Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

For NSERC office use only

Form 101 - Application for a Grant

Send to NSERC with your attachments, if applicable

Reference Number: 150683765

Applicant: Chris Metcalfe **NSERC PIN:** 15742

Trent

Program: Strategic Project Grants

Application Title: Impacts of nanosilver on a lake ecosystem

Chris Metcalfe

Form 101 - Application for a Grant

Electronic Attachments:

Cover Letter - Cover letter

Budget Justification - Budget justification

Contributions from Supporting Organizations - Attachment - Supporting organizations

Proposal - Proposal

References - References

Relationship To Other Research Support - Other research support

Other Documents - Letters of support

Chris Metcalfe

F100/Personal Data Form

Electronic Attachments:

Contributions - Metcalfe contributions

Paul Frost

F100/Personal Data Form

Electronic Attachments:

Contributions - research contributions

Holger Hintelmann

F100/Personal Data Form

Electronic Attachments:

Contributions - Form 100

Michael Paterson

F100/Personal Data Form

Electronic Attachments:

Contributions - Paterson Contributions

Marguerite Xenopoulos

F100/Personal Data Form

Electronic Attachments:

Contributions - 2011

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Trent

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Yasir Sultan

F183A/Org. Participating in RPP

Electronic Attachments:

Letter(s) of support - Metcalfe_Letter_Support

Chris D. Metcalfe
Environmental and Resource Studies Program
TRENT UNIVERSITY
Peterborough, ON, K9J 7B8, Canada
T: 705-748-1011 x7272; F: 705-748-1569; cmetcalfe@trentu.ca

April 10, 2011

Natural Science and Engineering Research Council
NSERC Strategic Grants Program
350 Albert Street
Ottawa, Canada, K1A 1H5

Subject: NSERC Strategic Proposal: Effects of nanosilver on a lake ecosystem

Dear Sir/Madame;

With this proposal to the NSERC Strategic Grants Program, we have proposed to study the effects of a model nanomaterial, nanosilver on a lake ecosystem. A large portion of this study will involve addition of nanosilver to a whole lake in the Experimental Lakes Area (ELA) in northwestern Ontario. We realize that this study, if approved, will be a high profile research project that will attract considerable national and international attention. This project will inevitably raise questions, which I will attempt to address here:

i) Why do we need to do it? Bench scale and even mesocosm scale research has shown that nanosilver and other nanomaterials can effect freshwater organisms; especially autotrophs, primary producers and primary consumers at the bottom of the trophic web. While we can speculate that these responses might produce ecosystem level effects, we cannot test these hypotheses without experimentation at the level of a whole lake ecosystem. Dr. Kaz Patalas, who was one of the leading researchers involved in the first whole lake addition study with phosphorus at ELA once said, "You can't answer big questions with tests in a bottle" (D. Schindler, Pers. Comm.). Arguably, the observations of the collapse of a fish population observed in a recent study completed at ELA with whole lake additions of a synthetic estrogen (i.e. EE2) have had more impact than all of the laboratory based studies that have shown effects on fish reproduction. Without the studies at ELA on the effects of phosphorus and acid rain on whole lake ecosystems, the international push for regulations on phosphorus discharges and sulfur emissions would have taken place much more slowly.

ii) Is this the right study team to do it? The study team of researchers from Trent University, Environment Canada and Fisheries and Oceans Canada is comprised of a multidisciplinary group of experienced and dedicated researchers. Among the members of the study team, several have previous experience on whole lake addition studies at ELA. Several members of the team also have a track record over the past 4 years of research on the ecological effects of nanomaterials; a considerable period of time in this field, considering that the first papers on this emerging issue in ecotoxicology began to appear in the literature in 2008. However, the study team recognizes that they will not have all of the answers and tools to plan and implement this complex project, so they have made considerable efforts to network with other researchers in Canada, the USA and in Europe to incorporate their expertise. We will also welcome complementary research projects at ELA that will take advantage of the "once in a lifetime" opportunity to participate in a whole lake addition study of nanomaterials. The Water Quality Centre at Trent University is a unique facility for studying the fate of contaminants in aquatic ecosystems. There is no other academic institution in Canada where researchers can access the range of analytical instrumentation, including ICP-MS, MC-ICP-MS, FFF, MIMS, stable isotope MS and flow cytometry equipment that will be necessary in order to conduct this study.

iii) Who will benefit? Environment Canada has invested considerable resources into preparing for this project, as well as a previous NSERC Strategic project (PI: Metcalfe) on the fate and effects of nanomaterials. As the lead agency responsible for assessing the environmental risks associated with new nanomaterials, Environment Canada is struggling to develop policies and procedures for risk assessment that will protect the environment, but not constrain this important economic sector. This project will help Environment Canada to understand whether or not nanomaterials are an important environmental issue that requires careful regulation. This project will also make a significant contribution to the international effort to study the environmental impacts of nanomaterials. Among the millions of dollars currently being invested on this research theme in the USA and in Europe, there are no whole lake addition studies in progress or planned. The OECD Working Group on Nanomaterials is aware of the development of this project and is keenly interested in following the progress of this study. Ultimately, however, this project is intended to protect the environment and the ecosystem services enjoyed by Canadians. Will we wait until there is evidence of effects of nanomaterials released into the environment, or will be pro-active and conduct this study before effects are observed?

Sincerely,

Chris Metcalfe, PhD
Professor, Environmental and Resource Studies
Trent University, Canada

Natural Sciences and Engineering Research Council of Canada
Conseil de recherches en sciences naturelles et en génie du Canada

Institutional Identifier	
System-ID (for NSERC use only)	150683765

FORM 101
Application for a Grant
PART I

Date
2011/04/14

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Metcalfe	Chris	D	**Valid** 15742

Institution that will administer the grant	Language of application	Time (in hours per month) to be devoted to the proposed research / activity
Trent	[X] English [ ] French	50

Type of grant applied for	For Strategic Projects, indicate the Target Area and the Research Topic; for Strategic Networks indicate the Target Area.
Strategic Project Grants	Environmental Science and Technologies / Enhancing Aquatic Ecosystem Services

Title of proposal
Impacts of nanosilver on a lake ecosystem

Provide a maximum of 10 key words that describe this proposal. Use commas to separate them.
nanomaterials, geochemistry, toxicity, bioaccumulation, productivity, nutrients, speciation, food web, lakes,

Research subject code(s) Primary	Research subject code(s) Secondary	Area of application code(s) Primary	Area of application code(s) Secondary
4705	4702	400	409

CERTIFICATION/REQUIREMENTS

If this proposal involves any of the following, check the box(es) and submit the protocol to the university or college's certification committee.

Research involving : Humans [ ] Human pluripotent stem cells [ ] Animals [X] Biohazards [ ]

Does any phase of the research described in this proposal a) take place outside an office or laboratory, or b) involve an undertaking as described in Part 1 of Appendix B?

[ ] NO [X] If YES to either question a) or b) – Appendices A and B must be completed

TOTAL AMOUNT REQUESTED FROM NSERC

Year 1	Year 2	Year 3	Year 4	Year 5
245,400	245,400	285,400	0	0

SIGNATURES (Refer to instructions "What do signatures mean?")

It is agreed that the general conditions governing grants as outlined in the NSERC *Program Guide for Professors* apply to any grant made pursuant to this application and are hereby accepted by the applicant and the applicant's employing institution.

Applicant

Head of department

Applicant's department, institution, tel. and fax nos., and e-mail

Environmental and Resource Studies
Trent
Tel.: (705) 748-1011 ext. 7272
cmetcalfe@trentu.ca

Dean of faculty

President of institution
(or representative)

The information collected on this form and appendices will be stored in the Personal Information Bank for the appropriate program.
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Personal identification no. (PIN)	Family name of applicant
Valid 15742	Metcalfe

CO-APPLICANTS

I have read the statement "What do signatures on the application mean?" in the accompanying instructions and agree to it.

PIN, family name and initial(s)	Research/ activity time (hours/month)	Organization	Signature
193144, Hintelmann, H	40	Trent	
189734, Xenopoulos, MA	40	Trent	
303666, Frost, C	40	Trent	
201183, Paterson, J	40	Government of Canada	

Use additional page(s) if necessary.

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CO-APPLICANTS' ORGANIZATIONS AND/OR SUPPORTING ORGANIZATIONS (if organization different from page 1)

It is agreed that the general conditions governing grants as outlined in the NSERC *Program Guide for Professors,* as well as the statements "What do signatures on the application mean?" and "Summary of proposal for public release" in the accompanying instructions, apply to any grant made pursuant to this application and are hereby accepted by the organization.

Family name and given name of signing officer, title of position, and name of organization	Signature
Enei, George, G Director General, Science & Risk Assess. Environment Canada Ramlal, Patricia, P Division Manager, Environ. Sci. Div. Fisheries and Oceans	

 Use additional page(s) if necessary.

Personal identification no. (PIN)	Family name of applicant
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Before completing this section, read the instructions for the definition of collaborators in the Eligibility Criteria section of the Program Guide for Professors.

COLLABORATORS

PIN, family name and initial(s)	Research/ activity time (hours/month)	Organization / Department
11344, Muir, D	30	Environment Canada,
Dabek-Zlotorzynska, E	30	Environment Canada,
Rennie, M	30	Fisheries and Oceans,
Higgins, S	20	Fisheries and Oceans,

Use additional page(s) if necessary.

Personal identification no. (PIN)	Family name of applicant
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SUMMARY OF PROPOSAL FOR PUBLIC RELEASE (Use plain language.)

This plain language summary will be available to the public if your proposal is funded. Although it is not mandatory, you may choose to include your business telephone number and/or your e-mail address to facilitate contact with the public and the media about your research.

Business telephone no. (optional): 1 (705) 748-1011 Ext. 7272

E-mail address (optional): cmetcalfe@trentu.ca

There is potential to apply nanotechnology to almost every economic sector, including consumer products, agriculture, biomedical, transportation and energy. While nanotechnology has the potential to produce societal benefits, it should be a priority to better understand the ecological risks from the release of nanomaterials (NMs) into the environment, including impacts upon ecological services. Our previous laboratory research has shown that NMs in the aquatic environment first affect organisms at the bottom of the food chain, including bacteria, algae and zooplankton. These responses may have devastating effects upon aquatic ecosystems by reducing overall productivity and altering the cycling of nutrients. There may be compensatory mechanisms within aquatic ecosystems that mitigate these responses, but it is impossible to predict these responses using laboratory studies. We propose to conduct a study at the Experimental Lakes Area (ELA) in northwestern Ontario by adding nanoform silver to a small lake over two field seasons. Nano-silver is a widely used NM that is used as a bacteriocide in hundreds of products, including surgical bandages, food wrappers, baby bottles, slippers, socks, underwear, shoe-liners and even in washing machines. The nanoform silver will be added daily at a location along the lakeshore to simulate point source inputs from a wastewater treatment plant, and we will monitor the lake along an exposure gradient for changes to nutrient cycling and biological effects within the lake food chain. However, before starting the additions of silver, we will refine our approach by determining what happens in mesocosms (i.e. plastic tubes) that are deployed in lakes. ELA has been used for over 40 years as a living laboratory to study the effects of pollutants in the environment, including past studies of the impacts of pollution from phosphorus, acid deposition, mercury and endocrine disruptors. While we do not take lightly the potential impacts of nano-silver on the study lake in ELA, this approach is the only way to determine ecosystem level impacts. In turn, this information will influence regulatory policy to limit the ecological risks from the release of NMs into the environment.

