Supporting Information

Effects of Microplastic on fitness and PCB bioaccumulation by the Lugworm *Arenicola marina* (L.)

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Results of pilot experiment
Seven Tables
Seven Figures

Pilot experiment: Methods, results and discussion

In this work two experiments were performed, a pilot experiment and the main bioassay. The pilot experiment followed previously published procedures and is addressed here. The bioassay is described in the main paper. The overall discussions in the main paper cover the outcomes of both experiments.

Materials and Methods

Prior to the pilot experiment, the test organisms cleared their guts in clean sea water overnight and were randomly assigned to the test beakers, such that each beaker contained a group of 5 *Arenicola marina* individuals with a known weight. The average group weight was 26.5 g and the variation (SD) among groups was 5.2 g. For the pilot experiment, we used closed 2 L glass test beakers with a diameter of 19 cm and a height of 9 cm. Due to the use of small test beakers, the water characteristics were variable over time. Additional to the plastic effect, the impact of these water quality variables on survival, activity and weight could be established. The test beakers contained ±2.2 kg sediment (WW, 5 cm thick layer) and ±0.7 L sea water (water layer of 2.5 cm). The lower two-third of the sediment did not contain plastic. The upper one-third of the sediment (1.7 cm, 0.72 kg) contained the polystyrene microplastic (PS), because the lugworm feeds on the upper sediment layer [1]. Effects of PS were assessed by exposing *A. marina* to a range of PS concentrations: 0, 1, 3, 10, 30 and 100 g PS/L in PCB contaminated sediment. These concentrations agree to 0, 0.074, 0.22, 0.74, 2.2 and 7.4 % DW PS in the sediment. Mixing of the sediment occurred during four weeks prior to the pilot experiment. The systems stabilized during one day, before addition of the lugworms. All treatments were performed in quadruplicate and randomly assigned to the test beakers. We applied aeration and refreshed the water twice a week. The dissolved oxygen saturation, temperature, pH, salinity, NH_4^+ and NO_2^- averaged 7.9 mg/L (79 % saturation), 13.4 °C, 8.0, 31 ‰, 6.4 mg/L and 0.07 mg/L respectively. Analysis of the water quality variables and the endpoints were done as in the bioassay, described in the main paper.

Results and Discussion

Effects of water quality variables. The use of small test systems showed the sensitivity of the organisms to variable water conditions. There were significant effects of the water quality variables on the endpoints (Fig. S2). We found a positive relation between the average amount of days that an organism survived in the experiment and average oxygen

concentration (Regression, p=0.002). Furthermore, a positive relation between average activity and average oxygen concentration (p=0.012) was detected. Our findings of a significant negative effect of low oxygen concentrations on the activity are in accordance with Cadée [2] who mentions that feeding might stop at low oxygen levels in the overlying water. High mortality might have been an indirect effect, initiated by starvation as a result of the negative effect of oxygen deficiency on the feeding activity of *A. marina*. This would imply that the duration of the deficiency was crucial, which is in agreement with our observations that high mortality started after one and a half week of exposure. However, literature shows that *A. marina* is assumed to be tolerant to oxygen deficiency [3]. In this pilot experiment, the lowest measured concentrations were on the first day 2.5 mg/L and remained for the rest of the experiment above 5.5 mg/L, while *A. marina* can tolerate oxygen concentrations as low as 3.2 - 4.1 mg/L that occur during ebb [3]. Additionally, *A. marina* survived concentrations as low as tenths or even hundredths of mg/L in a laboratory experiment and calculations imply that *A. marina* can survive 71 minutes without external oxygen supply [3]. For wet, dry and AFD weight (WW, DW, AFDW) loss, a significant negative relation with salinity was discovered (p=0.031, p=0.050, p=0.002), which agrees to previous reports [4]. For AFDW loss, a significant positive relation with pH was determined (p=0.031). As far as we know, this has not been quantified before and might only count for the observed limited pH range (SI Table S1). We found no relation between the endpoints and temperature, NH_4^+ and NO_2^- concentration. Furthermore, there was no significant spatial pattern in water quality variables and endpoints. By using the significantly influential water quality variables as covariables, the relation between the investigated endpoints and the treatment did not change. Nevertheless, we conclude that a set up in which water quality variables can be maintained constant is required in order to detect effects of PS. This was implemented in the bioassay described in the main text.

Effects of microplastic on fitness and performance of *A. marina*. *Survival.* The total mortality was 48.3 % in the pilot experiment, which is much higher than the average annual mortality of 22 % observed in the Dutch Wadden Sea [5]. Irrespective of the plastic concentration (ANOVA, p=0.457) (Fig. S3.A), survival was low. Also, no relation between the treatments and the amount of days that an organism survived was revealed (p=0.460). The mortality rate did not significantly differ between the treatments (ANOVA, p=0.561) (Fig. S6.A), with K_{mort} = -ln(B/B0)/t with B=survival n at time t, B0=survival n at start of the experiment and t is time in days [6]. It was ascertained that the contamination with PCBs did not result in PCB toxicity or plastic avoidance behaviour by *A. marina*, because of the use of

low PCB concentrations (SI Table 2). The measured PCB concentrations were 350 times lower than toxicity thresholds [7].

