

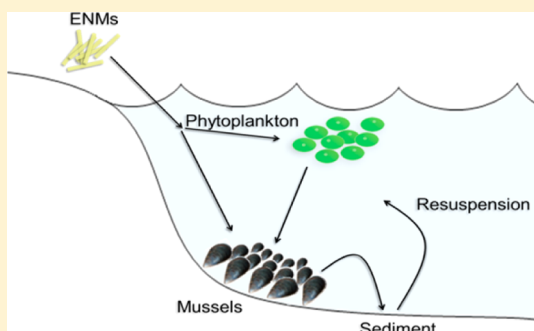
Effects and Implications of Trophic Transfer and Accumulation of CeO₂ Nanoparticles in a Marine Mussel

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S Supporting Information

ABSTRACT: Bivalves are hypothesized to be key organisms in the fate and transport of engineered nanomaterials (ENMs) in aquatic environments due to their ability to filter and concentrate particles from water, but how different exposure pathways influence their interactions with ENMs is not well understood. In a five-week experiment, we tested how interactions between CeO₂ ENMs and a marine mussel, *Mytilus galloprovincialis*, are affected through two exposure methods, direct and through sorption to phytoplankton. We found that phytoplankton sorbed ENMs in <1 h. The exposure methods used did not result in significantly different mussel tissue or pseudofeces Ce concentrations. Approximately 99% of CeO₂ was captured and excreted in pseudofeces and average pseudofeces mass doubled in response to CeO₂ exposure. Final mean dry tissue Ce concentration (\pm SE) for treatments exposed to 3 mg L⁻¹ CeO₂ directly was 33 \pm 9 μ g g⁻¹ Ce, and 0 \pm 0, 19 \pm 4, 21 \pm 3, and 28 \pm 5 μ g g⁻¹ for treatments exposed to 0, 1, 2, and 3 mg L⁻¹ CeO₂ sorbed to phytoplankton. Clearance rates increased with CeO₂ concentration but decreased over time in groups exposed to CeO₂ directly, indicating stress. These results show the feedback between ENM toxicity and transport and the likelihood of biological mediation in the fate and transport of ENMs in aquatic environments.



1. INTRODUCTION

With the rapidly rising use of engineered nanomaterials (ENMs) in consumer products and industry over the past 10 years,¹ concerns about their effects on human health and the environment have grown. The novel size-dependent properties that have made ENMs a growth industry also make their behavior in the environment and impacts on organisms difficult to predict based on knowledge of bulk materials. In conjunction with research into the physicochemical behavior and toxicological potential of relevant ENMs, environments, and organisms with high risks of exposure and key transport pathways need to be identified and investigated to properly manage and regulate this new class of materials.

The coastal marine environment is a probable sink for many ENMs as a consequence of patterns of use and disposal (e.g., TiO₂ in sunscreens,² paints,³ and other products;⁴ silver from fabrics and other household items;^{5,6} and CeO₂ catalyst in diesel fuel⁷) which will result in direct release to the ocean or indirect release through stream and urban runoff^{3,7} and wastewater treatment plant effluent.⁸ Once in the ocean ENMs will interact with organisms that inhabit the water column and benthic habitats. Two classes of marine organisms that are prevalent in coastal marine areas and have important ecological roles are phytoplankton, which are the base for many marine food webs, and bivalve filter feeders, including mussels, which feed on phytoplankton.

Mussels are highly efficient filter feeders, with individual mussels being able to filter more than 1 L of water per hour,⁹ and come into contact with large volumes of coastal seawater each day. Mussels and other marine bivalves have been targeted as a key model group for ENM studies due to their specialization in the collection and processing of particulate matter.¹⁰ A previous study¹¹ saw that mussels exposed to CeO₂ and ZnO ENMs over four days concentrated and excreted ENMs primarily in pseudofeces, a mucous-based secretion used to trap and remove inedible items from their gills before digestion occurs. The ENM-laden pseudofeces will then likely become incorporated into ocean sediments, where they may be bioavailable to a number of infaunal, grazing, and benthic organisms and may be resuspended in the water column by tidal action or bioturbation.

Here we explore the interactions between mussels, phytoplankton, and the common metal oxide ENM CeO₂¹² to investigate whether direct exposure to ENMs in the water column or indirect exposure through ENMs sorbed to live phytoplankton significantly alters this interaction. Several studies have looked at the trophic transfer and biomagnification of ENMs in terrestrial,¹³ freshwater,¹⁴ and marine¹⁵ food

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chains, but few have used marine bivalves at a subchronic time scale, i.e., lasting between 5 and 90 days.¹⁶ Marine bivalves are capable of filtering and selectively excreting inedible particles,¹⁷ and so may filter free CeO₂ from the water and capture it in pseudofeces while ingesting phytoplankton which have sorbed CeO₂. The interactions of phytoplankton with ENMs are not yet well understood, but sorption of CdSe quantum dots has been seen in a freshwater species.¹⁴ This suggests that transfer of other ENMs from phytoplankton to primary consumers and higher trophic levels is possible.

On the basis of this, we hypothesized that phytoplankton would sorb the majority of CeO₂ ENMs within three days, but would not suffer acute growth effects as a result of exposure.¹⁸ We also hypothesized that the rate at which mussels accumulate ENMs would be dependent on whether exposure was via trophic transfer or not. We predicted that mussels would accumulate more CeO₂ from trophic transfer than direct exposure due to increased bioavailability of CeO₂ ENMs sorbed to phytoplankton than free ENMs in the water through a “piggybacking” mechanism that allows the ENMs to bypass the mussels’ filtering processes. We also predicted that mussels exposed to phytoplankton-sorbed CeO₂ would show proportionally decreased feeding rates with increasing exposure concentration due to stress^{19,20} caused by the preferential accumulation of sorbed CeO₂. CeO₂ ENMs have an uncertain biological effect^{21,22} and did not result in a measurable physiological impact on mussels during an acute direct exposure,¹¹ but we hypothesized that with increasing accumulation toxicity may occur and depress feeding performance.

2. EXPERIMENTAL SECTION

2.1. Preparation and ENM Exposure. Detailed characterization of the CeO₂ ENMs and filtered seawater used can be found in Keller et al.²³ Briefly, CeO₂ ENMs were obtained from Melioris Technologies, Rochester, NY and were rod-shaped ($67 \pm 8 \times 8 \pm 31$ nm, $\leq 10\%$ polyhedra) with a surface area of $93.8 \text{ m}^2 \text{ g}^{-1}$. When suspended in $0.45 \mu\text{m}$ -filtered Santa Barbara seawater they aggregate rapidly to a stable agglomerate hydrodynamic diameter of approximately 800 nm as measured by dynamic light scattering (DLS, Zetasizer Nano ZS-90, Malvern Instruments), which agrees with previous findings.²³ Stock ENM suspensions of 1 g L^{-1} CeO₂ were prepared by adding dry CeO₂ ENM powder to filtered seawater, bath sonicating for 30 min, and shaking vigorously prior to use.

