to determine if plastic elicits an effect on the ability of flies to resist viral infection, we
176   performed a viral resistance assay across individuals that had been reared on each of the plastic
177   treatments. The survival of virally challenged adult flies was compared to those that were sham
178   challenged with a virus free inoculation ('sterile control') and those that were completely naïve
179   ('unchallenged control'). After two weeks, most virus-injected flies had died across all
180   treatments, while most non-injected and blank-injected flies were alive (Figure 1). To observe if
181   there was an effect of plastic treatment we looked at the interaction between the viral
182   treatment and plastic rearing environment, which appeared to be non-significant (Viral
183   Treatment x Plastics: $\chi2 = 10.55$, df = 710, p = 0.103).

184   Effect of plastic exposure on *D. melanogaster* life cycle

185   We recorded the number of pupae and adults, discerning for male and female adults, for each
186   treatment, as well as the time to pupation and the time of eclosion (in days). Having added the
187   same number of eggs per vial in each treatment (see materials and methods), we obtained 814
188   pupae and 810 adults for the control treatment. For the PVC treatment we obtained 691 pupae
189   and 672 adults, and for the PE treatment we obtained 691 pupae and 677 adults. There is an
190   asymmetry in the number of animals (pupae and adults) obtained from each treatment; plastic
191   treatments yield 0.85 flies for every fly in the control treatment. However, these differences
192   were non-significant (unpaired *t*-test between treatment pairs). The percentage of adults that
193   emerged from the pupae was equivalent in all treatments, with success rates 99.3% in the
194   control, 97.8% in 1% PE and 97.5% in 1% PVC treatments. The time of pupation follows a
195   distribution with a peak of individuals pupating on day 3, with no significant differences
196   between treatments, and the larval stage duration is similar for all treatments ($X^2 = 3.27$, p =
197   0.195, Figure 2 a). The same is seen for the time of eclosion of adults, also with a peak of
198   individuals emerging on day three for all treatments, and pupa duration similar for all
199   treatments ($X^2 = 1.73$ p = 0.421, Figure 2 b).

200   We also looked at the stage duration of larvae and pupae in the F2, where flies were reared
201   from treated flies in non-treated food. In this case, offspring of 4% PVC treated flies (not
202   included in the F1 life cycle experiments) had shorter larval stages (X^2 = 43.41, p = 2e-09, Figure
203   2 c), and offspring of all plastic treatments had shorter pupal stages than the control (X^2 =
204   11.31, p = 0.01, Figure 2 d).

205   We looked at the fertility per female in the F1 (see materials and methods). We found that
206   females reared with 1% PE laid more eggs than the controls, and those reared with 4% PVC laid
207   marginally more eggs than the control (difference among all groups X^2=14.4, p=0.002, Figure 3).
208   We saw no differences between 1% PVC treatments and controls.

209   The total number of females and males obtained for each treatment was recorded
210   (Supplementary Table 2). In the F1, while in the control treatment there was near equivalence
211   (50.2% males), in the plastic treatments there was a slight decrease in the proportion of males.
212   A binomial test indicated that the proportion of males of 46.4% for 1% PE and 44.5% for 1% PVC
213   were lower than the expected 50% (p=0.03 and p=0.002, respectively (1-tailed)). In the F2, were
214   larvae obtained from the treated animals were fed with food with no additives, however, we
215   saw no differences and sex ratios were at near equivalence in all treatments.

216   Adult fly size

217   Size of male flies resulting from larvae exposed to the different treatments was measured, as
218   well as F2 male flies not exposed to additives in their food. F1 males from exposed larvae did
219   not show any significant size differences between treatments and controls (X^2 = 2.47, p = 0.480
220   Figure 4 a). However, when the F2 individuals were measured, adults in both 1% and 4% PVC
221   treatments were significantly smaller than the controls and 1% PE treatments (X^2 = 14.78, p =
222   0.002, Figure 4 b).

223   **Discussion**

224   *Drosophila melanogaster* has been used in many toxicological studies and recently has been
225   utilised as a tool for the rapid assessment of microplastic mediated toxicity (Zhang et al., 2020).
226   Here we used *D. melanogaster* to assess the toxicity of two types of plastic: virgin microplastic
227   with no added chemicals (PE) and industrial plastics known to have hazardous chemical
228   additives (PVC). Leachates of the PVC particles used in this study have been shown to disrupt
229   sea urchin development and to contain harmful chemicals that can be released into sea water
230   (Rendell-Bhatti et al., 2020). Some plastic additives have been linked to changes in
231   developmental time, size and fitness in *D. melanogaster* (Quesada-Calderón et al., 2017; Chen
232   et al., 2019). In the current study, the plastics were added to a semisolid food matrix, and we
233   did not study the possible release of chemicals from the plastics to the food. Although we were
234   not able to test if the larvae had ingested the added plastics, the particle sizes were small
235   enough to be eaten by later instar larvae, though they may have selectively chosen to avoid
236   eating them. However, we saw the strongest effects in the offspring of flies treated with higher
237   percentage of plastics and those known to have chemicals adsorbed, and we therefore
238   hypothesise that the effects we saw may be caused by both these factors: a higher
239   concentration of particles and higher amount of chemical additives.

240   It is important to note that particle size is important for the strength of any plastic effects (Lee
241   et al., 2013; Haegerbaeumer et al., 2019; Silva et al., 2019). Using round 1 and 0.1 μm
242   polystyrene beads, a study in *D. melanogaster* found gut damage and locomotion disfunction,
243   amongst other effects (Zhang et al., 2020). Our study used a more environmentally relevant
244   plastic model: plastic fragments of irregular shape and sizes ranging from 23 to 500 μm. Despite
245   larger irregular particles being more likely to be found in the environment than nanobeads,
246   these may be less readily available to be ingested by the larvae. The particles used in two
247   studies on *C. riparius* had a similar size to those used in our study (Silva et al., 2019; Stanković et
248   al., 2020). Other studies had used environmentally relevant concentrations of plastics in
249   riverine sediments to a concentration of 0.04% (weight in volume) (Stanković et al., 2020), 25
250   times lower than the concentration used in our 1% treatments. However, our study provides an
251   account of what can happen in the case of food being taken from highly contaminated sites,
252   which are now widespread. The concentration of plastics added to the food in this study,
253   although high (1 or 4% weight in volume), is a plausible representation of such sites. This is the
254   case in landfill sites and other highly polluted settings. (municipal solid waste sent to landfills in
255   America contained 18.5% of plastics (United States Environment Protection Agency, 2020)) ,
256   where a wide variety of insects live off food items mixed with plastic and other inedible
257   materials (Qasim et al., 2020).

