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Effects of the polycyclic aromatic hydrocarbon anthracene on the survival of the semi-aquatic grasshopper *Cornops aquaticum* (Bruner, 1906): an experimental toxicological study

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Introduction: Environmental contamination by polycyclic aromatic hydrocarbons (PAHs) has increased in recent decades, intensifying the exposure of organisms in wetland ecosystems. Anthracene, due to its chemical stability and persistence, represents a potential risk to herbivorous insects associated with aquatic vegetation.

Methods: The toxicity of anthracene to the semi-aquatic grasshopper Cornops aquaticum (Bruner, 1906) was experimentally evaluated under controlled laboratory conditions using *Eichhornia crassipes* leaf fragments (49 cm^2) contaminated with increasing amounts (0.05, 0.10, and 0.20 g). Mortality, survival time, and bioaccumulation parameters, including daily uptake rate, were assessed.

Results: Anthracene exposure significantly reduced survival, confirming a dose-dependent toxic effect. A non-linear response was observed, with the 0.10 g treatment showing the most pronounced effect, resulting in only 10% survival at the end of the experiment. This pattern may be associated with reduced feeding or avoidance behavior at higher contamination levels. Sex-dependent differences were also identified: females exhibited greater tolerance at lower doses, whereas males showed higher survival at elevated concentrations. Body concentrations ranged from 0 to 4,022 μg g^{-1}, confirming effective uptake. Survival was more strongly associated with daily exposure rate than with total accumulated concentration, indicating a critical threshold between 450 and 550 μg g^{-1} day^{-1}.

Discussion: These findings demonstrate that exposure dynamics play a key role in anthracene toxicity and highlight Cornops aquaticum as highly sensitive to PAHs. The species shows strong potential as a bioindicator of organic contamination in wetland ecosystems, contributing to improved ecological risk assessment of persistent pollutants.

KEYWORDS
bioaccumulation, ecotoxicology, environmental contamination, organic pollutants, oxidative stress

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise a class of lipophilic organic compounds generated primarily through the combustion of coal and biomass, vehicular emissions, petroleum refining processes, and residential waste burning. These compounds are structurally characterized by the presence of two or more fused benzene rings (Cong et al., 2021; Sari and Esen, 2023).

The production of these organic micropollutants, widely used and generated during industrialization and global market expansion, has increased at an accelerated rate in recent decades (Vijayanand et al., 2023). Among them, anthracene—formed predominantly through incomplete combustion of organic materials—is a common organic contaminant in aquatic systems (Patel et al., 2020; Montano et al., 2025). It is classified as a priority pollutant due to its bioaccumulative capacity, mutagenicity, carcinogenicity, and overall toxicity, which justify growing environmental concern regarding its continued release (Thirumurugan et al., 2023; Vijayanand et al., 2023).

Anthracene is highly hydrophobic and exhibits low biodegradability due to its chemical stability (Rubio-Clemente et al., 2014). These properties promote its persistence in aquatic environments and facilitate deposition on aquatic plant surfaces. Contamination of aquatic and terrestrial ecosystems directly affects insects—the most diverse animal group globally—which perform essential ecological functions and contribute significantly to ecosystem services linked to the global economy (Paulson, Thakkar, and Parikh, 2020).

Environmental PAH contamination has been documented in studies involving bees and moths (Grčić et al., 2021; Sari and Esen, 2023), as well as in experimental research demonstrating significant physiological responses of insects exposed to structurally similar toxic compounds. Cardoso et al. (2024) reported that larvae of *Tenebrio molitor* accumulate and eliminate benzo[a]pyrene (a carcinogenic PAH), particularly in adipose tissues, evidencing bioaccumulation and toxic effects. Experimental investigations in grasshoppers further suggest that aromatic compounds may induce physiological and biochemical alterations, as demonstrated by Huang et al. (2020) in *Oedaleus asiaticus*.

Within this context, the semi-aquatic grasshopper *Cornops aquaticum*, the focus of the present study, plays a significant ecological role in freshwater ecosystems by acting as a natural regulator of aquatic vegetation (Franceschini et al., 2023) and serving as a food source for multiple predators. Consequently, exposure to anthracene may disrupt trophic dynamics and ecological balance in these environments.

Therefore, this study aimed to experimentally evaluate the effects of anthracene on the survival of *C. aquaticum* under different pollutant concentrations and to analyze the relationship between exposure and longevity reduction. To our knowledge, this is the first experimental study specifically addressing the toxicological effects of PAHs, such as anthracene, on grasshopper species, representing a novel contribution to environmental toxicology in semi-aquatic insects.

2 Materials and methods

2.1 Collection of biological materials: grasshoppers and host plants

The biological materials used in the experiment were collected in April 2024 at Água Preta Lake, located in the municipality of Belém, Pará State, Brazil. This lake is one of the main water supply reservoirs for the city and harbors abundant populations of the semi-aquatic grasshopper *C. aquaticum* associated with colonies of its host plant, *Eichhornia crassipes*.

