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Research article

Ecotoxicological effects of soft plastic fishing lures on the benthic amphipod *Hyalella azteca*

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ABSTRACT

Plastic pollution by lost, abandoned and discarded commercial fishing gear is well characterized for marine systems, whereas the environmental impact of lost recreational fishing gear in freshwater systems remains unclear. Especially soft plastic lures are increasingly used, potentially contributing to the pollution of waterbodies by the release of hazardous substances such as plasticizers. The objective of this study was to compare water- and sediment-borne acute toxicity of three types of commonly used soft plastic lures with the benthic amphipod Hyalella azteca. A standardized test setup was used to compare 96 h water-only and sediment acute toxicity as well as 14 d toxicity to determine leachate effects in a dilution series. Chemical composition of the lures and leachates in the water were qualitatively analyzed. All of the three tested soft lures including those claiming to be environmentally friendly - consisted of PVC and contained plasticizers, including highly concerning substances such as diethylhexyl-phthalate and dibutylphthalate, as well as other additives. Depending on tested lure type, even dilutions of the leachate to 1.5 % were highly toxic and caused up to 100 % mortality in H. azteca after 96 h exposures, especially in treatments without sediment. Strong differences in the chemical composition and resulting ecotoxicological effects among different lure types were identified. The results of this study demonstrate that the currently underestimated effects of soft plastic fishing lures require better documentation, awareness and regulation to reduce environmental pollution by recreational angling.

1. Introduction

The plastic pollution of aquatic ecosystems is currently recognized as a major threat to ecosystem functioning and health and is supposed to contribute to the global decline in biodiversity [1]. Plastic enters the environment via various sources, ranging from microand nanosized particles and fibres in sewage effluents, to discarded plastic products on the macroscale such as plastic waste and packaging. Lost, abandoned or discarded fishing gear is thereby recognized as one of the major input sources of plastic pollution into oceans [2,3]. However, in freshwater systems, this has hardly been considered, and the magnitude of emissions or potential harmful effects to freshwater organisms are widely unknown [4]. However, recently there is a raising awareness that commercial fisheries in inland waters also results in the emission of mostly passive fishing gears into the environment [3].

In contrast to large-scale commercial fishing in the marine environment, fisheries in freshwaters are often dominated by recreational angling, which was estimated to involve about 10 % of the population worldwide [5]. Recreational angling involves mostly the

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use of active fishing gear such as hook and line fishing, whereas commercial fisheries mostly use passive fishing gear such as nets and traps.

Recreational angling is in general not considered problematic to the environment and the aquatic life, and there are even some positive impacts reported that are related to a high level of awareness concerning environmental sustainability, conservation and animal welfare among recreational anglers [4,6,7]. However, several studies also point at problematic impacts of recreational fishing on aquatic ecosystems [8]. For instance, recreational angling can result in fish declines due to overfishing, fish injuries and catch selection, littering of the shoreline, trampling of shoreline vegetation and motor boating [9,10]. In contrast to these disturbance effects, there is little information on the extent of lost or discarded fishing tackle in freshwater ecosystems, but a few reports indicate a so far underestimated or unknown contribution to plastic pollution in freshwaters [4]. For instance, a recent study by Pander et al. [11] found that a total of 5442 items linked to recreational angling and almost 9.2 km of fishing line had accumulated over a 5-year period within a single 100 ha reservoir in Germany. The items that were quantified covered the full range of commercially available fishing tackle, including a broad variety of metal- and plastic based items in various degradation states. A meta-analysis based on sales statistics from Scandinavian countries report an estimated yearly input of 775 tons of fishing hooks and lures, up to 235 km fishing line and about one ton of net material into Scandinavian inland waters [12]. Although this study did not focus on the exact materials used, it is likely that many items consist of harmful materials widely used in the tackle industry such as lead, nickel, copper, polyvinyl chloride plastics, polyamide lines or silicone [11,13].

Lost fishing tackle can have direct impacts on fishes, e.g. ingested lures with hooks were shown to negatively influence the growth and body conditions of brook trout [14]. In addition, the gear itself, its degradation products and its leachates can potentially be toxic to the aquatic environment, which is to date not considered at all. While the toxicity of metal components of ammunition entering freshwater systems by hunting is relatively well studied [15], the composition and potential toxicity of the plastic-based components in fishing equipment are widely unknown. However, similar to other plastic waste, these items disintegrate over time and are therefore a potential source of pollution by micro- and nanoplastics to waterbodies. Another aspect is the high complexity of the used materials containing not only plastic polymers, but also a variety of additives such as fillers, stabilizers, pigments, plasticizers, flame retardants, fluorescent dyes, salts and flavors to increase their performance in the water and to be attractive for fish [16–18]. Once in the aquatic environment, some of those additives can leach out of the main polymer until reaching a concentration equilibrium depending on the respective chemical properties. These processes occur generally faster compared to the fragmentation of the main polymer.