Other Language Version of Summary (optional).

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ACTIVITY SCHEDULE
(Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)

Milestone	Description of activities	Anticipated starting date	Anticipated completion date
CONSULTATION AND APPROVALS	Consultation with research team and international colleagues on the objectives and study design, including discussions at a workshop scheduled for June 10, 2011. Once NSERC support is announced, approvals for ELA studies, animal care, will be finalized.	2011-06-10	2011-11-30
MESOCOSM STUDY	Studies with mesocosms at 3 ELA lakes, followed by data analysis. This phase will focus on refining methods to evaluate the fate and effects of nAg dosed into mesocosms. This phase will be followed by planning for the whole lake addition study.	2012-01-01	2012-12-31
WHOLE LAKE ADDITION STUDY	Additions of nAg during the ice free period to a lake at ELA for two field seasons and evaluation of ecosystem level responses and the fate of NM, followed by data analysis.	2013-01-01	2014-12-31
REPORTING PHASE	Reporting of the results of the whole lake addition study and teh mesocosm studies, including thesis defences. We anticipate that we will request an extension of the period of graduate support for the PhD students to cover this period.	2015-01-01	2015-12-31

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Before completing this section, **read the instructions** and consult the *Use of Grant Funds* section in the NSERC Program Guide for Professors for information about the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for),** and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only)

Effects of nAg on lake	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	77,000	77,000	77,000	0	0
b) Postdoctoral fellows	60,000	60,000	40,000	0	0
c) Technical/professional assistants	30,000	30,000	30,000	0	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	0	0	0	0	0
b) Operation and maintenance costs	11,000	9,000	9,000	0	0
c) User fees	13,000	26,000	26,000	0	0
3) Materials and supplies	64,000	45,000	45,000	0	0
4) Travel					
a) Conferences	7,200	7,200	7,200	0	0
b) Field work	43,200	43,200	43,200	0	0
c) Collaboration/ consultation	0	5,000	5,000	0	0
5) Dissemination costs					
a) Publication costs	0	3,000	3,000	0	0
b) Other activities	0	0	0	0	0
6) Other (specify)					
a)	0	0	0	0	0
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES	305,400	305,400	285,400	0	0
Total cash contribution from industry (if applicable)					
Total cash contribution from university (if applicable)					
Total cash contribution from other sources (if applicable)	60,000	60,000	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	245,400	245,400	285,400	0	0

Personal identification no. (PIN)	Family name of applicant
Valid 15742	Metcalfe

Before completing this section, **read the instructions** and consult the *Use of Grant Funds* section in the NSERC Program Guide for Professors for information about the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for),** and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (include cash expenditures only.)

Sum Total	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	77,000	77,000	77,000	0	0
b) Postdoctoral fellows	60,000	60,000	40,000	0	0
c) Technical/professional assistants	30,000	30,000	30,000	0	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	0	0	0	0	0
b) Operation and maintenance costs	11,000	9,000	9,000	0	0
c) User fees	13,000	26,000	26,000	0	0
3) Materials and supplies	64,000	45,000	45,000	0	0
4) Travel					
a) Conferences	7,200	7,200	7,200	0	0
b) Field work	43,200	43,200	43,200	0	0
c) Collaboration/consultation	0	5,000	5,000	0	0
5) Dissemination costs					
a) Publication costs	0	3,000	3,000	0	0
b) Other activities	0	0	0	0	0
6) Other (specify)					
a)	0	0	0	0	0
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH	305,400	305,400	285,400	0	0
Total cash contribution from industry (if applicable)					
Total cash contribution from university (if applicable)					
Total cash contribution from other sources (if applicable)	60,000	60,000	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	245,400	245,400	285,400	0	0

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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization

Environment Canada

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	60,000	60,000	0	0	0
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	1,000	1,000	1,000	0	0
2) Donation of equipment, software	0	0	0	0	0
3) Donation of material	0	0	0	0	0
4) Field work logistics	0	0	0	0	0
5) Provision of services	0	0	0	0	0
6)	0	0	0	0	0
Total of in-kind contributions to direct costs of research	1,000	1,000	1,000	0	0
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0	0	0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	1,000	1,000	1,000	0	0
Contribution to postsecondary institution overhead	0	0	0	0	0

Personal identification no. (PIN)	Family name of applicant
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Before completing this section, read the instructions for contributions from supporting organizations and consult the *Use of Grant Funds* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds, and *Guidelines for Evaluating Cost-Sharing Ratios and In-Kind Contributions in University-Industry Collaborations* regarding the eligibility of in-kind contributions.

Name of supporting organization

Fisheries and Oceans

CONTRIBUTIONS FROM SUPPORTING ORGANIZATIONS

	Year 1	Year 2	Year 3	Year 4	Year 5
Cash contributions to direct costs of research (Transfer amounts to page five (5); except those for the Ship Time program.)	0	0	0	0	0
In-kind contributions to direct costs of research					
1) Salaries for scientific and technical staff	24,000	24,000	24,000	0	0
2) Donation of equipment, software	10,000	0	0	0	0
3) Donation of material	0	0	0	0	0
4) Field work logistics	4,000	4,000	4,000	0	0
5) Provision of services	0	0	0	0	0
6)	0	0	0	0	0
Total of in-kind contributions to direct costs of research	38,000	28,000	28,000	0	0
In-kind contributions to indirect costs of research (not leveraged)					
1) Use of organization's facilities	0	0	0	0	0
2) Salaries of managerial and administrative staff	0	0	0	0	0
3)	0	0	0	0	0
Total of all in-kind contributions	38,000	28,000	28,000	0	0
Contribution to postsecondary institution overhead	0	0	0	0	0

Budget Justification:

1) Salaries and Benefits:

a) Students:

PhD: This project will support training of four PhD students and two MSc students. The PhD students and one MSc student will be enrolled in the Environmental and Life Sciences Graduate Program at Trent University and the other MSc student will be enrolled in the Biology Department at the University of Manitoba. We anticipate that we will be able to recruit one PhD student with external funding (e.g. NSERC, OGS) to the project. The six students will conduct thesis research on:

i) PhD: Effects of nAg on microbial communities (Supervisor: Xenopoulos)
ii) PhD: Effects of nAg on algal and zooplankton communities (Supervisor: Frost)
iii) PhD: Biomarkers of stress in fish exposed to nAg (Supervisor: Metcalfe)
iv) PhD: Speciation and fate of nAg (Supervisor: Hintelmann).
v) MSc (Jillian Fisher): Methods for the analysis of nAg in lake water (Supervisors: Metcalfe and Hintelmann).
v) MSc: Changes in bioenergetics in fish exposed to nAg (Supervisors: Paterson, Rennie).

One student (Fisher) will begin her studies in May, 2011. However, all other students will not start there studies until May, 2012 and it is anticipated, that the PhD students will not complete their studies until April, 2015. Therefore, we anticipate that we will apply to NSERC to extend graduate support for these PhD students for an additional 8 months after the close of the project (i.e. from September, 2014 to April, 2015). The students will spend a large proportion of the field season at ELA, and will return to Trent or Manitoba for course work, analysis and data manipulation.

- Year 1: $20,000 x 3 PhD = $60,000; $17,000 x 1 MSc
- Year 2: $20,000 x 3 PhD = $60,000; $17,000 x 1 MSc
- Year 3: $20,000 x 3 PhD = $60,000

Undergraduate: Salaries are required to hire two undergraduate students for 4 months in the summer of Year 3 to work primarily at ELA. The salary is calculated as $2,125 per month x 4 months x 2 students = $17,000 in Year 3.

b) Postdoctoral fellows:

Salaries are required for two PDFs, tentatively identified as Dr. Ehsanul Hoque (Supervisors: Metcalfe and Hintelmann) and Dr. Clayton Williams (Supervisors: Xenopoulos and Frost). For Dr. Williams, support is required for Years 1 and 2 and 3 of the study (36 month contract) for 100% of his annual salary ($40,000). He will conduct studies on then nutrient dynamics within mesocosms and the dosed lake, and he will divide his time between Trent and ELA. For Dr. Hoque, support will be needed for Years 1 and Year 2 (18 month contract) for 50% of his salary ($20,000). He will conduct research on the fate and distribution nAg in the mesocosms and in the lake; primarily as analytical work at the Water Quality Centre at Trent University. Dr. Hoque will also provide training on AF4-ICP-MS to Jillian Fisher (MSc) and a PhD student. The remainder of Dr. Hoque's salary in Year 1 will come from the NSERC-NRC-BDC grant to C. Metcalfe. The annual contribution from this project to PDF salaries will be $60,000 in Years 1 and 2, and $40,000 in Year 3.

c) Professional Assistants:

Professional assistants will be required to aid in the preparation and analysis of the large number of samples to be collected from the field sites. Samples of water, sediments, plankton, benthos, and fish tissues, will be shipped from ELA for processing by the research assistants. Because PhD students will not be recruited until May, 2011, this work by assistants will be especially necessary in the first year of the study because of the need to develop and validate the analytical methods and to conduct both base line monitoring and mesocosm work at ELA. In Years 2 and 3, professional assistants will be required to process the large number of samples from the whole lake addition study. Costs for the salary and benefits for a research assistant at Trent in Years 1, 2 and 3 were calculated as 35% of the time of the assistant x $40k x 2 = $28,000. Aging of fish collected at ELA will be done under contract at $2,000 per annum in years 1, 2 and 3.

2) Equipment or facility:

b) Operation and maintenance costs:

Costs are to maintain and operate analytical instruments at Trent University that are outside of the Water Quality Centre, including the MIMS, HPLC, C/N analyzer, TOC analyzer, ion chromatograph and fFFF. These costs cover replacement columns, channel membranes, analytical standards and certified reference materials, and maintenance (e.g. pump replacement). Costs are estimated at $9,000 in Years 2 and 3, and a larger amount of $11,000 in Year 1 to reflect startup costs.

c) User fees:

i) Water Quality Centre:

This analytical facility at Trent University is a shared use facility and researchers are charged for use of the analytical instruments in the Centre on an hourly basis, which covers the costs of operations, maintenance and training. The hourly costs for instruments to be used in this study for ICP analysis of Ag are: i) ICP-MS: $30 per h x 200 to 220 h; ii) MC-ICP-MS: $35 per h x 60 to 65 h. The hourly costs for analysis of isotopes of C and N using the stable isotope MS are $20 per h x 100 to 120 h.

We estimate that the user fees will be:

- Year 1: $ 6,000
- Year 2: $12,000
- Year 3: $12,000

ii) Aquatic Chemistry Lab:

The analytical lab at ELA conducts analysis of various water quality parameters on a fee for service basis, including nitrate/nitrite, ammonia, TDN, SRP, TDP, conductivity, in situ pH, in situ DIC and dissolved oxygen. Costs per sample vary from approximately $4 to $9 per sample, depending on the parameter.

