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Microplastic and Organic Fibres in Feeding, Growth and Mortality of *Gammarus pulex*

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Abstract: Microplastic fibres (MPFs) are a major source of microplastic pollution, most are released during domestic washing of synthetic clothing. Organic microfibres (OMF) are also released into the environment by the same means, with cotton and wool being the most common in the UK. There is little empirical evidence to demonstrate that plastic fibres are more harmful than organic fibres if ingested by freshwater animals such as *Gammarus pulex*. Using our method of feeding *Gammarus* MPFs embedded in algal wafers, we compared the ingestion, feeding behaviour and growth of *Gammarus* exposed to 70 µm sheep wool, 20 µm cotton, 30 µm acrylic wool, and 50 µm or 100 µm human hair, and 30 µm cat hair at a concentration of 3% fibre by mass. *Gammarus* would not ingest wafers containing human hair, or sheep wool fibres. Given the choice between control wafers and those contaminated with MPF, cat hair or cotton, *Gammarus* spent less time feeding on MPF but there was no difference in the time spent feeding on OMFs compared to the control. Given a choice between contaminated wafers, *Gammarus* preferred the OMF to the MPF. There were no significant differences in growth or mortality among any of the treatments. These results conclude that MPFs are less likely to be ingested by *Gammarus* if alternative food is available and are not more harmful than OMFs.

Keywords: microplastic; fibres; animal hair; wool; cotton; *Gammarus pulex*; feeding; growth

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1. Introduction

Microplastic pollution is no longer an obscure concern of environmental scientists. The level of public awareness and concern has resulted in changes to individual behaviours as well as governments updating legislation [1–3]. Microplastics were first discussed in the marine environment, but there are now a substantial number of studies on the presence and impact of MP in freshwater (FW) environments [4–6].

Microplastics (MPs) are defined as plastic particles of under 5 mm in size. They are either manufactured as such (primary MPs) or are produced when plastic products break down into smaller fragments (secondary MPs) [1]. Secondary MPs are categorised into fragments, fibres, foams films and pellets [7]. Microplastic fibres (MPF) are defined as more than twice as long as they are thick [8].

MPs in the freshwater environment originate from many sources including effluent from factories [9], surface water runoff [10], aerial dispersal [11,12] and slurry runoff. A significant contribution to MP pollution comes from microplastic fibres (MPF) which are copiously shed during machine washing of synthetic clothing [13,14]. The combination of vigorous machine washing, a massive shift from clothing materials made from natural fibres to plastics and disposable 'fast-fashion' has resulted in a serious pollution issue [15].

Studies on MPs tend to be divided into those looking for evidence of ingestion and those looking at the impact of the MP on some aspect of the organism's biology, with mixed and sometimes conflicting results [16–20]. While the majority of studies have focused upon MP particles, those which focus upon MPFs have found similar results with good evidence for ingestion in marine crustaceans, *Orchestia gammarellus*, *Carcinus*

maenas, *Carcinus aestuarii* and *Nephrops norvegicus* [21–24]. Meta analysis has shown that virtually every taxa investigated has been shown to ingest MPFs, and they have been shown to be harmful, especially to juveniles of both vertebrates and invertebrates [6]. An interesting question is why certain organisms would eat MPFs in the first place and there is an assumption that ingestion is accidental, indeed, this incidental feeding does seem to be the predominant cause of ingestion in fish [25,26]. This assumption was not fully upheld in our previous study, where we observed that *Gammarus pulex* selectively preferred food that was not contaminated with acrylic MPF [27]. That study concluded that the presence of the MPF was immediately detected and it was the presence of these fibres that deterred feeding, however, some fibres were still ingested suggesting incidental feeding did occur.

Most MPFs are released during domestic washing of synthetic clothing, where mechanical and chemical stress can cause the detachment of fibres [28]. However, organic microfibres (OMF) are also released into the environment by the same means [29,30], with cotton and sheep wool being the most common in the UK [31]. Both human and animal hair are commonly released into waste water and hair, wool and cotton are all similar in thickness to many MPFs [32].

Whilst wool can refer to the hair products of several taxa, in the UK the majority is from sheep in the genus *Ovis* which have a thickness of 70–90 µm [32]. Cotton fibres range from 10–20 µm [33] and pet hair such as dog or cat can range between a 19 and 120 µm [34]. Given that these fibres are in the same size range as MPFs, and that it is the physical presence of MPF in the guts of invertebrates displacing cause negative impacts impacts [27,35–37], we decided to use our previous methodology to investigate OMF ingestion in the freshwater shrimp *Gammarus pulex* [27]. *Gammarus pulex* is a standard ecotoxicological model organism and is important to many freshwater ecosystems across Europe and Asia [36,38,39], operating as a prey species, predator and shredder of organic material [38,40–42].

