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Acute and chronic exposure to diesel exhaust particles impairs the survival and development of the freshwater midge Chironomus riparius

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ABSTRACT

Diesel-powered vehicles contribute significantly to pollution through emission of fine particulate matter in the form of diesel exhaust particles (DEP). Significant amounts of DEP are introduced into freshwater bodies via road runoff after precipitation. These particles ultimately sink to the bottom of lakes and rivers and interact with macrobenthic invertebrates through ingestion or contact with the organisms' body surface, possibly leading to adverse effects. To date, little is known about whether DEP can have negative effects on benthic invertebrate species. We therefore exposed individuals of *Chironomus riparius*, a representative of the most abundant macroinvertebrate group and link in freshwater food webs, to DEP and examined their effects after acute, semi-chronic, and life-cycle exposure. DEP significantly affected the development and growth after sublethal 10-day exposure. The emergence ratio, development, and oviposition behaviour of *C. riparius* were significantly impaired after exposure to environmentally relevant DEP concentrations over a life cycle. We determined an LC_{50} of 85.3 mg/l for *C. riparius* exposed to DEP after 48 h. These results provide insight into how DEP may contribute to biodiversity and biomass loss in freshwater habitats acting as an additional stressor to drivers such as habitat loss and climate change.

Synopsis: The effects of diesel exhaust particles (DEP) on aquatic invertebrates are mostly unknown. This study reports acute and chronic negative effects of DEP on the survival and development of Chironomus riparius.

1. Introduction

Biodiversity loss is a major concern of the Anthropocene and affects all ecosystems. Nearly one in eight species on the planet is endangered by now and faces global extinction (Díaz et al., 2019). Drivers of this dramatic species loss are mainly anthropogenic and include climate change, habitat change, and pollution (Sánchez-Bayo and Wyckhuys, 2019). Pollution includes the introduction of pesticides, plastic waste, and airborne particulate matter, mainly from industrial plants and road traffic, into the environment and threatens the health of ecosystems (Barnes et al., 2009; Harrison, 2020). Freshwater ecosystems in particular, deliver crucial ecosystem services for both humans and animals but are overexploited and degraded for purely economic reasons (Arthington et al., 2010; Yeakley et al., 2016). In addition, rivers and streams in urban areas frequently experience the "urban stream syndrome" through sealed surrounding surfaces and higher runoff peaks, which additionally facilitates the introduction of nutrients and deposited airborne particulate matter into rivers and streams (Walsh et al., 2005).

Airborne fine particulate matter originating from diesel engines, hereafter referred to as diesel exhaust particles (DEP), belongs to the prevalent type of particles in the atmosphere around urban environments (Manisalidis et al., 2020).

Diesel exhaust particles (DEP) primarily consist of fine (PM2.5) and

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ultrafine particles (PM0.1) (Greim, 2019). Exhaust aerosols typically exhibit three particle size modes (Whitby and Cantrell, 1976). The nucleation mode, comprising over 90 % of the particle number but less than 10 % of the mass, includes particles ranging from 3 to 30 nm that primarily consist of VOCs condensed on sulfur compounds (Harrison et al., 2018; Kittelson and Kraft, 2014). The accumulation mode consists of particles between 20 and 500 nm, predominantly carbonaceous agglomerates and adsorbed materials, representing the majority of the particle mass (Kittelson and Kraft, 2014). The coarse mode, considered a subclass of the accumulation mode, includes particles ranging from 1 to 10 μm that result from re-entrained material deposited on exhaust surfaces, accounting for 5–20 % of the total mass (Kittelson, 1998; Harrison, 2020).

After precipitation, a considerable portion of DEP are introduced into freshwater ecosystems via road run-off and atmospheric deposition (Chin, 2006; Lamprea et al., 2011; Manisalidis et al., 2020).

These DEP are a major concern regarding the health of exposed organisms due to their small size and chemical composition (Vogt et al., 2003). It has been reported that DEP cause inflammation, respiratory diseases, and cancer in mammals (Ehsanifar et al., 2019; Oluyede et al., 2021). The chemical compounds supposedly taking a pivotal role in the toxicity of DEP are polycyclic aromatic hydrocarbons (PAH) (Meador, 2008). Some of these typical DEP associated PAH like Fluoranthene, Phenanthrene, and Pyrene are bioaccumulative due to their lipophilicity allowing them to accumulate in the fatty tissues of organisms (Honda and Suzuki, 2020). Additionally, some have carcinogenic properties due to their ability to form DNA-binding intermediates and induce mutations of oncogenes after their activation and can act as endocrine disruptors (Baird et al., 2005; Valavanidis et al., 2011). Research conducted on the effects of DEP on invertebrate organisms is scarce. Some studies cover the effects of DEP on terrestrial invertebrate pollinators like *Apis mellifera* and *Bombus terrestris* (Reitmayer. et al., 2019; Hüftlein et al., 2023; Seidenath et al., 2023). Studies of effects on aquatic organisms have described acute toxicity of DEP to brine shrimp larvae from 250 mg/l (Pikula et al., 2021). Additionally, some research was able to show developmental and organ toxicity of PM2.5 (particulate matter < 2.5 μm), which included DEP but also many other particulates to zebrafish larvae (functionally similar to invertebrates in ecotoxicological testing (Belanger et al., 2010, Duan et al., 2017; Manjunatha et al., 2021). This low data situation highlights the need for research into the effects of DEP on stream invertebrates, given that large amounts of road runoff are washed into nearby freshwater ecosystems (Gillis et al., 2022).

