Nanoparticle-mediated r-depression in the rotifer *Brachionus manjavacas*

A Thesis
Presented to
The Academic Faculty

by

Daniel Hicks

In Partial Fulfillment
of the Requirements for the Degree
Bachelor of Science in the
School of Biology

Georgia Institute of Technology
July 2008

Nanoparticle-mediated r-depression in the rotifer *Brachionus manjavacas*

Approved by:

Dr. Terry Snell, Advisor
School of Biology
Georgia Institute of Technology

Dr. Julia Kubanek
School of Biology & School of Chemistry and Biochemistry
Georgia Institute of Technology

Date Approved: 1 August 2008

ACKNOWLEDGEMENTS

I wish to thank Dr. Terry Snell for his constant guidance of my research, even from halfway across the world. I would also like to thank Dr. Tonya Shearer, Joey Bear , and Emily Weigel, each of whose humor and conversation brightened many a long day at the lab bench.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Nanotechnology research promises novel and practical applications of well-characterized materials. However, responsible development of the nanotechnology industry necessitates proactive research into the ecological responses of communities to the presence of nano-scale materials. I attempt to discern if and how the presence of inert nanoparticles at varying concentrations and size affects the fitness of populations of *Brachionus manjavacas* (Rotifera). Feeding fluorescently labeled 50 nm latex microspheres to rotifers produced dramatic fluorescence distributed throughout the females and their eggs. Fluorescent intensity was distinct from background epifluorescence exhibited by *B. manjavacas*, and increased with concentration, availability of food, and duration of exposure. Transfer of exposed maternal females and F1 offspring into nanoparticle-free environments demonstrated that these nanoparticles were rapidly cleared from the animals, and that the offspring suffered no significant effects from parental exposure. However, the population growth rate was depressed 50% in rotifer cultures exposed to 0.30 ug/mL of 50 nm particles, and 89% in cultures with nanoparticle concentrations of 1.14 μg/mL. Nanoparticles of identical composition but of larger diameter (up to 3000 nm, comparable to algae cells, a natural food source), caused no reduction in population growth rate. These larger particles remained confined in the gut, implicating nanoparticle size as a critical factor in bioactivity. Causes of growth rate depression include, but are not limited to, a marked decrease in feeding behavior. Mode of entry is suspected to be either epithelial digestive-tract phagocytosis or introduction through cellular pores.

CHAPTER 1

INTRODUCTION

Nanoscience is an emergent and multidisciplinary field that seeks to provide improved materials through an understanding of their properties on the nanoscale. Specifically, nanomaterials have at least one dimension measuring less than 100 nm, though some flexibility is allowed in the definition (Whitesides 2003). Structural manipulation at this level has led to innovative cancer treatments such as gold-tagged antibodies (El-Sayed et al. 2005) and liposome-mediated drug delivery (Park 2002, Ferrari 2005). In addition, the development of optical applications such as tunable nanoLEDs (Dai et al. 2002, Qian et al. 2005) and alternative fuel technology for superdense hydrogen storage (Dillon et al. 1997) has exemplified the manifold novel applications for even well-characterized materials and has given nanoscience tremendous potential.

But, for all this promise, unexpected nanomaterial effects on biological systems may limit permissible applications. Considering the adaptations of cells to the use of naturally occuring particles of similar size, nanoparticles may easily infiltrate or be incorporated into metabolic processes. Careful research into the possible detrimental effects of nanomaterials, then, is critical as nanoscience expands. McKenzie & Hutchison (2004) suggest a compelling rationale for "green nanoscience" research to develop environmentally friendly nanomaterials while avoiding the disruption of natural ecosystems. Strategies are also presented for decontamination devices that incorporate

nanotechnology breakthroughs to remove what contaminants may already be present in the environment. In a world humans have already polluted significantly, the ability of the nanoscience revolution to introduce a new class of contaminants mandates responsible development of nanotechnology from its inception.

To this end, cellular and ecological studies are required to assess the safety of nanoscale materials. The International Life Sciences Institute Research Foundation/Risk Science Institute outlines and summarizes the state of human- and animal-based nanoparticle testing and lists surveys of physicochemical characteristics, *in vitro* testing, and *in vivo* testing as three critical research thrusts (Oberdörster et al. 2005). Physicochemical research is sparse as yet, but existing data can at least provide preliminary pictures of high-profile nanomaterials. As an example, the recently discovered fullerenes, whimsically referred to as 'buckyballs', are notorious for their toxicity at low concentrations. (Sayes et al. 2004) demonstrated that their toxicity decreases dramatically with the attachments of substituents, and studied their hydrophobic aggregation in aqueous media. Such research has the appropriate aim of studying and minimizing nanoparticle toxicity, but lacks organismal context.

