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Abstract
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Keywords
nanoparticle, nantoxicology, aquatic toxicology, freshwater toxicology

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Differential uptake of gold nanoparticles by two species of tadpole, the wood frog (*Lithobates sylvaticus*) and the bullfrog (*L. catesbeianus*)

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Abstract

Engineered nanoparticles are aquatic contaminants of emerging concern exerting ecotoxicological effects on a wide variety of organisms. We exposed cetyltrimethylammonium bromide capped spherical gold nanoparticles to wood frog and bullfrog tadpoles with conspecifics and in combination with the other species continuously for 21 days, then measured uptake and localization of gold. Wood frog tadpoles alone and in combination with bullfrog tadpoles took up significantly more gold than bullfrogs. Bullfrog tadpoles in combination with wood frogs took up significantly more gold than controls. The rank order of weight normalized gold uptake was wood frogs in combination > wood frogs alone > bullfrogs in combination > bullfrogs alone > controls. In all gold-exposed groups of tadpoles, gold was concentrated in the anterior region compared to the posterior region of the body. The concentration of gold nanoparticles in the anterior region of wood frogs both alone and in combination with bullfrogs was significantly higher than their corresponding posterior regions. We also measured depuration time of gold in wood frogs. After 21 days in a solution of gold nanoparticles, tadpoles lost over 83% of internalized gold when placed in gold-free water for 5 days. After 10 days in gold-free water, tadpoles lost 94 % of their gold. After 15 days, gold concentrations were below the level of detection. Our finding of differential uptake between closely related species living in similar habitats with overlapping geographical distributions argues against generalizing toxicological effects of nanoparticles for a large group of organisms based upon measurements in only one species.

Introduction

Modern uses of nanoparticles encompass a wide variety of fields from catalysis and solar cells to drug delivery and cancer therapeutics.[1-3] This broad range of utility has been engendered by advances in synthetic control over the size, shape, and monodispersity of the nanoparticles. As these synthetic controls have continued to evolve, new applications and increased incorporation of nanoparticles into commercial and industrial products has become common. Many bulk materials exhibit new properties when confined at the nanoscale, essentially opening entirely new fields of materials research. One particularly poignant example is the new optical properties that emerge from gold when it is confined to the nanoscale. More specifically, gold nanoparticles are being investigated for their unique optical properties with applications in medicine, energy, and catalysis. Gold nanoparticles have one of the most varied and robust array of synthetic strategies incorporating spheres, cubes, pyramids, bipyramids, nanostars, octopods, and other variants.[4] Not only are the shapes well defined, but the surface coatings of gold nanoparticles are well characterized and can be specifically tailored for an intended use. One important consideration when dealing with nanomaterials outside of laboratory environments is that the surface chemistry is critical to determining the physiochemical properties of the nanomaterial that will be present in the environment. While the physiochemical identity of a nanomaterial may be well characterized in a laboratory, it is a poor assumption that the physiochemical identity will remain the same in the chemically complex environment. More specifically, the aquatic environment, and

the transient nature of environmental systems, will have dramatic influence on the aggregation state and surface chemistry of the nanoparticles. Given the increased research and applications of nanomaterials, it is likely that more and more nanoparticles will enter the environment as their usage in commercial products continues to increase.

Since the beginning of the 21st century nanoparticle-containing consumer products have been rapidly increasing. While gold nanoparticles have not been incorporated into consumer products at the same rate as other materials like silver, titanium, zinc, and carbon, they are an important class of materials that is widely studied across a variety of fields due to their unique optical properties.[5] As nanomaterials continue to make the transition from research labs to commercial production facilities, a legitimate concern is that engineered nanoparticles are contaminants of emerging concern in aquatic ecosystems. [6-12]

The environmental impacts of nanomaterials are not well understood especially in aquatic environments. Laboratory experiments have employed nanoparticles of various types, sizes, and coatings, and the test organisms have included macrophytes [13], invertebrates such as *Daphnia* [14,15] and bivalves [16], and vertebrates particularly the amphibian *Xenopus.*[17-20] More specifically, exposure to gold nanoparticles has been found to affect a number of different biochemical and morphological endpoints in a number of aquatic organisms[21], including algae [22], bivalves [21-23] *Daphnia* [24], fish[25,26] and to serve as a general toxicant.[27] Common themes that emerge from these studies indicate that gold nanoparticles are concentrated in the digestive systems, that gold nanoparticles increase the

formation reactive oxygen species, and that the smaller nanoparticles tend to be more toxic.

The toxicity of different metal and metal oxide nanoparticles on amphibian larval stages using a variety of endpoints has been previously described for *Xenopus laevis* [17-19,28-30], *Pelophylax perizi* [31] and *Lithobates catesbeianus.*[32] These studies span a wide range of nanomaterials including Fe_2O_3, CuO, ZnO, TiO_2, Ag, Carbon nanotubes, and CeO_2. While it is difficult to draw any firm conclusions across such a broad array of organisms and nanoparticles it is clear that these materials can inhibit growth, cause abnormalities, disrupt endocrine signaling, and both accelerate and retard metamorphosis. Clearly, there is ample room to further explore the impacts that nanomaterials have on the growth and development of amphibians.