Cultures of *Isochrysis galbana* (Prymnesiophyceae: Isochrysidales) were grown from axenic cultures obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, U.S.) in standard media (*f/2*) made with autoclaved filtered seawater. Cultures were grown for 13 days at 20°C on stir plates under cool white fluorescent lights ($100\text{--}120 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 14:10 light/dark), at which time logarithmic growth had been reached. Cultures were then exposed to 30 mg L^{-1} CeO₂ added from 1 g L^{-1} stock suspensions and incubated for 3 days to allow ample time for sorption to occur. This exposure concentration was chosen to reduce the total volume of phytoplankton required to be grown, while not causing significant toxicity at the time scales of this experiment. Cell densities were estimated using fluorescence (Trilogy, Turner Designs Model No.7200–043 with Chl-*a* in vivo module excitation wavelength 485 nm, emission wavelength 685 nm), which was converted to cell- mL^{-1} using a

standard curve made from hemacytometer counts (Reichert, Buffalo NY). Fluorescence interference from CeO₂ was negligible at the concentrations measured.

Mytilus galloprovincialis (Taylor Shellfish Farms Shelton, WA, U.S.) were acclimated under flowing, sand filtered seawater for 10 days prior to the start of the experiment. Mussels tested for CeO₂ accumulation ($n = 375$) were measured for total length and sorted into five size ranges of 3 mm from 45 to 60 mm in length (i.e., 45–48 mm, 48.1–51 mm, etc.) and kept in 750 mL filtered seawater with each cup having an individual from each size range. Mussels measured for clearance rate effects ($n = 25$) ranged from 48 to 51 mm and were kept individually in 150 mL filtered seawater. All sample cups received aeration and were kept at 15°C for the duration of the experiment. Every other day seawater was changed and mussels were fed a mixture of unexposed and CeO₂ ENM-exposed *I. galbana* cultures to keep a consistent concentration of phytoplankton of roughly 200 000 cells mL^{-1} while making total CeO₂ concentrations in the sample containers of 0, 1, 2, and 3 mg L^{-1} . As mentioned above, ENM-exposed phytoplankton cultures had a concentration of 30 mg L^{-1} CeO₂. One treatment was fed only unexposed *I. galbana* but received direct additions of a CeO₂ ENM stock suspension approximately 10 min after feedings for a final exposure concentration of 3 mg L^{-1} to simulate nonbiologically mediated exposure. While no direct data are available on CeO₂ ENM concentrations in coastal areas, the concentrations used here were chosen to be as close as possible to potential environmental concentrations near source zones,¹² while allowing for detectable levels in the tissue.

2.2. Clearance and Accumulation Rates. A previous study¹¹ showed low Ce tissue concentrations in *M. galloprovincialis* after four days of exposure, so a longer exposure time (37 days) was chosen to allow for greater accumulation to occur. Sampling was performed at the end of the first five days and then at the end of each week for the duration of the experiment. Maximum clearance rates, i.e., the rate at which mussels filter phytoplankton from the water column, were estimated by measuring the decrease in phytoplankton concentration every 10 min for 40 min post feeding using chlorophyll fluorescence. ENM filtration rate in the group fed CeO₂ directly was calculated from the clearance rate and assuming that $>99.9\%$ of CeO₂ was filtered from the water over a 2 day time period. In a previous study using *M. galloprovincialis* and CeO₂ ENMs at a similar concentration (1 mg L^{-1})¹¹ $<0.1\%$ of CeO₂ was left in the water after 24 h, which lends support to our assumption. Mussels sampled for Ce accumulation were allowed to clear their gut for 72 h in clean seawater with no added feed and were then dissected from their shells, rinsed in 5% HNO₃ and purified water (Barnstead Nanopure, Thermo Fisher Scientific, Waltham, MA, U.S., resistivity $>18 \text{ M}\Omega\text{cm}$), and dried for 72 h at 60°C . Tissue samples were weighed and digested in a 3:2 mixture of 30% H₂O₂/HNO₃ at 80°C for 2.5 h. Pseudofeces was allowed to settle in sample cups after aeration was removed then collected by pipet and triple washed with purified water. Identical drying, weighing, and digestion techniques were followed for pseudofeces samples. Since mussel feces dissolve in water, it was assumed that in the closed systems used in this experiment they were refiltered by the mussels and eventually the CeO₂ ENMs would be captured in tissue or pseudofeces. Tissue and pseudofeces samples were analyzed for Ce via inductively coupled plasma atomic emission spectroscopy (ICP-AES, iCAP 6300 Thermo Scientific, Waltham, MA),

with a detection limit of $50 \mu\text{g L}^{-1}$. Standard solutions were measured every 15 samples for quality assurance. Results are reported as Ce but samples are assumed to have contained Ce in the form of nanoparticulate CeO_2 before acid digestion.

2.3. Phytoplankton/ CeO_2 Interactions. Sorption of CeO_2 ENMs to *I. galbana* was determined by the separation of the fraction of phytoplankton with sorbed CeO_2 using centrifugal split-flow thin-channel (CSPLITT) fractionation (CFS 2000, Postnova Analytics, Salt Lake City, UT). Operational parameters (flow rates and centrifuge rpm) were calibrated for this experiment to give a roughly 50/50 split of both *I. galbana* and CeO_2 ENMs into the two fractions (lighter and denser) produced by this technique, which gave the greatest sensitivity to changes in either component or fraction. The cutoff density between the light and dense fractions is approximately 0.5 g cm^{-3} , the average density of an *I. galbana* cell. Sorption of CeO_2 ENMs to a cell will increase its density and increase the likelihood it will be captured in the dense fraction.

To perform a separation, 0.5 mL of phytoplankton culture exposed to CeO_2 ENMs was injected into the channel at 0.5 mL min^{-1} using a 1:7 filtered seawater/deionized water carrier solution with a flow rate of 4.5 mL min^{-1} and spun at 600 rpm for 15 min, followed by a second injection of a second 0.5 mL of exposed phytoplankton. Dense and light fractions were collected during these steps, which corresponded respectively to phytoplankton with and without sorbed CeO_2 . After an additional 15 min, the centrifuge was shut off and collection was routed entirely through the dense outflow for 20 min before stopping. Phytoplankton concentrations were determined through visual counts. Ce concentrations could not be reliably detected in either fraction via ICP-AES analysis due to the large dilution necessitated by this technique.