258   Our study showed no effect of the plastic treatments in the capacity to fight viral infections in
259   *D. melanogaster*. Previous studies had reported effects in the immune system of several models
260   by increase of immune cell numbers, physiological changes or protein or gene overexpression
261   (Canesi et al., 2015; Balbi et al., 2016; Greven et al., 2016; Sadler, Brunner & Plaistow, 2019;
262   Hirt & Body-Malapel, 2020). Other studies found injection of latex beads (composed of
263   polystyrene) used to block phagocytosis (Elrod-Erickson, Mishra & Schneider, 2000) can
264   increase susceptibility to some pathogens (Costa et al., 2009; Lamiable et al., 2016). To the best
265   of our knowledge, this is the first report testing the outcome of viral infection after microplastic
266   exposure. Despite our results showing no decrease in the capacity to resist viral infection in
267   plastic treated flies, we could not rule out a transgenerational immune effect, or an additive
268   effect after exposure for several generations, as it happens in other systems for exposures to
269   toxicants such as heavy metals (blow fly, (Pölkki, Kangassalo & Rantala, 2012)) or bisphenol S
270   (mouse, (Brulport et al., 2021). Further experiments with a fully-factorial design should be
271   considered to shed light on this point.

272   We observed significant differences in the male/female ratio in the 1% PE and 1% PVC
273   treatments in the F1. In contrast, no differences in sex ratios were found in *C. riparius* treated
274   with PE particles of equivalent size to the ones used in this study (Silva et al., 2019). Since
275   plastic additives often include endocrine disruptors, which are also known to affect *D.
276   melanogaster* (Quesada-Calderón et al., 2017; Chen et al., 2019), we initially hypothesised that
277   the male/female ratio could be affected by chemical additives in the PVC treatments. However,
278   we also found an effect in the PE treatment, which had no added chemicals, and hence
279   released chemicals may not be the only factor contributing to this effect. Nevertheless, we take
280   this result with caution as the pooling of all vials from each treatment to classify offspring by
281   sex may have confounded the results, as we could not control for vial effects.

282   We saw confounding changes in the fertility of treated flies. Both 1% PE and to a lesser extent
283   4% PVC treatments had more offspring than the control but the 1% PVC treatment showed no
284   differences with the control. Similar confounding results were seen in *D. magna*, where
285   exposure to 1-5μm spheres reduced fertility in successive generations after microplastic
286   exposure (Martins & Guilhermino, 2018), but animals exposed to 0.5μm PS beads showed
287   significant grater clutch sizes (Sadler, Brunner & Plaistow, 2019). These results were linked to
288   microplastic induced physical effects and chemical toxicity for the reduction in fertility, and
289   upregulation of haemocytes (immune response) in the case of the increased fertility. Future
290   work could inform whether these mechanisms are also responsible for the effects we saw in
291   our case.

292   We saw no effect in developmental time or adult size in the *D. melanogaster* exposed to
293   microplastics. In the dipteran *Chironomus riparius* exposure to mixed microplastics
294   (polyethylene-terephthalate, polystyrene, PVC and polyamide) resulted in increased average
295   body mass and length, as well as a significant increase of the development time from first instar
296   to adult (Stanković et al., 2020). In contrast, ingestion of polyethylene by the same dipteran *C.*
297   *riparius*, produced conflicting results showing reduced larval growth, but also a delay in the
298   emergence of adults (Silva et al., 2019). It is unclear why all these results differ but it could be
299   down to the aquatic medium in which *C. riparius* was exposed or due to the different properties
300   of the microplastic or microplastic mixes used.

301   Despite not seeing timing or size effects in the F1 generation of *D. melanogaster*, we saw
302   differences in the offspring of treated flies: the size of the F2 adults in PVC treatments (at both
303   concentrations tested) were smaller than control animals, and the life cycle was shorter in the
304   offspring of all treated flies. Similar results have been seen in the aquatic model *Daphnia*
305   *magna*, where individuals exposed to microplastics had smaller offspring but no other changes
306   in life history traits. This change in *D. magna* was linked to increased numbers of immune cells
307   under microplastic exposure (Sadler, Brunner & Plaistow, 2019). In our case, the flies were not
308   exposed to plastics, but were the offspring of exposed flies. In *D. melanogaster* and other flies,
309   the size of pupae and adult flies depends on the size of the larvae at the moment of pupation,
310   which is determined by the availability of food during larval development. Starvation usually
311   correlates with a longer larval stage, to attain the minimal viable size to survive metamorphosis,
312   and a shorter pupal stage, probably since smaller larvae means less tissue to be
313   metamorphosed (Alcaine-Colet, Wotton & Jimenez-Guri, 2015). In our case, the affected flies
314   were reared in plastic-free media, and therefore we did not expect that the food source would
315   trigger a change in their body size and developmental time. The changes to smaller size and
316   shorter life cycles in the F2 generation could be due to many potential factors such as an
317   epigenetic effect in the F1 which affects the F2, or a condition effect in the mothers, such as
318   physiological toxicity caused by physical effects (for example mechanical damage to the gut of
319   the mother) or chemical toxicity, that affects offspring development. Plastic particles are known
320   to cause oxidative stress (reviewed in (Pérez-Albaladejo, Solé & Porte, 2020)), neurotoxicity
321   (reviewed in (Prüst, Meijer & Westerink, 2020)) and developmental toxicity in other animals
322   (Martínez-Gómez et al., 2017a; Messinetti et al., 2018; Rendell-Bhatti et al., 2020), as well as
323   reduced gut function (Wright et al., 2013), which may have a negative knock on effect in
324   subsequent generations. In favour of this hypothesis, we saw that the offspring of flies with the

325   higher dose of plastics (4% PVC), which also are known to contain harmful chemicals (Rendell-
326   Bhatti et al., 2020), were the ones showing both the strongest changes in their life cycles, with
327   shorter larval and pupal stages, and the greatest reduction in body size.