The analytical lab at the Freshwater Institute (FWI) in Winnipeg conducts analysis of other water quality parameters, including suspended carbon and nitrogen, suspended phosphorus, DIC and DOC, chlorophyll and chlorophyll a

and TSS. The costs per sample vary from approximately $7 to $12 per sample, depending on the parameter. Because the water samples can be shipped in a timely manner to the FWI using the regular shuttle service, we will also use these analytical services.
Based upon analytical costs of approximately $130 per sample, we estimate that total user fees for these analyses will be:

- Year 1: 55 samples - $ 7,000
- Year 2: 110 samples - $14,000
- Year 3: 110 samples - $14,000

3) Materials and Supplies:

- Year 1: $64,000 ($19k for mesocosms + $20k for nAg + $25k for consumables)
- Year 2: $45,000 ($20k for nAg + $25k for consumables)
- Year 3: $45,000 ($20k for nAg + $25k for consumables)

i) Nanosilver:
Several assumptions must be made to estimate the costs for purchasing the nAg that is required for the whole lake addition study in Years 2 and 3. We assume that we will make additions to the lake on 400 days over the ice free periods in Years 2 and 3 at a daily load of 2 grams = 2 grams x 400 days = 800 grams. Mercator currently sells a 2 g stable dispersion of the PVP capped nAg for a price of approximately Cdn $150. Therefore, (800 g / 2) * $150 = $60,000. Spread over 3 years, this corresponds to $20,000 per year. The amounts of nAg required for additions to mesocosms in years 1, 2 and 3 are estimated to be <80 grams annually, for an estimated annual cost of $5,000.
ii) Consumables:
Radioisotopes of P and H, stable isotopes of C and N, sample bottles, containers and vials, reagents and filters. Annual costs of these consumables are estimated at $22,000 per year. Pit tags for marked recapture estimates of fish population sizes are valued at an additional $5,000 per year.
However, in Year 1, an additional $19,000 will be required to purchase the fitted plastic enclosures for the mesocosms. Note that the hoops and other hardware for the mesocosms are being provided by DFO as an in-kind contribution.

4) Travel:

a) Conferences:
Years 1, 2 and 3:
Attendance at one international conference per year by HQP to present the study results - $1800 per person x 4 HQP per year = $7,200
b) Field work:
i) Per diems at ELA of $65 per day x 500 people days (i.e. 3 PhD students, 2 summer students, 1 PDF, co-applicants) = $32,500 per year. Note that the per diem covers accommodation, meals, shuttle to Winnipeg, and use of field vehicles, ATVs and boats.

ii) Flights (round trip) to Winnipeg at $650 per person x 10 flights = $6,500 per year. Note that there is a weekly shuttle from Winnipeg to ELA during the field season.
iii) Vehicle rental and gas charges for vehicle to shuttle research equipment between Trent and ELA (e.g. MIMS) at the beginning and end of each field season = $4,200.
c) Collaboration: Travel and accommodation of collaborators and PAC to participate in annual 2 day project reporting/planning meetings at Trent University. Costs are estimated at $5,000 per year in Years 2 and 3.

5) Dissemination costs:

a) Publications:
Page charges for publication of 4-5 manuscripts in peer-reviewed journals in each of Years 2 and 3 of the study will be $3,000.

Contributions from Supporting Organization:

A) Environment Canada (Science and Risk Assessment, Science and Technologies Branch):

I) Cash Contributions

The Science and Technologies Branch of Environment Canada will contribute cash to the project in amounts of: Year 1: $60,000, Year 2: $60,000. In addition, Environment Canada contributed $80,000 in 2010-11 for research and a workshop to strengthen the proposal to the NSERC Strategic Grants Program in the 2011 competition. The focus of the workshop scheduled for June 10, 2011 is to plan the proposed whole lake addition study at ELA. The cash support provided by Environment Canada in Years 1 and 2 of the proposed study will be directed towards expenditures for research.

II) In-Kind Contributions to Direct Costs of Research:

1) Salaries for scientific and technical staff:

Environment Canada will contribute the salary of Y. Sultan for his work as a member of the Project Advisory Committee. This in-kind contribution is valued at $1,000 per annum for 2 days of his salary.

B) Fisheries and Oceans Canada (Environmental Science Division):

II) In-Kind Contributions to Direct Costs of Research:

1) Salaries for Scientific Staff:

a) Technical and professional assistants: Fisheries and Oceans Canada will contribute some of the salaries of the three researchers, Michael Paterson, Michael Rennie and Scott Higgins; all of whom will be collaborators on the project. This contribution is valued at $24,000 per year, which was calculated as the salary for the 3 researchers for 20 days per year, respectively. Dr. Paterson and Dr. Rennie will conduct field studies on fish at the ELA site and Dr. Higgins will contribute to studies of algae and epiphyton. All three researchers will analyze samples and data at the Freshwater Institute in Winnipeg, and will participate in organizational meetings with the study team.

2) Donation of Equipment:

a) Fisheries and Oceans will contribute to the project by donating the equipment for construction of the mesocosms, which is valued at $10,000 in Year 1 of the study.

3) Donation towards Field Work Logistics:

Fisheries and Oceans will contribute to the per diem costs for housing and transportation of the three collaborators, M. Paterson, M. Rennie and S. Higgins. This is valued at $4,000 per annum: Housing - $65 per day x 3 persons x 20.5 days = $4,000.

Introduction:

This proposed project addresses the target area of **Environmental Science and Technologies** within the NSERC Strategic Grants program, and specifically, the research topic of **Enhancing Aquatic Ecosystem Services**. We define aquatic ecosystem services to include nutrient cycling, food web processes that maintain productivity, maintenance of water quality, recreational services and fisheries. The proposed work focuses on the emerging issue of the environmental impacts of engineered nanomaterials. The increased used of nanomaterials (NMs) in consumer products and in industrial processes may lead to releases of nanoparticles into the environment, and particularly into surface waters. Currently, we have little understanding of the ways in which nanoparticles may impact aquatic ecosystems and we have few methods for detecting these responses before there is permanent damage. This project addresses the NSERC Strategic research priorities to develop "*methodologies or models that better explore and describe how anthropogenic activities affect aquatic ecosystems and the services that they provide*". This research will address the issue of the "*cumulative effects of various stressors, and of the interactions among these stressors, on the ability of aquatic ecosystems to provide services*". The project aims to characterize the techniques, procedures and policies that must be developed to assess changes to ecosystem function associated with the release of nanoparticles into the environment. Part of the project will involve additions of nanoform silver (nAg) to a lake in the Experimental Lakes Area (ELA) of northern Ontario.

We submitted a similar proposal in the 2010 NSERC Strategic Grants competition, and although it was not successful, the positive comments from the reviewers encouraged the project team to reapply. Over the intervening year between proposals, we made significant advances that address the original concerns of the reviewers. This work has been funded by $60,000 in financial support from Environment Canada that was targeted to address these concerns. We have developed analytical techniques for tracking the distribution of the model NM, nano-form silver (nAg) within sediments, water and biota. We have conducted bench-scale tests with sediment/water columns that indicate that nAg does not completely partition into sediment and a high proportion remains suspended in the water column. Through financial support of $20,000 from Environment Canada, we have scheduled a workshop for June 10, 2011 in Montreal to plan the details of a whole lake addition study with nAg. Invitees to the workshop include Canadian researchers working in this field and five invited international experts. A stable source of matching funds over 2012-14 ($120,000) has been secured from our supporting organization, Environment Canada. Collaborating researchers from Environment Canada have received independent funding for work on isotope ratio measurements of silver that will support this proposal.

Revenues from nanotechnology are expected to increase into the trillion dollar range, yet we have not developed appropriate policies and procedures for assessing the risks associated with the release of these materials into the environment. We do not know what changes to ecosystem services, including key ecological function and processes, to expect in surface waters impacted by NM and what methods we can use to monitor for these responses. In this proposed project, an interdisciplinary team will conduct the first whole ecosystem test of the effects of a model NM, nAg. This study will be conducted by a team of 4 co-applicants from Trent University, Peterborough, ON and a co-applicant from Fisheries and Oceans Canada, working with 2 collaborating scientists from Environment Canada and 2 collaborating scientists from Fisheries and Oceans. A Project Advisory Committee (PAC) consisting of international experts and a representative from Environment Canada will provide advice on project directions. It is anticipated that this project will attract international attention and researchers from other agencies and academic institutions will be encouraged to participate and add their expertise.

SECTION 1 - Research:

We propose to quantify the responses of a lake ecosystem after point source addition of a model NM, nAg. With this project, we plan to: i) assess the fate of nAg released into the lake, ii) evaluate the impacts of nAg on lake foodwebs, and iii) determine how key ecological processes respond to nAg.

The **specific objectives** of the proposed project are to:

- **a)** Characterize the chemical transformations and spatial movements of nAg within a natural lake ecosystem.
- **b)** Examine the responses of lower trophic levels (e.g., primary producers and microbial communities) in littoral and pelagic ecosystems to the addition of nAg and the impacts on ecological function
- **c)** Evaluate the ability of Ag to move through foodwebs and affect upper trophic levels, including fish populations

1.1 Background:

Nanoparticles with at least one dimension below 100 nm are similar in size to biological macromolecules such as proteins, DNA and phospholipids, so it is possible that these materials can cause disruptions at the molecular and cellular level [1]. NMs dispersed within the aqueous phase are more mobile, whereas NMs that are not dispersed and aggregate into larger particles are less mobile. Typically, NMs do not disperse well in water unless they are manufactured with functional groups bound to their surface (i.e. “capped” nanoparticles). There is little information available concerning the degradation and microbial transformation of NMs. “Aging”, or transformation of the surface capping agents caused by chemical or biological reactions may result in altered environmental behaviour of NMs [2]. The distribution of NM in porous media, such as soils and sediments is also governed by the size, shape and charge of the particles. Data from laboratory experiments indicate that NM may be relatively immobile in soils and sediments [3], or conversely, relatively mobile [4], depending on the characteristics of the NM.

Organisms occupying particular loci in trophic webs may be at increased risk of the toxic effects of NM. In toxicity tests, bacteria, plants and invertebrates are typically the most sensitive organisms to the biological effects of NMs [5]. There is evidence that a range of NMs exhibit anti-bacterial activity [6-8]. Overall, it is logical to assume that biological effects will be observed in the lower levels of the food web, including the primary consumers (zooplankton and benthic invertebrates), the algae and aquatic plants that comprise the primary producers, and the microbial communities intimately involved in decomposition and nutrient cycling. However, given the diversity of species within these lower trophic levels, it is unclear whether ecosystem resilience (e.g., shifts in community structure to more tolerant species) will mitigate the impacts of NM exposure [9]. Biological effects among aquatic vertebrates (fish, amphibians) may occur by direct toxic effects; especially, if there is biomagnification up through the food web [10]. Alternatively, there could be indirect effects on fish populations through reduced lake productivity, although previous food-web manipulations at ELA have shown that primary producers and consumers can be resilient to changes in food-web dynamics [11]. Thus, the system resilience imparted by biodiversity declines with increasing trophic level [12, 13]. As such, primary production and decomposition rates mediated by the lower trophic levels may not be altered appreciably after exposure to NMs, but the functions performed by higher trophic levels may disappear completely.

