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Accumulation of polystyrene nanoparticles and triclosan by a model tooth-carp fish, *Aphaniops hormuzensis* (Teleostei: Aphaniidae)

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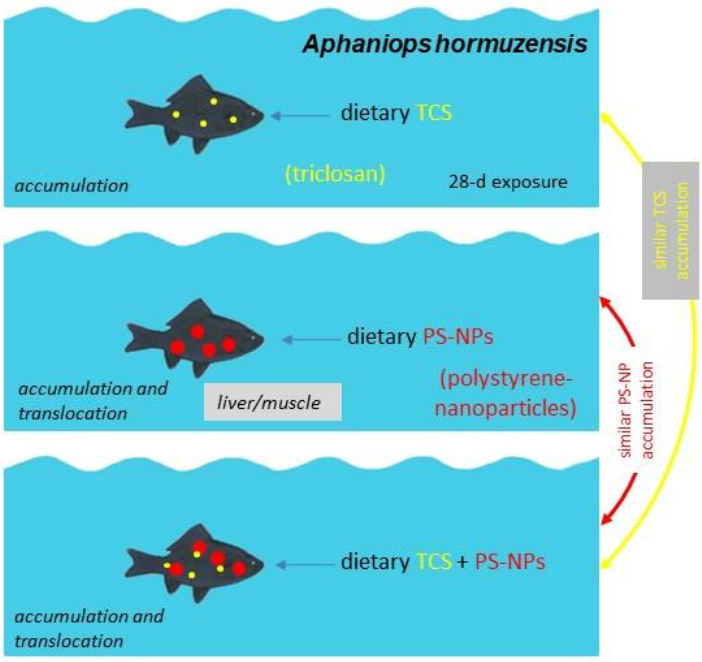
Graphical Abstract



Highlights

- *Aphaniops hormuzensis* exposed to dietary polystyrene nanoparticles (PS-NP)
- PS-NP accumulation observed in both digestive and non-digestive organs
- Presence of triclosan had no measurable effect on PS-NP accumulation and vice versa
- Results suggest PS-NPs and triclosan do not interact but former can be translocated on ingestion

Abstract

The presence and effects of nanoplastics (NPs; < 1 μm) in the aquatic environment are a growing concern. In this study, a model tooth-carp fish, *Aphaniops hormuzensis*, has been exposed to different concentrations of fluorescent polystyrene nanoplastics (PS-NP) in its diet (up to 5 mg kg^{-1}) over periods of 28 d and the particle accumulation in different tissues determined. Accumulation was observed in both digestive and non-digestive organs, with concentrations greater in the gut, liver and gill (up to 400 μg kg^{-1} dw) than in the skin and muscle (< 180 μg kg^{-1} dw), but no dependency on exposure time or dose was evident. The presence of the organic contaminant, triclosan (TCS), in the diet and at concentrations up to 0.5 μg kg^{-1} did not affect PS-NP uptake by *A. hormuzensis*, while TCS accumulation in the whole body increased with time (up to 10 μg kg^{-1}) and, likewise, appeared to be unaffected by the presence of PS-NPs. These observations suggest that the two contaminants do not interact with each other or that any interactions have no impact on accumulation. The results of this study add to the growing body of evidence that NPs can be translocated by aquatic organisms after ingestion, and reveal that, for the species and conditions employed, nanoparticles are accumulated more readily than a widely used organic chemical.

Environmental Implication

This study has shown that Polystyrene-Nanoplastic (PS-NPs) of 100-300 nm in size are accumulated by the tooth-carp fish, *A. hormuzensis*, over a period of dietary exposure of 28-days, with particle translocation observed to both digestive and non-digestive organs. Specifically, accumulation was greater in the gut, gill and liver than in the skin and muscle. The presence of the organic co-contaminant, triclosan (TCS), did not significantly affect the uptake of PS-NP; likewise, the accumulation of TCS appeared to be unaffected by the presence of PS-NPs, suggesting that little interaction (e.g., adsorption and desorption) takes place between the two

types of contaminant. The results of this study add to the growing body of evidence that NPs can be translocated by aquatic organisms after ingestion, and reveal that, for the species and conditions employed, nanoparticles are accumulated more readily than a widely used organic chemical.

Keywords: bioconcentration; exposure; nanoplastics; organic pollutants; tissue; translocation

1. Introduction

Long-term weathering (including abrasion and exposure to sunlight and microorganisms) causes plastic waste to be broken down into progressively smaller particles in the environment (Belzagui et al., 2020; Veerasingam et al., 2020). Microplastics (MPs), in the size range 1 μm nm to 5 mm, have, therefore, received extensive attention in the scientific literature and with respect to freshwater, seawater, sediments, groundwater, biota, soils, dusts and the atmosphere (Zhang and Liu, 2018; Abbasi et al., 2019; Panno et al., 2019; Batel et al., 2020; Evangeliou et al., 2020; Wang et al., 2020; Abbasi and Turner, 2021; Cincinelli et al., 2021; Tanentzap et al., 2021). Far fewer studies, however, have been conducted on nanoplastics (NPs; < 1 μm in size), largely because of analytical challenges in their identification and characterisation in aquatic and biotic matrices at realistic concentrations (Ter Halle et al., 2017). Nevertheless, the smaller size and surface area of NPs means that they have, potentially, more complex and harmful properties than MPs that relate to transport, interactions with light, reactivity, bioavailability and migration of additives (Gigault et al., 2021). Accordingly, NP research has focused on short-term interactions of relatively high concentrations of well-defined, commercial or customised NPs with organic or metallic pollutants or biota (or both) under controlled laboratory conditions (Liu et al., 2016; Liu et al., 2021; Matthews et al., 2021).

Results of such studies suggest that, in aquatic organisms, NPs are able to accumulate, translocate from digestive to non-digestive organs, including the brain, heart and gonads, exert many and varied adverse impacts on health, biomagnify, and interact with organic and inorganic co-contaminants (through adsorption) and affect the bioaccumulation of these chemicals (Trevisan et al., 2022). Specific information in this respect is, however, distinctly lacking for organisms from higher trophic levels, and in particular for freshwater fish (Barría et al., 2020; Brandts et al., 2022).

