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Transfer of gold nanoparticles from the water column to the estuarine food web

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Within the next five years the manufacture of large quantities of nanomaterials may lead to unintended contamination of terrestrial and aquatic ecosystems[1]. The unique physical, chemical and electronic properties of nanomaterials allow new modes of interaction with environmental systems that can have unexpected impacts[2,3]. Here, we show that gold nanorods can readily pass from the water column to the marine food web in three laboratory-constructed estuarine mesocosms containing sea water, sediment, sea grass, microbes, biofilms, snails, clams, shrimp and fish. A single dose of gold nanorods (65 nm length × 15 nm diameter) was added to each mesocosm and their distribution in the aqueous and sediment phases monitored over 12 days. Nanorods partitioned between biofilms, sediments, plants, animals and sea water with a recovery of 84.4%. Clams and biofilms accumulated the most nanoparticles on a per mass basis, suggesting that gold nanorods can readily pass from the water column to the marine food web.

The transport of contaminants to oceans through estuaries is often mediated by chemical and physical processes associated with mixing fresh water with sea water. As this region is also the habitat for many commercially and ecologically important shellfish and finfish, it could also be a critical point of nanomaterial contaminant entry into the marine food web. For example, salinity gradients, such as those found in tidal mixing zones, typically promote the flocculation and precipitation of organic matter and naturally occurring particulates[4,5]. Organic matter and particulates can be consumed by detritivores or shellfish, and they can also be a sink for anthropogenic material through burial in sediments[6]. At present little is known about the physicochemical behaviour of nanomaterial in the mixing zone, precluding prediction of their eventual environmental distribution. Measurement of nanomaterial distributions in model estuarine systems is a necessary first step towards the evaluation of the effects of nanoparticles on the environment.

This study used a series of three replicate estuarine mesocosms as laboratories for measuring the behaviour of nanoparticles in complex environments. These systems are representative of *Spartina* (cordgrass) dominated estuaries and have been successfully used for estimating the coastal impact of several other contaminants, including atrazine, fipronil, endosulfan and nutrients (Fig. 1)[7–10]. In this study, three replicates of a complex ecosystem were constructed to model the edge of a tidal marsh creek. The systems were made up from natural, unfiltered sea water from Cherry Point Boat Landing on Wadmalaw Island, South Carolina, USA

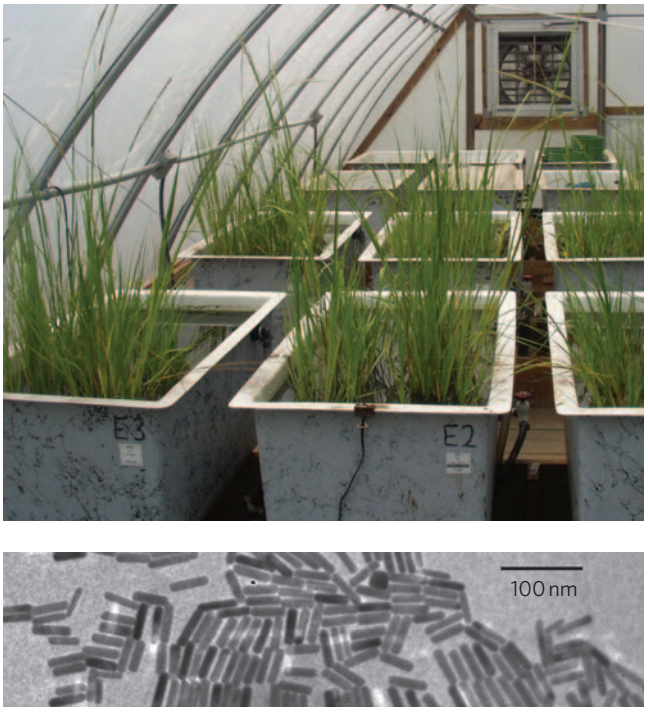

Figure 1 | Experimental setup. a, A series of replicate estuarine mesocosms (volume, 366 l each) modelling the edge of a tidal marsh creek was maintained in a greenhouse. Each tank contained natural sea water, sediment, biofilms, *Spartina alterniflora*, *Palaemonetes pugio*, *Cyprinodon variegatus*, *Ilyanassa obsoleta* and *Mercenaria mercenaria* and received a single dose of gold nanorods (65 nm × 15 nm). The exposure period was 12 days and the aqueous and sediment (top 2 cm) phases were monitored throughout. *Spartina* is shown growing in the sediment trays. **b**, Transmission electron microscopy (TEM) image of the gold nanorods used in the study.