Grasshoppers (*C. aquaticum*) were collected using an entomological net, following the methodology described by Braga et al. (2011), while *E. crassipes* plants were manually collected along the lake margins. Transportation within the lake was performed using a motorized boat. After collection, grasshoppers and plants were transported alive to the laboratory at the Universidade do Estado do Pará (UEPA), where the experiments were conducted.

All grasshoppers and host plants used in the experiment were maintained in water-filled tanks for acclimation. The tanks were kept under shaded conditions at ambient temperature, in an area adjacent to the insectary of the Dr. Joachim Adis Zoological Collection Laboratory at UEPA (CZJA–UEPA).

This study did not involve humans or vertebrate animals. All experimental procedures were conducted exclusively with insects. According to Brazilian regulations, experiments involving insects do not require approval by an institutional bioethics or animal experimentation committee.

2.2 Determination of the lethal dose of anthracene

The determination of the median lethal dose (LD_{50}) of anthracene was carried out through bioassays using adult grasshoppers, with *E. crassipes* leaves as the feeding substrate. The leaves were previously standardized to dimensions of 7 × 7 cm (49 cm^2). Anthracene (99% purity) was applied in powder form directly onto the adaxial surface of the leaf fragments at three distinct concentrations (0.05 g, 0.10 g, and 0.20 g per leaf). The compound was carefully distributed manually to ensure the highest possible homogeneity. Although minor variations in distribution cannot be entirely excluded, the same procedure was consistently applied across all treatments. The control group consisted of leaves without the addition of the compound, maintained under the same experimental conditions as the treated groups.

All experimental units were kept under tropical environmental conditions, with temperatures ranging approximately from 24 °C to 32 °C, natural photoperiod, and shaded conditions, in order to minimize environmental stress and experimental variability. Each treated leaf fragment was individually placed in a 1 L glass beaker containing a cotton swab moistened with water at the bottom and sealed with polyvinyl chloride (PVC) film to maintain humidity and preserve leaf physiological integrity. One adult grasshopper was then introduced into each experimental unit. Ten individuals were used per treatment (five males and five females), totaling 40 grasshoppers.

The experimental design followed a factorial scheme to assess potential differences associated with insect sex and anthracene concentration. The exposure period lasted 10 days, with leaf

replacement at the beginning of the sixth day to prevent contaminant degradation and ensure maintenance of the applied dose.

Observations were conducted three times daily, and mortality was recorded to allow temporal analysis of the toxic response. The collected data were organized for subsequent statistical analysis aimed at estimating the median lethal dose (LD_{50}) of anthracene, considering treatment and sex as variables.

Dead grasshoppers were weighed (fresh mass) using an analytical balance with 0.01 mg precision and subsequently stored in sealed containers for chemical analyses. These analyses were performed at the chemistry laboratory of the Haroldo Lisboa da Cunha Pavilion, Universidade do Estado do Rio de Janeiro (UERJ), Maracanã campus.

2.3 Analysis of anthracene absorption in grasshoppers

2.3.1 First step: extraction of anthracene from samples

This step aimed to establish an analytical methodology for detecting and quantifying anthracene in *C. aquaticum* specimens.

Anthracene extraction was performed using Soxhlet extraction. Grasshopper samples were weighed on an analytical balance and placed in cellulose extraction cartridges together with 1 mL of o-terphenyl at 100 ppm as a surrogate standard. The cartridges were inserted into the extraction chamber, and 70 mL of HPLC-grade dichloromethane, along with four glass beads, were added to a 150 mL round-bottom flask coupled to the extractor. After assembly of the condenser, heating was initiated, and the extraction time was set to 4 h following the first solvent reflux.

At the end of the extraction period, the extracts were transferred to 200 mL amber flasks using glass Pasteur pipettes and allowed to evaporate under a fume hood until complete solvent removal. The residues were then transferred to pre-weighed 2 mL chromatography vials and dried to constant weight.

This methodology was adapted from Wood et al. (1997) and Rinaldi et al. (2012) for application to grasshopper samples.

The dried extracts were reconstituted with 2 mL of HPLC-grade dichloromethane and filtered through a 0.45 µm filter using a glass syringe prior to analysis by high-performance liquid chromatography with diode array detection (HPLC–UV–DAD).

2.3.2 Second step: HPLC–UV–DAD analysis

Chromatographic analyses were performed using an Agilent Technologies 1,200 Infinity high-performance liquid chromatography system equipped with a degasser (1260 HIP Degasser G4225A), binary pump (1,260 Bin Pump G1312B), autosampler (1260 ALS G1329B), thermostatted column compartment (1260 TCC G1316A), and diode array detector (1260 DAD G4212B).

Additional analyses were conducted using another Agilent 1,200 Infinity system equipped with a quaternary pump (1,260 Quat Pump G1311B), degasser, autosampler (1260 ALS G1329B), thermostatted column compartment (1260 TCC G1316A), and multi-wavelength UV detector (1260 MWD VL G1365D).

The analytical methodology was adapted from EPA Method 1,654, Revision A, proposed by the United States Environmental Protection Agency (US EPA) in 1992. Method validation followed the guidelines established by Brazilian regulation RDC No. 166/2017 (ANVISA), using o-terphenyl as a surrogate standard and anthracene as the target analyte.

