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The Effects of Food on the Uptake and Excretion of Nano-Plastics by *Daphnia magna*

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Abstract: The effects of nano-plastics (NPs) on aquatic organisms have drawn significant attention. Understanding the uptake and excretion of NPs by aquatic organisms can provide clearer insights into their behavior within organisms. And the effect of different food on the processes is unclear. *Daphnia magna* (*D. magna*) is considered as a model organism for assessing the ecological risks of NPs. This work observed the uptake and excretion of NPs by *D. magna* under different food supply conditions. The effects of three different types of foods (*Chlorella* sp., *Euglena gracilis*, and yeast powder) on the uptake and excretion of two concentrations of NPs (1 mg/L and 3 mg/L) by the *D. magna* were compared. A Time-Gated Imaging technique was used to quantify the NPs uptake mass by *D. magna*. The study results showed the inhibitory effect presented by food on the uptake of NPs by *D. magna*. The inhibitory ability of different foods varies, with similar levels observed in *Chlorella* sp. and *E. gracilis*, while the inhibitory effect of yeast powder was slightly weaker. The facilitating effect was presented by food on the excretion of NPs. The time constant of excretion of NPs by feeding yeast powder was about 4–5 min longer than that of two types of algae. These effects can be attributed to food occupying the intestine tract of *D. magna* and supplying energy. This work emphasizes the important role of food in evaluating the ecological effects of NPs and provides support for future research on the long-term risks of pollutants to aquatic organisms and environmental sustainability.

Keywords: nano-plastics; food; uptake and excretion; *Daphnia magna*

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1. Introduction

Serious environmental problems are caused by the widespread use of plastic products [1,2]. They may be discarded randomly in rivers, lakes, marshes, and other water bodies. Aquatic ecosystems are becoming major sinks for plastics products [3,4]. Plastics break down into micro-plastics (<5 mm) and nano-plastics (<1 μm) under external effects such as photodegradation, chemical degradation, and biodegradation [5–8]. Nano-plastics (NPs) have attracted much attention because it is easier for them to penetrate into tissues and organs of organisms [9]. There is increasing evidence that NPs can be ingested by aquatic organisms with different nutrient levels, such as algae [10], zooplankton [11,12], and benthic species [13,14]. And a variety of detrimental effects can be generated after uptake, including decreased photosynthesis [15], physical damage [16], lower reproductive rates [17,18], and shortened lifespan [19].

In natural aquatic ecosystems, food must be considered when evaluating the effect of NPs. On the one hand, food exists in the aquatic ecosystems, and aquatic organisms will inevitably ingest food in the process of ingesting NPs [20,21], so more realistic results can be generated by food particles. On the other hand, aquatic organisms may be exposed to NPs

for a long time, so the long-term ecological risks of NPs to freshwater environments need to be addressed [22,23]. And food can provide support for long-term research because it can provide a large amount of energy for animals to maintain growth and reproduction [24,25]. In conclusion, it is necessary to study the effects of food on the uptake and excretion of NPs by aquatic organisms.

Several studies have demonstrated that food can influence the uptake and excretion of NPs by *D. magna*. For example, there was a study found that the uptake of NPs mass by *D. magna* can be decreased when a low concentration of *Chlamydomonas reinhardtii* was fed [26]. It has also been found that the accumulation of MPs and NPs in *Calanus sinicus* can be inhibited with the increasing of diatom concentrations [27]. Furthermore, it was reported that *D. magna* body burdens of MPs and NPs were significantly decreased by food during ingestion and egestion [28]. But, the food condition was single in these studies. It is important to note that *D. magna* live in aquatic systems where the types of food can vary both in the short term (weekly or daily) and in the long term [29]. This often results in *D. magna* being exposed to different food conditions. Therefore, studying the effects of different food on the uptake and excretion of NPs by *D. magna* is more consistent with the state of the natural environments.

For fluorescence-labeled NPs, a confocal microscope can be used to locate and quantify the NPs' uptake by organisms, but the background of autofluorescence cannot be eliminated completely [30]. In previous work [31], the problems of autofluorescence interference, low penetration, and destruction of biological tissue can be effectively solved by the combination of the rare earth up-conversion nanoparticles (UCNPs) and Time-Gated Imaging (TGI) technique [32–34]. Real-time in situ imaging and quantitative analysis of NPs were achieved by the TGI technique.

In this work, the main objective is to study the effects of the three representative foods (*Chlorella* sp., *Euglena gracilis* (*E. gracilis*), and yeast powder) on the uptake and excretion of NPs (3 mg/L and 1 mg/L) of *D. magna* through the TGI technique. The 24 h uptake and excretion processes of NPs by *D. magna* were mainly observed, and the distribution of NPs in the body was monitored. This study can help us to better reveal the potential effects of NPs on organisms in natural bodies and provide support for long-term research in the future.