Benthic arthropods play a vital role in freshwater ecosystems, as a key link between primary producers and secondary consumers and provide a food source for terrestrial predators via emerging imagines (Wallace and Webster, 1996). These invertebrates pose the most diverse group and the largest share of biomass in freshwater habitats (Thorp and Covich, 2009). Among benthic macroinvertebrates, the family of the Chironomidae represents the most widely distributed and often most abundant group of organisms (Armitage et al., 1995; Pinder, 1986). *Chironomus riparius* is among the most common chironomid species and is, as a key organism in European freshwaters, well suited for experimental effect analysis of environmental stressors due to its ease of handling and short generation times (Meregalli et al., 2000; OECD, 2011, 2004; Watts and Pascoe, 2000). To date, there is no knowledge of how DEP influence the survival and development of this benthic freshwater macroinvertebrate after acute and chronic exposure.

Therefore, we characterised the effects of DEP in multi-level exposure scenarios. We assessed the effects of DEP on *C. riparius* over acute exposure to a series of doses in a first step. Subsequently, we investigated the effects of DEP after chronic exposure on larval development over a 10-day period and finally the reproductive potential and emergence of imagines in a 30-day experiment. The acute toxicity test over 48 h included concentrations ranging from 3.9 to 250 mg/l which allowed us to establish LC values according to OECD 235 (OECD, 2011). To assess the toxicity of *C. riparius* towards larval development according to OECD 218, we exposed individuals of *C. riparius* to environmentally relevant doses of 4.5, 9, and 18 mg/l DEP over 10 days. We performed a life cycle test with the same concentrations used over the 10-day period with emergence ratio, the sex ratio of emerged adults, egg masses per replicate, and development rate as endpoints, to characterise the effects of DEP on larval development and imaginal reproduction and emergence. We hypothesized, that DEP would significantly affect the survival, development, and emergence of the larvae, and further the egg-laying behaviour of the imagines.

2. Methods

2.1. Production and characterisation of DEP

The production and characterisation of DEP were performed as previously described in Hüftlein et al., (2023). In brief, we used a four-cylinder diesel engine (OM 651, Daimler AG, Stuttgart, Germany) representing an average passenger car. The maximum injection pressure was 2000 bar, and the rated power was 150 kW at a maximum torque of 500 Nm (Lückert et al., 2011; Zöllner and Brueggemann, 2018). We simulated inner-city scenarios with acceleration, deceleration, and idle phases. The generated soot was collected through an electrostatic filter system (OekoTube Inside, Mels-Plons, Switzerland). The samples were collected over 15 inner city cycles of each 200 s. The DEPs were transferred in glass vials, stored with silica gel in the surrounding air to prevent moisture and stored at −20°C until further analysis or utilization in the exposure scenarios.

Measurements of the organic fraction were carried out by thermogravimetric analysis (TGA) (Fig. 2, supplemental material). A fraction of 72.2 % ± 1.1 % of the DEP mass was attributed to elemental carbon, 23.2 % ± 0.9 % w/w to organic fractions, and 4.6 % ± 0.7 % w/w to inorganic matter (Fig. 2, supplemental material). For the analysis of PAH content in the DEP, we used 1:1 cyclohexane: toluene (the latter as a keeper) as solvent. We weighed ~100 μg DEP and diluted it in the solvent to obtain 1 μg DEP/μl solvent to extract the PAH from the DEP. Subsequently, we centrifuged the suspension for 20 min at 14,000 turns/min at room temperature (Centrifuge 5415 C, Eppendorf SE, Hamburg, Germany). The supernatant was removed with a glass pipette and transferred in a conical 1 ml glass vial (Macherey-Nagel GmbH & Co.KG, Düren, Germany). A deuterated PAH standard (M-8272 Deuterated Analogs, Accustandard Inc., New Haven, USA) was added as an internal standard and used for calibration curves. PAH content was quantified with a Shimadzu Nexis GC2030/GCMS-QP2020 NX (Shimadzu, Kyoto, Japan) with Rtx-5MS fused silica (30 m* 0.25 mm* 0.25 μm, Restek, Bellefonte, Pennsylvania) and 280°C splitless injector. 1 μl of each sample was injected by an AOC-20s Plus Auto Sampler (Shimadzu, Kyoto, Japan). The column oven started with a temperature of 80°C holding for 5 min and increased by 20°C per minute until 280°C which was also held for 5 min.

