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Original article

Dietary tryptophan modifies the effects of developmental predator stress on lifespan in male *Drosophila melanogaster*

Sergejs Popovs [a,b], Tatjana Grigorjeva[b], Ronalds Krams[a,b,c], Giedrius Trakimas[b,d], Markus J. Rantala[e], Jorge Contreras-Garduño[f], Priit Jõers[g], Colton B. Adams[a,h,i], Indrikis Krams[a,b,i,j*] and Tatjana Krama[a,b,k]

[a]Latvian Biomedical Research and Study Centre, Riga 1067, Latvia
[b]Department of Biodiversity, Institute of Life Sciences and Technology, Daugavpils 5401, Latvia
[c]Institute of Food Safety, Animal Health and Environment "BIOR", Riga 1076, Latvia
[d]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius 10257, Lithuania
[e]Department of Biology, University of Turku, Turku 20014, Finland
[f]Laboratorio de Ecología Evolutiva, Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, Morelia 58190, México
[g]Institute of Molecular and Cell Biology, University of Tartu, Tartu 51010, Estonia
[h]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA
[i]Department of Psychology, University of Tennessee, Knoxville, TN 37998, USA
[j]Department of Ecology, Faculty of Medicine and Life Sciences, University of Latvia, Riga 1004, Latvia
[k]Chair of Plant Health, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu 51014, Estonia
*Address correspondence to Indrikis Krams. E-mail: indrikis.krams@ut.ee.
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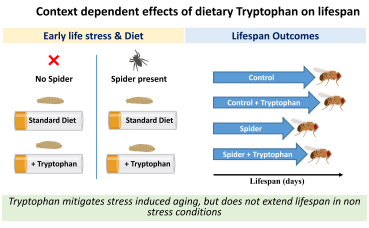
Abstract

Environmental stressors and diet both play key roles in shaping animal lifespan, yet their interactive effects remain poorly understood under ecologically realistic predation risk. We experimentally tested how predator-induced stress during early development and dietary tryptophan supplementation affect the lifespan of *Drosophila melanogaster* males. Tryptophan, an essential amino acid and precursor to multiple physiological pathways including serotonin biosynthesis, influences stress responses, and aging-related processes. Using a 2 × 2 experimental design (predator exposure ± tryptophan supplementation), we found that predator-exposed flies had the shortest lifespans, while those without predators lived longest. Tryptophan supplementation significantly increased lifespan in predator-exposed flies but did not alter lifespan in predator-free conditions. These results suggest that dietary tryptophan can mitigate some consequences of chronic developmental stress, but its effects depend on environmental context and do not imply a general mechanism of serotonergic lifespan extension. Instead, our findings support a broader ecological interpretation in which nutritional inputs interact with early-life stress to shape adult survival. Our results highlight the importance of diet–environment interactions in aging and demonstrate that ecologically relevant predator stress can modify life-history outcomes in a model organism. This study illustrates how nutritional manipulations can modify—but not eliminate—the survival costs of chronic stress.

Keywords developmental stress, diet–environment interaction, *Drosophila melanogaster*, lifespan, predator-induced stress, serotonergic precursor

Graphical abstract



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It is well established that lifespan can be influenced by dietary manipulation. For example, reducing total calorie intake affects behavioral and physiological responses and extends lifespan in the common fruit fly *Drosophila melanogaster* (Chippindale et al. 1993). Moreover, adding or removing specific nutrients influencing the nervous system can also shorten or prolong lifespan (Libert et al. 2007; Lee et al. 2008; Alcedo et al. 2013; Stefana et al. 2017; Sarmah et al. 2025). Dietary amino acids in particular play central roles in shaping life-history traits by influencing metabolism, reproduction, immune function, and stress responses. Among these, tryptophan is notable because it participates in multiple physiological pathways, including protein synthesis, immune regulation, metabolic signaling, and neuromodulation.