While there are no measured environmental concentrations of nanoparticles, there are probabilistic models for the release and subsequent concentrations of nanoparticles in sewage sludge and aquatic ecosystems[33,34] with predicted concentrations in freshwater environments ranging from 1-3000 µg/L.[35] Furthermore, trophic transfer of gold nanoparticles from soil to earthworms and finally to juvenile bullfrogs has been shown.[36] Since gold nanoparticles have been proposed as biosensors of pesticides[37] and as bioconjugates in the purification of polluted waters[38], the accumulation of gold nanoparticles in the aquatic environment including amphibian habitats is likely. Most of the previous ecotoxicological studies utilizing nanoparticles as toxicants have exposed them to either a single species or to different species that are not

closely related. Recently, our lab showed that long-term exposure to gold nanoparticles decreases time to metamorphosis without affecting mass in wood frog tadpoles.[39]

Based upon our previous work, we measured localization and uptake of gold nanoparticles in two species of anuran tadpoles, the wood frog (*Lithobates sylvaticus*) a species known to be sensitive to environmental stressors, and the American Bullfrog (*L. catesbeianus*), a species native to the eastern United States, but which has been introduced throughout North America, Europe, Asia, the Caribbean, and South America.[40] These two species are closely related but have differing life history strategies. We tested the hypothesis that competition or aggressive behavior between species should result in lower uptake of gold nanoparticles by the smaller wood frog tadpoles compared to bullfrog tadpoles. Additionally, we measured the depuration of gold nanoparticles from wood frog tadpoles. We hypothesized that gold nanoparticles would be concentrated in the anterior portion of the body as uptake is likely via the oral cavity or the gills as opposed to uptake through the skin.

Materials and Methods

Gold Nanoparticle Synthesis and Characterization

Cetyltrimethylammonium bromide (CTAB) capped gold nanoparticles were synthesized for the species specific uptake experiments utilizing the well-known seed mediated growth approach.[41] Briefly, gold seeds were synthesized by adding 600 µL of 10 mM $NaBH_4$ to a solution containing 9.75 mL of 100 mM CTAB and 250 µL of 10 mM $HAuCl_4$ while stirring vigorously. In a separate flask, 5 mL of 10 mM $HAuCl_4$ and 100 µL of 10 mM $AgNO_3$ were added to 95 mL of 100 mM CTAB. To this solution 550 µL of trisodium citrate were added and swirled until the solution became colorless. Once the solution turned colorless, 200 µL of seed solution was added and the solution was gently swirled. This solution was allowed to sit for a minimum of 2 hours before being purified via centrifugation. The resulting nanoparticle solution was spun at 14,000 xg for 20 minutes and the supernatant was discarded. The pellet was resuspended in 18.2 MΩ water. This centrifugation process was then repeated a second time. Approximately ninety 100 mL batches of nanoparticles were then mixed together to provide enough volume, at the appropriate concentrations, for the biological experiments. All reported data on particles is from the combined solutions.

For the depuration experiments CTAB capped nanoparticles were synthesized using a flow reactor system.[42] The flow reactor was utilized to streamline the synthesis and to address a growing trend towards flow based synthesis in the nanoparticle community.[43] Briefly, three input flasks were

connected to a peristaltic pump (Cole-Parmer Masterflex L/S) running at 30 rpm. The first flask contained 237 mL of 100 mM CTAB, 50 mL of 10 mM $HAuCl_4$, and 1 mL of 10 mM $AgNO_3$. The second flask contained 237 mL of 100 mM CTAB and 5.5 mL of 100 mM trisodium citrate. The third flask contained 475 mL of 100 mM CTAB and 1.2 mL of seed solution. The seed solution was prepared identically as above. The third flask was split into two lines that went through the peristaltic pump so that the usage of all solutions was matched. The solutions from flask one and two were mixed after the peristaltic pump and went through 18 feet of tubing before mixing with the seed solution. The seed solution had also gone through 18 feet of tubing before mixing with the combination of flask one and two. After the solutions were all mixed, they flowed through an additional 54 feet of tubing and were then collected into a large flask. Once all of the solutions had been collected into the large flask, the flask was kept in a water bath at 30 °C overnight and purified via centrifugation as described above. Multiple 1 L batches were synthesized and mixed after purification. All characterization is on the combined purified solutions of nanoparticles.

Nanoparticles were characterized via UV/Vis spectroscopy (Jasco v-670), DLS and Zeta potential measurements (Malvern NanoZS90), and TEM (Zeiss EM-109). The most prominent peak in the raw intensity distributions was used in all DLS measurements to measure the aggregation state of the particles. Particles were characterized after purification and at various time points after dilution into the dechlorinated tap water used in the experiments. Stock solutions of gold nanoparticles in MilliQ water were created at 500 pM, where the

concentrations were determined with Beer's law and a calculated molar absorptivity based on the absorption maximum. Note that this method returns a concentration of the nanoparticles in molarity where the nanoparticles are the unit of measure.

Gold Uptake and Localization by Wood Frog and Bullfrog Tadpoles

The collection and use of all animals was approved by the Institutional Animal Care and Use Committee (IACUC) of Gettysburg College. Wood frog (*Lithobabates sylvaticus*) egg masses were collected from vernal pools in Michaux State Forest, Adams County, PA, USA (39.91^{o} N, 77.56^{o} W) in early April, 2014, and were immediately transported (20 minutes) in pond water to the lab at Gettysburg College. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Eggs were maintained in pond water for two days, then transferred to a 50:50 mixture of pond water: dechlorinated tap water (for 1 day, and finally to dechlorinated tap water thereafter) (Stress Coat dechlorinator, Aquarium Pharmaceuticals Inc. Chalfont, PA). Dechlorinated tap water (pH 7.5, DO 7.2 ppm, temperature 21 °C, conductivity 772 µS/cm) has been used in laboratory experiments with anuran tadpoles with success.[39,44] Eggs from at least six different egg masses began to hatch four days after collection. Experiments began 11 days after collection when tadpoles reached Gosner stage 24-25.