We previously demonstrated that *Gammarus* will eat acrylic MPFs embedded in an algal wafer when given no choice (Supplementary Figure S1), but prefer not to eat the MPFs if uncontaminated wafers are available [27]. What is not known, and what this study aims to identify is whether the same behaviour of avoidance is observed when OMF are used rather than MPF. If it is simply the physical presence deterring feeding then it is expected that feeding will be indirectly proportional to the thickness of the fibres, OMF or MPF.

2. Materials and Methods

Gammarus pulex were gathered using kick sampling from a tributary of the River Lodden, Emm Brook (Decimal Degrees 51.440494, −0.874373 to 51.442274, −0.874359). This location provided a healthy population of *G. pulex* in a river with safe and easy access, reliable flow throughout the year and shallow depth. Hessian kick nets were used for collection and only individuals greater than 12 mm in length were taken and transported in plastic (PET) bottles from the collection site to the laboratory.

Once in the laboratory *G. pulex* were rinsed with reverse osmosis water to remove any contaminants from the brook and then placed into 45 L tanks of Organisation for Economic Co-operation and Development (OECD) reconstituted water [43], aerated with diffusion stones.

2.1. Fibre Preparation

A variety of different fibres that might be found in the aquatic environment following clothes washing were chosen (Table 1).

All fibres were soaked in RO water for 24 h and then rinsed with RO water to remove surface contamination. The cat and human hairs were twisted into a thread similar to the cotton and acrylic (Supplementary Figure S2), allowing them to be prepared using the methodology of [27], where by the thread is saturated with Reverse Osmosis (RO)

water frozen at −80 °C, and then inserted into a jig, allowing 500 µm lengths to be cut off and collected.

Table 1. Size, colour and source of fibres used.

Fibre	Size	Colour	Origin
100% cotton thread *Gossypium arboreum*	≈20 µm	Black	DMC black special embroidery thread product code 6404211000, Hobbycraft, Farnborough
100% acrylic wool	≈30 µm	Black	Hayfield Bonus DK product code 5723101001, Hobbycraft, Farnborough
Cat hair *Felis catus*	≈30 µm	Dark brown	Calico—author's cat.
Human hair *Homo sapiens*	≈50 µm	Dark brown	Female—author's mother
100% Jacob Wool yarn *Ovis aries*	≈70 µm	Black	West Yorkshire Spinners Brown Black Fleece Jacob Aran Yarn product code 6223481003, Hobbycraft, Farnborough
Human hair *Homo sapiens*	100 µm	Dark brown	Female—author's wife

The wafers were produced by homogenising 0.03 g of the manufactured MPF and OMFs with 0.97 g ground algal wafers (Wafer Algae Eater Fish Food, API) in a mortar and pestle. After 1 min 0.5 ml of RO water was added to reconstitute the mixture into a paste. This paste was then pressed to a 5 mm thick cake and dried on a hotplate. Once dried the cake was divided into 0.05 g wafers. Ten of each of the OMF wafers were selected, divided into quarters and crushed: the number of fibres within each wafer were recorded to calculate an average number of fibres per wafer.

2.2. Acute Exposures

Two methods were used to expose *G.pulex* to either one or two wafers. Fourteen *Gammarus* were placed into a 5 L aquarium and starved for 24 h before exposure. For the comparative ingestion study individual *Gammarus* were placed in a 5 L aquarium with 2 L reconstituted water and exposed to one of the six different fibre wafers (3%) or a control wafer (no fibres) for 4 h. After this the *Gammarus* were killed with 50 °C water, dissected under 10× magnification, the number of ingested fibres were clearly visualized and recorded. Each day two *Gammarus* were exposed to each treatment, this was repeated for 5 days providing 10 replicates a total of 70 *Gammarus*.

2.3. Choice Experiment

For the choice experiments the same experimental design was used, except *Gammarus* were exposed to each of the six different fibre wafers as well as a control for four hours, and the time spent feeding on each wafer and the number of visits made to each wafer were recorded, as were the number of fibres ingested. Due to the need for constant observations, and the length of time the experiments required, two rounds of experiments with three individuals in each could be performed per day. Each treatment was performed once per day and repeated for 10 days, giving 10 replicates.