A growing body of research has linked the serotonergic system to the regulation of mood, stress responses, feeding behavior, and lifespan across a variety of taxa (Margolis 2021; Hwang and Oh, 2025). Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from the essential dietary amino acid tryptophan, which can be experimentally manipulated in both vertebrates and invertebrates (van Galen et al. 2021). Manipulating serotonin levels—via dietary tryptophan or selective serotonin reuptake inhibitors (SSRIs)—alters behavioral and physiological traits in *D. melanogaster* (Kain et al. 2012; Krams et al. 2018, 2021). Elevated serotonin signaling has been associated with changes in locomotor activity, decision-making, stress responses, and behavioral predictability (Ries et al. 2017; Popovs et al. 2024).

However, the role of serotonin in modulating lifespan is complex: increased serotonergic activity can shorten lifespan under some conditions and alleviate stress-induced mortality under others (Ro et al. 2016; Lyu et al. 2021). Dietary tryptophan supplementation enhances serotonin synthesis and influences feeding, reproduction, and stress responses in both insects and vertebrates (Ries et al. 2017; Hori et al. 2018). Altering serotonin levels through genetic or pharmacological methods can modulate aging pathways, often via effects on insulin signaling, oxidative stress, and metabolic regulation (Libert et al. 2007; Alcedo et al. 2013; Munneke et al. 2022). Similarly, chronic SSRI treatment can reduce lifespan in *Caenorhabditis elegans* and flies (Petrascheck et al. 2007; Ries et al. 2017). Importantly, these diverse outcomes highlight that tryptophan supplementation does not act through a single linear serotonergic mechanism but instead reflects broader nutritional and physiological trade-offs.

Environmental stressors, including predation risk, can accelerate aging in prey species (Janssens and Stoks 2013; Pietrzak et al. 2015; Jermacz et al. 2020; Zhang et al. 2023), potentially through chronic activation of stress-response systems and altered energy allocation. Predator-induced stress during larval development in *Drosophila* reduces survival under both acute and chronic stress (Krama et al. 2023) and influences behaviors such as turning bias and phototaxis (Krams et al. 2021; Krama et al. 2023; Popovs et al. 2024). Predator stress can also induce a diabetes-like metabolic state marked by impaired glucose regulation (Krama et al. 2023).

Although tryptophan is the dietary precursor to serotonin, its effects on lifespan remain complex and context-dependent, varying across studies according to dosage, developmental stage, nutrient balance, and stress history. In some cases, tryptophan or serotonergic activation has been linked to reduced lifespan, whereas in others it interacts with environmental stressors in ways that alter survival outcomes (Kaneko et al. 2017; Dutta et al. 2022). These inconsistencies suggest that tryptophan's effects on aging may depend less on any single neurochemical pathway and more on interactions between nutrition, metabolism, and ecological conditions. In *D. melanogaster*, predator-induced developmental stress provides a realistic ecological framework for testing how such interactions shape lifespan.

Here, rather than inferring specific downstream neurochemical pathways, we test the broader ecological hypothesis that dietary tryptophan interacts with predator-induced stress to influence lifespan. Specifically, we ask whether tryptophan modifies the survival costs of chronic predator cues and whether its effects differ between stressful and benign environments. We selected dietary L-tryptophan as a nutritional manipulation because it serves as a precursor for multiple physiological pathways, including serotonin biosynthesis, immune regulation, and metabolic signaling, allowing us to test whether modest dietary supplementation can buffer life-history costs of chronic stress rather than targeting a single neurochemical mechanism.

To address these questions, we established 4 experimental groups: 1) a control group without predators or tryptophan, 2) a tryptophan-supplemented group without predators, 3) a predator-exposed group without tryptophan, and 4) a predator-exposed group with tryptophan supplementation. We expected predator-exposed flies to show the shortest lifespan, whereas the controls were expected to live the longest. We predicted that tryptophan supplementation would either have no effect or reduce lifespan in unstressed flies but would improve lifespan under predator-induced stress, consistent with earlier findings that tryptophan ameliorates stress-induced physiological dysfunction (Krama et al. 2023). By testing these predictions under a controlled but ecologically realistic predator-risk treatment in a genetically tractable model, we aim to bridge ecological and biological perspectives on how chronic stress and diet jointly shape aging.