Sub-micron particle size distributions were measured for each DEP sample by a fast response differential mobility particulate spectrometer DMS500 (Cambustion, Cambridge, England) at 10 Hz data rate (Symonds et al., 2007) (Fig. 1, supplemental material).

During the inner-city cycle, the median diameter of solid particles varied between 52.1 ± 1.8 nm and 101.9 ± 1.7 nm, depending on engine load and speed (Fig. 1, supplemental material).

2.2. Husbandry of *Chironomus riparius*

The culture of *C. riparius* was held in a breeding cage inside a Rubarth P 850 climate cabinet (Rubarth Apparate GmbH, Laatzen, Germany). Constant conditions were maintained with a temperature of 20°C and a day-night cycle of 12 h. The culture animals were reared in white bowls filled with quartz sand with a mean diameter of 0.16 mm (Quarzwerke GmbH, Frechen, Germany) and M4-medium (Elendt and Bias, 1990),

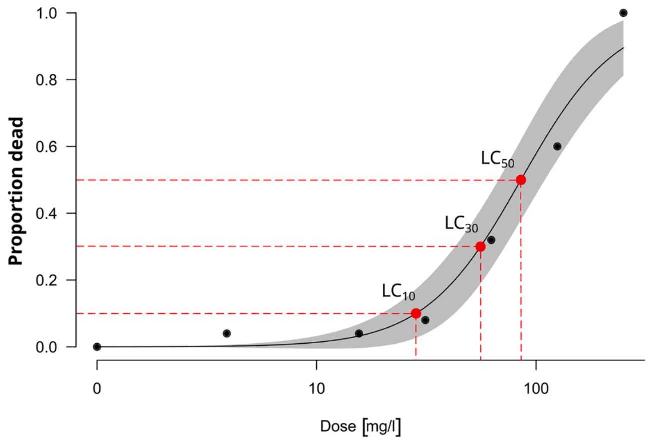

Fig. 1. Dose-response curve of the proportion of dead C. riparius individuals exposed to rising concentrations of DEP. Ribbons are showing the 95 % confidence intervals. Red points with dashed lines represent the LC values.

and fed with Tetramin® (Tetramin GmbH, Melle, Germany) *ad libitum*. The M4-medium was exchanged once a week.

2.3. Preparation of particle suspension for the acute toxicity experiment

We prepared concentrations from 250 mg/l followed by 125, 62.5, 31.25, 15.6, 7.8 and 3.9 as the lowest. DEP were weighed with a semi-micro-electronic balance Explorer (EX225D/AD, OHAUS Europe GmbH, Nänikon, Switzerland) using an antistatic kit (U-electrode PRX-U, Mettler-Toledo GmbH, Gießen, Germany) to avoid electrostatic charging during the process of weighing. After weighing, DEP particles were added to M4 together with 10 µl of 20 % Tween®20 (Merck Schuchardt OHG, Hohenbrunn, Germany) as an emulsifier, to a volume of 100 ml in each treatment except the negative control, to achieve a nearly homogeneous stock solution. For the solvent control (negative control) 10 µl of 20 % Tween®20 were added to the M4. Each stock solution was kept for 15 min inside an ultrasonic bath to improve the dispersion process.

2.4. Acute toxicity experiment

The acute toxicity of DEP was assessed by performing experiments according to the OECD guideline 235 (OECD, 2011). The experiments were conducted inside a climate chamber at 20 ± 0.1 °C, 14 h light and 9 h dark cycle with 30 min dusk/dawn each. Five days before the tests started, 10 freshly laid egg cases (not older than 24 h) were transferred to a glass dish filled with M4 Medium to guarantee one to two-day-old L1 larvae for the experiments. Directly after the hatching of the first individuals, a suspension of M4 Medium and pestled Tetramin® was added ad libitum to the glass dishes. This feeding prior to the experiments was important to ensure a survival rate of ≥ 85 % in the controls after exposure. However, during the acute toxicity tests, the larvae were not fed. Seven concentrations of DEP were used, each lower than the previous one by a factor of two, a solvent control containing 10 µl of 20 % Tween20® and a negative control containing only M4-medium. The highest DEP concentration was 250 mg/l followed by 125, 62.5, 31.25, 15.6, 7.8, and 3.9 as the lowest. The acute toxicity tests were performed in 6-well plates (Eppendorf AG, Hamburg, Germany) each well filled with 10 ml of the respective test solution. The larvae were randomly added to the wells the next day enabling the DEP to sink to the sediment. The finished prepared 6-well plates were then filled with five organisms in every single well resulting in 30 organisms per 6-well plate and a total of 900 organisms for all 30 plates. Positions of the wells as well as the placement of the well plates in the climate chamber were randomized and randomly repositioned every 24 h using the randomizer program Research Randomizer (Version 4.0) (Urbaniak and Plous, 2013) to prevent the influence of confounding factors such as differences in light availability or temperature. The mortality was recorded after 4 h, 24 h and 48 h. With the resulting data on the mortality, the LC10, LC30, and LC50 values were calculated for each time point.