Gammarus were exposed to the following combinations of fibre, using the methodology given above; cat/cotton, cat/acrylic, cotton/acrylic. The *Gammarus* were observed continuously number of feeding visits and the time spent feeding on each wafer were recorded, a feeding event was decided if a *Gammarus* could be seen feeding on the wafer, or removing part of the wafer and holding it while feeding. Each day two replicates for each combination could be run, this was repeated for 5 days for a total of 10 replicates. The human and sheep wool fibres were not used in the choice experiments because no fibres were ingested in the initial exposures.

2.4. Chronic Exposures

As before, *Gammarus* were starved and conditioned prior to initial weighing. Individuals were removed from the aquarium, dried by gently pressing them between paper towels and weighed so that only individuals between 0.1 g and 0.2 g were used. Fifty individuals were allocated randomly a treatment using the Excel RAND and ROUNDUP functions (Microsoft Office). The treatments used were acrylic 1%, acrylic 3%, cat 1%, cat 3% and control, with 10 replicates for each treatment.

Test aquariums were made using 250 mL round PET containers with 1 cm of aquarium gravel and 200 mL of reconstituted water. One *Gammarus* was placed in each aquarium alongside a 0.05 g wafer of whichever treatment was allocated. A rolling 7-day regime was followed for 28 days;

Day 1—Weigh *Gammarus*, clean aquarium and add wafer

Day 4—Remove old wafer and replace with new

Day 7—Remove wafer

Gammarus were dried prior to weighing. The aquariums were cleaned while the *Gammarus* were weighed, this was done by pouring the contents of the aquarium into a 1 mm sieve and then rinsing with tap water to remove remnants of MFs, wafer and waste. The aquarium itself was then wiped with a paper towel and rinsed with tap water, the contents of the sieve were then tipped back into the aquarium and 200 mL of reconstituted water was added along with a new suitable wafer, finally the *Gammarus* was replaced in the aquarium.

A block design was used: samples were divided into 5 groups A–E, with two of each treatment in each group. *Gammarus* within each group were allocated a number 1–10, thereby all 50 individual *Gammarus* could be identified with a number, e.g., B5. The 7-day regime was staggered by one day, day 1 group A was Monday, day 1 group B was Tuesday, etc. On day 1 it was also recorded if any *Gammarus* had died.

2.5. Statistical Analyses

All data was analysed using R Studio [44]. Number of fibres ingested were corrected for the number available, and presented as % of available fibres ingested. As the block design was consistent day on day and each individual was in its own aquarium and totally independent, all individuals were treated as true replicates. Shapiro–Wilk tests were used to test for normality within the data. The assumptions for normality were met in the comparative ingestion experiments and the time recordings for the choice experiments. The assumptions were also met in the acrylic/control and cotton/control fibre ingestion recordings. As such one-way ANOVA tests were used. The assumptions were not met for any of the choice experiment visit recordings or the cat/control fibre ingestion experiment so Kruskal–Wallace tests were used.

The chronic growth data was found to meet the assumptions for normal distribution, and one-way ANOVA tests were used. Due to the categorical nature of mortality results, the data was not normally distributed, as such McNamars test was used.

3. Results

3.1. Acute Fibre Ingestion

Gammarus pulex ingested wafers containing acrylic, cat hair and cotton but would not ingest wafers containing human hair, or sheep wool fibres. Where fibres were ingested, they were observed within the gut and faecal pellets (Figure 1). *Gammarus pulex* ingested significantly fewer cotton fibres than either acrylic or cat (Figure 1) $F_{2,27} = 5.737$ $p = 0.0084$ (acry/cat = 0.7491 acry/cott = 0.0047 cat/cott = 0.0103).

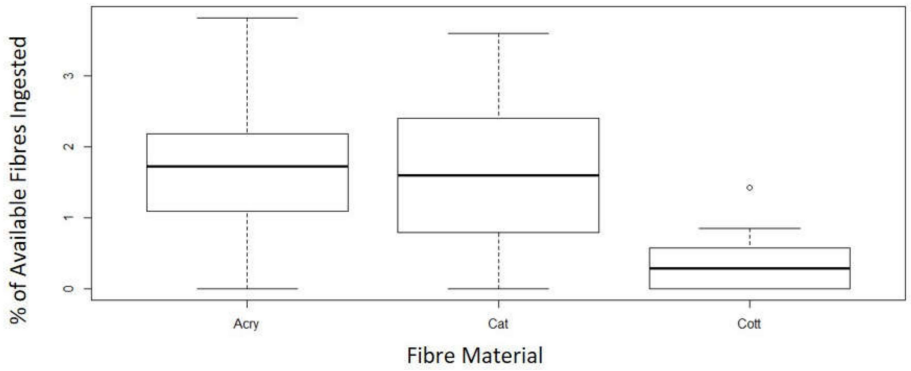

Figure 1. The percentage of available 200–500 µm fibres ingested by *Gammarus pulex* in 4 h. Acry = acrylic, Cat = *Felis catus*, Cott = cotton (n = 10).

