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Effects of New and Aged Polyethylenterephthalat and Polylactic Acid on *Gammarus fossarum* (Crustacea: Amphipoda) during Long-Term Exposures

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Abstract

The freshwater amphipod *Gammarus fossarum* (Koch, 1835) was exposed in four subsequent experiments of each 45 - 48 days to 1 cm^2 plastic foil (Polyethylenterephthalat (PET), Polylactic acid **(**PLA)) with mortality, feeding, and behavior in the Multispecies Freshwater Biomonitor© (MFB) (spontaneous locomotion, gill ventilation) being studied once a week. Mortality was generally high and similar in all experiments, with a slight increase in PLA treatments. PLA exposed animals showed higher ventilation, but spontaneous locomotion was equally high in all treatments during all experiments, showing high reproducibility. Feeding rates reached up to 50% of the provided alder leaf in all treatments and in all experiments. PLA foils started to age by surface cracks, deformation at the edges and loss of transparency esp. in the 4th run with 135 d old foils. Both types of foils were equally colonized with biofilm during 45 days' exposures, starting with ciliates and flagellates, followed by diatoms and green filamentous algae at last. Some taxa differed in absolute amounts in the 4 subsequent experiments due to seasonal succession. There was only marginal weight loss of the foils during 180 days of exposure. The biopolymer PLA started to degrade slowly after 180 d, whereas PET remained morphologically unchanged. The presence of the plastic foils in the beakers independent of their age had no significant effects on survival, behavior, and feeding of *G. fossarum* during 45 d of exposure.

Keywords

Toxicity, Plastic, Biopolymer, Biofilm, *Gammarus fossarum*

1. Introduction

During the last century, synthetic polymers have entered all aspects of human life. This is because they have long life-times, are chemically resistant and multifunctional in their applications, from e.g. toothbrush to medical implants and the automotive industry. Based on fossil fuels or gas components, synthetic polymers are generated by polymerization or poly-condensation [1]. Different types of additives, such as carbon, silicon, bisphenol A, phthalates, colors, and stabilizers are needed to improve the specific characteristics of the manifold products, e.g. the resistance against hydrolysis [2]. Between 1950 and 2015 about 8.3 tons of plastics have been produced in the world, out of which about 79% are deposited in landfills. Regarding all waste in the environment, about 60% - 80% are plastics [3] [4]. In the oceans, about 270.000 tons of plastic particles are estimated, out of which 35.000 t belong to the microplastics [5]. Plastic degradation into secondary microplastic particles (<5 mm) in the environment is a very slow process that takes several centuries [2] [4] including photolysis, thermal and mechanic degradation, catalytic and biological degradation [6]. The main source of secondary microplastics consumption is tire wear and textiles [5] [7]. On the other hand, primary microplastic particles have been used in personal care and cleaning products. However, many producers have recently started to reduce their usage where they serve only as a filling or abrasive material. The smaller the plastic particles, the more toxic they are due to higher bioaccumulation in tissues, the release of toxic byproducts, higher adsorption of other toxic compounds and microorganisms, resulting in additional toxicity due to oxidative stress, reduced immune responses, and inflammations [8].

Plastic consumption needs to be either replaced by alternative materials or recycled and, re-used. In most cases, their use must be eliminated where they are not essentially needed. The European Commission proposed a new directive to ban non-returnable plastic products from the personal care and household sectors. Recycling is difficult where a complex mixture of different polymers is being used. The new Single-Use Plastic Directive (EU/2019/9049 aims at a significant reduction in the consumption of single-use plastic products. Moreover, the European Chemical Agency (ECHA) proposes a significant reduction of microplastic particles in products starting from 2022.

Polyethylenterephthalat (PET) is the most frequently used polymer in transparent bottles used for carrying mineral water and drinks. It is resistant up to 80˚C and takes about 450 years to degrade in water [9]. Recently the bacterium *Ideonella sakaiensis* 201-F6 was found to catalyze the hydrolysis of PET into terephthalic acid and ethylene glycol [10] [11].

Bioplastic polymers originate either from renewable resources or biologically degradable polymers. These polymers might stem from either renewable or fossil resources, whereby polymers from renewable resources not necessarily show fast degradation [7] [12]. The most frequently used biologically degradable polymers are mixtures of thermoplastic starch and polylac-

tat (PLA), polycaprolactone (PLC), polybutylenadipatterephthalat (PBAT) and polyhydroxybutyrate (PHB) [13]. Regarding their environmental balance, these biopolymers are not better alternatives than fossil polymers, due to their high energy demand and fertilizer application during the production of their plant-based raw materials, such as corn, sugar cane, or potatoes. PLA is based on agricultural crop growing, biological fermentation, and chemical polymerization technologies, and is regarded as environmentally safe [1] [14]. PLA can be recycled and composted at high temperatures (ca. 60˚C) by hydrolysis within 45 - 60 days, however, no microbial degradation was observed in seawater, probably due to the circumneutral pH and low temperatures [15] [16]. Recently a combination of 4 microbes was found to support the microbial degradation of PLA at high temperatures [17]. Degradation of PLA in the environment might take several years [18], which appears to be an improvement compared to the lifetime of fossil polymers, which is estimated to several decades to centuries. The environmental performance of cassava starch-based PLA bottles is better than PET bottles regarding global warming, reduction of fossil energy use and human toxicity [19].

The aim of this study was to follow the natural degradation of PLA and PET foils in water under environmentally realistic conditions and to study potential effects on the freshwater amphipod *Gammarus fossarum* under longterm exposure to new and aged foils.

2. Methods

Gammarus fossarum (Koch 1835) are important key species in running water ecosystems, where they are often dominant in both numbers and biomass. They feed on leaves, detritus and other small invertebrates. Moreover, they are prey for other large invertebrates and fish. *G. fossarum* indicates water quality class II in the saprobic system and has proven to be sensitive towards various toxic substances [20] [21].