Bullfrog tadpoles (*Lithobates catesbeianus*) (up to 3 inches long at Gosner stage 25-28) were shipped from Carolina Biological Supply Co. and received in mid-April, 2014. These tadpoles were born in spring-summer 2013, overwintered

in the field, and collected in early spring, 2014. Tadpoles were maintained in their shipping medium for two days, then transferred to a 50:50 mixture of shipping medium: dechlorinated tap water for one day, then finally to dechlorinated tap water until experiments began 4 days after their arrival.

We designed an experiment to examine the uptake and location of gold nanoparticles in the presence and absence of congeneric tadpole species. We established 3 arrangements of tadpoles (wood frogs (WF) alone, bullfrogs (BF) alone, and WF + BF (3 of each species) in combination groups) with three treatments (in dechlorinated tap, in CTAB, and in gold nanoparticle solution with a final concentration of 20 pM) for a total of 9 groups with n=4 aquaria per group. Each aquarium initially contained 6 tadpoles. CTAB stock was 5 µM and to each CTAB tank we initially added 1936 ml of dechlorinated tap water, the tadpoles were added, then 64 mL of CTAB (5 µM) was added for a final CTAB concentration of 1.6×10^{-7} M (estimated concentration of free CTAB at the nanoparticle exposure dose).

For gold treatments, aquaria initially contained 1920 ml of dechlorinated tap water with 6 tadpoles per aquarium. Thereafter, we added 80 ml of gold nanoparticles (of stock 500 pM), to achieve a final gold concentration of 20 pM. Water controls received 1920 mL of dechlorinated tap water, the tadpoles were added, then 80 mL of dechlorinated tap water was added to each control tank. All solutions were changed twice a week and tadpole food pellets (Carolina Biological Supply Co.) were added after each solution change. If a tadpole died, it was removed and the volume in the aquarium reduced by accordingly per dead

tadpole to maintain the volume of media/tadpole. During the solution changing process the tanks were rinsed with dechlorinated tap water and wiped out with a paper towel. After three weeks, tadpoles were sacrificed by anesthetizing them in 30 % isopropanol and fixing them in 10 percent neutral buffered formalin.

Note: Eight tadpoles died before the end of the 25 day exposure period. These tadpoles were still processed and analyzed to quantify gold uptake but were not included in the reported findings. Two tadpoles were misplaced between exposure and processing and thus were not analyzed for gold content. Thus 4.6% of the initial sample size was not reported in the findings.

Fixed tadpoles were dried with a Kimwipe and a wet mass was measured. Each tadpole was placed in a teflon vial (Savillex Corporation) and 10.0 mL of trace metal grade nitric acid was added. Vials were heated to 200 °C until dryness, approximately 24 hours. Once dry and cooled to room temperature, 5.00 mL of 10% aqua regia (trace metal grade) was added to the vials with a volumetric pipette. Samples were sonicated in the teflon vials until the dried pellet was completely suspended. The resulting solutions were filtered using 0.22 µm syringe filters into 15 mL conical tubes. The tubes were capped and sealed with Parafilm and stored in the refrigerator until ready to be analyzed with ICP-OES (Optima Series, Perkin Elmer). The amount of gold in tadpoles was determined using the 267.595 nm emission line from gold and compared against a series of standard solutions that were prepared gravimetrically from a 1000 mg/L standard solution (Fluka) with $R^2 \geq 0.999$ in all cases. New calibration curves were prepared for each set of measurements. To establish baseline gold background

measurements for the digestion, the experimental protocols were followed starting with the addition of the 10.0 mL of nitric acid with an absence of tadpoles. The average value of the baseline experiments was subsequently subtracted from all measurements.

8 tadpoles from each group of the uptake experiment were selected at random from the gold exposed tadpoles groups. After being fixed in 10% formalin, tadpoles were carefully cut with a razor blade into an anterior half containing all viscera and a posterior half containing the tail. These sections were analyzed for gold as described above.

Gold Depuration from Wood Frogs:

Egg masses of wood frogs were collected on March 28th, 2015 from vernal pools in Michaux State Forest, Adams County, Pennsylvania (39.91^{o}N, 77.56^{o}W). At least 4 individual masses of eggs were transported (20 minutes) in pond water to the laboratory at Gettysburg College. Eggs were initially placed in 20-gallon aquaria with pond water. After 24 hours, room temperature dechlorinated tap water was added to the aquaria to create a 50:50 mixture. Thereafter, tadpoles were maintained in 100% declorinated tap water. The eggs hatched on 2 April 2015 and pooled embryos from the egg clutches were reared in dechlorinated tap water until Gosner stage 23 when experiments began.

We established four groups of tadpoles all of which received gold nanoparticles (final concentration 2 pM) for a minimum of 21 days. The first group was sacrificed on day 22, while subsequent groups were placed into 100%

dechlorinated tap water (no gold nanoparticles added) for 5, 10, and 15 days and then sacrificed. We used plastic, 3-liter aquaria as units of replication and randomly distributed tadpoles (initially 6 tadpoles per aquarium) into each. For each treatment group there were n=9 aquaria. Each aquarium had an initial volume of 2000 mL of test media. If a tadpole died, it was removed and the volume in the aquarium reduced by 333 mL per dead tadpole to maintain the volume of media/tadpole. Fresh media was added twice per week similar to the procedure described above. To 1800 mL of dechlorinated tap water, we added 200 ml of either gold nanoparticles at 20 pM for a final concentration of 2 pM or dechlorinated tap water. Aquaria with reduced volumes were adjusted accordingly.