3.2. Feeding Behaviour

When given a choice between a control or a contaminated wafer, there were no differences in the number of *Gammarus* visits made to any of the wafers (acrylic W = 5.193, p = 0.158, cat W = 4.886, p = 0.18, cotton W = 1.507 p = 0.680) or in the time spent feeding (cat $F_{1,18}$ = 0.487, p = 0.494, cotton $F_{1,18}$ = 0.076, p = 0.786) with the exception of the acrylic wafers. *Gammarus pulex* spent significantly less time feeding on the acrylic wafers (Figure 2A–C) ($F_{1,18}$ = 8.541, p = 0.0084).

When given the choice between cat and acrylic or cotton and acrylic, *G.pulex* spent significantly less time feeding on acrylic wafers $F_{1,18}$ = 19.59, p > 0.001 (cat/acrylic), $F_{1,18}$ = 20.71, p > 0.001 (cotton/acrylic) (Figure 3A,B, respectively). Choice experiments between contaminated wafers found no difference in time spent feeding between cat and cotton ($F_{1,18}$ = 0.077, p = 0.785) (Figure 3C).

It was found that given the choice between contaminated and control wafers, several organisms did not ingest any fibres 4/10 (acrylic & cat) and 6/10 (cotton), these were removed from the analysis. Several organisms also did not ingest any fibres even without a choice of non-contaminated wafers 1/10 (acrylic & cat) and 4/10 (cotton), these were also removed from the data set. When the results were analysed (Figure 4) it was found that significantly fewer acrylic fibres were ingested when given a choice $F_{1,13}$ = 8.524, p = 0.012, but there was no significantly difference in the number of OMF ingested with or without non contaminated wafers.