In this proposal we will focus on the fate and effects of a model metal-based nanomaterial. Capped nAg is currently used in hundreds of products as an odour-destroying, infection-fighting additive, including use in air fresheners, surgical bandages, food wrappers, baby bottles, cutting boards, slippers, socks, underwear, shoe-liners and even in washing machines. There is potential for nAg to be released from these products into the aquatic environment.

For instance, a recent study showed that nAg is washed into domestic wastewater by the laundering of socks and then enter the municipal sewage stream [14-16]. According to modeled environmental concentrations, nAg has the potential to enter surface waters through discharges from wastewater treatment plants at part per billion (ppb) concentrations that exceed the risk quotient for toxicity [17].

Our recent studies have shown that capped nAg at **low ppb levels** inhibits the growth of natural communities of bacteria [18] and algae (MS in prep) in water collected from lakes, rivers and ponds, although the microbial communities appear to recover somewhat after initial exposure (Fig. 1). On the other hand, nAg appears to totally disrupt sestonic algae (Fig.1). It is not known how microbial or algal communities will respond to repeated or continuous dosing with nAg. Given the extremely small size and reactivity of nAg, this NM may be more toxic than other forms of silver, such as silver ions [19]. However, it is also possible that the toxicity of nAg is related to the release of toxic silver cations from the NM surface into the aqueous matrix [6,20]. In the aquatic environment, the fate and toxicity of NM appears to be tightly linked to physico-chemical parameters that are important in lake ecosystems, including the concentrations and types of dissolved organic matter, pH and ionic strength, the redox environment and the presence of inorganic ligands [22-24]. A recent study showed that formation of nAg may occur under environmentally relevant conditions by reduction of silver ion (Ag^{+}) in the presence of humic acids [25], which introduces the possibility of *in situ* formation of nAg.

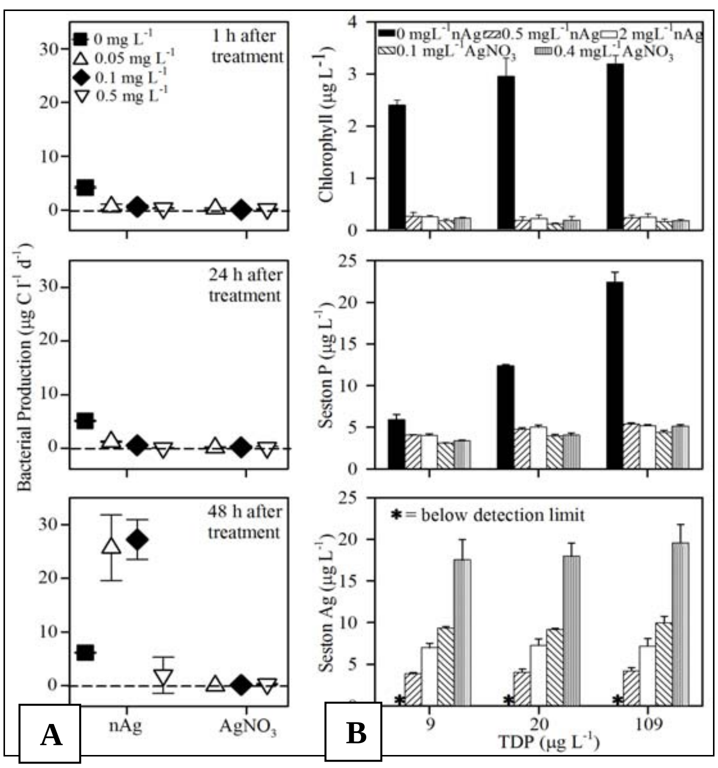


Fig. 1: Effects of exposure to different nominal concentrations of nAg and $AgNO_3$ (positive control) on: Panel A: production of natural aquatic bacterial communities at 0, 24 and 48 h after exposure, and Panel B: Algal growth bioassays for levels of chlorophyll and particulate phosphorus in natural lake seston. The lower portion of panel B shows the concentrations of total Ag levels accumulated in the seston in treatments with different total dissolved phosphorus levels in the bioassay bags. The data summarized in panel A is from Das et al. [20]. The data in summarized in panel B is from an MS in preparation.

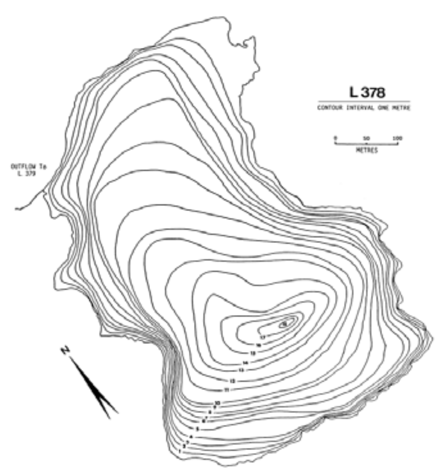
Although there are an increasing number of studies on the effects of NMs on aquatic organisms, *all* of these studies have been done over short temporal scales (i.e. < 1 month) under controlled laboratory conditions, and usually with only one test species. Many elements of ecosystems cannot be studied at small scales, or even in large-sized enclosures [26, 27].

There are likely to be many interactions between NMs and chemical and physical factors that are not represented by bench scale experiments or in mesocosms, including lake mixing, gas exchange, sediment-water exchanges, and species interactions (e.g., predation and herbivory). For example, NMs could quickly sediment out of the water column during calm conditions but may remain suspended by windy conditions and vigorous water mixing. The physiological and ecological effects of NMs on organisms may also be dictated by environmental conditions such as water temperature, nutrient condition and light regimes. Given the number and complexity of potential interactions, we currently cannot identify the most sensitive elements of lake ecosystems that are likely to be affected when NM are released into surface waters.

<u>1.2 Research Approach</u>

We propose to evaluate the fate and effects of NM in aquatic ecosystems through mesocosm studies and a whole lake addition study conducted at the Experimental Lakes Area (ELA) in northern Ontario. Only these types of field manipulations can provide information on the complex interactions that may occur as a result of inputs of NM into natural aquatic ecosystems. As a model NM, we will use PVP-capped (10-20 nm) nAg material with a particle size distribution of 10-20 nm. This NM is available in large quantities from Mercator Inc. (U.K.) and has been selected by an international working group of the OECD as a reference material for inter-laboratory testing.

During Year 1, we will complete baseline monitoring of the experimental lake and will conduct a mesocosm study. The baseline monitoring will provide important data on all response variables prior to addition of the NM in Year 2. The mesocosm experiment will allow us to test how nAg affects lake food webs at a smaller scale and to evaluate the distribution of Ag under field conditions. This work will show whether the nAg remains suspended in the water column or rapidly partition into the sediments, which will have important implications for implementation of the whole lake experiment. We will also use the mesocosms to examine responses of bacterial, algal and zooplankton communities to different doses of nAg over weeks to months of continuous exposure. In this experiment, we will study 6 duplicate mesocosms (2 control, 2 with a low dose, and 2 with a high dose) in each of L224 (low DOC) and L239 (high DOC) in the ELA from early June to mid-August. We have tentatively selected L378 at the Experimental Lakes Area (Fig. 2) for the addition of nAg during years 2 and 3 of the project, based on a number of criteria:



SELECTION CRITERIA
i) Ease of access for lake dosing,
ii) Small size to minimize the amount of nAg material required for lake dosing
iii) Sufficient depth to allow stratification and transient hypoxia in the hypolimnion
iv) pH <7; DOC at 3-9 ppm; phosphorus high enough for sufficient productivity
v) Presence of forage and/or predatory fish species

Figure 2: Bathymetric map of L378, tentatively identified for whole lake additions of nAg.

Based upon consultations with research personnel with Fisheries and Oceans Canada (Freshwater Institute, Winnipeg, MB), we identified L378 as a candidate lake for this study, but this selection is subject to change. L378 is deep enough (max depth = 18 m) to stratify and is of sufficient size (area = 24 hectares; volume = 1.8 million m^3) to have a diverse zooplankton community [28] and an extensive fish community that includes shiner species, pearl dace, burbot, white sucker, yellow perch and lake trout [29]. The water quality characteristics meet the criteria identified above [28, 29]. In addition, we have initiated the process of obtaining approval for a whole lake addition study at this location with the ELA Management Board.

During Year 2 of this study, the capped nAg will be added to L378 (or another suitable experimental lake) from ice-out in May to ice over in November. Unlike other whole lake addition studies at ELA where the stressor chemical was added evenly over the surface of the lake [30, 31], we will add nAg using a metering pump for a daily loading of 2 grams (i.e., 100 L/d of solution containing 20 mg/L nAg) through a diffuser at a single point on the lakeshore to simulate point source inputs from a municipal wastewater treatment plant (Fig. 3A). These nAg additions will simulate daily loadings in wastewater containing nAg at a concentration of 2 µg/L discharged at a daily rate of 1 million m^3; which would be typical of a treatment plant in Canada serving a municipality with >0.5 million people. This 2 ppb concentration has been chosen because our preliminary analysis of samples of municipal wastewater indicates that nAg (~10-40 nm in size) is present at concentrations in the range of 1-2 µg/L. This approach will simulate the types of exposures likely to occur in surface waters impacted by discharges of wastewater and will also allow us to study responses over an exposure gradient radiating out from the point source (Fig. 3B). We will repeat these additions in Year 3, given the probability that effects will be amplified by multiple years of addition.

We will institute a two tiered sampling regime to carefully monitor a range of lake responses to nAg over time (Fig. 3C) and distance (Fig. 3D). This sampling regime recognizes the need for intensive monitoring over time of localized responses within the littoral zone close to the point source (Fig. 3C), and less intensive monitoring of responses in the lake ecosystem that vary with distance from the source (Fig. 3D). . Sampling in the lake across this distance gradient from the point source will thus occur twice during the summer of Year 1 prior to addition of nAg and twice during each summer of Years 2 and 3.

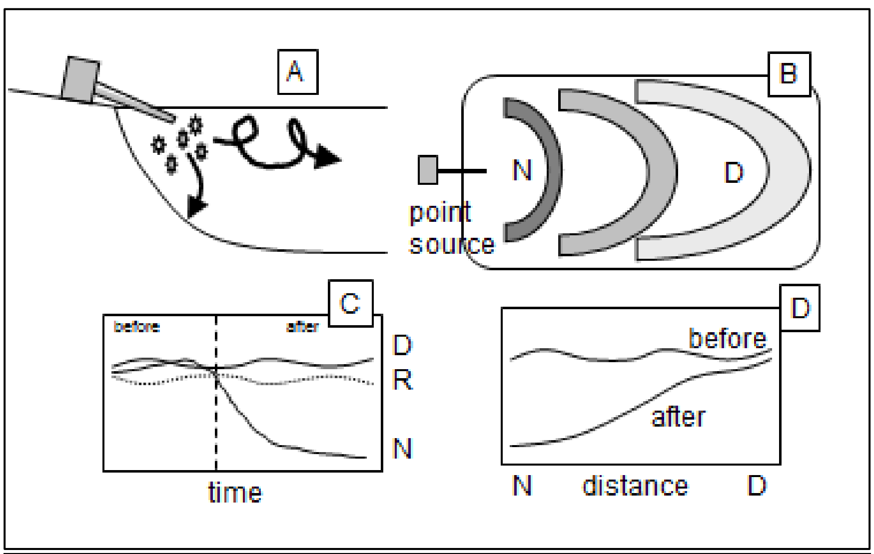


Fig. 3: Schematic of the proposed point source addition of nAg to the study lake (Panel A), expected spatial distribution of effects relative to the point source (Panel B), expected relationship between effects and time (Panel C) and expected relationship between effects and distance (Panel D). N = Near; D = Distant; R = Reference data from other ELA lakes.