Animals (7 - 9 mm) were taken from the laboratory culture and placed into glass beakers with 150 ml filtered water from lake Constance (100 µm), which is also used for the culture. The bottom of the beakers was covered with ash-dried pebbles (9 g) as substrate and hiding places. Moreover, 1 alder leaf (3 cm diameter, leached for 1 week) was added as food. The water in each beaker was individually aerated with aquarium pumps to provide high oxygen saturation. Four to eight beakers with 5 animals in each and with either 3 numbered pieces (I, II, III) of PET or PLA foils were run as replicates. Both types of foils were obtained from the producer, PLA, and PET-GAG. The latter represents a copolymer, created from 3 thin foils, PET-G (PET-Glycol) on the outside and PET-A (amorphous) inside. Both polymers are used in the packing sector of food, drinks, and cosmetics. Both foils were 0.2 mm thick, glossy and transparent. 1 cm^2 quadratic pieces were cut and marked with numbers I, II, III. The pieces were weighed before use in the experiments.

The four control beakers did not contain any foils. The experiments were performed in a thermostat at 10˚C in the dark, equal to the conditions of the culture. The experiments lasted for 45 - 48 days (6 weeks). Every week the water was renewed and feeding activity was determined as % leaf loss. The area of leaf loss was estimated in the following classes: 0%, 0% - 5%, 5% - 25%, 25% - 50%, 50% - 75%, 75% - 100%, *i.e.* for 0% that no feeding traces were seen, whereby at 100% the whole leaf was eaten. Moreover, twice a week, survival and the behavior of 8 randomly chosen animals from each test condition were selected from the 3 replicates for quantitative recordings in the Multispecies Freshwater Biomonitor© (MFB) for a period of 2 hours.

The MFB quantitatively records the behavior (e.g. locomotion, ventilation) and survival of aquatic animals, placed individually in a cylindrical flow-through test chamber (5 cm long, 2 cm inner diameter) sealed on both ends with nylon mesh (0.5 mm) screw lids. The gammarid moves completely free in the chamber and each movement is recorded by the non-optic quadrupole impedance conversion technology [22] [23] for a period of 4 Min. followed by a pause of 6 Min. Twelve subsequent recordings (*i.e*. 2 hours) were collected for 8 animals from PET, PLA and control simultaneously every week in the thermostat.

Once a week, before the renewal of the water the three foils in each beaker were microscopically investigated (400× magnification) on their upper surface, *i.e*. the side with the carved markings (I, II, III) regarding changes in surface structure, fissures, cracks, and biofilm colonization. One randomly chosen area in the middle of the foil was chosen under the microscope at 400× magnification and the number of different taxa occurring in this visual field was manually counted: ciliates, flagellates, diatoms, green algae, and filamentous algae.

At the end of the experiments, leaves were air-dried for 3 days and weighed to calculate the weight loss during the 45 d exposures. Foils were checked under the microscope, then rinsed with tap water (45˚ angle) and checked again under the microscope to estimate the number and taxa of tightly versus loosely attached biofilm. Moreover, the animals' gut filling content was estimated under a magnifying lens (400× magnification). The gut content was divided into the following classes: empty gut: 0%, 0% - 5%, 5% - 25%, 25% - 50%, 50% - 75%, 75% - 100% of the gut length filled with contents.

Whereas the first experimental run of 45 days was done with new foils, the following second run (with new gammarids) was performed on the aged foils, but otherwise performed exactly as the 1st experiment. After the 2nd experiment, two more experiments were subsequently performed with the foils of increasing age, *i.e.* at the end of the last (4th) experiment of 45 days, the foils were already 180 days (6 months) old. This procedure was carried out to estimate the progressive decay of the foils and the potentially increasing toxicity due to leaching of additives or mechanical decay.

The water from lake Constance was analyzed regularly during the whole experimental period of 6 months using rapid photometric test kits (Caldur®, WinLab®) for nitrite, nitrate, ammonium, phosphate, sulfate, and hardness. Moreover, conductivity and pH were recorded.

All data were treated with SigmaPlot 12.0 for graphical presentation and statistical analyses (two-tailed hypothesis testing) with non-parametric one Way ANOVA or RM ANOVA on Ranks for time-dependent data. Comparisons of 2 groups were done with paired t-tests (Biofilm colonization).

3. Results

3.1. Water Quality

The water quality of the water from Lake Constance used for the culture of the gammarids and the experiments remained equally good throughout the whole experimental period of 6 months (**Table 1**). The pH value did not change during the exposure time of 45 d in the experimental beakers, *i.e.* the PLA did not reduce pH by hydrolytic degradation.

3.2. Survival

In the 1st run with new foils the mortality in the PET group was significantly lower than that in the PLA or control groups over the 45 days (RM ANOVA on Ranks, p: 0.027). After 46 days of exposure 40% - 55% mortality was reached in all treatments (**Figure 1**). In the 2nd run with 45-day old foils this difference between the types of foils was supported, but not significant (p: 0.06). After 45 days of exposure 40% - 60% mortality was reached, with the lowest mortality in PET, followed by the control and PLA showing the highest mortality (60%) (**Figure 1**). In the 3rd run with 90-day old foils, mortality in the controls was significantly lower than in PLA (p: 0.018), whereas in the 4th run mortality was significantly lower in the control compared to both PET and PLA (p: 0.007).

3.3. Feeding

At the end of the 1st 45 d exposure, the weight loss of the alder leaves determined as dry weight loss did not significantly differ between the treatments (One-way ANOVA on Ranks, ns), (PET, PLA, control), whereby approximately up to 50% of the leaf was eaten (**Table 2**). At the end of the 2nd experiment with 45 d old foils, the same results were found *i.e.* no significant difference in feeding on the leaves between the treatments. In addition, approximately 50% of the leaf was consumed by the gammarids within 45 days. At the end of the 3rd experiment with 90 d old foils, up to 50% of leaves were eaten by the animals within 45 days of exposure. At the end of the 4th experiment with 135 days old foils, the feeding rates were similar to the previous experiments (**Table 2**). The differences in

Table 1. Chemical parameters in Lake Constance water each week before renewal in the experiments (N: 8).