2.5. Chronic exposure experiments

In a preliminary experiment, we tested the amount of DEP suspended in the water column and not sinking to the sediment. The amount was quantified, by adding the medium to the sand-DEP mixture and removing the medium the next day. The medium was then filtered through previously weighed cellulose filters (Carl Roth GmbH und CoKG, Karlsruhe, Germany) with a pressure pump. These cellulose filters were then dried in a drying cabinet at 60°C for 24 h (UFE600, memmert GmbH und CoKG, Schwabach, Germany). The dried filters were weighed again after 24 h and the first weighing value was subtracted from the second. As most DEP settle, the mean amount of DEP in the water column (n = 5 for all concentrations) was 1.22 + /- 0.44 mg in the 4.5 mg/l treatment, 1.37 + /- 0.31 mg in the 9 mg/l treatment and 1.38 + /- 0.55 mg in the 18 mg/l treatment (Supplemental Fig.7). The chronic exposure experiments were conducted in the same climate chamber with

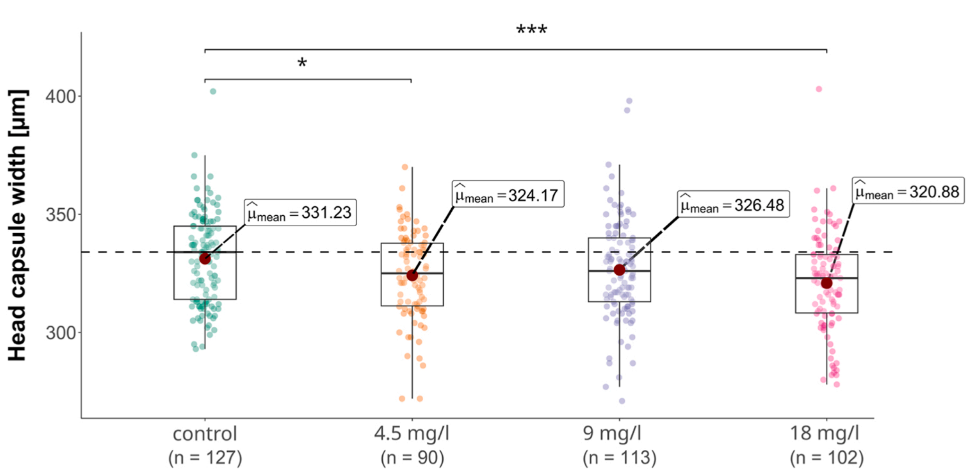

Fig. 2. Effect of 4.5, 9 and 18 mg/l DEP exposure on head capsule width of C. riparius larvae via sediment and water over a 10- day period, n represents the number of measured head capsules. The values in the boxes represent the mean head capsule width of the individuals from each treatment. Asterisks indicate significance between treatments (*** <0.001, * <0.05). Dashed line indicates median of the control treatment. A larger proportion of larvae had developed to the L4 stage in the 4.5 mg/L treatment, resulting in a smaller sample size (n = 90) of L3 larvae than in the other treatments.

the same day-night cycle. The setup for the (I) 10-day chronic toxicity test and the (II) life cycle test were identical. We chose concentrations below the LC10 of the acute toxicity test to avoid mortality in the larval stages. We exposed the larvae to sublethal doses of DEP, determined in the acute toxicity test, of 4.5, 9, and 18 mg/l and a negative control. To obtain these concentrations in a final volume of 670 ml 3.015, 6.03, and 12.06 mg DEP –were weighed and mixed with 230 g of quartz sand in a laboratory flask. The mixture of DEP and quartz sand correspondsto 0.01, 0.03, and 0.05 mg DEP per g dry weight. The quartz sand was weighed in a disposable weighing pan (41x41x8 mm, neoLab Migge GmbH, Heidelberg, Germany) with a precision scale (UW1020H, Shimadzu, Kyoto, Japan). The mixture was homogenized in an ultrasonic bath (TRANSSONIC 460, Elma Schmidbauer GmbH, Singen, Germany) for 15 min. The homogenized mixture was poured into a 1.5 l Weck® beaker (J. Weck GmbH u. Co. KG, Wehr-Öflingen, Deutschland). When adding 670 ml of M4-medium, we covered the sand with a glass lid to prevent splashing. The glass lid was removed after adding the medium. The beakers were prepared a day before adding the aeration-system, to allow settling of the quartz sand-DEP mixtures and the control sediment in the medium. The beakers were constantly but gently aerated through a pump hose system (Aqua forte air pump V-30, SIBO Fluidra, Veghel, Netherlands), with two pumps aerating 20 beakers at once through an air distributor and Y- shaped hose connections (12-way distributor, 6 mm diameter each, Osaga, Der-Koi-Shop GmbH & Co. KG, Buchholz / Aller, Germany). We added 2–5 grains of cetyl-alcohol (Carl Roth GmbH und CoKG, Karlsruhe, Germany) to the M4-Medium in every beaker to reduce the surface tension. The place for each replicate on the shelf was randomized with the randomizer program Research Randomizer (Version 4.0) (Urbaniak and Plous, 2013). After 24 h of aerating the medium, the larvae were added. Each treatment consisted of 10 replicates (beakers) with 20 larvae each. Two days old, L1 larvae from 10 different egg cases were randomly assigned to the replicates. The larvae were fed with 0.25 mg Tetramin® (Tetra GmbH, Melle, Germany) per larvae per day for the first ten days. After ten days, food was increased to 0.5 mg of Tetramin® per larvae per day. After five days each beaker was filled up to the starting level with approximately 100 ml distilled water, to compensate for the low amount of evaporated medium.

