FOCUSED STUDY ACTIVITY WORK PLAN

General Information

Work Plan Unique Identifier:	*B-IC-1-1718*	
Focused Study Activity Title:	**Monitoring Benthic-Macro Invertebrates: Investigation of Cause of Nutrients Signatures in the Athabasca River**	
Focused Study Category:	Investigation of Cause or Potential Ecological Impact	
Geographic Location (*choose from drop-down menu. If Project Location is in more than one area choose from second drop-down***)**	Lower Athabasca River	
Monitoring Site(s) Coordinates *(latitude and longitude)*	See Appendix D below	
Project Leader:	Joseph Culp	
Organization and contact information:	**Environment and Climate Change Canada** **Joseph.culp@canada.ca**	
Date Study initiated:	**2017**	
Monitoring Category: *(From OSM long-term plan; choose from drop-down menu)*	Biotic Response Monitoring	
Strategic Objective of Focused Study: *(From OSM long-term plan; choose from drop-down menu)*	Objective B2: Investigate the causal mechanisms of a known important biotic relationship in relation to Oil Sands Developments *The Lower Athabasca River generally has good ecological condition with intolerant taxa found in large abundances at all sampling sites. Nevertheless, the middle river reaches between M3 and M7C show early warning signs of environmental stress that needs to be examined more closely through focused studies designed to evaluate the combined effects of nutrient and contaminant stressors.* This focus study will evaluate the effects of municipal sewage discharge and oil sands activities on benthic macroinvertebrates in the Athabasca River using *in situ* monitoring methods and cutting edge biochemical analyses using NMR-(Nuclear Magnetic Resonance)-based metabolomics.	
Hypotheses: *(Briefly outline the specific hypotheses that your focused study is aiming to address)*	These field experiments will test the hypotheses that no differences exist among reaches along the mainstem Lower Athabasca River in either 1) benthic macroinvertebrate assemblage or 2) metabolite levels of dragonfly (Odonata) nymphs.	

Deliverables: *What tangible goal (s) and/or product(s) will the monitoring produce and when?*	Information on reach specific impacts of municipal sewage discharge by comparing benthic macroinvertebrate assemblages, physiological indicators of ecosystem health and cumulative effects inside and outside oil sands deposits using *in situ* caging methods, kick net sampling and cutting edge biochemical analyses using NMR-based (Nuclear Magnetic Resonance) metabolomics. Lastly, this information will also aid in the identification of the natural effects of bitumen exposure on benthic macroinvertebrates.

Detailed Study Plan

(Please provide detailed information on the specifics of your focused study including – (**keywords, hypothesis and the assumptions and constraints behind your hypothesis**)

Provide a maximum of 10 key words that describe this project. Use commas to separate them:

Athabasca River, benthic macroinvertebrates, bitumen, dragonfly, *in situ* studies, metabolomics, municipal wastewater, NMR, Odonata, oil sands

Describe how you will test your hypothesis:

Assessment of 2012-2015 FY JOSM data revealed that benthic macroinvertebrate assemblages showed a similar longitudinal pattern among all years. Reaches least disturbed by human activity (i.e., M0, M1 and M2) were separated from sites (M3, M3B, M4, M6, M7 and M7C) exposed to municipal sewage effluent (MSE) and oil sands development (M3B, M4, M6, M7 and M7C). The sites furthest downstream of the active oil sands mineable area (M8, M9) show a trajectory path trending towards the benthic macroinvertebrate assemblages of reaches M0, M1 and M2. Statistical analysis revealed that the most upstream sites (M0 – M2) had the highest abundances Hydropsychidae larvae, but fewer mayfly taxa (Heptageniidae), dragonflies and tolerant taxa, such as oligochaete worms or chironomid midges compared to middle reach sites (M3 – M7C). Middle reach sites tended to have lower abundances of EPT taxa, such as Perlodidae stoneflies and Hydropsychidae, but more tolerant taxa like oligochaete worms or chironomid midges than downstream sites. Downstream sites were different from upstream as they had higher abundances of Perlodidae stoneflies, Heptageniidae mayflies and dragonflies, but tended to have similar abundances of Hydropsychidae larvae and tolerant taxa, such as oligochaete worms and chironomid midges. These results suggest that benthic macroinvertebrate communities between M2 and M8 exhibited early warning signals of environmental stress (e.g., mild nutrient enrichment and/or contaminant exposure).