Materials and methods

Insects

In this study, we used Oregon-R-modENCODE (#25211) wild-type strains obtained from the Bloomington Drosophila Stock Center (Indiana University, Bloomington, IN, USA). Flies were maintained in humidity-controlled climate chambers (Panasonic MLR-352H; Panasonic Healthcare Holdings, Tokyo, Japan) at Daugavpils University under conditions of 25 ± 1 °C, approximately 40% relative humidity, and a constant 12:12 h light–dark cycle with neutral-white LED illumination. To generate experimental populations, ten F0 males and ten females were placed in a single polystyrene vial (Genesee Scientific, El Cajon, CA, USA) containing fresh food and allowed to oviposit for 24 h. Vials containing eggs were then transferred to free, ventilated plastic containers ($110 \times 90 \times 120$ mm) (Popovs et al. 2024). The density of eggs/larvae was averaged to 120 eggs/larvae per vial.

To induce predation stress, some containers contained spiders (*Pardosa pullata*) that were allowed to roam freely. One spider per container was used in this experiment. The spiders were kept in containers only during the fly development phase. Before the imagos hatched, the spiders were removed from the containers. Spiders were free to move and hunt within the containers, providing a continuous source of visual, olfactory, and tactile cues to the developing larvae and pupae, and occasionally captured larvae.

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However, predation rates were not quantified because the experimental aim was to impose chronic predator-associated stress rather than measure consumptive effects. Adult flies used for the lifespan experiment were never exposed to direct predation, because spiders were removed before adult emergence. This design follows previous work (Krama et al. 2023) and is widely used in ecological studies of predator-induced stress, while remaining fully compatible with standard *Drosophila* husbandry. Although spiders could potentially introduce chemical cues or waste products into the larval substrate, such effects are inherent to ecologically realistic predator-exposure paradigms and are considered part of the integrated stress environment experienced by developing flies. Importantly, in our system, predator exposure does not delay development but instead slightly accelerates larval–pupal transition (6–8 h; unpublished observations), indicating that cohort synchronization does not preferentially exclude stressed individuals.

Diet, drug treatment, and lifespan experiment

The standard diet was prepared following a recipe adapted from the Cold Spring Harbor Protocols (Lewis 1960): 100 mL of water, 4 g dextrose, 7 g cornmeal, 0.9 g agar, and 2 g deactivated yeast. To prevent mold growth, a 10% Tegosept (methyl-p-hydroxybenzoate; Genesee Scientific) solution was added. Each prepared tube contained approximately 9 g of food, which is sufficient to support larval development.

For the drug-feeding, the medium was supplemented with L-tryptophan to a final concentration of 50 mM (Krams et al. 2021). The standard diet contained deactivated yeast (2 g/100 mL), which provides trace background levels of tryptophan that were identical across all treatments. Thus, all groups received equivalent baseline dietary tryptophan, and experimental supplementation with free L-tryptophan (50 mM) constituted the primary source of variation in tryptophan availability.

The 50 mM tryptophan concentration was selected based on prior *Drosophila* studies showing that this dose produces measurable physiological effects while maintaining normal viability and development, whereas higher concentrations (≥100 mM) induce toxicity and reduced survival. This concentration therefore represents a biologically active but sub-toxic dietary manipulation rather than a nutritional optimization. The groups receiving tryptophan supplementation were treated during both the larval and adult stages of life. We emphasize that the supplemented compound was L-tryptophan (the dietary amino acid precursor of serotonin), rather than 5-hydroxytryptophan (5-HTP). Group labels in figures and tables therefore refer to “Trp” supplementation rather than 5-HTP to avoid confusion.

To exclude the possibility that predator stress altered food intake and thereby indirectly affected lifespan via dietary restriction, we note that feeding rates were quantified in the same *Drosophila*–predator system using a Blue FCF dye–based assay in our previous work (Krama et al. 2023). That study found no significant differences in food consumption between predator-exposed and control flies, indicating that predator stress does not modify feeding behavior or food palatability. Lifespan differences observed here are therefore unlikely to be driven by variation in caloric intake.