So far, there are knowledge gaps on the ecotoxicological effects of such complex mixtures of plastic additives and how they are released from soft plastic lures, especially since these progressively fragment from the meso- to micro- and nano-scale over time [19]. In this context, especially the flame retardants (brominated or phosphorylated substances) and plasticizers (phthalates) are of concern since they not only induce a direct toxic effect, but also disrupt hormonal regulation at very low environmental concentrations [20–22], with potential impacts on behavior, reproduction, development and immune response in a wide variety of organisms. For example, the central adrenergic nervous system of fish is affected by plasticizers [23]. Negative effect of plasticizers have also been shown in *Daphnia magna* [24], *Danio rerio* [25], and *Hyalella azteca* [26]. Such effects are slowly getting recognized by the fishing tackle industry, resulting in increased efforts to avoid certain plasticizers and develop biodegradable soft plastic lures [13], which also currently increase in their general market share. Accordingly, there are products labelled as "free of toxic additives", "BPA free" or "environmentally friendly", which suggests that a polymer mixture is used that contains no known harmful additive substances.



Fig. 1. The three different soft plastic lures type F (top), type N (middle), and type Z (bottom). Pictures are scaled to the same size. Information on dimensions is given in the main text. Photographs by Andreas Heddergott (TUM).

However, so far there is no information available about the potential environmental impact of the materials used in soft fishing lures and other plastic angling equipment. Given the large amount of discarded fishing gear and long retention times of the material, as shown by Pander et al. [11], this calls for a need to further investigate the toxicity of fishing gear, especially considering environmentally relevant organismic and population-level effects.

In order to provide novel insights into potentially harmful effects of soft plastic lures, we determined the toxicity of leachates from three commonly used types of plastic angling lures using the benthic invertebrate *H. azteca* as a model test species. We compared soft plastic lures covering a range of characteristics, including one cheap mass product without specific label, and two types that are labelled environmentally friendly (mid- and high-price). The specific kinetics of chemical leaching from these lures and the extrapolation to environmental concentrations were not the focus of this study. We rather followed the overall aim to test if lost or discarded soft plastic lures are a so far unknown or underestimated source of hazardous materials in freshwater ecosystems.

We tested the hypotheses that (a) the high-price products without harmful chemicals show no or less effects in comparison to the mid-price as well as low-price mass product, and (b) the toxicity of potentially released additives is lower in the presence of sediment.

2. Material and methods

2.1. Soft plastic lures

The soft plastic lures were selected according to the following criteria: First, we aimed to include brands being advertised as environmentally friendly and brands that do not make this claim. A second criterion for selection was that the lures should be of similar size, shape, color, and pigmentation. Third, the items should cover a wide price range (Fig. 1).

Type F lures had an average weight of 3.99 ± 0.01 g (mean \pm SD) and a length of 90.13 ± 1.75 mm (mean \pm SD). There is no information about the chemical composition provided. The manufacturer claims that their lure and especially the colors are ideal for attracting predators like pikeperch, trout, and bass. The only information provided about the material is that it is made of plastic and that the tail is extra soft. With a price of $22 \notin$ for 50 lures, they were the cheapest of the three. The extra soft tail could be an indicator for the use of plasticizers [14].

The second lure, type N had on average a weight of 5.93 ± 0.02 g (mean \pm SD) and a length of 85.13 ± 1.23 mm (mean \pm SD). The manufacturer has its own website and shop and their lure is sold to a variety of distributors. No further product specification is provided, but it is stated that the lure contains non-toxic plasticizers and that the lure is coated with garlic flavor. With a price of $10 \notin$ for five items, type N ranges in the upper price range for soft lures. The material is stated as polyvinyl chloride (PVC) with non-phthalate plasticizers.

The third soft fishing lure used is type Z, with a weight of 1.95 ± 0.05 g (mean \pm SD) and a length of 76.97 ± 1.02 mm (mean \pm SD). According to manufacturer's information, it does not contain toxic plasticizers like Bisphenol A (BPA) or phthalates. Rubber, mixed with garlic powder and (unspecified) amino acids, is used in combination with 15 % salt and a shrimp oil coating. The cost was in the medium price range with 7 \in for 10 pieces.

2.2. Test organisms

Hyalella azteca cultures were obtained from an online aquarist supplier (Interaquaristik.de Shop, Germany). *Hyalella* is a species complex, but the exact clade is not known for this study. The cultures were maintained according to Novak and Taylor [27] in SAM-S5 reconstituted water in 5 L beakers equipped each with a 10×10 cm gauze square. The culture was constantly aerated and maintained at 22 °C and a 16:8 h light:dark regime. The culture medium was exchanged weekly and animals were fed with crustacean food flakes (Crusta Menu, Tetra GmbH, Germany) *ad libitum*.