(I)After the 10-day chronic exposure experiment, the medium was discharged through a 120 µm plastic sieve, to retrieve any flushed-out larvae. The sediment with the remaining larvae were emptied into white plastic trays, handpicked, and fixated in 80 % EtOH. Every larva was photographed under a stereomicroscope (M50, Leica, Wetzlar, Germany) equipped with a digital camera (DP26 Olympus, Hamburg, Germany, light: KL 300 LED, Leica). All measurements were conducted using the Image Analysis Software cellSens Dimension (v1.11, OLYMPUS, Hamburg, Germany). Body length was measured through a polygonal line starting at the posterior end of the head capsule to the end of the last appendage (Fig. 3, supplemental material). The head capsule width is a measure of size, independent from the nutritional state, and therefore more reliable than body size (Watts and Pascoe, 2000). Therefore, the head capsule width was determined by first removing the head from the body and then measuring the length from the left to the right margin of the head capsule on the ventral side (Fig. 4, supplemental material).

(II)Throughout the life cycle experiment, the beakers were covered with gauze (mesh width: 1.5 mm) to prevent the imagoes from escaping. On the 20th day of the experiment, imagines began to emerge up until the 30th day. From this day onwards, the emergence of imagines, their sex, and the number of egg masses laid were recorded.

2.6. Statistical analysis

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022). The LC values were calculated by creating a binomial two-variable log-logistic model using the *drm()* function from the *drc* package (Ritz et al., 2015). The dose-response models were plotted using the ggplot2 package (Wickham et al., 2022)

Residual plots of response variables were used to test for homoscedasticity and normality using the R package *DHARMa* (Hartig, 2022). General linear models with body length, head capsule width, emergence as response variables, and treatment as a covariate were created using the base R *glm()* function. F-statistics were calculated with the function *Anova()* to assess p-values for differences between treatments. To compare treatment effects, we ran pairwise comparisons using the Tukey-HSD post-hoc test with Holm correction using the *multcomp* package (Hothorn et al., 2022). Head capsule widths of individuals from the different treatments were plotted using the *ggbetweenstats* function from the *ggstatsplot* package (Patil, 2021). Differences in larval stage distributions between treatments were determined using pairwise comparisons of proportions with Bonferroni correction using the *pairwise.prop.test()* function. Binomial general linear models with emergence ratio and sex ratio as response variables with treatment as a covariate were produced using the *glmer()* function to correct for overdispersion by adding a random effect on the observation level (Harrison, 2015). We only compared the head capsules of the L3 larvae, as they are the expected larval stage after 10 days. Additionally, including the L4 larvae in the statistical analysis could mask effects caused by the DEP treatment. For the sake of completeness, we provide data analysis with

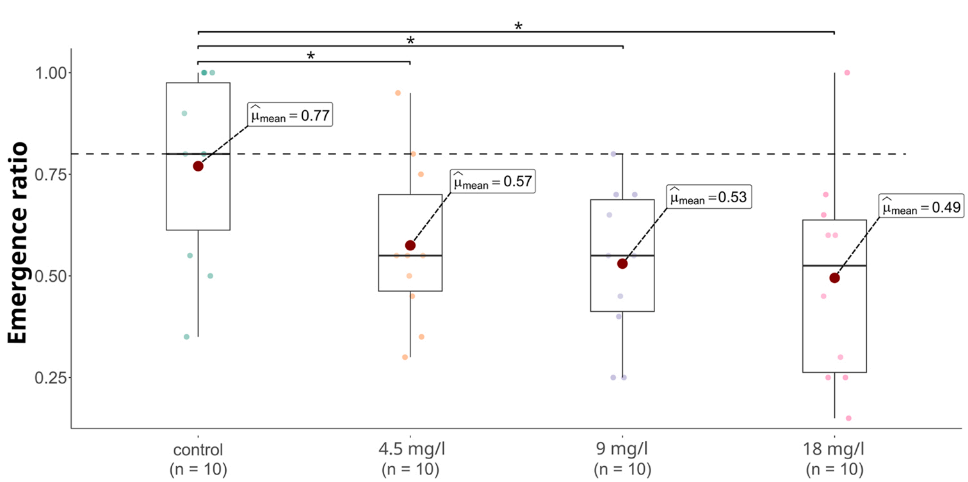

Fig. 3. Effect of 4.5, 9 and 18 mg/l DEP exposure on the emergence ratio of C. riparius via sediment and water over a 20- day period, n represents the number of replicates. The values in the boxes represent the emergence ratio per beaker from each treatment. Asterisk indicates significance between treatments (* <0.05). Dashed line indicates median of the control treatment.

