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Behavioural ecology meets oncology: quantifying the recovery of animal behaviour to a transient exposure to a cancer risk factor

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Wildlife is increasingly exposed to sublethal transient cancer risk factors, including mutagenic substances, which activates their anti-cancer defences, promotes tumourigenesis, and may negatively impact populations. Little is known about how exposure to cancer risk factors impacts the behaviour of wildlife. Here, we investigated the effects of a sublethal, short-term exposure to a carcinogen at environmentally relevant concentrations on the activity patterns of wild *Girardia tigrina* planaria during a two-phase experiment, consisting of a 7-day exposure to cadmium period followed by a 7-day recovery period. To comprehensively explore the effects of the exposure on activity patterns, we employed the double hierarchical generalized linear model framework which explicitly models residual intraindividual variability in addition to the mean and variance of the population. We found that exposed planaria were less active compared to unexposed individuals and were able to recover to pre-exposure activity levels albeit with a reduced variance in activity at the start of the recovery phase. Planaria showing high activity levels were less predictable with larger daily activity variations and higher residual variance. Thus, the shift in behavioural variability induced by an exposure to a cancer risk factor can be quantified using advanced tools from the field of behavioural ecology. This is required to understand how tumourous processes affect the ecology of species.

1. Introduction

Human activities are increasingly degrading the environment, often creating evolutionary mismatches between the risk of developing malignancies and the effectiveness of natural cancer defences [1]. As a result, many wildlife species are now developing cancers [2–6]. Because of the exacerbated need to activate anti-cancer defences [7], and/or because of the adverse effects of the disease itself [8], many phenotypic traits are substantially altered in these individuals, which may even ultimately influence ecosystem functioning [9,10]. For example, oncogenic processes can alter life-history traits in their host at both early and late stages of tumour development [11–14], increase the risk of predation [15,16] and modify their commensal, parasitic and microbiome communities [15,17–19]. By causing massive mortalities in populations of key ecosystem species, cancers can have major cascading effects on ecosystem biodiversity [20]. In addition, tumour progression can cause major modifications in the behaviour of the host [21]. Examples of this phenomenon include:

tumorous *Drosophila* selectively choosing their social environment to mediate tumour progression [22]; Tasmanian devils affected with devil facial tumour diseases (two transmissible cancers) reducing their social interactions with other conspecifics as tumour size increases [23]; and green sea turtles affected by fibropapillomatosis spending extended periods of time resting on the seabed compared to healthy individuals [24].

The studies of the effect of tumoural processes on the host's behaviour are currently mainly limited to late stages of tumour progression, because late-stage tumours are easier to observe and we generally lack the tools to detect early stage tumours in wildlife [25]. However, the exposure of species to cancer risk factors, and/or the very early stages of tumourigenesis are also predicted to have strong effects on the host behaviour [4,26,27]. For example, damage to the DNA must be repaired to avoid the accumulation of deleterious mutations leading to the emergence of tumorous cells (see for instance [28], an energetically costly process for the host [29]). These costs of being exposed to sublethal levels of cancer risk factors can be further increased when species are in an unfavourable ecological setting (for example under increased risk of predation [30], or intraspecific competition [31]).

The effects of tumoural processes on host behaviour are currently primarily quantified at the population level. This may be achieved by estimating the mean value of a given trait, for example distance travelled, number of contacts or distance between conspecifics [22,23], the duration of time spent resting [24] or the mean number of prey consumed [15]. A focus on the mean, while ignoring individual variation, necessarily limits our inferences and ultimate understanding because individuals are known to differ in susceptibility to cancer; some individuals have greater ability to fight cancer and this ability is often correlated with behaviour [21] (figure 1). For instance, recent conceptual advances and literature reviews indicate that individuals with a high energetic capacity are better able to fight cancer and that high capacity encourages higher activity, and vice versa, in humans and other animals [32,33]. In addition, and unlike most other animal attributes, behaviour is a highly labile trait with low repeatability [34], meaning that repeated measures of behaviour should be employed to estimate an individual's phenotype, otherwise biased estimates of population means may be obtained [35].

Individual differences in behaviour can be assessed using repeated measures of behaviour, and may be evident as individual differences in activity, individual differences in how activity changes through time, and even differences in how unpredictable an individual is—termed residual intraindividual variation (rIIV). An individual's rIIV may be viewed as a form of unexplained behavioural plasticity or represent within-individual endogenous factors such as energetic constraints, variation in epigenetics and hormonal cycles among others [36–38]. Given that more than 60% of overall behavioural variation is unexplained residual variation [34], individual differences in rIIV might be an important but mostly overlooked individual attribute to consider when studying behaviour [36]. Given the individual differences described above, and if they are linked to underlying physiology, we might expect to see a different expression of trait variance under stressful conditions as some individuals may be more affected than others (figure 1).

Here, we used wild planaria (*Girardia tigrina*), a common freshwater invertebrate, as a study species to study how

exposure to cancer risk factors may impact on animal behaviour, and individual behavioural variation at various hierarchical levels. The planaria model has several advantages to study the effects of exposures to sublethal doses of cancer risk factors. Early stages of carcinogenesis can be induced by exposing them to heavy metals or hydrocarbons [39–41]. Their DNA repair pathways and tumour suppressor genes are activated by an exposure to carcinogens which increase the costs associated with these functions for the animals [39,42,43]. They are also ecologically relevant, as *G. tigrina* has a widespread distribution, including in Australia, and is an invasive species which feeds on native invertebrates [44]. They are also easy to maintain and experiment with in the laboratory [45]. To simulate an exposure to a transient cancer risk factor, for the duration of one week we exposed planaria to cadmium, a heavy metal known to induce carcinogenesis in planaria [39,40]. Activity rates were monitored during this interval, and during a recovery interval of equal duration, to quantify the effect of both periods on the behaviour of the animals. This protocol aimed to mimic the early stage of tumourigenesis, which may not lead to malignant cancer in a wildlife species, but is likely to activate anti-toxicant and anti-cancer defences [28]. We then combined tools from behavioural ecology, statistical modelling and cancer ecology to explore the data both in terms of quantifying the effect of cadmium exposure and recovery on the population means of measured traits, but also on individual variation in means and individual variation in their rIIV. With this paper we aim to demonstrate the usefulness of this multidisciplinary approach to obtain valuable insights and a more complete picture on the role that tumoural processes play on the ecology of species [46]. Such insights are important considering how human activities are increasing the exposure of species to a range of cancer risk factors, likely contributing to the degradation of ecosystem functioning and altering the evolutionary course of species.