While such research provides a valuable portrait of nanomaterial chemistry, experimentation within natural systems adds a biological framework in which ecotoxicological data can be gathered. The majority of *in vitro* and *in vivo* research on the effects nanoparticle exposure has been performed on vertebrate subjects, particularly mammals, and many studies have demonstrated negative effects as a result of nanoparticle exposure. Data on human exposure is limited due to ethical considerations and the rarity of nanoparticles outside of research areas. *In vitro* human cell lines as

utilized by Ryan et al. (1994), however, may provide alternative testing methods. Research on human inhalation of airborne ultrafine particles in urban areas (Penttinen et al. 2000) provides valuable insight into this common route of exposure. In other vertebrates, however, much experimentation continues to increase understanding of the diverse adverse effects of nanoparticles on biosystems. Studies with rodents established pulmonary inflammation and oxidative stress as characteristic of introduced and inhaled nanoparticles (Lam et al. 2004, Bermudez et al. 2004). (Barlow et al. 2005) found that fetal bovine serum exposed to carbon black induced a size-differential immune response with nanoscale particles found to be more irritating. (Kashiwada 2006) demonstrated in localization tests that nanoparticles are adsorbed by Japanese killifish eggs and adults in a size-dependent trend, and that nanoparticles have the ability to cross biological membranes, including the blood-brain barrier. I build upon these studies through similar nanoparticle exposure protocols with aquatic invertebrates.

There is a striking need for research on invertebrate responses to nanoparticle exposure. Considering that estimates place the biomass of social insects alone at one-third of all terrestrial biomass (Hölldobler & Wilson 1994), the current trend of nearly exclusive research into vertebrates verges on negligence. Should certain nanoparticles prove deleterious to major invertebrate taxa, ecological communities worldwide could suffer a catastrophic loss of key species, and could collapse.

Phylum Rotifera is one such invertebrate taxon, comprising some 2,000 species inhabiting marine, freshwater, and moist to saturated terrestrial environments worldwide (Wallace & Snell 1991). Rotifers compete with copepods and cladocerans as important primary and secondary consumers, often having a high ingestion rate (greater than

50uL/animal/hr) and high primary production (up to 50% of zooplankton production) (Wallace & Snell 1991). Rotifers are outstanding model organisms for ecotoxicological research because (i) they are easily, rapidly cultured in the laboratory, and produce sensitive test results, (ii) rotifer population health is a good indicator of water quality (iii) their distinct phylogeny gives them toxicological sensitivity that differs from other groups, and (iv) their feeding and ability to exploit a wide variety of niches makes them important links in many ecosystems.

As such, rotifers have been the subject of a number of studies on aquatic signaling and water quality (Couillard et al. 1989, Capuzzo 1979, Snell et al. 1988, Snell et al. 2006). Monogonont rotifers are largely parthenogenic, but their periodic induction of sexual reproduction (mixis) illustrates the breadth of their sensory mechanisms, including dietary content (Gilbert 1980), photoperiod (Pourriot & Clement 1981), and quorum sensing related to population density (Snell et al. 2006). A wide variety of bioassays have been developed to assess the response of rotifers to several classes of environmental contaminants (Snell & Janssen 1995). Data including 24 hour LC50s have long been available for rotifers for heavy metals (Couillard et al. 1989), organic pesticides (Serrano et al. 1986), ammonia (Snell & Persoone 1988), chlorine-containing compounds (Capuzzo 1979), and petrochemicals (Rogerson et al. 1982). Nanoparticles represent a new class of environmental contaminants that have yet to be investigated in rotifers or any other zooplankter.

Current studies of the nanoparticle impact on biosystems, by focusing on vertebrates, lack significant investigation into the invertebrate, and specifically planktonic, response. Experimentation with the rotifer system can provide valuable data to fill this gap. Herein,

I report my efforts to characterize the immediate and long-term effects of inert nanoparticles on the reproduction of the rotifer *Brachionus manjavacas*, of the *B. plicatilis* species complex. To this end, I utilize fluorescent nanoparticles to observe how bioactivity, distribution within the body, reproductive rate, feeding behavior, and offspring fitness vary with nanoparticle concentration and size. It is hoped that this research on rotifer response to nanoparticle exposure will contribute to identifying margins of safety for the sustainable use of nanoparticles.

CHAPTER 2

MATERIALS AND METHODS

Rotifer Culture:

In order to initiate live rotifer cultures, approximately 20mg freeze-dried rotifer cysts were incubated in 15 ppt artificial seawater (ASW) at 25 °C with constant illumination for 24 hrs, generating between 150 and 300 rotifer neonate hatchlings. For procedures where feeding is required, Selco, an aquaculture food supplement consisting of dead yeast, was used as an alternative to chlorophyll-containing algae, which would affect fluorescence readings. Selco food suspensions were made by vortexing 0.1 mg Selco in 1mL ASW. Suspensions were re-vortexed immediately prior to usage and were always added to give final concentrations of 100 μg/mL. In order to produce ovigerous rotifers, a 24-well plate with each well containing 1000 μL ASW, 10 μL Selco suspension, and 2-3 neonates was incubated at 25 °C with illumination, generating between 50-65 ovigerous females.