Parameter	pH	NO_2 mg/l	NO_3 mg/l	NH_4 mg/l	PO_4 mg/l	SO_4 mg/l	Hardness °dH	Cond. (µS/cm)
Mean	6.81	0.02	1.74	0.26	0.04	44,62	8	444
SD	0.26	0.01	0.34	0.03	0.04	8.74	0	101

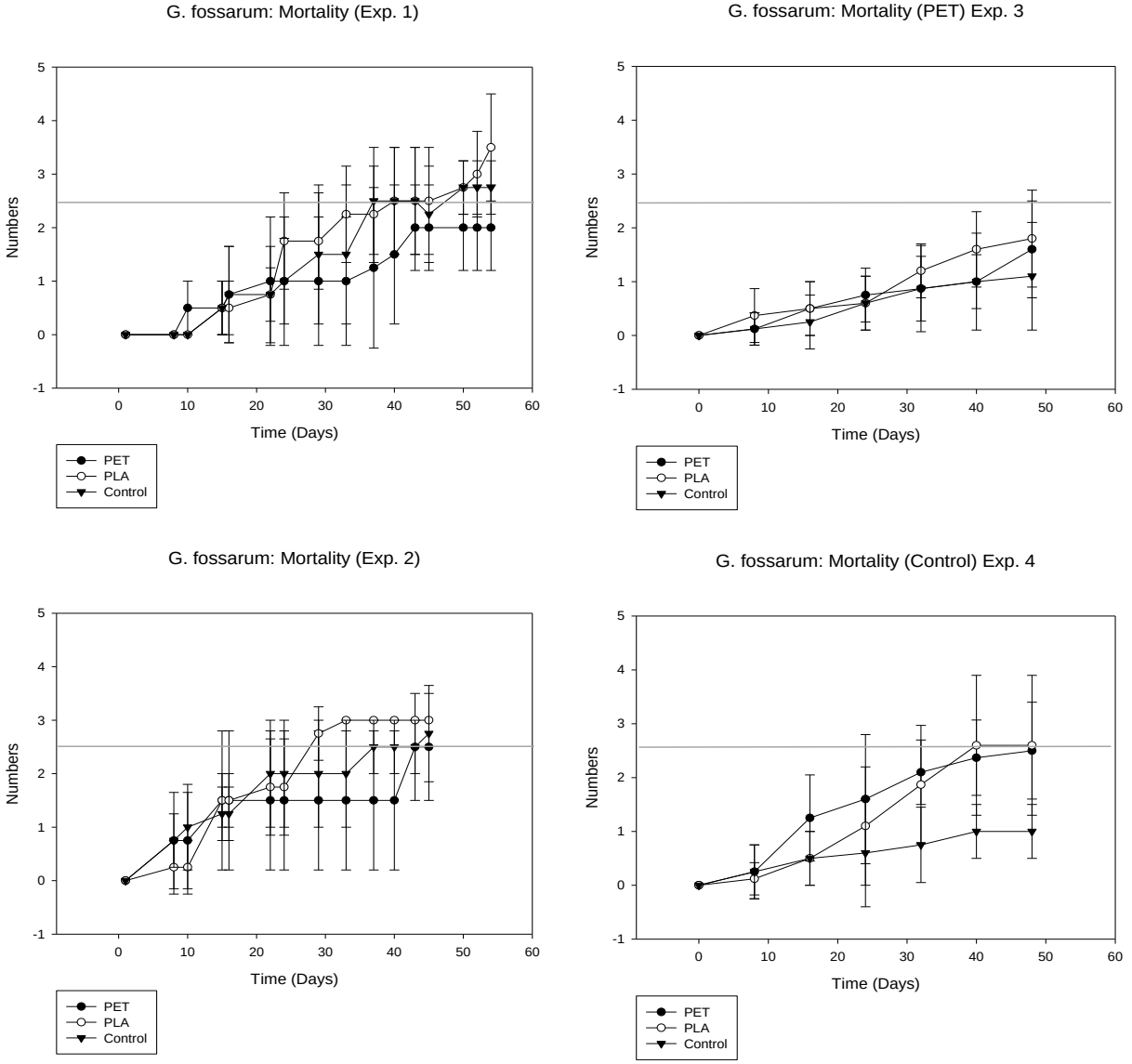


Figure 1. Mortality (Means and Sd) of *G. fossarum* exposed to plastic foils (PET, PLA) of different age (1: 0 d, 2: 45 - 48 d, 3: 90 d, 4: 135 d). Experimental conditions: Experiments 1 - 2: Number of animals per unit: 5, Replicates: 4; Experiments 3 - 4: Number of animals per unit: 5, Replicates: 8. Grey horizontal line indicates 50% mortality. In experiment 1 the plastic foils were added on day 10, whereas in the other experiments the foils were added on day 1.

Table 2. Feeding activity (Means and SD) as dry weight loss (%) at the end of the respective. Experiment (1 - 4) with plastic foils of different age (0 - 135 d).

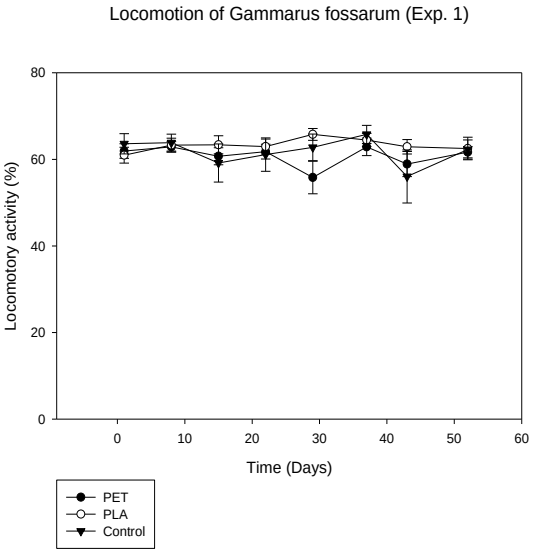
Experiment (foil age)	PET-x	PET-sd	PLA-x	PLA-sd	Control-x	Control-sd
1 (0 d)	41.44	9.33	34.87	22.18	43.04	22.73

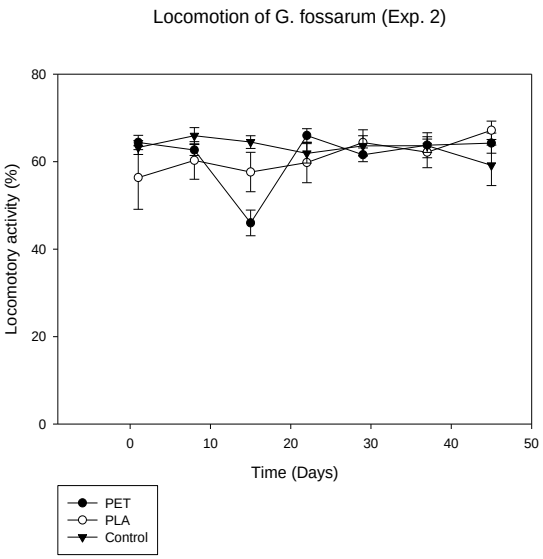
2 (45 d)	39.60	13.68	38.07	5.07	25.37	7.89
3 (90 d)	37.15	17.27	21.90	9.35	49.91	23.63
4 (135 d)	47.74	29.9	39.57	19.5	59.38	35.48