2. Material and methods

(a) Planaria trapping

Wild freshwater planaria (*G. tigrina*) were collected from Saint Augustine's lagoon (Highton, Victoria, Australia, 38°11'30"S, 144°18'48"N) in September 2022 using custom made planarian traps baited with beef liver and left in the water overnight. Saint Augustine's lagoon is a roughly triangular, 150 m long, and 140 m wide stormwater fed pond that receives urban runoff from the adjacent areas, with Australian eelgrass (*Vallisneria australis*) as the primary aquatic vegetation. It is regularly stocked with fish for recreational fishing. After capture, the planaria were transported to the laboratory and transferred into spring water (Coles Natural Water Spring) in 1 l food grade polypropylene plastic containers, at a density of approximately 1 individual per 10 ml of water and left in a dark cupboard in a laboratory at 20°C without feeding for one week to standardize their metabolism. The planaria were left unfed in the subsequent experiments.

(b) Cadmium exposure experiments

Cadmium is a heavy metal and non-essential element which is carcinogenic in humans and wildlife [47], including planaria, activating anti-cancer defences (e.g. tumour suppressor genes, DNA repair mechanisms) and causing tumours over long periods of time [39,40,48]. Cadmium contaminates aquatic ecosystems during the mining and processing of zinc or iron ore, the use of phosphate fertilizers in agriculture, waste incineration and