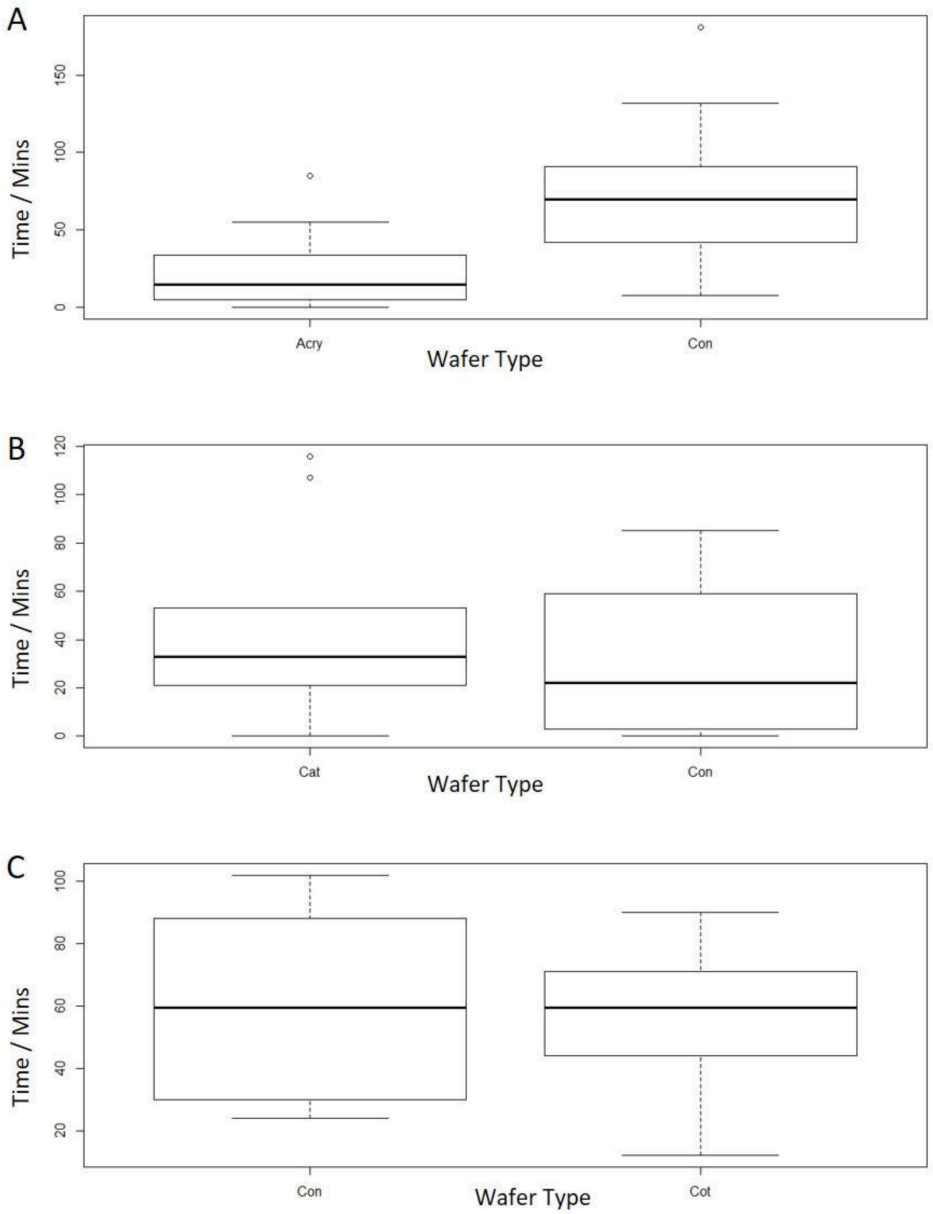

Figure 2. Time spent feeding on test or control wafers by *Gammarus pulex* in 4 h. Test wafers contaminated with 200–500 µm fibres Acry = acrylic (**A**), Cat = *Felis catus* (**B**), Cott = cotton (**C**) (n = 10).