Sorption of CeO_2 ENMs to phytoplankton cells was verified by exposing cultures to 1, 10, 20, and 30 mg L^{-1} CeO_2 ENMs for 30 min and separating sorbed and unsorbed fractions. Sorption kinetics were estimated by exposing phytoplankton cultures to 30 mg L^{-1} CeO_2 ENMs and measuring phytoplankton densities in each fraction in a time series, beginning immediately after exposure then at 6, 12, 24, 48, 72, and 96 h. Cell weight was calculated by comparing the mass of identical volumes of seawater and phytoplankton cultures of a known cell density.

Dissolved extracellular polymeric substances (EPS) were extracted from *I. galbana* cultures by centrifuging cultures at 1545 g for 30 min and collecting the supernatant.²⁴ EPS protein and carbohydrate content was measured using the modified Lowry Protein Assay Kit (Pierce, 23240, U.S.) and the anthrone method,²⁵ respectively.

2.4. Statistical Analyses. We tested whether clearance rate, Ce concentrations in pseudofeces, pseudofeces mass, and phytoplankton growth varied as functions of time and CeO_2 exposure treatment using two-way ANOVA. We also tested whether the sorption of CeO_2 to phytoplankton varied over time with a one-way ANOVA. When ANOVA showed significant differences, Tukey's tests were conducted posthoc. A linear regression was performed to test the dependence of clearance rate of mussels exposed to CeO_2 ENMs directly on the length of exposure. Levene's test was used to ensure homogeneity of variance between groups. Tissue Ce concentrations did not follow a normal distribution, therefore Kruskal–Wallis tests with posthoc Dunn's test for multiple comparisons were used to determine if Ce tissue concentrations

varied significantly as functions of time, exposure method, and exposure concentration. Hydrodynamic diameters of CeO_2 ENMs in dissolved EPS solution and phytoplankton growth media were compared using a two-sample *t* test. All statistical analyses were performed using the statistical software R (v. 2.11.1).

3. RESULTS AND DISCUSSION

3.1. Phytoplankton/ CeO_2 Interactions. In *I. galbana* cultures exposed to 30 mg L^{-1} CeO_2 ENMs for 8 days, we found that the impact of CeO_2 exposure on cell count depends on exposure time (ANOVA, $F_{2,8} = 2.46$, $p < 0.05$). Only on the eighth day were cell counts significantly lower in cultures exposed to CeO_2 than in control cultures (Tukey's test, $p < 0.05$), showing that CeO_2 ENMs cause subchronic toxic effects in these phytoplankton. However, phytoplankton cultures fed to mussels were only incubated with CeO_2 for 3 days, at which point cell concentrations between exposed and unexposed cultures were not significantly different (Tukey's test, $p > 0.05$, Figure 1).

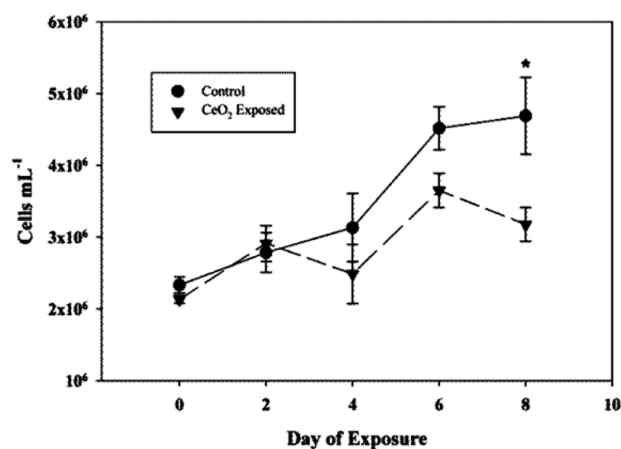


Figure 1. Mean \pm SE *I. galbana* growth with and without exposure to 30 mg L^{-1} CeO_2 in filtered seawater ($n = 30$, Two-way ANOVA with Tukey's test, asterisk represents statistically significant difference, $p = 0.01$).

We found that sorption of CeO_2 ENMs to phytoplankton varied significantly with time (ANOVA, $F_{1,7} = 12.98$, $p < 0.0001$) and occurred within 1 h after exposure to 30 mg L^{-1} CeO_2 ENMs (Tukey's test, $p < 0.0001$). This is shown by the separation of $>96\%$ of exposed phytoplankton into the denser fraction immediately after exposure, as compared to 65% in the control. We also found that sorption reached a steady state and did not vary after the first hour (Tukey's test, $p > 0.1$). The rapidity with which sorption occurred points to an electrostatic attraction between the charged surfaces of the ENMs and the phytoplankton cells, as opposed to a chemical process or active uptake by the phytoplankton. In cultures exposed to 0, 1, 10, 20, and 30 mg L^{-1} CeO_2 ENMs, we found that the proportion of phytoplankton present in the dense fraction increased with increasing exposure concentration (Figure 2).

From this, we can conclude that most or all of the CeO_2 ENMs sorbed to phytoplankton at the exposure concentrations tested. This is supported by the observation that no sedimentation of aggregates was seen in exposed phytoplankton cultures, whereas large ($>1 \mu\text{m}$) aggregates could be seen under a light microscope after 1 h when CeO_2 ENMs were suspended

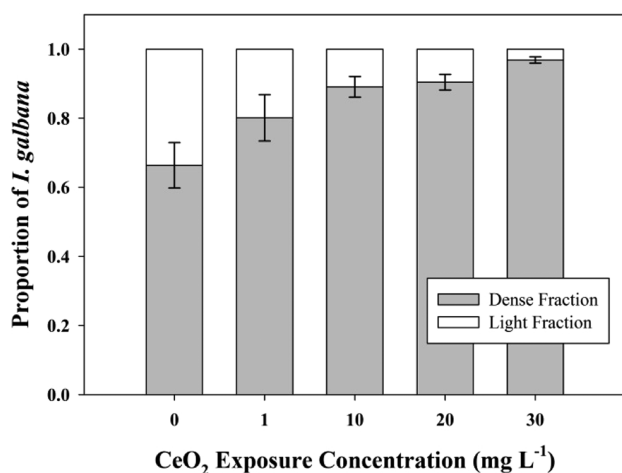


Figure 2. Mean \pm SE proportions of *I. galbana* cells in light and dense fractions separated by CSPLITT with increasing CeO₂ ENM exposure.

in seawater at an equivalent concentration. This is most likely a consequence of sorption and not disaggregation by EPS because when CeO₂ ENMs were suspended at a concentration of 30 mg L⁻¹ in a dissolved EPS solution (containing $2.3 \pm 0.1 \mu\text{g mL}^{-1}$ carbohydrates and $13.3 \pm 0.0 \mu\text{g mL}^{-1}$ proteins (\pm SE)) aggregate sizes measured by DLS were not significantly different (two-sample $t(2.558) = 0.5$, $p > 0.5$) from those in growth media only. Additionally, sedimentation of aggregates identical to that seen in seawater was observed. Due to the presence of phytoplankton, CeO₂ particle size in exposed cultures could not be determined through DLS to give a direct comparison, but together, these observations point to the conclusion that all or nearly all ENMs sorbed to phytoplankton. This gives a per cell mass load of 27 ± 1 picograms CeO₂ (\pm SE) at 30 mg L⁻¹ exposure concentration, which corresponds to a nonsteady state partitioning coefficient (K_s , the ratio of phytoplankton Ce concentration to the concentration of Ce in the surrounding media, akin to a bioconcentration factor), of 1.1×10^4 .