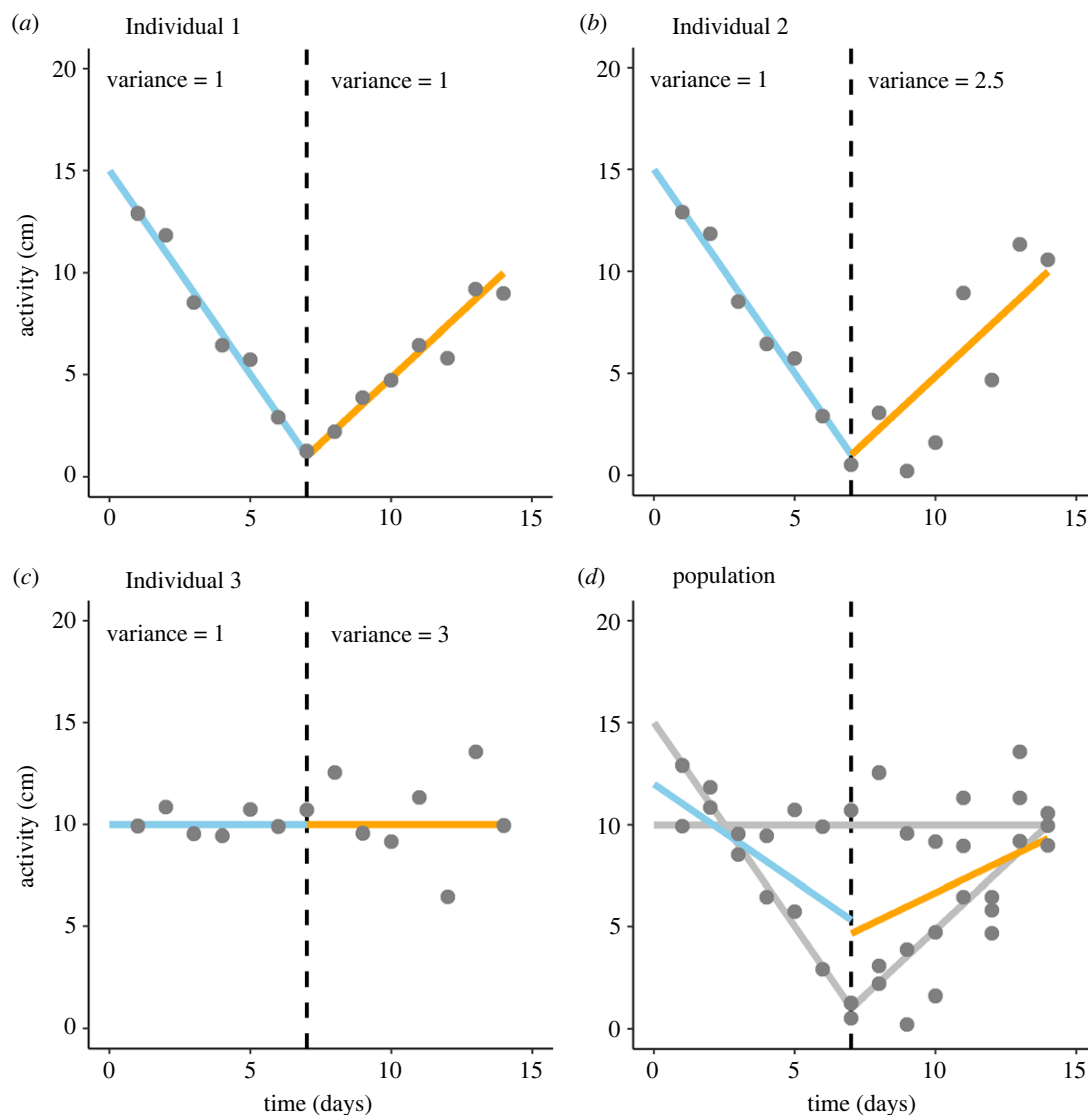


Figure 1. Hypothetical responses of three different animals in an experiment during which they are exposed to cadmium for one week and then recover in a clean environment for one additional week. During each day of the experiment each animal is subjected to a behavioural trial during which the distance travelled is measured. In all plots, the activity of animals during each behavioural trial are represented as grey dots, the blue lines represent the mean distance travelled by a planarian during the first 7 days of the experiment, and the orange lines represent the mean distance travelled by a planarian during the next 7 days. (a) As Individual 1 is damaged by cadmium (e.g. by tumoural processes), the mean distance travelled during the first half of the experiment decreases over time. Once the animal is moved into a clean environment the mean distance increases over time as it recovers. The variance of the residuals is the same for both phases of the experiment, thus the predictability is the same. (b) As Individual 2 is damaged by cadmium, the mean distance travelled during the first half of the experiment decreases over time. Once the animal is moved into a clean environment the mean distance increases over time as it recovers. The variance of the residuals also increases during the second phase of the experiment. Thus, the predictability decreases during the second phase. (c) The exposure of Individual 3 to cadmium has no effect on the mean distance travelled during each phase of the experiment; however, the variance of the residuals increases during the second phase of the experiment. The predictability decreases during the second phase. (d) The three individuals are pooled into a statistical population which may be obtained at the end of an experiment. All three individuals have different responses to exposure to cadmium with variations in their mean (solid grey lines) but also in their residual variance. This generates a relatively complex dataset in which both the information about the mean distance travelled by an animal and the residual intraindividual variability (rIIV) must be quantified to capture changes in residual variance to obtain an accurate picture.

disposal, and via natural emissions such as volcanic eruptions [47,49], and as a result bioaccumulates in the trophic chain [50,51]. Cadmium increases the risk of developing cancer by inducing oxidative stress and impairing major DNA repair pathways, thus increasing the probability of mutations in the genome [52,53]. In relatively undisturbed Australian aquatic ecosystems, the concentrations of cadmium in water are low, in the order of 0.1 ppb [54].

We first determined the lethal concentration 50 (LC50, measured at 96 h) of wild *G. tigrina* planaria exposed to cadmium. We exposed planaria to increasing nominal concentrations of 0, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8 and 9.6 ppm of Cd^{2+} prepared from a 1000 ppm Cd^{2+} stock solution obtained by dissolving CdSO_4

salts in spring water (Sigma Aldrich, catalogue no. 481882). We trialled 24 planaria (in six-well plates with one individual per well, Thermo Fisher Scientific, catalogue no. 140675) per treatment for a total of 168 animals and recorded their mortality over a period of 96 h. A LC50 of 2.08 ppm of Cd^{2+} (95% CI: 1.83–2.34 ppm) was estimated after analysing the data using a logistic regression (electronic supplementary material, S1). Based on this value we decided to use a nominal concentration of 0.5 ppm of Cd^{2+} (close to 25% of the LC50) to perform the behavioural experiments. This concentration was chosen in order to challenge the anticancer defences of planaria without causing mortality. The concentrations of cadmium in the water and sediments of Saint Augustine's Lagoon are not known. In urban environments,

Allinson *et al.* [55] found that surface sediments of 24 wetlands and retention ponds in Melbourne, located in the same state as our study site, and with relatively similar urbanism, have a median concentration of 2 ppm of cadmium (range: 2–3 ppm). Similarly, Sharley *et al.* [56] measured a mean concentration of 1.2 ppm of cadmium (range: less than 1–11 ppm) in 111 urban wetlands in and around Melbourne. Thus, we considered a nominal concentration of 0.5 ppm of Cd^{2+} as appropriate to simulate a sublethal exposure to a cancer risk factor.

To investigate the effects of cadmium exposure on behaviour, 60 planaria were transferred into ten six-well plates, with one individual per well, filled with either 10 ml of 0.5 ppm Cd^{2+} spring water solution (treatment) or 10 ml of spring water only (control) and then placed in the dark in a 20°C incubator (Infors HT Ectron) for 7 days. On day eight of the experiment, the cadmium solution was replaced in both the control and the treatment group with 10 ml of clean spring water after gently rinsing each well with water after which the plate was replaced in the incubator for an additional 7 days. Thus, in this publication we refer to the first 7 days of the experiment as the exposure phase, and the subsequent 7 days as the recovery phase. The experiment length was selected to simulate a short-term exposure to cadmium but also as a trade-off between the logistics involved in the experiment and the number of measurements required to quantify rIIV (here, seven repeated measurements per animal per phase, with an initial 60 individuals per treatment group, see [37]).

A behavioural assay was performed on every individual for each day of the 14-day long experiment. The whole experiment was repeated three times with 40 animals (20 in the control group and 20 in the cadmium exposed group) for a total of 120 individuals.

(c) Behavioural assay and movement recording

Once per day, each six-well plate was removed from the incubator and placed on a filtered light pad (Born LED Light Pad, model BOLEDLPA4, 279 lumens, white light) housed within a dark box that blocked out external light sources. When exposed to light, *G. tigrina* exhibit a photonegative response activity which stimulates their movement [57,58]. Planaria activity was filmed using a tripod-mounted Canon camera for 11 min before being replaced in the incubator.