Results and Discussion

Gold analysis and uptake of gold nanoparticles

Figure 1A shows the TEM micrograph of the CTAB capped gold nanoparticles used in the uptake studies. The particles are relatively monodisperse with a mean size of 22 ± 5 nm (n = 500). As shown in Figure 1B the UV/Vis data indicates that there is some aggregation occurring as the peak broadens and decreases in intensity over the course of 48 hours. It is important to note that the nanoparticles are not stable against aggregation at 50 pM in dechlorinated tap water. Figure 1C shows the DLS measurements of the particles at 0 and 48 hours which supports that particle aggregation is occurring. Combined together, the DLS and the UV-Vis data give an insight to the aggregation state of the particles in solution. The DLS data reported is the average size of the largest peak in the intensity distribution, which indicates that aggregation is occurring. However, the plasmon absorption band of the gold nanoparticles, which is very sensitive to the aggregation state, remains fairly stable indicating that there is a mixture of aggregated particles and particles that are primarily in the dispersed state. It is worth noting that the stability of CTAB particles is highly dependent on the concentration of free CTAB in solution.[45] It may be that the aggregation is not due entirely to the components of the dechlorinated tap water but that the dilution of CTAB plays a role as well. Even in the relatively well-controlled conditions we used, the particles still differ from their physiochemical identity in the lab. Zeta potential measurements show that the surface charge switches from + 30 mV to – 12 mV within fifteen minutes of

mixing with the dechlorinated tap water. The negative zeta potential persists on the particles throughout the exposure time.

To test the species-specific uptake of gold, we exposed wood frog and bullfrog tadpoles to gold nanoparticles either alone or in combination together. For the various tadpole groups in gold, there was a significant effect of exposure on gold uptake (one-way ANOVA, $F(4, 19) = 145.24$, $p < 0.0001$). While all groups of tadpoles exposed to gold showed some uptake, wood frogs showed significantly higher uptake per gram body mass than bullfrogs (Figure 2). Wood frogs in combination with bullfrogs and wood frogs alone took up the highest amounts of gold (144.6 and 102.9 µg Au/g body mass, respectively). Both groups of wood frogs took up significantly higher amounts of gold than all other groups (Tukey's test, $p < 0.01$ for all pairwise combinations, Figure 2). Bullfrogs in combination with wood frogs took up significantly higher amounts of gold compared to controls ($p< 0.05$), but not compared to bullfrogs alone.

While we do not have a definitive answer to why wood frogs take up more gold/body weight, the tadpoles in combination take up significantly more nanoparticles than when alone. Bullfrog tadpoles in combination with wood frogs also show an increase in the amount of nanoparticles taken up even though this difference is not significant. To our knowledge this is the first comparative study of nanoparticle uptake between closely related organisms of any kind. Why gold uptake was higher in wood frogs than in bullfrogs could be explained by several different mechanisms. One possibility is that gold nanoparticles were ingested with food particles. We did not measure ingestion rate, but Seale and Beckvar

measured ingestion rate of tadpoles of bullfrogs and wood frogs fed the cyanobacterium *Anabaena sphaerica*.[46] They found maximum ingestion rates to be very similar between the two species (and similar between five species in three different genera). Another possibility is that the presence of bullfrogs increased the activity and therefore the respiratory rate of wood frogs. Monello *et al.* found that tadpoles of the Pacific treefrog (*Pseudacris regilla*) increased activity and grew faster when grown in the presence of bullfrog tadpoles.[47] By contrast, Walston and Mullin showed that wood frog tadpoles that had no experience with bullfrog displayed reduced activity when grown in the presence of bullfrogs.[48] These possible mechanisms could explain why in our study, wood frogs in combination with bullfrogs took up the highest concentrations of gold, but not why wood frogs alone took up significantly higher concentrations than either bullfrogs in combination or bullfrogs alone.

Our work uses a diffuse exposure mechanism as opposed to a direct exposure method to the GI tract, however, the gross distribution of nanoparticles in the tadpole bodies is still of interest. Figure 3 shows that in all cases there is more gold in the anterior region of the tadpoles when compared to the posterior region. More specifically, there was a significant effect of condition (anterior vs. posterior) on mean gold uptake (one-way ANOVA, $F(1,16) = 28.46$, $p = 0.0001$). The anterior portions of wood frogs, both alone and in combination with bullfrogs showed significantly more uptake of gold than did their corresponding posterior regions (Tukey's test, $p < 0.01$ for both comparisons, Figure 3). The anterior portions of bullfrogs also had higher concentrations of gold than their

corresponding posterior regions, but not significantly. There was a significant combined effect of group x condition (anterior vs. posterior) on mean gold uptake (2-way ANOVA, $F(3,12) = 5.55$, $p = 0.01$) indicating that the anterior portions of wood frogs concentrated more gold than those of bullfrogs. These results are suggestive that the route of uptake is potentially through the oral cavity or through the gills rather than a passive adsorption through the skin.

