Microplastics and Nanoplastics

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Exposure to microplastic fibers does not change fish early life stage development of three-spined sticklebacks (*Gasterosteus aculeatus*)

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Abstract

Microplastic fibers are frequent contaminants of aquatic ecosystems. Early life stages of aquatic organisms are predicted to be especially vulnerable to microplastic pollution. We hypothesized that microplastic fibers in the water column might interfere with fertilization and embryonic development of fish. We tested this with an in vitro fertilization system with three-spined sticklebacks. Six egg clutches were divided and one half was fertilized and bread out in water with polyester fibers (PET fibers; mean diameter 9.7 ± 2.3 μm; mean length 245.6 ± 163.1 μm) at a concentration of 1×10^4 fibers/L while the other half served as control without fibers.
Observation with a dissection microscope revealed that some polyester fibers stuck to the outside of the eggs in the fiber treatments. Yet, overall 67.4 ± 12.9% eggs were fertilized from which 97.2 ± 4.2% larvae hatched without any significant difference between treatments. Mortality and abnormal development of larvae was low and was not changed by microplastic fibers, as was the heart rate of developing embryos five days post fertilization.
The present study illustrates that polyester fibers, even at concentrations three to four orders above levels reported from the environment, do not impair fertilization success, embryonic and early larval development of sticklebacks. Accordingly, concentrations of microplastic fibers currently observed in aquatic habitats do not appear to be harmful to early live stages of fish.

Highlights

First use of fish egg in vitro fertilization assay for microplastic fiber exposure
Fertilization and hatching success of fish was not altered by microplastic fibers
Fish early life stage development was unaffected by microplastic fiber presence

Keywords: Microplastic exposure, In vitro fertilization, Fish eggs, Early life stages, Polyester fiber, Embryonal development

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Introduction

Recent monitoring studies outline that microplastic fibers are the most prevalent type of microplastic debris in many aquatic habitats [1–4]. Accordingly, microplastic fibers often are the dominant microplastic shape that fish encounter [5–7]. Nevertheless, most effect studies of microplastics on fish were conducted with microplastic spheres and fragments, not with fibers [8]. Furthermore, the majority of exposure studies focused on adult life stages [8] although early life stages of aquatic organisms are generally more vulnerable to toxicants [9, 10]. With the present study, we wanted to test if the presence of microplastic fibers in the water column influences fertilization success and early development of three-spined sticklebacks. We suspected that the potential attachment of microplastic fibers to early life stages of fish affect their development.

Changes in embryonic development such as decreased hatching rates and delayed hatching time [11], and changes in blood circulation were reported for fish embryos exposed to polystyrene (PS) spheres and fragments [11, 12]. For example, in zebrafish (*Danio rerio*) exposed to microplastic fragments via the water column, accelerated blood flow velocities and heart rates were explained by hypoxic conditions in the eggs [12]. The microplastic fragments were not internalized but accumulated on the surface of the chorion. Thereby, externally adhered microplastic fragments covered the chorion pores and might have reduced oxygen availability for the embryos. The hypoxic microenvironment likely induced and established the observed alterations in the circulatory system [12].

Similarly, accelerated blood flow velocities and heart rates, and slightly inhibited hatching rates, were observed in a first study conducted with microplastic fibers (polyethylene terephthalate (PET), 3–5 mm) and zebrafish embryos [13]. For the present study, we chose smaller microplastic fibers (< 0.3 mm) similar to the fiber size class produced during household washing [14, 15], which enters the environment as laundry effluents [16]. Microplastic fibers < 300 μm slip through neuston nets commonly used for sampling fibers in environmental surveillance [17]. Smaller fibers are thus often neglected in monitoring studies [18] and little is known about their potential environmental impact. We used a concentration of 1×10^4 fibers/L, which is in the range of previous exposure studies conducted with adult life stages and microplastic fibers in the water column [19–22]. However, the concentration chosen for the present study is still higher than the concentrations reported from nature that are in the range of 1–10 fibers/L [17, 23, 24]. Yet, microplastic fiber concentrations used for exposure studies must be a compromise between environmental observations and concentrations that can be maintained as a reproducible and homogenous dispersion of fibers in the water column under laboratory conditions [20]. Furthermore, concentrations of microplastic fibers above currently reported levels can occur in local fiber contamination events, which might become more frequent in the future with rising plastic pollution [25].