Individual planaria trajectories were reconstructed from video recordings by applying a background subtraction method provided by the OpenCV python library (that we optimized for planaria activity), calculating the average coordinates of the pixels identified as being part of an animal (BackgroundSubtractorCNT method [59]). The trajectories were then standardized by interpolating them to one location per second and the activity of each planaria was calculated as the total distance (in mm) travelled by an individual during a trial [60]. The video footage was also used to measure planaria body length (mm). The first minute of the trajectory was discarded to allow the algorithm to accurately detect and track individual planaria (as it uses optical flow between different frames of the video to identify individuals) and the next 10 min were used to determine activity (a duration used in multiple studies, see for example [61–63]). Planaria can be slow moving and take several seconds to be initially detected by the algorithm. Since the video recordings were performed outside the incubator in the same room, we recorded the ambient air temperature (°C) to control for the potential confounding effects on the movement of the planaria.

(d) Statistical analyses of activity data

We used a Gaussian double hierarchical generalized linear model to quantify the effect of cadmium exposure on the activity of planaria [37]. This model allows for fixed and random effects to be specified for the mean level of the model that predicts the population average and interindividual variation in activity levels of

the planaria, and also in the residual part of the model that permits residual variation to vary with fixed effect predictors and to vary among individuals [37,64]. By contrast, most models assume a single residual variance that does not vary (the familiar constant variance assumption). We included a random intercept effect, and a random slope effect with respect to days elapsed within a phase (1–7); these effects capture individual variation in mean activity at day 1, and individual variation in how activity changes through time. We also included the treatment (control or cadmium exposed) as a categorical variable, an interaction between day and treatment group, the length of the planaria (in mm), the temperature (°C) and the batch number (categorical) as fixed effects. Before fitting the model, all continuous variables were mean centred. In the residual part of the model, we included the same fixed effect used in the average activity part, to determine if the predictability of planaria behaviour differs between treatment groups and phases. Additionally, we included a random intercept effect, which permits the model to capture individual differences in residual variation (rIIV). The model was specified with an unstructured correlation matrix, estimating all potential correlations between the intercepts, slopes and rIIV [36,37,64,65] and thus addressing any possible non-independence of the repeated measures within and across phases. The coefficient of variation of predictability (CV_p) was also used to compare the predictability between treatment groups and phases [37]. We ensured that the residuals at both the population and the individual level followed a normal distribution using histograms, boxplots and qq-plots to meet the model assumptions. Although activity rates of animals are often skewed, we found this was not the case here, and that log-transforming activity measurements negatively skewed the residuals, violating distribution assumptions. The zero activity values present in the data (9.6% of the total data) did not skew the residual distribution.

When needed, and to visualize the effect of a variable on the mean activity of planaria, or their mean residual variance at the population level, marginal effects were calculated by holding all parameters in the model constant except for the variable of interest [66].

All statistical analyses and modelling were performed using R v. 4.0.2 [67]. All linear models were fitted using the brms package with default priors [68]. Estimates are provided with their 95% credible intervals (95% CI). The model was run on 4 chains of 5000 iterations each with 500 iterations discarded as warmup and a thinning interval of 2. Diagnostic checks were also performed to ensure that the model converged appropriately ($\hat{R} = 1$, bulk effective sample size and tail effective sample size greater than 1000, random intercepts, and random slopes following a normal distribution [68]). All variance estimates reported in this study are expressed on the log scale. We used the posterior predicted values from the model to estimate differences between treatment means and variances, by calculating a difference value at each iteration to yield a distribution from which we obtained the mean difference and credible interval.

3. Results

We obtained a total of 1610 activity measurements, arising from 115 planaria. Over the course of the experiment, three planaria died in the control group and two in the treatment group and were excluded from the analysis. No tumours were observed during the experiment.

(a) Effect of cadmium on the average distance travelled by planaria at the population level

During the exposure phase the planaria exposed to cadmium were less active compared to the control planaria, showing an

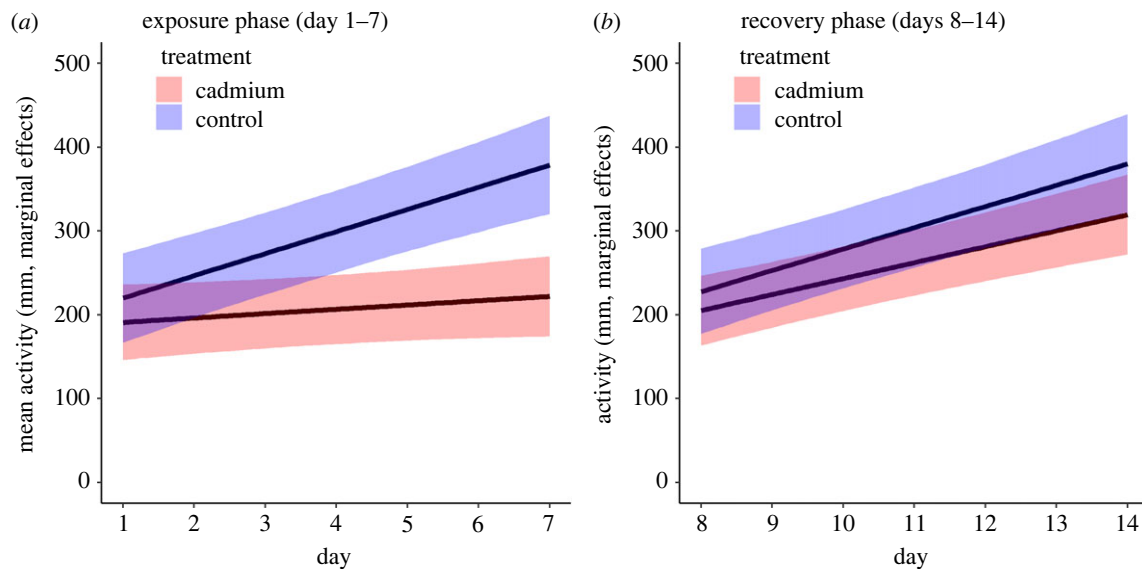


Figure 2. Effects of the exposure to cadmium, represented as their marginal effects, on the mean activity of planaria (solid lines, with their associated 95% CI error bands) during the (a) exposure and (b) recovery phases of the experiments. The planaria exposed to cadmium were less active in both phases compared to the treatment group.