1.3 Fate and speciation of nAg:

The residence time of nAg in the water column and its movement through the lake ecosystems will be a key determinant of the location and magnitude of its effects on lake food webs. For example, the immediate fate of added nAg may be governed by its rate of agglomeration or association with particles, which could lead to relatively rapid sedimentation in the littoral zone and greater toxicity on local benthic food webs (Fig. 3A). We will address the distribution of nAg in the water column and in association with particles, and will also determine the degree and rate to which added nAg is transported to sediments, or recycled to the water during re-mineralization of organic material.

Samples of water will be collected at different distances and depths (i.e. epilimnion, hypolimnion, sediment-water interface) from the point source. Particulate samples will be collected from littoral zones, the epilimnion, and with sediment traps deployed in the hypolimnion using methods that we have previously employed at ELA [32]. Different components of the food web will be sampled to determine the concentrations of Ag accumulated in biota. There are many challenges associated with analyzing nanoparticles in aqueous matrices at environmentally relevant concentrations [33]. However, we have developed methods for analysis of total Ag in sediments and biota using acid digestion and ICP-MS or ICP-OES, and analysis of nAg in water using asymmetric flow Field-Flow Fractionation (AF4) coupled with ICP-MS [34]. We are also using Amicon® centrifugal filtration units to isolate freely dissolved Ag for analysis. If pre-concentration of nAg is required, ultrafiltration [20], ultracentrifugation [35], or cloud point extraction [36] show potential for concentrating the nanoparticles prior to analysis. Analysis by ICP-MS will be conducted at the Water Quality Centre at Trent University. Methods for extraction, pre-concentration and storage of nAg from water samples without altering their size distribution will be optimized during Year 1.

In the whole lake and in mesocosms, we will monitor for concentrations of different organic and inorganic ligands for Ag, including the presence of sulfides [23]. We will determine the concentrations of free sulfide in oxic and anoxic water using a recently developed and optimized methylene blue method [23]. In combination with the general water chemistry (particularly chloride and dissolved organic matter), provided by the chemistry laboratory at ELA, this will allow us to model the speciation of dissolved Ag ions using the MINTEQ and/or WHAM models.

1.4 Effects on the lake ecosystem

1.4.1 Water quality:

Every 7-10 days during the ice-free season and every month during the winter, we will collect integrated water samples from the littoral zone near the addition site and from the epilimnion and hypolimnion in the centre of the lake. We will analyse these samples of dissolved nutrients and other water chemistry parameters, including pH, alkalinity, hardness, conductivity, dissolved oxygen, nitrate, nitrite, ammonia, TDN, TDP and DOC. Much of these analyses will be conducted at the Chemistry Laboratory at ELA or at the Freshwater Institute in Winnipeg. These data will be compared with data simultaneously be collected from nearby reference lakes.

1.4.2 Lake productivity, lower trophic levels and benthos:

To assess the responses of lower trophic levels to nAg additions, we will sample producers and consumers in the littoral and pelagic zones near and distant to the discharge point. In the littoral zone, periphyton will be collected with a syringe scrubber [37] from rock surfaces from a fixed depth. We will sample once per week to estimate algal abundance (chlorophyll and algal counts), species composition, stoichiometry (C, N, and P content), bacterial biomass, microinvertebrates consumers. In addition, we will assess the biomass and species composition of colonizing invertebrates using rock bags deployed each summer.

Depending upon the predominant substrate in littoral zone near the addition site, we will also assess responses of aquatic macrophytes (biomass and species composition) and sediment microbial communities. In the pelagic zone, we will sample phytoplankton using whole water passed through a 85 μm net and filtered onto GF/F filters. On these samples, we will analyse chlorophyll and particulate C, N, and P. Samples will be preserved for Lugol's for microscopic biomass and taxonomy composition analysis. Additional filters will be saved for pigments that will be extracted and processed by HPLC [38]. In addition, we will collect the <1.0 μm fraction on GF/F as an index of bacterial C, N and P concentrations. Additional water samples will be preserved for bacterioplankton and viruses.

Changes in viral and bacterioplankton abundance, bacterial membrane integrity and bacterial activity status (dead, dormant, active) will be assessed using flow cytometry [39]. We will follow standard limnological methods for whole-lake experiments at ELA [40]. The rotifer and zooplankton communities will also be evaluated by sampling on a weekly basis using Schindler-Patalas traps. These zooplankton samples will be taken both during day and night in the epilimnion using a 80-μm mesh. Zooplankton samples will be split into three parts in the laboratory, with one part preserved for microscopic analysis, one part saved for C, N analysis and one part used for zooplankton P analysis. Additional zooplankton tows using conical nets using a smaller mesh (30 μm) will be completed during day sampling to more effectively characterize rotifer abundance and biomass [41].

1.4.3 Effects on nutrient cycling and ecosystem function

<u>Carbon cycling</u>. We will investigate whether nAg affects carbon cycling using a number of methods, including injections of ^{13}C in short-term experiments with small bags incubated *in situ* and in benthic/periphyton chambers, using methods already established at ELA. Bacterial production will be measured as protein synthesis rate (^{3}H-leucine incorporation) using methods we have previously employed [42]. Rates of gross primary production and microbial community (autotrophs and heterotrophs) respiration will be determined from *in situ* changes of dissolved oxygen in sealed chambers (bottles) over a 24-hour period [43]. Microbial extra-cellular-enzymatic activity (alkaline phosphatase, aminopeptidase and ß-glucosidase) will also be assessed [44]. Leaf-litter and woody decomposition will be measured on pre-weighed leaf packets and wooden sticks (tongue depressors) after a 1-month *in situ* incubation. Rates of microbial respiration will be estimated on the colonized leaf packs and wooden sticks using measurements of oxygen consumption.

<u>Phosphorus and nitrogen cycling.</u> We will determine how N and P cycling respond to nAg in the littoral and pelagic zones. We will use small containers incubated *in situ* containing different water fractions and injected with radiophosphate, ^{33}P [45]. Nitrogen fluxes will be measured through short-term injections in bags of ^{15}N, as well as measurements of nitrogen fixation [46]. Water column and benthic denitrification (N_2 fluxes) will be estimated in water overlying lake sediment cores from measurements of N_2:Ar made using membrane inlet mass spectrometry (MIMS) [47].

1.4.4 Impacts upon fish and food webs

Cumulative Effects Assessment techniques [48] will be used to evaluate the biological health of fish in L378 and reference lakes. We will collect and assess fish for age distributions, size-at-age, condition index, gonadosomatic index, hepatosomatic index, indicators of disease (e.g. ecto- and endoparasites, external lesions), gut contents for diet analysis, and tissue contents of lipid, triglycerides and glycogen. The direct impacts on fish exposed to nAg will be evaluated by analyzing various biomarkers of stress. Candidate stress indicators include induction of metallothionein, release of heat shock proteins (e.g. HSP 70), modulation of hepatic glutathione and induction of hepatic cytochrome P450 activity [49]. Changes in population numbers in L378 and reference lakes will be assessed using marked recapture techniques with pit tags.

Twice a year during the whole lake experiment, we will examine changes in feeding relationships in both pelagic and benthic pathways by measuring stable isotopes (^{13}C and ^{15}N) in particulate organic matter, primary producers and consumers, and in fish [50]. Stable isotopes will be analyzed at the Water Quality Centre at Trent University. In addition, we will evaluate whether there is transfer of Ag from benthic invertebrates or zooplankton to benthic or pelagic fish, respectively by analysis of Ag in biological tissues using either ICP-OES or ICP-MS. In the case of species with low population numbers, such as lake trout, non-lethal sampling techniques (e.g. biopsies, whole body ultrasound) will be used to evaluate their condition and concentrations of Ag in tissues.

Through an independent funding source (i.e. CMP Research Fund of Environment Canada), our research Collaborators at Environment Canada and their project team will use multi-collector (MC) ICP-MS [51] to evaluate Ag isotope fractionation by measuring 107/109. Changes in these ratios will provide an isotopic "fingerprint" of biogeochemical processes, including trophic transfer of NM within food webs. MC-ICP-MS analysis will be conducted at the Institute for National Measurement Standards at NRC, Ottawa, or alternatively at the Water Quality Centre at Trent University.

We will also utilize data collected for smaller and more abundant fish species (e.g. shiners, pearl dace, yellow perch) to evaluate whether there have been changes in diet, changes in isotopic rations of carbon and nitrogen, changes in Ag isotopes, that reflect changes in the food webs. These data will be used to study the energy flows that support fish populations using bioenergetics models based on methods that we have used previously [52].

1.3 Project management, the research team and research linkages:

The Principal Investigator (PI), Chris Metcalfe will be responsible for managing the financial and scientific aspects of the research project. On an annual basis, members of the research team will submit reports on research progress, proposed research for the coming year and budget estimates. These reports/proposals will form the basis of the annual reports to be provided to the Strategic Grants program of NSERC. The PI will rely on advice provided from a **Project Advisory Committee** (PAC) to evaluate progress and the strategic value of proposed research. As shown in Table 1, the PAC will include a representative from the regulatory division of Environment Canada (Dr. Y. Sultan), as well as international researchers with expertise relevant to the proposed project: i) Dr. Michael Pace with expertise in limnology, ecosystem ecology and whole lake experiments, ii) Dr. Steve Diamond, with expertise in the effects of NMs, including nAg on aquatic organisms, iii) Dr. Alistair Boxall, with expertise on the environmental fate and risk assessment of contaminants, including NMs, iv) Dr. Mark Chappell, with expertise in materials science and geochemistry, and v) Dr. Lee Ferguson, with expertise in analytical techniques for NM. The members of the PAC will participate in a workshop in advance of the funding decision, in June, 2011 to provide the study team with advice on the design of the whole lake addition component of this proposed project. The PAC will review all written reports/proposals, and on an annual basis, will meet with the research team to review progress and to share research information. It is anticipate that these researchers will also become involved in this study through independent funding sources.

Table 1 also lists the members of the research team and their roles in carrying out the various components of the project. The research team includes 6 individuals (Frost, Hintelmann, Muir, Paterson, Xenopoulos, Higgins) who have previously been involved in either whole lake manipulations or mesocosm studies at ELA and know the area very well. This is a highly skilled and motivated group of researchers with international research reputations in freshwater ecology, ecotoxicology, water chemistry and fish biology.

All four co-applicants from Trent University have previously received (as PIs) NSERC Strategic Grants. The **Co-applicants** will direct funding from the NSERC Strategic Grants Program to support HQP and to conduct the field and laboratory components of the project. The **Collaborators** will not be directly supported by NSERC funds, but will participate as full members of the research team. Collaborating researchers will serve as co-supervisors or as members of the Supervisory Committees of graduate students working on the project.

Table 1: Affiliations and roles of co-applicants, collaborators and members of the Project Advisory Committee (PAC).