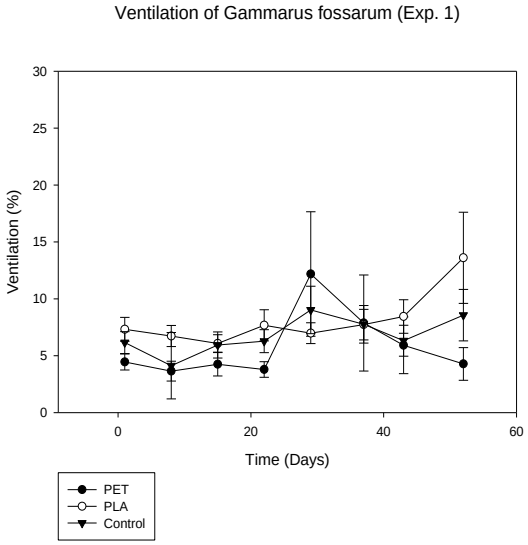
feeding activity between the treatments PET, PLA, and control were not significant in any of the 4 experiments (one Way ANOVA: ns). Moreover, there was no significant correlation between the thickness of the leaves at the start and the observed weight loss at the end of the experiments, *i.e.* thinner leaves were not better food sources than thicker leaves, as originally hypothesized (Spearman Rank Correlation, ns). The degree of gut filling in the animals at the end of the experiments was always between 60% - 80% and showed no significant difference between the treatment groups (one Way ANOVA: ns).

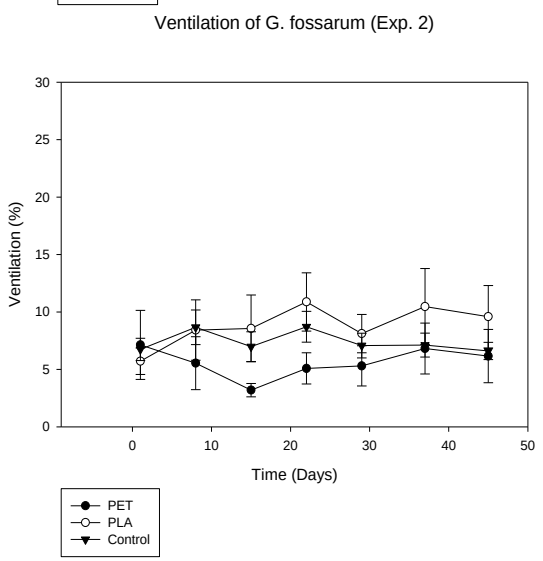
3.4. Behavior

The spontaneous locomotor activity of the gammarids was high during the whole experiment, both in the 1st with new foils and the 2nd with 45-day old foils, as well as in the 3rd (90 d old foils) and in the 4th (135 d old foils) runs (**Figure 2**). There was no significant difference between the treatments and the control in any of the 4 subsequent experiments (RM ANOVA on Ranks, ns.). Ventilation activity was significantly higher in PLA compared to other treatments (RM ANOVA on Ranks, $p < 0.05$) in the 1st and the 4th run (**Figure 2**).


Locomotion of Gammarus fossarum (Exp. 1)
Locomotory activity (%)
Time (Days)
PET
PLA
Control


Locomotion of G. fossarum (Exp. 2)
Locomotory activity (%)
Time (Days)
PET
PLA
Control


Ventilation of Gammarus fossarum (Exp. 1)
Ventilation (%)
Time (Days)
PET
PLA
Control