(salinity determined by conductivity and adjusted to 20‰ by the addition of deionized water) and contained a periodically submerged sediment tray in the primary tank and an attached reservoir for water storage (isolated with a screen) to simulate a tidal cycle[9–11]. Sediments were planted with *Spartina alterniflora*, 100 juvenile *Mercenaria mercenaria* (northern quahog clam) were added to the attached reservoir, and the primary tank was seeded with 50 *Ilyanassa obsoleta* (mud snails), 15 *Cyprinodon variegatus* (sheepshead minnow) and 100 *Palaemonetes pugio* (grass shrimp).

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Table 1 | Distribution of gold in estuarine mesocosms after aqueous introduction.

Phase (g)	Gold (μg kg^{-1})‡		$C_f^{\S}$	Per cent recovered gold in a given phase‖
	0 days	12 days		
Sea water (3.66×10^5)*	<LOD‖	0.42 ± 0.22	1.00	8.61 ± 4.51
Sediment (4.91×10^4)†	<LOD	13.9 ± 0.7	33.1	24.5 ± 1.23
Biofilm (1.01×10^3)*	12.2 ± 0.8	$6.41 \pm 0.28 \times 10^3$	1.53×10^4	61.0 ± 2.65
Spartina alterniflora (grass, 1.50×10^3)*	2.68 ± 2.01	3.45 ± 1.91	8.21	0.10 ± 0.06
Palaemonetes pugio (grass shrimp, 15.6)†	0.388 ± 0.30	48.1 ± 23.0	1.15×10^2	0.03 ± 0.01
Cyprinodon variegatus (GI tract and organs, sheepshead minnow, 22.5)†	0.964 ± 0.685	$1.99 \pm 2.34 \times 10^2$	4.74×10^2	0.31 ± 0.37
Ilyanassa obsoleta (snail, 5.5)*	<LOD	70.1 ± 33.2	1.67×10^2	0.05 ± 0.02
Mercenaria mercenaria (juvenile clams, 10.0)*	<LOD	$9.57 \pm 2.44 \times 10^3$	2.28×10^4	5.79 ± 1.48

*Estimated mass of a phase in grams. †Measured mass of a phase in grams. ‡Gold atom content in ppb at $t = 0$ and $t = 12$ days based on dry weight for non-aqueous samples. §Concentration factor: $C_f = C_{phase}/C_{water}$ at $t = 12$ days. ‖Mass balance and relative error estimated from measured mass of water and sediment, with an assumption of 2 mm photosynthetic biofilm thickness throughout, and water contents of 36% (sediment), 67% (biofilm), 64% (*Spartina*), 80% (*Palaemonetes*), 72% (*Cyprinodon*), 36% (*Ilyanassa*) and 46% (*Mercenaria*)[25–29]. Limit of detection (LOD) for this method is 18.0 ± 0.5 pg kg^{-1}. All concentration measurements report the grouped mean of three separate samples per tank ($n = 9$) averaged across the replicate tanks accompanied by the pooled standard deviation.

A stainless steel cage enclosing microscope slides was added to the primary tank and photosynthetic biofilms sampled off the slides. The mesocosms (sea water, sediments and *Spartina*) were established and allowed to equilibrate for approximately three months before exposure. All fauna were added to the mesocosms 5–10 days before the start of exposure.

Gold nanorods in the chemical form typical of their method of manufacture (cetyltrimethylammonium bromide (CTAB) stabilized, with a net positive charge)[12,13] were added to the outfall line of the reservoir during the 'rising tide' cycle of the primary tank to ensure rapid mixing. Previous work has shown that elemental gold is stable against oxidative dissolution in aerated sea water[14]. The aqueous and sediment (top 2 cm) phases were monitored throughout the course of a 12-day exposure experiment, which was sufficient to detect partitioning and mortality but not multigenerational effects in the test organisms. Initial and final gold concentrations were determined by inductively coupled plasma mass spectrometry for each phase and species (Table 1) using a method adapted from Falkner[15]. No calciferous shell material was analysed. Particle recovery was based on known and estimated masses of the wet weight of each phase in the mesocosms (biofilm and *Spartina* mass estimated, all others measured, Table 1). The mass of biofilm is a low estimate based on an estimated 2-mm thickness on the surfaces of the tanks and neglecting irregular sediment, leaf or filter surfaces. The corresponding mass balance for this system was 84.4% gold recovered after 12 days, which was comparable to the mass balances for mesocosm studies of organic molecules[8–10]. No overt organism mortality was detected. Partitioning from the aqueous phase is described by a concentration factor, C_f. C_f is operationally defined as the ratio of the concentration of gold (μg kg^{-1}) in the measured phase over the concentration in the aqueous phase (μg kg^{-1}) at the end of the experiment ($t = 12$ days).

