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Supplementary Information

Submission from Bruce Power Inc.

Renseignements supplémentaires

Mémoire de Bruce Power Inc.

In the Matter of

Bruce Power Inc. – Bruce A and B Nuclear Generating Station

Request for a ten-year renewal of its Nuclear Power Reactor Operating Licence for the Bruce A and B Nuclear Generating Station À l'égard de

Bruce Power Inc. - Centrale nucléaire de Bruce A et Bruce B

Demande de renouvellement, pour une période de dix ans, de son permis d'exploitation d'un réacteur nucléaire de puissance à la centrale nucléaire de Bruce A et Bruce B

Commission Public Hearing – Part 2

Audience publique de la Commission – Partie 2

May 28-31, 2018

28-31 mai 2018



Letter, F. Saunders to M. Leblanc, "Application for the Renewal of the Power Reactor Operating Licence: Supplemental Material", May 16, 2018, NK21-CORR-00531-14285 / NK29-CORR-00531-14980 / NK37-CORR-00531-02956 (excluding Enclosures 4, 5, and 6).

For the convenience of the Commission, the following document includes the portions of Bruce Power's submission which may be released to the public.



May 16, 2018

NK21-CORR-00531-14285 NK29-CORR-00531-14980 NK37-CORR-00531-02956

Mr. M. Leblanc Commission Secretary Canadian Nuclear Safety Commission P.O. Box 1046 280 Slater Street Ottawa, Ontario K1P 5S9

Dear Mr. Leblanc:

Application for the Renewal of the Power Reactor Operating Licence: Supplemental Material

The purpose of this letter is to supplement Bruce Power's application for the renewal of the Power Reactor Operating Licence provided in Reference 1, to address queries from Part One of the public hearing to consider Bruce Power's application for renewal of the Power Reactor Operating Licence.

The supplemental material in Attachment A provides updates on selected items discussed by the Commission at Part One of the public hearing.

Please note Attachment A includes an update on environmental research activities, including research on thermal effects (see Section 15.17 of Attachment A). With respect to this research, copies of material previously submitted to the Commission (Reference 2 and Reference 3) have been provided as Enclosure 1 and Enclosure 2, respectively.

A response to issues raised by Commission Member Document 18-H4.146 is provided in Enclosure 3.

Additionally, updates to the community interest reports, previously provided in Reference 4, are provided in Enclosure 4, Enclosure 5, and Enclosure 6.



If you require further information or have any questions regarding this submission, please contact Mr. Maury Burton, Department Manager, Regulatory Affairs, at 519-361-2673 extension 15291, or <u>maury.burton@brucepower.com</u>.

Yours truly,

Frank Saunders Vice President Nuclear Oversight and Regulatory Affairs Bruce Power

cc: CNSC Bruce Site Office (Letter only)

Attach.

Encl.

Reference:

- 1. Letter, F. Saunders to M.A. Leblanc, "Application for the Renewal of the Power Reactor Operating Licence", June 30, 2017, NK21-CORR-00531-13493 / NK29-CORR-00531-14085 / NK37-CORR-00531-02768.
- 2. Letter, F. Saunders to M. Leblanc, "Supplement to the Application for the Renewal of the Power Reactor Operating Licence: Whitefish Research Review", June 30, 2017, NK21-CORR-00531-13494 / NK29-CORR-00531-00531-14088.
- 3. Letter, F. Saunders to M. Leblanc, "Supplement to the Application for the Renewal of the Power Reactor Operating Licence: University Research Summary", June 30, 2017, NK21-CORR-00531-13587 / NK29-CORR-00531-00531-14219.

2

Attachment A

Application for the Renewal of PROL 18.00/2020: April 2018 Update to the Performance Review

APPLICATION FOR THE RENEWAL OF PROL 18.00/2020: MAY 2018 UPDATE TO THE PERFORMANCE REVIEW

May 2018

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0.0 INTRODUCTION

This supplemental submission has been prepared to provide updates on selected items discussed by the Commission at Part One of the public hearing to consider Bruce Power's application for renewal of the Power Reactor Operating Licence (Reference A1).

Section numbers align with the sections of the Performance Review as previously submitted. With respect to those sections for which no update is provided, the information previously submitted remains valid.

The 2017 Performance Review and the February 2018 update (Enclosure 1 and Attachment B to Reference A2) may be found <u>online</u>:

http://www.nuclearsafety.gc.ca/eng/the-commission/hearings/cmd/pdf/CMD18/CMD18-H4-1-SubmissionfromBrucePowerLicenceRenewalforBruceAandB-NGS.pdf

0.2 Highlights (2015-2017)

0.2.4 <u>Community</u>

In March 2018, Bruce Power released the results of public attitude research in order to understand and track attitudes and opinions from Bruce, Grey, and Huron County residents with respect to the following:

- Support for nuclear energy and refurbishment of the Bruce facility,
- Familiarity and impressions of Bruce Power, and,
- Communications with residents.

Telephone interviews were conducted among a representative sample of 850 local residents in Bruce, Grey, and Huron counties. The data was weighted by region, gender, and age to ensure the final sample reflected the population of all three counties. The margin of error was $\pm 3.4\%$ (19 times out of 20).

The survey indicated that <u>84% of the residents support the refurbishment of the Bruce nuclear</u> facilities (including 48% "strongly support" and 36% "somewhat support"). Residents are most likely to support the refurbishment of the facility to create jobs (21%) and to benefit the economy (9%).

The survey also indicated:

- 93% of the residents have confidence that Bruce Power operates safely,
- 90% of the residents feel that Bruce Power is a good community citizen,
- 89% of the residents feel that Bruce Power is positively involved in the local community,
- 87% of the residents have confidence in Bruce Power's security measures, and,
- 81% of the residents feel that Bruce Power updates the community regularly.

1.0 MANAGEMENT SYSTEM

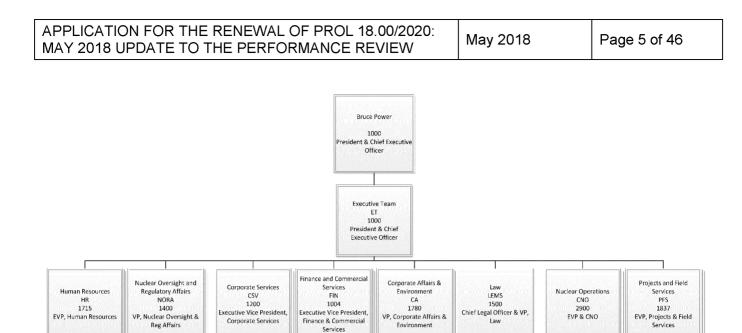
1.2 Organization

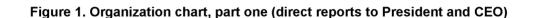
As described in the Performance Review, life extension activities include Major Component Replacement (MCR) and Asset Management (Reference A1 and A2). MCR will be executed primarily by Major Projects Division. Asset Management is integrated throughout Bruce Power's processes, but the Equipment Reliability Department (Engineering Division) has a primary role.

At time of preparation, the relevant reporting lines include:

- MCR: President and Chief Executive Officer Executive Vice President, Projects and Field Services Chief Vice President, Major Projects; and,
- Asset Management: President and Chief Executive Officer Chief Nuclear Officer Chief Engineering and Senior Vice President, Engineering Department Manager, Engineering (Equipment Reliability Support).

At the request of the Commission, an organization chart has been prepared to show these reporting lines, to the level of Department Manager (see Figure 1, Figure 2, and Figure 3).





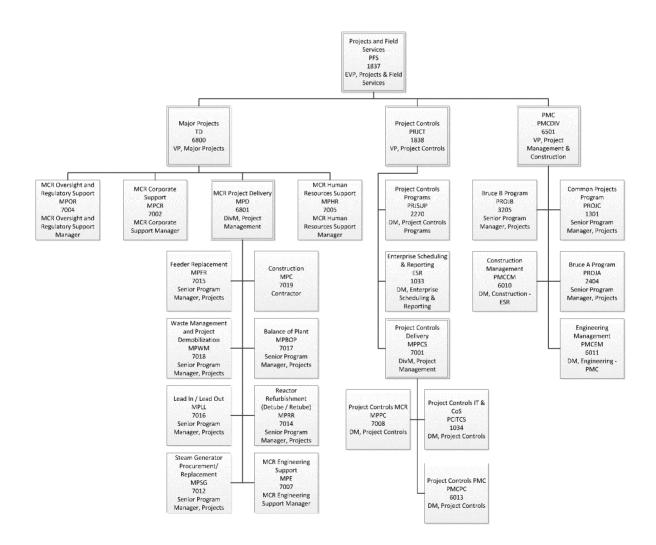


Figure 2. Organization chart, part two (Projects and Field Services)

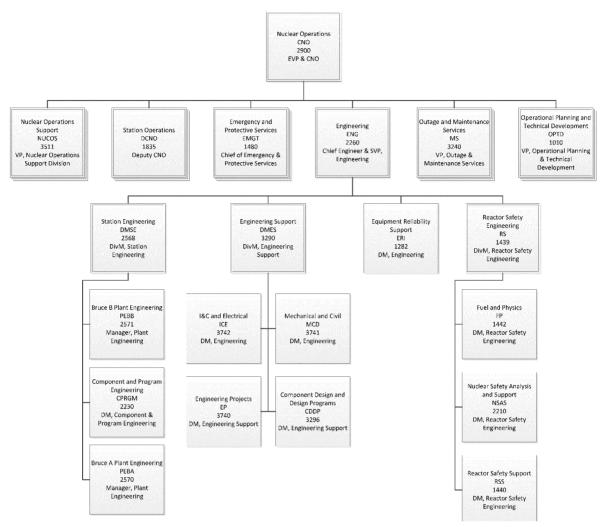


Figure 3. Organization chart, part three (Engineering)

2.0 HUMAN PERFORMANCE MANAGEMENT

2.6 Fitness for Duty

Bruce Power's existing Fitness for Duty program is robust, mature, and effectively ensures that workers are not under the influence of drugs or alcohol while at work. Bruce Power's observation and coaching program provides supervisors, managers, and security personnel with the tools and training to effectively observe workplace behaviours which might indicate impairment or a pattern of alcohol or drug use or abuse.

However, Bruce Power has prepared a preliminary plan to implement REGDOC-2.2.4, Fitness for Duty, Volume II: Managing Alcohol and Drug Use. This will introduce alcohol and drug testing into the Bruce Power workplace.

In order to implement the requirements of REGDOC-2.2.4 Volume II, a Third-Party Administrator and a qualified laboratory will be engaged to collect samples, verify specimen

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integrity, perform laboratory analyses, and ensure that chain-of-custody requirements are met. Additionally, it will be necessary to engage with the unions which represent impacted employees in order to jointly ensure compliance with REGDOC-2.2.4 Volume II.

Based on the time required to implement the various elements and engage with affected unions, Bruce Power plans to comply with all REGDOC-2.2.4 Volume II requirements by July 1, 2019, with the exception of random testing, for which compliance is planned by December 1, 2019 (Reference A3). However, these compliance dates may be impacted by legal challenges, particularly with respect to random alcohol and drug testing. Nonetheless, Bruce Power plans to provide regular updates to CNSC staff on progress with respect to compliance.

3.0 OPERATING PERFORMANCE

No update is provided.

4.0 SAFETY ANALYSIS

4.3 Probabilistic Safety Analysis

Single-unit and station aggregation results for the Bruce A and Bruce B Probabilistic Safety Assessments (PSAs) were provided in the Performance Review (References A1 and A2). For convenience, these results are shown here (Figure 4 and Figure 5), with the following updates:

- The risk of a hypothetical large release is compared with other risks¹. The intent is to provide a general understanding of how unlikely it is for large releases to occur. For example, the annual risk of dying for a single middle-aged individual (all causes) is more than 1 in 1000 per year—while the risk of a hypothetical large release (which would have limited health impact, even if doses exceeded regulatory limits for the public) is conservatively estimated as 100 to 1000 times less.
- The safety goal and administrative target are explicitly shown on the figure.

The PSA results show that the risk of a large radioactive release is extremely low. With risks typically between 10⁻⁵ and 10⁻⁶, this corresponds to a large release equivalent to one release every 100,000 or 1,000,000 years. For the traditional in-plant events, the aggregated station large release results are less than once in a million years and are on par with the world's best.

Needless to say, this is an extremely low probability, and reflects a large release frequency that continues to be reduced based on plant improvements as well as improved methodology. For additional details regarding the PSA results, please refer to the Performance Review (References A1 and A2).

¹ Comparative risks shown are approximate and intended to provide general context only.

Annual vehicle collisions (2007-2014): Transport Canada National Collision Database Online 1.0; vehicle kilometres driven (2009): Canadian Vehicle Survey: Annual 2009.

Train derailments, collisions, and million main-track train-miles (2007-2016): Transportation Safety Board of Canada Statistical Summary – Railway Occurrences 2016. Total accidents with a dangerous goods release include non-main-track accidents, but are compared with derailments and collisions per total main-track train miles in order to allow a comparison of frequencies with respect to overall levels train movements.

Mortality rate (all causes, 25-44 years; car accident): Statistics Canada Table 102-0601 and Table 102-0552.

Risk of a broken bone (20-64 years): Statistics Canada Canadian Community Health Survey Annual Component (2009-2010).

Note that the PSA results include:

- The application of EME: for purposes of modelling, EME was very conservatively assumed to reduce the frequency of severe core damage in applicable internal event sequences by approximately a factor of 10. This is considered a conservative estimate of the impact of EME (for clarity, a "conservative" estimate means the <u>actual</u> frequency of severe core damage is less than that determined through the PSA methodology with EME included). The EME credit was not applied for external hazards if the magnitude of the hazard could potentially impact the ability to implement and operate EME.
- A conservative credit for Containment Filtered Venting System (CFVS): for purposes of modelling, CFVS was assumed to reduce the frequency of a large release by a factor of 10, for event sequences that do not involve a bypass of containment. Most seismic and high wind events included this CFVS credit. For most fire event sequences, improvements related to a reduction in containment bypass events was assumed to reduce the frequency of a large release by a factor of 2. This is a conservative estimate; as noted previously (References A1 and A2), following final design and installation, refined CFVS calculations are expected to further reduce the large release frequency. As discussed later, the combination of EME and CFVS can result in no evacuation even for many four-unit events.

The PSA results are shown graphically and are summarized in Table 1. The results are aggregated in 4 groups for each of Bruce A and B. <u>Continued aggregation beyond the four groups of PSA results will yield a nonsensical result due to double-counting and due to the varying levels of uncertainty associated with each grouping</u>. However, even if that nonsensical aggregation were attempted, the sum of the unit and station aggregate results for each of Bruce A and Bruce B would still be below the safety goal of 10⁻⁵.

Figure 5 provides a clear view of site-wide results in a manner that allows changes to be easily seen. Additional aggregation would obscure relevant results by adding results that have significant variations in the level of uncertainty and thereby potentially obscuring changes in sub-indicators. (Recall that the PSA is intended to be a tool to monitor plant risk, optimize maintenance practices, and maximize the reliability of systems, structures, and components.) The at-power risk is determined mechanistically and has a low uncertainty. However, the modeling uncertainty is high for the external events, and the PSA results are therefore based on very conservative assumptions. As seen in the PSA results, the risks due to external events are much greater than that due to internal events—but this is at least in part an artifact of the conservative assumptions needed to incorporate the much greater uncertainties. As mentioned, the final design and installation of the CFVS is expected to further improve the PSA results for external events as the CFVS protects against radioactive releases, regardless of cause.

As noted previously (Reference A1), the results of the PSA are assessed against the goals defined in the governing procedures. The updated policy for considering enhancements to Bruce A and Bruce B, in the event that PSA results were in between the safety goal and the administrative target, was provided to CNSC staff in Reference A4.

An example of the application of this policy with respect to the Fire PSA is shown in the Integrated Implementation Plan through the Very Early Smoke Detection Apparatus (VESDA) fire prevention improvement projects at both Bruce A and B. In addition, Bruce Power constructed a state-of-the-art Fire Training Facility in 2015 that allows crews to train for plant

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fires in the manner that they would operate in an actual plant event. This facility can not be factored directly into the PSA results, but improves defence-in-depth.

Human interactions are modelled in the PSA using a standard methodology which considers the time and indications available to an operator to successfully complete a task as well as its relative difficulty. As actions become more complex to complete or involve multiple people in a coordinated action, very conservative assumptions are used to simplify the analysis. Actions related to some elements of the SAMG program are conservatively not credited at all. Detailed human reliability analysis for specific human interactions can be performed to acquire more realistic probabilities, but, due to practical cost and schedule constraints, this is usually done for the most risk significant human interactions. EME response is modelled with conservative assumptions, rather than specific actions, since detailed modeling with the current methodology would require a very significant effort—and the overall result of EME deployment clearly reduces risk, rather than the contrary.