The aim of the present study is to investigate the potential accumulation and toxicity of NPs constructed of polystyrene (PS-NPs) in a model tooth-carp fish, *Aphaniops hormuzensis* (order Cyprinodontiformes; family Aphaniidae), an endemic killifish found in the Persian Gulf Basin of southern Iran (Teimori et al., 2018). *Aphaniops hormuzensis* is known for its ability to adapt to widely different ecological conditions and, with a high reproductive rate and a physiological similarity with zebrafish (*Danio rerio*), serves as an attractive model in environmental studies (Motamedi et al., 2019). We determine PS-NP distributions in the digestive and non-digestive organs arising from different periods and concentrations of exposure, and study the impacts that particle exposure have on the accumulation of the organic pollutant, triclosan (TCS). TCS is an aromatic ether (5-chloro-2-(2,4-dichlorophenoxy)phenol; CAS 3380-34-5) with a solubility of about 10 mg L^{-1} at 20 ^{o}C and a log K_{ow} of 4.76 (Yalkowsky et al., 2010). It is used as a preservative and antimicrobial agent that has broad applications in clinical settings and in various personal care and consumer products, including soaps, shampoos, toothpastes, medical devices, plastics, textiles and shoes. Consequently, TCS is one of the more frequently detected and highly concentrated contaminants in aquatic and terrestrial environments (Dhillon et al., 2015).

2. Materials and methods

2.1. Fish sampling and acclimation

Aphaniops.hormuzensis, with an average wet weight of 0.544 ± 0.061 g and an average length of 3.40 ± 0.25 cm (and aged ~ 2 to 3 years based on scale ring counts of selected individuals), were obtained from the Mehran River in the Hormozgan province of Iran in June 2021. In the ichthyology laboratory at Shiraz University, Iran, fish were kept in dechlorinated tap water at 26 ± 1 ^{o}C and under a photoperiod of 12 h light:12 h dark in aquaria for two weeks, with feeding once a day with 3 to 6% of their body weight of a BioMar commercial food (protein = 56%, crude lipids

= 18%, carbohydrates = 8.9%; digestible energy = 19.7 MJ kg^{-1}). Water temperature, pH and dissolved oxygen concentration were maintained at 25 ± 1 ^{o}C, pH 7.5-7.9 and 6.9 mg L^{-1}, respectively.

2.2. Reagents and nanoparticles

All reagents used in the study were purchased from Sigma-Aldrich, Merck or Seastar, and pure, distilled-deionized water used for cleaning and dilution had a resistivity greater than 18 MΩ.cm. Working solutions and dilutions of 97% TCS were prepared in a 1:1 mixture of distilled water and ethanol.

Styrene was purified and neutralized by washing with a 5% *w/v* solution of NaOH followed by several washings with pure water and subsequent distillation under vacuum. Polymerization was performed in a three-port reactor, equipped with an additive funnel (to add styrene monomer droplets), cooler and thermometer. Briefly, 400 ml of benzoyl peroxide (1 to 3 % w/v) and polyvinyl alcohol (1 % *w/v*) in deionized water were mixed at 750-550 rpm for 20 min as nitrogen gas was passed through to remove dissolved oxygen. The temperature was then raised to 90 ^{o}C and styrene droplets were gradually added to the solution while being stirred for a period of 30 min. The polymerization reaction continued for 8 h before synthesized polystyrene nanoparticles (PS-NP) were fluorescently stained with rhodamine B (Vakili Tahami et al., 2016; Shohani et al., 2017). The identity of the PS-NPs was confirmed by attenuated total reflection Fourier Transform Infra-Red spectroscopy (FTIR) using a Bruker TENSOR II, and under an LEO-1455VT electron microscope PS-NPs appeared to be spherically shaped, with a minimum, maximum and average particle diameter of about 100 nm, 300 nm and 185 nm, respectively.

In order to load the fish diet with different concentrations of TCS and PS-NP, different quantities of the compound in a water-ethanol mixture (500 mg L^{-1}) and/or a colloidal slurry in pure water

149 (1000 mg L^{-1}) were sprayed on to the Biomar food before the contents were sealed with a layer
150 of gelatin and dried at room temperature (Ramos et al., 2016).
151
152 ***2.3. Exposures***
153 After a period of four days without feeding, 105 fish were randomly selected and seven individuals
154 were placed into a series of fifteen 10-L glass aquaria. The median lethal concentration (LC50) of
155 TCS was determined by exposing each aquarium to a different concentration of the compound
156 (0, 0.01, 0.1, 1, 1.5, 2, 2.5 mg L^{-1}), with controls based on corresponding volumes of ethanol as
157 the carrier solvent, for a period of 96 h and with water-contaminant changes performed daily.
158
159 The protocol above was repeated over a period of 96 h for fish in eight aquaria that were exposed
160 to different concentrations of PS-NP in water (0, 1, 5, 10, 25, 50, 100, 200 mg L^{-1}) and where
161 water (with PS-NPs) was changed daily, and in seven aquaria each that were fed different
162 concentrations of PS-NP or TCS in their daily diets (up to 200 mg kg^{-1} and 500 mg kg^{-1}
163 respectively) and where half of the water was changed daily.
164
165 In a second experiment, 221 specimens in 33 aquaria were fed daily diets, in triplicate, of 0
166 (control), TCS (0.5, 0.1, 0.01 mg kg^{-1}), PS-NP (5, 1, 0.1, 0.01 mg kg^{-1}), and PS-NP + TCS added
167 concurrently (0.5 + 0.5, 0.5 + 0.1, 0.5 + 0.01 mg kg^{-1}). Every three days the bottom third of water
168 in each aquarium was replaced, and replicates were terminated after 3 d, 14 d and 28 d. On
169 termination, three individuals were retrieved from each aquarium before being rinsed to remove
170 particles from the skin, anaesthetized and sacrificed by cervical transection.
171
172 ***2.4. Accumulation of PS-NPs***
173 To measure the accumulation of PS-NPs, tissue samples (skin, muscle, gut, liver, and gill) from
174 three individuals in each exposure were isolated and lyophilized in a freeze-dryer for 72 h before

being weighed. Different tissues from individuals or, where insufficient material was recovered, the pooled contents of a given tissue from the three individuals, were digested in 1M HNO_3 for 2 h at 70 ^oC, resulting in clear solutions with no particulate residue (Lu et al., 2016). Digests were diluted to 5 mL with pure water and PS-NP concentration was measured by fluorescent spectrophotometry (excitation: 450 nm; emission: 530 nm) using a Lambda 365 Perkin Elmer spectrometer that had been calibrated with serial dilutions of PS-NPs in HNO_3. Analysis of the digests of unexposed fish revealed no peak in the target wavelength of rhodamine b.