Ingestion of plastic. The organisms that survived the entire 28 days exposure period and were allowed to clear their guts had no plastic in their system, even those being exposed to the highest plastic concentrations. While in some of the organisms that died during the experiment, plastic was encountered after dissection. The difference in the amount of internal plastic particles between organisms that did or did not survive the exposure period was significant (Mann-Whitney U test, $p=3.21\times10^{-10}$). This supports the supposition that *A. marina* ingested PS particles of ≥400 µm but that these particles did not accumulate in this organism.

Gut content. The material that was egested during gut clearance overnight contained plastic particles. Here, no differentiation is made between internally detected plastic (in worms that died during the experiment) and plastic egested during gut clearance (by worms that survived the experiment), i.e. both are called gut content. By doing so, a negative relation between the amount of plastic particles in the gut content and survival was identified (Mann-Whitney U test, $p=9.34\times10^{-7}$) (Fig. S4.B). Linear regression showed that the amount of plastic particles in the gut content increased with the plastic concentration to which *A. marina* was exposed (1-sided P-value=0.023). Because of the non-normality of the data, we further investigated this relation with the Kruskal-Wallis test, which gave significant differences between treatments ($p=2.48\times10^{-4}$). As a post hoc test, pair-wise comparisons of the treatments were done with the Mann-Whitney U test, which revealed three significantly different treatment classes (1-sided p=0.004, $p=3.94\times10^{-5}$, p=0.025 respectively). Fig. S4.A shows that the amount of plastic particles in the gut content increased significantly over the following grouped treatments: low (treatment 0 and 0.074 %), middle (treatment 0.22 and 0.74 %), high (treatment 2.2 and 7.4 %). The findings imply a positive relation between environmental plastic concentration and ingestion of plastic. To see if the amount of plastic particles in the gut content was proportional to the exposure plastic concentrations, the gut volume was calculated. The average faeces production of 2.4ml/day during the winter and a defecation time of 20 minutes from Cadée [2] were used to calculate a gut volume of 3.33×10^{-5} L. The weight of our used plastic particles ranged from 3.5×10^{-8} - 1.2×10^{-6} g (radius of 0.2 - 0.65 mm, density of 1.05 g/L [8]). By using these numbers, the calculated gut concentrations ranged in the 0.22 % treatment up to 3.9×10^{-4} - 1.3×10^{-2} % (1 - 3 orders of magnitude lower than the exposure concentration), in the 0.74 % treatment up to 7.8×10^{-5} - 2.7×10^{-3} % (2 - 4 orders of magnitude

lower), in the 2.2 and 7.4 % treatments up to 7.8×10^{-4} - 2.7×10^{-2} % (2 - 4 orders of magnitude lower).

Activity. The activity averaged 0.43 heap/individual/day (SD 0.41). Average activity did not significantly differ between the treatments (ANOVA, p=0.708) (Fig. S1.B), also not by considering the activity in the first one, two or three weeks only. This was investigated because it could be speculated that in a later stage of the experiment, treatment effects become overwhelmed by captivity disadvantages, but this was not the case. The activity in the control treatment peaked after two weeks exposure, but was not significantly higher than the activity in the other treatments (Fig. S3.A). The lack of fit between treatment and activity in the pilot experiment might be explained by the impact of the water quality variables. The oxygen concentration had a significant impact on the activity. To compare the two experiments, we included the activity between the second and ninth day only and excluded the treatments that were not executed in both the pilot experiment and the bioassay (0.22 % and 2.2 % from the pilot experiment and treatment Oesterput from the bioassay). The calculated activity was 0.27 heap/individual/day in the pilot experiment and 0.42 heap/individual/day in the bioassay, which is a significant difference (Two samples t-test, $p=2.24\times10^{-4}$) (SI Fig. S5.A). This is interpreted as worms having a better condition in the bioassay.

Weight loss. Weight loss was observed in all but one group. The mean WW loss was 1.33 g/individual (25.1 %, SD 0.98), the mean DW loss was 0.31 g/individual (36.1 %, SD 0.12) and the mean AFDW loss was 0.25 g/individual (36.5 %, SD 0.098). There was no significant relation between plastic concentration and absolute WW/DW/AFDW loss (Regression, p=0.810, p=0.823, p=0.265 respectively) (Fig. S3.C), also not by taking the relative instead of the absolute loss. Some of the worms emerged from the sediment several days before the end of the experiment. Because this was assumed to be a sign of a weak condition, we investigated whether there was a relation between weight loss and the position in the test beaker (in or above the sediment), which was not the case (Two samples t test, p=0.541, p=0.518, p=0.693) (Fig. S6.B). The results did not change when worms in and above the sediment were analysed separately. Similar to the activity analysis, an effect of plastic on weight loss could be invisible in this pilot experiment because it might not have been the main stressor in this experiment. It could be that the weight losses were highly impacted by the variability of the salinity, which is in accordance with the findings of Spaargaren and Weber [4], and the pH. Mortality of heavy (adult) worms might, next to individual weight loss, explain the observed mean weight loss in the pilot experiment, but the latter one is expected to play a major role [9]. To compare the two experiments, the treatments 0.22 and 2.2 % from