Range-Finding Tests:

To determine reasonable concentration endpoints for visualization of nanoparticle exposure, 10 μL 50 nm nanoparticle suspension was pipeted into 990 μL ASW and vortexed to give a homogenous stock solution. A dilution series was performed to give three solutions of concentrations 28.7, 2.87, and .287μg/mL. In isolated wells of a 24-well plate, approximately 5 rotifers were added to 900 μL ASW, 100 μL nanoparticle dilution and allowed to incubate for either 2 hours or 2 days. Resultant fluorescence was

observed with an excitation wavelength of 485 nm and emission of 530 nm. Ideal concentrations give fluorescence that is bright enough to be clear and distinct from rotifer autofluorescence but dim enough not to hinder visibility. Repetition of this test at higher resolution was performed in order to further localize the threshold of activity and better observe the effects of changing concentration. The appropriate final nanoparticle concentration series was 5.74, 2.87, 1.43, 0.957, 0.718, and 0.000 μg/mL.

Nanoparticle Size	50 nm	100 nm	200 nm	500 nm	3000 nm
Mean diameter (nm)	37.7	83	217	546	2980
Standard deviation (nm)	4	11.1	15	18.3	83
Concentration (ug/mL)	28700	22000	14700	14000	23000
Standard deviation (ug/mL)	2890	1170	1780	667	2670
CAT# (Polysciences, Inc.)	17149	17150	17151	17152	17155

Table 1: Physical data for nanoparticles utilized in this procedure. Nanoparticles obtained from Polysciences, Inc.

Localization Testing:

For all nanoparticle sizes, detailed localization was performed to assess differential distribution throughout the rotifer body. Each well of a 24-well plate was filled with 900 μL ASW, 100 μL nanoparticle dilution (either 28.7 or 2.87 μg/mL) and approximately 5 rotifers and incubated at 25°C with illumination in four test groups differing by developmental stage and duration of exposure (neonate, 2 hr; neonate 2 d; ovigerous, 2 hr; ovigerous, 2 d). On completion of incubation, representative rotifers from all test conditions were isolated on 12-well slides in 25μL medium. To prepare for microscopy, 25 μL carbonated water was added to each well to anesthetize the rotifers, followed by 5 μL 20% formalin solution to fix them. Without anesthesia, on addition of formalin, the animals retract into their loricae, which causes them to appear unnatural and confounds localization. The beads of water in the wells were pipetted down as necessary for microscopy, and analyzed by use of a mercury lamp that produces epifluorescence to

localize nanoparticles within individual animals. Photographic documentation of the nanoparticle distributions was recorded.

Nanoparticle Bioassays:

For each size nanoparticle, at least 5 concentrations, (including 5.74, 2.87, 1.43, 0.957, 0.718 μg/mL) were tested for their effect on rotifer reproduction. For each size and concentration tested, each well of a 24-well plate was filled with 900 μL ASW, 100 μL nanoparticle dilution, 10 μL Selco suspension and approximately 5 rotifers and incubated at 25 °C with illumination for 2 d. After incubation, the numbers of rotifers in each well was counted and used to tabulate the average intrinsic rate of growth, r, for each size and concentration.

Generational Effect Studies:

At the conclusion of a 2-day bioassay, the members of the F1 generation are distinct by size and lack of eggs from the members of the P1 generation. From each concentration, 24 F1 rotifers were selected from the original 24-well plates, washed twice in ASW, and replaced individually in new 24-well plates in wells filled with 1000 μL ASW, 10 μL Selco suspension. The plates were replaced in illuminated 25 °C incubation for 2 further days, at which time the number of rotifers in each well was recounted, and the average intrinsic rate of growth calculated again for comparison with the exposed P1 values. This procedure is only meaningful for sizes where the intrinsic rate of growth was depressed.

F1 Clear Tests:

Concurrently to the generational effect study, approximately 5 other F1 individuals were isolated and transferred to a 12-well slide in 25μL medium. Anesthesia with 25 μL carbonated water but without formalin was administered, and visualization under a mercury lamp to induce epifluorescence was performed. Afterwards, the rotifers were isolated in individual wells of a 24-well plate containing 1000 μL ASW and 10μL Selco suspension. Every subsequent hour, rotifers were anesthetized and visualized again, and their clearing progress tracked until fluorescence disappeared. This procedure is only meaningful for sizes where nanoparticles were incorporated into tissues.

Feeding Bioassays:

The feeding bioassay is an adaptation of a procedure introduced by (Snell 2005) with the following modifications. For each nanoparticle size, a 24-well plate is prepared with 4 wells each of a control solution composed of 750 μL ASW and five test concentrations of nanoparticles composed of 675 μL ASW and 75 μL nanoparticle dilution (5.74, 2.87, 1.43, 0.957, 0.718 μg/mL final concentrations). Approximately 15 neonate rotifers were pipetted into each well to acclimate. After 45 min, 10 μL of a homogenized solution of 10 mg carmine in 2 mL ASW was added to each well. After an additional 15min, the rotifers were observed under a dissecting microscope to assay the proportion of feeding individuals in each well, indicated by the proportion of individuals with carmine visible in the gut. Rotifers were fixed with 10 μL 20% formalin to facilitate quantification.