average difference of 41.5 mm (95% CI: $-0.1, 82.4$ mm) over the whole phase. We observed an increase in the activity of planaria in both groups during the exposure phase of the experiment, but that increase was substantially smaller in exposed planaria compared to control planaria (mean increase of 5.2 mm per day, 95% CI: $-1.72, 12.29$ mm per day in cadmium exposed planaria compared to 26.5 mm per day, 95% CI: $17.5, 35.7$ mm per day in the control planaria). The water changes on the eighth day, at the start of the recovery phase, caused a drop in the activity in all groups to levels similar to the first day of the exposure phase suggesting a short-term stress response. During this second phase planaria in both groups increased their activity at nearly the same rate (19.1 mm per day, 95% CI: $11.9, 26.7$ for exposed planaria compared to 25.4 mm per day, 95% CI: $16.5, 34.9$ mm per day for control planaria; figure 2).

(b) Interindividual variation in the response of planaria to cadmium

During both phases of the experiment, individuals with higher initial activity tended to have higher increases in activity over time. The distance travelled by a planaria during a given day was on average larger when an individual had a high initial activity, albeit with some uncertainties around the effect sizes (figure 3).

The variance in the initial activity predicted by the model (i.e. the intercept variance predicted at day 1) was similar between the two groups during the exposure phase but larger in the control group during the recovery phase. During the exposure phase the variance was $\sigma^2 = 9.6$ (95% CI: $9.1, 10.1$) for the control group and $\sigma^2 = 9.2$ (95% CI: $8.6, 9.6$) for the cadmium exposed group, with an average difference between the two groups of 0.5 (95% CI: $-0.3, 1.2$). During the recovery phase the variance was $\sigma^2 = 9.5$ (95% CI: $9.1, 10.0$) for the control group and $\sigma^2 = 8.7$ (95% CI: $8.1, 9.3$) for the cadmium exposed group, with an average difference between the two groups of 0.8 (95% CI: $0.1, 1.5$) (figure 3).

We found little evidence that the variance in the daily change in activity of individual planaria (i.e. slope variance)

differed between the two phases of the experiment. During the exposure phase the variance was $\sigma^2 = 4.6$ (95% CI: $-1.1, 6.2$) for the control group and $\sigma^2 = 6.2$ (95% CI: $4.3, 7.1$) for the cadmium exposed group, with an average difference of -0.4 (95% CI: $-5.8, 3.9$). During the recovery phase the slope variance was $\sigma^2 = 4.7$ (95% CI: $0.8, 5.9$) for the control group and $\sigma^2 = 5.4$ (95% CI: $3.3, 6.4$) for the cadmium group, with an average difference of 0.8 (95% CI: $-1.3, 3.0$) (figure 3).

(c) Effect of cadmium on the residual intraindividual variability

At the population level, the average residual variance slightly decreased over time in both control and cadmium exposed groups, with a small difference during the exposure phase which disappeared during the recovery phase. Planaria length had a considerably stronger effect, whereby residual variance increased with length (figure 4).

There were significant interindividual differences in residual variation (inverse predictability) as given by CV_P values, but treatment differences were not observed. Individual variation in predictability was similar in the cadmium group ($CV_P = 0.29$, 95% CI: $0.19, 0.42$) and control group ($CV_P = 0.24$, 95% CI: $0.12, 0.38$) during the exposure phase. Similarly, the predictability was relatively similar between cadmium exposed ($CV_P = 0.25$, 95% CI: $0.14, 0.36$) and control group ($CV_P = 0.37$, 95% CI: $0.23, 0.52$) planaria during the recovery phase.

We found that the individual predictability was strongly correlated to the initial activity of planaria in the control group for both phases. Planaria with high initial activity levels tended to have lower predictability (= higher variation) in activity. This correlation was less pronounced for planaria exposed to cadmium during the recovery phase of the experiment (figure 5).

4. Discussion

To the best of our knowledge this study is the first to apply experimental designs and advanced statistical modelling