Relative sensitivity to a reference toxicant was determined using static 96h water-only exposure to $CuSO_4$ [27]. The LC_{50} was determined as 81.5 µg/L, which is in line with previously reported values ranging between 65 and 120 µg/L [28,29].

2.3. Toxicity tests

The ecotoxicological test setup was conducted following the standard procedure described in Novak and Taylor [27]. *H. azteca* individuals with an age range of 3–4 days were extracted from the maintenance culture by sieving through a 500 µm sieve. After sieving, the animals were collected in a Petri dish and individually transferred to the test vessels using a glass pipette. Tests were conducted under static conditions with continuous aeration. Test vessels of 400 mL beakers were filled with 275 mL of SAM-S5 medium and equipped with a 3 cm² gauze square if not indicated different in the specific descriptions below. For each experimental setup, 5 replicates per treatment and control were used, each containing 10 test organisms.

2.3.1. Toxicity tests with exposure to whole fish lures in water only and in water-sediment systems

2.3.1.1. Toxicity in water only systems. To test the toxicity of the fishing lures, *Hyalella azteca* were exposed to the lures for 96 h. One item per type of plastic lure was used per test vessel. Physico-chemical parameters (O₂ saturation and pH) were measured on day 0 and on day 4 using a multimeter Multi 3630 IDS (WTW GmbH, Germany). Mortality was recorded daily. The animals were fed with 0.9 mg of Tetra Crusta Menu (Tetra GmbH, Germany) suspended in deionized water on day 0 and on day 2 of the experiment. Test vessels were

sealed using Parafilm with airholes to reduce water evaporation and cross-contamination.

2.3.1.2. Toxicity in water-sediment systems. To simulate environmentally realistic conditions, the presence of sediment on the ecotoxicological effect was tested, since in most cases, a snag would occur close to the sediment, or a lost lure would sink to the bottom. To mimic natural conditions under which a lost lure would sink onto or into soft sediments, sediment-based toxicity was determined by placing one lure item into the sediment of each test vessel, so that it was completely covered. Standard sediment was used according to OECD Guidelines [30] with slight modifications. We used 2 % Kaolin clay and 5 % dried peat, which is slightly less than described in the original guideline. 100 mL of wet sediment were used per test vessel. For the 96 h exposure of the test organisms, a contraption was used according to Pyle et al. [31]. The experimental setup was slightly modified by using a 400 mL beaker and a glass tube with a 7 cm diameter gauze (500 μm) attached at the bottom. Since the gauze used was over 250 μm wide, an interaction with the sediment was not inhibited. Mortality was recorded daily.

2.3.2. Water-based toxicity tests on diluted leachates

To assess potential ecotoxicological effects of the three test lures, they were leached in medium (simulating the use of a lure in a freshwater system or that a lure would be lost when fishing, e.g. after a snag) and the leachate was then used in different dilutions for ecotoxicological testing with the amphipod *H. azteca*. Water-based toxicity tests were conducted on the leachates of the soft plastic lures up to 14 days. One item per type of plastic lure was incubated in 275 mL test SAM5S medium for 96 h without any animals and then removed. Since the lure types differed in weight and size, this resulted in slightly different initial mass/volume ratios. Accordingly, the initial 100 % test concentrations were 7.1 g/L for type Z, 21.58 g/L for type N and 14.53 g/L for type F. After the 96h incubation period the test medium was diluted by factor 2 until 1.5 % of the initial concentration. We did not attempt to adjust the mass/volume ratios by cutting the plastic items to avoid potential additional leaching by the destruction of the items. Toxicity tests were conducted using a dilution range 100 %, 50 %, 25 %, 12.5 %, 6.25 %, 3.125 % and 1.5 % for the type F lures. Preliminary tests showed a higher toxicity of type F than the other two lures, which is why type F had a wider test concentration range compared to Type Z and N lures where only 100 %, 50 %, 25 %, 12.5 % were tested. Mortality was recorded up to a maximum test duration of 14 d. The animals in the controls were fed twice a week. Depending on the mortality rate and feeding rate, the animals in the other treatments were fed *ad libitum*.

2.4. Chemical analysis

2.4.1. Material analysis

Qualitative material analyses of the soft plastic lure types were commissioned to a specialized commercial laboratory (Quality Analysis GmbH, Germany). According to the laboratory information, a combination of Fourier-Transform-Infrared (FTIR) spectroscopy with thermogravimetric analysis (TGA) and coupled gas chromatography and mass spectroscopy (GC/MS) was used to determine chemical identity of the materials of the respective lures. For identification of single elements in the plastic material, scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (SEM-EDX) was used. The analyses followed standardized and inhouse procedures according to the laboratories' information. Additionally, for the leaching experiment, a 50 mL water sample was collected after the 96h incubation period for each of the 3 fishing lure types. GC-MS was used to analyze water samples after the 96h incubation period with the fishing lure types.