2.5. Accumulation of TCS

From each exposure, three fish were retrieved, anaesthetized and freeze-dried. Whole fish were powdered in a porcelain pestle and mortar before 0.2 g were weighed into a 5 mL centrifuge tube with a screw cap. Ten mL of acetonitrile and 0.1 mL of 0.1 M ethylenediaminetetraacetic acid were added to the frozen fish and the contents were homogenized using an IKA Ultra Turrax T125 digital homogenizer before being shaken for 15 min and centrifuged at 4000 rpm for 30 min. The supernatant was transferred to a clean centrifuge tube and shaken for 10 min with 0.3 mL of *n*-hexane before the solvent layer was discarded.

Two-mL extracts were cleaned and the analyte concentrated on methanol-conditioned Waters Corp. Oasis HLB cartridges, with elution employing 1 mL of methanol/1% formic acid. Extracts were reduced to 0.5 mL under nitrogen at room temperature before being filtered through a 0.22 μm PTFE syringe filter and analysed by high performance liquid chromatography using an Agilent Technologies 1100 series HPLC system coupled with 6410 triple quadrupole liquid chromatography–mass spectrometry (LC/MS) (Waldbronn, Germany) (Pashael et al., 2022). Percentage TCS recoveries for *A. hormuzensis* homogenates were 98.4 ± 1.5, 95.6 ± 2.3, 91.2 ± 5.8, 93.4 ± 4.3 and 82.6 ± 7.5 for spiking levels of 2 μg kg^{-1}, 5 μg kg^{-1}, 10 μg kg^{-1}, 100 μg kg^{-1} and

500 μg kg^{-1}, respectively. The detection limit for TCS was about 0.001 mg mL^{-1}, or about 2 μg kg^{-1} on a dry weight tissue basis.

2.3. Statistical analysis

One-way ANOVA with Tukey's post-hoc test was employed in Minitab *v*19 to investigate significant differences (α = 0.05) between groups of data, while Pearson's moment correlations were performed in Excel 365. Estimates of LC50 by the probit model were undertaken in SPSS *v*19.0 software.

3. Results

3.1. Toxicity of PS-NPs and TCS

The estimated LC50 for *A. hormuzensis* exposed to aqueous TCS for 96 h was 0.924 mg L^{-1}, and controls revealed that mortality was not affected by the presence or concentration of ethanol present as a carrier solvent. The estimated LC50 for PS-NPs suspended in water for 96 h was 19.3 mg L^{-1}. In contrast, no mortality was observed for fish exposed to PS-NPs or TCS in their diet and up to concentrations of 200 mg kg^{-1} and 500 mg kg^{-1}, respectively.

3.2. Accumulation of PS-NPs

When *A. hormuzensis* were exposed to relatively low concentrations of PS-NPs in their diet, and both with and without TCS, particle accumulation was observed in all tissues considered. Figure 1 shows the accumulation of PS-NPs in the skin and muscle, or the tissues where sufficient material was obtained for replication (*n* = 3) in each aquarium. For individuals in each aquarium, variations (as relative standard deviation) were usually below 15%. However, mean concentrations (ranging from about 50 to 180 μg kg^{-1}) exhibited no clear dependence on time of exposure (3 to 28 d), concentration of PS-NPs, or presence or concentration of TCS.

Nevertheless, a significant correlation was observed between mean concentrations in muscle and skin ($y = 0.423\ x + 471$; $r = 0.619$, $p < 0.05$, $n = 21$) that was improved when exposures including TCS were excluded ($y = 0.590\ x$, $r = 0.787$, $p < 0.05$, $n = 12$).