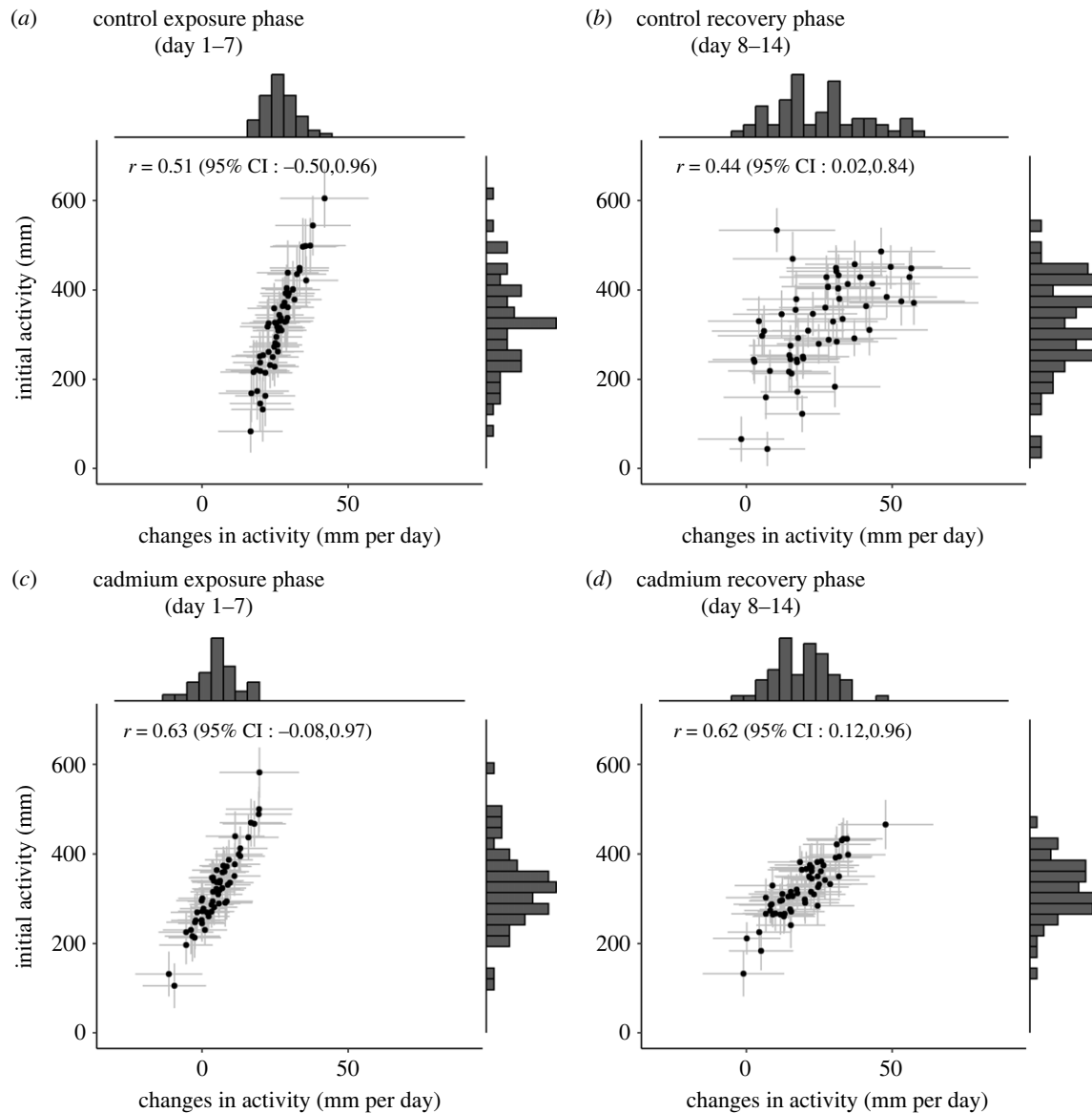


Figure 3. Effect of a sublethal exposure to cadmium followed by a recovery phase on the initial activity and change in activity (represented by black dots with their associated standard error as grey lines) on (a,b) control unexposed planaria and (c,d) exposed planaria. The histograms on the right of the plots visualize the variance in the predicted initial activity (in mm) of planaria during the two phases of the experiments (while accounting for temperature, body size and batch effects). The histograms on the top of the plots visualize the variance in the predicted change in activity (in mm per day) of planaria during the two phases of the experiments (while accounting for temperature, body size and batch effects). Exposure to cadmium reduced the variance of the initial activity of planaria during both the exposure and recovery phases of the experiment. No difference in variance in the daily change between phases was observed.

tools from behavioural ecology to study the effect of sublethal cadmium exposure, a cancer defence activator and subsequent recovery in freshwater planaria. Mortality during the experiment was relatively low, and overall, based on their activity levels, the planaria were able to recover from the toxic exposure. However, the behaviour of the planaria was altered.

Planaria in contact with cadmium were less active compared to the control group, probably because of the direct damage caused by cadmium and/or because the energy invested in repairing those damages cannot be invested in movement. This decrease in activity was also observed during short-term behavioural trials on planaria [63,69]. During the recovery phase, the planaria exposed to cadmium partially recovered, as evidenced by an increase in activity compared to the exposure phase. It is not impossible that, considering their impressive regeneration abilities, planaria would fully recover if given enough time [70].

Cadmium induced tumours were reported at high prevalence within 14 days in one laboratory strain of *G. tigrina* exposed to concentrations within the range of what we used in our study [40]. No such tumours were observed in our experiments. Thus, it is possible that planaria inhabiting urban stormwater ponds are exposed to pollution which applies selective pressures and are thus relatively resistant to a transient sublethal exposure to cancer risk factors. In addition, *G. tigrina* is a species native to North America that was introduced to Australia (and many other areas around the world [71,72]). Metal pollution is known to increase the colonization success of some aquatic invasive species. For example, exposure to copper decreases the species richness of native sessile marine species while having little effects on the species richness of invasive species [73]. Similarly invasive freshwater bivalves tend to be more resistant to heavy metal pollution compared to native ones (for example zinc [74]). Our results clearly indicate how an exposure to a transient