CHAPTER 3

RESULTS

Localization testing utilized epifluorescence microscopy to determine the fate of ingested nanoparticles in zooplankton. Differential localization was noted for changing rotifer developmental stage, nanoparticle concentration and nanoparticle size. Neonate rotifers tended to show less fluorescence than ovigerous females, and this is probably due somewhat to prior conditioning. Neonate rotifers entered the experimental treatment having never eaten, and the nanoparticle exposure, as discussed below, likely acted to inhibit their hunger. So, they refrain from eating in anticipation of less noxious conditions, where eating would be less likely to introduce toxins into the body. Neonates, however, must be fed to develop into ovigerous individuals, and so these individuals entered the test environment with some alternative edible material present, resulting in the ingestion of more nanoparticles. Higher concentrations correlated, predictably, to brighter fluorescence and similar distribution, as greater concentrations and longer exposure increased the probability that a given rotifer while swimming will come into contact with and ingest a nanoparticle. Changing nanoparticle size, in contrast to differential fluorescent intensity, led to differential absorption into tissues (Figure 1). For the 3000 nm particles, which are comparable in size to the green alga *Tetraselmis*, a natural food source for *B. manjavaca*s, fluorescence was confined to the gut, in the center of the animal. A similar pattern is seen for the 500, 200, and 100 nm nanoparticles, but

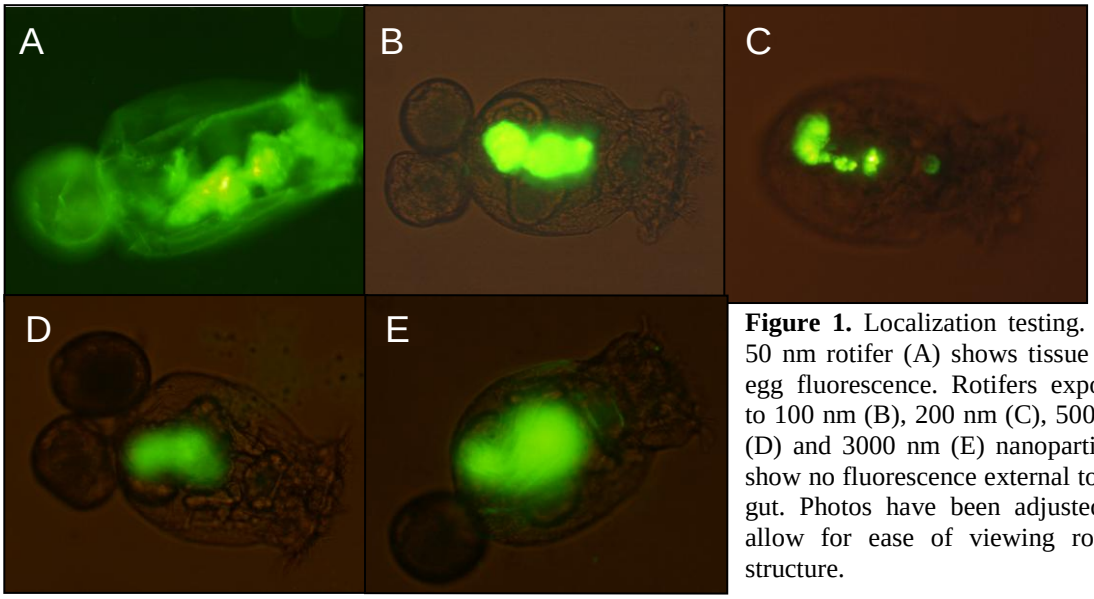

Figure 1. Localization testing. The 50 nm rotifer (A) shows tissue and egg fluorescence. Rotifers exposed to 100 nm (B), 200 nm (C), 500 nm (D) and 3000 nm (E) nanoparticles show no fluorescence external to the gut. Photos have been adjusted to allow for ease of viewing rotifer structure.

the 50 nm nanoparticles induced fluorescence throughout the rotifer body, infiltrating even the eggs. Because the nanoparticles used were of identical composition, this result indicates that below a certain threshold diameter (37.7-83 nm), nanoscale materials are able to pass through the gut, whether they escape through a pore or are taken up by epithelial phagocytosis.