3.2. Accumulation and Rejection of CeO₂ ENMs by *M. galloprovincialis*. Our primary hypothesis in this experiment was that mussels would accumulate more Ce when exposed to CeO₂ that was sorbed to phytoplankton than free CeO₂ in the water column, and that they would accumulate more Ce over time. This was refuted by our results as we found that the impact of exposure concentration on Ce concentration in mussel tissue varied significantly with time (Kruskal–Wallis, $H = 52.05$, $p < 0.0001$), but neither the method of exposure nor the exposure concentration resulted in a significant difference in general (Dunn's test, $p > 0.5$, Figure 3). Although there was a difference in accumulation based on exposure method on day 5 (Kruskal–Wallis, $H = 10.58$, $p < 0.01$), there was no significant difference over the longer term.

Final tissue Ce concentrations \pm SE for treatments exposed to 3 mg L⁻¹ CeO₂ free in the water column, and 1, 2, and 3 mg L⁻¹ CeO₂ sorbed to phytoplankton were 33 ± 9 , 19 ± 4 , 21 ± 3 , and $28 \pm 5 \mu\text{g g}^{-1}$ Ce in dry mussel tissue, respectively (Table S1 of the Supporting Information). Although mussels exposed to CeO₂ ENMs did reject large amounts of Ce in pseudofeces, we did not find a significant dependence of pseudofeces Ce concentration on time (ANOVA, $F_{2,3} = 0.680$, $p > 0.5$) or exposure (ANOVA, $F_{2,3} = 0.412$, $p > 0.1$) corresponding to concentration changes in pseudofeces over

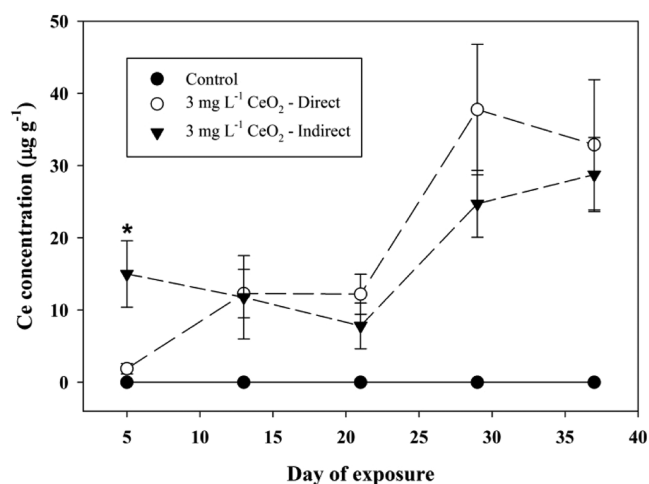


Figure 3. Mean \pm SE CeO₂ concentration in tissue of mussels exposed to 0 and 3 mg L⁻¹ CeO₂ ENMs directly in the water column or indirectly through trophic transfer. Direct and indirect treatments were not significantly different ($n = 135$, Dunn's test comparing direct and indirect treatments at 3 mg L⁻¹ CeO₂ exposure, $p > 0.05$) except at Day 5, marked by asterisk (Kruskal–Wallis, $H = 10.58$, $p < 0.01$). Treatments exposed to 1 and 2 mg L⁻¹ CeO₂ ENMs indirectly through trophic transfer are not shown for the sake of clarity.

time (Table S2 of the Supporting Information). This was due to the increased production of pseudofeces with CeO₂ exposure (Figure 4). An estimate of the mass balance for each treatment can be seen in Figure 5.

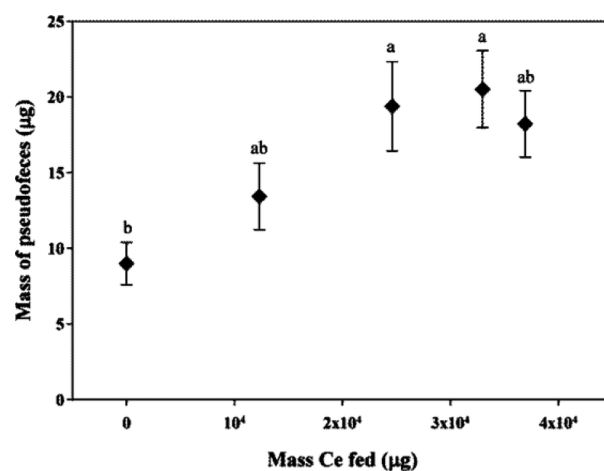


Figure 4. Mean \pm SE pseudofeces masses in μg from the final three sampling days as a function of the total mass fed in μg . From left to right, the points correspond to the control, 1 $\mu\text{g g}^{-1}$ CeO₂ indirect exposure, 2 $\mu\text{g g}^{-1}$ CeO₂ indirect exposure, 3 $\mu\text{g g}^{-1}$ CeO₂ direct exposure, and 3 $\mu\text{g g}^{-1}$ CeO₂ indirect exposure treatments. Letters represent statistical grouping from Tukey's test.

On the basis of these data, the nonsteady state partitioning coefficients (K_p , the ratio of tissue Ce concentration to the concentration of Ce in the surrounding media, similar to bioaccumulation factors) for each treatment were low, ranging from 8.8 to 18 with a median of 11. This corresponds well with the results of two other studies using marine invertebrates.^{14,26} For pseudofeces excreted by the mussels, a pseudofeces-water partitioning coefficient (K_p , the ratio of pseudofeces Ce concentration to the concentration of Ce in the surrounding

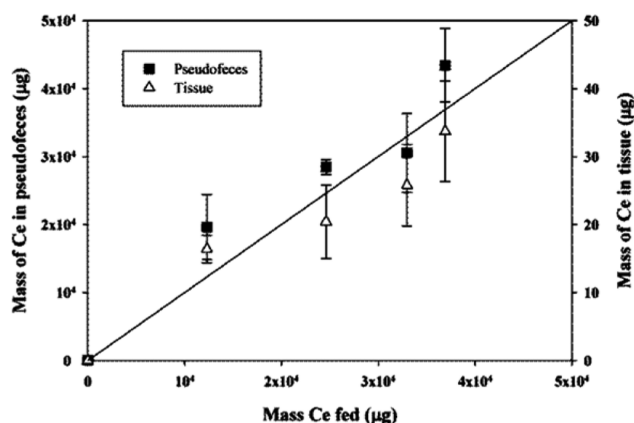


Figure 5. Mass balance of accumulation and excretion of CeO_2 by *M. galloprovincialis* showing the average masses of Ce in tissue and pseudofeces (\pm SE) as a function of the total mass fed in μg . From left to right, the points correspond to the control, $1 \mu\text{g g}^{-1}$ CeO_2 indirect exposure, $2 \mu\text{g g}^{-1}$ CeO_2 indirect exposure, $3 \mu\text{g g}^{-1}$ CeO_2 direct exposure, and $3 \mu\text{g g}^{-1}$ CeO_2 indirect exposure treatments. The diagonal line represents a 1:1 ratio for mass Ce fed vs mass of Ce in pseudofeces.

media) was also determined for each treatment. Expressed as $\log_{10} K_p$, the values we calculated have a range between 3.45 and 4.0 with a median of 3.6.