Bacchetta *et al.* found that nanoparticles were localized in the stomach and gut of larval *Xenopus laevis*.[18] Nations *et al.* reported nanoparticles in the gut coils of larval *X. laevis*.[29] Mouchet et al found double-wall carbon nanotubes concentrated in the intestine of *Xenopus laevis* tadpoles.[19] Unrine *et al.* demonstrated trophic transfer of gold nanoparticles by feeding adult bullfrogs with gold-exposed earthworms with the finding of gold accumulation in bullfrog internal organs including the stomach and intestine.[36] Our results, framed in the context of the literature on nanomaterial uptake in amphibians, are consistent with the most likely route of uptake of nanomaterials in amphibian larvae being through ingestion.

We measured gold nanoparticle uptake in wood frog and bullfrog tadpoles, two species that are closely related but have differing life history strategies. We found that their ability to accumulate gold nanoparticles is significantly different, at least in the laboratory. Carter *et al.* reported significantly different uptake and depuration of pharmaceuticals in two species of earthworm that live in slightly different layers of sediment.[49] These combined results indicate the importance of not generalizing toxicant sensitivity of aquatic and terrestrial organisms based

upon previous studies on closely-related species. More importantly further testing of non-target congeneric species with varying life history strategies is needed.

Depuration study

To further elucidate the route of uptake, we wanted to see if the particles would depurate from the tadpoles when the toxicant, gold nanoparticles, was removed from the tanks. CTAB capped gold nanoparticles, synthesized via flow reactor, were used for this study and their time dependent physiochemical properties when placed into dechlorinated tap water are shown in Figure 4. Gold nanoparticles with a mean diameter of 27 ± 12 were diluted in dechlorinated tap water and the aggregation was monitored with UV/Vis, DLS and Zeta potential. The decrease in the intensity of the plasmon band, indicates that the concentration of the particles is decreasing, and the shouldering of the peak indicates that the particles are aggregating (Figure 4B). Figure 4C shows that the particles very quickly increase in size and begin to decrease possibly due to large particles settling out of the solution. As noted previously, the combination of the UV-Vis and the DLS data is used to determine that while there is some degree of aggregation, the particles are primarily in the dispersed state. It is worth noting that the initial physiochemical properties of the nanoparticles in the uptake and depurations studies are very similar, yet they have somewhat different aggregation properties when they are diluted in dechlorinated tap water. As mentioned previously, the concentration of free CTAB may play a role in the different aggregation profiles of the gold nanoparticles. Additionally, the gold nanoparticles synthesized in the flow reactor are more polydisperse than the

particles synthesized via the traditional methods, which may also have an impact on the aggregation profile.

When the toxicant, gold nanoparticles, was removed from the wood frog tadpole's environment, the internalized gold was significantly depurated over time (one-way ANOVA, $F(3, 32) = 73.32$, $p << 0.00001$) (Figure 5). After 21 days in a solution containing 2 pM gold nanoparticles, the mean gold values in non-depurated tadpoles was 9.51 µg gold/g body mass. This value was significantly higher than those depurated for 5 days (1.37 µg/g), 10 days, (0.39 µg/g), and 15 days (below the level of detection) (Tukey's test, $p < 0.01$ for all pair-wise comparisons, Figure 5).

Overall, the literature on depuration of nanoparticles from organisms is not particularly robust, however there are a few papers that look at depuration in aquatic organisms. [24,50,51] To the best of our knowledge, there are no previously reported depuration data for amphibian larvae. In the common carp, Jang *et al.* reported that silver nanoparticles were reduced to control levels after 14 days of depuration.[51] Our work shows that while wood frog tadpoles do internalize gold nanoparticles after chronic exposure, they also depurate the nanoparticles in about two weeks. This suggests that these particular CTAB capped gold nanoparticles at around 30 nm might act as a transient toxicant. It is important to note that the particles here are relatively large and upon exposure to the dechlorinated tap water they aggregate to larger sizes.

The size of nanoparticles can significantly impact their biodistribution especially as you approach smaller nanoparticles. Recent work from Lavelle *et al.* shows that quantum dots (~12 nm) could enter the blood stream and be transported to other organs including the liver, gonads, spleen, and kidneys after nanoparticle exposure through oral gavage.[52] They postulate that there may be protein-mediated effects that have an impact on the uptake and internalization. This work may also help explain why our larger nanoparticles could be depurated. The increased size may lead to particles that cannot be transported into the bloodstream.

Conclusions

In this work we have shown that CTAB capped gold nanoparticles are taken up into both wood frog and bullfrog tadpoles. We found that their ability to accumulate gold nanoparticles is significantly different, at least in the laboratory. When the tadpoles are exposed to nanoparticles in the presence of one another, the uptake is higher indicating either a species dependent uptake or some competitive uptake regime. Uptake into the anterior region of the tadpoles was higher than the posterior suggesting that the route of uptake was through the oral cavity or through the gills rather than a passive uptake through the skin. Finally, gold was quickly and significantly depurated in wood frog tadpoles in as short as 5 days. Although the depuration starts within 5 days, it takes around 2 weeks time for the level of gold to fall below our limits of detection, indicating that gold nanoparticles may be a transient environmental toxicant. Finally, CTAB capped gold nanoparticles are susceptible to aggregation over time when in the presence of dechlorinated tap water and the free CTAB concentration is low enough. Our study points to the importance of not generalizing the sensitivity to toxicants of aquatic organisms based upon previous studies on closely-related species, and argues for further testing of non-target congeneric species with varying life history strategies.