The cause of the potential nutrient-contaminant signal observed in the benthic macroinvertebrate assemblage will be investigated by developing a focused study in the central Oil Sands (OS) area. The work will examine the association of the benthic community with the nutrient-contaminant signal using a combination of kick net samples of macroinvertebrates collected from field studies, reciprocal transplant of dragonflies in rock basket cages from reference to exposed sites (Brua et al. 2015), stable nitrogen and hydrogen isotope analyses of amino acids, and a cutting-edge NMR-based metabolomics analysis. The aim is to develop an understanding of the cumulative effects resulting from multiple stressors.

Metabolomics is the identification and characterization of small molecules called metabolites, which are derived from normal processes within an organism. Environmental metabolomics characterizes the biochemical products of interactions between living organisms and their environment. Metabolomics is a growing technique in ecotoxicology and has the potential to evaluate the effects of sub-lethal concentrations of contaminants on organisms. NMR-based metabolomics has the advantages of being a fast, non-selective, non-destructive technique with minimal sample preparation. Metabolite levels can be quantified to identify whether metabolites in exposed organisms have increased or decreased compared with unexposed organisms. The NMR spectra can be evaluated to determine if novel metabolites are present after contaminant exposure. Metabolites that increase or decrease in concentration after contaminant exposure (or new metabolites) can reveal responses induced by exposure and may have the potential to serve as indicators of environmental contamination.

Field studies over three years will compare benthic structural composition and functional health in six distinct areas of the mainstem Lower Athabasca River: 1) reference reach outside OS geology; 2) reference reach outside OS geology, but exposed to municipal sewage; 3) reference reach inside OS geology; 4) near field reach within OS geology exposed to municipal sewage, but out of OS development; 5) near field reach within central OS development; and 6) far-field recovery reach downstream of OS development. These field studies will test the hypotheses that no differences in: 1) benthic macroinvertebrate assemblages or 2) metabolite levels exist between dragonfly nymphs among the study reaches along the mainstem Lower Athabasca River. These field assessments will follow the ecological causal assessment approach outlined by Cormier et al. (2015).

Field Studies:

<u>Year 2017-18</u>

Kick net samples of macroinvertebrates will be collected in all six sampling reaches. In addition, dragonflies will be obtained at each sampling reach for assessment via NMR-based metabolomics and stable nitrogen and hydrogen isotope analyses of amino acids. This field study will determine if benthic macroinvertebrate assemblage structure, dragonfly metabolites and/or stable isotopes change with exposure to municipal sewage discharge, naturally occurring oil sands deposits, and/or oil sands activity. Moreover, if assemblage, metabolite or stable isotope composition in the far-field reach are similar to the reference values outside OS geology, there will be evidence of biological recovery. Finally, the dragonfly metabolite information will serve as a baseline for experiments in subsequent years.

<u>Year 2018-19</u>

Transfer experiments using dragonfly nymphs, a predacious benthic macroinvertebrate, will be undertaken to determine if nymphs moved to reaches of nutrient-contaminant exposure have different metabolic signatures than those in reference reaches. Differences in metabolic signatures can be indicative of exposure to environmental stressors. To establish the experiment, dragonfly nymphs will be collected at the reference site outside OS geology. Nymphs will be placed into 8 replicate rock basket cages (2 nymphs/cage) per reach. Sixteen nymphs will remain at the reference site outside OS geology, while the remaining individuals will be moved to the other five reaches along the Lower Athabasca River.