2.4.2. Leachate analysis

Leachate samples were stored at 4 °C until further processing. Samples were filtered through 0.45 μ m polyethersulfon (PES) filters. Major anion (F⁻, Cl⁻, NO₂⁻, Br⁻, NO₃⁻, PO₄²⁻) concentrations [mM] were measured using ion chromatograph ICS-1100 (ThermoFischer Scientific, Dreieich, Germany) based on USEPA standard procedure (Method 300.1) [32] equipped with a AG-23 as guard column and AS-23 separation column, using a carbonate eluent (consisting of 1.8 mM disodium carbonate and 1.7 mM sodium hydrogen carbonate). Major cation (Li⁺, Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺) concentrations [mM] were in parallel analyzed using an ion chromatograph ICS-1100 (ThermoFischer Scientific, Germany) equipped with a CG-16 as guard column and CS-16 separation column and 30 mM methane sulfonic acid as eluent. Samples were analyzed in triplicates (n = 3).

2.5. Statistical analysis

To determine effects of soft plastic lure and leachate exposures, we calculated survival estimates using the Kaplan-Meier procedure. A Log rank test was used to test for differences between the individual treatments. Additionally, to estimate LC_{50} values, non-linear modelling was used to fit a logistic regression function to the response data:

$$x = \frac{a}{1 + e^{-k(x-x_c)}}$$

In which a = amplitude, k = coefficient and x = centre.

Goodness of fit for model selection was determined by using the adjusted R_2 values *a priori*. All analyses were conducted using OriginPro 2023b (OriginLab Corporation, Northampton, USA). Significance was accepted at p < 0.05.

3. Results

3.1. Chemical composition of the fishing lures

The chemical composition of all the tested soft plastic lures was identical in terms of the basis polymer PVC. In each type, PVC was enriched with one or two phthalate plasticizers and their respective degradation products benzene, toluene and 1-chloro-undecane were found. The lure types varied in their composition of inorganic fillers, stabilizers and flame-retardants as well as the sub-stances used for the "glitter". Substances identified by FTIR and TGA coupled GC/MS are summarized in Table 1.

In the type F, two plasticizers were detected and identified as diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP). The sample additionally contained a mixture of other fillers such as a phosphorylated flame-retardant and a Barium-Zinc-stabilizer according to SEM-EDX results. The pigments in this soft lure are mainly based on melamine resin. For lure type N, the material was identified as PVC containing the plasticizer di-(2-ethylhexyl) terephthalate (DEHTP). Against the manufacturer's information, the sample was not free of plasticizers. No additional inorganic fillers were detected in contrast to the other lure types. The pigments in type N were mainly based on a type of polyester. In the brand type Z, the plasticizer diisononylphthalate (DINP) was detected, along with its degradation products. As inorganic filler of the polymer, sodium sulfate salt was the main compound. The manufacturer's statement that the product is bisphenol A free was confirmed. On the surface of the item, a NaCl coating was detected by SEM-EDX. The pigments in the soft lure were mainly based on melamine resin. Analysis of the water samples after the 96 h leaching period did not detect any organic compounds.

3.2. Ion concentrations in the leachates

The ionic composition of the water samples after the 96h leaching are listed in Table 2.

The ion concentrations and conductivity in the leachates did not increase significantly for type N and type F, so there was no substantial leaching of salts. Ion concentrations in the medium after 96 h of leaching of the fish lure type Z, showed an increased concentration of Na⁺, Cl⁻ and SO₄²⁻, which indicates leaching of NaCl and Na₂SO₄. Using the increased ion concentrations, one can deduce that, in 96 h, approximately 33 mg of Na₂SO₄ and 13 mg of NaCl leaches from the type Z lure, which is approximately 2 % of its overall weight. The leaching of NaCl and Na₂SO₄ in the lures consequently resulted in an increased conductivity in the type Z leachate compared to the aqueous medium up to 633 μ S/cm. The measurement of these ions in the water-phase therefore confirmed the REM-EDX measurements in the plastic item and the leaching of these salts into the water phase.