We found accumulation of CeO_2 in mussel tissues over time in all experimental groups, which when considered with the constant Ce concentrations in pseudofeces suggests that some ENMs were being stored either in the digestive gland or elsewhere in the organism and were not immediately captured in pseudofeces and excreted. It is unlikely that a significant amount remained in the gut as bivalves have been shown to excrete micrometer-scale particles after 30 h or less with and without additional food.^{27,28} In seawater, CeO_2 ENMs form aggregates near $1 \mu\text{m}$ and so will likely be egested well before the 72 h allowed for here. CeO_2 ENMs have been shown to disaggregate at lower pH,²⁹ so the presence of Ce in mussel tissues detected by elemental analysis could be due either to disaggregation or dissolution caused by the low pH encountered in the digestive gland. This would allow for passage of ions or individual ENM primary particles through the gut wall. Entry into body tissues of 10 nm amine-coated Au ENMs in a freshwater bivalve³⁰ and ionic zinc released by ZnO ENMs in *M. galloprovincialis*¹¹ has been seen, which suggests that Ce ions and/or CeO_2 primary particles may behave similarly.

3.3. Physiological Effects of CeO_2 Exposure. A small amount of mortality (<1%) was observed in this experiment, but was not correlated with any group. The effect of exposure

to CeO_2 ENMs on clearance rates of phytoplankton from the water varied significantly over time (ANOVA, $F_{2,16} = 1.90$, $p < 0.05$, Table 1). In general, clearance rates for trophic transfer treatments showed a positive correlation with exposure concentration over all time points, while in treatments exposed to CeO_2 directly, clearance rates decreased over time ($R^2 = 0.39$, $F_{1,16} = 10.21$, $p < 0.01$). This agrees with our hypothesis that exposure method influences clearance rate, but the response is different than our prediction that clearance rates in groups exposed to ENMs would be depressed due to stress—in fact, clearance rates in CeO_2 treated groups were higher than the control group. This increase in clearance rate is likely linked to the similar increase in pseudofeces production in all exposure treatments (Figure 4). A possible explanation is that the mussels perceive CeO_2 as being inedible and increase pseudofeces production to capture and reject the ENMs, which also increases the capture and rejection of phytoplankton. Decreasing food quality has been seen to result in increased clearance rate in another marine bivalve,²⁸ and so may account for this response. To counter the decreased amount of food ingested, the mussels compensate by increasing clearance rate. In food-limited environments like those simulated in this experiment, this could potentially lead to starvation and associated growth and reproductive effects.

As is shown in Table 1, clearance rates in treatments exposed to CeO_2 free in the water decreased over time, whereas those exposed to CeO_2 sorbed to phytoplankton remained consistently high. One possible explanation for this is that the mussels become desensitized to the presence of free CeO_2 ENMs over time and consequentially decrease their feeding rate. Alternately, exposure to free CeO_2 could be causing a mild toxic response that results in a gradual depression of the initially elevated clearance rate. Since both exposure methods resulted in accumulation of Ce within tissue but only unsorbed CeO_2 caused depressed feeding rates the toxicity of unsorbed CeO_2 ENMs may be expressed before storage in body tissue, perhaps in the form of irritation to the gastrointestinal lining.

Multiple studies of mussels and other bivalves have shown that clearance rates decrease in response to exposure to toxic substances,^{19,31,32} including one study that used another ENM, C60.³³ The mechanism for toxicity of CeO_2 ENMs is currently under debate.^{11,21,22,29,34,35} Two of the main toxicity paradigms for metal and metal oxide ENMs currently considered are release of toxic ions and reactive oxygen species (ROS) production,^{18,36,37} but these mechanisms are not likely to be responsible for the response we found in mussels or phytoplankton. CeO_2 has a very low rate of dissolution³⁸ and while possibly dissolving slightly at the lower pH of the mussel digestive gland will not release large amounts of toxic ions like ZnO and Ag ENMs^{39–41} during their short residence time in

Table 1. Mean \pm SE Clearance Rates ($\times 10^3$ cells $\text{mL}^{-1} \text{min}^{-1}$) over Time of *M. galloprovincialis* Exposed to 0, 1, 2, or 3 mg L^{-1} CeO_2 ENMs Indirectly through Trophic Transfer or 3 mg L^{-1} CeO_2 ENMs Suspended Freely in the Water (Direct)^{a,b}

[CeO_2] _{nominal} (mg L^{-1})	day of exposure				
	5	13	21	29	37
3 (direct)	11.2 \pm 1.0 ^a	10.4 \pm 1.7 ^{N.S.}	8.24 \pm 1.5 ^{ab}	8.31 \pm 1.1 ^b	5.19 \pm 2.1 ^{N.S.}
3	10.3 \pm 2.8 ^{ab}	9.16 \pm 0.9 ^{N.S.}	11.4 \pm 0.8 ^{ab}	13.0 \pm 0.5 ^{ab}	9.92 \pm 3.5 ^{N.S.}
2	4.35 \pm 2.1 ^{abc}	6.19 \pm 0.2 ^{N.S.}	7.55 \pm 0.3 ^{bc}	9.72 \pm 0.7 ^b	8.46 \pm 2.5 ^{N.S.}
1	4.11 \pm 1.9 ^{bc}	6.64 \pm 0.2 ^{N.S.}	7.57 \pm 0.2 ^{bc}	7.54 \pm 0.2 ^b	6.67 \pm 1.0 ^{N.S.}
0	1.95 \pm 0.8 ^c	5.24 \pm 1.6 ^{N.S.}	4.14 \pm 0.2 ^c	2.18 \pm 0.8 ^c	3.49 \pm 1.6 ^{N.S.}

^aLetters represent statistical groupings from Tukey's test by day of exposure. ^bN.S. = not statistically significant.

the gut. Additionally, there are several reports that CeO₂ ENMs can act as an antioxidant and anti-inflammatory by mimicking and outperforming the superoxide dismutase enzyme, thereby quenching ROS.^{39,42–45} However, CeO₂ ENMs may participate in several other toxicity mechanisms that have the potential to cause harmful effects at high enough concentrations or over long time scales due to their unusual electron configurations and redox capacities,⁴⁶ such as cell membrane disruption or energy transduction pathway interruption.⁷ Without further testing, however, hypotheses on the precise cause of the toxic response seen here remain speculation.