2.4 Statistical analysis

Statistical analyses were conducted to evaluate the effects of different anthracene treatments (control, 0.05 g, 0.10 g, and 0.20 g) on the survival of the semi-aquatic grasshopper *C. aquaticum* over time. The analysis was based on methods appropriate for censored survival data. Individual grasshoppers (males and females) were monitored from the beginning of the experiment until the occurrence of the event (death) or until censoring (survival at the end of the observation period), recording survival time and event status (1 = event; 0 = censored).

Survival probabilities were estimated using methods suitable for right-censored data, allowing inclusion of individuals that did not experience the event during the 10-day observation period. All statistical analyses were conducted using a significance level of 5% ($\alpha = 0.05$).

Survival curves were estimated using the Kaplan-Meier method for each experimental group (control, 0.05 g, 0.10 g, and 0.20 g) and stratified by sex (males and females). Differences between groups were assessed using the log-rank (Mantel–Cox) test and the complementary weighted Tarone–Ware test, as appropriate. Median survival times and their corresponding 95% confidence intervals (95% CI) were reported when estimable.

Associations between explanatory variables and event risk were evaluated using Cox proportional hazards regression models. Results were expressed as hazard ratios (HR) with corresponding 95% confidence intervals, in both univariate and multivariate analyses. The proportional hazards assumption was tested using Schoenfeld residuals, no significant violations were detected, and this assumption was considered in the interpretation of the results.

To reduce the impact of late censoring and improve estimate stability, a landmark time analysis was performed with the reference time fixed at 5 days (the period with the highest event density), including only individuals at risk beyond that time point. Model discrimination performance was assessed using Harrell's concordance index (C-index), which ranges from 0 to 1 and reflects the model's ability to correctly discriminate individuals with higher or lower risk (Harrell, 2015). Overall model fit was evaluated using the likelihood ratio test, and a pseudo-R^2 measure for survival models was also calculated.

All statistical analyses were conducted using Jamovi (version 2.7; https://www.jamovi.org), based on the R statistical environment (version 4.5; https://cran.r-project.org), employing the Survival Module of ClinicoPath for Jamovi (jsurvival 0.0.33), a dedicated package for survival analysis (R Core Team, 2025; The Jamovi Project, 2025).

Due to logistical and biological constraints associated with maintaining individual insects under controlled experimental conditions, the sample size was limited. However, survival analysis methods, such as Kaplan–Meier estimators and Cox proportional hazards models, are suitable for datasets with relatively small sample sizes and censored data. Therefore, the

TABLE 1 Median survival estimates and survival model parameters for *Cornops aquaticum* under different anthracene treatments, including total number of individuals (N), number of censored observations, observed events (deaths), expected number of events under the model, restricted mean survival time (rmean), standard error of the restricted mean (SE_rmean), and median time to event.

Events summary							
Group	N	Censored	Observed events	Expected events	Rmean	se_rmean	Median
Control	10	7	3	5.84	7.70	1.114	NaN
Anth. 0.05 g	10	5	5	5.73	7.00	1.020	6.00
Anth. 0.1 g	10	1	9	5.77	6.60	0.874	7.00
Anth. 0.2 g	10	4	6	5.65	6.70	0.872	5.00
Female	20	9	11	12.2	3.18	0.547	9.00
Male	20	8	12	10.8	3.43	0.587	7.00