2. Materials and Methods

In this study, UCNPs coated with polystyrene were used as biological probes, and TGI was employed to achieve visualization. The model organism *D. magna* (n = 10) was selected. And *Chlorella* sp., *E. gracilis,* and yeast powder were used as food to feed *D. magna*. The experiments mainly comprised two parts: (1) the effect of food on the uptake of NPs by *D. magna*; (2) the effect of food on the excretion of NPs by *D. magna.* The TGI imaging measurements were conducted at room temperature. Specific details are described in the following sections.

2.1. Synthesis of $NaLuF_4$: 20% Yb, 2% Er@$NaLuF_4$@SiO_2@PS (UCNPs@PS)

This study used polystyrene nanoparticles as representative NPs. The $NaLuF_4$: 20% Yb, 2% Er@$NaLuF_4$@SiO_2@PS was synthesized in four steps. First, $NaLuF_4$: 20% Yb, 2% Er@$NaLuF_4$ nanoparticles were prepared by a traditional solvent thermal method [35]. Then, SiO_2 was coated by a modified reverse microemulsion method. Finally, UCNPs@SiO_2@PS nanoparticles were synthesized by a two-step dispersion polymerization method [36]. Specific details were presented in the supporting information. A structural diagram of UCNPs@PS is depicted in Figure 1.

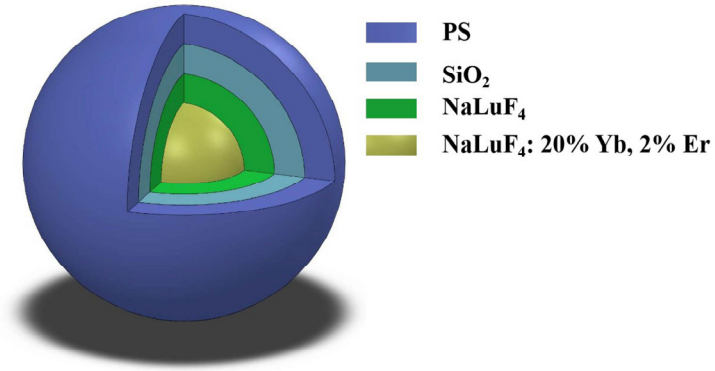

Figure 1. Structural diagram for UCNPs@PS.

2.2. Characterization of NPs

To ensure that the synthesized NPs can meet the requirements for subsequent observations, the materials were characterized. Morphology and particle size of UCNPs@PS were analyzed by transmission electron microscopy (TEM, Hitachi H-7650B, Japan). The zeta potential and hydrodynamic diameter of the material were measured at 25 °C by nano Zetasizer ZS90 (Malvern Instruments, Malvern, UK). The upconversion-luminescence (UCL) emission spectra were measured using a fluorescence spectrophotometer (FLS980, Edinburgh Instruments, Livingston, UK) under a continuous wave laser of 980 nm. The crystal structure and phase purity were characterized by X-ray diffraction (XRD, Shimadzu 7000, Japan). UCNPs@PS was sonicated in an ice bath for 30 min before use.

2.3. Time-Gated Imaging (TGI) and Quantification Method of NPs

To observe the distribution of NPs within the organism, TGI was employed. In previous research, a TGI system was built. The organisms were imaged under a 974 nm laser. The schematic diagram of the TGI is shown in Figure S1. In order to quantify NPs, different concentrations of NPs suspensions (0, 100, 500, 1000, 1500, and 2000 mg/L) were sucked into a quartz capillary with an inner diameter of 0.9 mm and an outer diameter of 1.2 mm, and luminescence intensity was captured using the TGI system.

2.4. Species

In this study, *Chlorella* sp., *E. gracilis,* and yeast powder were selected as three typical food sources for *D. magna*. The spherical-shaped *Chlorella* sp. belongs to the Chlorophyta phylum, with cell walls [37]. *E. gracilis* of spindle-shaped cells belongs to the Euglenophyta, without cell walls [38]. The size of *E. gracilis* is much larger than that of *Chlorella* sp. Moreover, the yeast powder is more different from the two kinds of algae.

Chlorella sp. (FACHB-5) and *E. gracilis* (FACHB-848) were cultured in SE medium and HUT medium, respectively, provided by the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB), National Aquatic Biological Resource Center. They were cultured at a temperature of 20 ± 0.5 °C and a 12 h light and dark cycle of 2000 lux cool white light. The conical bottle was shaken three times every day to prevent algae from sinking.

D. magna can be chosen as the test species because it is already recognized as a model organism for assessing the risks of environmental pollutants. *D. magna* were cultured in Elendt M4 medium. They were maintained at a temperature of 20 ± 1 °C, and the photoperiod was 1000 lux cool white light with 16: 8 h light and dark cycle. The culture medium was changed once a week to ensure optimal growth conditions. The whole experiments were conducted in dark conditions, and Neonates (<24 h) from the third generation were collected according to the OECD 211 [39].