In the present study, we used low concentrations of surfactant to facilitate the challenging issue to keep fibers dispersed in the water column, as described previously [26–28]. Furthermore, we used a setup with square-shaped glass bowls for breeding and constant agitation to promote irregular movement of the water column and thus fiber distribution.

We collected egg clutches from mature three-spined stickleback (*Gasterosteus aculeatus*) females and divided them in halves. One half was exposed to pristine polyester fibers (polyester = fibrous form of PET) from fertilization onwards, while the other half served as control. Biological endpoints were fertilization rates, heart rates of embryos, and hatching success. Furthermore, we investigated abnormal development rates and alterations in morphological features of hatched larvae. We hypothesized that microplastic fibers in the water might block the micropyle and thereby prevent fertilization. In addition, we hypothesized that microplastic fibers (< 0.3 mm), smaller than those tested previously [13], can also adhere to the chorion and possibly impair oxygen exchange, which might delay or disturb fish embryo development and lead to changes in heart rates.

Methods

Experimental design

Effects of microplastic fibers in the water column on early development of sticklebacks were tested with eggs from six breeding pairs of sticklebacks. In brief, each egg clutch ($N = 6$) obtained from mature females was divided in two halves before in vitro fertilization with sperm from one male. Half of the egg clutch (85–217 eggs each, Table S1) was fertilized and bread out in water containing polyester fibers (1×10^4 fibers/L; 200 mL total volume) and surfactant (Tween-80, final concentration 3.8×10^{-6}% (v/v)), while the other half served as control in water with surfactant only. Each egg clutch was subjected to complete water exchange every 48 h, whereby fiber treatments received water with the desired fiber concentration. Exposure lasted until three days post hatching (total experimental time of 12 days), the period for which the current EU animal welfare legislation does not apply for stickleback larvae [29]. Fertilization rates, hatching rates, mortality, and frequencies of abnormal body shapes of larvae were recorded. The heart rates of ten embryos per egg clutch half were determined at day five post fertilization, and three days post hatching 15

larvae from each egg clutch half were imaged to monitor potential differences in morphological development.

Microplastic material and quality control

Microplastic fibers were prepared in clean-room facilities from commercial pink polyester knitting yarn (diameter 9.7 ± 2.3 μm (mean ± standard deviation, N = 206), Fig. S1) with autofluorescence (excitation 511–551 nm, emission 573–613 nm). The polyester yarn was washed with water and ethanol and cut manually with scissors into small pieces, as described in Rebelein & Focken [30]. Briefly, to exclude large and very small fibers, cut pieces were washed twice through a 300 μm metal sieve (Retsch, Germany) and collected on a 25 μm metal sieve (Retsch, Germany) with pre-filtered 96% ethanol. Microplastic fibers were dried, and 50 mg/L were suspended in ultrapure water for a stock suspension. The stock suspension contained 0.001% (v/v) Tween-80 surfactant solution (Merck, CAS-Nr. 9005-65-6) to facilitate even dispersal of microplastic fibers [28]. We determined fiber concentration of the polyester fiber stock suspension using a Nikon fluorescence microscope (ECLIPSE, Ts2R-FL, Japan; filter setting: excitation 511–551 nm, emission 573–613 nm) with the software NIS-Elements AR (Nikon, 5.02.00). The fiber suspension (25 μL) was pipetted onto microscope slides (N = 25), covered with a petri dish while the water evaporated, and directly thereafter autofluorescent polyester fibers were counted under the microscope on the slide. Fiber size distribution was characterized from images of fibers filtered onto 0.8 μm polycarbonate membrane filters. The average size of the polyester fibers in the stock suspension was 245.6 ± 163.1 μm (mean ± standard deviation, N = 1446, Fig. S1) in length.

For the exposure of the egg clutches, we prepared experimental treatment suspensions from the stock suspension (2.63×10^6 fibers/L) to contain 10,000 polyester fibers per liter in pre-filtered, temperature-adjusted tap water (equivalent to a mass concentration of 0.19 mg/L). For control treatments, the same volume (761 μL) of ultrapure water that contains 0.001% (v/v) Tween-80 surfactant only was diluted in pre-filtered, temperature-adjusted tap water.