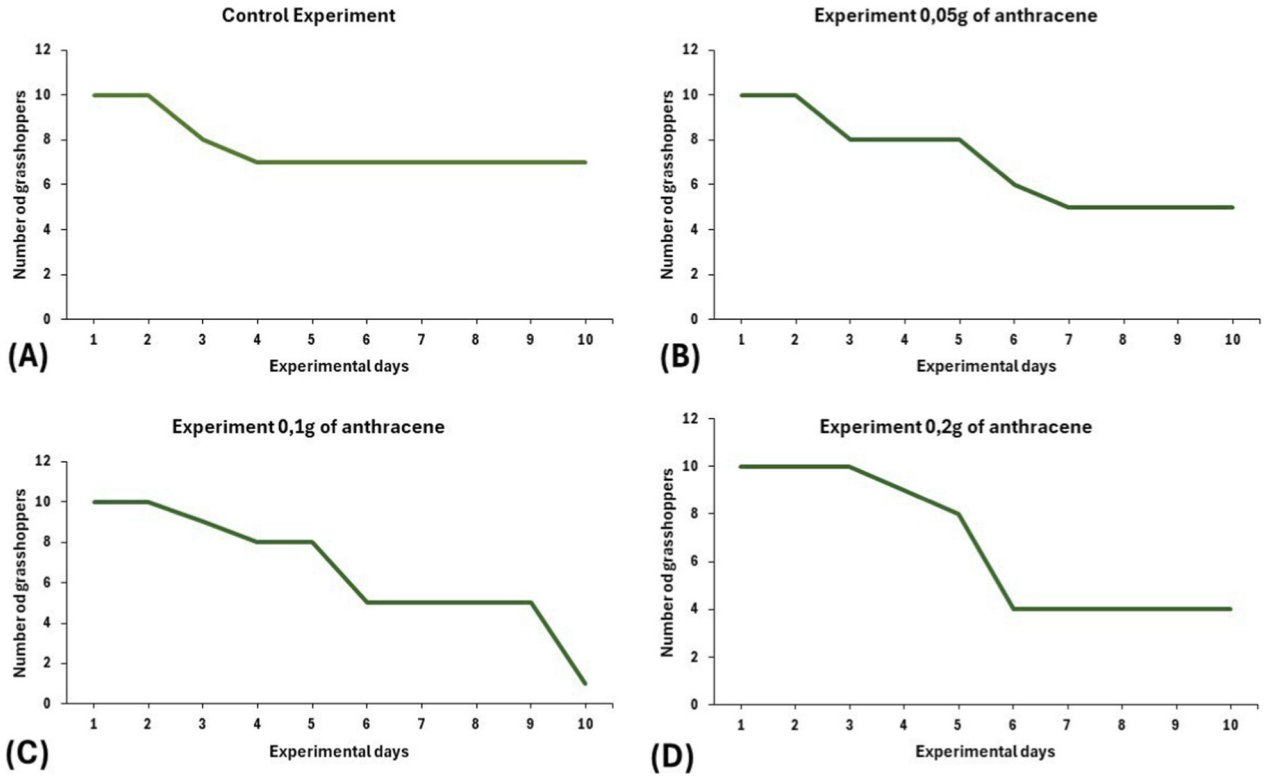


FIGURE 1
Survival time of *Cornops aquaticum* over a 10-day experimental period under different anthracene exposure conditions: **(A)** control (no contaminant), **(B)** 0.05 g treatment, **(C)** 0.10 g treatment, and **(D)** 0.20 g treatment of anthracene applied to *Eichhornia crassipes* leaves.

analytical approach adopted allows for robust inferences within the experimental context.

3 Results

The analysis of anthracene effects on the survival of *C. aquaticum* across experimental treatments (control, 0.05 g, 0.10 g, and 0.20 g) and by sex (males and females) revealed marked variation in the number of observed events (deaths) and censored observations (survivors) among groups (Table 1).

The 0.10 g treatment exhibited the highest number of observed events, whereas the control group showed a predominance of censored observations. Median survival times varied among treatments, ranging from 5 days (0.20 g) to 7 days (0.10 g), indicating treatment-dependent differences in survival dynamics (Table 1).