Importantly, we do not interpret our results as evidence for a single neurochemical mechanism. Although tryptophan is a precursor of serotonin, it also participates in multiple metabolic pathways, including kynurenine metabolism and protein synthesis, and may exert systemic effects on energy allocation and stress physiology. In the absence of direct neurochemical measurements, our findings are best viewed in the context of ecological trade-offs, whereby predator-induced developmental programming interacts with dietary perturbations to shape adult lifespan. We therefore emphasize a life-history perspective rather than pathway-specific causation.

Despite the use of synchronized eggs, some flies emerge significantly faster than others, on day 9. These individuals were removed, and only those that emerged on day 10, which is considered to be the normal rate of development, were used. This procedure ensured that all experimental males belonged to the same developmental cohort and reduced variation in age-structure, body size, and metabolic state that could otherwise confound lifespan comparisons across treatments.

The flies were briefly anesthetized with CO2 and transferred to narrow vials (95 mm × 25 mm, Genesee Scientific, El Cajon, CA, USA). Each treatment consisted of 10 independent vials (biological replicates), with 10 male flies per vial, yielding a total of 100 individuals per treatment group. Only male flies were used in the experiment. This choice was made due to the potential influence of female reproductive biology on the experimental outcomes. A significant portion of a female fly’s body consists of eggs and reproductive tissues, which could affect factors such as body mass, metabolic processes, stress-related metabolic processes (Burggren 2017), and serotonin-associated responses (Hibicke and Nichols 2022). By exclusively using males, we aimed to minimize these confounding variables and ensure more consistent data.

The flies were transferred to vials with fresh food every 3 days without the use of anesthesia. Mortality observations were made every day, approximately in the midafternoon (14:00).

Statistical analyses

The Kaplan–Meier method was applied to estimate survival probability with time (Kaplan and Meier 1958). The mean survival time was determined by calculating the area under the survival curve, which incorporates the full dataset. Data from flies that escaped, died before day 5, or died accidentally were censored and excluded from the analysis.

Mantel-Cox test (log-rank test) with 1 degree of freedom was used to compare overall survival functions between experimental and control groups.

To address potential non-independence among flies within vials, we reconstructed individual-level survival data from daily death counts and re-analyzed lifespan using Cox proportional hazards models. Vial identity was included as a clustering factor, and results were qualitatively identical to Kaplan–Meier estimates.

We report restricted mean survival time ± SE as a biologically meaningful and robust estimator of treatment effects (Han and Jung 2022). Restricted mean survival time (RMST) quantifies the average lifespan up to a specified cutoff and is recommended when survival curves do not converge or proportional hazards assumptions may be violated.

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All differences were considered significant if $P < 0.05$. All analyses were carried out using the Oasis 2 software (Han et al. 2016).

Results

Male flies that received tryptophan supplementation throughout their development and adult life but were not exposed to predators (Control + Trp group) lived on average 52.76 days (Fig. 1, Table 1). This did not differ significantly from the control group (50.88 days; Fig. 1, Table 1; $P = 0.509$, Table 2). The shortest lifespans were observed in flies reared in the presence of predators without tryptophan supplementation (Spider group, 44.26 days; Fig. 1, Table 1). However, when tryptophan was added to the diet of predator-exposed flies (Spider + Trp group), mean lifespan increased significantly (47.32 days, Table 1; $P = 0.023$, Table 2), although it remained shorter than in both the control group and the tryptophan-only group. Re-analysis of reconstructed individual-level survival data yielded median lifespans of 53 days (Control), 53 days (Control + Trp), 44 days (Spider), and 48 days (Spider + Trp), consistent with Kaplan–Meier estimates.