2.5. Uptake and Excretion Experiments of NPs for D. magna

2.5.1. Uptake of NPs Experiments

The experimental design is depicted in Figure 2. The experiments were conducted in 50 mL test beakers containing 20 mL Elendt M4 medium. Neonates (<24 h) were collected and cleaned to remove residual food in the body before experiments. The concentrations of NPs were 3 mg/L and 1 mg/L. The foods were algae (*Chlorella* sp. and *E. gracilis*) and yeast powder. The concentration of food used in the experiments was 19.42 mg/L (dry weight), which was equivalent to the densities of *Chlorella* sp. and *E. gracilis*, which were 5×10^5 cells/mL and 8×10^3 cells/mL, respectively. The groups were set up in this study as follows: (1) NPs groups, (2) NPs + *Chlorella* sp. (NPs + C) groups, (3) NPs + *E. gracilis* (NPs + E) groups, (4) NPs + yeast powder (NPs + Y) groups. *D. magna* were removed from the beakers at each timing (10, 20, 30, 60, 120, 240, 480, 720, 1200, and 1440 min) and were fixed with 10% formalin solution in an ice water bath. After collecting the samples, the *D. magna* were washed three times with Elendt M4 medium to remove attached NPs. Finally, the samples were imaged by the TGI system. Each beaker contained 5 individuals, with a total of 10 individuals per experimental condition. The beakers were covered with plastic wrap to prevent the water from evaporating, and the experiments were conducted in the dark.

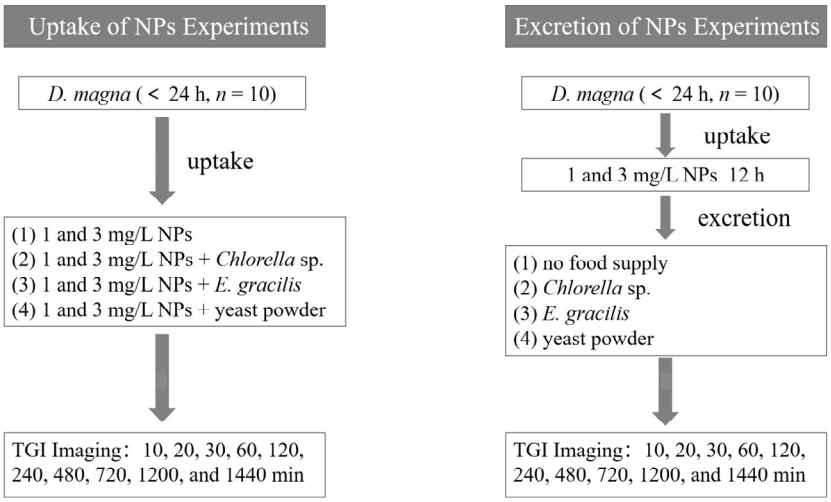

Figure 2. The experimental design for studying the uptake and excretion of NPs by *D. magna*.

2.5.2. Excretion of NPs Experiments

Neonates (<24 h) were exposed to Elendt M4 medium containing NPs at concentrations of 3 mg/L and 1 mg/L for 12 h, and then individuals were transferred to clean medium and medium with added food for excretion, respectively. The groups were set up in this study as follows: (1) no food supply, (2) *Chlorella* sp. (C) groups, (3) *E. gracilis* (E) groups, (4) yeast powder (Y) groups. Samples (n = 10) were collected at 10, 20, 30, 60, 120, 240, 480, 720, 1200, and 1440 min. The treatment methods were the same as above and were imaged by the TGI system (Figure 2).

2.6. Statistical Analysis

In this study, the samples (n = 10) were used in each group. TGI images and TEM images were processed using ImageJ 1.53c software. The experimental data were analyzed using Origin 2021. The significant difference for different foods was analyzed by One-Way Analysis of Variance or Kruskal–Wallis non-parametric test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate significance levels. The results are presented as mean ± standard deviation (SD). Significant difference analysis of the results was performed using the IBM SPSS Statistics 27.

3. Results

3.1. Characterization of NPs

As shown in Figure 3A,B, the average particle sizes of the UCNPs@PS were 264.69 ± 18.54 nm. The hydrodynamic diameter measured by nano Zetasizer ZS90 was 316.5 nm (Figure 3C), and the zeta potential was −27.2 mV in pure water. To further confirm the stability, the zeta potential was measured for 4 days in pure water and Elendt M4 medium, which showed good suspension stability of UCNPs@PS (Figure S2). Figure 3D shows strong characteristic emission peaks of Er^{3+} under the laser excitation of 980 nm. The XRD result (Figure 3E) shows a hexagonal phase for $NaLuF_4$: 20% Yb, 2% Er@$NaLuF_4$ nanoparticles, which was agreement with the standard card JCPDS No. 27-0726. For the quantification of UCNPs@PS, different masses of UCNPs@PS were added to a quartz capillary and captured by the TGI system. As shown in Figure 3F, the signal strength was linearly correlated with the UCNPs@PS mass. R^2 = 0.998.