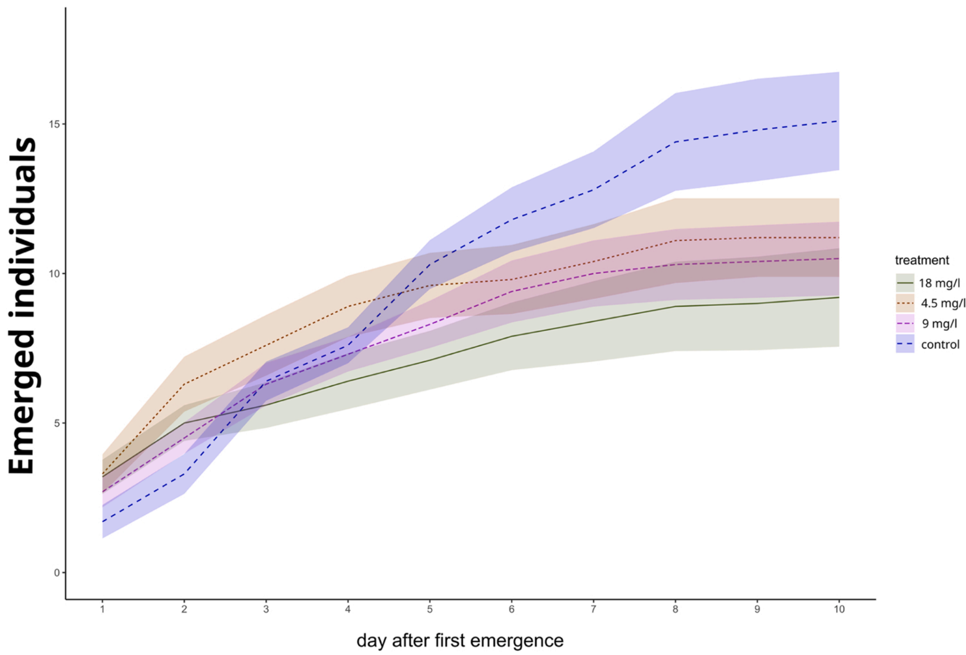

Fig. 4. Development rate of C. riparius individuals exposed to a control and 4.5, 9 and 18 mg/l DEP. Days after first emergence began 20 days after the start of the experiment.

both L3 and L4 in the supplements (Supplemental Fig. 5+ 6).

3. Results

3.1. Acute toxicity experiment

In order to estimate the toxicity of DEP after 48-hour acute exposure on the larvae of *C. riparius*, we calculated the lethal concentrations (LC) for 10, 30, and 50 % mortality in exposed individuals. The LC10 after 48 h was estimated to be 28.4 + /- SE 5.9 mg/l. The LC30 and LC50 were calculated to be 55.8 + /- SE 7.5 mg/l and 85.3 + /- 10.5 mg/l respectively (Fig. 1).

3.2. Chronic exposure experiments

Exposure to DEP for a 10-day period (I) had a significant effect on the head capsule width of *C. riparius* individuals (GLM with subsequent ANOVA, $X^2 = 16.412$, df = 3, $p < 0.001$). The head capsule width of the control individuals (mean width = 331.23 ± SD 19.0 μm) was significantly higher than the width of the head capsule of the individuals exposed to 4.5 mg/l (mean width = 324.17 ± SD 18.6 μm) (Tukey comparison: $p = 0.048$) and those exposed to 18 mg/l (mean width = 320.88 ± SD 20.5 μm) (Tukey comparison: $p < 0.001$) (Fig. 2).

Exposure to DEP for the entire larval period (II), had a significant effect on the emergence ratio of *C. riparius* individuals (GLMM with binomial distribution, $X^2 = 10.698$, df = 3, $p = 0.013$). Pairwise comparisons revealed, that the mean emergence ratio of individuals in the control group was 20 % higher than of individuals exposed to 4.5 mg/l (Tukey comparison: $p < 0.05$), 24 % higher than of individuals exposed to 9 mg/l (Tukey comparison: $p < 0.05$) and 28 % higher than of individuals exposed to 18 mg/l (Tukey comparison: $p < 0.05$) (Fig. 3).

The development rate (II), counted as days after the first individual emerged, was significantly affected when exposed to DEP (GLM with Poisson distribution, $X^2 = 50.54$, df = 3, $p < 0.001$). Holm-corrected pairwise comparisons revealed that the exposure to 18 mg/l significantly impaired the development of the *C. riparius* larvae compared to the control (Tukey comparisons with Holm correction: $p < 0.01$) (Fig. 4).