The comparison between the Spider + Trp group and the Control group approached but did not reach statistical significance ($P = 0.063$, Table 2). It is important to note that, despite some pairwise contrasts not meeting conventional significance thresholds, the differences in RMST between groups were substantial. For example, predator exposure reduced lifespan by approximately 6.6–8.5 days relative to controls, whereas tryptophan supplementation recovered approximately 3.1–5.4 days in predator-exposed flies (Table 2). These RMST differences indicate biologically meaningful effects even when statistical support for some contrasts is marginal, suggesting that effect sizes are appreciable despite limited power.

Discussion

Our study demonstrates that predator exposure and dietary tryptophan supplementation have context-dependent effects on the lifespan of male *D. melanogaster*. Flies exposed to predation risk showed the shortest lifespan, consistent with previous findings that chronic predation stress accelerates aging (Hawlena and Schmitz 2010; Krama et al. 2023). Notably, tryptophan supplementation extended the lifespan of flies reared under predation risk, even though high tryptophan intake is often associated with lifespan reduction through increased flux into the kynurenine metabolic pathway (Oxenkrug et al. 2011). Importantly, our goal was not to isolate serotonergic mechanisms but to examine whether a moderate, non-toxic nutritional intervention could mitigate developmental stress effects. Because tryptophan influences multiple downstream pathways, including neuromodulatory, immune, and metabolic processes, the observed lifespan effects likely reflect integrated physiological responses rather than a single biochemical route.

Although lifespan is the primary endpoint in the present study, predator stress in this experimental system has previously been shown to induce coordinated changes in metabolism, locomotor behavior, and antipredator performance (Krama et al. 2023). Importantly, feeding rates did not differ between predator-exposed and control flies in that work, ruling out dietary restriction as a driver of downstream phenotypic effects. The lifespan differences reported here should therefore be interpreted in the context of stress-induced physiological reprogramming rather than altered food intake. We acknowledge that predator presence may also introduce indirect effects via chemical cues, waste products, or microbial changes in the larval substrate; although these factors cannot be fully disentangled, they represent ecologically realistic components of chronic predator exposure. Together, these findings support the interpretation that predator stress alters life-history trajectories through active physiological adjustment rather than through reduced feeding or general nutritional deprivation.

However, we did not observe lifespan reduction in flies given tryptophan in the absence of predators. Although previous studies reported lifespan shortening from tryptophan exposure, these studies typically used higher effective doses (100–500 mM equivalents, probably reaching toxic concentrations) or restricted dosing to adults, where serotonergic elevations more strongly disrupt physiological homeostasis (Chakraborty et al. 2019; Lyu et al. 2021; Miller et al. 2021; Munneke et al. 2022). In contrast, our 50 mM supplementation began during larval development—a period when metabolic and serotonergic pathways may buffer or compensate for increased tryptophan availability. The physiological effects of tryptophan are nonlinear: low or moderate doses tend to support protein synthesis and mild neuromodulation, whereas higher doses promote kynurenine-pathway activity. Thus, the absence of lifespan reduction in our developmental treatment is consistent with the dose- and stage-dependent nature of tryptophan physiology rather than contradictory to earlier studies.

We emphasize that the 50 mM tryptophan supplementation used here was chosen to provide a robust yet sub-toxic perturbation rather than to define an optimal dietary range. Previous work in *Drosophila* indicates that substantially higher concentrations (100–500 mM) impair viability and development, reflecting pharmacological toxicity rather than physiological modulation. Accordingly, our aim was to test whether moderate dietary perturbation interacts with predator-induced developmental programming to influence adult lifespan. Although a full dose–response analysis would be informative, the present design focuses on establishing proof-of-principle effects within an ecologically relevant stress context.

Lifespan responses to dietary amino acids and protein are often non-linear, frequently exhibiting inverted U-shaped relationships in which moderate increases can be beneficial whereas higher levels are detrimental. Indeed, several studies have shown that increasing protein content within isocaloric diets can extend lifespan under specific ecological or physiological contexts. These findings reinforce our interpretation that tryptophan supplementation operates within a broader nutritional trade-off framework, with outcomes depending critically on dose, background diet, and environmental stress (Simpson and Raubenheimer 2009; Solon-Biet et al. 2014; Piper et al. 2017).