To prevent contamination, plastic labware was avoided and glass and metal labware used whenever possible. Ethanol and tap water were pre-filtered through a Whatman (Typ 1) cellulose filter to remove potential microplastic fiber impurities. All equipment was thoroughly rinsed with filtered deionized water followed by a rinse with filtered 96% ethanol to exclude microplastic contamination. Every workspace was wiped with filtered 96% ethanol before work and utensils were kept covered until use.

Furthermore, blank glass fiber filters (GF/C, Whatman) were placed in the experimental area and exposed to the ambient air for 48 h and one week to check for airborne fiber contamination (Fig. S2). Exposure bowls were kept loosely covered to minimize airborne contamination throughout the experimental period (Fig. S3).

Fish collection and in vitro fertilization

Three-spined sticklebacks in breeding condition were caught at the Luneplate estuary (53°28′37.3″N 8°31′08.9″E), Bremerhaven. Fish were transported to the lab and breeding pairs were subjected to in vitro fertilization as described by Barber & Arnott [31]. Briefly, egg clutches of six females were stripped and each of the six egg clutches was split in halves into two glass petri dishes. We used sperm from one male to fertilize both halves of the split egg clutches from a female (six males in total). Therefore, a drop of sperm buffered in Hank's Balanced Salt Solution was pipetted to each petri dish next to the egg clutch. Treatment suspensions with microplastic fibers or with surfactant only (control) were added and petri dishes swirled for mixing eggs and sperm. The clutches were left for 30 min and thereafter washed with pre-filtered tap water and transferred to glass bowls containing 200 mL of the experimental treatment suspensions.

The square-shaped glass bowls (base area 10 × 10 cm) facilitated homogeneous dispersion of microplastic fibers in suspension when placed in an angle to the movement direction on a shaker (GFL 1083, Germany) with continuous horizontal agitation (Fig. S3). This setup created an irregular movement of the water column and kept fibers dispersed in the water column, while regular stirring or swiveling induced fiber aggregation (tested in previous method tests). Bowls were maintained at an ambient temperature of 16 °C and treatment suspensions (200 mL) were exchanged every 48 h. Eggshells and dead larvae were removed daily (twice daily during hatching) to ensure good water quality. Daily records were taken of dead respectively unfertilized eggs. The amount of fertilized eggs was determined five days after fertilization when eyes of the embryos were visible. From fertilized eggs, hatching rates were determined. Abnormal development of larvae such as spinal deformities, pericardial edema or yolk sac edema were documented for each treatment according to the description of Cong et al. [32].

Morphometric measurements and heart rate determination

Videos of embryos were taken at day five post fertilization using a stereomicroscope (Nikon SMZ745T, Japan) equipped with a BRESSER MikroCam (SP 5.0, Germany) and Bresser MikroCamLabII software

(v3.7.13814, 2019, Germany). The video material was used to count the heart rates (for 60 s) of ten embryos per half egg clutch.

Three days after hatching the survival rate of stickleback larvae was recorded and 15 randomly chosen larvae per half clutch were measured and photographed under the stereomicroscope. Pictures of the larvae were analyzed with ImageJ 1.52r [33]. Total body length, head length, eye diameter, and length of the swim bladder were analyzed as described by Le Bihanic [34] and Ireland [35].

Microfiber treatment concentration

To check the microplastic fiber concentration as supplemented to the treatment bowls, five additional suspensions were prepared from the microplastic fiber stock suspension in glass bottles. As for the exposure treatments, 761 µL of fiber stock suspension were added to 200 mL pre-filtered, temperature-adjusted tap water in each glass bottle. The bottles were inverted ten times to homogenize the fiber suspension directly before two 50 mL subsamples were taken from each bottle. Subsamples were filtered onto 0.8 µm polycarbonate membrane filters. Filters were imaged under the fluorescence microscope as described for the microplastic fiber stock suspension and fibers counted using the software ImageJ 1.52r [33]. Furthermore, the amount of fibers in suspension was investigated in supplementary glass bowls without egg clutches with 200 mL pre-filtered tap water and either control or fiber experimental treatment suspensions as specified above. The fiber concentration was determined directly after preparation, after 24 h on the shaker, and after 48 h on the shaker from three bowls per control and fiber treatment respectively (18 bowls in total). We filtered two subsamples (50 mL) per bowl on membrane filters and counted the fibers under the microscope as specified above.