Additionally, the laying of egg masses by the emerged *C. riparius* females (II) was significantly impaired when being exposed to DEP during their larval stage (GLM with Poisson distribution, $X^2 = 12.7003$, df = 3, $p < 0.01$). The pairwise comparison revealed a significant reduction in laid egg masses of females emerged when exposed to 18 mg/l (mean = 1.4 egg masses per beaker) compared to all other treatments (control mean = 3.8 ± SE 0.8 egg masses per beaker, Tukey comparisons: $p < 0.01$, 4.5 mg/l treatment mean = 3.3 ± SE 1.04 egg masses per beaker, $p < 0.05$ and 9 mg/l treatment mean = 3.7 ± SE 1.89 egg masses per beaker, $p < 0.05$, see Fig. 5).

3.3. PAH content of DEP

In this study, the DEPs already used in Hüftlein et al., (2023) were used. The PAH content of the particles can be found in Table 1.

4. Discussion

Our results show that DEP cause elevated mortality after acute exposure but more importantly, impair the larval development and further the emergence of the imagines and number of laid egg masses by the females in chronic experiments. This impairment already occurs in environmentally relevant concentrations as the results of Elmquist et al., (2004) extrapolated that up to 6 mg black carbon per g dry weight, with 0.1 mg/g dry weight soot black carbon from total organic carbon, can be found in the Rhine River sediment. This is about twice the amount of the highest concentration used in both chronic exposure experiments – 0, 05 mg/g dry weight.

Further, after 10-day exposure (I) to 4.5 and 18 mg/l DEP, the head capsule width, a reliable proxy for body size, of the exposed individuals was significantly smaller than those of the control individuals. The reduction in size implies a biomass reduction, which could negatively impact freshwater ecosystems, as chironomids often represent the most abundant group of organisms in temperate freshwater ecosystems and essential food source for higher trophic levels (Armitage et al., 1995; Leszczyńska et al., 2021). As chironomid larvae use most of their nutrition for gametic and somatic growth (Péry et al., 2005), the reduced somatic growth might be caused by reallocated energy reserves used for

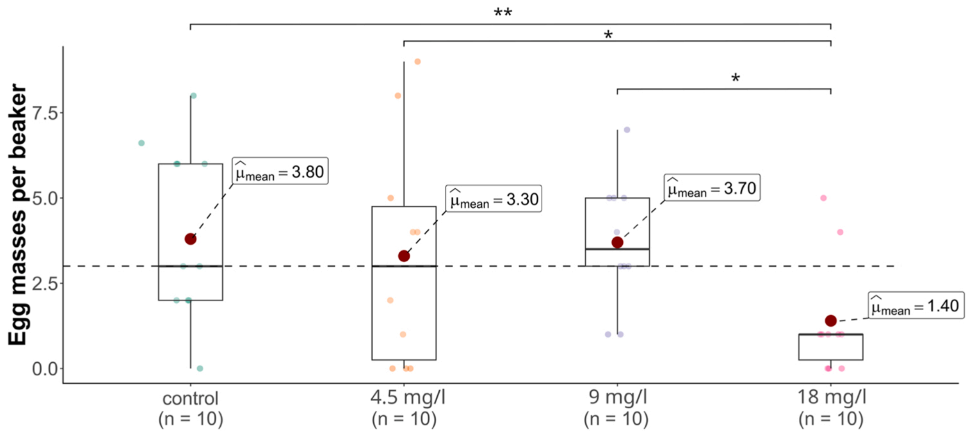

Fig. 5. Effect of 4.5, 9 and 18 mg/l DEP on the egg laying behaviour of emerged female C.riparius individuals. n represents the number of replicates. The values in the boxes represent the mean egg masses per beaker from each treatment. Asterisks indicates significance between treatments (**<0.01, * <0.05). Dashed line indicates median of the control treatment.

Table 1
Concentration of the analysed PAHs found in the DEP samples from Hüftlein et al. (2023). A total of three replicates were analysed via GC-MS. Naphthalene and 1-methylnaphthalene were below the limit of detection. (LOD = Limit of detection, LOQ = Limit of quantification).

PAH	n	Mean [µg/g]	Standard deviation	Standard error	95 % - CI
1-Methylnaphthalene	3	<LOD	0	0	0
Naphthalene	3	<LOD	0	0	0
Fluoranthene	3	107.03 (<LOQ)	39.16	22.61	97.28
Phenanthrene	3	220.47	49.04	28.32	121.84
Pyrene	3	444.05	130.22	75.18	323.49

detoxification as described for pesticides or by reduced food intake due to a false sense of satiation (da Silvia Pinto et al., 2021; Monteiro et al., 2019; Welden and Cowie, 2016). DEPs also have the ability to shift microbiome-associated microorganism communities in insects (Seidenath et al., 2023). Therefore, the reduced growth rates of the larvae in our study could also be a consequence of microbiome alterations, as these microorganisms are crucial for the growth and morphology of aquatic insect larvae such as *C. riparius* (Cai et al., 2022; Strand, 2018; Varg et al., 2021).