Conclusions

329   Exposure to microplastic particles affects sex ratios and fecundity in *D. melanogaster* within
330   exposed generation, but does not significantly change its life cycle nor affect the ability of male
331   flies to fight viral infection. However, non-exposed offspring of exposed flies from higher
332   content (4% w/v) PVC show a shorter larval stage compared to all other treatments and
333   controls, and non-exposed offspring from exposed flies of all plastic treatments show a
334   decrease in the duration of the pupa stage. Despite flies reared in supplemented food showing
335   no differences in body size, non-exposed offspring of exposed flies from PVC treatments show a
336   significant decrease in body size with respect to all other treatments. We saw the strongest
337   effects in the non-exposed offspring of flies treated with higher percentage of plastics and
338   those known to have chemicals adsorbed. Further investigation is needed into the effect of our
339   increasingly plastic-contaminated terrestrial environments on life history traits of exposed
340   animals.

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Bibliography

349   Al-Jaibachi R, Cuthbert RN, Callaghan A. 2018. Up and away: Ontogenic transference as a
350   pathway for aerial dispersal of microplastics. *Biology Letters* 14. DOI:
351   10.1098/rsbl.2018.0479.

352   Alcaine-Colet A, Wotton KR, Jimenez-Guri E. 2015. Rearing the scuttle fly Megaselia scalaris
353   (Diptera: Phoridae) on industrial compounds: implications on size and lifespan. *PeerJ*
354   3:e1085. DOI: 10.7717/peerj.1085.

355   Aljaibachi R, Laird WB, Stevens F, Callaghan A. 2020. Impacts of polystyrene microplastics on
356   Daphnia magna: A laboratory and a mesocosm study. *Science of the Total Environment*
357   705. DOI: 10.1016/j.scitotenv.2019.135800.

358   Allen S, Allen D, Phoenix VR, Le Roux G, Durántez Jiménez P, Simonneau A, Binet S, Galop D.
359   2019. Atmospheric transport and deposition of microplastics in a remote mountain
360   catchment. *Nature Geoscience* 12:339–344. DOI: 10.1038/s41561-019-0335-5.

361   Andrady AL. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62:1596–
362   1605. DOI: 10.1016/j.marpolbul.2011.05.030.

363   Arnold PA, Johnson KN, White CR. 2013. Physiological and metabolic consequences of viral

364   infection in Drosophila melanogaster. *Journal of Experimental Biology* 216:3350–3357.
365   DOI: 10.1242/jeb.088138.

366 Balbi T, Franzellitti S, Fabbri R, Montagna M, Fabbri E, Canesi L. 2016. Impact of bisphenol A
367   (BPA) on early embryo development in the marine mussel Mytilus galloprovincialis : Effects
368   on gene transcription. *Environmental Pollution* 218:996–1004. DOI:
369   10.1016/j.envpol.2016.08.050.

370 Bergmann M, Gutow L, Klages M. 2015. Marine anthropogenic litter. *Marine Anthropogenic
371   Litter*:1–447. DOI: 10.1007/978-3-319-16510-3.

372 Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R. 2011.
373   Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environmental
374   Science & Technology* 45:9175–9179. DOI: 10.1021/es201811s.

375 Brulport A, Lencina C, Chagnon MC, Le Corre L, Guzylack-Piriou L. 2021. Transgenerational
376   effects on intestinal inflammation status in mice perinatally exposed to bisphenol S.
377   *Chemosphere* 262:0–8. DOI: 10.1016/j.chemosphere.2020.128009.

378 Campanale C, Massarelli C, Savino I, Locaputo V, Uricchio VF. 2020. A detailed review study on
379   potential effects of microplastics and additives of concern on human health. *International
380   Journal of Environmental Research and Public Health* 17. DOI: 10.3390/ijerph17041212.

381 Canesi L, Ciacci C, Bergami E, Monopoli MP, Dawson KA, Papa S, Canonico B, Corsi I. 2015.
382   Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene
383   nanoparticles in the hemocytes of the marine bivalve Mytilus. *Marine Environmental
384   Research* 111:34–40. DOI: 10.1016/j.marenvres.2015.06.008.

385 Capolupo M, Franzellitti S, Valbonesi P, Lanzas CS, Fabbri E. 2018. Uptake and transcriptional
386   effects of polystyrene microplastics in larval stages of the Mediterranean mussel Mytilus
387   galloprovincialis. *Environmental Pollution* 241:1038–1047. DOI:
388   10.1016/j.envpol.2018.06.035.

389 Chen MY, Liu HP, Cheng J, Chiang SY, Liao WP, Lin WY. 2019. Transgenerational impact of DEHP
390   on body weight of Drosophila. *Chemosphere* 221:493–499. DOI:
391   10.1016/j.chemosphere.2018.12.193.

392 Cherry S, Perrimon N. 2004. Entry is a rate-limiting step for viral infection in a Drosophila
393   melanogaster model of pathogenesis. *Nature Immunology* 5:81–87. DOI: 10.1038/ni1019.

394 Christian PD. 1987. Studies on Drosophila C and A viruses in Australian populations of
395   Drosophila melanogaster.

396 Chtarbanova S, Lamiable O, Lee K-Z, Galiana D, Troxler L, Meignin C, Hetru C, Hoffmann JA,
397   Daeffler L, Imler J-L. 2014. Drosophila C Virus Systemic Infection Leads to Intestinal
398   Obstruction. *Journal of Virology* 88:14057–14069. DOI: 10.1128/jvi.02320-14.