Bruce Power has been working with an independent developer to develop a computer-based analytical tool that utilizes Monte Carlo methodology to predict EME deployment success in the variety of severe events. This tool (ARES All Hazard) is currently being installed at site but will require more development before results can be factored into analysis. Bruce Power plans to evaluate this tool to determine usage for training and emergency response. For more details, see Section 10.2.3.

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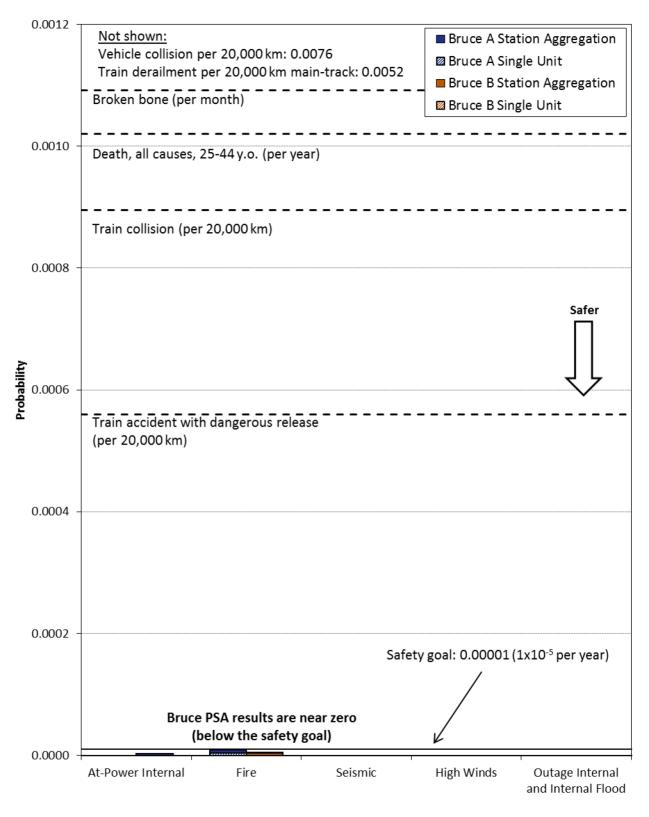


Figure 4. Probabilistic safety assessment results. (Single unit results include impacts of non-accident units on accident unit.)

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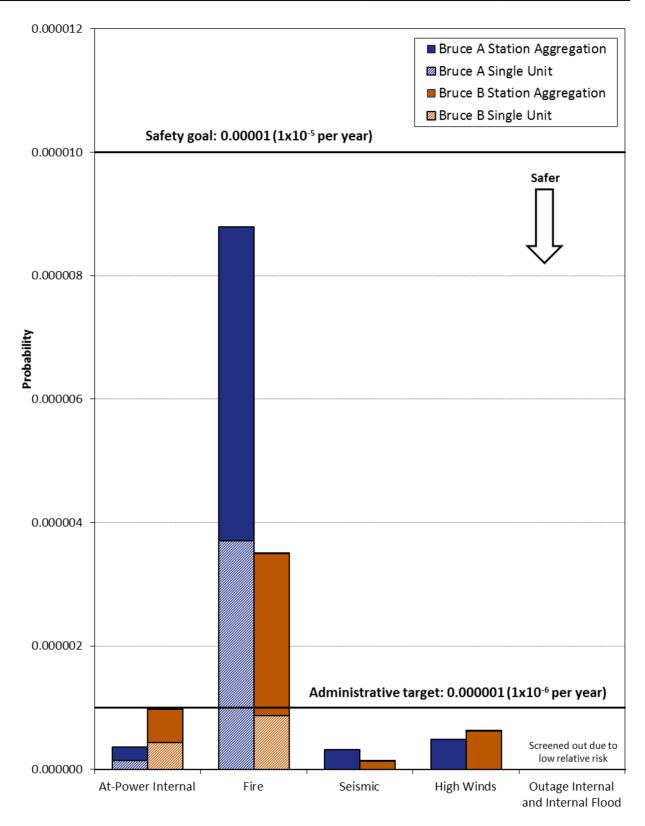


Figure 5. Probabilistic safety assessment results, magnified 100x. (Single unit results include impacts of non-accident units on accident unit.)

Table 1. Multi-unit aggregation of PSA Large Release Frequency results

Purpose

The purpose is to summarize the safety assessment based on the Large Release Frequency for each of Bruce A and Bruce B, defined as the frequency of accidents causing a release to the environment greater than 10^{14} Bq of Cesium-137.

Parametric uncertainty		Modeling uncertainty	Completeness uncertainty	
•	Mean values represented in results	 Low uncertainty for internal failures at power and during outage Conservative assumptions for 	• Comprehensive scope/screening of internal and external reactor hazards. PSA performed for hazards not screened out.	
		fire initiating events	 External hazards cutoff frequency 	
		 Conservative modeling for external hazards seismic and 	1x10 ⁻⁴ per year. Margins assessed for lower frequency events.	
		high winds	 Spent fuel events addressed deterministically and in the process of being screened for PSA. 	

Overall results characterization

- Individual hazard category results meet the LRF PSA goal of 1x10⁻⁵ per reactor per year
- Sum of internal events LRF = 3.8x10⁻⁶ per reactor per year for Bruce A and 1.3x10⁻⁶ per reactor per year for Bruce B. Both meet LRF PSA goal of 1x10⁻⁵ per reactor per year.
- LRFs for seismic and high winds are small in comparison to aggregated internal events LRF.

Multi-unit considerations

• Fire hazard category dominates both single unit and multi-unit results at both stations

Defence-in-depth characterization

• Defence-in-depth improvements implemented or in progress based on Fukushima lessons learned, including fire protection improvements such as early smoke detection

Safety margin characterization

- No vulnerabilities identified. No risk dominant sequences identified in Fire PSA.
- Seismic results do not increase significantly until severity exceeds that associated with 1 in about 40,000 year frequency.
- High wind SCDF doubles for event frequencies as low as 1 in 140,000 years.

Performance monitoring

- Bruce Power governance ensures potential design or operational changes are evaluated for impact on PSA results
- Bruce Power governance requires review if average or instantaneous PSA goals are exceeded
- PSAs are updated every 5 years or sooner if major changes occur

Integrated decision-making inputs

PSA goal met?	Defence-in-depth	Safety margins	Multi-unit implications	Performance monitoring
Yes	Confirmed	Dominated by fire hazard category	Fire still the dominant hazard category. No additional insights.	Annual average and instantaneous LRF monitoring

Conclusions

- Individual hazard categories and the sum of results for internal hazards meet the LRF goal
- Large margins for external hazards with frequencies 1 in 10,000 years or greater
- Fire dominates LRF. Results credit EME and improvements in fire detection equipment. There are no risk-dominant sequences.

5.0 PHYSICAL DESIGN

No update is provided.

6.0 FITNESS FOR SERVICE

6.7 Fuel channels

Detailed information on the fracture toughness of pressure tubes was provided in the Performance Review (Section 6.7, Reference A1), supplemental material (Reference A13), and February update to the Performance Review (Section 3, Reference A2).

Selected updates have been provided here for clarity with respect to ongoing discussions at the Commission hearings. **Hydrogen equivalent concentration** $[H_{eq}]$ Measurement of deuterium concentration in the pressure tube (converted into hydrogen concentration); parts per million.

Upper shelf temperature region At or above 250° C (the main mode of operation)

Transition temperature region Below 250° C (a mode of operation with very limited duration)

Cohesive zone model

Fracture toughness model that applies in the transition temperature region (< 250° C)

6.7.1 <u>Deuterium uptake</u>

As discussed previously, based on conservative predictions, a limited number of Bruce pressure tubes are predicted to have hydrogen equivalent concentrations of 120 ppm or greater, in a small portion of the pressure tube (<50 mm), at the time of the MCR outage (see Figure 6).

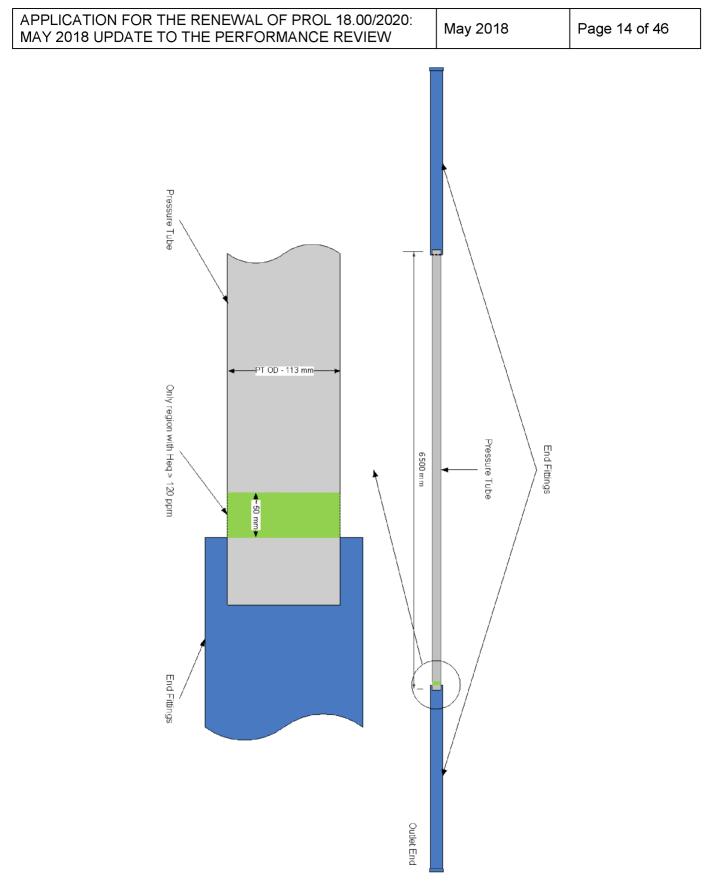


Figure 6. Schematic of pressure tube and end fittings. The small region for $[H_{eq}]$ is predicted to reach 120 ppm is shown (green) in the expanded view.

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Based on these conservative predictions (using a deterministic methodology), Unit 6 and Unit 5 are the first two units for which some tubes are predicted to reach 120 ppm [H_{eq}] (November 2019 and February 2020, respectively). Note that Unit 6 will undergo an MCR outage in 2020; the Unit 5 MCR outage is scheduled for 2026. This is followed by Unit 7 (January 2022) and Unit 8 (October 2026). See Table 2; for comparison, the MCR outage schedule is shown in Figure 7.

Table 2. Dates upon which [<i>H</i> _{eq}] will reach 120 ppm
(with deterministic and probabil	listic methodologies)

Unit	Deterministic	Probabilistic
3	Beyond MCR	Beyond MCR
4	Beyond MCR	Beyond MCR
5	Feb 2020	Beyond MCR
6	Nov 2019	Beyond MCR
7	Jan 2022	April 2028
8	Oct 2026	Beyond MCR

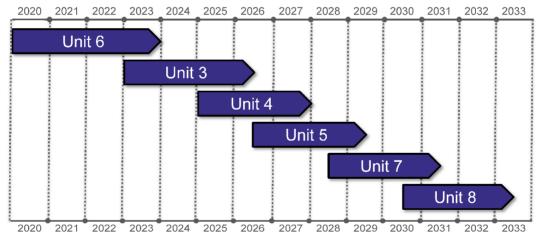


Figure 7. Planned schedule for MCR outages (may be subject to change)

The predicted $[H_{eq}]$ at the time of the MCR outage is shown in Table 3 (this table was previously provided in Reference A2, but has been re-ordered to show the units in order of MCR outage).

Table 3. MCR outage date, target operating life (EFPH), and projected
maximum [<i>H_{eq}</i>] (with deterministic and probabilistic methodologies)
at the outlet rolled joint region for Bruce units 3-8.

MCR outage date	Unit	Projected operating life	Deterministic	Probabilistic
2020	6	245,000 EFPH	121 ppm	99 ppm
2023	3	245,000 EFPH	102 ppm	92 ppm
2025	4	255,000 EFPH	104 ppm	81 ppm
2026	5	300,000 EFPH	151 ppm	120 ppm
2028	7	300,000 EFPH	147 ppm	120 ppm
2030	8	300,000 EFPH	139 ppm	103 ppm

Note: projections of $[H_{eq}]$ were made for each individual fuel channel assembly, based on the temperature and pressure in that channel.

The predictions for $[H_{eq}]$, shown above and as discussed in previous submissions to the Commission, are based on a conservative deterministic calculation. Bruce Power will be discussing with the CNSC the use of a more realistic, probabilistic methodology. This probabilistic approach predicts that $[H_{eq}]$ will not exceed 120 ppm in any unit until April 2028 (Unit 7).

At the end of the proposed licensing period (September 2028), only Unit 8 will not yet have a Major Component Replacement outage completed or in progress. The predicted $[H_{eq}]$ for Unit 8 in 2028 is 129 ppm at 286,000 EFPH (deterministic methodology). Using the probabilistic approach, the predicted $[H_{eq}]$ is 96 ppm.

6.7.2 Burst test program

In order to demonstrate fitness-for-service of the pressure tubes, Bruce Power developed fracture toughness models. These models are key inputs into the leak-before-break and fracture protection assessments, which demonstrate the safe operation of pressure tubes in the unlikely event of a through-wall crack penetration. (These assessments are provided to CNSC staff.)

The fracture toughness models have been thoroughly validated in the upper shelf temperature region (which is the predominant mode of operation). With respect to the lower shelf temperature region, the model has been validated, and additional data is planned to be collected to more thoroughly demonstrate validity.

A schematic showing the relationship between fracture toughness, temperature, and the fracture toughness model is provided in Figure 8. This figure shows how the models for fracture toughness (upper shelf and cohesive-zone) bound observed fracture toughness data. As deuterium concentrations increase, observed fracture toughness decreases, but the cohesive-zone model changes shape to continue to conservatively bound the observed data.

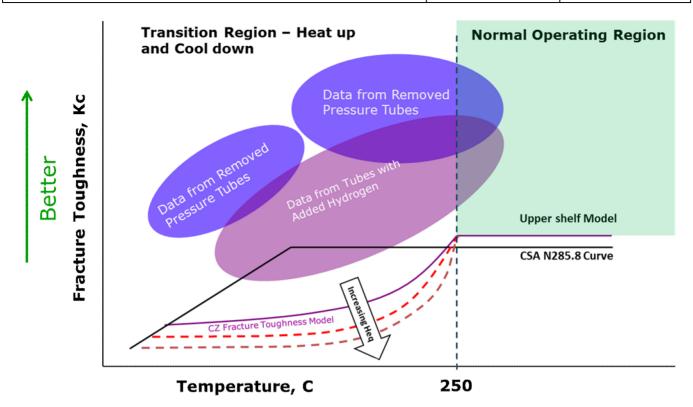


Figure 8. Relationship between fracture toughness, temperature, and the fracture toughness models

A robust research program was initiated several years ago to improve knowledge of pressure tube behavior as hydrogen levels increase. A key element of this research is the burst test program, described in Reference A2. (It is important to note that the burst test program is not the only research program that supports validation of the fracture toughness models. An extensive suite of experimental and analytical R&D is underway, and updates are provided to the CNSC regularly.)

Burst tests are planned through 2022; more may be planned for 2023 and beyond, following evaluation of the test results. A current (revised) summary of the planned burst tests through 2019 is provided in Table 5.

Overall, the burst test program is expected to extend the limit of the upper shelf fracture toughness model to 140 ppm by the end of 2018 and to 160 ppm by Q2 2019. The limit of the cohesive-zone fracture toughness model is expected to be extended to 160 ppm by the end of 2019. The timing and test conditions may be adjusted, based on test results. As mentioned above, improvement to the predictions of $[H_{eq}]$ in pressure tubes is underway.

Target hydrogen concentration	Primary goal
160 ppm	Evaluate fracture toughness at replacement date for Bruce tubes (additional tests needed to address tube-to-tube variability)
60-70 ppm	Evaluate effect of chlorine and orientation of deposited hydrides (most relevant for lower $[H_{eq}]$ concentrations at the inlet or front end section)
As received	Evaluate baseline fracture toughness in tubes removed from operating reactors

Table 4. Summary of planned burst test program goals

Table 5. Current pressure tube burst test program (2018-2019 only);subject to change.

Year	Test #	Target [<i>H_{eq}</i>] (ppm)	T(° C)
2018	n/a	As-received	250
	BT-33	80	250
	BT-34	80	< 250
	BT-35	80 or TBD	< 250
	BT-36	160	250
	BT-37	160	< 250
2019	BT-38	160	< 250
	BT-39	160	< 250
	BT-40	160	< 250
	BT-41	160	< 250
	BT-42	80 or TBD	< 250

7.0 RADIATION PROTECTION

No update is provided.

8.0 CONVENTIONAL HEALTH AND SAFETY

No update is provided.

9.0 ENVIRONMENTAL PROTECTION

9.5 Environmental Risk Assessment

References to the Quantitative and Predictive Environmental Risk Assessments are provided in Section 15.16.