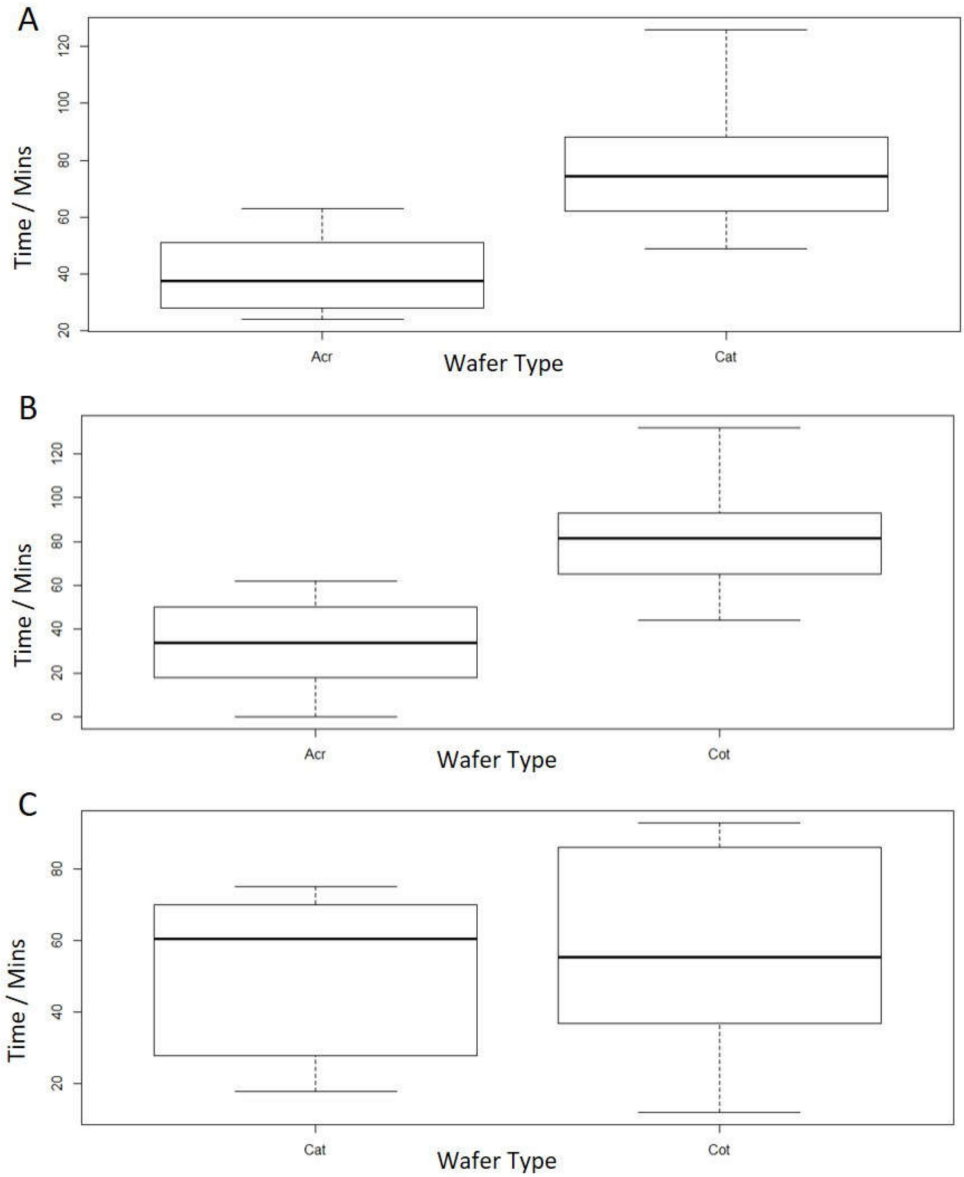

Figure 3. Time spent feeding on wafers by *Gammarus pulex* when given a choice between acrylic & cat (**A**), acrylic & cotton (**B**) and cat & cotton (**C**) (n = 10). Contamination was 3% by mass 200–500 μm fibres.