3.3. Toxicity of fish lures in water and in water-sediment systems

All three tested fish lures were toxic to *H. azteca*. The 96 h acute toxicity of the different lure types was generally higher in wateronly exposures compared to sediment exposures, suggesting that toxic compounds partially interact with the sediment (Fig. 2). The cumulative survival in the controls ranged above 90 % at 96 h and was therefore within the validity range [27]. Highest mortality was observed for the lure type F with a cumulative survival of 0 % for both, water-only and sediment exposure after 96 h. In addition, in water-only exposures all animals died within 24 h, and the sediment exposure had only 44 % survival at 72 h. Pairwise comparison of the effects of type F exposure showed significant (Log-rank test) differences from the control in water (χ^2 (1,80) = 74.87, p < 0.001) and in sediment (χ^2 (1,80) = 68.64, p < 0.001). The second highest effect on survival was observed for the lure type Z with a

Table 1

List of organic substances identified in the fishing lure types. Information on the parameters Log K_{ow} and water solubility of the substances are based on PubChem Database entries (https://pubchem.ncbi.nlm.nih.gov, last accessed March 15, 2024).

Lure type	Compound	CAS – Nr.	Description	Log K _{ow}	Solubility [mg/L]	
Type F	Benzene	71-43-2	Degradation product PVC	2.13	1790	
	2-Ethylhexyl alcohol	104-76-7 Degradation product Plasticizer		2.73	880 (25 °C)	
	Phthalic anhydride	85-44-9	Degradation product Plasticizer	1.60	6000 (25 °C)	
	1-Chloro-undecane	2473-03-2	Degradation product PVC	4.76		
	Dibutyl phthalate (DBP)	84-74-2	Plasticizer	4.50	Insoluble in water ^a	
	Toluene	108-88-3	Degradation product PVC	2.73	526 (25 °C)	
	Diethylhexyl phthalate (DEHP)	117-81-7	Plasticizer	7.60	0.27 (25 °C)	
Type N	Benzene	71-43-2	Degradation product PVC	2.13	1790	
	2-Ethylhexyl alcohol	104-76-7	Degradation product Plasticizer	2.73	880 (25 °C)	
	1-Chloro-undecane	2473-03-2	Degradation product PVC	4.76	а	
	Toluene	108-88-3	Degradation product PVC	2.73	526 (25 °C)	
	Di-(2-ethylhexyl) terephthalate (DEHTP)	6422-86-2	Plasticizer	8.39	4.0 (20 °C)	
Type Z	Benzene	71-43-2	Degradation product PVC	2.13	1790 ^a	
	1-Chloro-undecane	2473-03-2	Degradation product PVC	4.76	а	
	Diethylhexyl adipate	103-23-1	Degradation product Plasticizer	6.80	0.78 (22 °C)	
	Toluene	108-88-3	Degradation product PVC	2.73	526 (25 °C)	
	Phthalic anhydride	85-44-9	Degradation product Plasticizer	1.60	6000 (25 °C)	
	Diisononylphthalate (DINP)	20548-62-3	Plasticizer	9.37	0.2a	

^a Note: No further information available.

Table 2

Ionic concentrations (mM), mean electrical conductivity (EC: μ S/cm at 25 °C) and mean pH value of the tests medium (SAM) without and after 96 h incubation of the fishing lures. Standard deviation is given in italics. bdl = below detection limit. Samples were measured in triplicates (n = 3).

	Li^+	Na ⁺	NH_4^+	\mathbf{K}^+	${\rm Mg}^{2+}$	Ca ²⁺	Cl^{-}	NO_2^-	Br^-	NO_3^-	PO ₄ ³⁻	SO_4^{2-}	\mathbf{F}^{-}	EC	pН
SAM	bdl	0.89 <i>0.01</i>	bdl	0.04 0.01	0.22 0.00	0.95 <i>0.03</i>	2.13 <i>0.03</i>	bdl	0.02 0.01	bdl	bdl	0.24 0.00	bdl	394 <i>0.47</i>	7.98 0.00
Type F	bdl	0.89 <i>0.01</i>	bdl	0.03 <i>0.00</i>	0.22 0.01	1.01 <i>0.01</i>	2.15 <i>0.02</i>	bdl	0.02 <i>0.00</i>	bdl	bdl	0.27 0.03	bdl	395 <i>0.47</i>	7.73 0.02
Type N	bdl	0.89 <i>0.01</i>	bdl	0.04 <i>0.01</i>	0.22 0.00	0.98 0.04	2.10 <i>0.01</i>	bdl	0.01 <i>0.00</i>	bdl	bdl	0.24 <i>0.00</i>	bdl	400 <i>0.94</i>	7.74 0.03
Type Z	bdl	3.31 <i>0.11</i>	bdl	0.04 <i>0.00</i>	0.22 0.00	1.00 <i>0.02</i>	2.92 <i>0.07</i>	bdl	0.03 <i>0.01</i>	0.01 <i>0.01</i>	bdl	1.11 0.07	bdl	633 <i>24.52</i>	7.20 <i>0.03</i>

cumulative survival of 8 % in water-only exposure with a significant difference from the control (χ^2 (1,80) = 53.75, p < 0.001), followed by the lure type N with 16 % survival after 96 h (χ^2 (1,80) = 45.72, p < 0.001). In the treatments where the lures were buried in sediment, type Z and type N showed similar effects, with 57 % and 55 % cumulative survival, respectively. Both treatments were significantly different from the control (Type Z: χ^2 (1,80) = 8.56, p < 0.01; Type N: χ^2 (1,80) = 9.52, p < 0.01).