This escape from the digestive tract, which is certainly not the normal course for ingested material, prompted investigation into whether or not the fitness of rotifers ingesting nanoparticles was affected by their presence in the tissues. The 2-day bioassays (Figure 2) served in this regard to measure for a single size particle the differences in reproductive rate of *B. manjavacas* across concentrations. Overall, exposure to increasing concentrations of 50 nm nanoparticles was associated with a depression in reproductive rate. Considering that exposures for this size consisted of 2.29, 1.14, 0.574, 0.287, 0.191, 0.144 and 0.00 μg/mL of the nanoparticle, it should be noted that these are comparable to noxious concentrations of such well-recognized toxicants as mercury and selenium

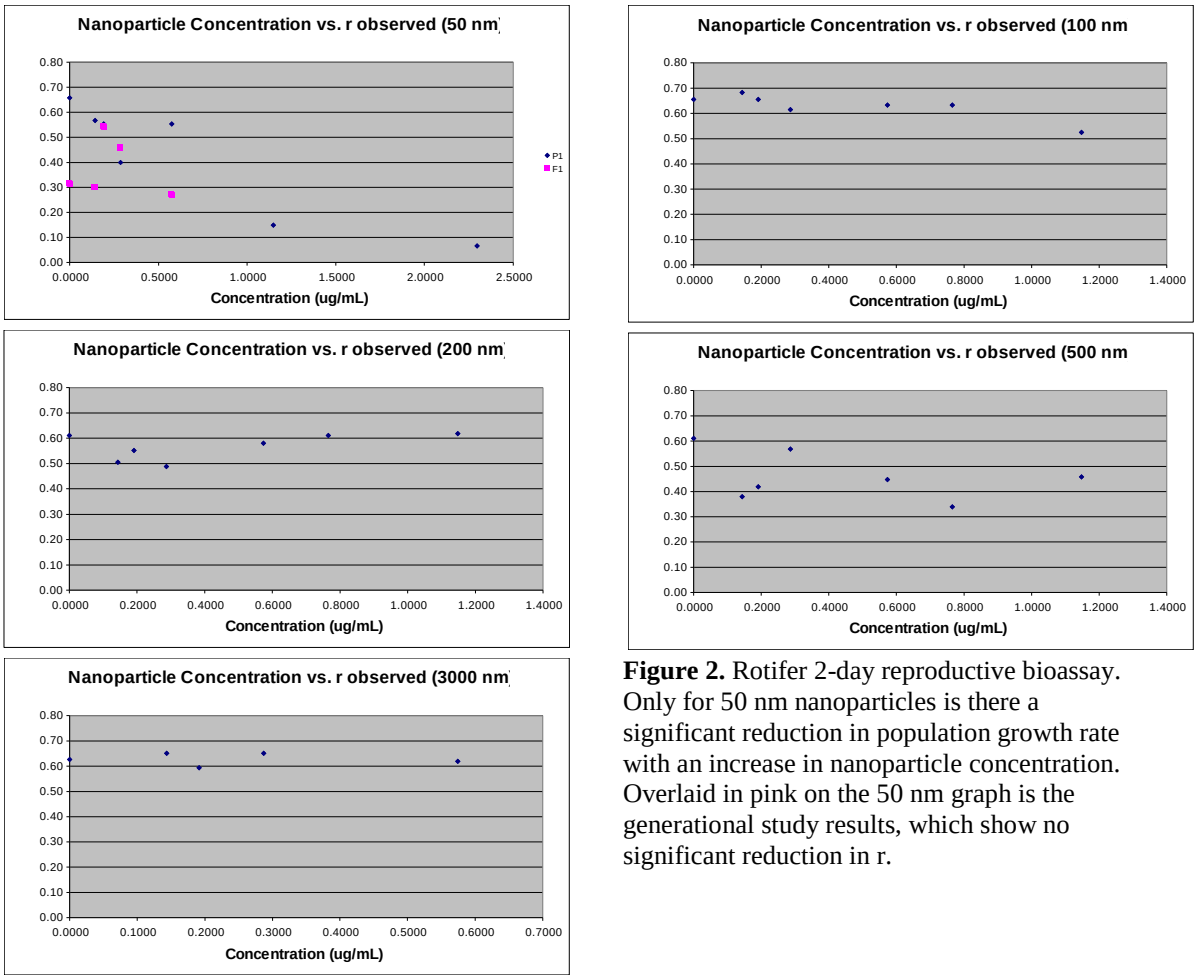

Figure 2. Rotifer 2-day reproductive bioassay. Only for 50 nm nanoparticles is there a significant reduction in population growth rate with an increase in nanoparticle concentration. Overlaid in pink on the 50 nm graph is the generational study results, which show no significant reduction in r.

(Couillard et al. 1989). Control populations had an observed r of 0.66, while nanoparticle concentrations of 2.29 μg/mL produced an r of 0.07. 2-Day Bioassays for 100, 200, 500, and 3000 nm nanoparticle exposures all showed no statistically significant reduction in r. These results imply that the materials the nanoparticles consist of were also not bioactive with respect to reproductive rate at the concentrations tested and that the depression in r was another effect due to the size of the nanoparticle itself, occurring below the threshold diameter of (37.7-83 nm).