<u>Year 2019-20</u>

A transplant study using dragonfly nymphs will also be completed in Year 3. However, this experiment will incorporate the use of reciprocal transfer methods such that nymphs from each of three reaches will be moved to each of the other reaches. The three study locations include a reference reach inside OS geology, a near field reach within OS geology that is exposed to municipal sewage but not OS development, and a near field reach within the central area of OS development. This experiment will allow us to assess if the dragonfly

NMR-based metabolomics signal recovers from exposure to nutrient-contaminant stressor regimes.

Assumptions and Constraints behind the hypothesis and the testing method:

(1) Nutrient inputs from Ft. McMurray and Oil Sands Developments will likely affect biological processes in the river and have the potential to confound the effects of OS stressors including contaminants;

(2) Contaminant inputs to the mainstem arise from several possible pathways (including atmospheric transport, tributary inflows, groundwater flux, etc.). These contaminant inputs likely act as stressors that modify biological composition of benthic food webs; and

(3) Nutrient and contaminant effects on benthic food webs should be detectable through a suite of diagnostic, bioassessment indicators.

References:

Brua, R. B., J. M. Culp, and A. C. Alexander. 2015. Assessment-Specific Field Study Designs and Methods. Pages 203-212 *in* Norton, S. B., S. M. Cormier and G. W. Suter II (eds). Ecological Causal Assessment. CRC Press, Boca Rotan, FL.

Cormier, S.M., S.B. Norton and G.W. Suter III. 2015. Conducting causal assessments: our approach for identifying causes. pp. 79-88 *in* Norton, S. B., S. M. Cormier and G. W. Suter II (eds.). Ecological Causal Assessment. CRC Press, Boca Rotan, FL.

Data Management

If this work generates data please summarize your project-level data management plan.

Deliverables	Timeframe
Data Collection Period: *Field work*	Start : 2017-08-21 End: 2017-09-11
Data Analysis Period: *Laboratory analysis and QA/QC of data*	Start : 2017-10-16 End: 2018-03-30
Data Release Date: *Metadata and data consistent, complete and meet basic standard format for publication in Open Data; on or linked to JOSM portal*	2018-11-01

Reporting and Publications

Provide information on the anticipated reports / publications. (Insert additional rows if needed)

Expected Subject/Titles of Publications or Reports	Short Description of Publication or Report	Expected Year of Publication
Benthic Macroinvertebrate Assemblage Response to Municipal Sewage, Naturally-Occurring Bitumen and Oil Sands Development: A Field Experiment	A field study was performed that examined how benthic macroinvertebrate assemblages from reference reaches in the Lower Athabasca River responded to exposure to naturally-occurring bitumen, municipal sewage discharge and oil sands development.	2019
NMR-Based Metabolomics of a Predatory Benthic Macroinvertebrate Exposed to Naturally-Occurring Bitumen, Municipal Sewage and Oil Sands Activities	An *in situ* field experiment was performed to evaluate the effects of naturally-occurring bitumen, municipal sewage and oil sands activity that aids in assessing cumulative effects along the Lower Athabasca River.	2021

Technical / Professional Roles and Responsibilities

Identify members of the monitoring team/organization, their roles and responsibilities. Identify monitoring organization leads if different from overall monitoring activity lead. (Insert additional rows if needed)

Role	Responsibilities
Dr. Robert Brua Co-Project Lead	Design of focus study, field work, analysis of data, writing and interpretation of focus study
Dr. Joseph Culp Co-Project Lead	Design of focus study, analysis of data, writing and interpretation of focus study
Science Team Support	Design of focus study, writing and interpretation of focus study
Field and Lab Technical Lead and Support	Design of focus study, field work, lab analyses of focus study

Deliverables (Year 1) If your Focus Study is longer than 1 year then complete **Appendix C** for multi-year deliverables breakdown

Provide a summary of tangible quarterly deliverables. Identify major project areas (deliverables) and results that can be identified as a tangible goal. This could include: field work, lab work/ analysis, evaluation, data, reports, publications, SOPs etc. Do not define process as your Deliverable e.g. 'fly to Ft. McMurray to conduct fieldwork' or 'seek Director approval for report'.