Exposure to type N lures in the sediment resulted in a significantly higher survival over time compared to the water-only tests (p < 0.001). At 96 h, there was also a significant higher survival between sediment exposure of type Z lures, and for type F sediment exposure at the 24 h time point compared to the water-only tests (both p < 0.001). The type Z lure sediment exposure also resulted in significantly higher survival rates at the 48 h and 72 h time point (p < 0.001). Survival curves are shown in Fig. 2.

3.4. Toxicity of the lures' leachates

In general, the exposure of *H. azteca* to leachates of the three lure types tested caused mortality in the test organisms. For type F, the most severe effects were observed, while type Z and type N caused a significantly lower effect on mortality in *H. azteca*.

There was no significant difference at the highest exposure scenario in terms of mortality between the exposures with one lure item present in the test medium compared to the exposure to the leachate water without the lure. Maximum mass concentrations were 7.1 g/L for type Z, 21.58 g/L for type N and 14.53 g/L for type F in case of one lure per test vessel. Subsequent dilution of the media containing the leachate showed a dilution dependent concentration-effect relationship (Fig. 3). Overall, the mortality increased in every treatment for every concentration over time. The treatment with the fishing lure type F always caused highest mortality rates in every dilution. Referring to the initial lure mass per volume during the pre-exposure leaching period, the following LC_{50} values were calculated: the 24 h LC₅₀ (95 % lower confidence limit LCL, 95 % upper confidence limit UCL) for type F was determined as 0.41 g/L (LCL: 0.38, UCL: 0.45), which corresponds to 2.6 % dilution of the leachate. The mortality for type N and type Z did not exceed 50 % at this time point. In addition, every dilution with the fishing lures type F resulted in 100 % mortality after 96 h. Second highest response was seen for type Z with a 96 h LC₅₀ of 6.22 g/L (LCL: 5.11, UCL: 7.09), which corresponds to a 42.6 % dilution of the leachate, followed by type N with 7.3 g/L (LCL: 6.31, UCL: 8.84), which corresponds to 40.9 % dilution. In case of the type F lure, the experiment could not be extended to 14 d since the tested dilutions already resulted in 100 % mortality. The 14d LC₅₀ values for type Z and type N were 1.56 g/L (LCL: 1.36, UCL: 1.81) and 1.30 g/L (LCL: 0.89, UCL: 1.71), which corresponds to 10.8 % and 6.1 % dilution of the leachate, respectively. Pairwise comparisons showed highly significant differences to the control for all tested dilutions at the level p < 0.001 with the exception of the 6.25 % dilution of type Z (χ^2 (1,3) = 6.37, p < 0.05). Survival in the control group was 93.4 % after the 14 d experimental period.

4. Discussion

In this study, we demonstrate that commonly used plastic soft lures used in recreational angling contain hazardous materials that result in harmful acute effects in a benthic invertebrate under controlled laboratory conditions. The toxicity of the tested materials was significantly higher in the water-only than in the sediment exposure tests, indicating that interactions with sediment particles (covered by sediment versus open in the water column) play a major role for their harmfulness. Over the 14 day exposure period, leachates of the three lure types tested caused concentration- and time-dependent mortality in Hyalella azteca. Products labelled as "environmentally friendly" were less toxic, but all types tested contained a suite of potentially harmful substances that can partition into the water phase over time. Toxicity was most pronounced in the cheapest product with the highest chemical complexity of the matrix within the tested lure types. This was also the product where least transparency about the used materials was available. The main component of each of the items was PVC. This polymer is enriched with a range of additives to meet the desired product properties as a fishing lure. We found different types of plasticizers, inorganic fillers and melamine or polyethylene pigments, and, in one brand even a flame retardant. Depending on the brand, coatings with salt and flavors were also used. In general, the toxicity was linked to the complexity of the matrix and the respective additives. The highest toxicity was found in the cheapest product type F, which also contained the plasticizers DBP and DEHP, both being regulated under EU legislation as substances of very high concern due to their toxic effects on reproduction and endocrine disrupting properties [33]. In addition, this product contained a phosphorylated flame retardant and a metal based inorganic filler, which are known to be toxic to aquatic organisms [34-37]. In each of the products advertised as more environmentally friendly, only one plasticizer was found and no flame retardant, but ecotoxicological effects on our



Fig. 2. Kaplan Meier survival estimates for the different fish lures in water and sediment within 96 h exposure period. Red lines indicate 95 % confidence limits. Log-rank test results in each panel indicate differences to the respective control group.