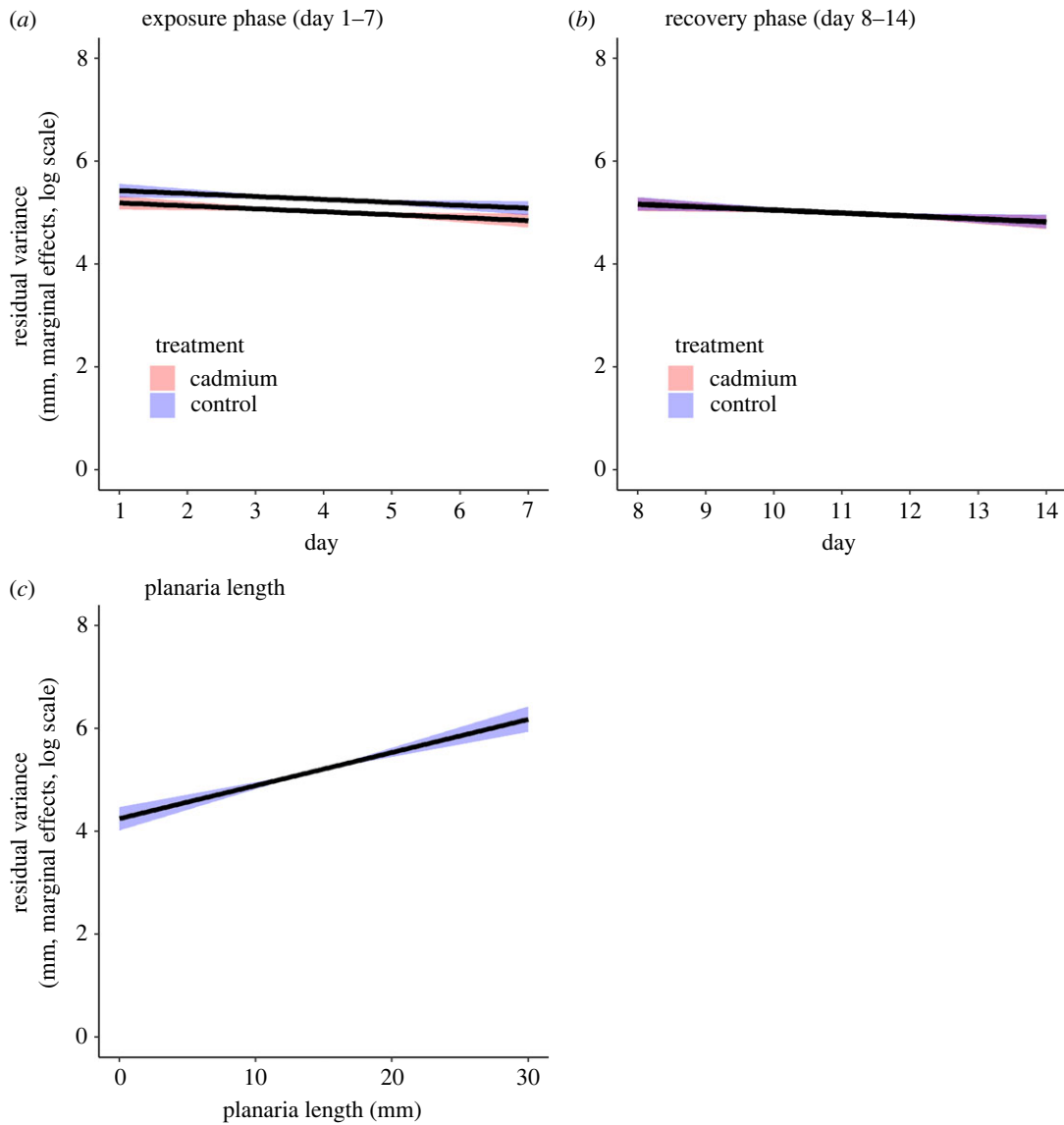


Figure 4. Effects of cadmium exposure on the mean residual variance of the activity of planaria (solid lines, with their associated 95% CI error bands) during (a) the exposure and (b) recovery phases of the experiments. (c) Effect of planaria length on the mean the mean residual variance of the activity of planaria.

cancer risk factor can temporarily affect the mean activity levels of an animal population.

We observed that both the rate at which the activity increased over time and the rIIV of planaria were correlated with the predicted initial activity at the start of a phase. This pattern linking high activity and rIIV has been observed in different species. For example, wild elephants (*Loxodonta africanus*) and barn owls (*Tyto alba*) with high average activity also have a high rIIV [65,75], and bold sticklebacks (*Gasterosteus aculeatus*), which explore their space extensively around their shelter, will be less predictable compared to shy individuals [76]. In the case of planaria, an interpretation of these results is that high activity is energetically costly and difficult to consistently maintain over long period of times, thus explaining the high residual variance in the control group. When exposed to cadmium, damaged planaria are not able to perform short bursts of activity (the mean activity and the variance in their initial activity was lower than the control group) and their activity is more consistent over time. The effect of a contaminant on the predictability of behaviour seems to dependant on the molecule that planaria are exposed to. For example, the predictability of the behaviour of hermit crabs (*Pagurus bernhardus*) is similar

between individuals exposed to copper and that of control crabs [77], yet individuals of this same species exposed to microplastics have reduced predictability compared to non-exposed individuals [78]. We also observed that the average residual variance increases with planarian length. As the size of the planaria increases, and, therefore, their ability to move large distances increased, they become less predictable. Thus, experiments investigating the effect of a pollutant on the activity of wild planaria should either use planaria of the same size, to reduce the introduction of this additional residual variance but with the cost of having a sample that is less representative of wild populations, or explicitly include planaria length using a double hierarchical mixed effect model to account for this effect.

The increase in activity in all groups, albeit to a lesser extent in the cadmium exposed planaria, over the course of the experiment suggests a lack of habituation to light exposure [58,79] and planaria maintained their photonegative behaviour. The effect of light exposure on the behaviour of planaria has often been studied for a relatively short period of time (e.g. [62,80]) and studies investigating how planaria respond to repeated light exposure over a period of time of more than a few days are more limited. Our results contrast with previously