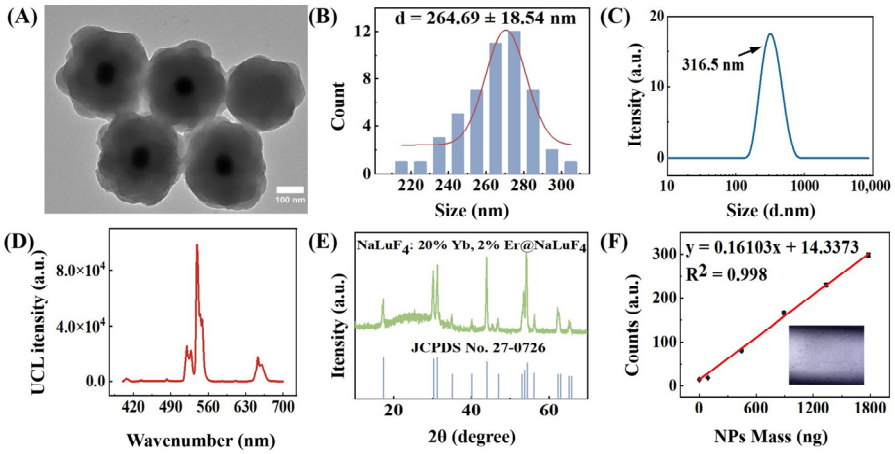

Figure 3. (**A**) TEM image of the UCNPs@PS. Scale bars, 100 nm; (**B**) TEM particle size distribution statistical chart of UCNPs@PS; (**C**) dynamic light scattering (DLS) result of UCNPs@PS colloidal; (**D**) UCL emission spectra of the UCNPs@PS at a continuous wave of 980 nm under excitation light (2 W/cm^2); (**E**) XRD pattern of $NaLuF_4$: 20% Yb, 2% Er@$NaLuF_4$ nanoparticles; (**F**) linear fitting function for counts and UCNPs@PS mass.

3.2. Uptake Experiments

To assess uptake of NPs, *D. magna* were exposed to different concentrations of NPs (1 mg/L and 3 mg/L). Figure 4A shows that the higher the concentrations of NPs, the more NPs were ingested by *D. magna*. The uptake of NPs by *D. magna* depended on the exposure time. At 10 min of exposure, NPs have been observed in *D. magna*. NPs mass in animal body continuously increased, and the body burdens in animals reached a peak at ~8 h; there were 1499.82 ± 209.79 ng NPs and 598.27 ± 112.29 ng NPs ingested by *D. magna* when the concentrations of NPs were 3 mg/L and 1 mg/L. After 8 h, NPs in *D. magna* were constantly fluctuating and almost reached a state of equilibrium (Figure 4A).

During the experiments, the same dry weight food was fed to *D. magna*. It was found that the reduced uptake of NPs mass and the slower uptake rate of NPs were caused by food, regardless of the concentration of NPs (Figure 4B–D). The inhibitory effect was presented by food on the uptake of NPs by *D. magna*. In addition, more NPs were ingested by *D. magna* in 3 mg/L NPs groups than at 1 mg/L NPs groups, and it reached its peak at ~8 h, which was consistent in the absence of food. At 8 h, *D. magna* ingested 458.71 ± 68.72 ng NPs when exposed to 3 mg/L NPs and fed yeast powder (Figure 4B). After feeding algae, there were 133.34 ± 48.65 ng and 123.68 ± 30.67 ng NPs ingested by *D. magna* when the foods were *Chlorella* sp. and *E. gracilis*, respectively (Figure 4C,D). More NPs were ingested in the yeasty diet groups compared with algal diet groups. These results suggested that the inhibitory ability of different foods varied in the uptake of NPs by *D. magna*.

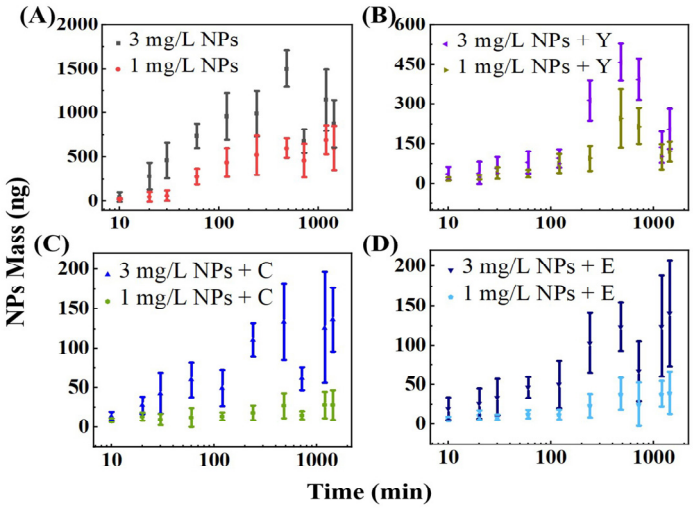

Figure 4. *D. magna* ingested NPs under different exposure conditions. (**A**) Body burdens of NPs in *D. magna* when exposed to NPs during the 24 h; (**B**–**D**) body burdens of NPs in *D. magna* when exposed to NPs + food during the 24 h (mean ± SD; $n = 10$). The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