Conc. leachate [g/L]

Fig. 3. Concentration-response models of mortality in *H. azteca* at three time points after exposure to dilutions of aqueous media after a 96h incubation period with the three tested fishing lures. Reduced Chi-Square (χ^2) and corrected R² are indicated. Dotted red lines indicate upper and lower 95 % confidence intervals.

test organisms were still evident. The variety of additives added to the PVC was lower in those that claim to be more environmentally friendly, which most likely leads to a lower environmental impact in the long term. It is likely that differences in the chemical composition of the individual lure types used here are the reason for different magnitudes of our observed effects, whereas interactions of the additives may have resulted in increased toxicity. However, given the extremely high diversity of soft lure products on the market and the numerous substances they are made of, this can potentially not be limited to the substances detected in this study. If these items are lost in aquatic habitats, a complex mixture of chemicals is released over the time-course of their degradation, which can affect benthic organisms.

4.1. Environmental relevance and risk assessment

Currently, no sound risk assessment of soft plastic fishing lures in freshwater environments can be performed, since environmental concentrations and emission rates are largely unknown. More research is therefore needed for a comprehensive environmental risk assessment of this highly diverse group of plastics in the environment. However, as exemplarily shown herein, we can demonstrate negative effects on survival of our test organism indicating potential environmental issues in freshwaters heavily used for recreational angling. This is particularly evident since Raison et al. [10] reported a constant rate of deposition of approximately 12,000 soft plastic fishing lures throughout the year in Charleston Lake, USA. As estimated by Unsbo et al. [12], the emission of fishing lures can reach up to several hundreds of tons in Scandinavia. Furthermore, Pander et al. [11] reported more than 13 kg of hard and soft plastic items in Lake Eixendorf, Germany, which is one of the few reports on environmental concentrations. This amount of plastic lures found in the shoreline of the reservoir would represent a mass concentration of about 0.1 mg/L considering the whole water volume. However, these items are not homogenously distributed in the environment, but rather accumulated in distinct areas with higher fishing activity, accessibility and increased risk for snags. Furthermore, due to ongoing fishing activities, a cumulative input of lure items over time can continuously increase the environmental concentration. Initially after being lost or discarded, the lures presumably remain in the water phase and can be buried in soft sediments over time. The release of leachates can therefore potentially lead to spatially higher

concentrations. The toxic responses could be clearly related to leachates, since there were no significant differences in mortality comparing the whole fishing lure present in the test medium with just the water phase after the 96 h incubation period.

4.2. Components driving toxic responses

Soft plastic fishing lures containing a variety of different substances are a good example for the complexity that comes along with assessing effects of plastic in the environment [19,38]. Qualitative analytical on chemical composition data did not explain the overall effects, which demands to promote further research into the synergies of chemical effects of additives leaching from plastic matrices. As shown previously, additives derived from PVC plastics can induce acute toxic responses, cytotoxicity, oxidative stress as well as endocrine disruption [4,39–41]. For some PVC products, up to 1213 chemical features were found by non-target analysis, indicating that there is a higher amount of potentially harmful chemicals contained than previously assumed [41]. A recent study by Lewin et al. [4] found up to 45 additive substances in the leachates of 16 different types of soft plastic fishing lures that can be classified as persistent, mobile and toxic leachates.

Common features of the plastic soft lures tested here were the PVC degradation products benzene and toluene. Benzene is a highly toxic substance, and its presence in aquatic environments can have detrimental effects on aquatic organisms. The toxicity of benzene to aquatic life is well-documented, and regulatory bodies set standards to limit its concentration in water to protect ecosystems (https://echa.europa.eu/de/registration-dossier/-/registered-dossier/16102/6/2/10). It is acutely toxic to a variety of aquatic organisms, including *Hyalella azteca* in the mg/L range [42]. Toluene is however considered to have low acute toxicity to aquatic organisms such as fish and invertebrates. Differences in the toxicity effects that we observed for the leachates from the three lure types can be related to additional compounds that were identified. For instance, lure type N, which had the least toxic leachate, was not enriched with additional salts or inorganic fillers. In contrast, type Z leachate was characterized by high concentrations of NaCl and Na₂SO₄, which might have contributed to an elevated mortality over time in our test organisms. However, the salt concentration alone cannot be seen as main cause for mortality, since effect thresholds for aquatic organisms generally range in the lower g/L range [43] that were by far not reached in this study. There was a substantial higher variety of additive compounds detected in type F that could contribute to the overall strong effects. A putative phosphorus-containing flame retardant (PFR) was found only in the type F lure, a substance class that is known to be toxic to aquatic organisms depending on their chemical structure and properties [34]. However, the analytical procedures available in this study could not identify the PFR leachate in the water or sediment phase.