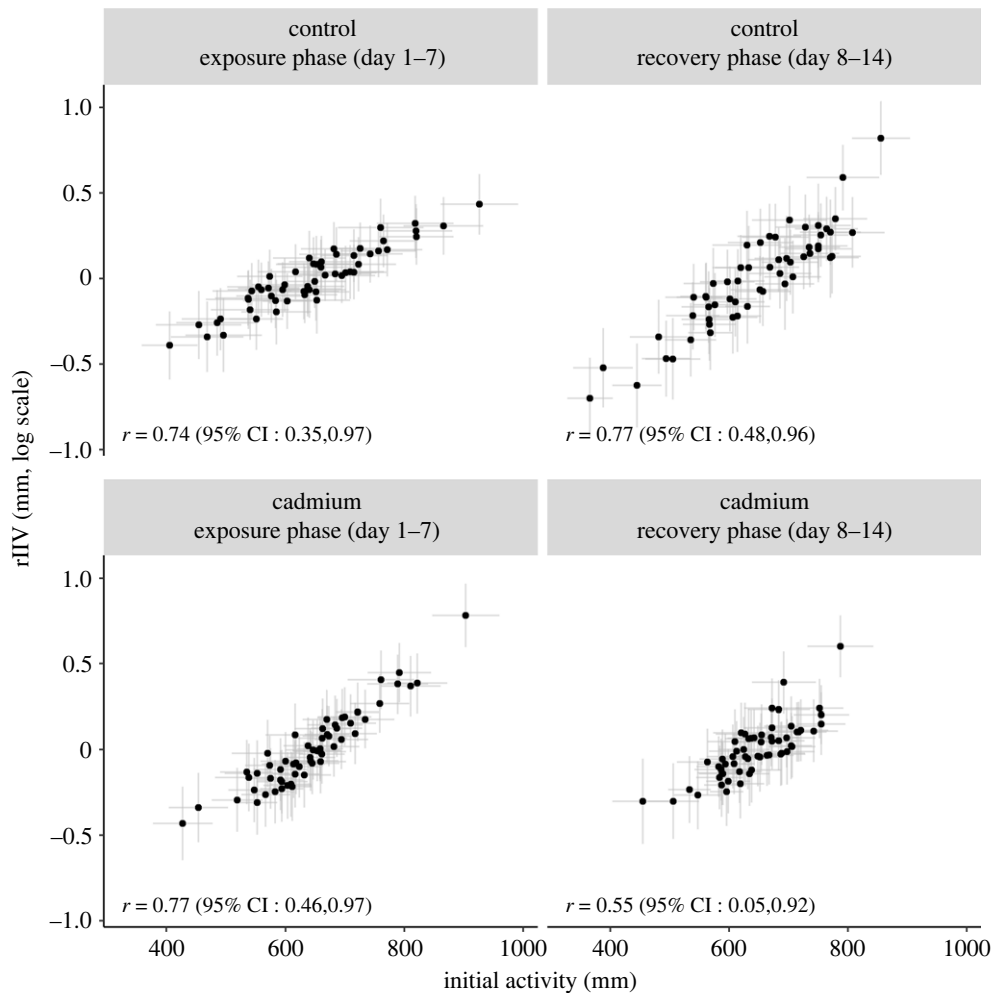


Figure 5. Residual interindividual variability (rIIV, black dots) in function of the initial activity levels of planaria during the exposure and recovery phases of the experiments. Planaria with higher initial activity levels in the control group exhibit a higher rIIV, and are thus less predictable, often with large variations in day-to-day activity. Grey lines represent the 95% CI of each estimate.

published studies. For example, Prados *et al.* [58] and Westerman [79] found that the average distance travelled by *G. tigrina* repeatedly exposed to light decreased over time. A potential explanation for these differences is that it is likely that those two studies used planaria strains cultivated in the laboratory for extended period of times with food provided ad libitum multiple times per week (often beef liver or egg yolk) [81], thus reducing the selection pressure for predatory behaviour. In the wild, planaria actively hunt for a wide range of invertebrate preys with varying temporal and spatial densities [82]. Thus, it is possible that, as the duration of their starvation period increases, planaria actively seek food sources. It is not possible to have a definitive answer with the experimental design we used and further studies will be required to understand behavioural variation between planaria strains. Our results highlight the importance of comparing the ecology and behaviour of laboratory raised strains of planaria to those of wild populations of the same species.

In this study, which is a proof of concept, we only used one concentration of cadmium which simulated a sublethal exposure. Our approaches could be replicated across multiple cadmium concentrations and modelled using a double hierarchical generalized linear model to investigate the shape of the behavioural response of planaria in relation to increasing exposure to cancer risk factors, data which is lacking for nearly all wildlife species. It is possible that as the concentration increases differences in predictability appear between

treatment groups, highlighting individuals more resistant to the exposure. This approach could also be applied to a range of other risk factors likely to activate anti-cancer defences and that are predicted to have an impact on animal behaviour [4,9]. In addition, it may be interesting to apply this approach using a combination of both tumour initiators (such as cadmium) and promoters, which could increase the speed at which tumorous processes damage the host (as observed in certain species of planaria) [48]. Finally, a direct measure of damage caused by the exposure to the chemical, for example damage to DNA [83], the production of oxygen reactive species [84] or the expression of genes [85] associated with the exposure to cadmium could be included in the model to provide mechanistic insight on the effect of cadmium on the behaviour of the animal. The inclusion of damage measurements would be particularly useful to investigate how the damage-repair ability of individuals, for example in relation to ageing or reproduction, affects the variance and correlates with behavioural traits and the likelihood of tumours appearing (e.g. as observed in humans [86]). Overall, our results highlight the importance of considering the effect of the exposure to cancer risk factors on the behaviour of animals.

With this study we demonstrate the value of using behavioural ecology modelling tools to investigate the effect of the exposure to a sublethal cancer risk factor on the behaviour of animals. As environments are increasingly degraded, and

cancer risk landscapes are changing, understanding how oncogenic effects perturb ecosystems is becoming increasingly important. Studies at the interface of ecology and oncology will contribute significantly to predict the negative effect that cancers can have on the biosphere.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The data are provided on Dryad [87] and in the electronic supplementary material [88].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. H.K.: conceptualization, data curation, investigation, methodology, writing—review and editing; S.T.: conceptualization, methodology, validation, writing—review and editing; J.M.: methodology, resources, validation, writing—review and editing; J.B.: writing—review and editing; A.M.: investigation,

writing—review and editing; P.A.B.: investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; D.J.M.: methodology, validation, writing—review and editing; B.U.: writing—review and editing; A.S.: conceptualization, methodology, writing—review and editing; F.T.: funding acquisition, writing—original draft, writing—review and editing; A.M.D.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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