Statistics

Statistical tests were performed with RStudio v1.1.463 [36]. Normality distribution and homogeneity of variances of the data were checked with Shapiro-Wilk's test and Levene's test, respectively. Developmental rates were normalized to the total amount of eggs or embryos hatched (Table S1). Potential differences between treatments were analyzed with a Wilcoxon signed-rank test. Biometric and heart rate data were analyzed with a two-way ANOVA (factors treatment and egg clutch) followed by a post-hoc Tukey test, when data were normally distributed. With non-normally distributed data, non-parametric Kruskal-Wallis tests were performed for factor treatment and factor egg clutch with a subsequent post-hoc Wilcoxon rank sum test. A $p < 0.05$ was considered statistically significant.

Results

Exposure with microplastic fibers

We exposed stickleback eggs and larvae to microplastic fibers at a nominal concentration of 1×10^4 fibers/L. Counting of microplastic fibers in additional prepared suspensions ($N = 5$, measured in duplicates) revealed concentrations of 9236 ± 552.7 (mean ± standard deviation) polyester fibers per liter. In the additional square-shaped glass bowls prepared with treatment suspensions but no fish eggs, we quantified 10,924 ± 1701.8 fibers (mean ± standard deviation, $N = 3$, measured in duplicates) directly after preparation and found no polyester fibers on the control filters. After 24 h, on average 36.4% of the polyester fibers were still dispersed in the water column (4297.4 ± 1376.2, mean ± standard deviation, $N = 3$, measured in duplicates), which was similar to fiber counts after 48 h (34.4%, 3761.4 ± 1321.5, mean ± standard deviation, N = 3, measured in duplicates). We detected only one PES fiber on one subsample filter from the controls at 48 h, which presumably resulted from handling during the filtering procedure. On the other control filters and additional blank filters exposed to the ambient air for one week, we detected only fibers that had a clearly distinguishable appearance in color, shape, or fluorescence intensity to the PES fibers used in the experiment (Fig. S2; maximum of four other fibers per filter compared to > 150 PES fibers on fiber treatment filters). Thus, the level of fiber contamination was low. As fibers tended to aggregate as soon as any irregular shapes, such as (broken) eggshells, dead larvae, or protein aggregates were present, such debris was removed daily and treatment suspensions were exchanged every other day. The treatment bowls were placed on a shaker and agitated throughout the experiment. Together these measures ensured a consistent exposure of egg clutches and larvae to floating microplastic fibers during the experiment. We did not observe microplastic fiber aggregates on the water surface or walls of the treatment bowls. The individual fibers were floating in the water column and we noticed a small proportion moving on the ground of the exposure bowls due to the irregular movement of the water column during agitation. Under the microscope, we observed some fibers that attached to the chorion of the eggs. Yet, fibers tended to attach rather on unfertilized or damaged eggs than fertilized healthy ones (Fig. S4).

Egg & Larval survival and development

Fertilization rates and hatching rates in fiber treatments were not significantly different from control treatments (Table S1, Fig. S5). The mean (± standard deviation) egg fertilization rate was 71.8 ± 11.4% for fiber treatments and 63.7 ± 12.3% for control treatments and the hatching rate was 96.5 ± 5.0% and 96.7 ± 6.8% for fiber and control

treatments, respectively. Mortality (range 0–5.6%) and abnormal development (range 0–2.4%) of embryos and larvae were generally low (Table S1, Fig. S5) except for one clutch that showed higher mortality (17.1 and 12.6%) and higher abnormal development rates (4.8 and 9.6%) in control and fiber treatment halves of the clutch, respectively. Overall, mortality and abnormal development did not significantly differ between treatments.

Morphological parameters

The morphological parameters measured (body length, head length, eye diameter, swim bladder length, and head-to-body length ratio), did not differ significantly between larvae exposed to polyester fibers and control animals (Table S2). Yet, morphological parameters (except head-to-body-ratio) differed between egg clutches ($p < 0.05$), which demonstrates a greater natural variability between egg clutches of different breeding pairs than between treatments with and without microplastic fibers. The length of the larvae ranged between 6.16 mm and 7.69 mm and the head length ranged between 1.23 mm and 1.81 mm (Table S2).