10.0 EMERGENCY MANAGEMENT AND FIRE PROTECTION

10.2 Nuclear Emergency Preparedness and Response

10.2.1 <u>Provincial Nuclear Emergency Response Plan</u>

In December 2017, the Ontario Fire Marshall and Emergency Management (OFMEM) implemented a new revision of the Provincial Nuclear Emergency Response Plan (PNERP). This revision provided enhancement to allow for further alignment to Canadian standards (CSA N1600 and REGDOC-2.10.1) and international guidance (IAEA GSR Part 7, *Preparedness and Response for a Nuclear or Radiological Emergency*). The changes also included a review of the technical analysis of the potential hazards associated with highly unlikely multi-unit events within a CANDU plant. The review provided opportunity for public comment.

The changes to the PNERP include:

- Creation of a new Contingency Planning Zone (10-20 km from the nuclear facility), clarifying how local protective actions could be implemented if needed;
- Updated descriptions of accident scenarios, including severe accidents;
- Clear descriptions of key emergency response activities for various accident scenarios;
- Formalized review and public consultation requirements;
- Guidelines for protective actions, roles and responsibilities for stakeholder organizations, and updated training and exercise requirements consistent with national and international standards; and,
- A more detailed rationale behind key features of the plan, including planning zone sizes.

For comparison, the planning zones associated with the former PNERP 2009 are shown in Figure 9, while the updated planning zones associated with the PNERP 2017 are shown in Figure 10.

The PNERP 2017 has aligned intervention levels to recent changes in Health Canada guidelines. The PNERP 2017 defines Generic Intervention Levels (formerly known as Protective Action Limits, or PALs) and an associated suite of Operational Intervention Levels. The PALs defined in the former PNERP 2009 are shown in Table 6, and the updated Generic Intervention Levels associated with the PNERP 2017 are shown in Table 7.

Bruce Power is working with the local designated and host municipalities (Kincardine and Saugeen Shores, respectively) to finalize municipal implementing plans that meet the new requirements of PNERP 2017. More specifically, Bruce Power is supporting the municipalities in meeting the Contingency Planning requirements for alternate facilities beyond the contingency planning zone (>20km), evacuation activities, radiological monitoring, decontamination, and emergency worker support.

Municipal plans have been drafted and are ready to be finalized and implemented. Some additional work is still required to identify alternate facilities, Mennonite populations, schools, and retirement homes.

In addition, Bruce Power plans to enhance its mobile decontamination capability, as discussed in the Performance Review (Reference A1 and Reference A2). Bruce Power also plans to update plume modelling analytical software with the new Generic Intervention Levels identified in the PNERP 2017.

In 2019, Bruce Power plans to again participate in a major inter-utility corporate exercise (Huron Resilience). This will be the first exercise to validate the new PNERP 2017, and will involve the province, municipalities, OPP, Grey-Bruce Health Unit, and many others.

Following final design and installation, refined CFVS calculations will be undertaken: the CFVS will change the amount and distribution of isotopes relevant to a hypothetical large release. Note that the combination of EME and CFVS is expected to generally result in no evacuation needed, even for most four-unit events. Bruce Power plans to provide the relevant data to the province in order for the impact of CFVS to be taken into account with respect to emergency planning.

	Lower level		Upper level	
Protective measure	Effective dose	Thyroid dose	Effective dose	Thyroid dose
Sheltering	1 mSv	10 mSv	10 mSv	100 mSv
Evacuation	10 mSv	100 mSv	100 mSv	1 Sv
Thyroid blocking	n/a	100 mSv	n/a	1 Sv

Table 6. Former Protective Action Levels (PNERP 2009)

Table 7. Current Generic Intervention Levels (PNERP 2017)		
Projected dose	Protective Actions	
10 mSv whole body in the first 2 days	Sheltering	
50 mSv to thyroid in the first 7 days	lodine thyroid blocking	
100 mSv whole body in the first 7 days	Evacuation	



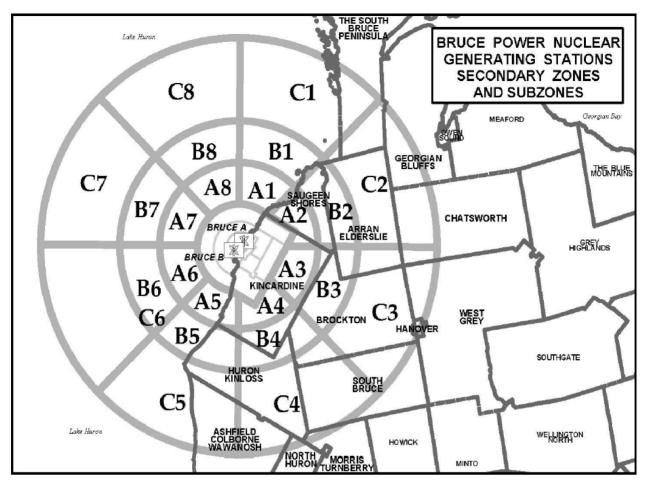


Figure 9. PNERP 2009 planning zones

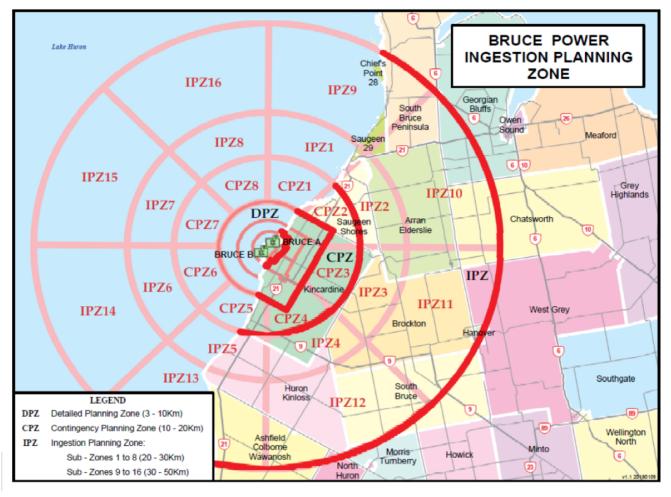


Figure 10. PNERP 2017 planning zones

10.2.2 Fukushima enhancements

Based on lessons learned from Fukushima, Bruce Power implemented numerous enhancements to emergency preparedness, including physical **Emergency Mitigating Equipment** (EME) Fire pumper trucks, generators, and support equipment that can be used to prevent and/or mitigate severe accidents through the provision of cooling water and alternate sources of electrical power to key systems.

enhancements to the nuclear facilities and supporting infrastructure, enhancements for data retrieval and sharing, and training. The enhancements were described in the Performance Review (Reference A1 and A2). In this section, the significance of Emergency Mitigating Equipment (EME) for preventing and mitigating an accident is discussed.

Following the Fukushima incident, Bruce Power developed enhancement plans to include short-term enhancements (providing maximum safety benefits in the shortest time), longerterm enhancements (requiring additional analyses, assessments, and modifications), and enhancements to emergency response facilities and processes. These enhancements are intended (in order of importance) to improve the ability of Bruce Power to:

1. Prevent severe accidents,

- 2. Mitigate severe accidents that are already in progress, and,
- 3. Prevent the release of radioactivity to the public from a severe accident.

<u>The actions designed to prevent severe accidents were implemented immediately following</u> <u>Fukushima and were completed primarily in 2012, with some completed in 2013</u>. These short-term enhancements focused on providing additional make-up water and included installation of dry hydrants, installation of redundant EME connections to steam generators and to irradiated fuel bays, and procurement of EME.

Based on these modifications, EME (fire pumpers) can draw water from the Condenser Cooling Water outfall via dry hydrants, and then deliver that water to systems as required through EME connections. Two off-site fire pumpers are dedicated to each of Bruce A and Bruce B, with an additional spare truck. The pumpers are supported by a fuel truck and by equipment capable of clearing debris. These enhancements made in 2012 and 2013 provided the ability to completely prevent many severe accidents.

- Accident prevention: in a severe, multi-unit accident scenario, over 5 hours will be available for the deployment of EME to provide additional cooling water. This deployment can be accomplished within 30 minutes. Once deployed, the EME will provide the cooling water needed to terminate the accident and fully prevent the release of radioactivity.
- Accident mitigation: implementation of enhancements aimed at mitigating severe accidents, should they occur, has made significant progress and are planned to be be complete by 2019² (Reference A2). At this time, all units have at least one means of utilizing EME to limit progression of an event, and Severe Accident Management procedure guides are in place. Additional means to mitigate an event through use of EME quick connections has been completed in 4 units³, with 3 more due in 2018 and the last in 2019. In conclusion, in the unlikely event that a severe accident were to progress to the point of a radioactive release, the application of EME currently installed would significantly mitigate that release.

To prevent increased releases to the public in a severe event, Bruce Power has developed a design for a passive containment filtering system that will ensure releases are filtered by maintaining containment pressures well below design pressure, even in a severe multi-unit event. Bruce Power plans to finalize the design by late 2019/early 2020 and to install the system at both stations by 2022.

In summary, the Fukushima event was thoroughly reviewed, a clear understanding of the lessons learned was achieved, actions were developed and a rapid properly prioritized implementation program undertaken. To understand the impact of these improvements, Bruce Power and OPG implemented a third-party analysis with respect to the impact of EME on five case studies, including some beyond design basis accidents (Reference A8). The summary of the case studies (Table 9, Reference A2) is provided in Table 8 below.

³ See previous footnote.

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 $[\]frac{2}{3}$ The final tie-in for the Unit 6 installation is planned for the MCR outage beginning in 2020.

Case	Accident	EME	Result	Details
1	Single-unit	None	Within sheltering PALs (<10 km) Below sheltering PALs (>10 km) Evacuation PALs not exceeded	Rapidly-developing large Loss of Coolant Accident (LOCA)
2	Two-unit	None	Within sheltering PALs (<10 km) Below sheltering PALs (>10 km) Evacuation PALs not exceeded	Loss of heat sink (corium relocates to the fueling machine duct)
3	Four-unit	Limited	Within sheltering PALs (<10 km) Below sheltering PALs (>10 km) Evacuation PALs not exceeded	Loss of heat sink (station blackout) with no station response that requires power. Assumes limited EME application to mitigate consequences only (emergency moderator makeup two hours after core is uncovered, and end shield tank makeup after 24 hours), and does not include prevention.
4	Four-unit	Very limited	Exceed evacuation PALs (<10 km) Exceed evacuation PALs (>10 km)	Loss of heat sink (station blackout) with no station response that requires power. Assumes very limited EME (end shield makeup after 24 hours).
Note: application of additional EME will reduce dose levels to levels similar to Case 3.				
5	Four-unit	None	Exceed evacuation PALs (<10 km) Exceed evacuation PALs (>10 km)	Four-unit loss of heat sink (station blackout) with unrealistically-minimal station response.

Table 8. Case studies for accident analysis to support emergency planning

Note: application of additional EME will reduce dose levels to levels similar to Case 3.

Case 5 is considered a worst-case scenario, which assumes an unrealistic and highly limited amount of operator action, with no deployment of EME to prevent or to mitigate the accident. If EME designed for prevention was applied, the accident can be completely prevented. If even limited EME is applied, as in Case 5, hours after the accident has begun, the accident and release are terminated, resulting in public dose levels that do not require evacuation and are similar to that of a design basis accident.

The deployment of EME is rapid. As stated above, to prevent a release, over 5 hours are available before additional cooling water is required—but EME can be deployed in a much shorter time frame. In 2013, Bruce Power demonstrated the application of EME to four units through individual couplings in each unit—starting from off-site deployment and including the clearing of debris along the path of deployment, this was accomplished in approximately one hour. The deployment time was reduced through installation of the inter-unit feedwater tie, and currently (2017) the time to deploy EME to all four units is approximately 30 minutes.

Prevent accidents: again, Bruce Power emphasizes that the enhancements made in 2012 and 2013 provide the ability <u>to completely prevent many severe accidents</u>, as well as <u>to mitigate severe accidents</u> in progress.

Mitigate accidents: mitigation of severe accidents is provided through Severe Accident Management Guideline (SAMG) toolkits (completely installed by 2016): these toolkits provide the specialized hardware and tooling to allow application of EME to the Primary Heat Transport System, Moderator System, and Shield Tank. The toolkits are intended to be flexible and allow for responders to physically install new connections for EME when needed for emergency response. The toolkits provide additional provisions for make-up water, above and beyond the permanent EME couplings that were installed in 2012 and 2013.

Although SAMG toolkits allow for application of EME to the Primary Heat Transport System, Moderator System, and Shield Tank, Bruce Power is currently installing permanent connections (quick connections) for the EME to provide a redundant path that can be connected very quickly. All operating units will be completed by 2020.

Prevent releases: the planned installation of passive Containment Filtered Venting System (CFVS) is intended to <u>prevent the release of radioactivity to the public</u>, even in the event of a multi-unit severe accident. The CFVS will provide a completely passive system for filtration of radioactive releases from containment. While the final analysis will not be completed until the CFVS is installed and tested, the CFVS is expected to reduce—on the order of 10,000 times—the quantity of radionuclides that could be released in an event that would have assumed a containment failure which will now be prevented. As discussed earlier, the application of EME and CFVS is expected to result in no need to evacuate even in many four-unit accidents; Bruce Power plans to provide the relevant information to the province when the final analysis is complete.

A more complete discussion of post-Fukushima physical modifications was provided in the Performance Review (References A1 and A2).

10.2.3 ARES 3D simulation software

Bruce Power is currently working with ARES to enhance emergency response analytics and training through development and implementation of three-dimensional simulation capability. A three-dimensional site model is used as part of a Monte Carlo simulation to analyze emergency response during a severe weather event, including tornados. The software is designed to simulate a wide variety of potential barriers to deployment of EME.

The simulation and analytic software has been developed. Installation is planned for 2018 Q2. Following installation, Bruce Power plans to evaluate the potential for this novel software for integration within the emergency response program, for enhancement of the PSA results, and for support of training and emergency response planning.

11.0 WASTE MANAGEMENT

Further information on Bruce Power's response to the SON's written submission to the CNSC (CMD 18-H4.146) in relation to the PROL renewal application is provided as Enclosure 3 of this submission.

12.0 SECURITY

No update is provided.

13.0 SAFEGUARDS AND NON-PROLIFERATION

No update is provided.

14.0 PACKAGING AND TRANSPORT

No update is provided.

15.0 OTHER MATTERS OF REGULATORY INTEREST

15.12 Indigenous engagement

An update with respect to the status of engagement and consultation efforts of Bruce Power and Indigenous groups is provided below. Bruce Power will be providing separate reports on its engagement efforts with each community that will supplement the community interest reports previously provided to the Commission (Reference A15); see Enclosures 4, 5, and 6 of this submission.

The Site is located within the traditional territory of the Saugeen Ojibway Nation (SON) and the traditional harvesting territories of the Historic Saugeen Métis (HSM) and the Métis Nation of Ontario (MNO). Since December 2015, Bruce Power has been providing information to the SON, the HSM, and the MNO about the application for renewal of the Power Rector Operating Licence (PROL) and Major Component Replacement (MCR). Bruce Power has had several meetings with each community to discuss the application and associated regulatory approvals and any questions or concerns. The HSM has indicated their support for the application, and the SON and MNO have raised a number of concerns which Bruce Power has discussed with them, and measures have been proposed by Bruce Power and/or CNSC staff to address these issues.

Bruce Power has proposed further steps to address concerns raised by the SON and the MNO. These concerns and the information provided to Bruce Power to date have not changed Bruce Power's conclusion that the continued operation of the site (including the life extension activities) will likely not have an appreciable impact on asserted or established Aboriginal or treaty rights. Any such impact would be, at most, minimal and no different from the current impact of the Bruce site (which has been operating safely for decades).

In addition, it is important to underscore that Bruce Power is applying for a ten-year operating licence. The continued operation of the Site after 2028 is contingent on further CNSC reviews and Commission approval of a renewed operating licence. These further reviews will take into account the results of additional studies, research, and environmental monitoring programs, any changing environmental conditions, and the impact of these conditions on operations at the Bruce site. These reviews will also provide further consultation opportunities for Indigenous groups.

The CNSC, in its role as a life-cycle regulator, will be responsible for annually reviewing the performance of Bruce Power, including Bruce Power's environmental protection program. In order to continue operating during the Licensing Period, Bruce Power will also be required to meet a range of other provincial and federal regulatory requirements that are relevant to key areas of concern of the SON and MNO.

15.12.1 Saugeen Ojibway Nation

Since January 1, 2018, Bruce Power and the SON have continued their dialogue with respect to the application for renewal of the PROL, employment, training, and business opportunities. This dialogue has occurred through face-to-face meetings, letter exchange, emails, and

telephone conversations. Dialogue prior to January 1, 2018 was previously reported to the Commission in the lead-up to the Part 1 Hearing.