Accumulation of 50 nm particles in amictic eggs suggested that the offspring of exposed individuals exposed to these nanoparticles might also suffer reduced fitness even in an environment free of contamination. The Generational Effect Study and the F1

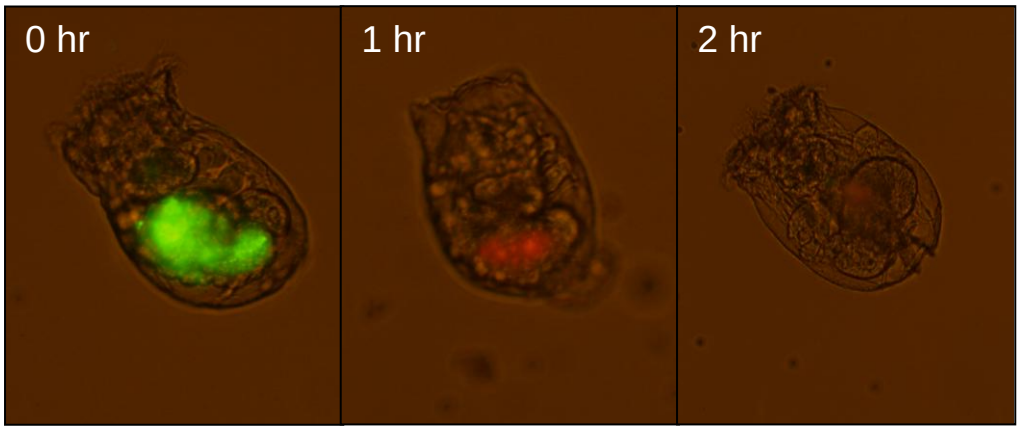

Figure 3. F1 clear testing. Bright green fluorescence at t= 0 hr is processed to red fluorescence by t= 1 hr, and has nearly vanished at t= 2 hr.

Clearing tests were undertaken to assess the strength and duration, respectively, of the possible detrimental effects of this accumulation. However, the Generational Effect Study showed no statistically significant difference in r between the offspring of individuals exposed to nanoparticles and the offspring of individuals who were not. In the F1 Clearing Test, the characteristic yellow-green fluorescence that was observed at t= 0 hr had been altered to red fluorescence at t= 1 hr, and had disappeared by t= 2 hr (Figure 3). Initially, algal contamination of the testing plate was suspected as the source of the red fluorescence, but rejected on further analysis because the fluorescence of chlorophyll is typically more orange in color. Rather, it is suggested that the breakdown of the epifluorescent nanoparticles in the rotifer body leads to the production of a red-fluorescing intermediate. Overall it seems that the high metabolic rate of rotifers quickly degrades incorporated nanoparticles and effects a short residence time for nanoparticles in tissues once exposure is halted. It is probable that there is some depression intitially, but as the nanoparticles are quickly eliminated, the population regains vigor, and the small initial disturbance is not noted after two days. It was initially inferred from the results of the localization testing and the 2-day bioassay that that nanoparticle migration

into tissues solely effects the observed depression in r for 50 nm particle exposure. In order to support this assumption, the feeding bioassay, which tests for aversion to

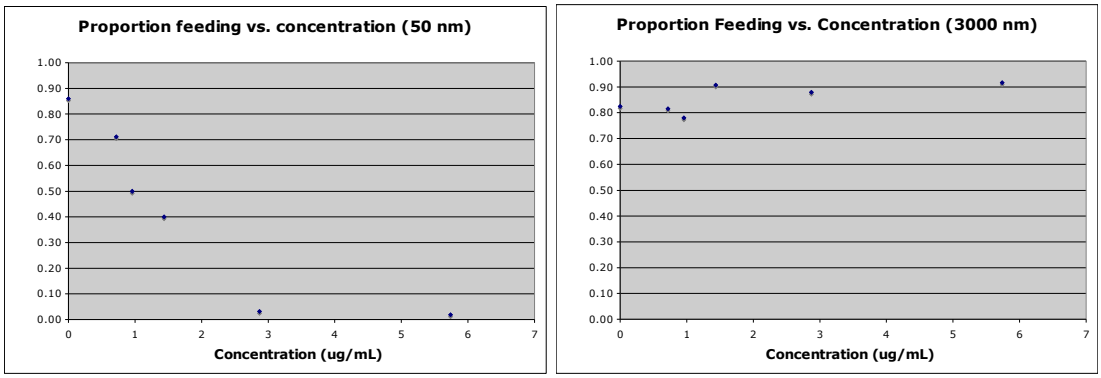

Figure 4. Feeding bioassay. Increasing concentrations of 50 nm nanoparticles are inversely correlated with the proportion of feeding rotifers. This trend is absent for 3000 nm nanoparticles.

feeding in the presence of a suspected noxious substance, was conducted. A noxious response is evolutionarily favored because it prevents an organism from consuming substances it can chemically detect to be poisonous, but by reducing food intake it can also cause a decrease in secondary production. Contrary to expectation, the feeding bioassay (Figure 4) showed significant aversion to feeding as 50 nm nanoparticle concentration increased. A control group exhibited 86.0% of individuals feeding, while at 5.74 μg/mL 50 nm nanoparticles, only 1.82% of individuals were found to be feeding. No such noxious response was found with 3000 nm nanoparticles. This indicates that while nanoparticle-mediated metabolic stress is highly implicated by the other testing methods, reduced food intake is also a factor in the observed depression of r.