Deliverable(s) (please provide enough information to support status reporting)
Q1 – April to June
Field and lab logistics: Equipment maintenance, contract preparation, materials acquisition, planning
Q2 – July to September
Field work & lab logistics: Equipment maintenance, contract preparation, materials acquisition, planning, field sampling
Q3 – October to December
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry analyses, sample preparation for metabolomics and stable isotope analysis, benthic processing
Data Product: Progress reporting
Q4 – January to March
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry analysis, metabolomic and stable isotope analysis, benthic processing
Data Product: Progress reporting

Detailed Financial Breakdown – Year 1 of 3 (2017-2020)

Also complete **Appendix B** for the multi-year financial breakdown

Budget requirements – List areas that require budget expenditures: (ADD OR DELETE BUDGET CATEGORIES AS REQUIRED)	**OS Funding**	**External Funding (*outside JOSM*)**
O&M - Operations and Maintenance:		
Helicopter Costs	**$20,000**	**$**
Field Costs	**$**	**$**
Fleet Use	**$**	
Data Management	**$**	**$**
Internal Lab Analysis	**$5,200**	**$**
Consumable Materials & Supplies	**$23,500**	**$**
Sub-Total	**$48,720**	**$**
O&M – Travel:		
Field Work	**$2,800**	**$**
Conferences (*identify conference*)	**$**	**$**
Meeting (*identify meeting*)	**$2,200**	**$**
Sub-Total	**$5,000**	**$**
O&M - External Contracts:		
Goods and Services Contract (*Benthic macroinvertebrate sorting & taxonomy and NMR service*)	**$30,000**	**$**
External Lab Analysis	**$**	**$**
Sub-Total	**$30,000**	**$**
Salaries:		
Principal Investigators	**$**	**$**
Technical / Professional Assistants	**$**	**$**
Field Staff	**$1,200**	**$**
WSTE Analytical Support Services	**$8,000**	
Sub-Total	**$9,200**	**$**

Budget requirements – List areas that require budget expenditures: (ADD OR DELETE BUDGET CATEGORIES AS REQUIRED)	OS Funding	External Funding (*outside JOSM*)
2017-2018 GRAND TOTAL* (BEFORE OTHER RELATED COSTS	**$92,920**	**$**

Appendix A – Approvals

Project Submitted by:		
Name:		
Organization:	Signature:	Date:

Project Approved by:		
Dr. Monique Dubé (AEP)		**Dr. Kevin Cash (ECCC)**
Signature		Signature
Date		Date

APPENDIX B – Detailed Multi-year Financial Breakdown (Complete the following detailed financial breakdown; add or delete categories as required)

Budget requirements	Year 1 (2017- 2018) APPROVED		Year 2 (2018- 2019) NOT APPROVED		Year 3 (2019- 2020) NOT APPROVED	
	Cash	In-kind	Cash	In-kind	Cash	In-kind
1) Salaries and benefits						
a) Investigators						
b) Technical/professional assistants						
c) Field Staff	1,200		1,200		1,200	
d) WSTD Analytical Services	8,000		8,000		8,000	
2) Operations and maintenance						
a) Facilities						
b) Equipment						
c) Lab analysis	5,200		5,200		5,200	
d) Data management						
e) Field work						
f) Helicopter Cost	20,000		20,400		20,808	
g) Shipping						
h) Fleet Use						
3) Consumable Materials and supplies						
a) Lab supplies, field gear, chemicals, filters, bottles, nets	23,520		23,990		24,470	

b)						
4) Travel						
a) Conferences and meetings					2,000	
b) Field work	2,800		2,856		2,913	
c) Project-related travel	2,200		2,244		2,289	
5) Dissemination & Engagement						
a) Publications/Reports					1,500	
b) Translation (if required)					500	
c) Communications						
d) Stakeholder Engagement						
e) Indigenous Peoples Engagement						
6) External Contracts						
a) Benthic macroinvertebrate sorting & taxonomy, NMR service	30,000		18,000		18,360	
Grand Total* (Before other related costs)	$92,920		$81,890		$87,240	