399 Costa A, Jan E, Sarnow P, Schneider D. 2009. The Imd pathway is involved in antiviral immune
400   responses in Drosophila. *PLoS ONE* 4. DOI: 10.1371/journal.pone.0007436.

401 Cozar A, Echevarria F, Gonzalez-Gordillo JI, Irigoien X, Ubeda B, Hernandez-Leon S, Palma AT,

402   Navarro S, Garcia-de-Lomas J, Ruiz A, Fernandez-de-Puelles ML, Duarte CM. 2014. Plastic
403   debris in the open ocean. *Proceedings of the National Academy of Sciences* 111:10239–
404   10244. DOI: 10.1073/pnas.1314705111.

405   Détrée C, Gallardo-Escárate C. 2018. Single and repetitive microplastics exposures induce
406   immune system modulation and homeostasis alteration in the edible mussel Mytilus
407   galloprovincialis. *Fish and Shellfish Immunology* 83:52–60. DOI: 10.1016/j.fsi.2018.09.018.

408   Driedger AGJ, Dürr HH, Mitchell K, Van Cappellen P. 2015. Plastic debris in the Laurentian Great
409   Lakes: A review. *Journal of Great Lakes Research* 41:9–19. DOI: 10.1016/j.jglr.2014.12.020.

410   Eerkes-Medrano D, Thompson RC, Aldridge DC. 2015. Microplastics in freshwater systems: A
411   review of the emerging threats, identification of knowledge gaps and prioritisation of
412   research needs. *Water Research* 75:63–82. DOI: 10.1016/j.watres.2015.02.012.

413   Elrod-Erickson M, Mishra S, Schneider D. 2000. Interactions between the cellular and humoral
414   immune responses in Drosophila. *Current Biology* 10:781–784. DOI: 10.1016/S0960-
415   9822(00)00569-8.

416   Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser
417   J. 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces
418   Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* 9:1–15. DOI:
419   10.1371/journal.pone.0111913.

420   Espinosa C, Cuesta A, Esteban MÁ. 2017. Effects of dietary polyvinylchloride microparticles on
421   general health, immune status and expression of several genes related to stress in gilthead
422   seabream (Sparus aurata L.). *Fish and Shellfish Immunology* 68:251–259. DOI:
423   10.1016/j.fsi.2017.07.006.

424   Ferreira ÁG, Naylor H, Esteves SS, Pais IS, Martins NE, Teixeira L. 2014. The Toll-Dorsal Pathway
425   Is Required for Resistance to Viral Oral Infection in Drosophila. *PLoS Pathogens* 10. DOI:
426   10.1371/journal.ppat.1004507.

427   Greven A-C, Merk T, Karagöz F, Mohr K, Klapper M, Jovanović B, Palić D. 2016. Polycarbonate
428   and polystyrene nanoplastic particles act as stressors to the innate immune system of
429   fathead minnow (Pimephales promelas). *Environmental Toxicology and Chemistry*
430   35:3093–3100. DOI: 10.1002/etc.3501.

431   Haegerbaeumer A, Mueller MT, Fueser H, Traunspurger W. 2019. Impacts of micro- and nano-
432   sized plastic particles on benthic invertebrates: A literature review and gap analysis.
433   *Frontiers in Environmental Science* 7. DOI: 10.3389/fenvs.2019.00017.

434   Hirt N, Body-Malapel M. 2020. Immunotoxicity and intestinal effects of nano- and microplastics:
435   a review of the literature. *Particle and Fibre Toxicology* 17:1–22. DOI: 10.1186/s12989-020-
436   00387-7.

437   Holland ER, Mallory ML, Shutler D. 2016. Plastics and other anthropogenic debris in freshwater
438   birds from Canada. *Science of the Total Environment* 571:251–258. DOI:
439   10.1016/j.scitotenv.2016.07.158.

440   Huerta Lwanga E, Gertsen H, Gooren H, Peters P, Salánki T, Van Der Ploeg M, Besseling E,

441   Koelmans AA, Geissen V. 2016. Microplastics in the Terrestrial Ecosystem: Implications for
442   Lumbricus terrestris (Oligochaeta, Lumbricidae). *Environmental Science and Technology*
443   50:2685–2691. DOI: 10.1021/acs.est.5b05478.

444   Hwang J, Choi D, Han S, Jung SY, Choi J, Hong J. 2020. Potential toxicity of polystyrene
445   microplastic particles. *Scientific Reports* 10:1–12. DOI: 10.1038/s41598-020-64464-9.

446   Jin Y, Xia J, Pan Z, Yang J, Wang W, Fu Z. 2018. Polystyrene microplastics induce microbiota
447   dysbiosis and inflammation in the gut of adult zebrafish. *Environmental Pollution* 235:322–
448   329. DOI: 10.1016/j.envpol.2017.12.088.

449   Jousset FX, Plus N, Croizier G, Thomas M. 1972. [Existence in Drosophila of 2 groups of
450   picornavirus with different biological and serological properties]. *Comptes rendus*
451   *hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles*
452   275:3043–6.

453   Kim SW, An YJ. 2020. Edible size of polyethylene microplastics and their effects on springtail
454   behavior. *Environmental Pollution* 266. DOI: 10.1016/j.envpol.2020.115255.

455   Lamiable O, Arnold J, de Faria IJ da S, Olmo RP, Bergami F, Meignin C, Hoffmann JA, Marques JT,
456   Imler J-L. 2016. Analysis of the Contribution of Hemocytes and Autophagy to Drosophila
457   Antiviral Immunity. *Journal of Virology* 90:5415–5426. DOI: 10.1128/jvi.00238-16.

458   Lee K, Shim WJ, Kwon OY, Kang J. 2013. Size-Dependent E ff ects of Micro Polystyrene Particles
459   in the Marine Copepod Tigriopus japonicus. *Environmental science & technology*
460   47:11278–11283. DOI: dx.doi.org/10.1021/es401932b.