The concentration of nanoparticles in open water samples rose rapidly as the tanks mixed and then fell to a constant level of ~0.4 μg kg^{-1} after 24 h (Fig. 2). It was not possible to determine in this study if aqueous nanoparticles were in dynamic equilibrium with the other phases or if they represented a static, solubilized reservoir of suspended particles[4,5]. Nanoparticles partitioned slowly into the sediments and were essentially equilibrated at the end of 12 days (Fig. 2).

Water and sediment represented 99%+ of the total mass of the system and were significant 'sinks' for nanoparticles, accounting for ~35% of the total mass of particles recovered. However, biofilms were the most important sink overall. Although the estimated mass of photosynthetic biofilms was less than 0.5% of the mass of the complete system, their high C_f value (1.53×10^4) resulted in roughly 60% of the mass of recovered nanoparticles entrained in

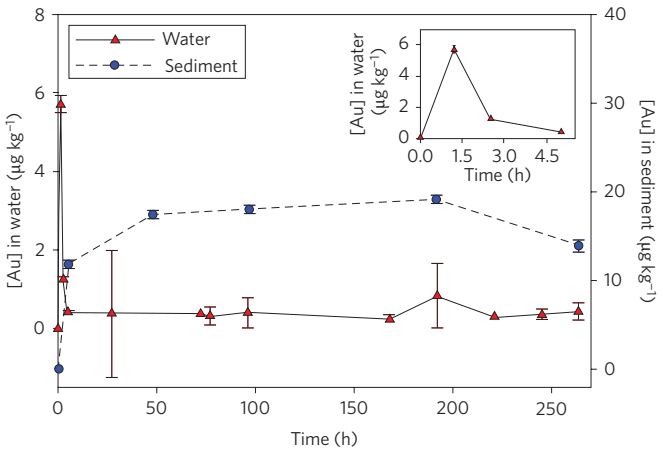

Figure 2 | Nanoparticle concentration versus time. As the tanks mixed, the concentration of gold in sea water (red triangles) rose rapidly from below detection limits to a maximum of 5.7 μg kg^{-1} (see inset for higher resolution). Within 5 h, the concentration of gold became constant at ~0.4 μg kg^{-1}. The concentration of gold in sediments (blue circles) reached an apparent plateau within 48 h. The nominal concentration of 'zero' reflects a measurement below the 18 pg l^{-1} detection limit for the ICP-MS method. Error bars correspond to pooled standard deviation for three replicate samples drawn from each of the three mesocosms ($n = 9$).

biofilms (the conservative method used to estimate biofilm mass in this study may have significantly depressed the measured total gold recovery). One explanation for this high affinity is that multispecies marine biofilms can incorporate significant amounts of charged monomers in the polysaccharides and proteins that are part of their structure[16]. This leads to negatively charged surfaces that should display a high affinity for positively charged CTAB-stabilized nanorods. Regardless of mechanism, the overall result is intriguing because biofilms simultaneously offer a route into the food web through grazing by detritovores and also a route for mineralization through biofilm calcification[16].

Juvenile *Mercenaria mercenaria*, the only filter feeder in the study, had the highest C_f (2.28×10^4) and took up ~5% of the total nanoparticles added even though they only accounted for less than 0.01% of the total mass of the system. Adult *Mercenaria* are commercially important shellfish for human consumption, and this result indicates that filter feeders may in general be important routes into the human food chain for these materials.

Cyprinodon variegatus were dissected before analysis, and samples were subdivided as brain tissue, gills, musculoskeletal (skin, muscle and bones), and combined body cavity organs and

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gut contents. Gold levels were below detection limits in the brain tissue, gills and musculoskeletal samples. Gold was only detected in the combined organ and gut content samples, suggesting it was not moving through the circulatory system of the animal or being absorbed through skin or gill contact. The corresponding C_f for *Cyprinodon variegatus* organs was 4.74×10^2. *Ilyanassa obsoleta* also contained gold (excluding shell) with a C_f of 1.67×10^2. This is intriguing, because *Ilyanassa obsoleta* feed primarily on biofilms, which themselves had a much higher partitioning constant, and suggests that biofilms may stabilize gold against metabolism or direct uptake. *Palaemonetes pugio*, which are omnivorous, had a C_f of 1.15×10^2 (whole body sample). *Spartina alterniflora* had the lowest C_f among tested biota and accounted for only 0.20% of the total recovered gold. This was surprising given reports of other vascular plants taking up nanomaterials[17,18]; however, it is possible that the nanorods in this study were either too large for uptake or were unable to penetrate the sediments to reach the *Spartina* roots on the 12-day timescale.