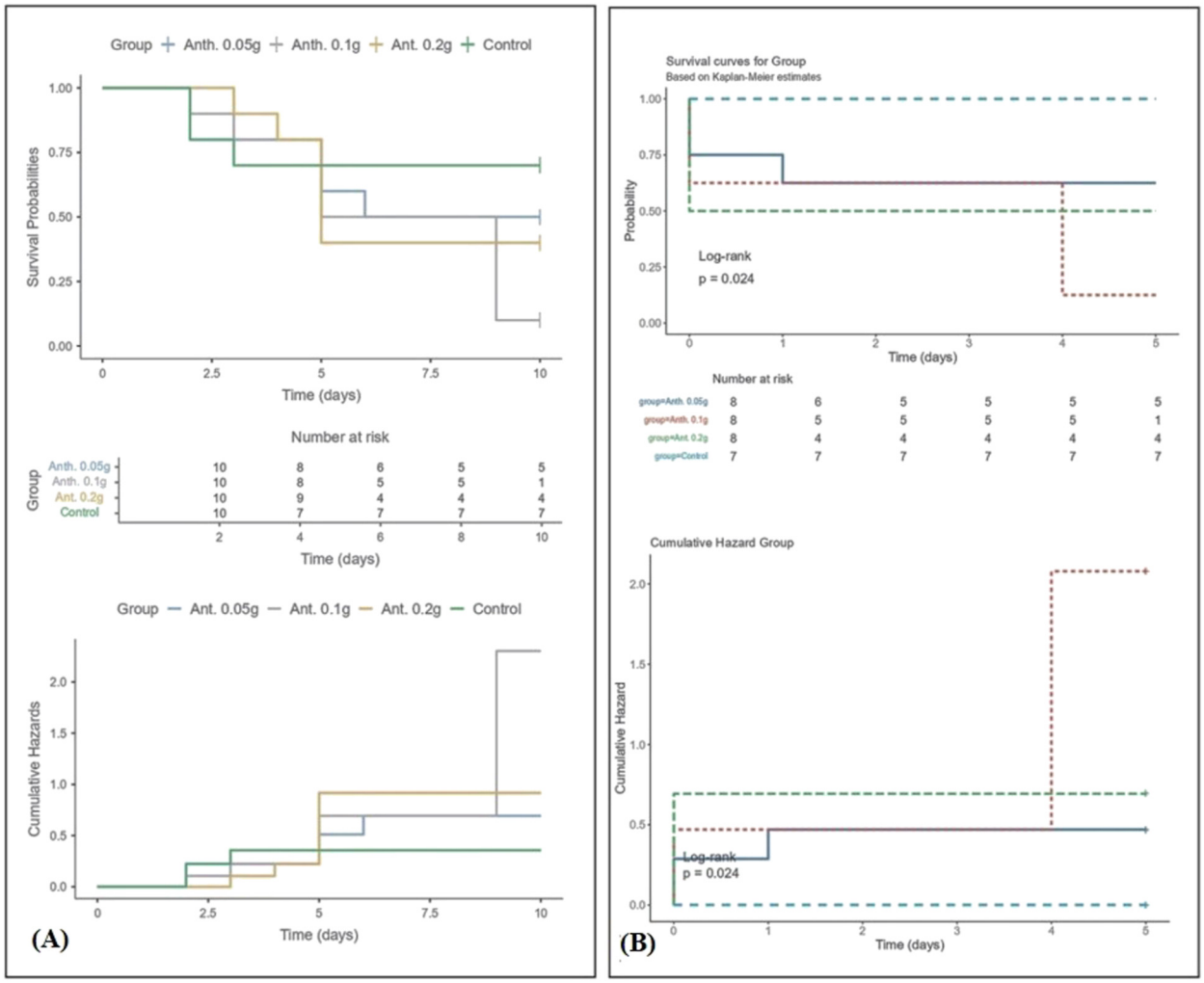


FIGURE 2
Kaplan–Meier survival estimates of *Cornops aquaticum* exposed to increasing anthracene concentrations (0.05 g, 0.10 g, and 0.20 g) compared to the control group over a 10-day experimental period. Panel **(A)** shows survival probability curves with corresponding numbers at risk at each time point and cumulative hazard functions for each treatment group. Panel **(B)** presents early survival dynamics (0–5 days), highlighting the period with the highest event density and corresponding cumulative hazard patterns. Significant differences among treatments were detected using the log-rank test (p = 0.024; IC = 95%). Survival probabilities declined progressively with increasing anthracene exposure, with the 0.10 g treatment exhibiting the lowest final survival probability. Tick marks indicate censored observations.

Sex-stratified analysis revealed comparable survival patterns between females (11 events) and males (12 events). However, females exhibited a longer median survival time (9 days) compared to males (7 days), suggesting potential sex-related differences in tolerance to anthracene exposure (Table 1).

For the control group, median survival could not be estimated due to the absence or very low frequency of events, as most individuals survived until the end of the experimental period. In contrast, in all anthracene-treated groups, 50% or more of the individuals experienced the event before the end of the 10-day observation period (Table 1).

3.1 Survival under anthracene exposure

Anthracene exposure induced both acute and cumulative toxic effects in *Cornops aquaticum*, resulting in a significant reduction in survival when insects were fed *Eichhornia crassipes* leaves contaminated with anthracene (Tarone–Ware test: $\chi^2 = 7.94$; df = 3; p = 0.047).

Considering 10 individuals per treatment (five males and five females), mortality reached 50% in the 0.05 g treatment, 90% in the 0.10 g treatment, and 60% in the 0.20 g treatment (Figure 1). Survival declined progressively with increasing exposure, although the response was not strictly linear between the two highest concentrations. In the control group, survival remained high throughout the experimental period, with only isolated early mortality events followed by stabilization through day 10.

In the anthracene-treated groups, a marked temporal pattern was observed, with a significant increase in mortality between the third and seventh days of exposure, indicating a cumulative toxic response. This pattern was confirmed by risk estimates derived from the Kaplan-Meier method, whose survival curves demonstrated

TABLE 2 Survival probabilities of *Cornops aquaticum* exposed to increasing anthracene concentrations (0.05 g, 0.10 g, and 0.20 g) and control treatment across the 10-day experimental period. For each time point (1, 5, and 10 days), the table presents the number of individuals at risk (i.e., alive and under observation immediately prior to the time point), the number of observed events (deaths) occurring within each interval, and the corresponding cumulative survival probability (%). Survival estimates reflect right-censored data and demonstrate treatment-dependent reductions in survival over time, with the 0.10 g group exhibiting the most pronounced decline.

Group	Time (days)	Number at risk	Number of events	Survival
Control	1	10	0	100.0%
	5	7	3	70.0%
	10	7	0	70.0%
Anth. 0.05 g	1	10	0	100.0%
	5	8	4	60.0%
	10	5	1	50.0%
Anth. 0.1 g	1	10	0	100.0%
	5	8	5	50.0%
	10	1	4	10.0%
Anth. 0.2 g	1	10	0	100.0%
	5	8	6	40.0%
	10	4	0	40.0%

significant differences among treatments (log-rank test: p = 0.024) (Figure 2).

The control group exhibited the highest survival probability throughout the experimental period and did not reach 50% mortality. In contrast, all contaminated treatments showed progressive survival declines, with the 0.10 g group presenting the lowest survival probability, reaching only 10% at the end of the 10-day observation period (Table 2). The univariate Cox proportional hazards model, adjusted using landmark time analysis with the reference time fixed at 5 days, showed global statistical significance (Likelihood Ratio Test = 12.256; df = 3; p = 0.007), indicating that anthracene treatments significantly influenced mortality risk. Model discrimination was considered good, with a concordance index (C-index) of 0.746 (SE = 0.063). The coefficient of determination indicated that 32.7% of the variability in mortality risk was explained by treatment (R^2 = 0.327). The proportional hazards assumption was not violated (p = 0.206), supporting the interpretation of hazard ratios (HR) as constant effects over time.