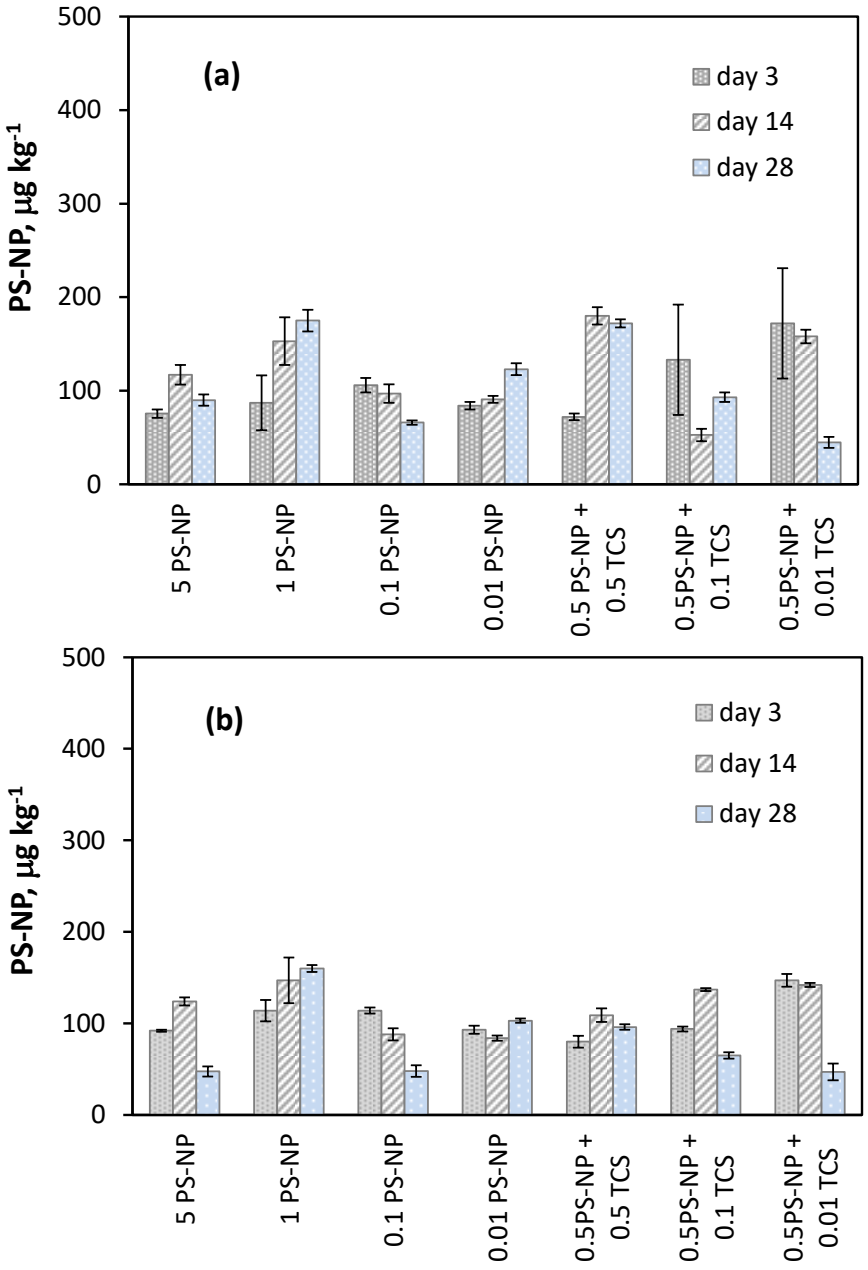
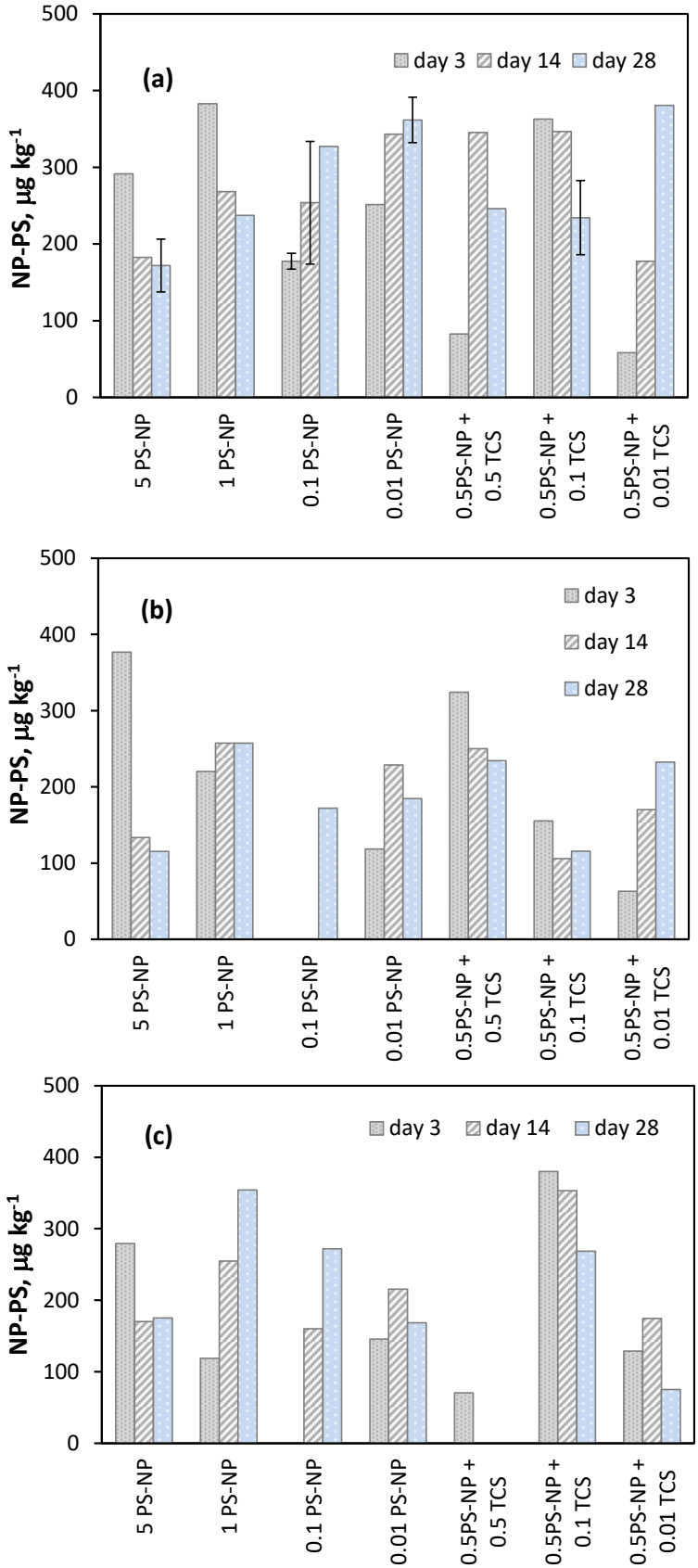


Figure 1: Dry weight concentrations of PS-NP in the (a) skin and (b) muscle tissue of *A. hormuzensis* under the different exposure conditions. Errors represent one standard deviation about the mean of three measurements.


(a)
day 3
day 14
day 28
NP-PS, µg kg-1
500
400
300
200
100
0
5 PS-NP
1 PS-NP
0.1 PS-NP
0.01 PS-NP
0.5PS-NP + 0.5 TCS
0.5PS-NP + 0.1 TCS
0.5PS-NP + 0.01 TCS
(b)
day 3
day 14
day 28
NP-PS, µg kg-1
500
400
300
200
100
0
5 PS-NP
1 PS-NP
0.1 PS-NP
0.01 PS-NP
0.5PS-NP + 0.5 TCS
0.5PS-NP + 0.1 TCS
0.5PS-NP + 0.01 TCS
(c)
day 3
day 14
day 28
NP-PS, µg kg-1
500
400
300
200
100
0
5 PS-NP
1 PS-NP
0.1 PS-NP
0.01 PS-NP
0.5PS-NP + 0.5 TCS
0.5PS-NP + 0.1 TCS
0.5PS-NP + 0.01 TCS

232

Figure 2: Dry weight concentrations of PS-NP in the (a) gut, (b) gill and (c) liver of *A. hormuzensis* under the different exposure conditions. Errors represent one standard deviation about the mean of three measurements of a pooled sample. Where no errors are shown, data represent a single measurement or the mean of two measurements; where no data are present, insufficient material was recovered for analysis.

Figure 2 shows the accumulation of PS-NPs in the gut, gill and liver of *A*. *hormuzensis* under different exposure conditions. Note here that data are more limiting, with triplicates only present from the pooled gut contents in five cases and measurements absent in two (gill) or three (liver) cases. As above, accumulation exhibited no clear dependence on exposure period, concentration of PS-NPs, or presence or concentration of TCS. However, compared with the skin and muscle, accumulation was more variable, and no statistically significant correlations were observed between the tissue types. According to one-way ANOVA, mean concentrations of PS-NPs (for all exposure conditions and exposure times) in the skin and muscle, in the liver and gill, and in the gut and liver were not significantly different, but concentrations in the gut, gill and liver were significantly greater than concentrations in the skin and muscle, and concentrations in the gut were significantly greater than in the gill.