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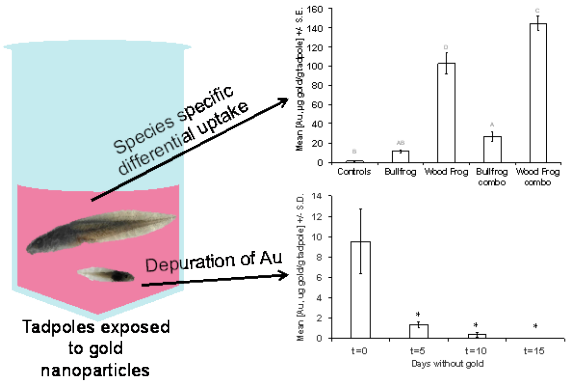
TOC graphic



Uptake of gold nanoparticles is species dependent and generalizing the impacts of nanomaterials to aquatic organisms should be avoided.

Figures

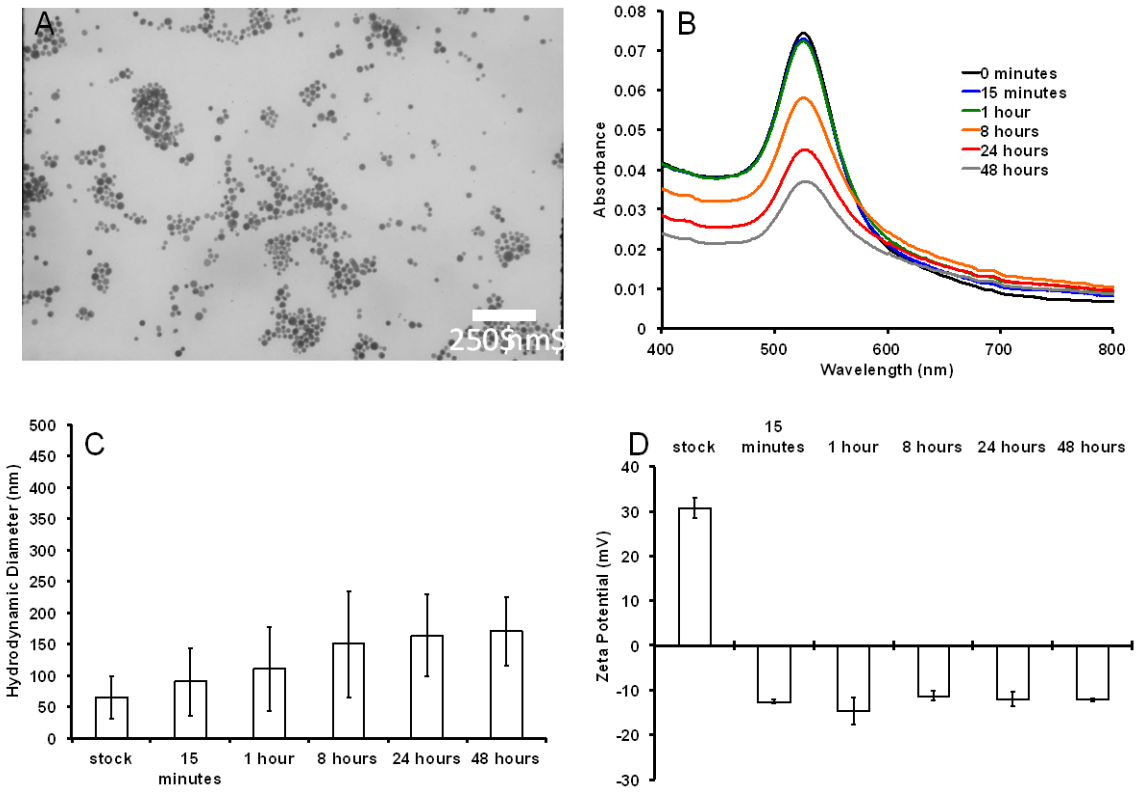


Figure 1: Nanoparticle characterization for the uptake and localization experiments. a) TEM micrograph of CTAB capped AuNPs (22 ± 5 nm). B) aggregation over time diluted 1-10 from stock solution in dechlorinated tap water c) DLS data at 0 and 48 hours showing aggregation in dechlorinated tap water. D) Zeta potential measurements of a 500 pM nanoparticles diluted 10 x in dechlorinated tap water.