4. ENVIRONMENTAL SIGNIFICANCE

We hypothesized here that close association of ENMs with phytoplankton may potentially bypass the mussels' filtering processes through a "piggybacking" mechanism. Figure 3 shows that this may have been causing enhanced uptake in the first 5 days of exposure, and although this effect disappeared after two weeks, this finding has significant implications for acute environmental exposure scenarios. Mussels in the environment may uptake ENMs rapidly upon introduction into the environment (where ENMs will be associated with phytoplankton or natural sediments⁴⁷), which may not be predicted by studies that do not take dietary exposure into account. This and other studies have shown differential effects between dietary and direct exposure to toxicants in aquatic organisms, with dietary exposure generally exhibiting greater toxic effects.^{48–50} Even though we did not find a clear indication of toxicity due to association with food items, the multifold increase in clearance rate seen in all exposures followed by a subsequent decrease in the direct exposure has environmental significance for both the health of the mussels and the fate and transport of these ENMs in aquatic environments.

Additionally, phytoplankton in this experiment were seen to sorb all available CeO₂ ENMs within 1 h of exposure and remain in this sorbed state for at least five days. The rapid rate with which sorption occurs means that some amount of sorption may have occurred before the mussels took up the phytoplankton in the exposure group we have been referring to as direct or unsorbed, despite the high clearance rates typical of mussels and other bivalve filter feeders. This issue was at least partially mitigated as a consequence of the ~10 min period between the addition of phytoplankton and the addition of CeO₂ to the sample containers, which allowed for the majority of the phytoplankton to be filtered from the water before the introduction of the ENMs. The fact that we found a toxicological response in the direct exposure treatment supports the distinction between the two exposure scenarios, although the high affinity of CeO₂ ENMs for phytoplankton suggests the environmental relevance of a direct exposure method may in fact be low.

When this rapid sorption is taken into consideration, along with the effects of CeO₂ ENM exposure on mussel accumulation and physiology, a coherent model of the interactions between the components of our system begins to emerge. With increasing exposure concentrations, mussels increase their clearance rates as a response to what they perceive as a low-quality food source as well as increasing their production of pseudofeces in order to prevent the ingestion of ENMs. Due to these responses Ce concentrations in the tissue and pseudofeces remain constant with increasing exposure concentrations, although as can be seen in the mass balance (Figure 5), total Ce mass in the pseudofeces is positively

correlated with exposure concentration. Over time, the direct exposure to CeO₂ begins to exert toxic effects, resulting in a lowering of clearance rate. This may or may not lead to an eventual increase in bioconcentration compared to dietary exposure, depending on whether pseudofeces production also declines. If so, mussels may be less able to reject CeO₂ ENMs and will ingest and bioconcentrate more of the ENMs.

A model summarizing the processes and rates calculated in this study is shown in Figure 7. Despite not being at steady

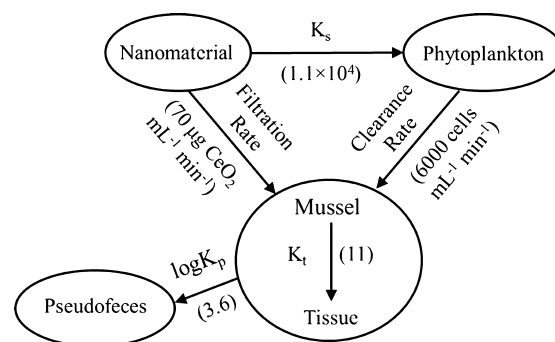


Figure 7. Summary of processes with rates calculated based on the results of these experiments. Filtration and clearance rates are means and are for ENMs and phytoplankton respectively, K_s , K_y , and $\log K_p$ are the unitless nonsteady state partitioning coefficients for phytoplankton, mussel tissue, and pseudofeces, respectively.

state, the partitioning coefficients calculated can still provide information on the environmental significance of these results. For example, the low K_t and high K_p values we saw for mussels in this study lead to the conclusion that ENM rejection in pseudofeces resulting in buildup in marine sediments will be a primary fate for insoluble metal oxide ENMs in ecosystems characterized by large populations of filter-feeding bivalves, such as rocky intertidal zones or shellfish farms. Additionally, based on what we have shown here, it is likely that a significant portion of the fate and transport of ENMs in aquatic environments will be biologically mediated in some way. This could either occur through sorption to phytoplankton and other algae or by exposure to concentrated organic deposits like pseudofeces, which has been verified in other studies looking at marine and estuarine systems.^{51,52} The next step as indicated by this and other research is to evaluate the uptake of ENMs by decomposers and other benthic organisms and any toxic effects they may have to continue to build a more complete picture of the fate and consequences of ENMs in the marine environment.

■ ASSOCIATED CONTENT

📄 Supporting Information

Mean \pm SE mussel tissue and pseudofeces Ce concentrations ($\mu\text{g g}^{-1}$) over time (Tables S1 and S2). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

BCF	bioconcentration factor
CSPLITT	centrifugal split-flow thin-channel
ENM	engineered nanomaterial
K_{pf}	pseudofeces-water partitioning coefficient