To further evaluate the effects of food on uptake of NPs by *D. magna*, differences in the mass of NPs ingested under different food supply conditions were analyzed. Despite the significant differences in cellular structure and chemical compositions between *Chlorella* sp. and *E. gracilis*, it is not observed that there are notable variations in the mass of NPs ingested by *D. magna* at all the tested time points ($p > 0.05$). The inhibitory ability of *Chlorella* sp. and *E. gracilis* was similar. It is concluded that various types of algae may not have a substantial effect on the uptake of NPs by *D. magna*. However, it was observed that there were significant differences in the uptake of NPs mass at all exposure times ($p < 0.05$) when the yeast powder and algae were compared in 1 mg/L NPs groups. There were not significant differences only at 20 min, 30 min, 1 h, and 20 h in 3 mg/L NPs groups ($p > 0.05$). Figure 5 showed the differences of the NPs mass ingested by *D. magna* at 2 h, 4 h, and 8 h during the time when *D. magna* were exposed to different food supply conditions. These results showed that the inhibitory ability of yeast powder was slightly weaker compared with *Chlorella* sp. and *E. gracilis*.

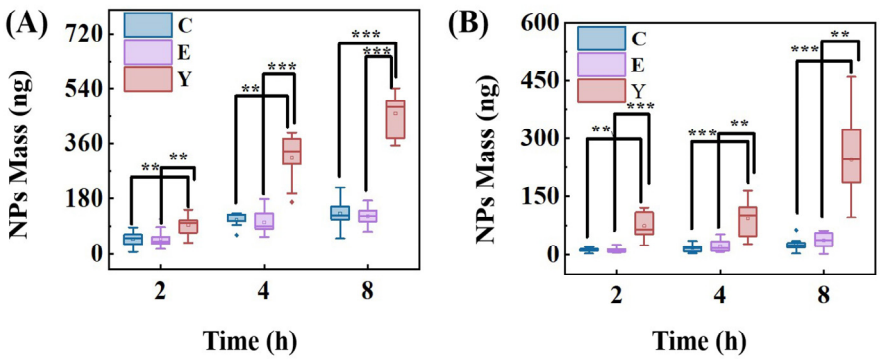

Figure 5. Box plots of body burdens of NPs in *D. magna* exposed to different food conditions for 2 h, 4 h, and 8 h. (**A**) The concentration of NPs was 3 mg/L; (**B**) the concentration of NPs was 1 mg/L. Statistically significant differences were indicated using ** $p < 0.01$, *** $p < 0.001$. The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

Figure 6 shows the distribution of NPs in *D. magna* in the uptake stage. The NPs were mainly found in the intestine tract of *D. magna*. For further analysis, *D. magna* were exposed to 3 mg/L NPs for 24 h and were sectioned using TEM (Figure S3). The NPs also were

mainly observed in the intestine tract. Remarkably, some suspected NPs were also found in the carapace. This indicated that NPs might translocate within the body parts of *D. magna*.

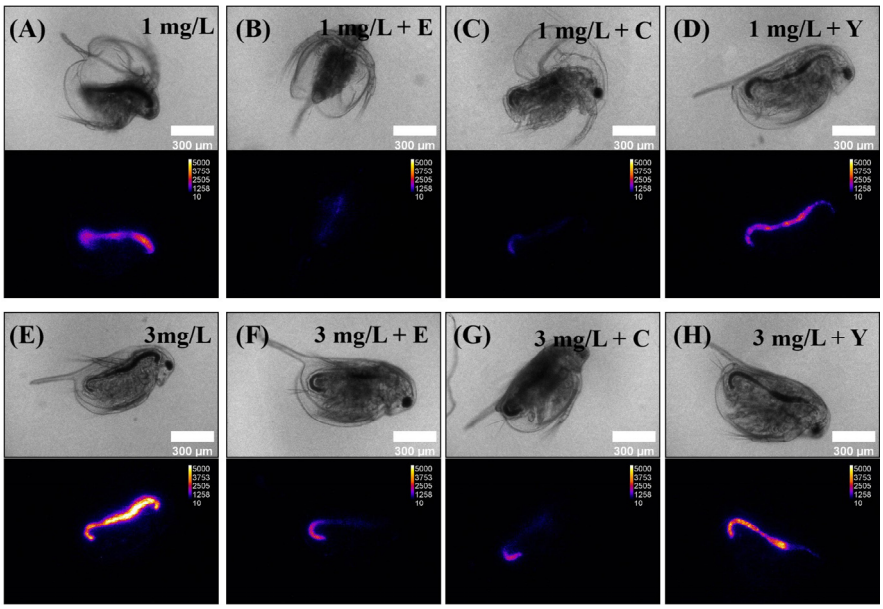

Figure 6. Bright field and dark field images of *D. magna* that ingested NPs under different conditions for 12 h. (**A**–**D**) The concentration of NPs was 1 mg/L; (**E**–**H**) the concentration of NPs was 3 mg/L. The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