Gold nanoparticles, including nanorods in this size range, have applications in industrial catalysis, chemical sensing and pharmaceutical technologies, and it is known they can be taken up by cells in culture[19–23]. This work reports the fate of gold nanoparticles in a complex ecosystem containing sediments, biofilms, primary producers, filter feeders, grazers and omnivores. On a per mass basis the filter feeders (*Mercenaria mercenaria*) were the most effective sink for nanoparticles, followed closely by biofilms. The results indicate that knowledge of the ratio of filter feeder mass to biofilm mass may be critical to predicting the direct uptake of some nanomaterials into the estuarine food web.

Methods

Mesocosms. Mesocosms were maintained in a greenhouse at the Center for Coastal Environmental Health and Biomolecular Research in Charleston, South Carolina. Three separate mesocosms were constructed, charged with sea water, sediment, flora and fauna in accordance with procedures outlined in NOAA Technical Memorandum NOS NCCOS 62 (ref. 11). Intertidal estuarine sediments (top 2–4 cm) were collected from a reference site located on Leadenwah Creek (32° 38.848′ N, 080° 13.283′ W), Wadmalaw Island, South Carolina. Sediments were sieved with a 3-mm sieve, homogenized and dispensed into sediment trays. Four sediment trays were placed into each tank and elevated ~5 cm to allow for drainage. Each sediment tray contained ~12.3 kg sediment. The mesocosm had a semi-diurnal tidal cycle, with high tide occurring at 10:00 and 22:00, and low tide occurring at 4:00 and 16:00. The tides were driven by submersible pumps set to timers. Tanks were monitored continuously ($n = 50$ per day) for several water quality parameters, including temperature (°C), pH, dissolved oxygen (mg l^{-1}) and salinity. Salinity was held constant with a collective average of 20. The other parameters varied diurnally in accordance with daytime heating and photosynthetic activity; however, these differences were within the established norms for this system (see Supplementary Figs S1–S4)[7,11,24].

Nanoparticles. Gold nanorods (65 nm × 15 nm, relative standard deviation in aspect ratio of 3.5%) were grown according to published procedures developed in the Murphy group[12,13]. Particles were purified before use by centrifugation, discarding the supernatant, followed by resuspension in 150 ml ultrahigh-purity water. Resuspended nanorods were present as single particles[12,13]. The final concentration of purified nanorod stock solution was 4.30×10^{-10} M nanorods. Each nanorod was composed of ~500,000 gold atoms. Nanorod solutions were injected by means of gravity feed through a Teflon capillary into the water feed line of the mesocosms during the rising tide cycle to ensure the best possible mixing. The addition required less than one minute. The final theoretical particle loading in the 366 l tank, assuming good mixing, was 7.08×10^8 particles ml^{-1}, which is of the same order of magnitude particle loading for combined bacterial, planktonic and viral particles expected in estuarine waters.

Sampling and measurement. Samples were withdrawn at regular time intervals throughout the experiment and frozen for later work-up and analysis. Water samples were treated with an aqua regia solution, diluted and spiked with an iridium internal standard for later analysis on a Finnigan Element High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS). The results for the digestion experiments were compared with spiked NRC Nearshore Sea water Reference Material CASS 4. Sediment samples were dried, finely ground and weighed into a Teflon digestion vial. Additions of Optima Grade concentrated HF–HCl–HNO_3 were made and the vial heated to dryness. The residue was taken up in aqua regia, diluted and spiked with an Ir internal standard for analyses on the ICP-MS. Biofilms were grown on replicate, pre-cleaned glass slides incubated in mesocosms. After incubation, biofilms on slides and their sorbed nanoparticles were digested with concentrated nitric acid. Tissue samples were washed to separate adsorbed nanoparticles and/or sediments before drying and were then digested with concentrated nitric acid. The digests for biofilms and tissues were heated to dryness and the residue taken up in aqua regia, diluted and spiked with the internal standard before analysis on the ICP-MS (ref. 15).

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Author contributions

J.L.F., T.J.S., P.L.P. and M.H.F. conceived and designed the experiment. P.S. and C.J.M. synthesized the nanoparticles. P.C., R.F. and P.L.P. dosed and sampled from the mesocosms. I.G.S., M.H.F. and P.L.P. maintained the mesocosms. C.H., P.C. and T.J.S. digested the samples and performed the ICP-MS analyses. A.W.D. provided biofilm sampling apparatus and procedures. S.K. performed *Cyprinodon* dissections. J.L.F., C.J.M. and T.J.S. wrote the manuscript. All authors contributed to materials and analysis tools, discussed the results and contributed to the manuscript.

Additional information

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