Co-Applicants	**Affiliation**	**Role**
Dr. Chris Metcalfe, Environmental Resource Studies (ERS)	Trent University, Peterborough, ON	Project coordination (PI); Fate and trophodynamics of Ag Biomarkers of stress in fish
Dr. Holger Hintelmann, Dpt. Chemistry and ERS		Fate and speciation of Ag
Dr. Paul Frost, Dpt. Biology		Responses of zooplankton and benthos, limnology
Dr. Marguerite Xenopoulos, Dpt Biology		Primary production Biogeochemistry, limnology
Dr. Michael Paterson	Fisheries & Oceans Canada, Winnipeg, MB	Population level responses in fish; Food web dynamics
Collaborators	**Affiliation**	**Role**
Dr. Derek Muir Dr. Ewa Dabek-Zlotorzynska	Environment Canada, Burlington, ON Environment Canada, Ottawa, ON	Trophodynamics of Ag; Isotopic profiling
Dr. Michael Rennie Dr. Scott Higgins	Fisheries & Oceans, Winnipeg, MB	Bioenergetics responses in fish Responses of algae & epiphyton
PAC	**Affiliation**	**Role**
Dr. Yasir Sultan	Emerging Priorities Division, Environment Canada, Ottawa	Advice on project priorities and approaches International liaison
Dr. Michael Pace	University of Virginia, VA, USA	
Dr. Steven Diamond	US EPA, Duluth, MN, USA	
Dr. Alistair Boxall	University of York-Central Science Laboratory, U.K.	
Dr. Mark Chappell	US Army Corps of Engineers, MS, USA	
Dr. Lee Ferguson	Duke University, NC, USA	

This project will also benefit from close cooperation with other national and international projects on the environmental impacts of NMs. Dr. Nathalie Tufenkji (McGill University) and Dr. Kevin Wilkinson (Université de Montréal) received support in 2010 from the NSERC Strategic Grants program for work on the fate and effects of nanomaterials in the terrestrial environment. Dr. Tufenkji has provided a letter (attached) that confirms that this proposed project is highly complementary and that she looks forward to continuing the collaborative relationship that has developed as a result of support of our previous respective NSERC Strategic Grants (2007-10). Dr. Metcalfe, Dr. Tufenkji and Dr. Wilkinson are also co-applicants on the NRC-NSERC-BDC Nanotechnology Initiative research project on “Technologies and Strategies for the Assessment of Aquatic Toxicity of Manufactured Nanomaterials” (2009-2011) with Greg Goss as PI, which provides linkages to a network within Canada for research on the fate and effects of NMs.

Members of the PAC from the USA, Dr. Diamond, Dr. Ferguson and Dr. Chappell of the PAC will provide an important liaison role for work being conducted on the ecological impacts of NMs by the US EPA, Duke University and the US Army Corps of Engineers, respectively. We have a collaborative linkage with work on NMs supported by the EU being conducted by Dr. Alistair Boxall at the University of York-Central Services Laboratory in the U.K. We also have linkages to the work on the environmental fate of NMs being conducted at the Swiss Federal Institute for Water Research (EAWAG); especially, with Dr. Michael Burkhardt.

SECTION 2 - Training Plan:

This project will contribute to the training of two MSc students, four PhD students, two PDFs and 2 summer students. We anticipate that we will be able to recruit one of these graduate students with external funding (e.g. NSERC, OGS). Graduate students at Trent University supported by the project will be enrolled in the Environmental and Life Sciences Graduate Program, and one MSc student co-supervised by M. Paterson and M. Rennie will be enrolled in the graduate program of the Department of Biology at the University of Manitoba (see Budget Justification section for research roles). We have already recruited one MSc student, Ms. Jillian Fisher to start her studies in May, 2011. Once there is a funding announcement from NSERC in October, 2011, we will immediately recruit graduate students to begin their studies in May, 2012. Given the funding horizon for this project (i.e. 2011-2014), we anticipate that it will be necessary to apply to NSERC for an extension of support for the PhD students to April, 2015. Two PDFs, tentatively identified as Dr. E. Hoque and Dr. C. Williams will also be part of the study team.

Our HQP will be trained in an interdisciplinary environment. Two PIs on this proposal (Frost and Xenopoulos) benefited tremendously by conducting their PhD dissertation work at ELA, first through access to the excellent field facilities, and secondly by experiencing the intellectual atmosphere among the other HQP, visiting researchers and resident scientists at the site. This opportunity will be passed on to a new generation of HQP. Each student will be in charge of a specific aspect of the proposal (see Budget Justification section). However, interactions between the students, PIs and across disciplines will be highly encouraged. These interactions across disciplines are necessary to fully develop the linkages between the biological, chemical and physical effects of nAg in lakes. As well, our government collaborators will participate in the training of HQPs as committee members, examiners, and co-supervisors. The PDFs will be in charge of the more complex and integrative aspects of this proposal (e.g., food web linkages) and will be expected to interact and participate in mentoring the more junior researchers.

We are excited at the training opportunities for upper year undergraduate summer students in Year 3 of this project, when we expect to observe effects in the lake ecosystem and there will be the most intensive period of sampling.

We anticipate that we will have success in attracting undergraduate students with NSERC research awards to this project. This project represents a unique training opportunity for HQP in an emerging area of environmental science. In addition, the exposure of HQP to concepts related to ecological risk assessments and the regulatory challenges of our government partners will provide them with a balanced view of this emerging issue.

SECTION 3 - Interactions with supporting organizations.

As indicated by the letters of support from Environment Canada and Fisheries and Oceans Canada, this proposed project addresses the strategic needs of both of these federal organizations. For this reason, these agencies will be directly linked to the project through the participation as members of the research team (i.e. Muir and Dabek-Zlotorzynska with EC; Paterson, Rennie and Higgins with DFO), or as members of the PAC (Sultan with EC), thus ensuring that there will be continuous sharing of information with the supporting organizations. Fisheries and Oceans Canada will invest significantly in the project through the in-kind contribution of the time of their research staff, field equipment and facilities. Environment Canada has already invested and will continue to invest in the project through both in-kind and cash contributions. The Ontario Ministry of the Environment (OMOE) has also provided a letter of support for this project (attached). Although this provincial agency cannot make a cash or in-kind commitment at this time, the OMOE is keenly interested in this project and we intend to consult regularly with this agency and include their research and regulatory personnel in meetings and workshops. Health Canada also prepared a letter of support for this project, which unfortunately was not received in time for this submission. Health Canada and Environment Canada are jointly responsible for developing regulatory policy and procedures for nanomaterials, and are interacting with the OECD to coordinate Canada's approach with other jurisdictions.

SECTION 4 – Benefits to Canada and the Supporting Organizations:

It is anticipated that the development of NM products and technologies in Canada and worldwide will grow exponentially over the next decade. Environment Canada and Health Canada recently informed the private sector that companies must seek approval of all new NMs introduced into the Canadian market under the Canadian Environmental Protection Act (CEPA). However, there are currently no specific regulatory guidelines for assessing and managing the risks of NMs to the environment. Both Health Canada and Environment Canada are participating in the work of the OECD Working Party on Manufactured Nanomaterials, which is coordinating international efforts to develop procedures for assessing ecological risks. This whole lake addition study will make a unique contribution to the effort to understand the risks associated with the release of NM into the environment, and will also contribute to the development of tests and procedures that can be used to assess the effects of NMs on ecosystem function.

There are few places in the world where one can conduct a whole lake experiment. ELA provides a unique opportunity to test for the effects of NMs at the whole ecosystem scale. Past whole lake addition and mesocosm studies conducted at the ELA (e.g., eutrophication, acidification, mercury, flooding, endocrine disruptors), generated data that resulted in the development of regulatory policies, and also contributed to a better understanding of human effects on lake ecosystems. The results generated from this proposed research will benefit not only to Canadians but will make an enormous contribution the international scientific and regulatory community. This will place Canadian researchers and Canada at the forefront of research on the environmental impacts of nanomaterials.

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RELATIONSHIPS TO OTHER RESEARCH SUPPORT:

1) Environment Canada (2010-13)

Title: Effects of nanosilver on a lake ecosystem.

Applicant: Metcalfe

Year 1: $80,000 (Environment Canada)

Year 2: $60,000 (Environment Canada)

Year 3: $60,000 (Environment Canada)

The project on "Effects of nanosilver on a lake ecosystem" is a priority for the New Substances Assessment Branch of Environment Canada. In order to address the concerns of the reviewers of the proposal submitted to the NSERC Strategic Grant program in 2010, Environment Canada provided $60,000 in 2010-11 to support research on the development of analytical methods and bench-scale testing of the fate of nAg. A further $20,000 was provided in 2010-11 to support the travel costs of international experts that are invited to a workshop on June 10, 2011 to plan a whole lake addition study. Environment Canada has also pledged cash support in the amount of $60,000 per annum as matching funds for each of the first 2 years of a follow up study, regardless of whether the NSERC Strategic proposal is successful. If the Strategic proposal is successful, the cash contribution from Environment Canada will be directed towards hiring Postdoctoral Fellows to assist with the whole lake addition study.

2) NSERC Strategic (STPGP) project (2007-2010):

Title: Fate and effects of nanomaterials in the aquatic environment.

Co-applicants: Metcalfe (PI), Xenopoulos and 5 other researchers at the University of Alberta, University of Winnipeg and University of Ottawa.

Year 1: $271,000 (NSERC), $60,000 (Environment Canada)

Year 2: $285,000 (NSERC), $60,000 (Environment Canada)

Year 3: $282,000 (NSERC), $60,000 (Environment Canada)

With permission from NSERC, this project was extended to March 31, 2011. Support from this project has helped Dr. Metcalfe and Dr. Xenopoulos to lay the groundwork for the investigation on the fate and effects of nanosilver in a lake ecosystem. Through support from this project, Xenopoulos and Metcalfe have conducted laboratory tests to determine the toxicity of nAg and $nTiO_2$ to amphibians, fish, cladocerans, algae and mixed bacterial populations, and have developed analytical methods for these nanomaterials in water (see attached progress report for Year 2). This project also consolidated the collaborative relationship between the co-applicants and the New Substances Division of Environment Canada, which provided additional support for the project in the amount of $60,000 per year.

3) NRC-NSERC-BDC Nanotechnology Initiative project (2009-2011):

Title: Technologies and strategies for assessment of aquatic toxicity of manufactured nanomaterials.

Co-applicants: Goss (PI), Metcalfe and 9 other researchers at the University of Alberta, University of Victoria and University of Montreal and Wilfrid Laurier University.

Year 1: $90,000 (NSERC), $90,000 (NRC), $90,000 (Industry)

Year 2: $90,000 (NSERC), $90,000 (NRC), $90,000 (Industry)

Year 3: $90,000 (NSERC), $90,000 (NRC), $90,000 (Industry)

This project will potentially overlap by 6 months with the proposed NSERC Strategic project. Support from the NRC-NSERC-BDC project in the amount of $50,000 per annum that is directed to Dr. Metcalfe has provided support for the PDFs, Dr. G. Paterson (Year 1) and Dr. E. Hoque (Years 2 and 3), who have been involved in research to develop methods for the analysis of nAg in water using fFFF-ICP-MS techniques. This analytical work will be of direct benefit to the proposed project by providing the methods for determining the fate of nAg in lake ecosystems. This project also lead to the development of a collaborative network of researchers in Canada that can be called upon to provide advice and ideas.