3.3. Excretion Experiments

D. magna were exposed to different concentrations of NPs (3 mg/L and 1 mg/L) for 12 h without the addition of food, and then animals were transferred to clean medium. Figure 7A shows the behavior of *D. magna* excreting NPs in the absence of food. The average load of NPs in individuals decreased with the increase in exposure time. A similar trend was observed after the addition of food during the excretion phase (Figure 7B–D). And it declined rapidly within the first 1 h in all exposure conditions. For example, when *D. magna* excreted at the low concentration, it was observed that the NPs in each body of the animal decreased by more than 90% when food was provided. In contrast, there was only about 65% reduction of the NPs burdens in the absence of food. The facilitating effect was presented by food on the excretion of NPs by *D. magna*. Over time, it became more difficult for *D. magna* to excrete NPs. And the NPs were still in the intestine tract during the excretion stage (Figure 8). It is worth noting that NPs were not completely excreted during the 24 h excretion period regardless of whether food was added. However, after the addition of food, the residual mass of NPs in *D. magna* was less than that in the absence of food. Table 1 summarizes NPs mass after 24 h excretion by *D. magna* under different exposure conditions.

Table 1. The NPs mass after 24 h excretion by *D. magna* under different exposure conditions.

Concentration of NPs (mg/L)	Food	NPs Mass (ng)
3	/	54.26 ± 26.42
	Chlorella sp.	7.14 ± 0.94
	E. gracilis	14.04 ± 10.41
	yeast powder	25.04 ± 20.57
1	/	35.64 ± 54.82
	Chlorella sp.	6.71 ± 7.78
	E. gracilis	4.83 ± 3.91
	yeast powder	11.44 ± 9.09

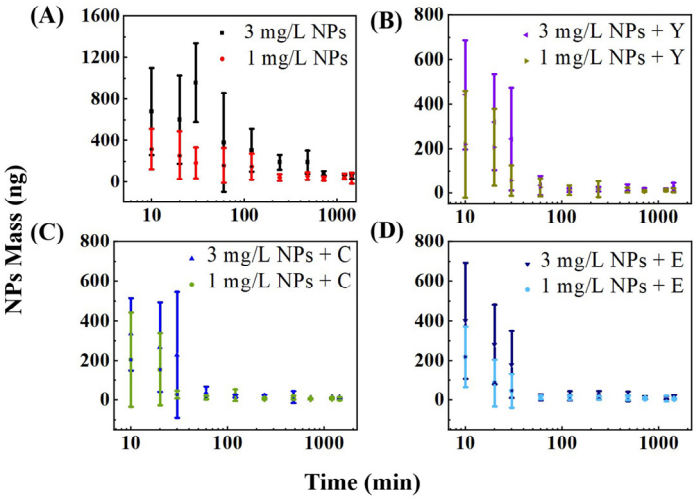

Figure 7. *D. magna* excreting NPs under different exposure conditions. (**A**) Body burdens of NPs in *D. magna* when exposed to NPs during the 24 h. (**B**–**D**) body burdens of NPs in *D. magna* when exposed to NPs + food during the 24 h (mean ± SD; *n* = 10). The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

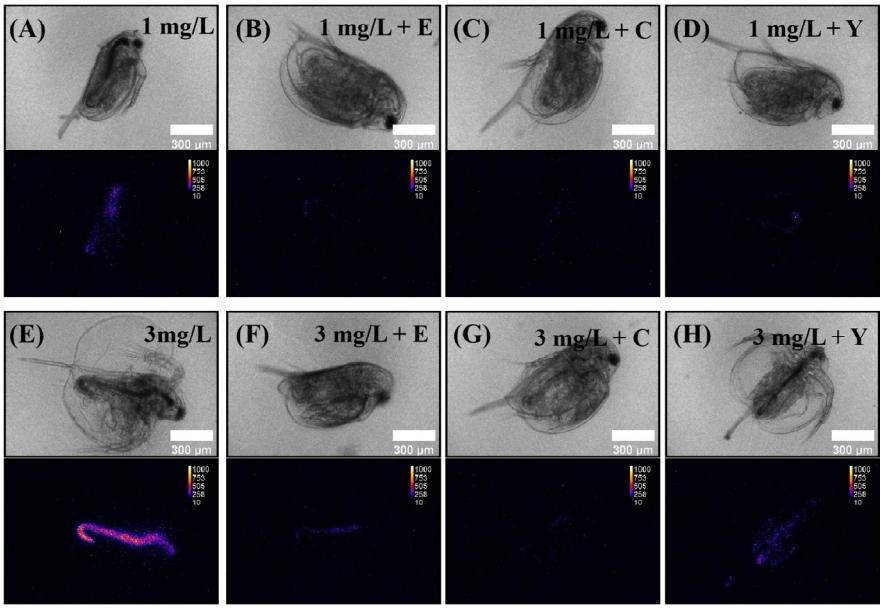

Figure 8. Bright field and dark field images of *D. magna* excreting NPs under different conditions for 12 h. (**A**–**D**) The concentration of NPs was 1 mg/L; (**E**–**H**) the concentration of NPs was 3 mg/L. The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