The concentrations of PS-NPs in different tissue of *A. hormuzensis*, [PS-NP-tissue], were normalised to concentrations in amended food, [PS-NP-diet], as dimensionless assimilation efficiencies, AEs (Chong and Wang, 2000):

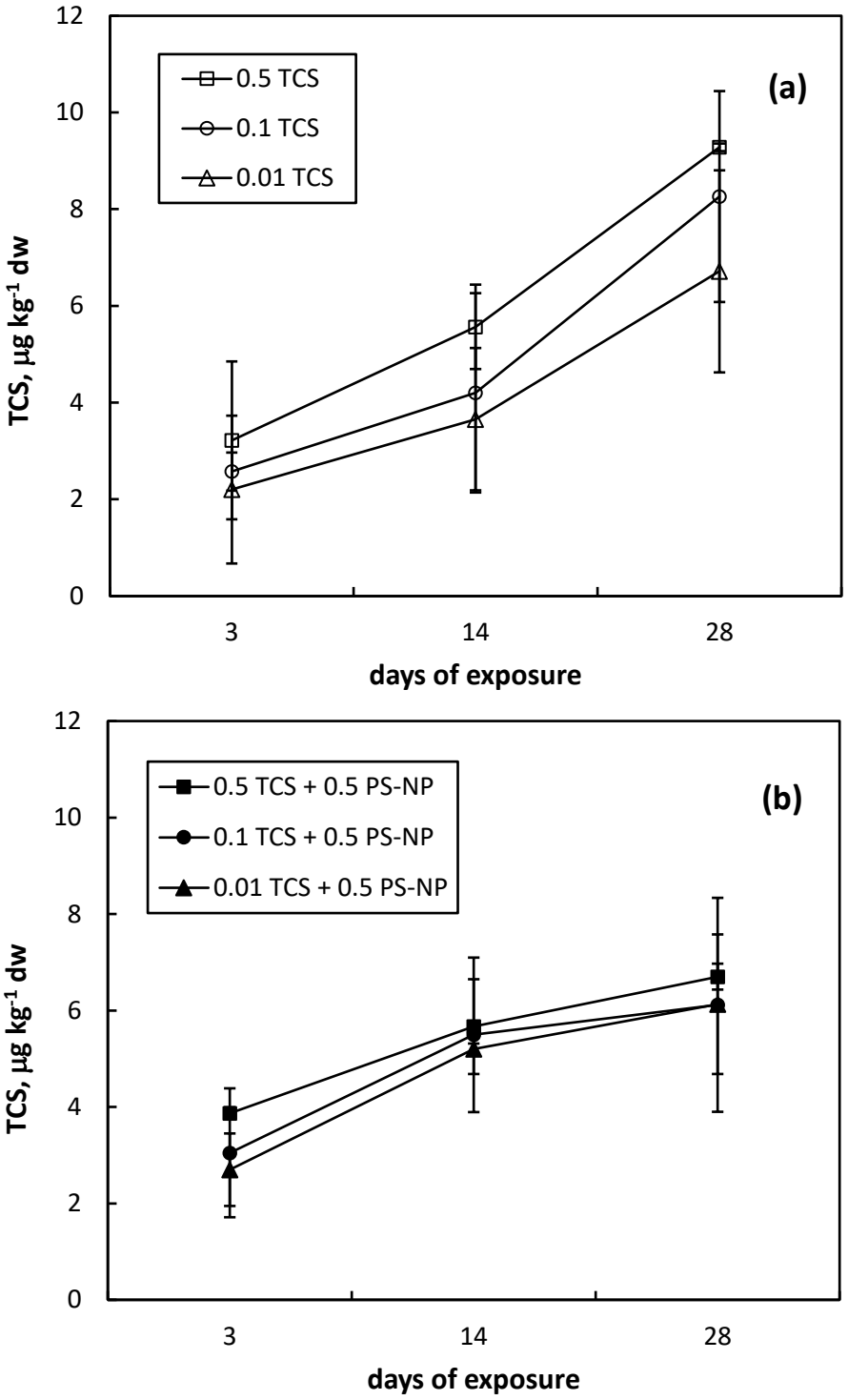
$$AE = [\text{PS-NP-tissue}]/[\text{PS-NP-diet}] \quad (1)$$

Table 1 provides values of AE for each tissue, averaged over the three exposure times, for the four concentrations of dietary PS-NPs employed (in the absence of TCS). There is a clear

increase in AE with decreasing dietary concentration in all tissue types but that is greatest for the gut.

Table 1: Mean assimilation efficiencies for PS-NPs in different tissues of *A. hormuzensis* exposed to different dietary concentrations and calculated according to Equation 1.

	PS-NP-diet, mg kg^{-1}			
tissue	5	1	0.1	0.01
skin	0.019	0.138	0.895	9.92
muscle	0.018	0.141	0.835	9.31
gut	0.043	0.296	2.53	31.9
gill	0.042	0.245	0.573	17.7
liver	0.042	0.243	1.44	17.7



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272 Figure 3: Dry weight concentrations of TCS in individuals of *A. hormuzensis* exposed to different

273 concentrations of TCS in the (a) absence and (b) presence of PS-NPs. Errors represent one

274 standard deviation about the mean of three measurements.

3.3. Accumulation of TCS

The accumulation of TCS by *A. hormuzensis* in exposures with and without PS-NPs is shown in Figure 3. Note that no significant impacts on growth were observed in the presence of either contaminant. TCS was not detected in the control exposures (< 0.2 μg kg^{-1}) and in the absence of PS-NPs the mean concentration of accumulated TCS exhibited a progressive increase with increasing time of exposure and a (non-significant) increase with increasing exposure concentration. Likewise, in the presence of a constant concentration of PS-NPs, mean TCS accumulation increased with increasing time of exposure and exhibited a (non-significant) increase with increasing exposure concentration. After 28 d, mean concentrations of accumulated TCS were lower in the presence of PS-NPs than in their absence, but at each TCS exposure concentration differences were non-significant. At this time point, assimilation efficiencies for TCS in whole *A. hormuzensis*:

AE = [TCS-fish]/[TCS-diet] (2)

ranged from about 0.01 to 1 and are comparable with the lower values of AE for PS-NPs reported in Table 1.