Heart rates

The heart rates at day five post fertilization did not differ significantly between fiber-exposed and control embryos (Fig. 1). However, the heart rates between egg clutches differed significantly ($p = 0.0117$). The mean heart rate per egg clutch ranged from 86.4 ± 4.2 to 96.1 ± 4.8 beats per minute.

Discussion

The present study addressed possible effects of microplastic fibers in the water column on fertilization of eggs and early development of embryos and larvae of three-spined sticklebacks. We assessed fertilization and hatching success, heart rates of embryos, and morphological features of three-day-old larvae during a laboratory exposure experiment with microplastic fibers. Exposure in square-shaped bowls with slight and irregular movement of the water column, together with frequent water exchanges and fiber replacement, was applied to facilitate that fibers were kept in suspension throughout the experiment. We did not observe significant effects of the microplastic fibers on the vitality parameters investigated here and natural variation between offspring of different adult breeding pairs was higher than treatment effects. This suggests that relatively small microplastic fibers, even at three to four orders higher concentrations than currently observed in the wild, are not harmful to fertilization success and early development of fish larvae.

Environmental relevance of the used microplastic fibers

We chose polyester fibers for the present study, since they are predominantly used in the global textile production in fabrics for apparel, garments, and other finished textiles [37]. Accordingly, polyester fibers are the most common fiber polymer polluting natural water systems [1, 38–40]. We used red-pink polyester fibers, which also showed strong autofluorescence with red filter settings (excitation 511–551 nm, emission 573–613 nm), since they are easy to distinguish in color and shape from other natural or worn fibers that might occur as

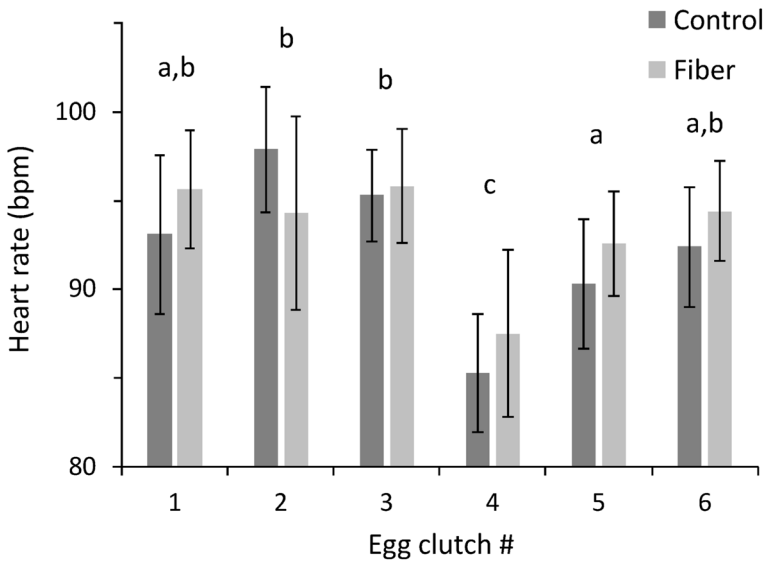

Fig. 1 Heart rates of stickleback embryos at day five post fertilization in beats per minute (bpm). Data show mean values (± standard deviation) of 10 embryos per half egg clutch. Different letters indicate statistical differences between egg clutches (ANOVA with post-hoc Tukey test ($p < 0.05$))

contaminants in the laboratory. For the present study, we filtered fibers through a < 300 μm sieve to resemble the fiber size class that is released during household washing, which can reach the environment as laundry effluent (93% of the released fibers were below 500 μm in length [14]).

The nominal concentration of 1×10^4 fibers/L, as used in the present study, is about three to four orders above values reported from the wild, which were collected with small mesh sizes (0.7 μm and 20 μm) [17, 23, 24]. Yet, higher concentrations might occur in local events of microplastic accumulation or contamination, and globally with expected increases of plastic pollution in the environment [41].