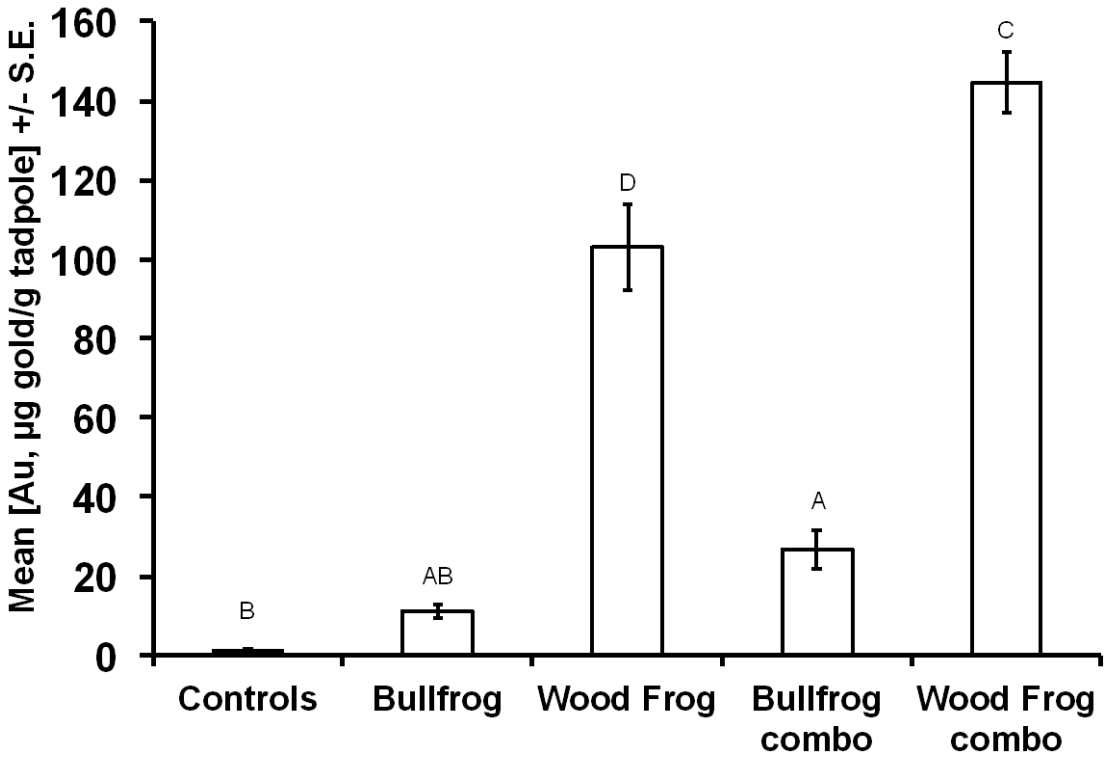


Figure 2. Concentration of gold nanoparticles (mean+/- S.E.) detected in wood frogs, bullfrogs, and those in combination (e.g. wood frogs combined with bullfrogs) (n = 4 tanks per group). Controls were pooled, non-gold exposed groups (n= 32 tanks). Bars that share letter notations are not significantly different.

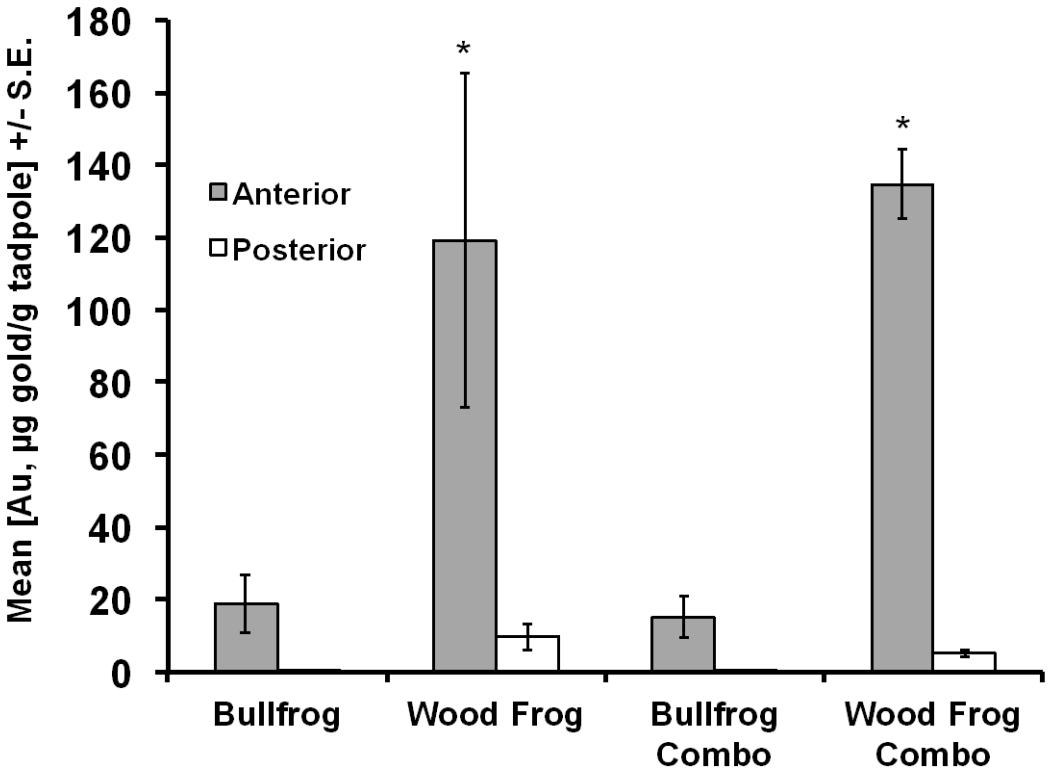


Figure 3. Concentration of gold nanoparticles (mean +/- S.E.) detected in anterior vs. posterior body regions of wood frogs (WF), bullfrogs (BF), and those in combination with the other (WF combo and BF combo). Sample sizes were n = 4 tanks per group. *: $p < 0.01$