Excretion of NPs in *D. magna* could be well described by single exponential fitting: $y = y_0 + A \cdot \exp(-t/\tau)$. τ refers to the time constant of excretion. When no food was added, the time constant was 134.4 min (3 mg/L) and 33.8 min (1 mg/L), respectively (Figure 9). The results showed that the time constant was related to NPs mass in *D. magna*. In the 3 mg/L NPs groups, the τ for *Chlorella* sp., *E. gracilis*, and yeast powder were 21.1 min, 20.6 min, and 25.0 min, respectively (Figure 9A). In the 1 mg/L NPs groups, the time constants were 13.7 min, 12.4 min, and 17.0 min, respectively (Figure 9B). The excretion rates were found to be accelerated after adding food to the medium, especially in the 3 mg/L groups. The time constant of feeding yeast powder was about 4–5 min longer than that of the two types of algae. These results suggested that food played an important role in the excretion of pollutant particles by aquatic organisms.

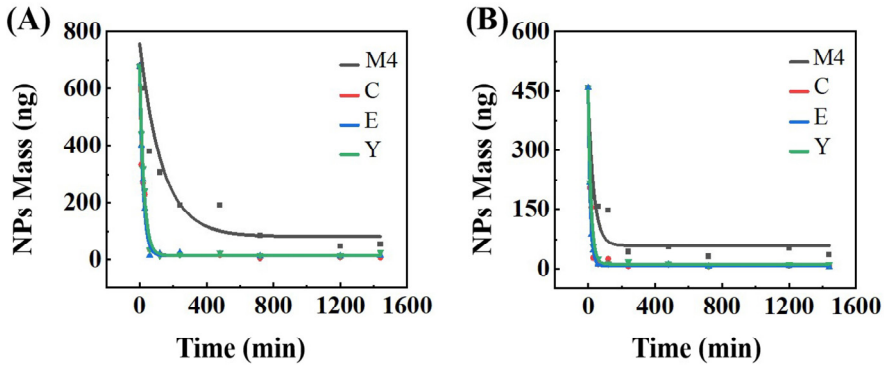

Figure 9. Single exponential fitting for *D. magna* excreting NPs. (**A**) The concentration of NPs was 3 mg/L; (**B**) the concentration of NPs was 1 mg/L. The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y).

4. Discussion

4.1. The Mechanism for the Effect of Food on the Uptake of NPs

In this work, it was clearly observed that the uptake of NPs by *D. magna* was related to the concentration of NPs, exposure time, and food (Figure 4). The number of particles increased with higher concentrations of NPs, which explained why more NPs were ingested by *D. magna* in the high concentration. Fluorescence signals were observed within 10 min and were found in the intestine tract of *D. magna*, which confirmed that NPs can be rapidly ingested by *D. magna* [40]. It can be explained by the behavior of *D. magna*. *D. magna*, as filter feeders, are capable of capturing NPs and food particles to their mouths in the form of "drinking water" by forming a stream of water [41].

Food plays a crucial role in the uptake of NPs mass by *D. magna*, not just providing conditions for their long-term survival. The results have shown that food supply can inhibit the uptake of NPs mass and slow down the uptake rate of NPs by *D. magna*. There was a study that also reported that when *D. magna* were exposed to 200 nm AIE-NH_2-NPs and 200 nm AIE-COOH-NPs, the uptake of NPs mass decreased by 69.3% and 81.9% after the algae were added, respectively [42].

For this inhibitory effect presented by food, it is speculated that there is an interaction between food and NPs. NPs can be adsorbed on the surface of food to form hetero-aggregates, which will settle to the bottom of the beaker [43,44]. Therefore, the bioavailability of NPs was reduced. To verify the reason for heterogeneous aggregation, the fluorescence intensity of NPs was measured after 24 h. It was found that the addition of yeast powder caused sedimentation of 25.5% NPs (Figure S4). There were 35.7% and 34.1% of NPs sedimented when there were additions of *Chlorella* sp. and *E. gracilis*, respectively (Figure S4). This may partially explain why *D. magna* ingested more NPs when yeast powder was used as a food compared with *Chlorella* sp. and *E. gracilis*. However, it is worth noting that the difference in uptake of NPs mass is not solely attributable to sedimentation. In the previous discussion, it was clearly found that the uptake of NPs mass by *D. magna* was significantly reduced under different food conditions, which could not be brought about by aggregation.

Some studies thought *D. magna* are selective filter-feeding animals. With the addition of food, they tend to ingest food particles [45]. This predatory preference may affect the uptake rate of NPs, but the specific degree of its contribution cannot be determined.