the pilot experiment and treatment Oesterput from the bioassay were excluded from analysis. The DW and AFDW loss in the bioassay turned out to be significantly lower than in the pilot experiment (Two samples t test, $p=3.74\times10^{-6}$, $p=8.24\times10^{-9}$) (SI Fig. S7.B). The WW losses did not significantly differ between the two bioassays ($p=0.267$). The organisms in the bioassay did not preliminary clear their guts, while the organisms in the pilot experiment did. As a result, the start weight in the bioassay was overestimated and the difference in weight loss compared to the pilot experiment even larger than noted.

References

1. Retraubun, A.S.W., M. Dawson, and S.M. Evans, *The role of the burrow funnel in feeding processes in the lugworm Arenicola marina (L.).* Journal of Experimental Marine Biology and Ecology, 1996. **202**(2): p. 107-118.
2. Cadée, G.C., *Sediment reworking by Arenicola marina on tidal flats in the Dutch Wadden Sea.* Netherlands Journal of Sea Research, 1976. **10**(4): p. 440-460.
3. Alyakrinskaya, I.O., *Some ecological features of the lugworm Arenicola marina L. (Annelida, Polychaeta) and its morphological and biochemical adaptations to burrowing.* Biology Bulletin, 2003. **30**(4): p. 411-418.
4. Spaargaren, D.H. and R.E. Weber, *Osmotic responses in the coelomic fluid of Arenicola marina subjected to salinity change.* Netherlands Journal of Sea Research, 1979. **13**(3-4): p. 547-561.
5. Beukema, J.J. and J. De Vlas, *Population parameters of the lugworm, Arenicola marina, living on tidal flats in the Dutch Wadden Sea.* Netherlands Journal of Sea Research, 1979. **13**(3-4): p. 331-353.
6. Gourley, S.A. and Y. Kuang, *A stage structured predator-prey model and its dependence on maturation delay and death rate.* Journal of Mathematical Biology, 2004. **49**(2): p. 188-200.
7. MacDonald, D.D., L.M. Dipinto, J. Field, C.G. Ingersoll, E.R. Lvong, and R.C. Swartz, *Development and evaluation of consensus-based sediment effect concentrations for polychlorinated biphenyls.* Environmental Toxicology and Chemistry, 2000. **19**(5): p. 1403-1413.
8. Andrady, A.L., *Microplastics in the marine environment.* Marine Pollution Bulletin, 2011. **62**(8): p. 1596-1605.
9. Beukema, J.J., *Seasonal changes in the biomass of the macro-benthos of a tidal flat area in the Dutch Wadden Sea.* Netherlands Journal of Sea Research, 1974. **8**(1): p. 94-107.
10. Van Noort, P.C.M., J.J.H. Haftka, and J.R. Parsons, *Updated Abraham Solvation Parameters for Polychlorinated Biphenyls.* Environmental Science and Technology, 2010. **44**: p. 7037-7042.

Table S1: Water quality variables in the pilot experiment and the bioassay.

Water quality variables	Pilot experiment		Bioassay	
	Mean	**Range**	**Mean**	**Range**
Oxygen (mg/L)	7.87	2.50-10.51	10.07	9.66-11.15
Oxygen (%)	78.9	29.5-98.7	94.2	91.7-103.2
Temperature (°C)	13.4	11.2-15.1	12.3	11.2-13.5
pH	8.03	7.61-8.30	8.16	8.07-8.25
Salinity (‰)	30.9	23.4-33.3	32.1	31.7-33.0
NH_4^+ (mg/L)	6.4	2-10	0.2	0-1
NO_2^- (mg/L)	0.07	0.0-0.6	0.03	0.0-0.2

Table S2: Concentrations of most abundant PCBs and sum of all PCBs (ΣPCBs) in the contaminated sediment (µg/kg DW) (mixture of Diemen and Oesterput sediment).

PCB congener	**Concentration** (µg/kg DW)
PCB 28	0.11
PCB 52	0.25
PCB 101	0.62
PCB 118	0.33
PCB 138	0.75
PCB 149	0.76
PCB 153	0.93
PCB 170	0.28
PCB 180	0.56
ΣPCBs	5.28

Table S3: Statistics PCB analysis[a].