Bruce Power and the SON have held five meetings since January 1, 2018 to discuss issues and questions related the PROL renewal application and other regulatory approvals, including:

- Mitigation measures (for impingement, entrainment, and thermal effects) and fisheries offset projects as proposed in the Fisheries Act Authorization application;
- The methodology used to prepare the Environmental Risk Assessment (ERA) and Predictive Effects Assessments (PEA);
- Climate change and cumulative effects;
- The SON's and Bruce Power's respective and separate meetings with respect to engagement with the CNSC, Fisheries and Oceans Canada, and Ontario Ministry of Environment and Climate Change;
- The assessment of thermal effects and the Thermal Environmental Compliance Approval Application; and,
- Environmental monitoring and stewardship initiatives.

In addition, there have also been three meetings between the leadership of the SON and Bruce Power since January 1, 2018, to discuss employment, training, and business opportunities from the ongoing operation of the Bruce site and from life extension investments and ways to enhance the SON's involvement in regulatory decision-making, environmental monitoring, and stewardship activities, which included some discussions relating to the Application.

The SON were also provided with a tour of the facility in March 2018 to help further explain the processes of impingement and entrainment. Additionally, the SON attended a meeting between Bruce Power, CNSC, and Fisheries and Oceans Canada to discuss the Fisheries Act Authorization application, with a particular focus on the current draft offset plan and the monitoring for these offsets. These meetings are in addition to the meetings that the SON have had with the CNSC regarding the PROL renewal application in 2017 and 2018.

The SON have not requested any other meetings with Bruce Power to discuss issues relating to the application for renewal of the Power Reactor Operating Licence in advance of the Commission Hearing, Part Two. There are, however, further meetings planned to continue dialogue and progress issues that were discussed in the leadership meetings.

In meetings with Bruce Power, the SON have expressed concerns with respect to:

- Impacts to fish from entrainment, impingement, and thermal effects and the adequacy of current mitigation measures;
- The adequacy of environmental monitoring and fisheries data that was relied upon by Bruce Power;

- The adequacy of the approach to assessing impacts in the PROL renewal application, including ERA/PEA methodology, the assessment cumulative effects, and the need to assess impacts from climate change;
- Legacy issues and nuclear waste; and,
- SON employment, training, and financial participation in the Bruce site.

The SON have also taken issue with Bruce Power's characterization of certain information in the SON community interest report and Bruce Power's assessment of impacts on the SON's asserted and established Aboriginal and treaty rights from the continued operations and life extension of the Bruce site. The SON have not provided any information that would change Bruce Power's assessment that the continued operation of the Site and the life extension activities will likely not have an appreciable impact on the SON's asserted and established rights, and that any impact would at most be minimal.

This assessment is consistent with previous assessments carried out since 2001. Bruce Power is, however, willing to work with the SON on additional monitoring measures to verify these assessments. The company has also advanced a number of projects that continue to enhance the health of the fishery and will, through an offset program associated with other regulatory requirements, offset the minimal impacts which have been quantified.

In response to concerns and questions raised by the SON, Bruce Power has provided further information with respect to current mitigation measures for impingement, entrainment, and thermal effects, the alternative mitigation measures that have been considered by Bruce Power, and why these additional measures are not feasible, appropriate, and/or necessary to deploy. Bruce Power has also provided further information with respect to its approach to assessing impacts in the PROL renewal application, including the PEA and the assessment of cumulative effects and discussed the SON's concerns relating to this and future impacts of climate change.

While the issues that the SON have raised have not changed Bruce Power's conclusions regarding environmental impacts or impacts to SON Aboriginal and treaty rights, Bruce Power and/or CNSC staff have proposed a number of measures in response to the SON's concerns or identified existing or future mechanisms available to the SON to address their concerns:

- Environmental Study and Monitoring Programs: Bruce Power and the CNSC staff have proposed or will be undertaking several measures to continue to enhance already robust environmental monitoring, expand the SON's involvement in environmental monitoring, and reduce uncertainties in the current fisheries data:
 - Bruce Power has offered capacity funding to the SON and has begun discussions with the SON to develop a joint environmental monitoring and stewardship program that will enhance the SON's involvement in the design and implementation of monitoring measures. Bruce Power and the SON Environment Office recently met and agreed to an action plan for the development of a joint environmental monitoring program focused on three key areas of concern raised in this engagement: impingement/entrainment, thermal, and climate change;
 - The CNSC staff have invited the SON to be involved in CNSC's Independent Environmental Monitoring Program (IEMP) and will be asking the Commission to

direct CNSC staff to work with the SON on the planning and sampling for the IEMP. CNSC have asked the SON to identify any special foodstuffs or other environmental aspects of significance to the SON that could be included in the program and invited the SON to assist in gathering the samples to be monitored.

- The CNSC staff will be proposing a thermal effluent study and analysis program which will focus on enhancements to environmental monitoring to support the development of a winter plume model and reduce uncertainties surrounding the impact of the thermal plume. Bruce Power will also be consulting on the development of an updated thermal monitoring plan for the Environmental Compliance Approval Application;
- The CNSC staff will be proposing a fish impingement and entrainment study and analysis program that is focused on enhancing the previous impingement and entrainment monitoring plan. Bruce Power will be consulting with the SON on the development of an updated impingement and entrainment monitoring plan for the Fisheries Act Authorization application. This plan will be designed to understand and reduce uncertainties in the data collected and will be reviewed by both the CNSC and DFO; and
- Bruce Power will be consulting with the SON on the development of any additional enhancements to the environmental monitoring program beyond the above that are recommended or required by the CNSC.
- Offset Measures for Impingement and Entrainment: Bruce Power has repeatedly asked the SON since February 2016 to identify potential projects to offset impacts from impingement and entrainment as part of the Fisheries Act Authorization. Bruce Power has not received any proposals to date and has included proposed offset projects in the Fisheries Act Authorization. In an effort to advance dialogue with the SON about additional projects, Bruce Power proposed an additional five projects to the SON in February 2018 that would benefit the SON fishery and the environment as a whole and further offset any impacts from impingement and entrainment. These additional projects were identified through discussions with Saugeen First Nation community members. Bruce Power reiterated at a meeting with SON Leadership on May 2, 2018 that Bruce Power is interested in undertaking jointly developed environmental stewardship projects in the SON's traditional territory whether these are part of the Fisheries Act Authorization or not. Bruce Power remains open to other proposals from the SON and is eager to work with the SON on projects that could help benefit the SON fishery.
- Alternative Mitigation Measures Assessment: CNSC staff will be proposing a requirement in Bruce Power's proposed Licence Conditions Handbook that would require it to conduct a further assessment of feasible mitigation measures for thermal effluent and fish impingement/entrainment by December 31, 2019. This assessment is in addition to work that Bruce Power has already done to consider alternative mitigation measures as part of the ECA Application and the Fisheries Act Authorization application, and previous environmental assessments. It is being proposed in the event the level of risk changes in the future and notwithstanding the position of CNSC staff that additional mitigation measures are not necessary at this time, and that Bruce Power has and will continue to make adequate provision for the protection of the environment. This information can be incorporated into the next Environmental Risk Assessment, which will be submitted to the CNSC in 2022.

- **Climate Change:** Bruce Power has invited the SON to participate in and shape the scope of a 3-year climate change study that was recently announced by Bruce Power and the Council of the Great Lakes Region. This study will provide insight into, among other things:
 - The state of climate change science in the Great Lakes Region;
 - The impact of a changing climate on various ecosystems and sectors in the Great Lakes, including the region's aquatic environment, fisheries and Bruce Power's operations;
 - The knowledge and decision-making systems companies and communities need to better manage changing risks as a result of climate; and,
 - The role that Bruce Power and other sectors might play in tackling climate change on a local and regional level, and how companies can adjust their corporate sustainability strategies to limit their impact.

The data gathered through these additional measures will be used to inform future applications for CNSC licence renewals, Fisheries Act Authorizations, ECA Applications, Permits to Take Water, and any changes required to monitoring program. It will also be incorporated into future ERAs and PEAs, which are updated at least every five years, or earlier if there are significant changes in operations or in the science on which the ERA is based. The next ERA and PEA will be submitted to the CNSC in 2022, prior to the restart of the first refurbished reactor in December 2023 under the current proposed schedule. As a lifecycle regulator, CNSC staff can direct Bruce Power to take further action in response to any updated information.

While Bruce Power and the SON have differing views over the adequacy of the assessments and baseline data included in the PROL application, Bruce Power is eager to work jointly with the SON to reduce areas of disagreement and build SON confidence in the assessments and monitoring programs. Discussions between Bruce Power and the SON on these issues remain ongoing. Bruce Power is committed to strengthening its relationship with the SON and working with the SON on the various measures that have been proposed to address their concerns.

Further information on Bruce Power's response to the SON's written submission to the CNSC in relation to the PROL renewal application (CMD 18-H4.146) is provided as Enclosure 3 of this submission.

15.12.2 Métis Nation of Ontario

Since January 1, 2018, Bruce Power and the MNO have continued their dialogue with respect to the PROL renewal application, as well as with respect to employment, training, and business opportunities. This dialogue has occurred through face-to-face meetings, emails, and telephone conversations.

Bruce Power and the MNO have held four meetings since January 1, 2018 to discuss issues relating to the PROL renewal application, other regulatory approvals (the Fisheries Act Authorization and the Environmental Compliance Approval applications), and employment, training, and business opportunities for the MNO relating to the Site. Through these meetings,

correspondence, and written submission to the Commission (CMD 18-H4.57), the MNO have expressed concerns about a number of issues, including:

- A lack of incorporation and assessment of MNO Valued Components in the Environmental Risk Assessment (ERA) and Predictive Effects Assessment (PEA), including perceptive effects of the project on MNO rights and interests;
- Gaps in baseline data in the ERA and PEA with respect to predicting impacts on Métis rights and interests and the need for MNO involvement in monitoring;
- Cumulative effects and climate change, particularly the combined effects of thermal emissions, rising lake temperatures, other thermal influences, and nutrient loading on important aquatic life and habitats;
- A lack of involvement of the MNO in emergency response plans; and,
- The adequacy of the proposed fisheries offset projects.

In the course of the engagement, Bruce Power responded to 143 written comments or questions relating to the Application from the MNO's consultant, MNP. The vast majority of the comments were responded to on March 26, 2018, and the remaining three were responded to on April 9, 2018. While Bruce Power and the MNO have differing views over the adequacy of the assessments and baseline data included in the Application, Bruce Power is eager to work collaboratively with the MNO to address three specific recommendations that they made during the engagement. Specifically, Bruce Power and the MNO have agreed to co-develop:

- 1. A MNO monitoring program to ensure that MNO Valued Components are being appropriately monitored, assessed, and are incorporated into future ERAs;
- 2. A MNO-specific diet survey to further assess and understand any impacts of the Site on MNO citizens' health, which will verify the conclusions of the existing Human Health Risk Assessment; and,
- 3. A MNO Emergency Communications and Management Plan.

Bruce Power and the MNO have identified preliminary tasks to implement these measures, and Bruce Power is looking forward to working with the MNO on these important initiatives. The MNO monitoring program and diet survey will be used to supplement existing data and verify Bruce Power's assessments of the impacts of the Site and proposed life extension on MNO citizens' health and MNO harvesting rights, which are discussed in Section 7 of the MNO community interest report and have not changed.

With respect to climate change and the fisheries offset projects, Bruce Power has invited the MNO to participate in and shape the scope of the 3-year climate change study discussed above and has invited the MNO to identify alternative fisheries offset projects for impacts from impingement and entrainment and other ideas for general fisheries improvement projects that can be considered outside of the Fisheries Act Authorization application. Bruce Power has also invited the MNO to tour the Truax Dam Removal Project site (scheduled for May, 2018).

Bruce Power has also met with the MNO to discuss opportunities in the area of economic development. Further information on these discussions and Bruce Power's engagement with

the MNO on the PROL application will be provided in a supplementary community interest report in May 2018.

Bruce Power looks forward to continuing to strengthen its relationship with the MNO by working through the identified recommendations and priority items, in order to better incorporate the Métis way of life, thinking, and insights in future submissions, and looks forward to working together to advance other shared priorities.

15.12.3 Historic Saugeen Métis

Since January 1, 2018, Bruce Power and the HSM have continued their dialogue with respect to the PROL renewal application, employment, training, and business opportunities relating to the site. This dialogue has occurred through face-to-face meetings and email.

Bruce Power and the HSM have held four meetings since January 1, 2018 to discuss issues and questions related to the PROL renewal application, other regulatory approvals (the Fisheries Act Authorization and the Environmental Compliance Approval applications), and employment, training, and business opportunities for the HSM relating to the Bruce site. This has also included a tour of the Truax Dam, which is one of the proposed offset projects in the Fisheries Act Authorization.

Through meetings with Bruce Power and the HSM's written submission to the Commission (CMD 18-H4.55), the HSM indicated that they support the Application and have a significant interest in ongoing environmental monitoring and a profound need for ongoing involvement and engagement throughout the licence period given their traditional use of the lands and waters surrounding the Site. The HSM have concluded, based on current assessments, that there is no anticipated adverse effects to their harvesting rights, culture, or way of life in their traditional territory, which includes Lake Huron and the lands and waters in the counties of Bruce, Grey and Huron. The HSM have stated that through ongoing monitoring and open communications, they hope to continue to have confidence that the operations of the Site will either (a) not adversely impact HSM's traditional harvesting activities or (b) if adverse impacts arise, the parties will work together in a timely manner to identify measures to mitigate these impacts.

Bruce Power remains committed to continuing to share information with the HSM on Bruce Power's operations and monitoring measures throughout the licence period and to engage the HSM on any changes to Bruce Power's monitoring programs. Bruce Power has also invited the HSM to participate in and to shape the scope of the three-year climate change study discussed above.

Further information on Bruce Power's engagement with the HSM is included in a supplementary community interest report; see Enclosure 6 of this submission. Bruce Power remains committed to sustaining and strengthening its relationship with the HSM by continuing to share information with the HSM on the PROL renewal application and all other regulatory matters, and to working together to advance shared priorities.

15.12.4 Indigenous employment

Bruce Power is committed to providing training, education, and employment opportunities to Indigenous peoples. Bruce Power's annual goals include a commitment to providing

employment to local Indigenous community members and to increasing Indigenous employment.

Bruce Power is also committed to creating a culturally safe and inclusive work environment in which Indigenous peoples' cultures, beliefs, and values are acknowledged and respected. As part of new manager training, we speak to new Bruce Power leaders about local Indigenous culture and the importance this has to Bruce Power. In 2018, Bruce Power plans to enhance its Indigenous Cultural Awareness Training through integration of this training into the leadership development program for non-management workers.

Bruce Power has hired two full-time workers who are dedicated to increasing Indigenous employment at Bruce Power and with Bruce Power suppliers and building trades. These workers have the full support of the Human Resources team, as well as the broader organization when required. In addition, Bruce Power has introduced a number of measures designed to address barriers to increasing Indigenous employment through various education and training initiatives. A summary of these initiatives and the results are provided below.

The Indigenous Employment Team manages the Indigenous talent pool from local SON, MNO, and HSM community members, as well as Indigenous people across the country.

The process for managing the Indigenous Talent pool is as follows:

- A team member speaks with each candidate and reviews the candidate's résumé to obtain an understanding of that candidate's desired career paths and roles;
- The team reviews the candidate's qualifications against the requirements of the role. If the candidate does not meet the requirements of the role, the candidate's qualifications are reviewed against other opportunities, including those that are indirect to Bruce Power (e.g. opportunities with suppliers). A team member discusses potential education or experience programs that may assist with upgrading the candidate's education or experience;
- Each candidate is provided an opportunity for a practice interview prior to any official interview. If tests are part of the hiring process, the team also provides the opportunity for appropriate support beforehand;
- The Indigenous Employment Team works with the recruitment team to understand the upcoming recruitment needs of the business. Once roles become available, the candidate pool is reviewed, and Indigenous candidates are invited to apply to relevant positions; and,
- Throughout this process, the Indigenous Employment Team maintains communication with each candidate to inform the candidate with respect to process steps and milestones.

In order to increase the number of Indigenous candidates in the talent pool, we actively reach out to the communities and discuss employment at Bruce Power with potential candidates. We remain active in the communities and schools and keep the candidate pool informed of current and future opportunities. A number of additional initiatives are also under discussion and development with Indigenous communities to build on this progress to leverage the increase in employment expected on the Site during the Licensing period.

The numbers of Indigenous individuals hired directly or indirectly as a result of this effort is shown in Table 9.

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	2015	2016	2017	2018 (YTD)	Total
Permanent worker	5	3	18	1	27
Temporary worker	9	5	3	6	23
Contractor/supplier (indirect hire)	0	1	2	8	11
Development student placement	7	7	7	9	30
Work placement	0	0	3	1	4
Total	21	16	33	25	95

Table 9. Indigenous hiring (2015-2018)

Note: temporary worker includes "Appendix A" and augmented staff.