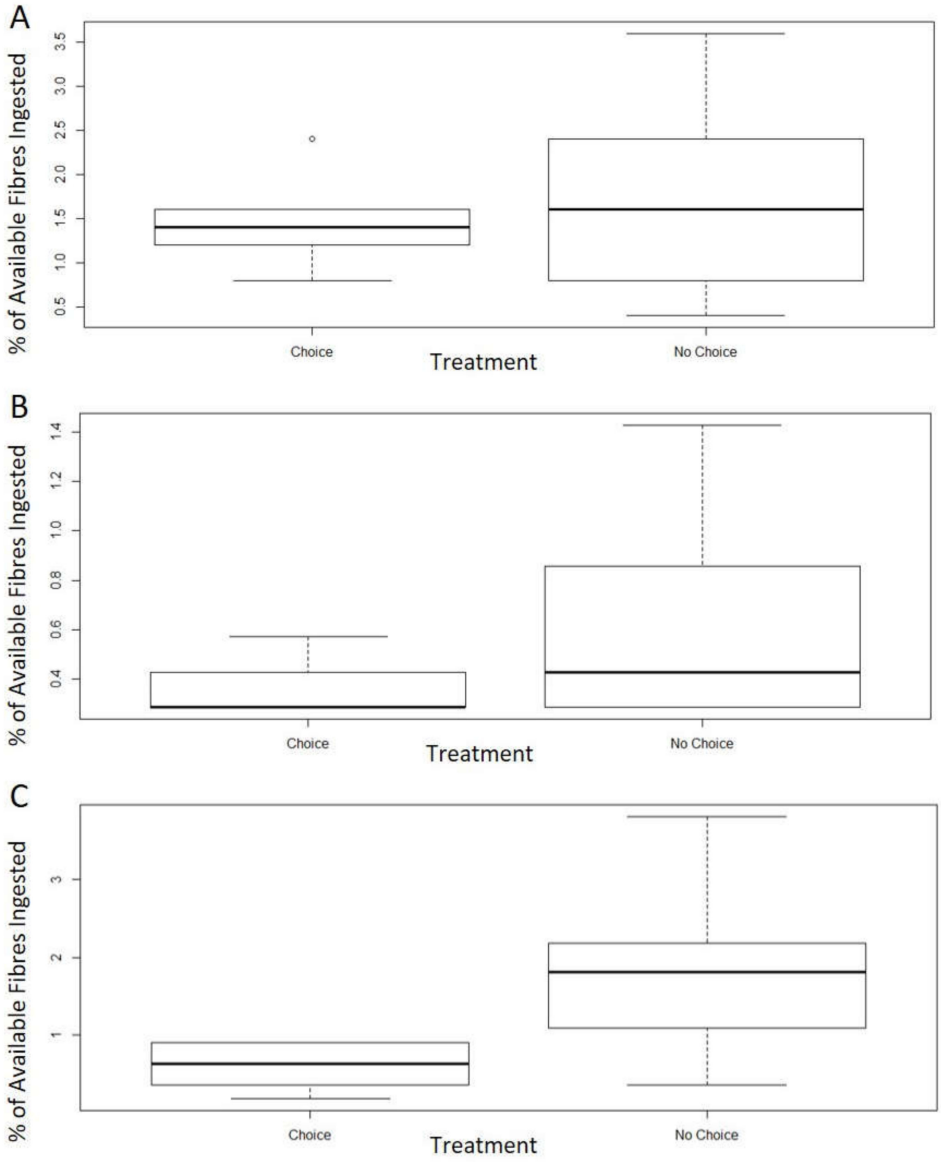

Figure 4. % of available fibres ingested with and without the choice of uncontaminated food sources by *Gammarus pulex* (n = 10). Contamination was 3% by mass 200–500 µm fibres from cat (**A**) cotton (**B**) and acrylic (**C**).

3.3. Chronic Ingestion

There was no significant difference in the starting mass of individual *Gammarus* between treatments ($F_{4,45}$ = 0.312, p = 0.869). After the 28 days, there was still no significant difference in mass ($F_{4,45}$ = 0.812, p = 0.524), or growth (change in mass) of *Gammarus* between treatments (Figure 5) ($F_{4,42}$ = 0.761, p = 0.557). The greatest growth was found in the control (7.7 mg ± 3.8, n = 9) and cat 3% (7.7 mg ± 4.0, n = 10), followed by acrylic 3% (7.2 mg ± 3.1, n = 10), acrylic 1% (5.3 mg ± 3.8, n = 9), with smallest growth in cat 1% (4.6 mg ± 6.0, n = 9).

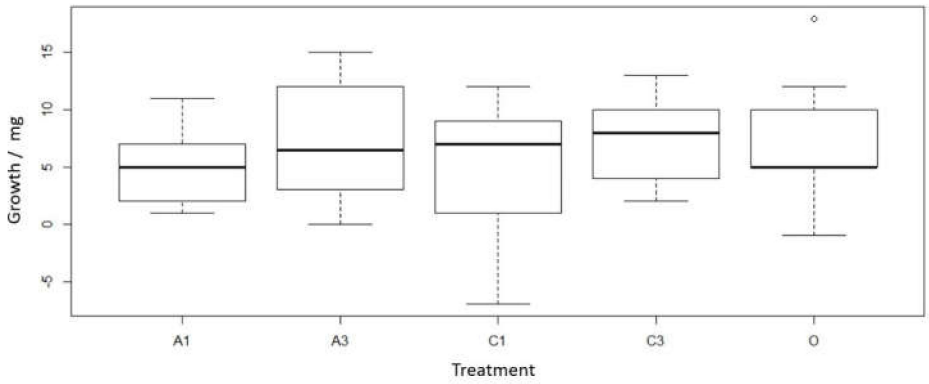

Figure 5. Growth as change in mass/mg of *Gammarus pulex* after 28 day exposure to different fibre treatments. A1—acrylic 1% by mass, A3—acrylic 3% by mass, C1—cat 1% by mass, C3—cat 3% by mass, 0—control.

While there was greater mortality in the acrylic treatments compared to cat treatments (1% 2/10 vs. 1/10, 3% 4/10 vs. 1/10) these were found to be not significantly different (1% $Chi^2_1 = 1$, $p = 1$, 3% $Chi^2_1 = 1.33$, $p = 0.248$).

Data can be found in Supplementary Materials Tables S1–S3.

4. Discussion

When given a choice between food contaminated with acrylic fibres and those without, *G. pulex* avoided eating the acrylic-contaminated wafers. This is a repeat of the result published by Yardy and Callaghan [27] using the same technique applied here. However, unlike our previous study, there was no evidence in a reduction in the number of visits to contaminated wafers, only the number of fibres ingested and the time spent feeding on contaminated wafers.

In contrast, the *Gammarus* would not eat wafers containing human hair or sheep wool, preferring to starve. These OMFs were larger in diameter than MPFs, cat hair or cotton, which were all ingested. This suggests either avoidance or a functional size limit to ingestion of fibres, with a potential maximum thickness of between 30 µm and 50 µm. The latter seems most likely and is similar to the findings of [35] who found PET fragments <53 µm were most ingested. A study on *Gammarus fossarum* [36] also found that MP 20 µm were readily ingested.