	$logK_{OW}$[b]	ANOVA[c] p-value	# values >dl[d]	Treatment with all values < dl[e]
PCB congener:				
PCB 18	5.43	7.095×10^{-7}	23	-
PCB 20	5.58	1.287×10^{-5}	21	Oesterput
PCB 28	5.58	8.133×10^{-8}	25	-
PCB 29	5.58	0.236	20	0 %
PCB 31	5.58	8.663×10^{-8}	27	-
PCB 44	6.02	1.456×10^{-8}	27	-
PCB 52	6.02	1.504×10^{-8}	27	-
PCB 101	6.42	6.097×10^{-8}	27	-
PCB 105	6.51	4.840×10^{-9}	27	-
PCB 118	6.51	5.064×10^{-8}	27	-
PCB 138	6.82	7.485×10^{-7}	27	-
PCB 149	6.66	3.096×10^{-8}	27	-
PCB 153	6.82	6.324×10^{-7}	27	-
PCB 155	6.50	3.818×10^{-5}	24	-
PCB 170	7.21	3.326×10^{-7}	27	-
PCB 180	7.21	7.120×10^{-7}	27	-
PCB 194	7.61	0.540	8	Start, Oesterput, 0 %
PCB 204	7.39	0.583	12	Sediment, Start, Oesterput
PCB 209	8.27	0.001	12	Start, Oesterput, 0 %
ΣPCBs		5.112×10^{-8}	27	-

[a] Analysis of differences between treatments.

[b] From [10]

[c] Appearance of differences between treatments, investigated with ANOVA.

[d] Total amount of values: 27.

[e] In some cases, the outcomes of all quadruplicates within a treatment were below the detection limit.

Table S4: SumPCB (ΣPCB) concentrations in the various treatments of the bioassay.

	Mean (µg/kg)	**SD**	**%SD**
Sediment	1.84	0.22	11.7
Non-exp. lugworms	2.43	0.22	9.3
Treatment C	2.40	0.61	25.5
Treatment 0 %	7.00	1.35	19.2
Treatment 0.074 %	9.01	1.76	19.5
Treatment 0.74 %	8.54	1.48	17.4
Treatment 7.4 %	8.31	2.17	26.1

Table S5: BSAFs.[a)]

PS Treatment	**0 %**	**0.074 %**	**0.74 %**	**7.4 %**
PCB congener:				
PCB 18	14.80	22.69	18.30	20.15
PCB 20	12.33	14.06	18.39	17.94
PCB 28	35.29	40.21	26.56	46.14
PCB 29	-	0.40	3.08	6.89
PCB 31	21.03	26.64	22.47	27.04
PCB 44	31.52	31.92	39.26	34.75
PCB 52	31.35	39.40	35.92	40.45
PCB 101	29.59	32.48	31.73	32.71
PCB 105	10.60	19.36	18.91	13.90
PCB 118	27.52	29.01	29.80	29.95
PCB 138	33.86	36.20	33.79	37.27
PCB 149	22.75	24.73	24.93	24.87
PCB 153	27.23	29.46	29.91	30.45
PCB 155	82.31	106.72	97.10	100.57
PCB 170	11.35	12.61	12.48	11.80
PCB 180	10.29	10.53	10.56	10.96
PCB 194	-	20.72	10.51	10.41
PCB 204	-	-	-	-
PCB 209	-	2.60	2.47	4.23
ΣPCBs	20.47	23.40	22.84	24.48

[a)] Concentration in the organism (µg/kg) / concentration in sediment (µg/kg), both on a DW basis.

Table S6: Tissue concentration ratios (Contaminated sediment exposed / clean sediment exposed).

PS Treatment	**0 %**	**0.074 %**	**0.74 %**	**7.4 %**
PCB congener:				
PCB 18	4.32	7.49	7.98	5.80
PCB 20	-	-	-	-
PCB 28	4.58	5.80	3.71	5.93
PCB 29	-	0.16	0.81	1.70
PCB 31	5.33	7.47	6.28	6.81
PCB 44	3.71	4.23	5.07	4.08
PCB 52	8.07	11.46	10.14	10.41
PCB 101	4.28	5.30	5.02	4.70
PCB 105	3.77	7.75	7.36	4.89
PCB 118	2.40	2.86	2.85	2.59
PCB 138	2.13	2.56	2.32	2.32
PCB 149	3.35	4.10	4.01	3.64
PCB 153	2.11	2.58	2.53	2.34
PCB 155	2.26	3.30	2.90	2.72
PCB 170	3.51	4.37	4.22	3.61
PCB 180	5.38	6.19	6.02	5.68
PCB 194	-	-	-	-
PCB 204	-	-	-	-
PCB 209	-	-	-	-
ΣPCBs	2.92	3.76	3.56	3.47

Table S7: Tissue concentration ratios (Contaminated sediment with PS / Contaminated sediment without PS).