The negative effects of DEP exposure are not limited to the larval stage. The number of emerging imagines (II) was also significantly affected after exposure to DEP during their complete larval stages. If the 30 % reduction in emergence in every DEP treatment compared to the control presented here were transferable to populations in the environment, then a massive reduction in the biomass of flying imagines would be a result. Additionally, female *C. riparius* individuals laid significantly fewer egg cases when exposed to 18 mg/l DEP during their larval stage than females from the control treatment, which will lead to a reduced number of larvae in the next generation. This reduction in the number and biomass of chironomid larvae could possibly lead to an increase in algal growth rates, as chironomid grazers in particular, have been shown to have significant impacts on algal and bacterial growth (Botts, 1993; Nogaro et al., 2008). They can influence epiphytic algal assemblages through selective grazing, reducing the biovolume of preferred foods and construction materials (Botts, 1993). Neury-Ormanni et al. (2020) further demonstrated that chironomids exhibit selective grazing behaviour on microalgae, with their feeding behaviour being influenced by pesticide contamination. Especially aquatic invertebrate predators, but also early fish stages are dependent on chironomid larvae as a food source (Armitage, 1995). These reductions of primary food sources for secondary consumers can lead to bottom-up effects and, consequently, influence population dynamics of predators and biodiversity through intra-guild predation like Odonata larvae hunting larvae of the same order (Allgeier et al., 2019; Gerstle et al., 2023). Imagines of chironomids play a major role as an important food source for invertebrate and vertebrate predators associated with riparian habitats (Armitage, 1995). Emerging insects comprise between 50 % and 100 % of the diet of invertebrate and vertebrate predators, whereas chironomids represent between 60 % and 90 % of occurring imagines respectively (Alberts et al., 2013; Kautza et al., 2016; Paetzold et al., 2005; Sanzone et al., 2003). The availability of biomass to terrestrial consumers derived from aquatic ecosystems can be up to 3 times greater than the terrestrial counterpart, demonstrating the importance of aquatic macroinvertebrates, not only to aquatic but also to adjacent ecosystems (Kautza et al., 2016). Hence, the significant reduction in biomass of larvae and imagines of chironomids exposed to DEP may lead to a disruption in ecosystem functioning.

Additionally, we were able to establish a dose-response relationship for the acute 48-hour exposure of DEP on the survival of *C. riparius* larvae, which may now be utilized for further guidelines in aquatic systems. The LC50 and LC10 were calculated to be 85.3 mg/l and 28.4 mg/l, respectively. One aquatic study by Pikula et al., (2021) tested the effects of DEP on the nauplius larvae of the brine shrimp *A. salina*. In contrast to our results, Pikula et al., (2021) found significantly reduced viability after 96 h and at a concentration of 250 mg/l, which is three times higher than the LC50 for *C. riparius* established in this study. Studies with 120 h post-fertilization old zebrafish larvae on the effect of PM2.5 (including DEP) at environmental concentrations resulted in significantly increased mortality between 15 µg/ml (Manjunatha et al., 2021) and 200 µg/ml (Duan et al., 2017). This discrepancy could, of course, be attributed to the fact that the toxicity of DEP can differ strongly when originating from different regions (Li et al., 2019) or be caused by different amounts of PAHs due to different combustion parameters. This is why we used characterised particles (Supplemental Fig.2 + 3) to enable comparability, as both physical properties (size, shape) and chemical properties (attached organic and inorganic substances) can significantly influence the toxicity of the particles (Schmidt et al., 2019). The presence of metals and PAHs in DEP for example significantly contributes to their toxicity (Longhin et al., 2016).

5. Conclusion

We investigated the effects of acute increasing concentrations and chronic environmentally relevant doses of DEP on the survival, development, and reproduction of *C. riparius* individuals. We demonstrated impaired development, emergence, and reproduction after 10 days and entire life cycle exposure with sublethal concentrations of DEP. Additionally, we were able to establish a first dose-response relationship of *C. riparius* to DEP after 48-hour exposure. These results indicate that DEP could be a contributing factor to the current loss of insect biomass and biodiversity and may have negative effects on aquatic ecosystems.

Future research should include multi-generational studies in mesocosms as well as field studies to provide more realistic insights into the effects of DEP, exploring interactions with natural sediments and a wider range of species. Furthermore, the individual effects of the various chemical and physical components of DEP as well as controlled concentrations of different PAH should be approached in future research. Finally, the development of strategies to mitigate the effects of DEP on freshwater ecosystems, including pollution reduction and ecological restoration techniques, will be essential to address these environmental challenges.

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CRediT authorship contribution statement

Matthias Schott: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis. **Christian Laforsch:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Elisa Nickl:** Validation, Methodology, Investigation. **Jona Schmitt:** Methodology, Investigation, Formal analysis. **Dieter Brüggemann:** Supervision, Resources, Funding acquisition, Conceptualization. **Thomas Hillenbrand:** Supervision, Resources, Conceptualization. **Andreas Mittereder:** Methodology, Investigation, Formal analysis. **Frederic Hüftlein:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118808.

Data availability

Data will be made available on request.

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