461   Longdon B, Cao C, Martinez J, Jiggins FM. 2013. Previous exposure to an RNA virus does not
462   protect against subsequent infection in Drosophila melanogaster. *PloS one* 8. DOI:
463   10.1371/journal.pone.0073833.

464   Longdon B, Day JP, Alves JM, Smith SCL, Houslay TM, McGonigle JE, Tagliaferri L, Jiggins FM.
465   2018. Host shifts result in parallel genetic changes when viruses evolve in closely related
466   species. *PLoS Pathogens* 14:1–14. DOI: 10.1371/journal.ppat.1006951.

467   Martínez-Gómez C, León VM, Calles S, Gomáriz-Olcina M, Vethaak AD. 2017a. The adverse
468   effects of virgin microplastics on the fertilization and larval development of sea urchins.
469   *Marine Environmental Research* 130:69–76. DOI: 10.1016/j.marenvres.2017.06.016.

470   Martínez-Gómez C, León VM, Calles S, Gomáriz-Olcina M, Vethaak AD. 2017b. The adverse
471   effects of virgin microplastics on the fertilization and larval development of sea urchins.
472   *Marine Environmental Research* 130:69–76. DOI: 10.1016/j.marenvres.2017.06.016.

473   Martins A, Guilhermino L. 2018. Transgenerational effects and recovery of microplastics
474   exposure in model populations of the freshwater cladoceran Daphnia magna Straus.
475   *Science of the Total Environment* 631–632:421–428. DOI: 10.1016/j.scitotenv.2018.03.054.

476   Messinetti S, Mercurio S, Parolini M, Sugni M, Pennati R. 2018. Effects of polystyrene
477   microplastics on early stages of two marine invertebrates with different feeding strategies
478   *. *Environmental Pollution* 237:1080–1087. DOI: 10.1016/j.envpol.2017.11.030.

479   von Moos N, Burkhardt-Holm P, Koehler A. 2012. Uptake and E ff ects of Microplastics on Cells
480   and Tissue of the Blue Mussel Mytilus edulis L. after an Experimental Exposure.
481   *Environmental science & technology* 46:327–335. DOI: 10.1021/es302332w.

482   Navarro R, Pérez Perrino M, Gómez Tardajos M, Reinecke H. 2010. Phthalate Plasticizers
483   Covalently Bound to PVC: Plasticization with Suppressed Migration. *Macromolecules*
484   43:2377–2381. DOI: 10.1021/ma902740t.

485   Parenti CC, Binelli A, Caccia S, Della Torre C, Magni S, Pirovano G, Casartelli M. 2020. Ingestion
486   and effects of polystyrene nanoparticles in the silkworm Bombyx mori. *Chemosphere* 257.
487   DOI: 10.1016/j.chemosphere.2020.127203.

488   Pérez-Albaladejo E, Solé M, Porte C. 2020. Plastics and plastic additives as inducers of oxidative
489   stress. *Current Opinion in Toxicology* 20–21:69–76. DOI: 10.1016/j.cotox.2020.07.002.

490   Pölkki M, Kangassalo K, Rantala MJ. 2012. Transgenerational effects of heavy metal pollution on
491   immune defense of the blow fly Protophormia terraenovae. *PLoS ONE* 7. DOI:
492   10.1371/journal.pone.0038832.

493   Prüst M, Meijer J, Westerink RHS. 2020. The plastic brain: Neurotoxicity of micro- And
494   nanoplastics. *Particle and Fibre Toxicology* 17:1–16. DOI: 10.1186/s12989-020-00358-y.

495   Qasim M, Xiao H, He K, Noman A, Liu F, Chen MY, Hussain D, Jamal ZA, Li F. 2020. Impact of
496   landfill garbage on insect ecology and human health. *Acta Tropica* 211:105630. DOI:
497   10.1016/j.actatropica.2020.105630.

498   Quesada-Calderón S, Bacigalupe LD, Toro-Vélez AF, Madera-Parra CA, Peña-Varón MR,
499   Cárdenas-Henao H. 2017. The multigenerational effects of water contamination and
500   endocrine disrupting chemicals on the fitness of Drosophila melanogaster. *Ecology and*
501   *Evolution* 7:6519–6526. DOI: 10.1002/ece3.3172.

502   R Core Team. 2019. R: A language and environment for statistical computing. *R Foundation for*
503   *Statistical Computing, Vienna, Austria. Http://Www.r-Project.Org/Index.Html.*

504   Rendell-Bhatti F, Paganos P, Pouch A, Mitchell C, D'Aniello S, Godley BJ, Pazdro K, Arnone MI,
505   Jimenez-Guri E. 2020. Developmental toxicity of plastic leachates on the sea urchin
506   Paracentrotus lividus. *Environmental Pollution* 269:115744. DOI:
507   10.1016/j.envpol.2020.115744.

508   Rillig MC. 2012. Microplastic in terrestrial ecosystems and the soil? *Environmental Science and*
509   *Technology* 46:6453–6454. DOI: 10.1021/es302011r.

510   Rodriguez-Seijo A, Lourenço J, Rocha-Santos TAP, da Costa J, Duarte AC, Vala H, Pereira R. 2017.
511   Histopathological and molecular effects of microplastics in Eisenia andrei Bouché.
512   *Environmental Pollution* 220:495–503. DOI: 10.1016/j.envpol.2016.09.092.

513   Sadler DE, Brunner FS, Plaistow SJ. 2019. Temperature and clone-dependent effects of
514   microplastics on immunity and life history in Daphnia magna. *Environmental Pollution* 255.

515   Sharma MD, Tregenza T, Hosken DJ. 2011. Sex combs, allometry, and asymmetry in Drosophila.
516   *Biological Journal of the Linnean Society* 103:923–934.

517   Silva CJM, Silva ALP, Gravato C, Pestana JLT. 2019. Ingestion of small-sized and irregularly
518   shaped polyethylene microplastics affect Chironomus riparius life-history traits. *Science of*
519   *the Total Environment* 672:862–868. DOI: 10.1016/j.scitotenv.2019.04.017.