Where fibres were ingested, they were observed within the gut and faecal pellets. When given no choice of food, *Gammarus pulex* ingested significantly fewer cotton fibres than either acrylic or cat hair. When not given a choice *Gammarus* did not ingest fewer acrylic fibres that cat hair, this suggests as with our previous study and others that *Gammarus* will readily ingest PMF [27,36]. Cotton fibres, which were both the thinnest and as plant fibres, arguably the closest to the natural diet of *G. pulex* were ingested at the lowest rates. Size is unlikely to be a factor. Blarer and Burkhardt-Holm [36] found that 20 µm PA fibres were readily ingested by *G. fossarum*. Other than the potential upper size limit, thickness does not explain fibre ingestion rates.

Chemical cues are important factors in *G. pulex* feeding [45]. It is possible that the cotton contained unpalatable chemicals, possibly the black dye. While it has been shown that sublethal exposure to dyes does trigger a stress response in *G. pulex* [46], this was at concentrations 1/16th of LD50, far higher than would have been experienced in this study. There was nothing within the behavioural data that suggested that *Gammarus* were repelled from feeding on cotton contaminated wafers, and so there must be another factor. As already stated, cotton fibres were identified in both gut and faeces, without any signs of degradation, therefore it cannot be that cotton was simply digested, hence its apparent absence. Another possibility is that the cotton fibres have a higher tendency than the other fibres to clump together, thereby making them easier to avoid, although none of these clumps were observed post feeding, in the gut or remaining wafer. It is possible

that there were pollutants adsorbed onto the purchased fibres (acrylic, cotton and sheep wool), however, these were purchased new and stored in sealed containers, therefore any adsorbed substances would have been from manufacturing. The human and cat hairs were washed without soap prior to collection and then rewashed in the laboratory. In either case, any residual pollutants would be expected to remain had the fibres been in freshwater.

Studies with MP beads have demonstrated that size does matter. Whilst *G. pulex* ingested 90 µm polystyrene (PS) beads, smaller 1 µm beads that were ingested at lower rates [47]. The relationship between size and ingestion frequency was explained by the larger beads settling on the food rather than being suspended in the water column, so this cannot explain the differences of fibre ingestion in wafers.

Fibres of all types that were ingested in this study remained intact and were excreted whole in the faecal pellets of *G. pulex*. The literature suggests that the negative impacts from MP and MPF in *G. pulex* are related to their taking up space in the gut, thereby reducing food intake and influencing growth [8,35,47]. Based on this we predicted that OMFs that are not digested, but egested from the gut, will have a similar impact on *Gammarus* growth as MPF.

The chronic ingestion data supported this and although there was lower growth in animals fed MPFs, there were no significant differences in growth between the fibre treatments and there was no evidence for increased mortality following MPF exposure. This study supports the findings of a previous study on *G. fossarum* [36] where a significant difference was found in assimilation efficiency between those exposed to PA fibres and control. While this study found no significant difference in mortality, the discrepancy between acrylic and cat at 3% should not be ignored, and it is possible that at greater concentrations there would be a greater mortality. Importantly it is unknown whether this increased mortality would be found if uncontaminated food was available as well as acrylic contaminated, this would be more applicable to environmental conditions.

Gammarus pulex show an avoidance to MPF when given a choice, but no avoidance to OMF, and as these are far more numerous [30,31] it follows that they are more likely to ingest OMFs [48]. If OMFs are more likely to be ingested and they do not elicit avoidance behaviour, yet they have a similar impact, it could be argued that it is the lower diameter OMF not MPF are a greater risk to at least some invertebrate species. We have been releasing processed OMF into the environment for centuries, and in very high rates through WWTPs for decades [49–51]. Many individuals and organisations have suggested that as a society we should swap to organic based fabrics rather than synthetic to limit the release of microfibres. Whilst this seems to be a worthy goal, this study has found no significant difference in mortality between MPF and OMF, it is easy to assume that plastics are always more of a problem in freshwater, however this may not be the case.

5. Conclusions

The current, quite relevant, concern over the release of MPFs into our waterways [13,14], assumes that MPFs will have a more detrimental impact than organic fibres released through the same processes. However, there is little empirical evidence to demonstrate that plastic fibres are more harmful than organic fibres which have been released from washing machines for as long as they have existed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/environments8080074/s1, Figure S1: Fibres in faecal pellets, Figure S2: Hair twisted into thread, Table S1: Movement data, Table S2: Ingestion data, Table S3: Chronic Data.

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Conflicts of Interest: The authors declare no conflict of interest.

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