***The Total Salary Costs for ECCC ($9,200) in 2017-18 with other related costs is $12,652. Total O&M for ECCC ($83,720) in 2017-18 with other related costs is $88,349. The Grand Total for ECCC ($101,000) in 2017-18 with other related costs is $101,000**

APPENDIX C –Years 2 and 3 Deliverables (Complete the following detailed breakdown. Provide a summary of tangible quarterly deliverables. Identify major project areas (deliverables) and results that can be identified as a tangible goal.)

Year 2 (2018- 2019)
Deliverable(s) (please provide enough information to support status reporting)
The following deliverables are based on projected activities at the present time (Dec 2016). ECCC will provide updated deliverables and budget estimates for 2018-19 as work continues in the next fiscal years. Changes will be made depending on the previous years' progress and new science questions arise.
Q1 – April to June
Field and lab logistics: Equipment maintenance, contract preparation, materials acquisition, benthic macroinvertebrate, water, SPMD and sediment chemistry QA/QC, metabolomic and stable isotope analyses, planning
Q2 – July to September
Field work and lab logistics: Equipment maintenance, contract preparation, materials acquisition, planning, benthic macroinvertebrate, water, SPMD and sediment chemistry QA/QC, metabolomic and stable isotope analyses
Q3 – October to December
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry, NMR metabolomics processing, stable isotope analyses
Data Product: Benthic assemblage, NMR metabolomics, water, SPMD and sediment chemistry **data set from 2017-18 ready for release; Progress Reporting for year 1 and 2**
Q4 – January to March
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry, NMR metabolomics sample preparation
Data Product: Progress Reporting for year 1 and 2

Total Annual Budget

Year 3 (2019- 2020)
Deliverable(s) (please provide enough information to support status reporting)
The following deliverables are based on projected activities at the present time (Dec 2016). ECCC will provide updated deliverables and budget estimates for 2018-19 as work continues in the next fiscal years. Changes will be made depending on the previous years' progress and new science questions arise.
Q1 – April to June
Field and lab logistics: Equipment maintenance, contract preparation, materials acquisition, planning, NMR metabolomics analyses, benthic macroinvertebrate, water, SPMD and sediment chemistry QA/QC
Q2 – July to September
Field work and lab logistics: Equipment maintenance, contract preparation, materials acquisition, planning, NMR metabolomics analyses, benthic macroinvertebrate, water, SPMD and sediment chemistry QA/QC
Data Product: NMR metabolomics, water, SPMD and sediment chemistry **data set from 2018-19 field season ready for release**
Q3 – October to December
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry analyses, NMR metabolomics sample preparation
Data Product: Stable isotope data set from 2017-18 ready for release; Progress Reporting for year 2 and 3
Data Product: Manuscript based on year 1 (benthic assemblages) of focus study ready for submission
Q4 – January to March
Lab logistics: Equipment maintenance, materials acquisition, planning, water, SPMD and sediment chemistry analyses, NMR metabolomics analysis

Data Product: Progress Reporting for year 2 and 3
Total Annual Budget

Appendix D– Site Locations for Focus Study (Athabasca Mainstem)

Latitude and longitude in decimal degree format for each study reach along the mainstem Athabasca River.

Sampling Location	Latitude	Longitude
M0	54.7265	-113.3006
M1A	56.6219	-111.65542
M2A	56.6927	-111.4623
M3	56.7938	-111.4042
M4	57.098	-111.5649
M9	58.0666	-111.3681