Because our design did not include behavioral, metabolic, or neurochemical assays, we do not attribute lifespan changes to specific downstream pathways. Instead, our findings support a broader, ecologically grounded conclusion: nutritional context can shift how organisms respond to chronic developmental stress. The discrepancy with previous lifespan studies may therefore reflect differences in dose, timing of supplementation,

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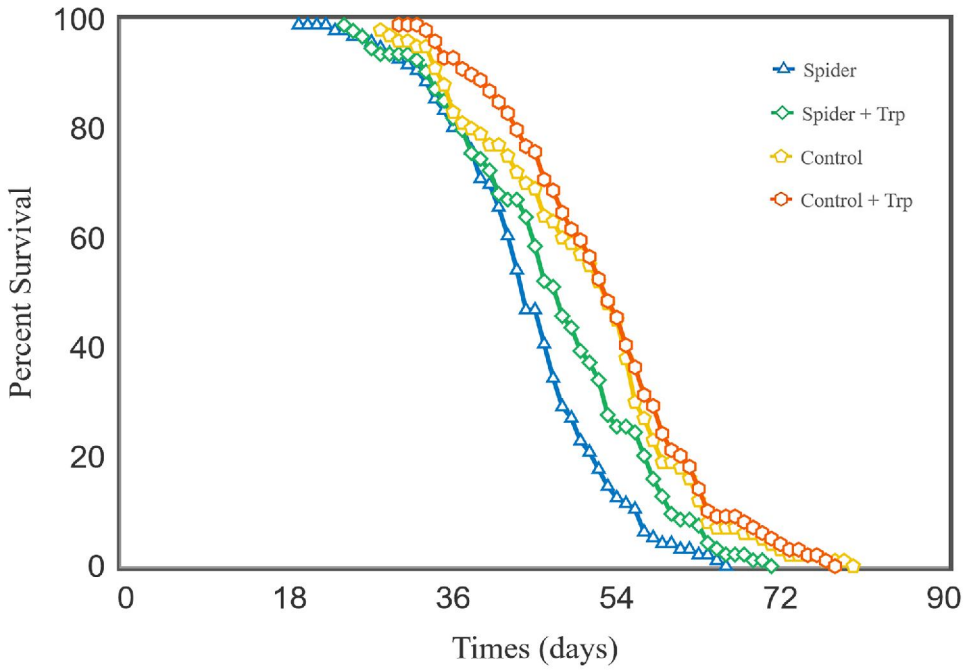

Figure 1 Mortality plot for *Drosophila melanogaster* males living on a standard diet (Control group, yellow pentagons), on a diet supplemented with tryptophan (Control + Trp group, orange hexagons), exposed to predators and living on a standard diet (Spider group, blue triangles), and exposed to predators and living on a diet supplemented with tryptophan (Spider + Trp group, green diamonds). These treatment groups differed significantly: Control vs. Spider ($P < 0.001$), Control + Trp vs. Spider ($P < 0.001$), Control + Trp vs. Spider + Trp ($P = 0.005$), and Spider vs. Spider + Trp ($P = 0.023$).

Table 1 Number of subjects for each treatment group, restricted mean of days lived, standard error, and 95% confidence intervals.

Name	Number of subjects	Restricted mean survival time		
		Days	SE	95% CI
Control	100	50.88	1.15	48.63–53.13
Control + Trp	99	52.76	1.05	50.70–54.82
Spider	96	44.26	0.93	42.44–46.08
Spider + Trp	94	47.32	1.10	45.16–49.48

Table 2 Mantel-cox (log-rank) test statistics for pairwise comparisons between groups. RMST difference indicates the difference in restricted mean survival time (days) between first vs. second group in comparison.