Overall, it is estimated a total of 135 employees currently working at Bruce Power have selfidentified as Indigenous. Since these are self-identification numbers there it is possible that there are additional Indigenous employees who have not self-identified. This number does not reflect Indigenous people who are employed through a Building and Construction trade or with one of suppliers and contractors. The company continues to make progress in this area and is committed to working with Indigenous communities to have greater numbers of Indigenous peoples as part of its workforce in the years to come.

This progress has been made possible with the support of other organizations which share Bruce Power's principles and goals. Highlights include:

- Power Workers' Union (PWU) Indigenous Internship Program: in 2016, Bruce Power and the PWU launched a new program to provide employment opportunities to Indigenous community members to gain mentored work experience at Bruce Power. This program is available to candidates who meet the educational qualifications for PWU positions, but require some additional experience to meet the requirements for a permanent PWU position. This program has been very well received across the organization and helps prepare candidates for future permanent positions within Bruce Power.
- Indirect hiring: the Indigenous Employment Team works closely with contractors and suppliers to identify their current hiring needs. The team provides potential candidates (with their permission) to the suppliers and follows up with the supplier to discuss next steps. The Indigenous Employment team also has a strong connection with the local Indigenous community and encourages and invites members of the community to be added to the pool of candidates. The team continues to build the network of suppliers by assisting with development of a hiring and training strategy focused on Indigenous candidates. The team reinforces Bruce Power's expectation that contractors and suppliers employ local Indigenous candidates within their organizations. These hires would not be reflected in the company's Indigenous employment numbers.
- Organization of Canadian Nuclear Industries (OCNI) partnership: the OCNI provides support to suppliers of the nuclear industry by organizing supplier days and delivering workshops that are relevant to industry partners. Bruce Power is proud to partner with the OCNI and actively participates as a member of OCNI's First Nations, Métis and Inuit (FNMI) committee to assist with Indigenous hiring strategies in the broader nuclear industry across the country.

 Building Trades: Bruce Power continues to strengthen its relationship with the Building Trades Unions. Members of the Bruce Power Labour Relations Team and Indigenous Employment Team have met with the trades unions to develop a strategy to increase the number of local Indigenous community members joining the trades halls. Bruce Power also actively participated on the Electrical Power Systems Construction Association Indigenous Board with the goal of increasing the number of Indigenous members throughout the association.

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While employment opportunities are an important component in Bruce Power's commitment to the Indigenous communities, equally important are opportunities that build knowledge, skills, and experience for community members—particularly the youth. Developing and executing education and training programs also means bridging gaps in accessibility to such programs. Bruce Power is committed to providing educational opportunities through tuition reimbursement and community outreach programs. Highlights of the education and outreach programs include:

- Indigenous Education and Work Experience Opportunity: this program assists Indigenous people with obtaining the minimum educational requirements necessary to compete for entry-level positions at Bruce Power. This program offers full tuition sponsorship for up to 10 Indigenous students annually in power engineering technology (Georgian College), office administration (Georgian College), and applied nuclear science radiation safety (Algonquin College). Despite this focus, if applicants are interested in other programs, Bruce Power will nonetheless consider the applicants for tuition reimbursement. This program includes a four-month, paid work placement at Bruce Power.
- Local school visits: a focus on local Indigenous youth is critical to ensuring Bruce Power helps build a deep pool of local Indigenous talent for the future. The Indigenous Employment Team works closely with local schools and speaks to students to discuss future career opportunities at Bruce Power. Topics include college/university student programs, opportunities within the skilled trades, the importance of Science, Technology, Engineering, and Math (STEM), the Indigenous Education and Work Experience Opportunity, Indigenous scholarship opportunities, and Bruce Power's high school co-op program.
- High school co-op opportunities: these co-op opportunities provide a full semester on site at Bruce Power for senior students from Bruce Grey Catholic District School Board and Bluewater District School Board. The four-credit program provides valuable experience, essential skills, and career exploration for students on a pathway to post-secondary apprenticeship, college, or university.
- Algonquin College satellite campus: Bruce Power and Algonquin College are in the final planning stages of bringing a satellite campus to host the Applied Nuclear Science and Radiation Safety program locally by Fall 2019. Transportation will be arranged for local Indigenous community members wishing to participate, if required, and where eligible, tuition will be sponsored through the Indigenous Education and Work Experience Opportunity.
- Summer and Development Student Programs: Bruce Power provides employment opportunities to local Indigenous students as part of the Summer and Development Student Program. Bruce Power ensures that each local student who meets the criteria of the program is provided with employment opportunities for term.

- Huronia Area Aboriginal Management Board (HAAMB) Skills Ready Program: Bruce Power was integral in the development of this unique three-year partnership, which gives participants exposure to Bruce Power through a six-week job shadow placement after completion of a 15-week training program offered through Georgian College.
- Skills Ontario: Bruce Power supports and participates in Skills Ontario programs with the goal of supporting youth—including Indigenous Youth—in gaining valuable insights into skilled trades and in highlighting the various career opportunities. Bruce Power is a proud partner and sponsor of the First Nations, Métis, and Inuit Student Conference as part of this year's Skills Canada Competition.

15.12.5 Additional updates

In June 2017, Bruce Power launched the Indigenous Relations Supply Network (IRSN) as part of its commitment to invest in the community; to increase Indigenous employment, training, and education initiatives; and to leverage potential business development opportunities for new and existing Indigenous-owned local businesses. To date, the IRSN has 25 members, and membership is growing.

ISRN members are asked to participate in the Progressive Aboriginal Relations (PAR) program of the Canadian Council for Aboriginal Business to allow for best practices to be used by our partner companies. IRSN members are committed to supporting the involvement of local Indigenous people in the economic spin-off activities and benefits produced through ongoing operations and future Major Component Replacement developments.

Bruce Power, through the IRSN, collaborated with the Organization of Canadian Nuclear Industries (OCNI) to establish a dedicated human resource and office space in Port Elgin. This physical presence will be the initial point of contact for the public, business members, and the local Indigenous communities to indicate our collective support of Indigenous business development and to promote interactions of community members with Bruce Power and its supplier network.

In order to build community capacity and to generate wealth through sustainable and longterm business ventures, Bruce Power plans to support the following strategies:

- To develop and implement a comprehensive Indigenous Procurement Policy;
- To work through the IRSN to increase the engagement between suppliers and the local Indigenous communities, including exploring opportunities in procurement processes;
- To enhance key reporting metrics in order to track progress and improvements; and,
- To support Indigenous business development initiatives that will provide economic opportunities to the SON, HSM, and MNO.

Indigenous procurement policies will drive the implementation of a local Indigenous preferential grading in order to evaluate proposals and to promote local Indigenous businesses. The modifier, planned to be in place by May 30, 2018, will favour proposals that involve local Indigenous ownership through the Bruce Power's supplier network and/or value-added work performed by local Indigenous people. The modifier will result in a benefit to local

Indigenous communities through direct contracts from Bruce Power and through contracts issued by Bruce Power's supplier network.

The economic benefit to Indigenous communities is summarized in Table 10.

Supplier	Value	Notes
Local Indigenous busines	ses	
Abraflex	\$6.1 million	Radiation protection suits, seals, gaskets, O-rings. Local Indigenous owned/staffed development supported by Bruce Power Supply Chain. Annual spending of \$1.5 M in 2016- 2017 and \$4.6 M in 2018.
EMC	\$200,000	Bruce Power Supply Chain and Outage and Maintenance Services, along with EMC Power, piloted the use of removable insulation blankets fabricated by an Indigenous consortium. Indigenous spending estimate: \$200,000 over one year (2018-2019). Extension options are in place for additional spending.
Bruce Peninsula Transit	\$140,000	At the Request for Proposal stage; Bruce Peninsula Transit has been identified as a potential provider for these services.
Non-local Indigenous bus	inesses	
Wahta Springs	\$41,000	Bottled water (Wahta Mohawk Territory). Annual spending of \$1000 in 2015 and \$20,000 in 2017-2018.
Cameco	\$2 billion	Cameco is Gold Level PAR member of CCAB. Exclusive fuel supply arrangement with a total value estimated to be \$2 billion to 2030.

Table 10. Economic benefit to Indigenous businesses

These economic benefits are in addition to the indirect economic benefits that the SON receive from the operation of the Site through its equity interest in the Bruce to Milton Transmission Reinforcement Project, which was constructed to transmit electricity from the Site and certain wind projects in the area to Hydro One's Switching Station in Milton.

15.16 Elements of the licence application package

As initially described in Reference A1, the licence renewal application package was structured as two primary documents: the licence renewal application and the performance review. These documents were supported with the following supplementary submissions to the Commission (technical material submitted directly to CNSC staff on request is not listed here):

- Integrated Implementation Plan (Reference A5),
- Unit 6 Return to Service Plan (Reference A7),
- Unit 6 Project Execution Plan (Reference A7),
- Environmental Risk Assessment (Reference A8),
- Final report for the U12 Environmental Assessment follow-up monitoring program (Reference A9),

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- Summary report of whitefish research activities (Reference A10),
- Summary report of low-dose radiation and environmental research activities (Reference A11),
- Security program description (Reference A12),
- Fitness-for-service of pressure tubes (Reference A13),
- Updated Environmental Risk Assessment (Reference A14),
- Indigenous community interest reports (Reference A15),
- Supplemental application for consolidation of selected 13152 licences (Reference A16),
- Supplemental material and update to the performance review (Reference A2),
- Community interest reports (Reference A17), and,
- April 2018 update to the Performance Review (this submission).

Environmental Risk Assessment: an Environmental Risk Assessment was submitted in June, 2017, and included an Environmental Quantitative Risk Assessment and a Predictive Environmental Risk Assessment (Reference A8). The Quantitative Risk Assessment was updated in December 2017 (Reference A14), to incorporate additional detail. No update to the Predictive Environmental Risk Assessment was needed.

As noted in Reference A14, the impact of the Bruce site on the surrounding environment is very low and does not cause any significant adverse environmental impact.

The Environmental Quantitative Risk Assessment and Predictive Environmental Risk Assessment are available <u>online</u>:

http://www.brucepower.com/wp-content/uploads/2016/08/Supplement-3-ERA-NK21-13620-NK29-14261-NK37-02787.pdf

Additionally, the December 2017 update to the Environmental Quantitative Risk Assessment is available <u>online</u>:

http://www.brucepower.com/environmental-risk-assessment-data-update/

The Environmental Risk Assessment identified no likely human health and ecological radiological effectives relative to Bruce site operations. Specific outputs of the Environmental Risk Assessment include the following:

- The human health risk assessment for chemical and physical stressors identified no unacceptable risks for people using the lands around the Bruce site for recreational or residential/agricultural purposes.
- There is no radiological risk to human health for members of the public resulting from normal operation at the Bruce site. The radiological human health risk assessment

predicted radiation doses to be less than 1% of the legal dose limit for a member of the public.

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- The ecological risk assessment for chemicals and physical stressors identified elevated risks might be present for some terrestrial biota at some industrial locations on the Bruce site.
- There is no radiological risk to non-human biota resulting from normal operations on the Bruce site.

The Predictive Environmental Risk Assessment incorporated future activities associated with the life extension program, including MCR, outages, and routine operations. The Predictive Environmental Risk Assessment identified no unacceptable risks to humans nor to the environment during future Bruce site activities (including MCR).

It is important to note that the *Canadian Environmental Assessment Act, 2012*, <u>does not</u> <u>require</u> an environmental assessment for renewal of the PROL. However, the existing Power Reactor Operating Licence requires completion of an Environmental Risk Assessment on a 5-year cycle, pursuant to PROL 18.00/2020, Licence Condition 9.2 and Licence Condition 3.3, and pursuant to REGDOC-3.1.1, *Reporting Requirements for Nuclear Power Plants*.

The Environmental Risk Assessment was completed in accordance with REGDOC-2.9.1, *Environmental Principles, Assessments and Protection Measures*, and with CSA N288.6-12, *Environmental Risk Assessment at Class I Nuclear Facilities and Uranium Mines and Mills.*

Relevant engagement efforts were described in the Environmental Risk Assessment, as well as in the Performance Review (Reference A2) and Community Interest reports (Reference A17).

Summary report of whitefish research activities: a review of scientific research with respect to Lake Whitefish and Round Whitefish was submitted in June 2017 (Reference A10).

Summary report of low-dose radiation and environmental research activities: a summary of university-based research results, resulting from research funded by Bruce Power, was submitted in June 2017 (Reference A11).

15.17 Environmental research activities (including thermal effects)

As discussed above, the application for renewal of the PROL included a review of scientific research with respect to effects on Lake Whitefish and Round Whitefish (Reference A10), as well as other low-dose radiation and environmental research activities (Reference A11). Copies of these previous submissions are provided, for the convenience of the Commission, as Enclosure 1 and Enclosure 2.

As research findings are published in peer-reviewed academic journals, these findings are available to all interested parties.

The whitefish research review (Enclosure 1) includes summaries of historical research, surveys, and monitoring, as well as summaries of independent, university-based research activities funded by Bruce Power.

The research results additionally support the general understanding that the direct impact of the thermal discharge on the survival of Lake Whitefish embryos and juveniles is likely to be limited. A list of relevant publications and theses is provided in Table 11 (thermal effects on whitefish).

The research has shown that no additional effects are expected to be caused by chemical and radiation exposure at the very low levels that result from Bruce Power operations. A list of relevant publications and thesis is provided in Table 12 (combined stressors on whitefish).

Additionally, the research has conclusively demonstrated that spawning-condition Lake Whitefish near the Bruce site are members of a larger genetic and ecological group (see Table 13, population structure of whitefish).

Finally, a list of publications and theses related to other low-dose radiation effects is provided in Table 14.

Table 11. Scientific reports on thermal effects (white	fish)
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Mitz, C., Thome, C., Cybulski, M.E., Laframboise, L., Somers, C.M., Manzon, R.G., Wilson, J.Y., Boreham, D.R., 2014. A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture. <i>Journal of North American Aquaculture</i> 76, 179-184.	Publication
O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the mountain whitefish, <i>Prosopium williamsoni</i> and cross amplification in the Round Whitefish, <i>P. cylindraceum</i> , using paired end Illumina shotgun sequencing. <i>Conservation Genetics Resources</i> 5, 89 91.	Publication
Sreetharan, S., Thome, C., Mitz, C., Eme, J., Mueller, C.A., Hulley, E.N., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., 2015. Embryonic development of Lake Whitefish (<i>Coregonus clupeaformis</i>): a staging series, analysis of growth and impacts of fixation. <i>Journal of Fish Biology</i> 87 3:539-558.	Publication
Cellular response to changes in temperature	
Sessions, K. Plasticity of the heat shock response and development of thermotolerance during embryonic development of Lake Whitefish (<i>Coregonus clupeaformis</i>). MSC thesis University of Regina, Saskatchewan. 2015.	MSc Thesis
Stefanovic, D.I. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish (<i>Coregonus clupeaformis</i>). MSc thesis University of Regina, Saskatchewan. 2015.	MSc thesis
Stefanovic, D.I., Manzon, L.A., McDougall, C.S., Boreham, D.R., Somers, C.M., Wilson, J.Y., Manzon, R.G, 2016. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish. <i>Comparative Biochemistry and Physiology, Part A</i> 193:1-10	Publication
Growth, development and survival responses to changes in temperature	
Eme, J., Mueller, C.A., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., 2015. Critical windows in embryonic development: shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (<i>Coregonus clupeaformis</i>) embryos and hatchlings. <i>Comparative Biochemistry and Physiology, Part A</i> 179, 71-81.	Publication

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-	Lee, A.H., Eme, J., Mueller, C.A., Manzon, E.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., 2016. The effects of cumulative acute heat shock exposures on morphology and survival of Lake Whitefish (<i>Coregonus clupeaformis</i>) embryos. <i>Journal of Thermal Biology</i> 57:11-20.	Publication
	Lim, M. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (<i>Coregonus clupeaformis</i>) and Round Whitefish (<i>Prosopium cylindraceum</i>). MSc thesis, McMaster University, Hamilton, ON. 2016.	MSc thesis
	Lim, M., Manzon, R., Somers, C., Boreham, D., Wilson, J., 2017. The effects of fluctuating temperature regimes on the embryonic development of lake whitefish (<i>Coregonus clupeaformis</i>). <i>Comparative Biochemistry and Physiology, Part A</i> 2014:19-29.	Publication
	Mitz, C. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (<i>Coregonus clupeaformis</i>). PhD thesis, McMaster University, Hamilton, ON. 2016.	PhD thesis
	Mitz, C., Thome, C., Thompson, J., Manzon, R., Wilson, J., Boreham, D., 2017. A method to transform a variable thermal regime to a physiologically equivalent effective temperature. <i>Journal of Thermal Biology</i> 65:21-25.	Publication
	Mueller, C., Doyle, L., Eme, J., Manzon, R., Somers, C., Boreham, D., Wilson, J., 2017. Lipid content and fatty acid profile during Lake Whitefish embryonic development at different incubation temperatures. <i>Comparative Biochemistry and Physiology, Part A</i> 203:201-209.	Publication
	Mueller, C.A., Eme, J., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., accepted. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in Lake Whitefish (<i>Coregonus clupeaformis</i>). <i>Journal of</i> <i>Comparative Physiology B</i> 185, 3:315-331.	Publication
	Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (<i>Coregonus clupeaformis</i>). PhD thesis McMaster University, Hamilton, Ontario. 2015.	PhD thesis
	Thome, C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R., Wilson, J.Y., 2016. Incubation of Lake Whitefish (<i>Coregonus clupeaformis</i>) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development. <i>Canadian Journal of Fisheries and Aquatic Sciences</i> 73, 1213-1221.	Publication
	Whitehouse, L., McDougall, C., Stefanovic, D., Boreham, D., Somers, C., Wilson, J., Manzon, R., 2017. Development of the embryonic heat shock response and the impact of repeated thermal stress in early life stage lake Whitefish (<i>Coregonus clupeaformis</i>) embryos. <i>Journal of Thermal Biology</i> 69:294-301.	Publication