In case of our exposure scenario, we observed less pronounced signs of toxicity in the presence of sediment, which can be explained by a slower release of the leachates into the water phase when the lure items are buried in sediment and a potential partitioning to the sediment phase by more hydrophobic additives [44]. Besides the flame retardant, a barium-zink stabilizer was found in the type F lure, which is used as a heat stabilizer in the processing of PVC. Toxic barium levels for *H. azteca* have not been widely reported, although one study showed that the acute toxicity of barium was at least 8 times lower than zinc [45], which agrees with Zhang et al. [36], who found that the toxicity of barium for some aquatic organisms was at least 10 times lower than zinc. An EC_{50} for Zn for *H. azteca* was reported as 99 µg Zn/L [37], but could not be determined in our study.

In addition, the several types of plasticizers found in the soft lures used here cannot be seen as the cause of acute toxicity as there was no analytical confirmation of the phthalates in our test media after the 96 h incubation period. Since the partitioning kinetics of phthalates from the lure items into the water phase are controlled by their respective water solubility and ambient temperature [46], migration rates are rather slow and given the relatively low toxicity in is unlikely that effect concentrations are reached within the 96 h leaching period [47]. However, endocrine-disruptive effects such as estrogenicity caused by phthalate plasticizers in the leachate of a plastic fishing lure soft were detected using *in vitro* bioassays [4].

4.3. Environmental fate and long-term implications

In addition to the short-term ecotoxicological effects characterized in this study, potential long-term effects need to be further evaluated. First, we can assume a certain pseudo-persistence as "fresh" fishing lures are continuously emitted by ongoing losses. However, this strongly depends on fishing activities and lure types used in the respective environment. We can therefore assume that areas with highly frequented fishing spots and a greater snag risk are more susceptible to long-term enrichment of hydrophobic chemicals in sediment and biota. Sediment can work as a primary sink for toxicants and therefore the toxicity is reduced and dependent on the composition and the amount of the sediment [48]. The release of additives from the plastic lure items is of environmental concern, as degradation is very slow in the environment and lures are consequently often found as a whole [10]. Second, depending on the temperature and turbidity of the water, the plasticizers can leach up from several months to years [47,49]. The leachate rate thereby showed to be constant, indicating PVC additives as a long-term contaminant. The long-term effects of a continuous leaching of PVC additives into the surrounding media needs to be further considered under different environmental scenarios. A third aspect is the plastic weathering that makes lost fishing lures a long term source for micro- and nanoplastics. Thereby, the particles can cause lethal and mostly sublethal effects along the food chain [50,51].

5. Conclusion

Recreational fishing lures are a currently hardly considered aspect in terms of environmental pollution, despite their increased use and presence, especially in areas with high fishing pressure. This study is not meant to mimic environmental scenarios in their whole variety, but demonstrates that leachate from lures containing harmful chemicals are highly toxic under standardized laboratory conditions. We demonstrated that leachates from cheaper mass products of commonly used soft plastic lures are more acutely toxic to *Hyalella azteca* than those from higher-quality products marketed as "environmentally friendly". The cheaper lure also contained a greater number of organic chemicals than the more expensive lures, but assigning cause to the observed effects was beyond the scope of this study. This is of particular importance given the high environmental awareness of most recreational anglers, who have a choice among different brands and products. They thus need to know about hazardous substances in lures and the resulting pollution from different types of lure products that are available on the market. The identified differences between the lure types also demands better information from the manufacturers on the compounds used in these items, and a more strict regulation on their use in products that may end up in the environment. Ideally, the chemical composition should be lawfully mentioned during sale, similar to food product labels. A comprehensive assessment of further long-term effects needs to be considered. Besides the toxicity of individual components and the long-term effect of soft plastic lures, we need to get more insight into interaction effects of the various chemical components of fishing gear with the aquatic environment as well as the bioaccumulation of these substances in aquatic organisms.

CRediT authorship contribution statement

Beggel S: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Kalis EJJ:** Writing – review & editing, Verification. **Gilb KM:** Investigation, Writing – review & editing. **Pander J:** Conceptualization, Writing – review & editing. **Geist J:** Conceptualization, Supervision, Resources, Writing – review & editing.

Data availability statement

No research-related data are stored in publicly available repositories, and the data are available from the corresponding author on request.

Ethics declaration

Review and/or approval by an ethics committee was not needed for this study because this work involved invertebrates for which no animal ethics approval is required according to the European Communities Council Directive 2010/63/EU (article 1, paragraph 3).

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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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