Ventilation of G. fossarum (Exp. 2)
Ventilation (%)
Time (Days)
PET
PLA
Control

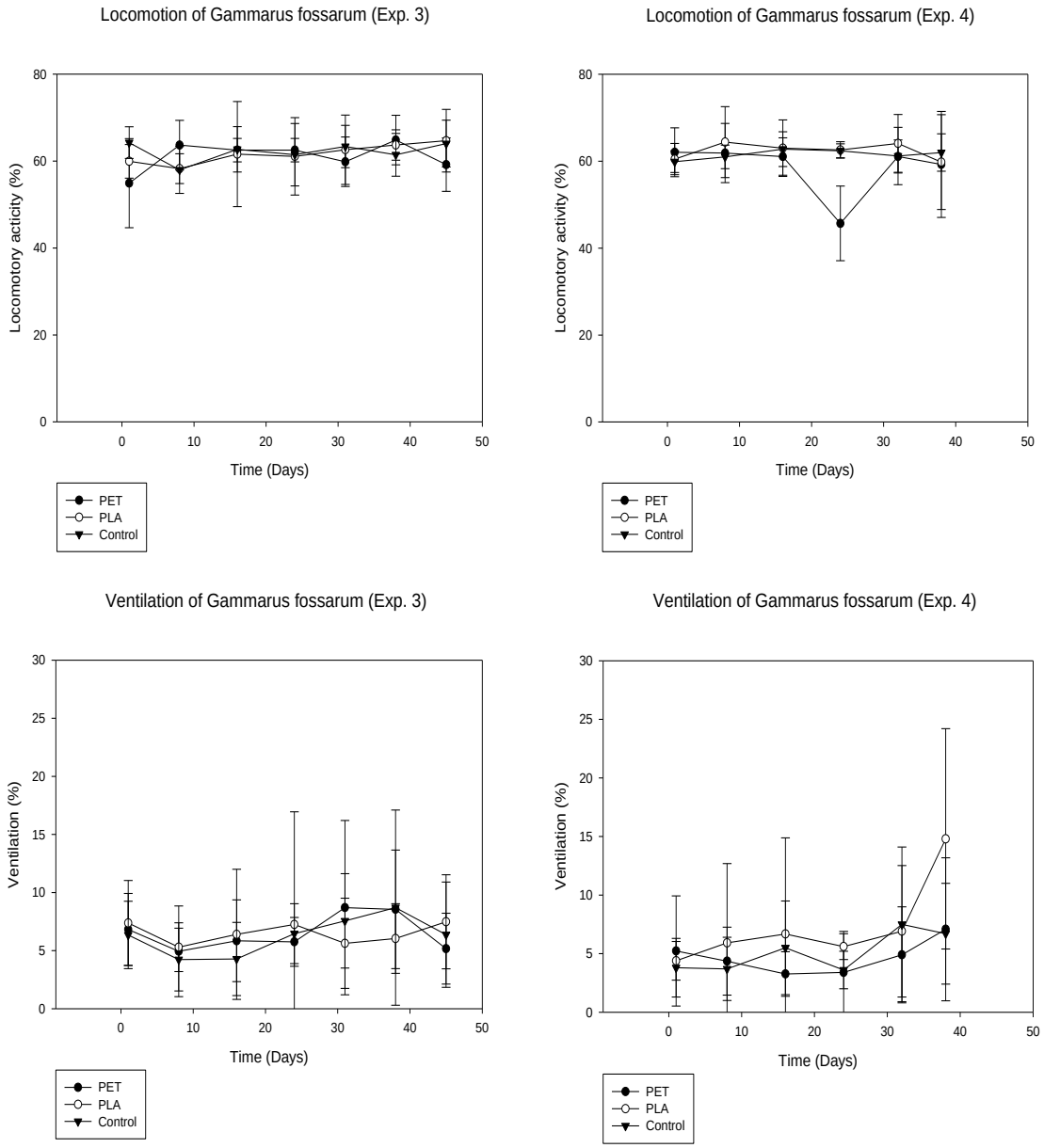


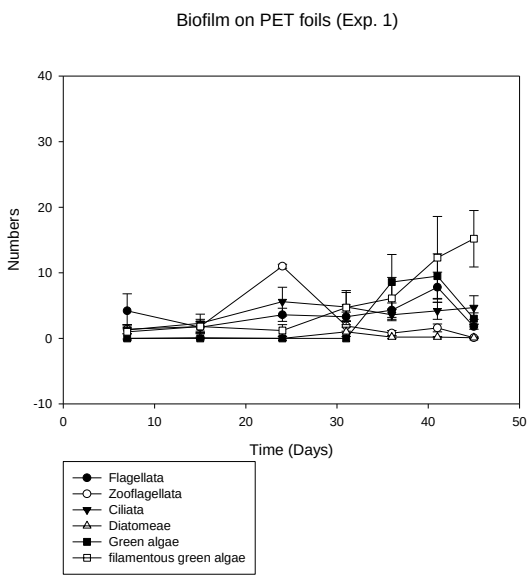
Figure 2. Behavior of *Gammarus fossarum* (Means and Sd: locomotion, ventilation; N: 8) in the course of the experiments (1 - 4) exposed to plastic foils (PET, PLA) and the controls.

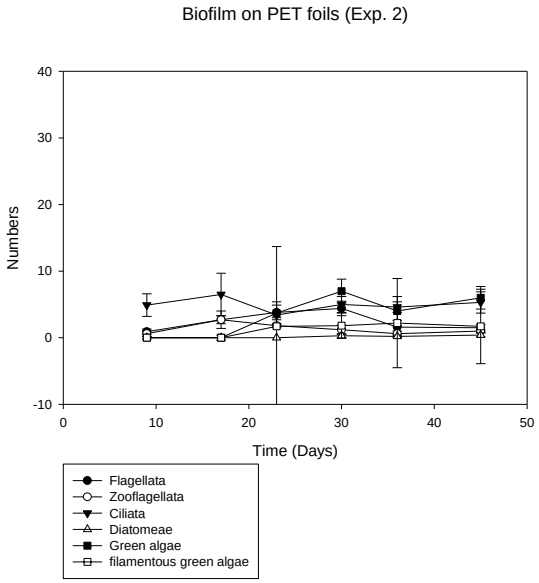
3.5. Non-Bacterial Biofilm and Aging of the Foils

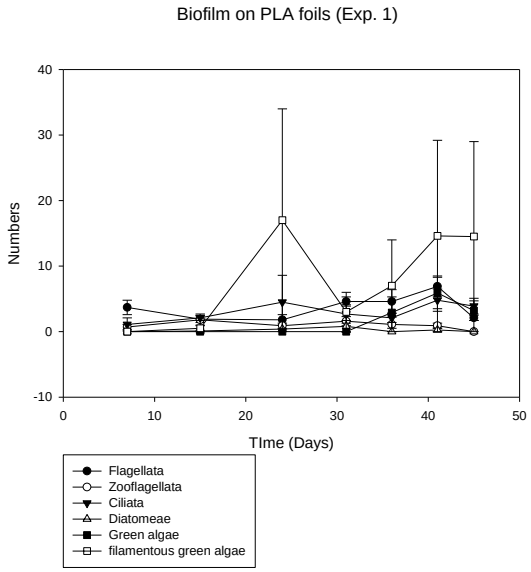
In the 1st run with the new foils, early colonization of the foils with biofilm within one week was observed, consisting of ciliates and flagellates. Diatoms and green algae, and esp. filamentous algae started to colonize the foils after more than 35 days (**Figure 3**). There were slightly more ciliates on PET foils (p: 0.07; paired t-test). For the other taxonomic groups, there were no differences between the type of foils. During the 1st experiment, the colonization with diatoms was significantly lower than that with ciliates, flagellates and filamentous algae on both types of foils (one Way ANOVA on Ranks, PET: p: 0.008; PLA: p: 0.002). After 45 days some material degradation at the

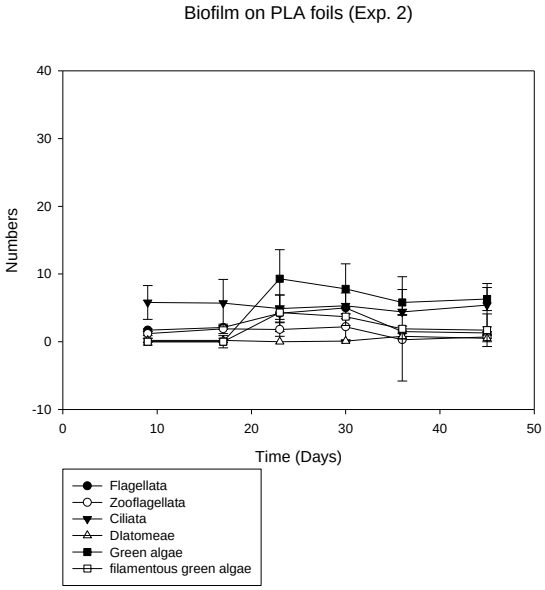
scratches for the numberings was observed on some PLA foils. The colonized biofilm did not differ in the composition before and after rinsing with tap water. However, the absolute numbers of the taxa were lower after rinsing, *i.e*. biofilm was partly loosely attached to both types of foils. Moreover, there was no weight loss of the foils (%; N = 12: PET: x −0.566, SD: 1.39 and PLA: x: 0.036, SD: 1.49), the high variation might be due to weighing errors.