4. Discussion

4.1. Accumulation and translocation of PS-NPs

We did not observe any dependence of accumulation of PS-NPs in *A. hormuzensis* on time of exposure, possibly because variations amongst conditions in the aquaria and concentrations in amended food outweigh those arising from differences in accumulation. Nevertheless, the results of this study suggest that PS-NPs of average diameter 100 nm are able to enter both digestive

and non-digestive organs and both contact and internal tissues of *A. hormuzensis* when the animal is exposed to particles as part of its diet. Accumulation in internal organs requires ingested PS-NPs to cross the intestinal barrier and enter the circulatory system via haemolymph. Specifically, PS-NPs in the gastrointestinal tract would be delivered to the liver via the portal vein, with particles not retained by the liver distributed elsewhere (Brandts et al., 2022). This would explain the high levels of PS-NPs in the liver relative to the muscle, for example. Additionally, it is also possible that PS-NPs in contact tissues like the gill and skin are captured more directly from water. This would require a fraction of ingested PS-NPs to be egested or ventilated (through the gill) and mobilized into the aqueous medium, with captured particles evading detachment during washing. The role of epidermis infiltration in translocation is unclear but evidence from field studies of larger plastic particles in fish muscle tissue than in the blood (Ma et al., 2022) and infiltration of NPs (about 50 nm) through the epidermis into fish eyes (Sendra et al., 2021) suggests that it is possible. The correlation between PS-NP accumulation in the skin and muscle of *A. hormuzensis* that we have observe supports this possibility.

Translocation of nanoplastic and microplastics of (mainly) polystyrene or polyethylene construction has been reported widely in the literature for aquatic invertebrates and vertebrates and from both laboratory exposures and field studies (e.g., Browne et al., 2008; Brennecke et al., 2015; Zhao et al., 2017; Bhargava et al., 2018). Regarding fish, microplastics have been reported in different organs (including those not involved in digestion) from various freshwater and marine species caught from the wild (Collard et al., 2017; Abbasi et al., 2018; McIlwraith et al., 2021). Experimental studies have demonstrated the passage of PS-NPs of a similar size range to those used herein across the intestinal epithelium (Clark et al., 2022; Vagner et al., 2022) and have found evidence for their translocation in the Crucian carp, *Carassius carssius* (to the brain; Mattsson et al., 2017), and the fathead minnow, *Pimephales promelas* (to the liver and kidney; Elizalde-Velázquez et al., 2020). Lu et al. (2016) exposed zebra fish (*Danio rerio*) to 20 mg L^{-1} of

fluorescent, waterborne PS-NPs and found that 5 μm particles accumulated in the gut, gills and liver but 20 μm particles were not detected in the liver, while Zhang et al. (2019) treated red tilapia (*Oreochromis niloticus*) with 0.1 μm PS-NPs and reported accumulation in the gut, gill, liver and brain. This suggests that suspended PS-NPs can also be taken up through the gills and translocated but that the process is limited by particle size. These studies also demonstrated increasing, non-linear PS-NP accumulation over time in each organ. Brandts et al. (2022) exposed goldfish (*Carassius auratus*) to waterborne PS-NPs of 44 nm in diameter and, via size exclusion chromatography-mass spectrometry, found accumulation in the liver and muscle, but not in the digestive tract.

Recent research has raised some concerns about the extent of translocation and the particle sizes involved. For instance, Catarino et al. (2019) and Triebskorn et al. (2019) suggest that markers of nanoplastic and microplastic particles, including fluorescent dyes, may leach into the aqueous medium during exposures or during histological procedures when solvents are applied. Triebskorn et al. (2019) also surmise that particles may be unintentionally relocated through the dissection process in experimental studies, particularly when using high concentrations, or through more general contamination in field studies. In carefully conducted exposures using *D. rerio*, Batel et al. (2020) found that polyethylene particles above 4 μm in diameter were restricted to the gut lumen. The authors argued that the translocation of larger particles was physiologically unlikely but that smaller, nano-sized particles might have the propensity to cross the intestinal epithelium.

In the present study, we observed no fluorescence in control fish, took care to avoid cross contamination during dissection, and used particles smaller than the upper limit capable of being taken up actively by tissues (about 1 μm; Triebskorn et al., 2019). Moreover, the lack of a dose-

dependence on PS-NP accumulation (across two orders of magnitude) that we observed is not consistent with leaching of a mass-dependent concentration of fluorescent dye. Rather, lack of dose-dependency may reflect a limit to the quantity of plastics able to pass the intestinal epithelium or the availability of active transporter sites (Clark et al., 2022), or a concentration-dependent aggregation of PS-NPs. In the environment, however, where considerably lower concentrations of nanoparticles are likely, such limiting factors are not predicted to be important.

4.2. PS-NP-TCS interactions

The role of micro- and nanoplastics in the accumulation of co-contaminants, including TCS, by aquatic biota has received increasing attention over the past decade but results of laboratory studies and theoretical modelling are often inconclusive or contradictory (Triebskorn et al., 2019). Moreover, and in particular for fish, the focus has generally been on the desorption of adsorbed or additive organic chemicals and metals from plastics and other engineered particles in the digestive environment and their subsequent potential for uptake rather than the co-administration of contaminants and plastics as part of the diet (Rochman et al., 2013; Chen et al., 2017; Yan et al., 2017; Zhang et al., 2019).

Concentrations of TCS accumulated by *A. hormuzensis* exposed to sublethal concentrations in the diet showed a dependence on time of exposure, but mean concentrations were not significantly different among the different exposure concentrations. This suggests that some maximum (saturated) concentration is attained in the fish overall, although any shifts in concentration among the different organs are unknown, and that percentage TCS bioavailability decreases with increasing amount in the diet. No significant differences in mean TCS accumulation between equivalent exposures in the presence and absence of PS-NPs are partly consistent with the limited, relevant information available in the literature regarding freshwater and marine fish. Thus, in a study on *D. rerio* exposed to waterborne polyethylene beads (~ 10 to

100 μm) and Ag, Khan et al. (2015) showed that co-exposure had no impact on Ag accumulation but when Ag had adsorbed to the polyethylene surface, reduced uptake occurred. In the European seabass (*Dicentrarchus labrax*), Granby et al. (2018) showed that the uptake of various polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers in the diet was similar in the presence and absence of polyethylene particles (~ 100 to 250 μm). However, enhanced accumulation (and bioavailability) was observed when the organic contaminants had been pre-sorbed to the microplastics for reasons that are unclear. By contrast, Grigorakis and Drouillard (2018) found a reduction in accumulation of dietary PCBs by the goldfish, *C. auratus*, when polyethylene beads (100 to 500 μm) were added to food.