While other studies on the effects of nanoparticles on aquatic biosystems have not established their effects on population growth, a comparison of trends of nanoparticle activity and distribution can be made. In comparing the bioactivity of C_{60} (mean diameter .683 nm) in the fathead minnow and *Daphnia* systems, (Zhu et al. 2006) found that environmental conditions play a role in determining the toxicity of nanoparticles, and can

have opposite effects on different species. Fullerenes solubilized by the addition of tetrahydrofuran proved more than an order of magnitude less toxic (LC_{50}= 0.8 ppm) to *Daphnia* than than fullerenes solubilized by continuous agitation (LC_{50}> 35 ppm). Conversely, fathead minnow larva exposed to 0.5 ppm C_{60} with THF experienced 100% mortality, while larva exposed to 0.5 ppm C_{60} with agitation showed no adverse effects after 48 hours. In addition, the minnow larva showed increased levels of oxidized lipids in their gills, brains, and livers. While chemical conditions were not manipulated in my investigation, the results presented by Zhu et al. parallel my finding that relatively low concentrations of nanoparticles below the threshold diameter (approximately 50 nm) are toxic to zooplankton. These results also support the assumption that oxidative stress, as observed in many prior studies (Lam et al 2004, Oberdörster et al 2005), is a major contributor to nanoparticle-mediated stress on biosystems.

The differential adsorption of fluorescent nanoparticles across sizes and salinities into medaka (Japanese killifish) eggs by (Kashiwada 2006) further establishes that environmental conditions can affect nanoparticle adsorption. As Kashiwada increased the concentration of embryo rearing medium from 1 to 30 times the normal value, the resultant nanoparticle concentration in medaka eggs reduced six-fold. (Kashiwada 2006) also provides a medaka-based comparison for levels of nanoparticle adsorption into rotifer tissues. Nanoparticles of diameter 494 nm were adsorbed much more readily than smaller or larger particles (42,000, 18,600, 932 , 39.4 nm), which all expressed about the same level of fluorescence. This trend differs from rotifer observations, but not without likely explanation. For eggs, the only mode of entry available to nanoparticles is adsorption, while for adult rotifers direct ingestion is possible. However, considering that

494 nm nanoparticles concentrated highly in medaka eggs, the observation that 500 nm nanoparticles were not adsorbed into rotifer eggs indicates distinct surface structure. Both sets of findings do demonstrate that at least above certain threshold diameters, few nanoparticles are able to cross biological membranes.

Caution is warranted in the disposal of nanoparticles with a diameter below an approximate threshold of 50 nm. While exposed populations have been shown to quickly eliminate contamination and return to normal growth rates, continuous exposure causes severe retardation of growth rate and disrupts the productivity of populations. This effect would clearly alter predator-prey cycles and lead to ecosystem instability. Because *B. manjavacas* serves as both a primary consumer and a detritivore, decreased growth rates could also lead to uncharacteristic algal blooms and crippled nutrient cycling.

As this is a primary study in a largely unexplored field, there are of course many possibilities for further investigation raised by this inquiry. One simple expansion would be to test the rotifer response to more diverse types of nanoparticles in an attempt to identify trends in observed responses. The nanoparticle responses of groups such as cladocerans that occupy similar niches could be compared as a test of the predictive value of the findings presented. Perhaps most usefully, a multi-trophic level microcosm could be constructed, ideally with a predator whose metabolic rate is slower and allows for biomagnification of nanoparticles, so that lasting effects might be better observed than in *B. manjavacas*. Such a system would not only shed light on the characteristics of chronic exposure, but also on how nanoparticle introduction into a stable ecosystem might shift predator-prey equlibria.

REFERENCES

Barlow, P., K. Donaldson, et al. (2005). "Serum exposed to nanoparticle carbon black displays increased potential to induce macrophage migration." Toxicology Letters. 155(3): 397-401.

Bermudez, E., J. Mangum, et al. (2004). "Pulmonary Responses of Mice, Rats, and Hamsters to Subchronic Inhalation of Ultrafine Titanium Dioxide Particles." Respiratory Toxicology 77: 347-357.

Capuzzo, J. (1979). "The effect of halogen toxicants on survival, feeding, and egg production of the rotifer Brachionus plicatilis." Estuarine and Coastal Marine Science 8: 307-316.

Colvin, V. (2003). "The potential environmental impact of engineered nanomaterials." Nature Biotechnology 21(10): 1166-1170.

Couillard, Y., P. Ross, et al. (1989). "Acute toxicity of six metals to the rotifer Brachionus calyciflorus." Toxicity Assesment 4: 451-462.

Dai, Y., Y. Zhang, et al. (2002). "Synthesis and optical properties of tetrapod-like zinc oxide nanorods." Chemical Physics Letters 358(1-2): 83-86.