Table 12.	Scientific	reports on	combined	stressors	(whitefish)	
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Kulesza, A. The combined effects of thermal and radiological stress on the emb development of Lake Whitefish (<i>Coregonus clupeaformis</i>). MSc thesis, McMast Hamilton, ON. 2017.		is
Lim, M. Thermal, morpholine, and radiation stressor effects on the embryonic of Lake (<i>Coregonus clupeaformis</i>) and Round Whitefish (<i>Prosopium cylindraceum</i> McMaster University, Hamilton, ON. 2016.		sis
Mitz, C. The cost of hormesis: Trade-offs in an energetically constrained envir Embryonic development of the Lake Whitefish (<i>Coregonus clupeaformis</i>). PhD University, Hamilton, ON. 2016.		is
Mitz, C., Thome, C., Cybulski, M.E., Somers, C., Manzon, R., Wilson, J., Boreh there a trade-off between radiation-stimulated growth and metabolic efficiency? <i>Research</i> 188(4.2):486-494.		on
Sreetharan, S., Thome, C., Tsang, K., Somers, C.M., Manzon, R.G., Boreham, J.Y., 2017. Micronuclei formation in rainbow trout cells exposed to multiple streat shock, and ionizing radiation. <i>Toxicology In- Vitro</i> 47:38-47.		on
Thome, C. The effects of thermal, chemical and radiological stressors on embr development in Lake Whitefish (<i>Coregonus clupeaformis</i>). PhD thesis McMast Hamilton, Ontario. 2015.		is
Thome, C., Mitz, C., Hulley, E., Somers, C., Manzon, R., Wilson, J., Boreham, I Characterization of the Growth Stimulation and Heat-Shock Induced Adaptive F Developing Lake Whitefish Embryos after Ionizing Radiation Exposure. <i>Radiatio</i> 188(4.2):475-485.	Response in	on
Thome, C., Mitz, C., Sreetharan, S., Mitz, C., Somers, C., Manzon, R., Borehar 2017. Developmental effects of the industrial cooling water additives morpholine hypochlorite on Lake Whitefish (<i>Coregonus clupeaformis</i>). <i>Environmental Toxic Chemistry</i> 36(7):1955-1965.	e and sodium	on

Table 13. Scientific reports on population structure (whitefish)

Eberts, R. Dietary niche partitioning by two sympatric whitefish species in Lake Huron: stable isotope analysis at three temporal scales. B.Sc. (Hon.) thesis. University of Regina, Saskatchewan. 2013.	B.Sc. thesis
Eberts, R. Resource use and ecological population structure of Lake Whitefish (<i>Coregonus clupeaformis</i>) spawning aggregations in Lake Huron. MSc thesis. University of Regina, Saskatchewan. 2015.	MSc thesis
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Eberts, R., Wissel, B., Simpson, G., Crawford, S., Stott, W., Hanner, R., Manzon, R., Wilson, J., Boreham, D. and Somers, C., 2017. Isotopic structure of Lake Whitefish in Lake Huron: Evidence for regional and local populations based on resource use. <i>North American Journal of Fisheries Management</i> 37, 133-148.	Publication

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Morgan, T., 2016. Genetic population structure of the Round Whitefish (<i>Prosopium cylindraceum</i>) in North America: multiple glacial refugia and regional subdivision. MSc thesis, University of Regina, November 2016.	MSc thesis
Morgan, T., Graham, C., MacArthur, A., Raphenya, A., Boreham, D., Manzon, R., Wilson, J., Lance, S., Howland, K., Patrick, P., Somers, C., 2017. Genetic population structure of the round whitefish (<i>Prosopium cylindraceum</i>) in North America: multiple markers reveal glacial refugia and regional subdivision. In Press.	Publication
O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 201 Development and characterization of twenty two novel microsatellite markers for the mountain whitefish, <i>Prosopium williamsoni</i> and cross amplification in the Round Whitefish, <i>P.</i> <i>cylindraceum</i> , using paired end Illumina shotgun sequencing. <i>Conservation Genetics</i> <i>Resources</i> 5, 89 91.	

Table 14. Scientific reports on the effects of low-dose radiation

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Lemon, J., Phan, N., Boreham, D., 2017. Single CT scan prolongs survival by extending cancer latency in Trp53 heterozygous mice. <i>Radiation Research</i> 188(4.2):505-511.	Publication
Lemon, J.A., N. Phan, D.R. Boreham. 2017. Multiple CT scans extend lifespan by delaying cancer progression in cancer-prone mice. <i>Radiation Research</i> 188(4.2):495-504.	Publication
McDonald, A., Smith, N., 2017. Foreword. Radiation Research 188(4.2):469-469.	Foreword
Pirkkanen, J., Boreham, D., Mendonca, M., 2017. The CGL1 (HeLa × normal skin fibroblast) human hybrid cell line: A history of ionizing radiation induced effects on neoplastic transformation and novel future directions in SNOLab. <i>Radiation Research</i> 188(4.2):512-524.	Publication
Puukila,S., Lemon, J., Lees, S., Tai, T., Boreham, D., Khaper, N., 2017. Impact of ionizing radiation on the cardiovascular system: A review. <i>Radiation Research</i> 188(4.2):539-546.	Publication
Puukila, S., Thome, C., Brooks, A.L., Woloschak, G., Boreham, D.R., 2017. The role of radiation induced injury on lung cancer. <i>Cancers</i> 9:89.	Publication
Puukila, S., Thome, C., Brooks, A., Woloschak, G., Boreham, D., 2017. The role of Radiation induced injury on lung cancer. <i>Cancers</i> 9(89).	Publication
Shephard, A.M. 2017. Effects of early life ionizing radiation exposure on the life-history of the cricket, <i>Acheta domesticus</i> . McMaster University, Hamilton, ON, September 2017.	MSc thesis
Sreetharan, S. Prenatal ionizing radiation exposure effects on cardiovascular health and disease in C57B1 mice. MSc thesis, McMaster University. 2017.	MSc thesis
Sreetharan, S., Thome, C., Tharmalingam, S., Jones, D.E., Kulesza, A.V., Khaper, N., Lees, S.J., Wilson, J.Y., Boreham, D.R., Tai T.C., 2017. Ionizing radiation exposure during pregnancy: effects on postnatal development and life. <i>Radiation Research</i> 187(6):647-658.	Publication
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Thome, C., Tharmalingam, S., Pirkkanen, J., Zarnke, A., Laframboise, T., Boreham, D., 2017. The REPAIR project: Examining the biological impacts of sub-background radiation exposure within SNOLAB, a deep underground laboratory. <i>Radiation Research</i> 188(4.2):470-474.	Commentary
Tran, J. Impacts of low-dose ionizing radiation on life history and immunity in the cricket, <i>Acheta domesticus L</i> . MSc thesis, McMaster University, Hamilton, ON. 2017.	MSc thesis
Tharmalingam, S., Sreetharan, S., Kulesza, A., Boreham, D., Tai, T., 2017. Low-dose ionizing radiation exposure, oxidative stress and epigenetic programming of health and disease. <i>Radiation Research</i> 188(4.2):525-538.	Publication

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- A2. Letter, F. Saunders to M. Leblanc, "Application for the Renewal of the Power Reactor Operating Licence: Supplemental Material", February 12, 2018, NK21-CORR-00531-14126 / NK29-CORR-00531-14817 / NK37-CORR-00531-02906.
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(This document contains prescribed information, pursuant to the General Nuclear Safety and Control Regulations, Section 2 1(c), and cannot be released to the public.)

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Enclosure 1

B-REP-07015.01-00003 Whitefish Research Review

(Enclosure 1 of Letter, F. Saunders to M. Leblanc, "Supplement to the Application for the Renewal of the Power Reactor Operating Licence: Whitefish Research Review", June 30, 2017, NK21-CORR-00531-13494 / NK29-CORR-00531-00531-14088)



WHITEFISH RESEARCH REVIEW

B-REP-07015.01-00003

Rev 000

February 2017

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ABSTRACT OF PRESENT REVISION:

Initial issue.

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EXECUTIVE SUMMARY

Lake Whitefish represent the majority of the commercial harvest in the Canadian waters of Lake Huron. In 2014, the commercial fishery for Lake Whitefish in Lake Huron was valued at \$642,283 and accounted for 85% of the Lake Huron commercial harvest. From 1997 to 2014, the commercial harvest of Lake Whitefish from Lake Huron has ranged from 127,093 kg to 592,972 kg annually [R-76].

Given their commercial importance, Bruce Power has completed significant work to measure potential interactions with Lake Whitefish. These interactions include the possibility of impingement and entrainment in the cooling water intake and through the return of warmed waters to Lake Huron.

Lake Whitefish (coregonus clupeaformis) are a cold-water, benthic-oriented fish species that typically occupy deeper (15-55 m) cold water during spring, summer, and fall. Lake Whitefish spawn in late fall (October-December, with peak in November) and hatch following ice breakup in mid-April. Mature Lake Whitefish continue to occupy shallow near-shore water for feeding throughout the winter.

Lake Whitefish spawning sites (depth of 1-8 m) typically have cobble substrate. Successful recruitment is believed to be improved with protective winter ice cover, the presence of high-quality substrate, and suitable spring water temperatures. However, water temperature (cold fall and gradual warming in the spring) may be less important for Lake Huron than for warmer lakes which represent more marginal habitat.

Lake Whitefish larvae use near-shore nursery habitats, including steep rocky shorelines and shallow embayments with reed beds. It is believed that larvae are dispersed into nursery habitats primarily due to currents. Subsequently (1-2 months following hatching), juvenile Lake Whitefish disperse into deeper, offshore habitats.

Round Whitefish were selected as a second species of interest because they share areas of similar habitat and have similar life histories as Lake Whitefish. Preliminary evidence indicated they may be more thermally sensitive than Lake Whitefish. Although historic catches of both species were low near the Bruce Power site, Round Whitefish were more common than Lake Whitefish [R-11].

Round Whitefish (*prosopium cylindraceum*) are also cold-water benthic-orientated fish found in freshwater lakes at depths of 6-36 m. Spawning occurs on cobble substrate in late November and early December, with hatch occurring early to mid-April depending on temperature conditions. Round Whitefish larvae remain close to the bottom after hatching and occupy depths of 3-7 m.

The potential for interaction of Bruce Power site cooling water intake and discharge with Lake and Round Whitefish has been recognized since the late 1990s. These potential interactions include the entrainment and impingement of Whitefish and possible effects from the return of warmer water to Lake Huron. Since that time, two Environmental Assessments [R-12] [R-14] have concluded that the potential for harm to Lake and Round Whitefish populations is low. However, uncertainties remained in the scientific understanding of Lake and Round Whitefish, and in the assessment of potential impacts.

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Since 2011, Bruce Power has expended significant effort to improve the scientific understanding of Lake and Round Whitefish, particularly with respect to potential impacts of Bruce Power site operations. In partnership with Saugeen Ojibway Nation, Bruce Power-funded independent, multi-year university-based research programs to address key research questions with respect to Lake and Round Whitefish (Box A) [R-26] [R-69].

The University of Guelph research program was funded entirely by Bruce Power. The McMaster/Regina University research program was funded by Bruce Power, with matching funds obtained through a rigorous competitive peer-reviewed NSERC collaborative research and development grant. Under the terms of the funding contracts, researchers are free to publish their findings in the peer-reviewed scientific literature.

Over 5 field seasons, researchers have collected nearly 2000 adult Lake and Round Whitefish and raised over 550,000 Lake Whitefish and more than 40,000 Round Whitefish embryos. The program at the University of Guelph has generated two theses and one peer-reviewed publication. The joint program between McMaster and Regina Universities has generated seven theses and 13 peer-reviewed publications

This independent university-based research and development has endeavored to identify Whitefish populations in Lake Huron and model these populations. Laboratory and *in situ* studies have been completed to identify both thermal and combined radiological, chemical and thermal stressor effects on developing Lake Whitefish embryos and juveniles.

Box A – Key Research Questions

Population discrimination: How many whitefish populations exist in Lake Huron, and is there any likelihood that these populations will be impacted by Bruce Power operations based on spatiotemporal distribution?

Population modeling: What is the most appropriate mathematical model(s) that can describe Lake Huron whitefish population dynamics and estimate any potential effects of Bruce Power?

Entrainment effects: Are there any significant effects (stress response/mortality) of Bruce Power entrainment on local or lake-wide whitefish populations?

Thermal effects: Are there any effects (negative or beneficial) of the Bruce Power thermal plumes from cooling water discharges on the whitefish population(s) in terms of stress response or mortality?

Combined stressors: Are there any combined effects (negative or beneficial) of Bruce Power radiological and conventional emissions on the whitefish population(s) in terms of stress response or mortality?

The Whitefish research programs have supported the training of 3 post-doctoral fellows, 4 PhDs and 9 MSc students. An additional 3 MSc and 2 PhD students are in the process of completing their studies. The training of highly qualified personnel and the development of expertise in Lake Huron Whitefish has added valuable resources to tackling the myriad of ecological challenges facing the Great Lakes.

This report summarizes the extensive published scientific results that derive from these university-based research programs up to November 2016. Additionally, we have placed these results into context, with reference to historic or parallel monitoring programs conducted by Bruce Power and/or independent consultants.

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Population Discrimination

Prior to the initiation of the research program, little was known with respect to the population structuring of Lake Whitefish in Lake Huron, and almost nothing was known with respect to Round Whitefish in Lake Huron. Some evidence of limited population structuring along the Bruce peninsula had been observed previously through genetic analysis, while interim results from a lake-wide mark/recapture study did not shed additional light on the nature of whitefish population structuring in the vicinity of the Bruce site.

While extensive spawning grounds were known to be used by Lake Whitefish along the coast of the Bruce Peninsula well to the north of the Bruce Power site, there was almost no direct evidence of Lake Whitefish spawning near the Bruce Power site itself. However, annual gillnetting studies demonstrated the presence of ripe Lake and Round Whitefish, indicating at least the possibility of spawning near the Bruce Power site. It was assumed that, if spawning activity took place, the Lake and Round Whitefish individuals spawning near the Bruce Power site were members of a much larger population.

The research program was intended to challenge this assumption. University of Regina and University of Guelph researchers collaborated with respect to collecting and sampling some of nearly 2000 Lake and Round Whitefish from spawning grounds around the main basin of Lake Huron as well as the North Channel and Georgian Bay from 2010 to 2014.

University of Regina researchers used genetic analysis to examine the relationships between spawning fish, and stable isotope analysis to examine the movement and resource use of adult fish at different locations relative to Bruce Power. Changes in stable isotope ratios in fish tissue reflect differences in the isotopic baselines of specific lake locations, the use of different habitats and specific prey choices. They completed both local (near Bruce Power) and lake-wide assessments.

The local assessment found no evidence for the presence of distinct genetic groups of Lake or Round Whitefish within the vicinity of Bruce Power. Similarly, stable isotope analysis shows no evidence for distinct Lake or Round Whitefish feeding behavior in this vicinity, as there was high resource use overlap between fish at different sampling areas. However, their research did show that Lake and Round Whitefish occupy separate ecological niches, defined as differential resource use. The period of greatest overlap occurred during the spawning period.

The lake-wide assessment found that Lake Huron is a genetic source population for Round Whitefish for Lakes Michigan, Superior and northern Georgian Bay as well as for Lake Nipigon through Lake Superior. Sampling was not wide-spread enough within Lake Huron to draw conclusions regarding population structure within the lake.

Also on lake-wide scale, stable isotope analysis shows that six ecological groups of Lake Whitefish are present across Lake Huron and Georgian Bay. In the area local to Bruce Power, the main basin of Lake Huron could be divided into Southwestern and Northeastern (including the Bruce Power site) ecological groups with heavy population mixing within each of these groups. No small distinct ecological groups are identified within the area near the Bruce Power site (i.e., Scougall Bank and McRae Point). These results are consistent with previous genetic analysis suggesting weak structuring within the eastern main basin.