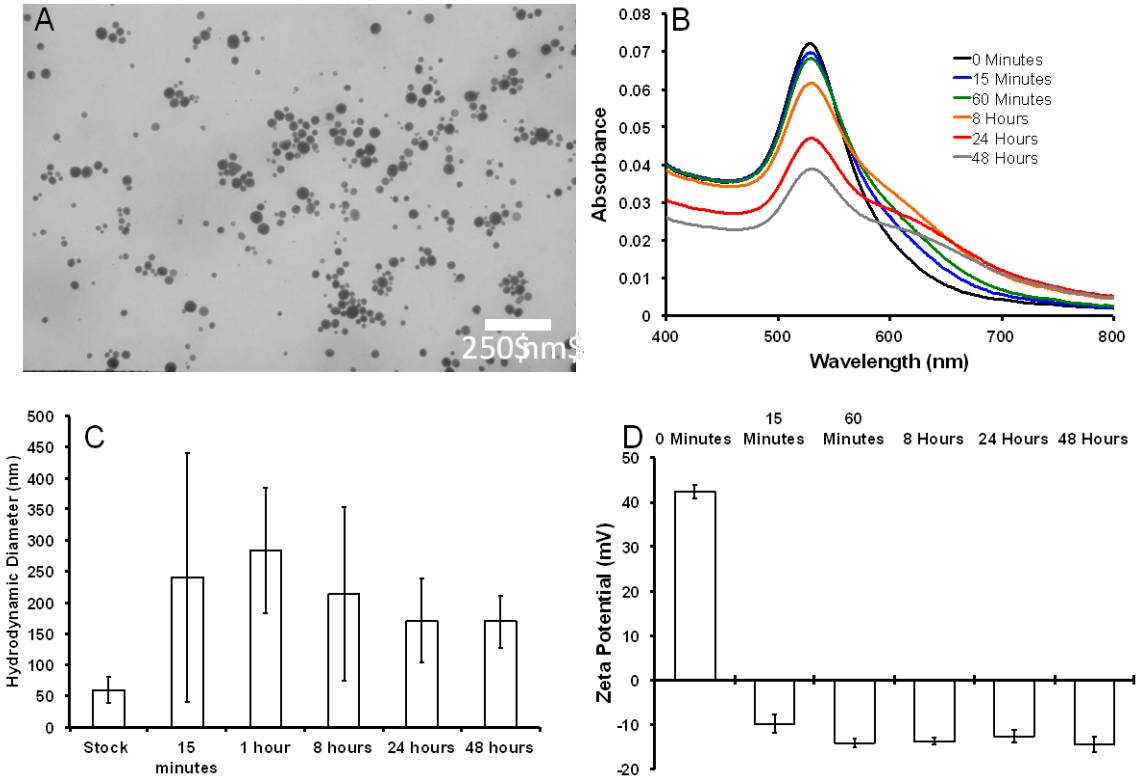


Figure 4: Nanoparticle characterization for the depuration experiments. a) TEM micrograph of CTAB capped AuNPs (27 ± 12 nm). B) aggregation over time diluted 1-10 from stock solution in dechlorinated tap water c) DLS data at 0 and 48 hours showing aggregation in dechlorinated tap water. D) Zeta potential measurements of a 500 pM nanoparticles diluted 10 x in dechlorinated tap water.

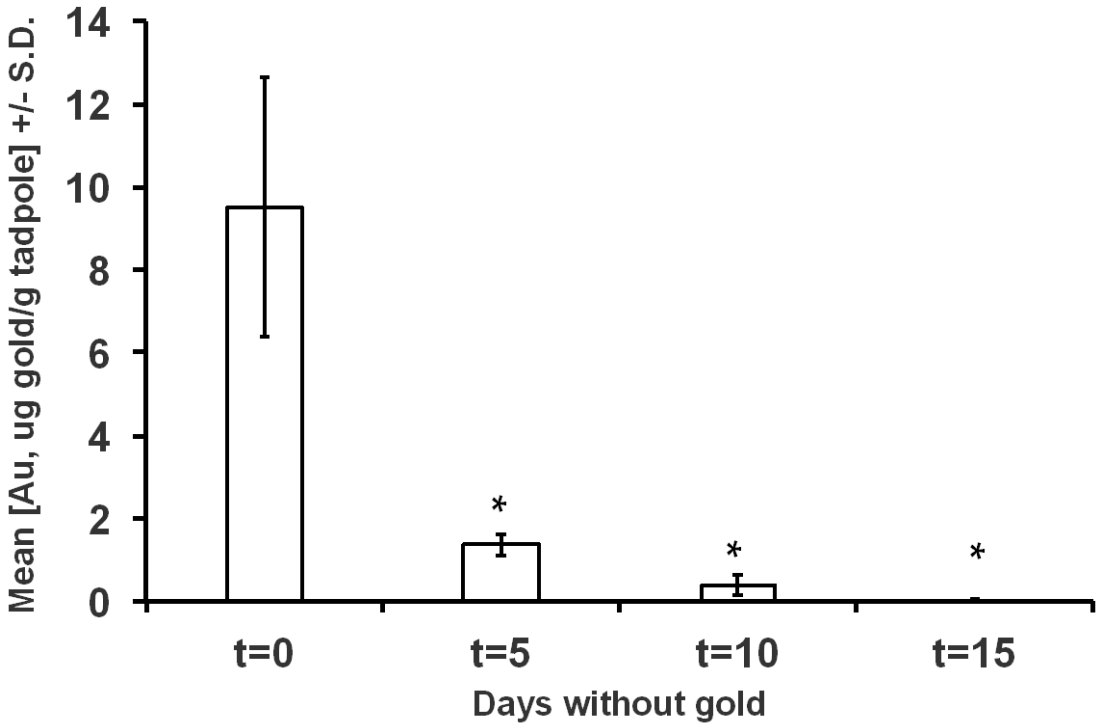


Figure 5. Concentration of gold nanoparticles (mean +/- S.E.) detected in wood frog tadpoles exposed to gold for 21 days, then depurated for 5, 10, and 15 days. Sample sizes were n = 9 tanks per group *: $p < 0.01$ vs. all three depuration groups.