Unlike the studies above, our investigation employed much smaller plastics with higher surface areas and that are able to cross the intestinal epithelium and translocate. Despite using more reactive and bioavailable particles, however, there is no evidence that PS-NPs facilitate or inhibit the uptake of TCS, at least by *A. hormuzensis*.

5. Conclusion

This study has shown that PS-NPs of 100-300 nm in size are accumulated by the tooth-carp fish, *A. hormuzensis*, over a period of dietary exposure of 28-days, with particle translocation observed to both digestive and non-digestive organs. Specifically, accumulation was greater in the gut, gill and liver than in the skin and muscle, but accumulation did not display a dose-dependence. The presence of the organic co-contaminant, TCS, did not significantly affect the uptake of PS-NP; likewise, the accumulation of TCS appeared to be unaffected by the presence of PS-NPs, suggesting that little interaction (e.g., adsorption and desorption) takes place between the two types of contaminant. While the understanding of the interactions between NPs

and aquatic organisms (with or without co-contaminants) is improving, future studies are recommended that involve more realistic plastic concentrations, coupled with a greater range in their shape, condition (e.g., aging, fouling) and polymeric construction.

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References

Abbasi S, Soltani N, Keshavarzi B, Moore F, Turner A, Hassanaghaei M. 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. Chemosphere. 205, 80–87.

Abbasi, S., Keshavarzi, B., Moore, F., Turner, A., Kelly, F.J., Dominguez, A.O., Jaafarzadeh, N., 2019. Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran. Environmental Pollution 244, 153-164.

Abbasi, S., Turner, A., 2021. Dry and wet deposition of microplastics in a semi-arid region (Shiraz, Iran). Science of the Total Environment 786, 147358.

Barría, C., Brandts, I., Tort, L., Oliveira, M. Teles, M., 2020. Effect of nanoplastics on fish health and performance: a review. Mar. Pollut. Bull. 151, 110791.

Batel, A., Baumann, L., Carteny, C.C., Cormier, B., Keiter, S.H., Braunbeck, T., 2020. Histological, enzymatic and chemical analyses of the potential effects of differently sized microplastic particles upon long-term ingestion in zebrafish (*Danio rerio*). Marine Pollution Bulletin 153, 111022.

Belzagui, F., Gutiérrez-Bouzán, C., Álvarez-Sánchez, A., Vilaseca, M., 2020. Textile microfibers reaching aquatic environments: a new estimation approach. Environ. Pollut. 265, 114889.

Bhargava, S., Lee, S.S.C., Ying, L.S.M., Ying, Neo, M.L., Teo, S.L.M., Valiyaveettil, S., 2018. Fate of nanoplastics in marine larvae: A case study using barnacles, *Amphibalanus Amphitrite*. ACS Sustainable Chemical Engineering 6, 6932-6940.

Brandts, I., Cánovas, M., Tvarijonaviciute, A., Llorca, M., Vega, A., Farré, M., Pastor, J., Roher, N., Teles, M., 2022. Nanoplastics are bioaccumulated in fish liver and muscle and cause DNA damage after a chronic exposure. Environmental Research 212, 113433.

Brennecke, D., Ferreira, E.C., Costa, T.M., Appel, D., da Gama, B.A., Lenz, M., 2015. Ingested microplastics (> 100 μm) are translocated to organs of the tropical fiddler crab *Uca rapax*. Marine Pollution Bulletin 96, 491–495.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). Environmental Science and Technology 42, 5026–5031.

Catarino, A.I., Frutos, A., Henry, T.B., 2019. Use of fluorescent-labelled nanoplastics (NPs) to demonstrate NP absorption is inconclusive without adequate controls. Science of the Total Environment 670, 915–920.

Chen, Q.Q., Yin, D.Q., Jia, Y.L., Schiwy, S., Legradi, J., Yang, S.Y., Hollert, H., 2017. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 609, 1312–1321.

Chong, K., Wang, W.X. 2000. Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environmental Toxicology and Chemistry 19, 1660–1667.

Cincinelli, A., Scopetani, C., Chelazzi, D., Martellini, T., Pogojeva, M., Slobodnik, J., 2021. Microplastics in the Black Sea sediments. Science of the Total Environment 760, 143898.

Clark, N.J., Khan, F.R., Mitrano, D.M., Boyle, D., Thompson, R.C., 2022. Demonstrating the translocation of nanoplastics across the fish intestine using palladium-doped polystyrene in a salmon gut-sac. Environment International 159, 106994.

Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E. 2017. Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). Environmental Pollution 229, 1000–1005.

Elizalde-Velázquez, A., Crago, J., Zhao, X., Green, M.J., Cañas-Carrell, J.E., 2020. In vivo effects on the immune function of fathead minnow (*Pimephales promelas*) following ingestion

and intraperitoneal injection of polystyrene nanoplastics. Science of the Total Environment 735, 139461.

Evangeliou, N., Grythe, H., Klimont, Z., Heyes, C., Eckhardt, S., Lopez-Aparicio, S., and Stohl, A., 2020. Atmospheric transport is a major pathway of microplastics to remote regions. Nature Communications 11, 1–11.

Gigault, J., El Hadri, H., Nguyen, B., Grassl, B., Rowenczyk, L., Tufenkji, N., Feng, S., Wiesner, M., 2021. Nanoplastics are neither microplastics nor engineered nanoparticles. Nat. Nanotechnol. 16, 501–507 (2021).

Granby, K., Rainieri, S., Rasmussen, R.R., Kotterman, M.J.J., Sloth, J.J., Cederberg, T.L., Barranco, A., Marques, A., Larse, B.K., 2018. The influence of microplastics and halogenated contaminants in feed on
toxicokinetics and gene expression in European seabass (*Dicentrarchus labrax*) Environmental Research 164, 430-443.

Grigorakis, S., Drouillard K.G., 2018. Effect of microplastic amendment to food on diet assimilation efficiencies of PCBs by fish. Environmental Science and Technology 52, 10796–10802.

Jovanović, B., 2017. Ingestion of microplastic by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13, 510–515.

Khan, F.R., Syberg, K., Shashoua, Y., Bury, N.R., 2015. Influence of polyethylene microplastic beads on the uptake and localization of silver in zebrafish (*Danio rerio*) Environmental Pollution 206, 73-79.

Liu, L.; Fokkink, R.; Koelmans, A.A., 2016. Sorption of polycyclic aromatic hydrocarbons to polystyrene nanoplastic. Environ. Toxicol. Chem. 35, 1650–1655.