Comparison	χ^2	Bonferroni *p*-value	RMST difference
Control vs. Control + Trp	0.44	0.509	−1.88
Control vs. Spider	24.20	< 0.001	6.62
Control vs. Spider + Trp	5.34	0.063	3.56
Control + Trp vs. Spider	34.26	< 0.001	8.5
Control + Trp vs. Spider + Trp	9.97	0.005	5.44
Spider vs. Spider + Trp	7.13	0.023	−3.06

genotype, or diet composition rather than conflicting biological mechanisms. Another possible explanation relates to interactions between serotonergic and insulin-producing neurons (Kaplan et al. 2008; Krama et al. 2023), which jointly regulate energy balance and stress physiology. Although we did not measure serotonergic activity, insulin signaling, or feeding behavior, early exposure to tryptophan may allow developmental compensation within these systems, buffering lifespan costs that appear in adult-only supplementation paradigms.

Tryptophan supplementation did not fully restore lifespan in predator-exposed flies. We therefore interpret this pattern at a life-history level: nutritional inputs can modify—but not eliminate—the survival costs of chronic developmental stress. Prior work shows that predator-exposed flies develop metabolic dysfunction resembling insulin resistance (Krama et al. 2023), and tryptophan supplementation was associated with partial mitigation of lifespan reduction. Whether this reflects changes in nutrient allocation, stress physiology, or amino-acid sensing remains to be tested.

Our findings suggest that diet × environment interactions play an important role in shaping aging trajectories in *Drosophila* (Rose et al. 1992). The partial rescue of lifespan under predation risk indicates that nutritional modulation can buffer—but not erase—the physiological consequences of chronic threat. From an ecological perspective, such interactions reflect trade-offs between somatic maintenance and stress coping, paralleling “live fast, die young” strategies under high-risk conditions.

A limitation of the present study is the exclusive use of male flies. Females typically experience stronger energetic trade-offs due to egg production and reproductive investment, which may modulate the balance between stress responses, metabolism, and survival. Predator-induced metabolic programming may therefore differ quantitatively or qualitatively between sexes. Future studies incorporating females will be essential to determine how reproductive allocation interacts with chronic predation

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risk and dietary modulation. Additionally, collecting data on feeding, locomotion, metabolic reserves, and kynurenine-pathway intermediates would clarify the mechanisms underlying the observed lifespan patterns. Direct physiological measurements are necessary to evaluate mechanistic hypotheses about serotonergic signaling, insulin regulation, or metabolic stress.

Overall, our results do not attempt to infer molecular causation but highlight a general interaction between environmental stress and diet. Dietary tryptophan mitigated some—but not all—effects of predator-induced developmental stress on lifespan in male *D. melanogaster*. Tryptophan did not extend lifespan under benign conditions, underscoring that its effects are dose-dependent and strongly shaped by environmental context. By combining ecologically realistic predator cues with a simple nutritional manipulation, our study illustrates how diet and environment interact to shape aging outcomes. This work provides an ecological foundation for future mechanistic research on how amino-acid metabolism, stress physiology, and neural signaling jointly contribute to lifespan variation in animals.

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Authors' contributions

Colton Adams: Conceptualization, Methodology, Validation, Writing—review & editing. Giedrius Trakimas: Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—review & editing. Indrikis Krams: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing—original draft. Jorge Contreras-Garduño: Conceptualization, Methodology, Validation, Writing—review & editing. Markus J Rantala: Conceptualization, Methodology, Validation, Writing—review & editing. Priit Jõers: Conceptualization, Methodology, Validation, Writing—review & editing. Ronalds Krams: Investigation, Resources, Supervision, Writing—review & editing. Sergejs Popovs: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Writing—review & editing. Tatjana Grigorjeva: Formal Analysis, Investigation, Software, Validation, Writing—review & editing. Tatjana Krama: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

This work did not require ethical approval from a human subject or animal welfare committee in the Republic of Latvia.

Data availability

The data supporting the results have been archived in the Zenodo public repository; https://doi.org/10.5281/zenodo.17689419.

Artificial intelligence (AI)-assisted technologies

We used AI-assisted technologies (ChatGPT 5.2) to improve the clarity of our text.

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