520   de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC. 2018. Microplastics as an emerging
521   threat to terrestrial ecosystems. *Global Change Biology* 24:1405–1416. DOI:
522   10.1111/gcb.14020.

523   Stanković J, Milošević D, Savić-Zdraković D, Yalçın G, Yildiz D, Beklioğlu M, Jovanović B. 2020.
524   Exposure to a microplastic mixture is altering the life traits and is causing deformities in
525   the non-biting midge Chironomus riparius Meigen (1804). *Environmental Pollution* 262.
526   DOI: 10.1016/j.envpol.2020.114248.

527   Therneau TM. 1999. A Package for Survival Analysis in S. :1–83.

528   United States Environment Protection Agency. 2020. *Advancing Sustainable Materials*
529   *Management: 2018 Fact Sheet*.

530   Vale PF, Jardine MD. 2017. Infection avoidance behavior: Viral exposure reduces the motivation
531   to forage in female Drosophila melanogaster. *Fly* 11:3–9. DOI:
532   10.1080/19336934.2016.1207029.

533   Veneman WJ, Spaink HP, Brun NR, Bosker T, Vijver MG. 2017. Pathway analysis of systemic
534   transcriptome responses to injected polystyrene particles in zebrafish larvae. *Aquatic*
535   *Toxicology* 190:112–120. DOI: 10.1016/j.aquatox.2017.06.014.

536   Wright SL, Rowe D, Thompson RC, Galloway TS. 2013. Microplastic ingestion decreases energy
537   reserves in marine worms. *Current Biology* 23:R1031–R1033. DOI:
538   10.1016/j.cub.2013.10.068.

539   Zhang Y, Wolosker MB, Zhao Y, Ren H, Lemos B. 2020. Exposure to microplastics cause gut
540   damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity
541   in Drosophila. *Science of the Total Environment* 744:140979. DOI:
542   10.1016/j.scitotenv.2020.140979.

543   Zhao S, Zhu L, Li D. 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai,
544   China: Not only plastics but also natural fibers. *Science of the Total Environment* 550:1110–
545   1115. DOI: 10.1016/j.scitotenv.2016.01.112.

546

Figure 1

Survival of *D. melanogaster* after rearing on food containing plastics and challenged with viral pathogen (DCV).

Survival shows as mean proportion alive per day across the replicates. Red dashed line and circle: control food, yellow crosses and line: 1% virgin polyethylene (PE) , green line and triangle: 1% Polyvinyl Chloride (PVC), blue line and triangle: 4% PVC. Panels are split into the three viral treatments Control- no challenge, Sterile- flies challenged with a sterile infection and Virus- challenged with DCV virus.
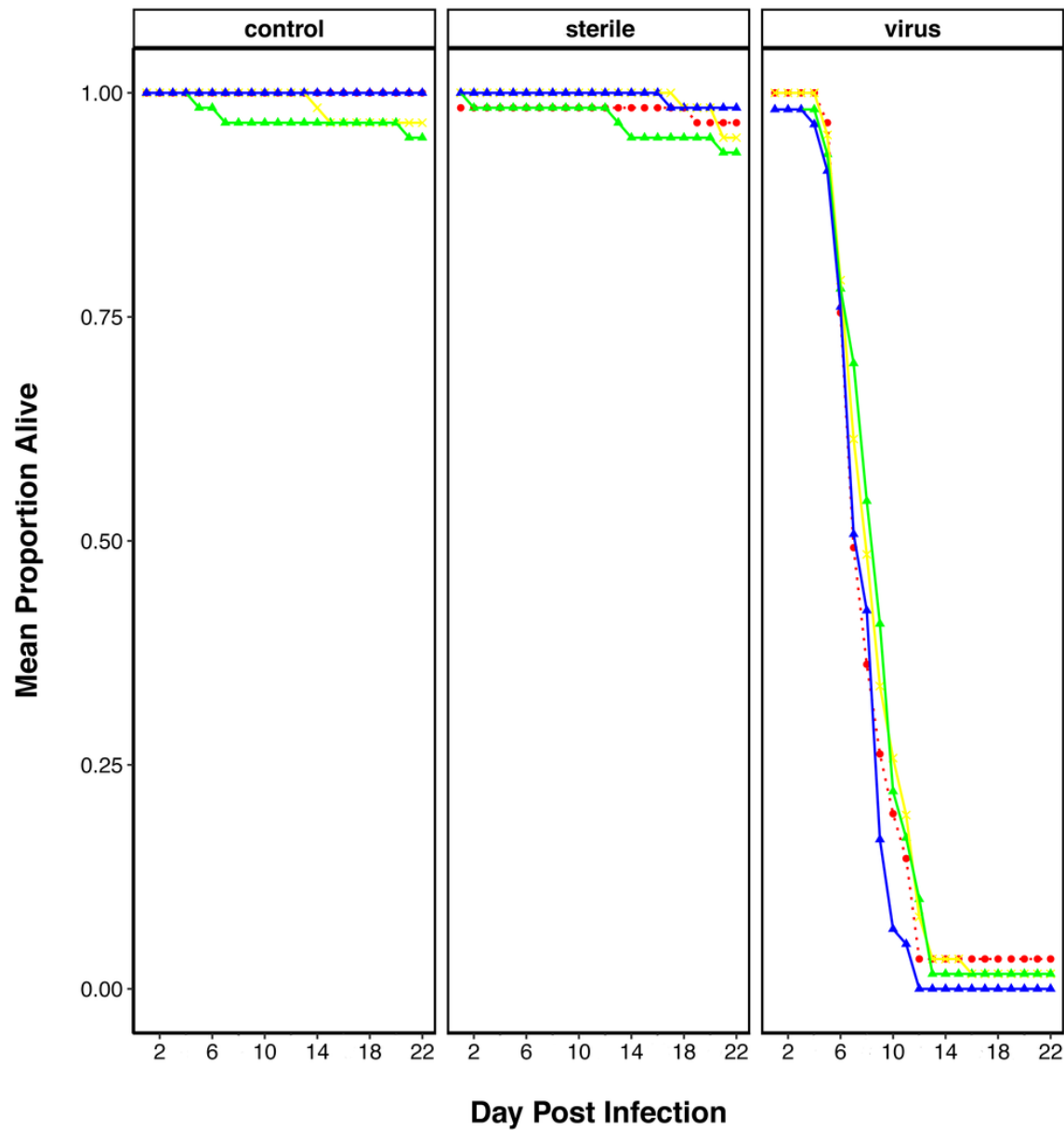
Figure 2

Stage duration under each condition.