In the 2nd run on the 45-day aged foils the colonization scheme was similar to that of the 1st run: ciliates and flagellates as early colonizers, followed by diatoms and green algae (**Figure 3**). Filamentous algae colonized after more than 30 days. Both types of foils were colonized equally with no statistical difference for any of the taxa. During the 2nd experiment, the colonization with diatoms was significantly lower than that with ciliates and filamentous algae on both types of foils (one Way ANOVA on Ranks, PET: p: 0.002; PLA: p: 0.008). By the end of the


Biofilm on PET foils (Exp. 1)
Numbers
Time (Days)
Flagellata
Zooflagellata
Ciliata
Diatomeae
Green algae
filamentous green algae


Biofilm on PET foils (Exp. 2)
Numbers
Time (Days)
Flagellata
Zooflagellata
Ciliata
Diatomeae
Green algae
filamentous green algae


Biofilm on PLA foils (Exp. 1)
Numbers
TIme (Days)
Flagellata
Zooflagellata
Ciliata
Diatomeae
Green algae
filamentous green algae


Biofilm on PLA foils (Exp. 2)
Numbers
Time (Days)
Flagellata
Zooflagellata
Ciliata
Diatomeae
Green algae
filamentous green algae

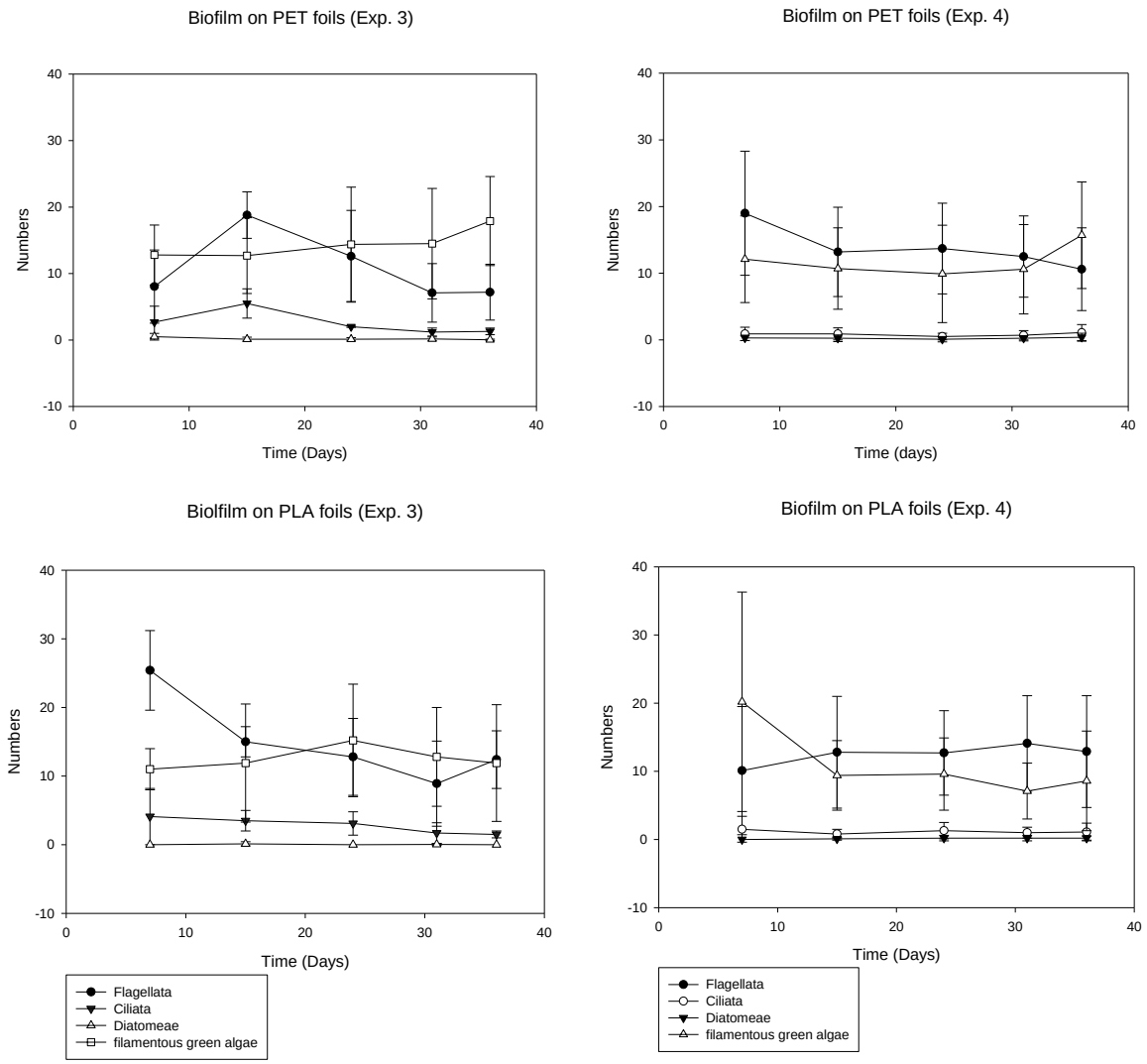


Figure 3. Non-bacterial biofilm colonization (Means and Sd) of the PET and PLA foils throughout the 4 subsequent experiments with weekly change of water from Lake Constance. Determination of selected taxa on a randomly selected part in the middle of the foils (400× magnification).

experiment, both foils were densely covered with filamentous algae as in the 1st run. Rinsing with tap-water at the end of the 2nd experiment did not change the biofilm taxa composition, but the absolute numbers were lower, *i.e*. the biofilm was loosely attached to both types of foils. The weight loss (%) of the foils at the end of the experiment was again very low and variable (N = 12: PET: Mean: 0.12; SD: 0.97 and PLA Mean: 0.84, SD: 0.81), Again, some further fissures at the numbering scratches in a few PLA foils could be seen in the 2nd run.