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The research results conclusively demonstrate that spawning-condition Lake Whitefish near the Bruce Power site are members of a larger genetic and ecological group. There is no evidence of any distinct ecological or genetic population of Lake or Round Whitefish utilizing spawning grounds near Bruce Power.

Population Modeling

University of Guelph led the research program with respect to population modeling. The University of Guelph research team reviewed the available scientific literature for estimates of Lake Whitefish mortality and life history parameters. Initial technical reports were provided that led to two theses and one publication with the involvement of two students.

The primary result from the University of Guelph research program is the development of chemical probes for the identification of Lake Whitefish (the DNA "barcoding" technique). Identification of larval Lake Whitefish through the use of a key-based technique is challenging, while identification of eggs is virtually impossible. The DNA-based probes may allow for more accurate quantification of impacts in the future, through accurate and rapid identification of the species for collected larvae and embryos.

The research team was not able to identify population structure using the DNA barcoding technique.

Initial efforts were made to determine appropriate mortality and life-history parameters, and to develop appropriate models for spatial data. However, the key research question (what is the most appropriate mathematical model to describe Lake Huron whitefish population dynamics, and to estimate the potential effects of Bruce Power) was not answered through this research program.

Nonetheless, we have made significant advances in understanding potential impacts on Lake Whitefish since 2011. First, the overall research program has definitively shown no distinct ecological or genetic population of Lake or Round Whitefish in the immediate vicinity of the Bruce Power site. Second, extensive monitoring conducted by Bruce Power demonstrated minimal Lake Whitefish losses from impingement and entrainment: less than 1% of the Zone 1 quota from impingement and entrainment.

Effects of Impingement and Entrainment

The EA FUP included an extensive analysis of the effects of entrainment and impingement on Lake Whitefish. An environmental consulting firm conducted entrainment monitoring for two years at the Bruce A intake, supplemented with larval tows in the vicinity of the intake, and performed quality assurance checks on the routine Bruce Power impingement monitoring program. Two years of data was analyzed to estimate the cumulative impact of impingement and entrainment of Lake Whitefish at Bruce Power relative to the commercial fishing quota.

Overall, the results demonstrated a minimal effect of impingement and entrainment relative to the commercial fishing quota. The total effect of impingement and entrainment for Bruce Power site operations (including Bruce A and Bruce B generating stations) is the annual loss of less than 0.5% of the commercial fishing quota (0.33% of quota in 2013 and 0.15% of quota in 2014). In other words, the impact of Bruce Power site operations is very small and the combined effect of the commercial fishing harvest (191,155kg in 2013 and 149,275kg in 2014) and Bruce Power site operations (988kg in 2013 and 443kg in 2014) is well below the 2013 and 2014 commercial fishing quota of 292,287kg. The quota is established to maintain a sustainable fishery in Lake Huron and is meant to be protective of the long term health of the population.

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Thermal Effects

Survival and Development of Lake Whitefish Embryos

Prior to the initiation of the research program, the effects of warmer temperatures on incubating Lake and Round Whitefish embryos was not fully understood. Several published studies indicated that warmer temperatures decreased time-to-hatch and increased the probability of embryos mortality, with the specific details dependent on incubation techniques and/or source of Lake Whitefish embryos. However, a detailed study indicated that Lake Whitefish embryos nonetheless were able to withstand cyclic heat shocks of up to 7°C for 6-h each day throughout development, with virtually no impact on survival, although hatch could occur up to several weeks earlier than when incubated at lower temperature (0°C for Lake Whitefish).

No scientific information was available with respect to the impact of warmer temperatures on whitefish cellular responses. Additionally, it was not known whether an earlier hatch would be beneficial or harmful to Lake Whitefish; whitefish that hatch early may have access to more or less food, or may be more or less susceptible to predation.

Bruce Power has funded extensive research into the impacts of thermal and combined thermal, radiological and chemical stressors on developing Lake and Round Whitefish embryos. This has included examining stressor effects on cellular responses and growth, development and survival.

From 2011 to 2016, the McMaster University and University of Regina research teams incubated over 550,000 Lake Whitefish embryos and over 40,000 Round Whitefish embryos in ongoing efforts to define thermal and combined stressor effects on Whitefish. Novel incubation and irradiation methods were developed to support this research. Heat shock protein gene sequences were identified to aid in the identification of cellular stress responses in Round and Lake Whitefish.

Key results from this research indicate that Lake Whitefish embryos and young of the year juveniles mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. This level of heat shock exceeds the temperature changes seen in the temperature loggers placed in the *in situ* incubation near the discharge channel.

Lake Whitefish embryos were slower than young of the year juveniles to show a heat shock response but once they showed a response, it continued over at least 2 days following the heat shock. This response suggests that embryos and young of the year juveniles are likely able to protect themselves from subsequent exposure to heat, and may provide an explanation at the biological level for the observation that survival is not affected by intermittent exposure to heat.

Lake Whitefish hatch earlier and have reduced survival with increasing constant temperatures. Infrequent exposure of embryos and young of year juveniles to warmer water has little effect on survival or development. Lake Whitefish embryos and hatchlings incubated at higher constant temperatures showed increased heart rate and oxygen consumption. Incubation at higher constant temperatures earlier in incubation had a very small lasting effect even when the temperatures later in incubation were cooler. Higher constant temperatures during incubation also resulted in a shorter incubation period, higher yolk area and mass and lower body lengths and mass at hatching. Higher constant incubation temperatures also decreased the hatch window, survival, and increased oxygen needs. Regular weekly heat shocks had no effect on time-to-hatch, survival, yolk mass, body mass, length or head width.

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Incubation of Lake Whitefish embryos in chambers on the lake bottom in the Bruce Power discharge areas resulted in an average 10% advance in development. Embryos incubated in the discharge area were slightly longer with a smaller yolk area but showed no increase in developmental abnormalities. These chambers were deployed on the lake bottom, above the cobble substrate, whereas normal Lake Whitefish incubation tends to occur down within the cobble. There is the potential for the cobble substrate to provide a thermal buffer which may reduce the temperature effects observed in the *in situ* study. This is currently being investigated using a simulated thermal flume at Laurentian University.

Embryos hatching earlier in warmer temperature conditions have larger yolk sacs and smaller bodies. Embryos incubated at higher temperatures may be less developed and have greater capacity to maintain themselves without external food sources than embryos hatching after long incubation at cooler temperatures. The possibility of a mismatch between plankton availability and hatch timing produced by exposure to warmer temperature may be reduced because these embryos may be hatching at an earlier stage of development with greater yolk reserves. The exact mechanism that triggers embryo hatch remains unknown but may be related to a change in the movement of embryos, temperature changes and/or light exposure. In addition, hatch windows (i.e., the time from first to last hatch) increase with cooler incubation temperatures.

It is still not known whether Lake Whitefish use spawning grounds near Bruce Power. Although direct evidence of spawning has almost never been observed, the potential for spawning has been inferred from catches of ripe adult Lake Whitefish and the potential suitability of the substrate [R-47]. Nonetheless, the research program results support the general understanding that the direct survival impact of the Bruce Power site thermal discharge on Lake Whitefish embryos and juveniles, if present near the Bruce Power site, is likely to be limited.

Survival and Development of Lake Whitefish Young of the Year

Bruce Power has also funded research into the impacts of thermal stressors on Lake Whitefish Young of the Year (YOY). This has included examining effects on cellular responses and growth, development and survival.

Lake Whitefish YOY were incubated at several different temperatures and were exposed to regular heat shocks throughout development in multiple experiments by researchers at McMaster and Regina Universities. They found that YOY could mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. Lake Whitefish YOY quickly mounted a cellular heat shock response and it dropped much more rapidly than in embryos, peaking at 2-hours after the heat shock. Subtle differences in the physiology of YOY exposed to heat shocks during embryonic development were observed. Although these differences are not expected to impact survival of YOY, the link between heat exposure during embryogenesis and post-hatch effects is not yet fully clear.

Survival and Development of Round Whitefish Embryos

Prior work indicated that Round Whitefish embryos were able to withstand cyclic heat shocks of up to 5.5°C for 6-h each day throughout development, with virtually no impact on survival, although hatch could occur up to several weeks earlier than when incubated at a constant 1.7°C.

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Bruce Power-funded research showed that Round Whitefish embryos had increased mortality when exposed to rapid warming near the end of development and when incubated at a constant 8°C. There was no change in survival with exposure to 1-hour 3°C heat shocks throughout development. Seasonal temperature changes increased the development rate and shortened hatch timing. Embryos incubated at lower temperatures had increased body mass and decreased yolk mass. Exposure to regular temperature spikes had little effect on development stage onset.

Thermal Effects Summary

The outflow from Bruce Power site facilities may generate a sinking thermal plume in the winter, with temperatures reaching a maximum of 4°C warmer than the lake bottom temperature. Results from the *in situ* incubation of Lake Whitefish support this range of temperature change. Our research program has demonstrated that Lake Whitefish are capable of surviving a 4-hour, 9°C heat shock and that Round Whitefish can survive regular 1-hour 3°C heat shocks and incubation at a constant 5°C. In light of these results, no impact on embryo survival is expected in the range of temperature changes related to a sinking thermal plume near the discharge area.

Combined Stressors

Prior to the research program, it was not known whether multiple external stressors could affect whitefish embryos synergistically or antagonistically. Assessment of potential impacts on Lake Whitefish considered each stressor separately, without consideration for the possibility of interactions between cellular or organism response to multiple stressors.

Box B – Key Research Results

Population discrimination: No local genetic or ecological populations of Lake Whitefish (LWF) or Round Whitefish (RWF) have been identified in the area near Bruce Power. The LWF and RWF in the area surrounding Bruce Power belong to genetic and ecological groups that encompass large parts of Lake Huron.

Population modeling: DNA barcode identification developed for LWF.

Entrainment effects: The impact of Bruce site operations on LWF has been shown to be less than 0.5% of the quota set for a sustainable fishery.

Thermal effects: Intermittent temperature increases throughout LWF development have no impact on embryo mortality. LWF and RWF embryos exposed to regular heat shocks throughout development do experience a small advance in hatch and some changes in size and metabolism.

LWF embryos are able to mount a response to intermittent heat shocks that ensure survival of a 4-hour 9°C heat shock. RWF showed no change in survival with regular 1-hour 9°C heat shocks from a constant 5°C base. These intermittent heat shocks exceed the temperature changes recorded by the temperature loggers from *in situ* incubation chambers near the discharge channels. Temperature changes of the magnitude seen in the vicinity of the thermal discharge have been shown to have no significant effect on the mortality or stress response of LWF embryos.

Combined stressors: The relationship between radiation and morpholine exposure and LWF and RWF embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and different for LWF and RWF. LWF are more sensitive to radiation exposure whereas RWF appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for LWF.

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The Bruce Power-funded research program exposed Lake Whitefish embryos to constant or variable temperature, as well as one of acute or chronic gamma radiation or chemical stress (morpholine or sodium hypochlorite). Doses of chronic radiation of 0.06-4.4 mGy/day resulted in an earlier hatch. High doses of acute radiation increase embryo mortality. Morpholine concentrations of 1 or 10 mg/L resulted in increased larval size at hatch. There were no significant effects of morpholine at 100mg/l. From 500-1000mg/L there was a decreased larval size, earlier hatch and increased embryo mortality.

Lake Whitefish embryos were then exposed to a combination of thermal, chemical and radiological stressors. A heat shock administered prior to irradiation had a protective effect on Lake Whitefish embryos. The same heat shock administered prior to exposure to morpholine had no protective effect. A heat shock of 2-hours duration (temperature increase of 3°C or 9°C) administered six hours prior to irradiation decreased embryo mortality by up to 25%.

Compared to Lake Whitefish, Round Whitefish are likely more sensitive to morpholine exposure, with developmental and mortality effects starting at 500mg/l. No consistent effects are seen at levels between 0 and 100mg/L. Conversely, Round Whitefish are likely more resistant to low dose radiation than Lake Whitefish, with a 20% reduction in post-hatch mortality for Round Whitefish embryos exposed to chronic gamma radiation doses between 0.1 and 0.3 mGy/day.

The relationship between radiation and morpholine exposure and Lake and Round Whitefish embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and are different for Lake and Round Whitefish. Lake Whitefish are more sensitive to radiation exposure whereas Round Whitefish appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for Lake Whitefish.

The Bruce Power ECA limit for morpholine is 2.5 mg/L measured at the cooling condenser water duct. Any morpholine released from the cooling condenser duct would be rapidly diluted in the discharge channel. Morpholine monitoring at Bruce Power has a Minimum Detectable Level (MDL) of 0.02 mg/L. Monthly monitoring from January 2011 to November 2016 ranged from the MDL to 1 mg/L, with 90% of monitoring results less than the MDL. Mortality effects on embryos in this study occurred at morpholine doses at least 200 times greater than the ECA limit at Bruce Power. No measurable effects on Lake or Round Whitefish embryos were seen at doses up to 40 times greater than the ECA limit at Bruce Power.

The International Commission on Radiological Protection lists natural background levels of radiation exposure for fish up to 0.01 mGy/day. Dose rates of 0.1 to 1 mGy/day are considered low dose rates for fish [R-66]. Fish are thought to be more resistant to radiation exposure than humans and dose recommendations for aquatic organisms have been set at 3.5 Gy/yr, compared to the human limit of 0.05 Gy/yr [R-65].

Results from the combined stressor research showed that effects from morpholine and gamma radiation were only seen at levels substantially higher than those seen operationally at the Bruce Power site.

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Summary

Bruce Power has funded extensive independent university-based research and development surrounding potential interactions with Whitefish. This independent, university-based research has added to a limited knowledge base regarding population discrimination and the effect of thermal and combined radiological, chemical and thermal stressors on Lake and Round Whitefish. Results of this research are publically available for use by industrial, scientific and regulatory groups. The research has also supported the training of highly qualified personnel with expertise in Lake Huron Whitefish.

Key research results are summarized in Box B for each the identified research questions.

It is unclear how the changes in Lake and Round Whitefish embryo size and metabolism in response to heat shocks impact the fitness, behaviour and survival of Lake and Round Whitefish at 6 months and 1 year of age. Bruce Power expects to fund further research to explore these uncertainties in Lake and Round Whitefish outcomes.

Future research plans to examine the potential protective insulating effect of the cobble substrate for incubating embryos. There remain several uncertainties in the outcomes of Lake and Round Whitefish Young of the Year (YOY) and juveniles exposed to thermal stressors, particularly with respect to behaviour and fitness. It is not known whether increased incubation temperatures and/or heat shock exposure impacts the hormonal stress responses (i.e., blood sugar, adrenaline, electrolyte levels and metabolic rate) of larval and juvenile Whitefish. It is also not known how increased incubation temperatures and/or heat shock exposure impacts swim performance, foraging capability and behaviour in larval and juvenile Whitefish. Warmer incubation temperatures may lead to hatching at an earlier development stage where the larvae are potentially less able to capture prey or avoid predators and where their shorter lengths impose greater energy demands due to viscosity.

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1.0 INTRODUCTION

Lake Whitefish (*coregonus clupeaformis*) are a cold-water, benthic-oriented fish species that typically occupy deeper (15-55 m) cold water during spring, summer, and fall [R-35]. Lake Whitefish spawn in late fall (October-December, with peak in November) with hatch following ice breakup in mid-April [R-8] [R-56]. Mature Lake Whitefish continue to occupy shallow near-shore water for feeding throughout the winter [R-35].

Spawning sites (1-8m) typically have cobble substrate. Successful recruitment is believed to be improved with protective winter ice cover, the presence of high-quality substrate, and suitable spring water temperatures [R-35] [R-10] [R-102] [R-42] [R-1]. However, water temperature (cold fall and gradual warming in the spring) may be less important for Lake Huron than for warmer lakes which represent more marginal habitat [R-70].

Lake Whitefish larvae use near-shore nursery habitats, including steep rocky shorelines and shallow embayments with reed beds [R-64]. It is believed that larvae are dispersed into nursery habitats primarily due to currents. Subsequently (1-2 months following hatching), juvenile Lake Whitefish disperse into deeper, offshore habitats [R-64].

Round Whitefish (*prosopium cylindraceum*) are also cold-water benthic-orientated fish found in freshwater lakes at depths of 6-36m. Spawning occurs on cobble substrate in late November and early December, with hatch occurring early to mid-April depending on temperature conditions. Round Whitefish larvae remain close to the bottom after hatching and occupy depths of 3-7m. No information is available about the habitat of juvenile Round Whitefish [R-64].