3.2 Sex-specific survival patterns

A multivariable Cox proportional hazards model was applied to simultaneously evaluate the effects of treatment (control, 0.05 g, 0.10 g, and 0.20 g) and internal anthracene concentration on the survival of male and female grasshoppers. The model demonstrated adequate discrimination performance (C-index = 0.698; SE = 0.068) and was globally significant (Likelihood Ratio Test = 12.174; p = 0.032).

The control group exhibited a significantly lower risk of death compared to contaminated treatments (HR = 0.13; p = 0.025). Internal anthracene concentration significantly influenced mortality risk (HR = 1.00; p = 0.006), indicating a dose–response relationship. Males showed a tendency toward a higher hazard ratio under anthracene exposure (HR = 2.85; p = 0.057), suggesting increased vulnerability relative to females.

Model-derived survival patterns revealed clear sex-dependent differences across treatments. At the lowest anthracene concentration (0.05 g), females exhibited higher survival rates than males. Conversely, at higher concentrations (0.10 g and 0.20 g), males demonstrated greater survival compared to females. In the control group, both sexes showed similar survival probabilities, indicating that sex-related differences emerged primarily under chemical stress conditions (Figure 3).

3.3 Anthracene bioaccumulation and daily exposure

Body concentrations of anthracene increased with exposure level, reaching values of up to 4,022.272 μg g^{-1} (Table 3). Considerable interindividual variability was observed within treatments, in both males and females. Low anthracene concentrations were occasionally detected in individuals from the control group.

Analysis of daily exposure revealed that individuals receiving doses exceeding approximately 450–550 μg g^{-1} day^{-1} exhibited reduced survival, frequently dying within 5 days of exposure. In contrast, individuals with lower daily exposure rates often survived throughout the entire experimental period, even when final body concentrations were high.

3.4 Relationship between daily dose and survival time

Survival time was more strongly associated with daily anthracene intake than with total accumulated body concentration. Individuals exposed to daily doses above

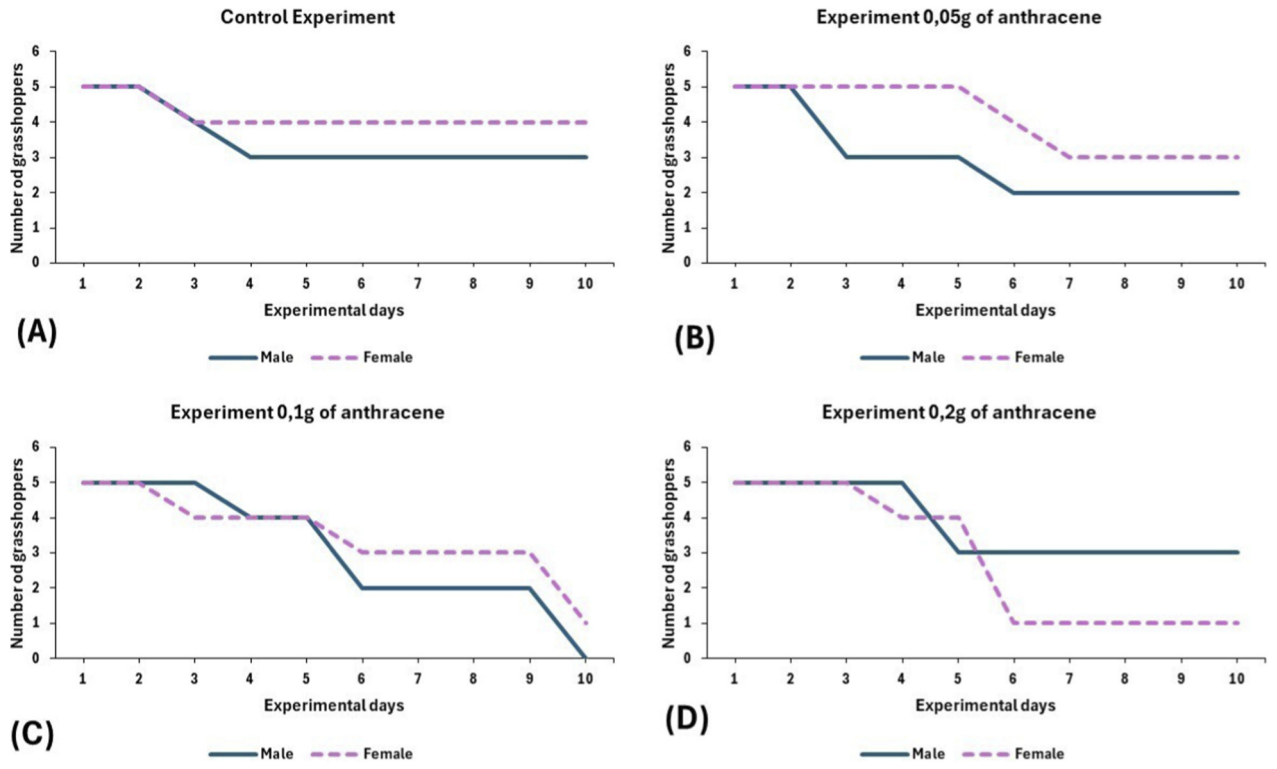


FIGURE 3
Survival of male and female *Cornops aquaticum* over a 10-day experimental period under different anthracene exposure conditions: **(A)** control (no contaminant), **(B)** 0.05 g, **(C)** 0.10 g, and **(D)** 0.20 g of anthracene applied to *Eichhornia crassipes* leaves.