Liu, Z.; Li, Y.; Sepúlveda, M.S.; Jiang, Q.; Jiao, Y.; Chen, Q.; Huang, Y.; Tian, J.; Zhao, Y., 2021. Development of an adverse outcome pathway for nanoplastic toxicity in *Daphnia pulex* using proteomics. Sci. Total Environ. 766, 144249.

Lu Y, Zhang Y, Deng Y, Jiang W, Zhao Y, Geng J, Ding L, Ren H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environmental Science and Technology 50, 4054-60.

Ma, C., Chen, Q., Li, J., Li, B., Liang, W., Su, Lei, Shi, H., 2022. Distribution and translocation of micro- and nanoplastics in fish. Critical Reviews in Toxicology https://doi.org/10.1080/10408444.2021.2024495

Matthews, S.; Mai, L.; Jeong, C.B.; Lee, J.S.; Zeng, E.Y.; Xu, E.G., 2021. Key mechanisms of micro- and nanoplastic (MNP) toxicity across taxonomic groups. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 247, 109056.

Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Scientific Reports 7, 1–7.

McIlwraith, H.K., Kim, J., Helm, P., Bhavsar, S.P., Metzger, J.S., Rochman, C.M., 2021. Evidence of Microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. Environmental Science and Technology 55, 12372-12382.

Motamedi, M., Teimori, A., Masoumi, A.H., Shaghooei, P.M., Mousavi, S.E., 2019. Early embryonic development of brackish water Killifish *Aphanius hormuzensis* (Teleostei, Aphaniidae) inhabiting coastal environment in Southern Iran. Journal of Applied Ichthyology 35, 1260-1268.

Panno, S.V., Kelly, W.R., Scott, J., Zheng, W., McNeish, R.E., Holm, N., Hoellein, T.J., Baranski, E.L., 2019. Microplastic contamination in karst groundwater systems. Groundwater 57, 189-196.

Pashaei R., Dzingelevičienė, R., Abbasi, S., Szultka-Młyńska, M., Buszewski, B., 2022. Determination of 15 human pharmaceutical residues in fish and shrimp tissues by high-performance liquid chromatography-tandem mass spectrometry. Environmental Monitoring and Assessment 194, 325.

Ramos, M., Valdés, A., Beltrán, A., Garrigós, M.C., 2016. Gelatin-based films and coatings for food packaging applications. Coatings 6, 41. https://doi.org/10.3390/coatings6040041

Rochman, C.M., Hoh, E., Kurobe, T., The, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports 3, 3263.

Sendra, M., Pereiro, P., Yeste, M.P., Mercado, L., Figueras, A., Novoa, B., 2021. Size matters: Zebrafish (*Danio rerio*) as a model to study toxicity of nanoplastics from cells to the whole organism. Environmental Pollution 268A, 115769.

Shohani, N., Pourmahdian, S., Shirkavand Hadavand, B., 2017. Response surface methodology for design of porous hollow sphere thermal insulator. IOP Conference Series: Materials Science and Engineering 269, 012073.

Tanentzap, A.J., Cottingham, S., Fonvielle, J., Riley, I., Walker, L., Woodman, S.G., Kontou, D., Pichler, C.M., Reisner, E., Lebreton, L., 2021. Microplastics and anthropogenic fibre concentrations in lakes reflect surrounding land use. PLoS Biol 19, e3001389. https://doi.org/10.1371/journal.pbio.3001389

Teimori, A., Esmaeili, H.R., Hamidan, N., Reichenbacher, B., 2018. Systematics and historical biogeography of the *Aphanius dispar* species group (Teleostei: Aphaniidae) and description of a new species from Southern Iran. Journal of Zoological Systematics and Evolutionary Research 56, 579-598.

Ter Halle, A.; Jeanneau, L.; Martignac, M.; Jardé, E.; Pedrono, B.; Brach, L.; Gigault, J., 2017. Nanoplastic in the North Atlantic Subtropical Gyre. Environ. Sci. Technol. 51, 13689–13697.

Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P., Krais, S., Müller, Y.K., Pittroff, M., et al. 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. Trends in Analytical Chemistry 110, 375–392.

Trevisan, R., Ranasinghe, P., Jayasundara, N., Di Giulio, R.T., 2022. Nanoplastics in aquatic environments: Impacts on aquatic species and interactions with environmental factors and pollutants. Toxics 10, 326.

583 Vagner, M., Boudry, G., Courcot, L., Vincent, D., Dehaut, A., Duflos, G., Huvet, A., Tallec, K.,
584 Zambonino-Infante, J.L., 2022. Experimental evidence that polystyrene nanoplastics cross the
585 intestinal barrier of European seabass. Environment International 166, 107340.
586
587 Vakili Tahami, S.H., Pourmahdian, S., Shirkavand Hadavand, B., Azizi, Z.S., Tehranchi, M.M.,
588 2016. Thermal tuning the reversible optical band gap of self-assembled polystyrene photonic
589 crystals. Photonics and Nanostructucure – Fundamentals and Applications 22, 40-45.
590
591 Veerasingam, S., Al-Khayat, J.A., Aboobacker, V.M., Hamza, S., Vethamony, P., 2020.
592 Sources, spatial distribution and characteristics of marine litter along the west coast of Qatar.
593 Mar Pollut Bull. 159, 111478.
594
595 Wang, T., Hu, M., Song, L., Yu, J., Liu, R., Wang, S., Wang, Z., Sokolova, I.M., Huang, W.,
596 Wang, Y., 2020. Coastal zone use influences the spatial distribution of microplastics in
597 Hangzhou Bay, China. Environ. Pollut. 115137.
598
599 Yalkowsky, S.H., He, Yan, Jain, P. Handbook of Aqueous Solubility Data Second Edition. CRC
600 Press, Boca Raton, FL 2010, p. 844
601
602 Yan, Z., Lu, G., Sun, H., Ma, B. 2017. Influence of multi-walled carbon nanotubes on the effects
603 of roxithromycin in crucian carp (*Carassius auratus*) in the presence of natural organic matter.
604 Chemosphere 178, 165–172.
605
606 Zhang, G., Liu, Y., 2018. The distribution of microplastics in soil aggregate fractions in
607 southwestern China. Sci. Total Environ. 642, 12–20.
608

609 Zhang, S., Ding, J., Razanajatovo, R.M., Jiang, H., Zou, H., Zhu, W., 2019. Interactive effects of
610 polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the
611 freshwater fish red tilapia (*Oreochromis niloticus*). Science of the Total Environment 648, 1431–
612 1439.
613
614
615