PS Treatment	**0.074 %**	**0.74 %**	**7.4 %**
PCB congener:			
PCB 18	1.73	1.85	1.34
PCB 20	1.29	1.63	1.43
PCB 28	1.27	0.81	1.29
PCB 29	-	-	-
PCB 31	1.40	1.18	1.28
PCB 44	1.14	1.36	1.10
PCB 52	1.42	1.26	1.29
PCB 101	1.24	1.17	1.10
PCB 105	2.06	1.95	1.30
PCB 118	1.19	1.19	1.08
PCB 138	1.20	1.09	1.09
PCB 149	1.22	1.20	1.08
PCB 153	1.22	1.20	1.11
PCB 155	1.46	1.28	1.20
PCB 170	1.25	1.20	1.03
PCB 180	1.15	1.12	1.06
PCB 194	-	-	-
PCB 204	3.64	1.24	1.46
PCB 209	-	-	-
ΣPCBs	1.29	1.22	1.19

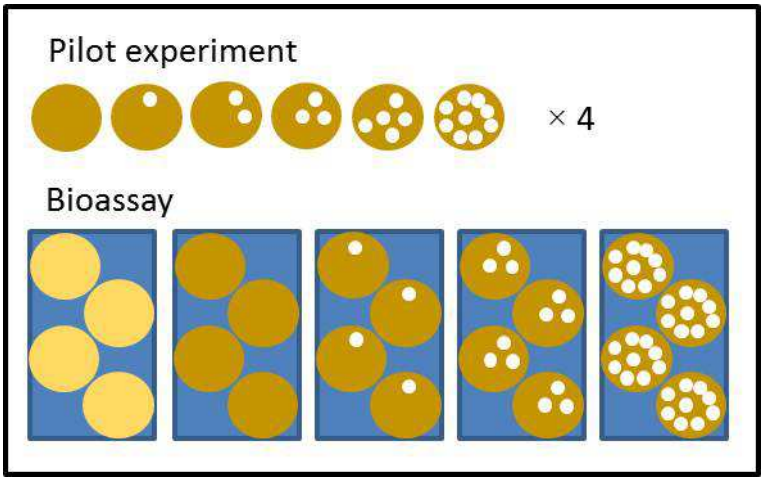

Figure S1: Schematic presentation of the experimental set up. The pilot experiment consisted of six treatments in 2 L test beakers. The bioassay used five treatments of which one in a PCB clean environment and four in a PCB contaminated environment and was carried out in 2L test beakers in large aquaria. The amount of white dots visually indicates differences in plastic concentration.