Stage duration in the F1 for (a) larvae and (b) pupa, and in the F2 for (c) larvae and (d) pupa for each treatment. Squares represent the mean and whiskers represent the standard error of the mean (SEM). Red: control, yellow: virgin polyethylene (PE), green: 1% polyvinyl chloride (PVC), blue: 4% PVC. Note that y axis extent is different between a/c and b/d. Asterisks correspond to significant differences ($p < 0.05$) between the treatment and the control in a multiple comparisons test.
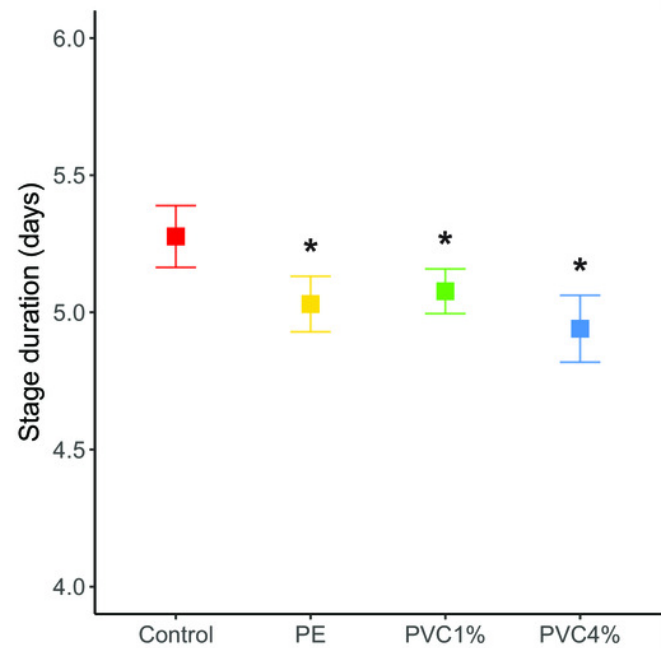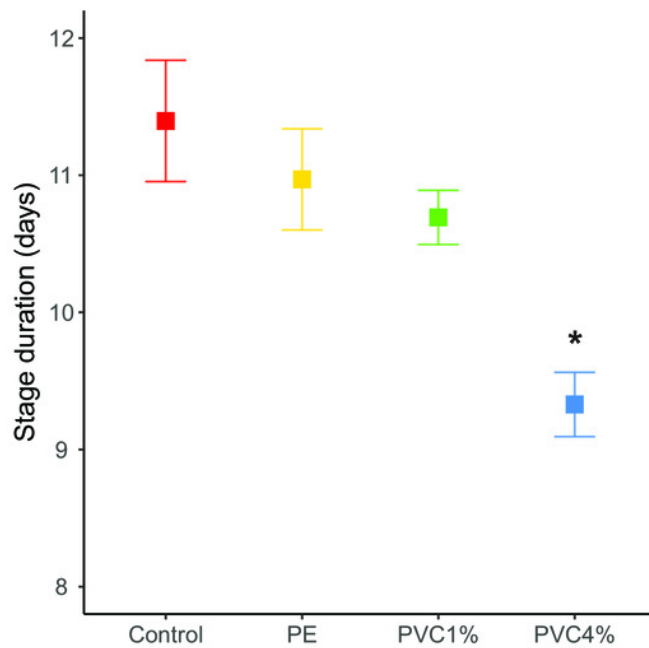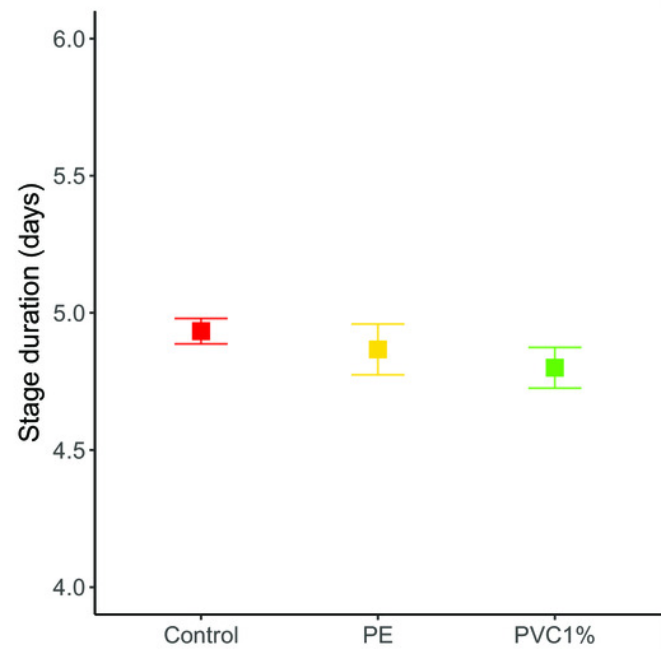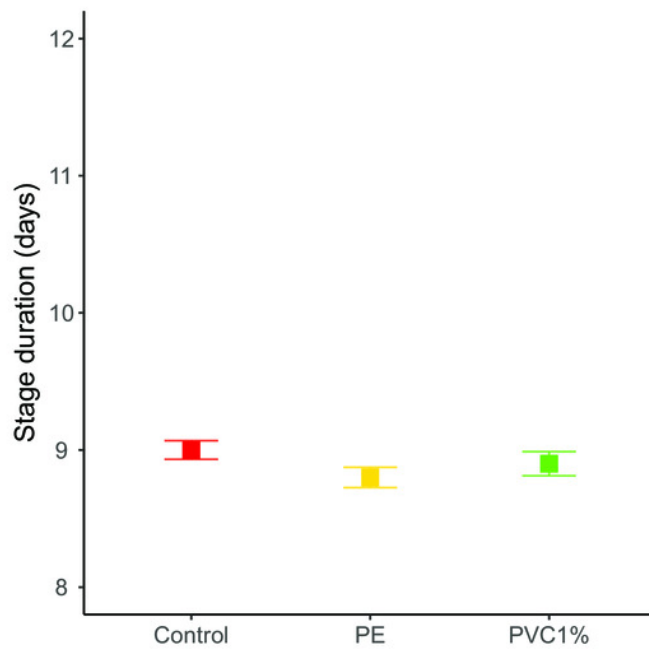
Figure 3