Furthermore, the European Marine Strategy Framework Directive (MSFD) [42] is aiming to reach the good environmental status (GES) in European seas. MSFD covers microplastic as environmental indicator for GES and demands that „The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned". To reach this goal the MSFD Commission Decision on Methodological Standards [43] demands the development of threshold values for possible adverse effects of microplastic on marine animals. To our knowledge, such threshold values do not exist for microplastic beads, fragments, and fibers in fish yet. Effect studies that cover concentration levels that might occur in nature in the future, like the present one, are crucial to develop such threshold values for microplastic fibers.

Experimental handling of microplastic fibers in the lab

A major concern for aquatic exposure studies with microplastics is to achieve a rather homogeneous distribution within the water column, which is often supported by the use of surfactant [44]. Previous exposure studies with microplastic fragments and spheres used higher surfactant concentrations than the present study and did not observe impacts on the development of zooplankton or fish and sea urchin embryos [26, 27, 45]. Continuous movement of the water in the experimental tanks can also promote homogeneous distribution of microplastics. This was previously achieved in exposure studies with adults and microplastic fibers by mixing the water in the experimental tanks by aeration, thus keeping the fibers in suspension [19, 21]. Yet, strong aeration, which also whirls around the egg clutches and yolk-sac larvae, is not ideal for sensitive embryonic and larval stages. In the present study, we therefore used a setup with square-shaped glass bowls, which were placed diagonal on a horizontal shaker. The slight but irregular movement of the water column kept fibers in motion while not disturbing the egg clutches and hatched larvae.

In laboratory exposure studies, microplastic fibers tend to aggregate, settle to the bottom and adhere to the exposure vessels, and very little fibers stay suspended in the water column at low concentrations [20]. These difficulties often lead to the use of high concentrations of microplastics in exposure studies. For example, a previous study with PET fibers, exposed zebrafish embryos to fibers 3–5 mm in length at a concentration of 20 mg/L [13]. For the present study, we chose shorter fibers (< 0.3 mm) and a much lower concentration of 0.19 mg/L. Methodological tests showed that after 48 h more than a third of the polyester fibers were still dispersed in the water column (equal to more than 600 fibers in the 200 mL exposure volume). The other fibers presumably attached as individual fibers to the bottom and walls of the exposure bowls, since no fiber aggregates were visible. We could not observe fiber aggregates in the exposure bowls when additional obstacles such as the egg clutches were present. Overall, a considerable amount of fibers stayed dispersed in the water column in the present study, even at 100 times lower concentration than used in previous exposure studies.

Effects of microplastic fibers on early life stages

In the present study, individual polyester fibers attached to the chorion of eggs in the fiber treatments. We did not observe internalization of microplastic fibers into eggs. Similar observations of an efficient barrier function of the chorion were made with fish embryos exposed to microplastic and nanoplastics fragments and spheres in the water column [12, 34, 46]. In the present study, we observed that more fibers got stuck to broken eggshells and debris than to intact eggs (Fig. S4). The question is if this observation means that the fibers have caused egg damage or if fibers are simply more adhesive to egg shells and eggs that were damaged for other reasons. Given the absence of difference between treatments with and without fibers, it is unlikely that the fibers had damaged the eggs. Thus, we propose that fibers predominantly stick to broken eggshells and irregular shaped material, and healthy egg clutches with smooth egg surfaces are less susceptible to fiber attachment.

Our results demonstrate that in vitro fertilization rates did not differ between control and fiber treatments. The data indicate that polyester fibers in the water column at the concentration used here do not hinder sperms to reach an egg and enter in through the micropyle. Similarly, in zebrafish in vivo fertilization rates did not change in the presence of small PS spheres (diameter of 1 μm) at concentrations of 1.82×10^7 spheres/L and higher [47]. The present study illustrates that also larger-sized microplastic fibers in the water column do not impair (in vitro) fertilization rates of eggs. Yet, with adult Japanese medaka (*O. latipes*) that were exposed to

polyester fibers in the water column in vivo, slightly increased fertilization rates were seen after two weeks of exposure [19]. Leaching additives that interfere with the endocrine system were suggested as explanation, but not further tested [19]. Thus, in nature chronic exposure of parental life stages with microplastic fibers and/or their additive leachates might affect fertilization rates. However, the present study suggests that the fertilization process itself is not altered by the presence of microplastic fibers in the water column.