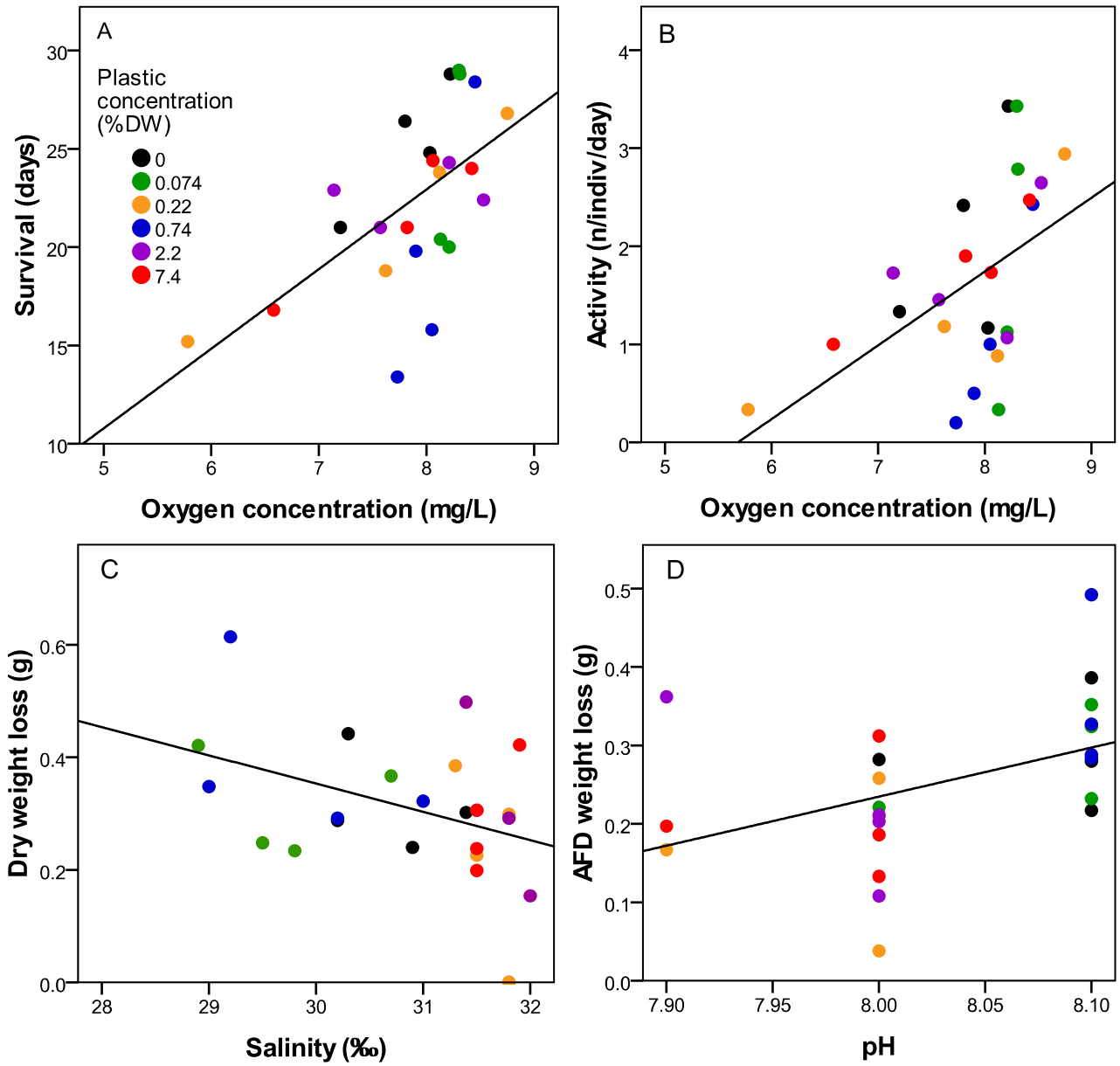

Figure S2: **Pilot experiment**; Influence of water quality variables. A. Relation between oxygen concentration and amount of days of survival. B. Relation between oxygen concentration and mean activity. C. Relation between salinity and DW loss. D. Relation between pH and AFDW loss.

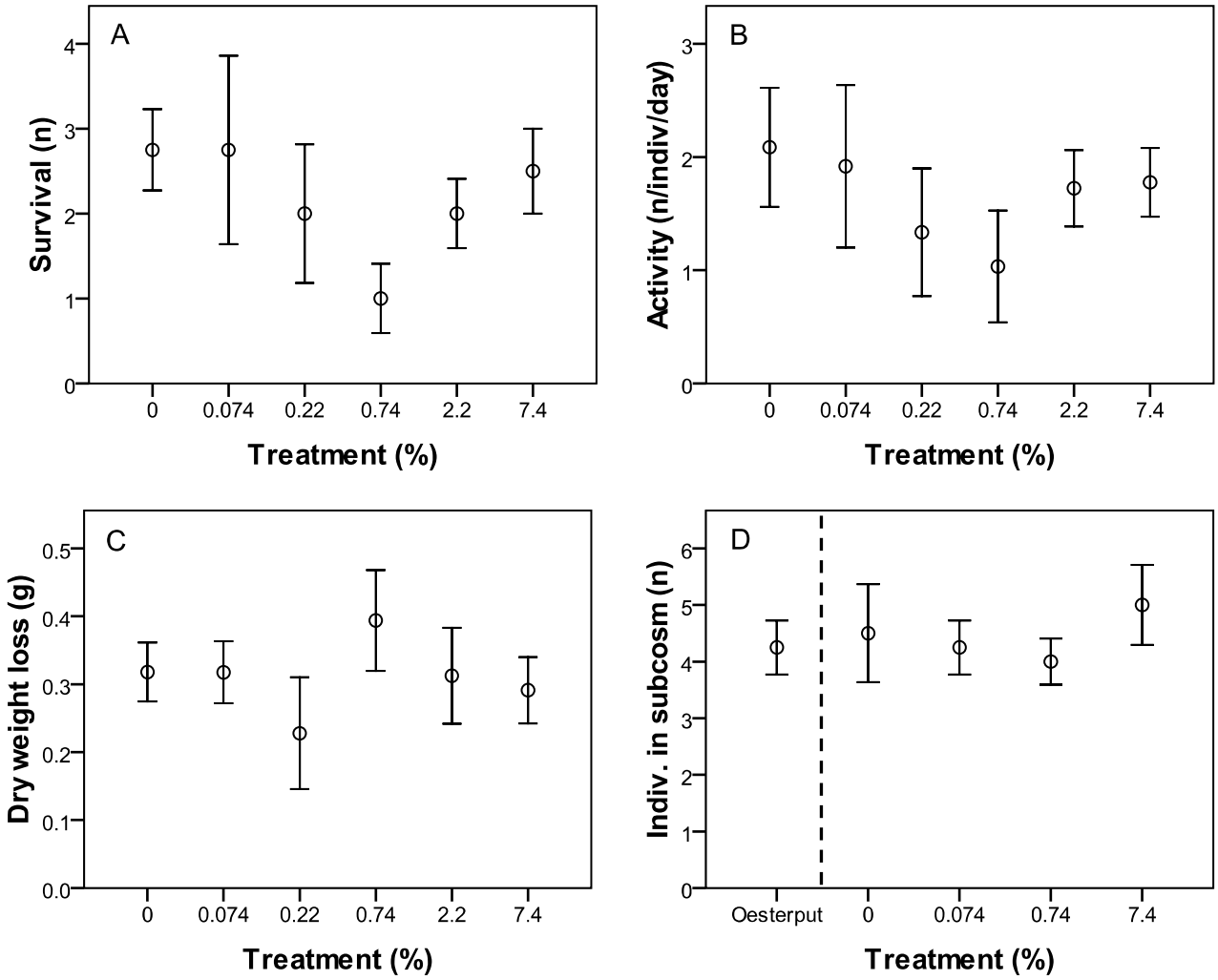

Figure S3: A. Pilot experiment; Difference in total amount of organisms that survived between treatments. B. Pilot experiment; Difference in activity between treatments. C. Pilot experiment; Difference in DW loss between treatments. D. Bioassay; Difference in the amount of organisms that remained in their test beaker between treatments. The bars indicate mean ± standard error (SE).