REFERENCES

- Peralta-Videa, J. R.; Zhao, L. J.; Lopez-Moreno, M. L.; de la Rosa, G.; Hong, J.; Gardea-Torresdey, J. L. Nanomaterials and the environment: A review for the biennium 2008–2010. *J. Hazard. Mater.* **2011**, *186* (1), 1–15.
- Lewicka, Z. A.; Benedetto, A. F.; Benoit, D. N.; Yu, W. W.; Fortner, J. D.; Colvin, V. L. The structure, composition, and dimensions of TiO₂ and ZnO nanomaterials in commercial sunscreens. *J. Nanopart. Res.* **2011**, *13* (9), 3607–3617.
- Kaegi, R.; Ulrich, A.; Sinn, B.; Vonbank, R.; Wichser, A.; Zuleeg, S.; Simmler, H.; Brunner, S.; Vonmont, H.; Burkhardt, M.; Bollner, M. Synthetic TiO₂ nanoparticle emission from exterior facades into the aquatic environment. *Environ. Pollut.* **2008**, *156* (2), 233–239.
- Weir, A.; Westerhoff, P.; Fabricius, L.; Hristovski, K.; von Goetz, N. Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol.* **2012**, *46* (4), 2242–2250.
- Benn, T.; Cavanagh, B.; Hristovski, K.; Posner, J. D.; Westerhoff, P. The release of nanosilver from consumer products used in the home. *J. Environ. Qual.* **2010**, *39* (6), 1875–1882.
- Benn, T. M.; Westerhoff, P. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.* **2008**, *42* (11), 4133–4139.
- Klaine, S. J.; Alvarez, P. J. J.; Batley, G. E.; Fernandes, T. F.; Handy, R. D.; Lyon, D. Y.; Mahendra, S.; McLaughlin, M. J.; Lead, J. R. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1825–1851.
- Kiser, M. A.; Westerhoff, P.; Benn, T.; Wang, Y.; Perez-Rivera, J.; Hristovski, K. Titanium nanomaterial removal and release from wastewater treatment plants. *Environ. Sci. Technol.* **2009**, *43* (17), 6757–6763.
- Foster-Smith, R. L. The effect of concentration of suspension of the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.), and *Venerupis pullastra* (Montagu). *J. Exp. Mar. Biol. Ecol.* **1975**, *17*, 1–22.
- Canesi, L.; Ciacci, C.; Fabbri, R.; Marcomini, A.; Pojana, G.; Gallo, G. Bivalve molluscs as a unique target group for nanoparticle toxicity. *Mar. Environ. Res.* **2012**, *76*, 16–21.
- Montes, M. O.; Hanna, S. K.; Lenihan, H. S.; Keller, A. A. Uptake, accumulation, and biotransformation of metal oxide nanoparticles by a marine suspension-feeder. *J. Hazard. Mater.* **2012**, *225*, 139–145.
- Keller, A. A.; McFerran, S.; Lazareva, A.; Suh, S. Global life cycle releases of engineered nanomaterials. *J. Nanopart. Res.* **2013**, *15*, (6), (13) Judy, J. D.; Urine, J. M.; Bertsch, P. M. Evidence for biomagnification of gold nanoparticles within a terrestrial food chain. *Environ. Sci. Technol.* **2011**, *45* (2), 776–781.
- Bouldin, J. L.; Ingle, T. M.; Sengupta, A.; Alexander, R.; Hannigan, R. E.; Buchanan, R. A. Aqueous toxicity and food chain transfer of quantum Dots (TM) in freshwater algae and *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1958–1963.
- Jackson, B. P.; Bugge, D.; Ranville, J. F.; Chen, C. Y. Bioavailability, toxicity, and bioaccumulation of quantum dot nanoparticles to the amphipod *Leptocheirus plumulosus*. *Environ. Sci. Technol.* **2012**, *46* (10), 5550–5556.
- Hanna, S. K.; Miller, R. J.; Muller, E. B.; Nisbet, R. M.; Lenihan, H. S. Impact of engineered zinc oxide nanoparticles on the individual performance of *Mytilus galloprovincialis*. *PLoS One* **2013**, *8* (4), e61800.
- Urban, E. R.; Kirchman, D. L. Effect of kaolinite clay on the feeding activity of the Eastern Oyster *Crassostrea virginica* (Gmelin). *J. Exp. Mar. Biol. Ecol.* **1992**, *160* (1), 47–60.
- Miller, R. J.; Lenihan, H. S.; Muller, E. B.; Tseng, N.; Hanna, S. K.; Keller, A. A. Impacts of metal oxide nanoparticles on marine phytoplankton. *Environ. Sci. Technol.* **2010**, *44* (19), 7329–7334.
- Moore, M. N.; Widdows, J.; Cleary, J. J.; Pipe, R. K.; Salkeld, P. N.; Donkin, P.; Farrar, S. V.; Evans, S. V.; Thomson, P. E. Responses of the Mussel *Mytilus edulis* to copper and phenanthrene—Interactive effects. *Mar. Environ. Res.* **1984**, *14* (1–4), 167–183.
- Eertman, R. H. M.; Groenink, C.; Sandee, B.; Hummel, H.; Smaal, A. C. Response of the Blue Mussel *Mytilus edulis*-L following exposure to PAHs or contaminated sediment. *Mar. Environ. Res.* **1995**, *39* (1–4), 169–173.
- Van Hoecke, K.; Quik, J. T. K.; Mankiewicz-Boczek, J.; De Schampelaere, K. A. C.; Elsaesser, A.; Van der Meeren, P.; Barnes, C.; McKerr, G.; Howard, C. V.; Van De Meent, D.; Rydzynski, K.; Dawson, K. A.; Salvati, A.; Lesniak, A.; Lynch, I.; Silversmit, G.; De Samber, B.; Vincze, L.; Janssen, C. R. Fate and effects of CeO₂ nanoparticles in aquatic ecotoxicity tests. *Environ. Sci. Technol.* **2009**, *43* (12), 4537–4546.
- Zhang, H. F.; He, X. A.; Zhang, Z. Y.; Zhang, P.; Li, Y. Y.; Ma, Y. H.; Kuang, Y. S.; Zhao, Y. L.; Chai, Z. F. Nano-CeO₂ exhibits adverse effects at environmental relevant concentrations. *Environ. Sci. Technol.* **2011**, *45* (8), 3725–3730.
- Keller, A. A.; Wang, H. T.; Zhou, D. X.; Lenihan, H. S.; Cherr, G.; Cardinale, B. J.; Miller, R.; Ji, Z. X. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environ. Sci. Technol.* **2010**, *44* (6), 1962–1967.
- Zhang, S. J.; Jiang, Y. L.; Chen, C. S.; Spurgin, J.; Schwehr, K. A.; Quigg, A.; Chin, W. C.; Santschi, P. H. Aggregation, dissolution, and stability of quantum dots in marine environments: Importance of extracellular polymeric substances. *Environ. Sci. Technol.* **2012**, *46* (16), 8764–8772.
- Daniel Luzon, M. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science* **1948**, *107* (2775), 254–255.
- Holbrook, R. D.; Murphy, K. E.; Morrow, J. B.; Cole, K. D. Trophic transfer of nanoparticles in a simplified invertebrate food web. *Nat. Nanotechnol.* **2008**, *3* (6), 352–355.
- Brillant, M. G. S.; MacDonald, B. A. Postingestive selection in the sea scallop (*Placopecten magellanicus*) on the basis of chemical properties of particles. *Mar. Biol.* **2002**, *141* (3), 457–465.
- Cranford, P. J.; Emerson, C. W.; Hargrave, B. T.; Milligan, T. G. In situ feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. *J. Exp. Mar. Biol. Ecol.* **1998**, *219* (1–2), 45–70.
- Van Hoecke, K.; De Schampelaere, K. A. C.; Van der Meeren, P.; Smagghe, G.; Janssen, C. R. Aggregation and ecotoxicity of CeO₂ nanoparticles in synthetic and natural waters with variable pH, organic matter concentration and ionic strength. *Environ. Pollut.* **2011**, *159* (4), 970–976.