600–800 μg g^{-1} day^{-1} generally survived only 2–5 days, whereas those with lower daily intake completed the 10-day exposure period.

4 Discussion

4.1 Dose-dependent and cumulative toxicity of anthracene

The observed reduction in survival confirms that anthracene exerts pronounced toxic effects on *Cornops aquaticum*, consistent with the known properties of low-molecular-weight polycyclic aromatic hydrocarbons (PAHs). The delayed onset of mortality followed by a rapid decline in survival supports a cumulative toxic mechanism, in which intracellular accumulation precedes physiological collapse—a pattern commonly reported for PAHs in aquatic and terrestrial invertebrates (Patel et al., 2020; Honda and Suzuki, 2020).

The non-monotonic response observed between the 0.10 g and 0.20 g treatments may be associated with behavioral and physiological responses to higher contamination levels, where organisms reduce feeding activity or exhibit avoidance behavior, leading to lower effective exposure despite higher nominal doses; such responses are widely reported in behavioral ecotoxicology and are recognized as important mechanisms modulating exposure and toxicity (Weis et al., 2011; Araújo and Blasco, 2019).

In addition, interindividual variability in detoxification capacity may contribute to this pattern. Although this result should be interpreted with caution, similar non-linear survival patterns have been reported for grasshoppers and other insects exposed to aromatic compounds and secondary metabolites (Huang et al., 2017; Huang et al., 2024). This pattern may also reflect experimental variation and should be further investigated in future studies.

4.2 Toxicokinetic relevance of daily exposure rate

One of the central findings of this study is that mortality was more strongly associated with daily exposure rate than with final accumulated body burden. The identification of a critical daily exposure threshold between 450 and 550 μg g^{-1} day^{-1} suggests metabolic saturation, beyond which detoxification pathways and antioxidant defenses become overwhelmed. This toxicokinetic behavior has been demonstrated in experimental and modeling studies involving insects and aquatic invertebrates exposed to hydrophobic organic contaminants (Ong et al., 2011; Szczybelski et al., 2019; Bauer et al., 2024).

The occurrence of early mortality in individuals with relatively low final body concentrations but high daily intake reinforces that toxic influx, rather than total accumulation alone, is the primary determinant of lethality consistent with previous findings on PAH uptake and depuration dynamics (Girardin et al., 2020; Honda and Suzuki, 2020). This finding is consistent with recent advances in ecotoxicology that emphasize the importance of exposure dynamics over static concentration metrics (Szczybelski et al., 2019; Redman et al., 2022; Bauer et al., 2024). In this context, the contaminant

TABLE 3 Body concentration of anthracene (μg g^{-1}), daily dose, and survival time of male and female *Cornops aquaticum* exposed to different treatments.

Tratamento	Male grasshopper (μg g^{-1})	Daily dose (μg g^{-1} day^{-1})	Male lifespan (days)	Female grasshopper (μg g^{-1})	Daily dose (μg g^{-1} day^{-1})	Female lifespan (days)
Control 1	0.000	0.000	10	0.000	0.000	10
Control 2	0.647	0.323	2	0.422	0.042	10
Control 3	0.000	0.000	10	0.626	0.313	2
Control 4	0.000	0.000	10	0.000	0.000	10
Control 5	18,523	6,174	3	0.000	0.000	10
Anth. 0.05 g	993,941	496,970	2	1,834,334	183,433	10
Anth. 0.05 g	2,366,849	473,370	5	277,270	46,212	6
Anth. 0.05 g	2,686,369	268,637	10	1,151,127	115,113	10
Anth. 0.05 g	1,670,422	167,042	10	2,397,028	479,406	5
Anth. 0.05 g	232,836	116,418	2	752,735	75,273	10
Anth. 0.1 g	2,246,206	249,578	9	845,807	422,903	2
Anth. 0.1 g	2,704,533	540,907	5	346,343	38,483	9
Anth. 0.1 g	2,718,997	543,799	5	2,107,940	210,794	10
Anth. 0.1 g	2,905,333	322,815	9	1,308,265	145,363	9
Anth. 0.1 g	1,486,314	495,438	3	1,525,963	305,193	5
Anth. 0.2 g	3,178,632	317,863	10	1,864,464	372,893	5
Anth. 0.2 g	2,835,148	283,515	10	959,546	191,909	5
Anth. 0.2 g	1,713,104	342,621	5	1,408,063	469,354	3
Anth. 0.2 g	3,239,670	809,918	4	3,574,542	357,454	10
Anth. 0.2 g	4,022,272	402,227	10	2,212,302	442,460	5

Anth, Anthracene.

intake rate may be a more reliable predictor of toxicity than total body burden, particularly for compounds with rapid absorption and limited detoxification capacity.