Government of Canada / Gouvernement du Canada
Fisheries and Oceans / Pêches et Océans

March 29, 2011

Natural Sciences and Engineering Research Council of Canada
Strategic Grant Program,
350 Albert St,
Ottawa, ON, K1A 1H5

Dear Sir/Madame,

I am writing this letter of support for the proposal submitted to the NSERC Strategic Grants program by the Principal Investigator, Dr. Chris Metcalfe for the project entitled, "Fate and Effects of Nanosilver in a Lake Ecosystem". The proposed research is to be undertaken in part at the Experimental Lakes Area (ELA), which is a federal government facility jointly operated by Fisheries and Oceans Canada (DFO) and Environment Canada. At the ELA, DFO staff and scientists conduct research on the effects of human activities on food webs and fish habitat, including the effects of toxic chemicals on fish populations. As such, the proposed research to examine the effects of nanosilver on fish and fish habitat is timely and important for the DFO. One of the missions of Fisheries and Oceans Canada is to deliver healthy and productive aquatic ecosystems to Canadians. In support of this mission, DFO has identified priority research areas, 5 of which are applicable to this topic including 1) aquatic animal health, 2) ecosystem assessment and management strategies, 3) habitat and population linkages, 4) emerging and enabling technologies for regulatory and policy responsibilities and, 5) fish population and community productivity.

The ELA facility has been the location for multiple whole-ecosystem manipulations, mesocosm experiments, and long-term studies. It is funded by the Government of Canada, but must partially offset expenses incurred by external users by charging per diem rates. These rates provide access to the experimental lakes, laboratory space, use of boats and equipment, access to long-term chemical, hydrological, and meteorological data, and food and accomodations for visiting researchers.

Fisheries and Oceans Canada works with a joint federal-provincial Management Board to obtain final approval for new projects at ELA. The Management Board will work with Dr. Metcalfe to seek the necessary provincial and federal approvals to allow the project to move forward. We do not expect any problems with this approval process and anticipate that it will be completed within the next 6 months. Recent discussions with the Management Board have indicated preliminary support.

DFO staff will be directly collaborating with the researchers involved in the project to provide the greatest ecosystem context for the results. DFO scientists will be involved in project

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501 University Crescent
Winnipeg, Manitoba
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(204) 983-5000

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planning, selection of lakes to be studied, and the development of the experimental design and methods for assessing the fate and effects of nanosilver in lakes. It is likely that over the project duration, the principle staff involved, on a part-time basis, will include Drs. Michael Paterson, Michael Rennie, Scott Higgins, and other research technicians and staff.

To help ensure the success of this important research project, DFO will provide in-kind contributions of at least $38,000 in year 1 and $28,000 in years 2 and 3. This contribution includes: labour time of Dr. Michael Paterson, Dr. Michael Rennie, and ELA support staff, utilization of equipment owned by DFO (e.g. experimental mesocosms), and per diem expenses of DFO staff while working on the project. Our investment reflects the importance we place on this research and we look forward to working with Dr. Metcalfe and to the results of this collaboration.

In closing, we would like to emphasize the DFO's strong support for this proposal, which would enable all partners to achieve jointly well beyond the sum of their individual capabilities and produce great benefits for the Canadian environment.

If you have questions, please contact me at 204-983-5173 or Patricia.Ramlal@dfo-mpo.gc.ca.

Yours truly,

Patricia Ramlal

Dr. Patricia Ramlal
Division Manager, Environmental Science Division
Central and Arctic Region
Fisheries & Oceans Canada

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

FORM 183A

Information Required from Organizations Participating in Research Partnerships Programs

Read the instructions before completing the Form.

GENERAL INFORMATION ON THE ORGANIZATION

Name of organization: Fisheries & Oceans Canada	Name and title of contact person at the organization: Michael Paterson
Mailing address: 501 University Cres. Winnipeg, MB R3T 2N6	Mailing address for the contact person (only if different):

Telephone number:	Facsimile number:	Telephone number: 204-984-4508	Facsimile number: 204-984-2404

E-mail address:	E-mail address: michael.paterson@dfo-mpo.gc.ca

Is your organization ☐ Private sector? ☒ Government owned? ☐ Government agency/department?

Industry/Products and Services Code:

Is your organization ☐ Profit-motivated? ☐ Not-for-profit?

Web site: www.dfo-mpo.gc.ca

Canadian ownership (in percentage) (If Applicable) %	Date of incorporation in Canada (If Applicable) /	Total number of employees in Canada ~10,000
Types of products sold and/or services offered		Total annual sales for previous year (If Applicable)
		Net profit (loss) for previous year (If Applicable)

Is your organization ☐ a parent company? ☐ a subsidiary of? (specify) Government of Canada

RESEARCH AND DEVELOPMENT ACTIVITIES

Does your organization have an R&D department? Yes ☐ No ☐

If not, does it undertake R&D within the organization's premises? Yes ☐ No ☐

Annual R&D expenditures (previous/ current / next year)

/ /

Number of R&D staff in Canada
Scientists and technicians:

R&D staff with a PhD:

APPLICANT INFORMATION

Family name: Metcalfe	Given names: Chris	Initial(s) of all given names:
Title of proposal: Fate and effects of nanosilver in a lake ecosystem		Personal identification no. (PIN) 15742
		Appl ID (for NSERC use only)

ORGANIZATION'S CONTRIBUTIONS

Contributions to the direct costs of research	Year 1	Year 2	Year 3	Year 4	Year 5
a) Cash contribution					
b) In-kind contribution	38,000	28,000	28,000		

Has your organization received publicly-funded support for R&D directly related to the proposed project? Yes ☐ No ☒

Are the applicant and co-applicant(s) at arm's length from your organization? Yes ☒ No ☐

Name, title and telephone number of authorized representative of the organization	Signature	Date
Patricia Ramlal, Division Manager, Environmental Science Division, 204-983-5173	Patricia Ramlal	30 March 2011

Canada

Ministry of the Environment

Standards Development Branch

40 St. Clair Ave. West
7th Floor
Toronto ON M4V 1M2

www.ene.gov.on.ca

Tel.: 416 327-5519
Fax: 416 327-2936

Ministère de l'Environnement

Direction de l'élaboration des normes

40, avenue St. Clair ouest
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Ontario

April 8, 2011

Natural Sciences and Engineering Research Council
Strategic Grant Program
Ottawa, Canada

Dear Sir/Madame;

The Ontario Ministry of Environment (MOE) is writing this letter of support for the proposal submitted to the NSERC Strategic Grants program by the Principal Investigator, Dr. Chris Metcalfe for the project entitled, "Fate and Effects of Nanosilver in a Lake Ecosystem".

MOE protects Ontario's environment using regulations, targeted enforcement and a variety of innovative programs and initiatives. MOE addresses environmental issues that have local, regional and/or global effects and is responsible for protecting clean and safe air, land and water to ensure healthy communities, ecological protection and sustainable development for present and future generations of Ontarians.

Nanoparticles are a contaminant of emerging concern to the Ontario Ministry of the environment. The proposed project to evaluate the environmental fate and toxic effects of nanosilver added to a lake in the Experimental Lakes Area (ELA) of northern Ontario will contribute to a better understanding of the potential environmental impacts of these substances. Past whole lake addition studies with phosphorus, acidification, mercury and endocrine disrupting substances have confirmed and brought relevancy to laboratory studies. Results from whole lake studies provide the most compelling arguments for action and in the past have led to regulatory responses at both federal and provincial levels.

The Ontario ministry of the Environment anticipates that this proposed study may have a similar effect upon policy regarding risk assessment of nanomaterials. The Ministry believes that the team assembled by the applicant brings strong scientific experience and credentials to the project and will contribute to resolving a number of important questions regarding the fate and effects of nanosilver. We strongly support this proposal to NSERC.

If there is any additional information required relative to our interests as a supporting organization for this proposal, please contact Sonya Kleywegt (416-212-1525 or sonya.kleywegt@ontario.ca).

Sincerely,

Tim Fletcher
Supervisor, Water Quality Standards
Standards Development Branch

McGill

Department of Chemical Engineering

Département de génie chimique

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April 4, 2011

NSERC Strategic Project Grants Selection Committee
350 Albert Street
Ottawa, ON, Canada
K1A 1H5

RE: Mutual collaboration on the NSERC Strategic project *"Toxicity, transformations and transport of engineered nanoparticles in soils: New approaches to detect and characterize environmental risks (Tufenkji et al.)"* and the proposed project on, *"Fate and Effects of Nano-Silver in a Lake Ecosystem (Metcalfe et al.)"*.

To Whom It May Concern,

In the 2010 competition of the NSERC Strategic Grants program, myself and my research colleagues were successful in receiving support for a project that focuses on evaluating the environmental risks associated with the release of nanomaterials into soils. In the 2011 Strategic competition, Dr. Chris Metcalfe and his research team are submitting a proposal for NSERC support of a project on the fate of nanoAg in natural waters. During the 2007-10 funding cycle, we both also received support from the NSERC Strategic Grants program for projects related to the fate and effects of nanomaterials in the environment and we found that it was highly advantageous for both groups to keep each other up-to-date on our mutual progress. Group members and group leaders attended each others' project workshops and our research efforts were coordinated whenever possible.

Should the proposal submitted by Dr. Metcalfe in 2011 be funded, we fully intend to maintain and increase this high level of collaboration, including the use of common nanoparticles (nanoAg), common and complementary experimental conditions (e.g. pH, ionic strength, organic matter content, etc.) and the use of complementary analytical techniques. As in the previous projects, both teams are in close contact with Environment Canada to ensure that there is no duplication of efforts in this rapidly expanding field. Through our reporting to Environment Canada, we are both contributing to the work of the OECD Working Group on Nanomaterials to develop a coordinated approach to assessing the environmental risks associated with the development of nanomaterials.

We strongly believe that funding of Dr. Metcalfe's project will allow us to re-establish a mutually beneficial and highly synergistic research network that is likely to make significant advances in the field.

Yours sincerely,

Nathalie Tufenkji

Nathalie Tufenkji, PhD
Associate Professor and Canada Research Chair

SEND ONE ORIGINAL ONLY DO NOT PHOTOCOPY

APPENDIX A (Form 101)
Environmental Impact

Complete this Appendix if you have checked the "YES" box under Certification/Requirements on page 1, Form 101. Include activities that will take place in Canada **and/or abroad.** This information will assist NSERC in determining whether a screening is required under the *Canadian Environmental Assessment Act.* (See the "Requirements for Certain Types of Research" in the NSERC *Program Guide for Professors*.)