In addition, considering that the intestine tract is one of the primary sites of interaction for NPs in *D. magna* [46], it is necessary to pay attention to the interaction between food particles, NPs, and the intestine tract. The interaction between food and the intestine tract may have an effect on the load of NPs in the body. During the experiment, when *D. magna* were exposed to the NPs + food groups, foods were found in the intestine tract of *D. magna* (Figure S5). This indicated that some spatial sites in the intestine tract may be occupied by

food, resulting in the available spaces for NPs being reduced. Therefore, the occupation of the intestine tract may be the primary reason for the reduced uptake of NPs mass.

4.2. The Mechanism for the Effect of Food on the Excretion of NPs

In the excretion experiments, in the 3 mg/L groups, the time constant of excretion was reduced from 134.4 min to 20–25 min after adding food. In the 1 mg/L groups, the time constant was reduced from 33.8 min to 12–17 min (Figure 9). Food accelerated the excretion of NPs. It was reported that the 100 nm fluorescent polystyrene particles within the body of *D. magna* were significantly reduced in the excretion stage when algae were added [28]. This is consistent with our findings.

The intestine tract is the primary location of nutrient absorption [47]. The normal digestive processes are interfered with by NPs, leading to a decrease in animal energy assimilation [48]. So, for the facilitating effect presented by food on the excretion of NPs by *D. magna*, it is speculated that the nutritional components of these foods can provide a certain amount of energy for *D. magna*. *Chlorella* sp., *E. gracilis*, and yeast powder not only contain nutrients such as protein, amino acids, and lipids but also contain cellulose or dietary fiber [49–52]. Cellulose and dietary fiber can also promote intestinal peristalsis, which may also be the reason for the faster excretion of NPs by *D. magna*. To further verify the reason for energy, the starch solution without cellulose was chosen as food [53]. The results of uptake and excretion of NPs are consistent with those observed when feeding other foods. The addition of starch solution also reduced the NPs mass ingested by *D. magna* (Figure S6). In the excretion phase, the starch solution reduced the time constant of excretion to 27.0 min and 17.5 min in the 3 mg/L NPs groups and 1 mg/L NPs groups, respectively (Figure S7). Therefore, the energy supply of food may be the primary mechanism. The slight difference between yeast powder and algae may be because of certain nutrients contained in the microalgae diet, which can reduce bacterial communities. And because of the high enzyme and water content, microalgae can be ingested and digested very efficiently by organisms [54]. At the same time, we should also be aware that the energy provided by food to *D. magna* can also affect the process of NPs uptake, as food greatly accelerated the excretion of NPs.

Meanwhile, NPs were not completely excreted within 24 h. It was found that there was still a small amount of $nTiO_2$ remaining in the intestine tract during the 24 h excretion stage [55]. One explanation is that NPs may be stuck in the surface structure of microvilli in the fore intestine tract of *D. magna* [56].

In summary, the effects of food on the uptake and excretion of NPs by *D. magna*, which can be attributed to the combined effects of food occupying the intestine tract of *D. magna* and supplying energy. According to results, they further indicated that the occupation of the intestine tract by food may be the primary mechanism in the uptake NPs stage, and the energy supply may be the primary mechanism in the excretion NPs stage.

5. Conclusions

In this work, the processes of NPs uptake and excretion by *D. magna* were observed by the TGI system, and quantitative analysis was carried out to obtain NPs mass in *D. magna*. This study has shown that the uptake and excretion of NPs by *D. magna* were closely related to food supply. In the process of uptake of NPs, the inhibitory effect was presented by food. The inhibitory ability of different foods varies. The effect can be attributed to food occupying the intestine tract of *D. magna* due to foods being found in the intestine tract. And food can accelerate the excretion of NPs by *D. magna*. Starch solution serves as evidence that the food supplying energy is the cause of the results. This work aims to create more realistic conditions and help us to understand the importance of food supply in study of the effect of NPs on aquatic organisms. In the future, environmental conditions must be fully considered, and it is important to note that specific mechanisms of action of different foods remain to be fully understood.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16103941/s1, Figure S1: Schematic diagram of TGI system; Figure S2: Zeta potential of UCNPs@PS with 100 mg/L in pure water and Elendt M4 medium was measured by nano zetasizer ZS90; Figure S3: Different tissues of TEM images of the *D. magna* when exposed to NPs (3 mg/L) for 24 h. (A,B) Intestine tract; (C,D) carapace. Microvilli (MV), Nucleus (N), white arrows refers to NPs; Figure S4: Sedimentation of NPs under different conditions was measured by FLS980. The foods were *Chlorella* sp. (C), *E. gracilis* (E), and yeast powder (Y); Figure S5: Food was ingested by *D. magna* in uptake of NPs experiments; Figure S6: *D. magna* ingested NPs when the food was starch during the 24 h; Figure S7: (A) *D. magna* excreting NPs when the food was starch during the 24 h; (B) single exponential fitting for *D. magna* excreting NPs; Table S1: Chemical compositions of HUT medium for the cultivation of *E. gracilis*; Table S2: Chemical compositions of SE medium for the cultivation of *Chlorella* sp.; Table S3: Chemical compositions of Elendt M4 medium for the cultivation of *D. magna*.

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