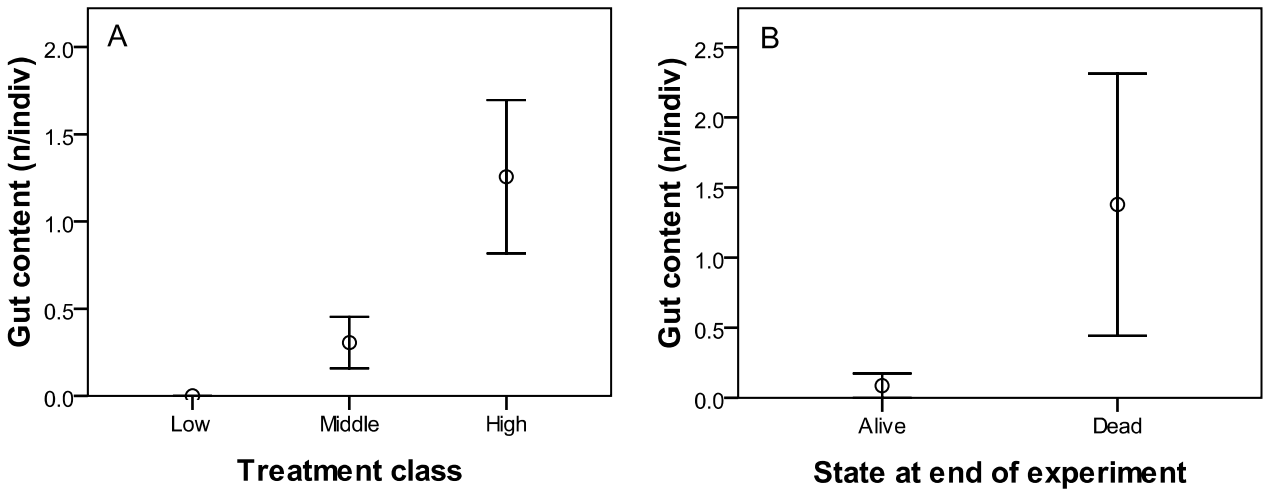

Figure S4: **Pilot experiment.** A. Difference in amount of plastic particles in gut content between exposure plastic concentrations. Expressed in treatment classes (Low = 0 and 0.074 %. Middle = 0.22 and 0.74 %. High = 2.2 and 7.4 %). B. Amount of plastic particles in the gut content of organisms that died during the experiment. compared to the organisms that survived. The bars indicate mean ± standard error (SE).

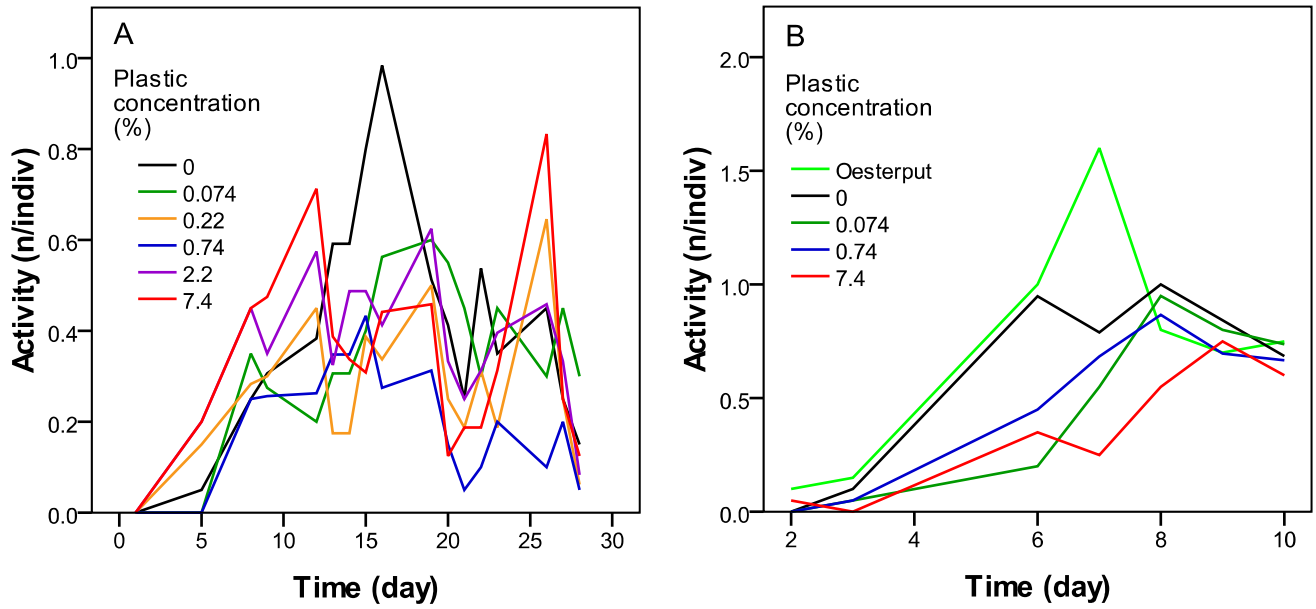

Figure S5: Variation of the activity over time. A. Pilot experiment. B. Bioassay.