Previous studies that used higher microplastic concentrations than the present study reported delayed hatching time, decreased hatching rates, and also altered heart rates of medaka and zebrafish embryos exposed to PS and PET microplastics [11, 13, 48]. This was presumably caused by hypoxic conditions in the eggs due to aggregation of microplastics on the egg surface that hindered the gas exchange [12, 13]. However, significant effects were detected only in treatment groups exposed to relatively high concentrations of 1×10^6 particles/L to 1×10^9 particles/L, which is at least five orders higher than currently observed in nature [17, 23, 24]. In general, toxicity of microplastics seems to increase with rising numbers of particles in the water [49]. Additionally, the present study used shorter microplastic fibers than a previous study [13], which might also have less impact on fish embryos in terms of surface area and adherence to the eggs, and consequential physiological implications to the embryo. Zhao et al. [50] recently demonstrated that intestinal toxicity was more severe when zebrafish were exposed to 200 μm long microplastic fibers than shorter fibers (50 μm) and suggested the aspect ratio of fibers to influence fiber toxicity. A limitation of our study in this respect is that we used only one type and size class of (pristine) fibers at only one concentration to investigate microplastic fiber toxicity on early life stages of fish. In nature, embryos encounter a mix of microplastic fiber polymers, sizes, with and without additive components. Microplastic fibers also interact with the environment and processes such as weathering and biofouling change their characteristics and thereby potentially their impact on organisms.

With the present study, we demonstrated that pristine polyester fibers are not toxic to early life stages of sticklebacks and do not inhibit their development, even at concentrations three to four orders higher than reported from nature. Furthermore, we observed that differences in heart rates of embryos and morphological features of larvae were higher between clutches from different breeding pairs than between half clutches if one half was exposed to microplastic fibers. Our results suggest that natural variability in early life stage development of sticklebacks is bigger than the effect of microplastic fibers in the water column.

Abbreviations

EU: European Union; GES: good environmental status; MSFD: Marine Strategy Framework Directive; PET: polyethylene terephthalate; PS: polystyrene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43591-021-00015-x.

Additional file 1 Table S1. Total egg number per half clutch, rates of fertilization and hatching success of egg clutches, and development and mortality of early life stages up to day three post hatching. Fertilization rate refers to fertilized eggs of the total egg number, hatching rate refers to hatched eggs of fertilized eggs, abnormal development refers to the number of abnormal developed embryos and larvae of all fertilized eggs that did survive, and mortality refers to the number of dead embryos and larvae of all fertilized eggs. **Table S2.** Morphometric parameters of stickleback larvae three days post hatching (mean ± standard deviation). Different letters indicate significant differences between egg clutches of the same breeding pair (Kruskal-Wallis test with post-hoc pairwise t-test, $p < 0.05$). **Fig. S1.** Polyester fiber length ($N = 1446$) (**A**) and width ($N = 206$) (**B**) distribution of manual cut pieces after sieving. **Fig. S2.** Polyester fibers with autofluorescence that we used in the study (left) are clearly distinguishable from fibers detected on the filters exposed to the ambient air in the experimental area for one week (right). Size bar marks 500 μm. **Fig. S3.** Experimental setup with square-shaped glass bowls placed in an angle towards the movement direction on the shaker to facilitate irregular movement of the water column (left). Bowls were kept loosely covered with lids, which were previously washed with filtered water and ethanol to prevent air-borne contamination during the experiment (right). **Fig. S4.** Fibers stuck to broken eggshells of sticklebacks (left) and occasionally to the chorion of embryos (right). Pictures were taken at day five post fertilization. Scale bar = 0.5 mm. **Fig. S5.** Fertilization (**A**) and hatching rate (**B**) of stickleback eggs, abnormal development (**C**) and mortality (**D**) of stickleback early life stages up to day three post hatching. Coordinates on the abscissa show the percentage values observed in the control half of the egg clutches and the coordinate on the ordinate gives the percentage observed in the respective fiber treatment half egg clutch.

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Authors' contributions

AR: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. UK: Writing – review & editing, Project administration, Funding acquisition. JPS: Conceptualization, Writing - review & editing, Supervision. The author(s) read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusion of this article is included within the article and its additional files.

Declarations

Competing interests

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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