- (30) Renault, S.; Baudrimont, M.; Mesmer-Dudons, N.; Gonzalez, P.; Mornet, S.; Brisson, A. Impacts of gold nanoparticle exposure on two freshwater species: a phytoplanktonic alga (*Scenedesmus subspicatus*) and a benthic bivalve (*Corbicula fluminea*). *Gold Bull.* **2008**, *41* (2), 116–126.
- (31) Mafra, L. L., Jr; Bricelj, V. M.; Ouellette, C.; Bates, S. S. Feeding mechanics as the basis for differential uptake of the neurotoxin domoic acid by oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*. *Aquat. Toxicol.* **2010**, *97* (2), 160–171.
- (32) Chandurvelan, R.; Marsden, I. D.; Gaw, S.; Glover, C. N. Impairment of green-lipped mussel (*Perna canaliculus*) physiology by waterborne cadmium: Relationship to tissue bioaccumulation and effect of exposure duration. *Aquat. Toxicol.* **2012**, *124*, 114–124.
- (33) Al-Subiai, S. N.; Arlt, V. M.; Frickers, P. E.; Readman, J. W.; Stolpe, B.; Lead, J. R.; Moody, A. J.; Jha, A. N. Merging nanogenotoxicology with eco-genotoxicology: An integrated approach to determine interactive genotoxic and sub-lethal toxic effects of C-60 fullerenes and fluoranthene in marine mussels, *Mytilus* sp. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2012**, *745* (1–2), 92–103.
- (34) Falugi, C.; Aluigi, M. G.; Chiantore, M. C.; Privitera, D.; Ramoino, P.; Gatti, M. A.; Fabrizi, A.; Pinsino, A.; Matranga, V. Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. *Mar. Environ. Res.* **2012**, *76*, 114–121.
- (35) Garcia, A.; Delgado, L.; Tora, J. A.; Casals, E.; Gonzalez, E.; Puentes, V.; Font, X.; Carrera, J.; Sanchez, A. Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. *J. Hazard. Mater.* **2012**, *199*, 64–72.
- (36) Thomas, C. R.; George, S.; Horst, A. M.; Ji, Z. X.; Miller, R. J.; Peralta-Videa, J. R.; Xia, T. A.; Pokhrel, S.; Madler, L.; Gardea-Torresdey, J. L.; Holden, P. A.; Keller, A. A.; Lenihan, H. S.; Nel, A. E.; Zink, J. I. Nanomaterials in the environment: From materials to high-throughput screening to organisms. *ACS Nano* **2011**, *5* (1), 13–20.
- (37) Miller, R. J.; Bennett, S.; Keller, A. A.; Pease, S.; Lenihan, H. S. TiO₂ nanoparticles are phototoxic to marine phytoplankton. *PLoS One* **2012**, *7*, (1).
- (38) Cornelis, G.; Ryan, B.; McLaughlin, M. J.; Kirby, J. K.; Beak, D.; Chittleborough, D. Solubility and batch retention of CeO₂ nanoparticles in soils. *Environ. Sci. Technol.* **2011**, *45* (7), 2777–2782.
- (39) Xia, T.; Kovichich, M.; Liang, M.; Madler, L.; Gilbert, B.; Shi, H. B.; Yeh, J. I.; Zink, J. I.; Nel, A. E. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *ACS Nano* **2008**, *2* (10), 2121–2134.
- (40) Kittler, S.; Greulich, C.; Diendorf, J.; Koller, M.; Epple, M. Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. *Chem. Mater.* **2010**, *22* (16), 4548–4554.
- (41) Miao, A. J.; Zhang, X. Y.; Luo, Z. P.; Chen, C. S.; Chin, W. C.; Santschi, P. H.; Quigg, A. Zinc oxide engineered nanoparticles dissolution and toxicity to marine phytoplankton. *Environ. Toxicol. Chem.* **2010**, *29* (12), 2814–2822.
- (42) Bennett, S. W.; Keller, A. A. Comparative photoactivity of CeO₂, γ -Fe₂O₃, TiO₂, and ZnO in various aqueous systems. *Appl. Catal. B-Environ.* **2011**, *102* (3–4), 600–607.
- (43) Karakoti, A. S.; Monteiro-Riviere, N. A.; Aggarwal, R.; Davis, J. P.; Narayan, R. J.; Self, W. T.; McGinnis, J.; Seal, S. Nanoceria as antioxidant: Synthesis and biomedical applications. *J. Miner.* **2008**, *60* (3), 33–37.
- (44) Hirst, S. M.; Karakoti, A. S.; Tyler, R. D.; Sriranganathan, N.; Seal, S.; Reilly, C. M. Anti-inflammatory properties of cerium oxide nanoparticles. *Small* **2009**, *5* (24), 2848–2856.
- (45) Korsvik, C.; Patil, S.; Seal, S.; Self, W. T. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chem. Commun.* **2007**, No. 10, 1056–1058.
- (46) Sun, C.; Li, H.; Chen, L. Nanostructured ceria-based materials: Synthesis, properties, and applications. *Energy Environ. Sci.* **2012**, *5* (9), 8475–8505.
- (47) Zhou, D. X.; Abdel-Fattah, A. I.; Keller, A. A. Clay particles destabilize engineered nanoparticles in aqueous environments. *Environ. Sci. Technol.* **2012**, *46* (14), 7520–7526.
- (48) Lotufo, G. R.; Farrar, J. D.; Bridges, T. S. Effects of exposure source, worm density, and sex on DDT bioaccumulation and toxicity in the marine polychaete *Neanthes arenaceodentata*. *Environ. Toxicol. Chem.* **2000**, *19* (2), 472–484.
- (49) Hook, S. E.; Fisher, N. S. Sublethal effects of silver in zooplankton: Importance of exposure pathways and implications for toxicity testing. *Environ. Toxicol. Chem.* **2001**, *20* (3), 568–574.
- (50) Fisher, N. S.; Hook, S. E. Toxicology tests with aquatic animals need to consider the trophic transfer of metals. *Toxicology* **2002**, *181*, 531–536.
- (51) Hull, M. S.; Chaurand, P.; Rose, J.; Auffan, M.; Bottero, J. Y.; Jones, J. C.; Schultz, I. R.; Vikesland, P. J. Filter-feeding bivalves store and biodeposit colloiddally stable gold nanoparticles. *Environ. Sci. Technol.* **2011**, *45* (15), 6592–6599.
- (52) Ferry, J. L.; Craig, P.; Hexel, C.; Sisco, P.; Frey, R.; Pennington, P. L.; Fulton, M. H.; Scott, I. G.; Decho, A. W.; Kashiwada, S.; Murphy, C. J.; Shaw, T. J. Transfer of gold nanoparticles from the water column to the estuarine food web. *Nat. Nanotechnol.* **2009**, *4* (7), 441–444.