Similar results were found in the 3rd and 4th run. Aggregation of organic matter associated with biofilm organisms increased during the 45 d of exposure, Filamentous green algae and flagellates were dominant on both types of foils and independent on the age of the foils (**Figure 3**). Throughout the 6 months of experiments, the biofilm colonization was reproducible in the 4

subsequent exposures of 45 d and did not differ between PLA and PET foils as substrate (paired t-test).

However, comparing one taxa group throughout the course of the 4 experiments on one type of foil, significant differences occurred for PET foils in the number of flagellates (p: 0.001), ciliates (p: 0.006) and filamentous green algae (p: 0.007). For PLA, foils significant differences occurred in the amount of flagellates (p: 0.001) and ciliates (p: 0.001) (RM ANOVA on ranks). This shows that there were seasonal differences in the quantitative composition of the biofilms throughout the 180 days in the water from Lake Constance used for the experiments (**Figure 4**).

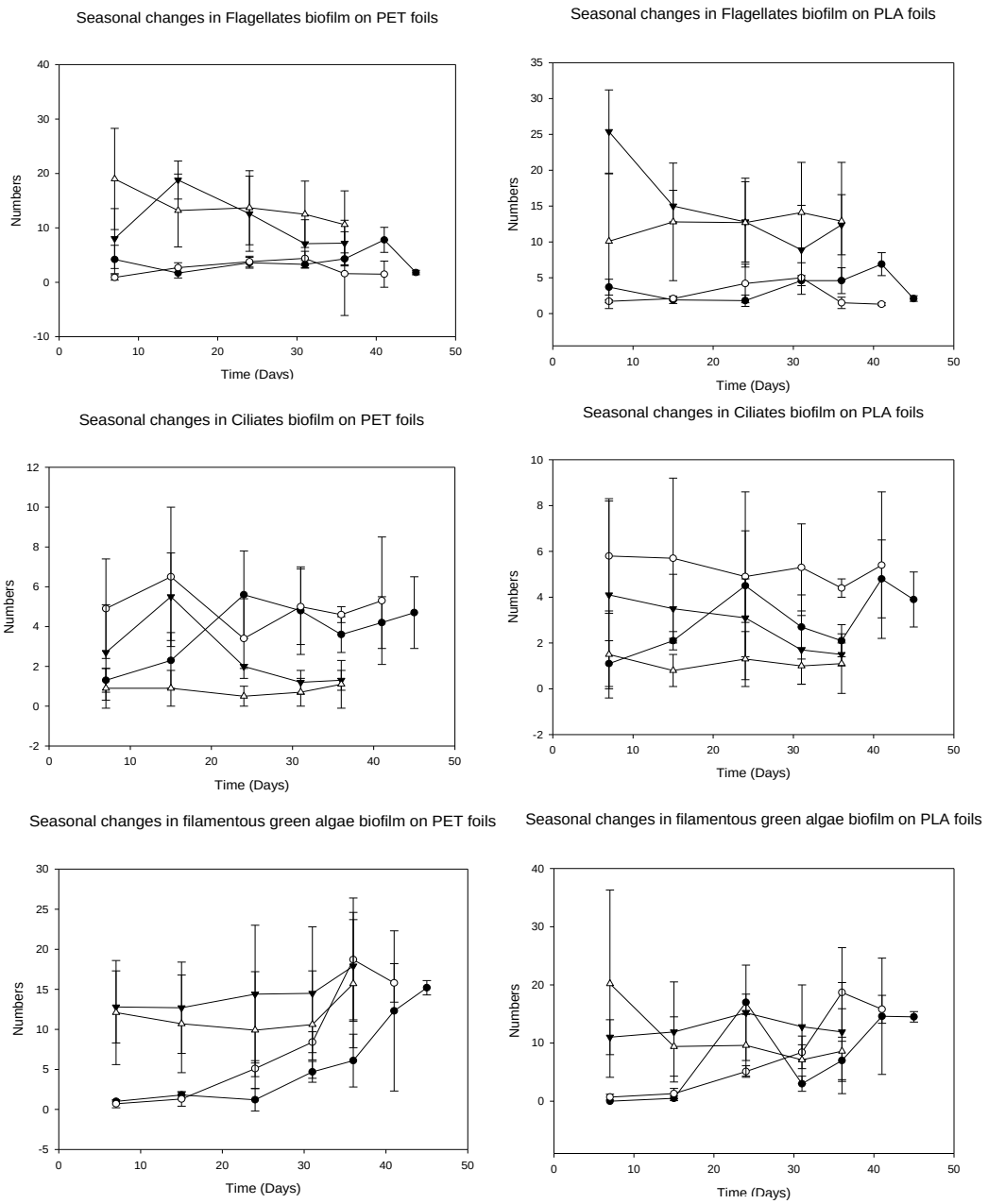


Figure 4. Seasonal changes in biofilm composition on both PET and PLA foils: Exp 1 (spring): black cir-

cles, Exp. 2 (early summer): white circles, Exp. 3 (summer): black triangles, Exp. 4 (autumn): white triangles.

The PLA foils especially showed damage due to handling, such as fissures at the scratches for numbering and deformation at the edges. This observation can be attributed to the fact that the PLA foil was a monolayer, hence degradation might start faster than in the PET GAG 3-layer foil. During the 4th experiment, some PLA foils became intransparent, which might be due to hydrolysis. However, the visually observed damages were rare and of a minor extent, *i.e*. even the biopolymer PLA could not significantly be degraded within 180 days (at the end of exp. 4). The dry weight loss after the 4th experiment showed slight increases such as 0.05% (SD: 0.02%) for PLA and 0.04% (SD: 0.06%) for PET, which might be due to weighing errors and/or biofilm colonization.

4. Discussion

4.1. Toxicity

The potential toxicity generated by plastic foils (PLA, PET) was studied over 180 days in 4 subsequent experiments of 45 days. Mortality of *G. fossarum* exposed to new or aged (45, 90, 135 d) PLA foils was slightly higher compared to PET foils and controls. During the 4th experiment with 135 d old foils, mortality was equally high in the treatments with PLA or PET foils, whereas control mortality was low.