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Metcalfe	Chris	D	**Valid** 15742

Name of applicant's organization
Trent

Title of proposal
Impacts of nanosilver on a lake ecosystem

Name of other participating organizations (if applicable)
Fisheries and Oceans Canada
Environment Canada

Name of Location (Please complete an additional copy of Appendix A for EACH location at which research will be undertaken.)
Experimental Lakes Area, ON

1. Main characteristics of the location (i.e., physical description & coordinates)

Both mesocosm and whole lake addition studies will take place at the Experimental Lakes Area (ELA), which is located in northwestern Ontario near the town of Kenora, ON. For most of its history, the ELA was administered and supported by the offices of the Department of Fisheries and Oceans Canada (DFO) at the Freshwater Institute in Winnipeg, MB. However, the site is now jointly managed and supported by both DFO and Environment Canada. All research activities at the site are overseen and approved by the ELA Management Board. This study will require approvals and permits from both federal and provincial (i.e. Ontario) regulatory agencies.
The ELA is unique in the world in providing a dedicated research facility for ecosystem-scale investigations and long-term monitoring of ecosystem processes. The ELA is located on the granitic geology of the Canadian Shield and provides access to over 100 small lakes that are relatively unaffected by external human influences and industrial activities. Most water bodies at ELA are oligotrophic soft water lakes, and the surrounding watersheds are located in temperate coniferous forests that were historically partially logged.
According to the ELA website, the stated aims of the ELA are:
o To better understand global threats to the environment through knowledge gained from whole-ecosystem, experimental, scientific research
o To monitor and demonstrate the impacts of human activities on watersheds and lakes
o To develop appropriate environmental stewardship strategies for the preservation, restoration and enhancement of ecosystems
o To educate and promote environmental protection and conservation through an integrated approach to ecosystem stewardship

Whole lake studies have taken place at ELA from 1968 to 2005 to study the impacts of eutrophication, atmospheric deposition, aquaculture, etc.

Continue on page 3 of this Form (if necessary).

NOTE: There is a potential to generate several Appendices A. **Please ensure that all Appendix A pages are numbered consecutively in the space provided in the upper right corner of the form. IF YOU FORESEE THE NEED FOR MORE THAN 3 (THREE) APPENDICES A, PLEASE CONTACT NSERC'S ENVIRONMENTAL ASSESSMENT UNIT BY TELEPHONE AT (613) 992-3612 OR (613) 995-8079, OR BY E-MAIL AT enviro.assess@nserc-crsng.gc.ca.**

The information contained in this form will be used by NSERC to determine any potential environmental effects.
(See the "Requirements for Certain Types of Research" in the NSERC *Program Guide for Professors*.)

Personal identification no. (PIN)	Family name of applicant
15742	Metcalfe

APPENDIX A (Form 101) CONTINUED

2. Principal activity(ies) and activity component(s).

There will be two types of studies conducted at the ELA: i) mesocosm studies in which plastic lined circular structures with diameters of 1 or 2 meters extending from the lake surface to the bottom will be deployed over field seasons from May to November, ii) whole lake addition studies that are tentatively planned for L378, which is a small lake of 24 hectares, a maximum depth of 18 m and a volume of 1.8 million litres, which when last sampled in the 1970s, had an extensive fish community. In both the mesocosms and in the lake, the aquatic environment will be dosed daily with nanoform silver (nAg) over field seasons from May to November. Mesocosm studies will be conducted over the first field season in 2012, with <100 g of nAg spiked into the mesocsoms. The whole lake addition study will take place over the field seasons of 2013 and 2014, with spiking of an estimated 800 g of nAg over the two field seasons. Following dosing with nAg, the water, sediments and aquatic organisms living in the mesocosms or in the lake will be sampled to evaluate the impact at the levels of individuals, populations and communities. In cases where population numbers will sustain this sampling effort, fish will be sacrificed for analysis of Ag and indicators of effects. In cases where population numbers are low, non-lethal sampling methods (e.g. tissue biopsies, ultrasound) will be used.

Continue on page 3 of this Form (if necessary).

3. For each principal activity and activity component, list the environmental elements affected and provide a description of those effects.

The impacts of additions of nAg to mesocosms or to a whale lake are not yet known. However, we can speculate that the impacts will include reductions in communities of bacteria and algae, resulting in reduced productivity within the lake. These changes in lower trophic levels may cause reduced numbers or diversity of primary consumers (e.g. zooplankton) and reduced condition and possibly numbers of aquatic vertebrates (i.e. fish and amphibians). In addition, there may be changes in the biogeochemical cycling of nutrients, such as nitrogen, carbon and phosphorus, but the exact nature of these changes is difficult to predict.
It is anticipated that effects noted in mesocosms deployed in the lakes will not cause significant stress to the lake ecosystem or any long-term impacts. However, additions of nAg to the whole lake may cause impacts that persist over a period of months or perhaps years after cessation of nAg addition. Other whole lake experiments conducted at ELA have shown that the impacts of whole lake manipulations persist for a maximum of 3 years after the experimental period, until the ecosystem adjusts to a new equilibrium.

Continue on page 3 of this Form (if necessary).

4. Mitigation measures.

No mitigation measures are planned for these studies. It is anticipated that the mesocosm studies will have minimal impact on the lakes in which the mesocosms were deployed because of the relatively small volume of water that will be dosed and the relatively small amounts of nAg that will be added to the mesocosms. For the whole lake addition study, it is anticipated that, over time, the nAg will precipitate to the sediments and will be buried through natural sedimentation processes. Any possible mitigation measures within the lake, such as sediment dredging or capping, or the addition of chelating agents could potentially result in more environmental harm than the original nAg addition.

Continue on page 3 of this Form (if necessary).

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

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APPENDIX B (Form 101)
Canadian Environmental Assessment Act
Pre-Screening Checklist

Complete this Appendix if you have checked the "YES" box under Certification/Requirements on page 1, Form 101. Include activities that will take place in Canada **and/or abroad.** This information will assist NSERC in determining whether a screening is required under the *Canadian Environmental Assessment Act.* (See the "Requirements for Certain Types of Research" in the NSERC *Program Guide for Professors.*)

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Metcalfe	Chris	D	**Valid** 15742

Name of applicant's organization
Trent

Applicants are responsible for verifying whether permits are required for any of the activities listed below. Please indicate yes (Y), no (N) or unknown (U) by checking the appropriate box for EACH of the listed activities.

Y	N	U	DESCRIPTION OF ACTIVITY
			Part 1. - Determination of Physical Work under the CEAA
	X		Does any phase of the proposal involve the **construction, operation, modification, decommissioning, abandonment or other activity** in relation to a built structure that has a fixed location and is not intended to be moved frequently?
			Part 2. - Determination of Assessable Activities under the CEAA
	X		Activity takes place in a National Park or National Nature Reserve in Canada
	X		Activity takes place on First Nation lands
	X		Activity takes place in the North (Yukon, Nunavut, or the Northwest Territories)
	X		Activity takes place in or within 30 metres of the right-of-way of a power line, a natural gas line, or a railway line
X			Activity takes place in or adjacent to a water body, resulting in harmful alteration, disruption or destruction of fish habitat (including the removal or damaging of aquatic vegetation)
X			Destruction of fish other than by fishing
	X		Sampling or prospecting for ores or minerals
	X		Disposal of a prescribed nuclear substance other than in a laboratory equipped for such disposal
X			Deposit of a deleterious or other substance into the environment (in the earth, air, or water)
	X		Any kind of remediation of contaminated land
	X		Deposit of oil, oil wastes or any other substances harmful to migratory birds in waters or in areas frequented by migratory birds
	X		Killing or removal of migratory birds, their nests, eggs, or carcasses or other physical activities that may require a permit or other authorisation under the *Migratory Birds Regulations* or *Migratory Bird Sanctuary Regulations*
	X		The removal or damaging of vegetation and/or the carrying on of agricultural activities or the disturbance or removal of soil in a wildlife area that requires a permit under section 4 of the *Wildlife Area Regulations* under the *Canada Wildlife Act*
	X		Physical activities that are carried on in Canada and that are intended to threaten the continued existence of a biological population in an ecodistrict, either directly or through the alteration of its habitat
	X		Establishment or operation of a field camp in a single location that will be used for 200 person-days or more within a calendar year
	X		Seismic surveying involving more than 50 kg of chemical explosive in a single blast; or marine or freshwater seismic surveying, if during the survey the air pressure measured at a distance of one metre from the source would be greater than 275.79 kPa (40 lbs/sq in)

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Personal identification no. (PIN)	Family name of applicant
Valid 15742	Metcalfe

APPENDIX B (Form 101) continued

Are any authorizations, permits, or licences required to undertake any activity for any phase of the proposal? If **yes**, list them below, along with the name of the issuing agency(ies). If **no**, please state "None required" and submit this page with the rest of your proposal.

Permit for whole lake additions by Experimental Lakes Area management committee
OMNR fishing for scientific purposes permit
Animal Care approval from Trent University

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APPENDIX C
Referee Suggestions
(Form 101)

Complete Appendix C for all types of grants (except Discovery Grants, Research Tools and Instruments - Category 1, Major Resources Support Grants and Partnership Workshops Program). Read the instructions before completing the appendix.

Date: 2011/04/14

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Metcalfe	Chris	D	**Valid** 15742

Title of proposal
Impacts of nanosilver on a lake ecosystem

	Referee	Area(s) of expertise		PIN	Lang.
A	Bernhardt, E (Emily) Biology Department Duke University FFSC 3313 Durham, NC UNITED STATES 27788 1 (646) 825-1278 ebernhar@duke.edu	ecology, biogeochemistry	1		
B	Kidd, K (Karen) Biology Department University of New Brunswick at Sint John Tucker Park Saint John, NB CANADA E2L4L5 1 (596) 648-5500 kiddk@unbsj.ca	Ecotoxicology	2		
C	Handy, R (Richard) School of Biological Sciences University of Plymouth Drake Circus Plymouth UNITED KINGDOM 44 (170) 5258460 R.Handy@plymouth.ac.uk	Ecotoxicology	3		
D	Klaine, S (Stephen) Institute of Environmental Toxicology Clemson University Pendleton Clemson, SC UNITED STATES 29634 1 (864) 646-2961 SKlaine@clemson.edu	Ecotoxicology	4		
E	Behra, R (Renata) Environmental Toxicology EAWAG (Swiss Federal Inst. for Water Res) Ueberlandstrasse 133 8600 Dubendorf SWITZERLAND 41 (440) 823-5199 renata.behra@eawag.ch	Ecotoxicology	5		

NSERC reviewing committee	Committee reviewer	Personal identification no. (PIN)
	1st committee reviewer	
	2nd committee reviewer	
	3rd committee reviewer	

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APPENDIX C
Referee Suggestions CONTINUED
(Form 101)

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Date: 2011/04/14

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Metcalfe	Chris	D	**Valid** 15742

Title of proposal
Impacts of nanosilver on a lake ecosystem

F	Wood, CM (Chris) Biology Department McMaster University 1280 Main Street West Hamilton, ON CANADA L8S4K1 1 (905) 525-9149 ext 23537 woodcm@mcmaster.ca	Area(s) of expertise Fish physiology	6	PIN: Lang.:
G	Cardinale, B (Bradley) Ecology, Evolution and Marine Biology University of California, Santa Barbara Building 408 Santa Barbara, CA UNITED STATES 93106 1 (805) 893-4157 cardinale@lifesci.ucsb.edu	Area(s) of expertise Ecology, freshwater biology	7	PIN: Lang.:

NSERC reviewing committee	Committee reviewer	Personal identification no. (PIN)
	1st committee reviewer	
	2nd committee reviewer	
	3rd committee reviewer	

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