Offspring per female in the F1

Total offspring per female, where flies reared in plastic supplemented or control media laid eggs on non-supplemented food. Boxes correspond to the interquartile range (IQR), whiskers extend to the largest value no further than 1.5 * IQR from the limit of the box, black dots represent the mean, black bars represent the median, and empty dots represent outlier values (exceeding 3 standard deviations from the mean). Profiles at the right of each panel illustrate the distribution density of each treatment. Red box/profile: control, yellow box/profile: 1% virgin polyethylene (PE), green box/profile: 1% polyvinyl chloride (PVC), blue box/profile: 4% PVC. Asterisks correspond to significant differences (p<0.05) between the treatment and the control in a multiple comparisons test.
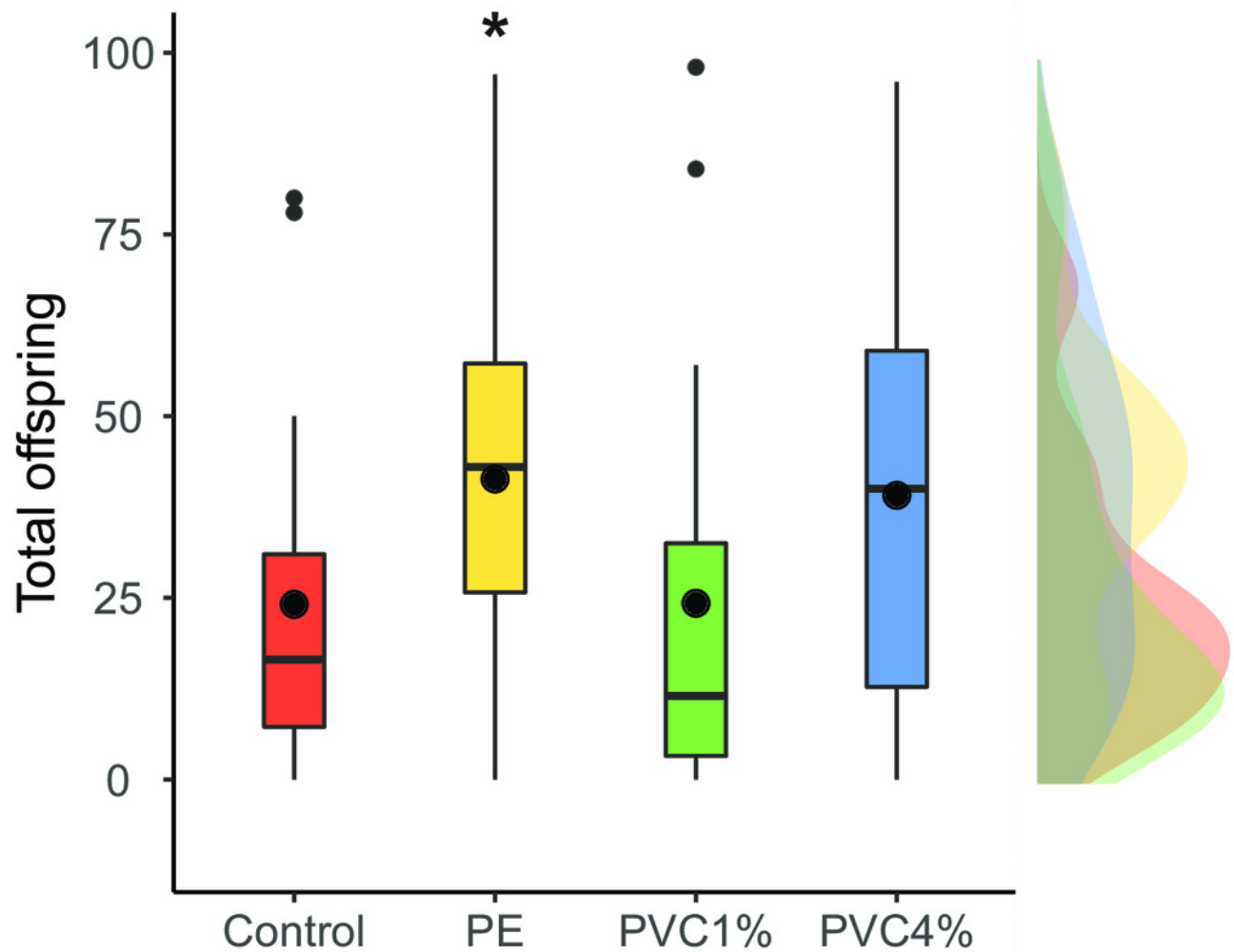
Figure 4

Comparison of wing size under each condition

Right wing sizes for F1 (a) and F2 (b) male flies. Boxes correspond to the interquartile range (IQR), whiskers extend to the largest value no further than 1.5 * IQR from the limit of the box, black dots represent the mean, black bars represent the median, and empty dots represent outlier values (exceeding 3 standard deviations from the mean). Profiles at the right of each panel illustrate the distribution density of each treatment. Red box/profile: control, yellow box/profile: virgin polyethylene (PE), green box/profile: 1% polyvinyl chloride (PVC), blue box/profile: 4% PVC. Asterisks correspond to significant differences (p<0.05) between the treatment and the control in a multiple comparisons test.