4.3 Sex-related differences in tolerance

Sexual dimorphism in anthracene tolerance was evident throughout the experiment. The higher survival of females at low exposure levels may be associated with greater lipid reserves, which can enhance the bioaccumulation of hydrophobic PAHs and temporarily buffer their toxic effects. However, at higher exposure levels, these lipid reserves may act as reservoirs for contaminant accumulation, intensifying oxidative stress and accelerating mortality (Huang et al., 2017; Girardin et al., 2020).

Additionally, higher reproductive metabolic demands in females may contribute to increased production of reactive oxygen species, reducing resistance to chemical stress under high-dose conditions (Gomes et al., 2023; Xu et al., 2025). This pattern may also reflect differences in detoxification efficiency, as enzymatic systems involved in xenobiotic metabolism, such as cytochrome P450, may respond differently between sexes.

In contrast, males, possibly due to lower lipid content and different metabolic allocation, may exhibit greater resistance at higher anthracene concentrations, in agreement with previous ecotoxicological observations in Orthoptera (Huang et al., 2024).

Overall, these results suggest that physiological and metabolic differences between sexes can influence responses to chemical stress. More broadly, they indicate that sex-related variation in bioaccumulation, detoxification, and energy allocation may significantly affect toxicological outcomes (Gomes et al., 2023; Xu et al., 2025).

Reinforcing the importance of considering sex as a key biological variable in ecotoxicological risk assessments. However, these interpretations should be treated with caution, as the underlying physiological mechanisms were not directly evaluated in this study.

4.4 Ecological implications and bioindicator potential

The high sensitivity of *C. aquaticum* to anthracene, even at relatively low exposure levels, highlights its potential as a bioindicator species of organic contamination in wetland ecosystems. Field and laboratory studies have demonstrated that grasshoppers and other insects experience significant population-level impacts in contaminated environments, including reductions

in survival, abundance, and reproductive success (Soliman et al., 2017; Messi et al., 2025).

The strong dependence of survival on exposure rate rather than final accumulation underscores the ecological relevance of continuous or episodic PAH inputs into aquatic systems. These findings emphasize the importance of incorporating toxicokinetic parameters into environmental risk assessments and support the use of semi-aquatic insects as sentinel organisms for monitoring organic pollution in freshwater environments.

From an ecological perspective, reductions in *C. aquaticum* populations may generate cascading effects within aquatic ecosystems. Insects—particularly aquatic and semi-aquatic species—play critical roles in nutrient cycling and energy transfer across trophic levels and are widely recognized as sensitive bioindicators of environmental contamination (Chowdhury et al., 2023). Furthermore, as PAHs and other persistent organic pollutants accumulate in aquatic organisms and transfer across trophic levels, anthracene bioaccumulation in *C. aquaticum* represents a potential pathway for contaminant movement to predators such as spiders, fish, amphibians, insectivorous birds, and other organisms, thereby increasing ecological risk in wetland environments. These effects are especially relevant in semi-aquatic systems, where contaminants deposited on vegetation surfaces are efficiently incorporated into food webs through herbivorous insects.

Taken together, these findings suggest that even moderate levels of PAH contamination may cause disproportionate effects on key herbivorous insects, potentially altering trophic interactions and ecosystem functioning in wetland environments.

Although the sample size was relatively limited, the statistical analysis confirmed that anthracene exerted a lethal effect on the grasshoppers, particularly at the 0.10 g and 0.20 g doses. However, further studies using larger sample sizes and additional dose ranges are needed to corroborate the observations of the present study.

5 Conclusion

Anthracene significantly reduced the survival of *C. aquaticum*, with toxicity more strongly determined by the daily exposure rate than by total accumulated concentration. This finding underscores the importance of exposure dynamics in shaping toxicological effects and suggests that static concentration-based metrics may underestimate ecological risk.

Sex-related differences in tolerance further indicate that physiological and metabolic traits play a central role in responses to PAHs, reinforcing the need to incorporate biological variability into ecotoxicological assessments. Although conducted under controlled laboratory conditions that do not fully capture the complexity of natural environments, this study provides important evidence of the sensitivity of semi-aquatic insects to organic contaminants. *C. aquaticum* emerges as a promising bioindicator of organic pollution in aquatic ecosystems.

Future studies should validate these findings under field conditions, investigate the underlying physiological and molecular mechanisms, and assess whether similar patterns occur across other PAHs and insect species.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

WL: Data curation, Methodology, Investigation, Writing – original draft, Writing – review and editing, Visualization. MC: Methodology, Supervision, Writing – review and editing. AG: Methodology, Writing – review and editing, Supervision. MB: Visualization, Writing – review and editing. VS: Visualization, Writing – review and editing. CB: Data curation, Writing – review and editing, Methodology, Supervision.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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