Dillon, A., K. Jones, et al. (1997). "Storage of hydrogen in single-walled carbon nanotubes." Nature 386: 377-379.

Donaldson, K., L. Tran, et al. (2005). "Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure." Particle & Fibre Toxicology 2(10).

El-Sayed, I., X. Huang, et al. (2005). "Surface Plasmon Resonance Scattering and Absorption of anti-EGFR Antibody Conjugated Gold Nanoparticles in Cancer Diagnostics: Applications in Oral Cancer." Nano Letters 5(5): 829-834.

Ferrari, M. (2005). "Cancer nanotechnology: opportunities and challenges." Nature Reviews Cancer 5: 161-171.

Gilbert, J. (1980). "Female polymorphisms and sexual reproduction in the rotifer Asplanchna: evolution of their relationship and control by dietary tocopherol." American Naturalist 116(375-379).

Hölldobler, B. and E. Wilson (1994). Journey to the Ants. Cambridge, Belknap Press.

Kashiwada, S. (2006). "Distribution of Nanoparticles in the See-through Medaka (Oryzias latipes)." Environmental Health Perspectives 114(11): 1697-1702.

Kishikawa, H., S. Miura, et al. (2002). "Transmural pressure induces IL-6 secretion by intestinal epithelial cells." Clinical & Experimantal Immunology 129(1).

Lam, C., J. James, et al. (2004). "Pulmonary Toxicity of Single-Wall Carbon Nanotubes in Mice 7 and 90 Days After Intratracheal Instillation." Respiratory Toxicology 77: 126-134.

Lürling, M. and M. Scheffer (2007). "Info-disruption: pollution and the transfer of chemical information between organisms." Trends in Ecology & Evolution 22(7): 374-379.

McKenzie, L. and J. Hutchison (2004). "Green Nanoscience: An integrated approach to greener products, processes, and applications." Chemistry Today 22(5): 25-28.

Oberdörster, G., A. Maynard, et al. (2005). "Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy." Particle & Fibre Toxicology 2(8).

Park, J. (2002). "Liposome-based drug delivery in breast cancer treatment." Breast Cancer Research 4: 95-99.

Penttinen, P., K. Timonen, et al. (2000). "Ultrafine particles in urban air and respiratory health among adult asthmatics." European Respiratory Journal 17: 428-435.

Pourriot, R. and P. Clement (1981). "Action de facteurs externes sur la reproduction et le cycle reproducteur des Rotifers." Acta Oecologia Generale 2: 135-151.

Qian, F., S. Gradečak, et al. (2005). "Core/Multishell Nanowire Heterostructures as Multicolor, High-Efficiency Light-Emitting Diodes." Nano Letters 5(11): 2287-2291.

Rogerson, A., J. Berger, et al. (1982). "Acute toxicity of ten crude oils on the survival of the rotifer Asplanchna sieboldi and sublethal effects on rates of prey consumption and neonate production." Environmental Pollution (Series A) 29: 179-187.

Ryan, M., G. Johnson, et al. (1994). "HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney." Kidney International 45: 48-57.

Sayes, C., J. Fortner, et al. (2004). "The Differential Cytotoxicity of Water-Soluble Fullerenes." Nano Letters 4(10): 1881-1887.

Serrano, L., M. Miracle, et al. (1986). "Differential response of Brachionus plicatilis (Rotifera) ecotypes to various insecticides." Journal of Environmental Biology 7: 259-275.

Snell, T., J. Kubanek, et al. (2006). "A protein signal triggers sexual reproduction in Brachionus plicatilis (Rotifera)." Marine Biology 149: 763-773.

Snell, T. (2005). Rotifer ingestion test for rapid toxicity assessment of fresh and marine waters. Small-Scale Freshwater Environment Toxicity Test Methods. C. Blaise and J. Ferard (eds). Kluwer. Dordrecht, The Netherlands, 1. 323-335.

Snell, T. and G. Persoone (1988). "Acute toxicity bioassays using rotifers. I. A test for marine and brackish environments with Brachionus plicatilis." Aquatic Toxicology 14: 65-80.

Stringer, B. and L. Kobzik (1998). "Environmental particulate-mediated cytokine production in lung epithelial cells (A549): role of preexisting inflammation and oxidant stress." Journal of Toxicology and Environmental Health Part A 55(1): 31-44.

Wallace, T. and T. Snell (1991). Phylum Rotifera. Ecology and Classification of North American Freshwater Invertebrates. J. Thorp and A. Covich (eds), Academic Press: 195-254.

Whitesides, G. (2003). "The 'right' size in nanobiotechnology." Nature Biotechnology 21(10): 1161-1165.

Zhu, S., E. Oberdörster, et al. (2006). "Toxicity of an engineered nanoparticle (fullerene, C_{60}) in two aquatic species, Daphnia and fathead minnow." Marine Environmental Research 62(1): S5-S9.