Gammarids used the plastic foils as substrate and hiding places but did neither graze nor destroyed them. Toxicological studies on the effects of macroplastic on freshwater invertebrates are lacking, to our knowledge. Toxicity studies with microplastics are only of restricted relevance as microplastic toxicity is mostly caused by mechanical effects, such as clogging the gut system and tissue inflammation, where small microplastics pass the gut membranes [24] [25]. Weber *et al*. [26] studied the effects of PET microplastic (10 - 150 µm; 0.8 - 4000 g/ml) on *Gammarus pulex* and did not find any effects on survival after 48 days of exposure. Mateos-Cardenas *et al*. [27] showed that *G. duebeni* took up microplastic particles (1 - 1000 µm) adhered to *Lemna minor* during feeding, but to a lower extent (1 - 2 particles in the gammarid gut of 28.6% of the exposed gammarids). In a previous study, PLA particles (0.7 - 3 mm, grey, irregular shape) were not taken up by *G. fossarum*, probably due to the large size, compared to microplastic particles of PE, PP, and PBT, which were smaller in size (ca. 0.5 mm) [28]. During a 4 week' exposure to these three different microplastic particles, no differences in mortality, feeding, and the locomotor activity of *G. fossarum* were found compared to the control group [28]. In the current study, spontaneous locomotor activity did not differ during any of the four subsequent experiments between the treatments, while ventilation activity was slightly higher in the PLA treatments. Increased ventilation is often regarded as toxic stress in aquatic

animals, due to low pH or elevated metal levels [29]. This might indicate some migration of lactic acid out of the biopolymer; however, no changes in pH could be recorded. Feeding activity on the alder leaves did not differ between the treatments, which indicates no effects on the two types of plastic foils.

It appeared that especially in the beginning, during the 1st experiment with new foils, PLA generated more potential for toxicity than PET. This might be due to the fact that PLA which is a monolayer biopolymer and might be more susceptible to chemical attacks as compared to the 3-layer copolymer PET-GAG. Potential migrants from PLA are monomers and dimers of lactic acid, which are regarded as environmentally safe as they represent common cellular components with rapid degradation [30]. Moreover, PLA foils lost transparency over 180 days of exposure and became opaque. This has been described as a change in the degree of crystallinity and was observed by Maiza *et al.* [31] in plasticized PLA experiments at 100˚C.

The toxicity of macroplastics might mainly arise from the release of toxic substances into the water. Evidence has been provided by studies on mineral water bottles, where some authors found traces of acetaldehyde and formaldehyde in water stored in PET bottles [32] [33]. Xeno-estrogens found in PET bottled mineral water showed effects in both the Yeast Estrogen Screen (YES hERa) test and in chronic exposures to the gastropod *Potamopyrgus antipodarum* (56 d). However, this might be due to contamination from the source of the water, as this finding could not be reproduced later [34]. PET caused cytotoxic and cytostatic effects on human lymphocytes, but no genotoxicity after 8 weeks of water storage [35]. Also, Ceretti *et al.* [36] did not find any genotoxicity (Microtoxtest, Micronucleustest, Comet assay) from migrating substances after 10 days in PET bottled and sparkling mineral water. PET-bottled water extracts (Solid Phase Extraction, SPE) did not induce either toxic or endocrine effects in *in-vitro* tests [37]. Migration of toxic compounds from plastics highly depends on the conditions and clearly increased with temperature, sunlight exposure and carbonation of the water [36] [37].

4.2. Degradation

PLA showed faster degradation and was more prone to fissures than PET, also showing in-transparency and deformation of the edges within 180 days, whereas the 3-layer PET-GAG foil remained morphologically unchanged. PLA has been reported to adsorb water, followed by loss of transparency and increasing fragility [38]. PET is reported to be very stable for 15 years [39], with degradation times of 35 mm thick PET estimated to occur in 27 years [40] to 93 years [41]. The slow degradation of PLA during 180 days in our experiments might be due to low temperatures (10˚C) and neutral pH in the water from Lake Constance as well as the lack of sunlight during the exposures. Karamanlioglu *et al*. (2017) [15] reported a rapid microbial degradation of PLA in compost at 50˚C - 60˚C for 60 days, supported by findings of PLA-depolymerase activity at 60˚C in *Bacillus* strains [42].

Mutsuga *et al*. [43] found that pure PLA sheets lost about 0.28 - 15 µg/cm weight due to lactic migration after 180 d (at 40˚C). Weight loss of the foils was low in our experiments (<1%) and decreased from the 1st to the 4th experiment, *i.e*. with increasing age of the foils. The material loss might have been compensated by increased weight due to the adsorption of biofilm and water molecules.

4.3. Biofilm

The non-bacterial biofilm on PET and PLA foils consisted of early colonizers such as ciliates and flagellates followed by diatoms and filamentous green algae at last. Also, conglomerates of organic matter associated with biofilm organisms and extracellular polymers increased over time. There was no significant difference in the colonization of these taxa between the two types of glossy plastic foils.

Biofilm communities adhered to plastic polymers contribute to its degradation by microbial activities, such as degrading ester bonds via enzymatic hydrolysis. Microbes excrete metabolic enzymes, *e.g.* depolymerase and extracellular substances [44]. About 90 microbial genera have been found to degrade plastics, *e.g*. for PLA include *Tritirachium album* (proteinase K) which favors hydrolysis whereas a strain of *Commamonas testosterone* degrades terephthalate to protocatechuate, which is further degraded by *Pseudomonas putida* [44]. The composition of bacterial communities on plastics exposed to seawater differed between different polymers (esp. PVC compared to other plastics) and stages of biofilm succession, but these differences disappeared with an exposure time of 2 months [45]. However, Kirstein *et al*. [46] found only slight differences in biofilm on different types of synthetic polymers during 15 months' exposure in seawater, with the exception of some polymer substrate-specific taxa.

5. Conclusion

During 45 days of exposure of *G. fossarum* to PET or PLA foils, no effects on mortality, feeding, and behavior could be seen independent on the age and stage of degradation of the foils. No significant weight loss of the foils was found, indicating the high stability of both types of plastic polymers. Both plastics serve as a substrate for similar biofilm communities. PLA started to degrade morphologically while PET remained unchanged for 180 days. However, the biopolymer PLA could not be degraded to a significant extent within 180 days of exposure to natural lake water at environmentally relevant temperatures.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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