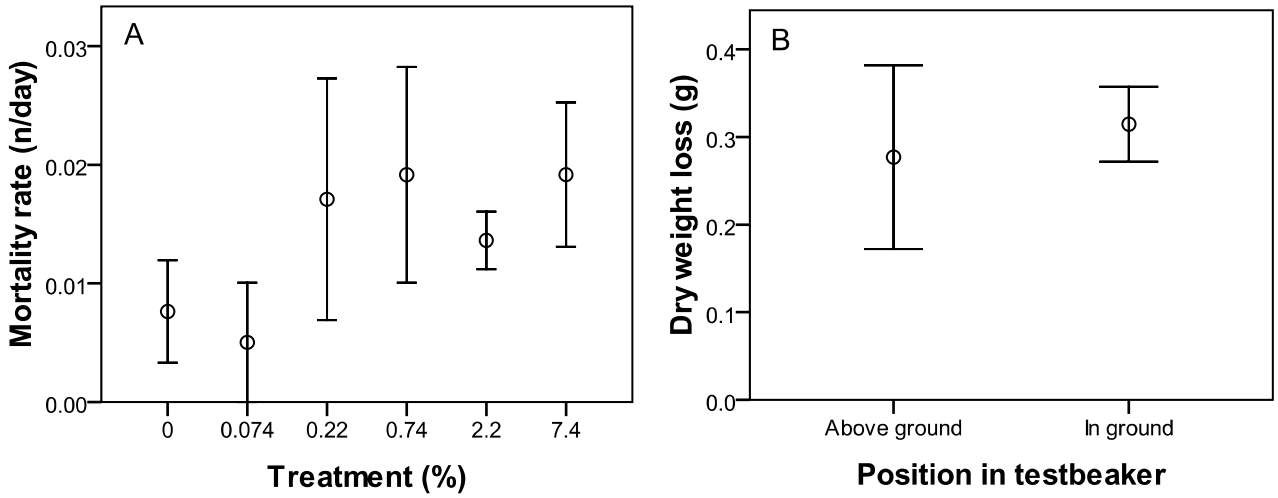

Figure S6: **Pilot experiment**. A. Difference in mortality rate between treatments. B. Difference in DW loss between worms that were in and above the sediment at the end of the experiment. The bars indicate mean ± SE.

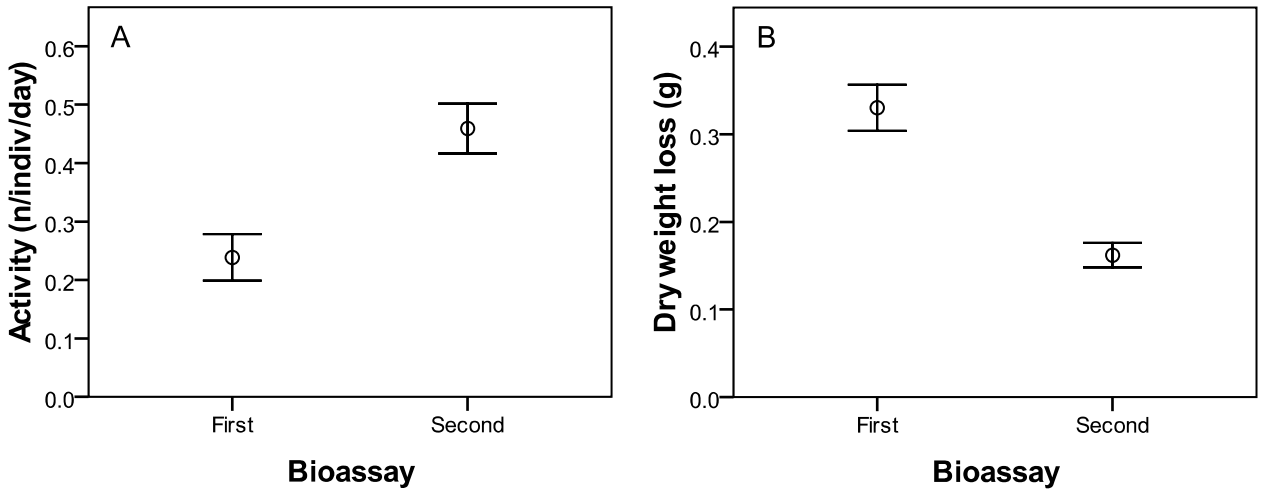

Figure S7: A. Activity of *A. marina* in the pilot experiment compared to the bioassay. B. DW loss of *A. marina* in the pilot experiment compared to the bioassay. The bars indicate mean ± SE