1.1 Lake Whitefish Populations in Lake Huron

Lake Whitefish is by far the most important commercially-harvested species in the Canadian waters of Lake Huron [R-35]. For example, in 2013, Lake Whitefish represented 72% (41.4 million kg) of the commercial Canadian harvest by mass and 75% (\$15.1 million) by landed value [R-84]. Similarly, Lake Whitefish represented 86% (0.6 million kg) of the commercial United States Lake Huron harvest by mass and 95% (US\$2.66 million) by landed value in 2013 [R-30].

Management of commercial Lake Whitefish harvest in both US and Canadian waters is based on putative spawning stocks, although it is known that harvest areas may encompass multiple spawning stocks due to migration of Lake Whitefish [R-35] [R-33] [R-34]. Reported spawning locations are shown in [R-27]; note that Douglas Point is not indicated as a known spawning location, while the western side of the Bruce Peninsula includes numerous reported spawning sites.

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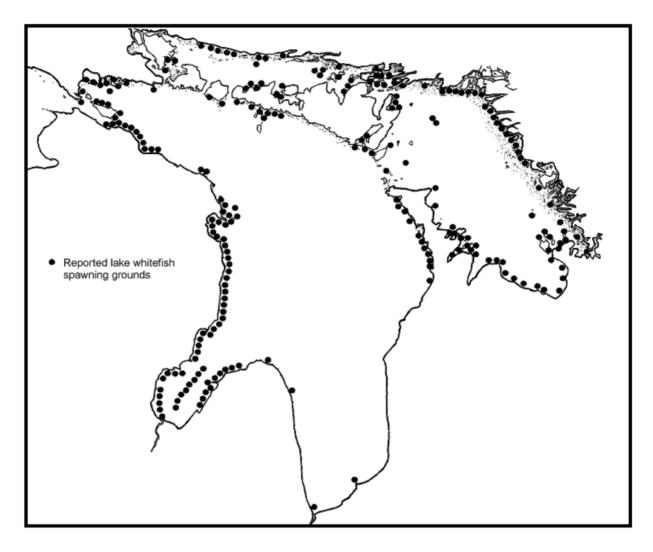


Figure 1 Map of Lake Huron showing locations of reported spawning areas for Lake Whitefish (*coregonus clupeaformis*), from Crawford (2003) [R-27]

1.2 Lake Whitefish and Bruce Power

Bruce Power operates in Zone 1 (formerly Quota Management Areas 4-4 and 4-7), an administrative region of the Canadian side of Lake Huron which runs roughly from Goderich to tip of the Bruce peninsula. Zone 1 is co-managed by Indigenous groups and the Ontario Ministry of Natural Resources and Forestry. In 2013, the Lake Whitefish harvest in Zone 1 represented 14% (191,155 kg; \$722,000) of the Canadian commercial harvest of Lake Whitefish in Lake Huron (1,407,071kg; \$5,161,150) [R-84]. Commercial harvest data does not include quantities harvested for subsistence, ceremonial, or recreational purposes.

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Because of the value of the commercial fishery and its importance to stakeholders, including Indigenous groups, interactions between Lake Whitefish and Bruce Power have been included in historical and current environmental assessments and approvals. Both the Whitefish Interactions with Nuclear Generating Stations (WINGS) report series and the Lake Whitefish Technical Working Group (TWG) contributed to the scope of research and monitoring conducted by Bruce Power.

The purpose of this document is to summarize Lake Whitefish knowledge acquired by Bruce Power through research studies, population and habitat surveys and entrainment and impingement monitoring.

1.3 Round Whitefish and Bruce Power

Round Whitefish were selected as a second species of interest because they share areas of similar habitat and have similar life histories as Lake Whitefish. Preliminary evidence indicated they may be more thermally sensitive than Lake Whitefish [R-57]. Round Whitefish were also historically more common than Lake Whitefish near the Bruce Power site [R-11].

1.4 WINGS Recommendations

The Whitefish Interactions with Nuclear Generating Stations (WINGS) reports reviewed existing scientific knowledge with respect to Lake and Round Whitefish, conducted field studies, identified potential effects of Bruce Power operations to Lake and Round Whitefish, and recommended monitoring studies to address these potential effects [R-6] [R-59] [R-60] [R-61] [R-62] [R-63] [R-64] [R-73].

The WINGS reports noted two important scientific information gaps with respect to Lake and Round Whitefish [R-61].

First, it was not known whether Lake and Round Whitefish in the main basin of Lake Huron were part of a panmictic (single) population, or whether Lake and/or Round Whitefish could be divided into phenotypically and genetically distinct populations (stocks).

Second, it was not known whether Lake or Round Whitefish were spawning in the area near Douglas Point. Based on field monitoring conducted during the WINGS research program, the potential for Lake and Round Whitefish to use spawning habitat near Douglas Point was identified. However, "strong evidence" of local spawning was not identified [R-61].

The WINGS program additionally identified a set of general hypotheses with respect to potential effects of Bruce Power operations on Lake and Round Whitefish:

- Survival and development of Lake and Round Whitefish embryos and larvae are affected by Bruce Power thermal plume(s).
- Lake and Round Whitefish are affected by impingement and entrainment.
- Spawning of Lake and Round Whitefish is affected by Bruce Power thermal plume(s). This may include delayed spawning near Bruce Power, altered distribution of spawning (crowding at spawning sites), or delayed migration past Douglas Point on route to the Fishing Islands.

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• The Bruce Power thermal plume(s) attract Lake and Round Whitefish during the winter and spring.

The WINGS report recommended a phased monitoring program, with the initial phase focused on (a) determination of the population structure of Lake and Round Whitefish in the main basin of Lake Huron, (b) identification of the use of local shoals for reproduction, and (c) determination of larval survival in local nursery habitats [R-61] [R-6]. Tasks for subsequent monitoring phases were identified but specific recommendations were dependent on the result from the initial intensive monitoring program.

1.5 Lake Whitefish Technical Working Group

A Technical Working Group (TWG) was formed to develop, separately, a follow-up work plan for elements related to effects on whitefish habitat and biota as well as effects on the Indigenous Lake Whitefish fishery.

An ad hoc Technical Working Group (2003-2005) was formed in order to develop the U34 follow-up work plans for Lake Huron whitefish. These follow-up program elements were focused on effects to whitefish habitat and biota as well as effects on the Indigenous Lake Whitefish fishery, as required by the CNSC during review of the U34 Restart EA Screening Report. TWG participants included representatives from CNSC (chair), Chippewas of Nawash First Nations, Saugeen Métis Council, Fisheries and Oceans Canada, OMNR, CFA, OPG, SENES, Bruce Power and Golder Associates. Members included participants in the WINGS study.

The three-year TWG follow-up plan included elements for (1) defining thresholds for effect following development of population models by University of Guelph and SON, (2) participation in a lake-wide mark/recapture study led by the Chippewa Ottawa Resource Authority, (3) spawning condition surveys, embryo surveys (if triggered by sufficient numbers of ripe females), and substrate temperature monitoring, (4) monitoring for impinged whitefish, and (5) monitoring for entrained whitefish [R-61].

1.6 Summary

Based on the known biology of the Lake Whitefish, potential effects of Bruce Power operations have been hypothesized, particularly with respect to impacts of the thermal plume as well as entrainment and impingement.

This document will outline efforts by Bruce Power and external parties to address the questions raised by the WINGS reports and the TWG. This summary will include:

- Historical population and thermal effects research (Section 2.0).
- Lake Whitefish Research Partnership results to date (Section 3.0).
- Population and habitat surveys (Section 4.0).
- Temperature monitoring (Section 5.0).
- Impingement and Entrainment monitoring (Section 6.0).
- Other relevant research and reports (Section 7.0).

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This report synthesizes published scientific research and monitoring results in order to summarize current knowledge and understanding with respect to Lake Whitefish and potential impacts on Lake Whitefish and to identify outstanding gaps in knowledge and understanding.

2.0 HISTORICAL RESEARCH

2.1 Population Discrimination of Lake Whitefish in Lake Huron

Research efforts to discriminate distinct populations (stocks) of Lake Whitefish in Lake Huron have been underway for more than a decade, including a lake-wide mark/recapture program as well as genetic analysis.

Final results for a lake-wide mark/recapture study (to identify Lake Whitefish stocks in the main basin of Lake Huron) have not been reported. Preliminary results for northern Lake Huron (not including Douglas Point) have been reported [R-33] [R-34] [R-67]. Population data (life history parameters) for main basin Lake Huron populations were reported [R-25].

Genetic (DNA microsatellite) analysis of Lake Whitefish from Lake Huron has been ongoing [R-99] [R-100] [R-101]. Historic results indicate relatively low genetic diversity within Lake Huron, although some structuring is observed [R-99] [R-101].

Three publications and one thesis have included samples from presumed spawning aggregations at or near Douglas Point [R-99] [R-54] [R-86] [R-38]. Analysis of Lake Whitefish DNA microsatellites found weak population structuring at spawning sites within the main basin of Lake Huron. The Douglas Point and the Fishing Islands aggregations were found to represent "one to two" populations. All studies considered did not to find evidence of a distinct genetic population of Lake Whitefish in the Douglas Point area.

2.2 Effects of Temperature on Lake Whitefish Embryos

Impacts of incubation temperature on Lake Whitefish embryological survival and development have been studied since the 1920s [R-9] [R-8] [R-56] [R-58] [R-90] [R-93].

Initial efforts to study Lake Whitefish incubation were limited. For example, Hall incubated Lake Whitefish eggs at 10-11°C with varying oxygen concentration and pH [R-58]. No embryos survived to hatching when incubated under experimental conditions within a few days of fertilization.

Price incubated 120,000 Lake Whitefish eggs from Lake Erie over three winter seasons at constant temperatures of 0.5° C to 10° C [R-93]. Price found generally uniform survival (approximately 60%) and post-hatch abnormality at 2° C to 6° C. However, 0.5° C was proposed as the optimal incubation temperature. Incubation time ranged from 141 d at 0.5° C to 30 d at 10° C. Price proposed a model of the time required to reach developmental stages of the form M/A^{T} . Price observed that hatching was a result of movement of the embryo as well as the release of hatch enzymes.

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Brooke conducted a similar experiment with embryos from Lake Michigan and found optimal survival with constant temperature incubation at $4-8^{\circ}$ C [R-9]. Over this temperature range, incubation period ranged from 59-112 days. A polynomial equation was derived to compute time to development stage, including hatch. Daily mean temperatures were used to compute daily development rates for each of 20 developmental stages. Note that model development rates were not computed sequentially; in other words, the time to stage *i* is based on a model that is distinct from the time to stage *j*. Brooke observed longer incubation periods and a greater optimum temperature range than Price. However, differences in methodology render this study difficult to compare to the Price study.

The Brooke model was developed based on constant temperature incubation experiments [R-9], but verified with experiments that mimicked natural, variable temperature conditions for an inshore site in southern Lake Michigan [R-8]. Under these natural conditions, Brooke model development times were within 14% at most of actual development times [R-8]. The Brooke model predicted 50% hatch in 140 days, with observed 50% hatch in 135 days.

Similar experiments with Lake Whitefish eggs incubated with water drawn from Lake Huron again resulted in consistent predicted and observed hatch dates [R-8]. Under natural, variable temperature conditions, the Brooke model predicted hatch in 142 and 140 days in each of two years, with 151 days observed for each year.

The Brooke model was the first developmental rate model for Lake Whitefish which was shown to be applicable for both constant and variable temperatures. The model was used to predict hatch advancement based on a hypothetical continuous increase in temperature due to warm-water effluent. With model temperatures 1-3°C greater than natural temperatures, Lake Whitefish hatch was predicted to occur 8-21 days earlier than under natural conditions [R-8].

The first Lake Whitefish incubation experiment with controlled temperature change was conducted by Griffiths [R-56]. In this study, Griffiths observed Lake Whitefish survival and development rate based on 16 combinations of base (18-h daily) and cycle (6-h daily) temperatures [R-56]. Analysis considered survival, development rate, and developmental abnormalities throughout each of three periods (blocks) of development.

Griffiths found optimum constant incubation temperature of 4-6°C, with survival of approximately 75%. However, survival at a constant incubation temperature 7°C was similar (71%). A sharp decline in survival was assumed to occur at constant incubation temperatures above 8°C (as determined by analysis of contour intervals), and almost no survival was observed at 10°C (5% survival). Temperature excursions of up to 10°C for 6 hours per day every day throughout development resulted in essentially no impact on survival [R-56] but did reduce time to complete hatch.

Data was used to develop polynomial models of survival and development rate for each of three developmental blocks, as well as an overall model of survival and development rate from fertilization to hatch. The Griffiths block models were verified with experiments that involved incubation of Lake Whitefish embryos at temperatures corresponding to ambient Lake Ontario temperatures. Eggs were observed to hatch 1.5 to 3 weeks early when exposed, respectively, to a 3°C temperature increase (greater than ambient) for 1 day out of every 4 or continuously throughout development.

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When compared with the Brooke experiments, the Griffiths study advanced understanding with respect to survival and development rate throughout development (the earliest period of development was considered the most sensitive to temperature). Additionally, the Griffiths study included experiments with controlled temperature changes that were much greater than natural variable temperatures observed by Brooke. Additionally, Griffiths considered developmental abnormalities throughout development (although in the final analysis, all abnormalities were assumed to be fatal). A limitation of the Griffiths study is that analysis was based on complete hatch, rather than 50% hatch.

All reported experimental results and development models suffer from one consistent limitation: hatch timing is not a developmental milestone. Rather, hatch may be triggered by external stimuli, including mechanical agitation and temperature excursions. Although the mechanism of hatch has not been studied in detail, experimenters have consistently reported induction of hatch through handling (mechanical agitation) and application of experimental treatments (acute temperature changes).

For example, Griffiths reports "upward temperature fluctuations and mechanical agitation advanced hatch of near-term embryos." Price makes a similar observation: "from both observation and experiments, the hatching of whitefish eggs results from a combination of mechanical movements of the fish within the shell and the action of hatching enzymes" [R-93]. Hall notes that, for some experimental treatments, hatching occurred within "an hour or two" [R-58].

It is challenging to compare these results due to differences in experimental methodology (such as agitation of the eggs due to aeration and water flow). Applying the results to natural conditions is even less certain, as experiments which include naturally variable temperatures (i.e., [R-8] [R-56] [R-90]), did not incorporate the potential hatch-triggering mechanism of environmental cues, such as wave action. In other words, all results and models for hatch time may not be directly applicable to in-lake conditions.

Additionally, the studies conducted so far do not fully consider the possible range of temperature excursions. While over-wintering embryos are more likely to be exposed to small temperature excursions (as in the case of near-freezing water being replaced by a sinking plume of 4°C), some potential spawning grounds may be exposed to much larger temperatures on occasion. None of the studies to date have considered the effects of large but infrequent temperature excursions.

Note: Additional published and unpublished research reports sponsored by Bruce Power or CANDU Owners Group are discussed in Section 3.0.

2.3 Population Discrimination of Round Whitefish in Lake Huron

No historical studies were available on Round Whitefish population distribution in Lake Huron [R-64].

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2.4 Effects of Temperature on Round Whitefish Embryos

The first Round Whitefish incubation experiment with controlled temperature change was conducted by Griffiths. In this study, Griffiths observed Round Whitefish survival and development rate based 4 base (18-h daily) and cycle (6-h daily) temperatures. Analysis considered survival, development rate, and developmental abnormalities throughout each of three periods (blocks) of development [R-57].

Griffiths found an optimum constant incubation temperature of 1.7°C, with survival of approximately 89%. However, survival at a constant incubation temperature 5 °C was similar (>80%). A sharp decline in survival occurred at constant incubation temperatures above 5°C and no survival was observed at 10°C. The greatest sensitivity to temperature changes occurred during early and late embryonic development. Regular cyclical temperature changes above 7°C produced sharp drops in survival [R-57].

3.0 LAKE WHITEFISH RESEARCH PARTNERSHIPS

3.1 Lake Huron Lake Whitefish Distribution Study

3.1.1 Scope Development

The Lake Huron Lake Whitefish Distribution Study (lake-wide mark/recapture program) had the following objectives:

- 1. Determination of spatial distribution and movement of Lake Whitefish populations in Lake Huron.
- 2. Determination of contribution of Lake Whitefish stocks to commercial fishery yields.
- 3. To determine whether Lake Whitefish stocks display fidelity to spawning grounds.

The study resulted from a partnership between the Chippewa/Ottawa Resource Authority, Michigan Department of Natural Resources, US Fish and Wildlife Service, Chippewas of Nawash, Saugeen First Nation, and Ontario Ministry of Natural Resources. Bruce Power joined the partnership in 2003.

3.1.2 Accomplishments

Adult Lake Whitefish were tagged and released from six spawning areas in the main basin of Lake Huron. Bruce Power contributed tagging effort for Lake Whitefish near Douglas Point and Sarnia, in the southern main basin (well south of any known major spawning grounds on the eastern main basin of Lake Huron).

Over 35,000 Lake Whitefish were tagged and released from 2003-2006. More than 2000 tags were recovered by 2012.

Preliminary results from the Lake Whitefish Distribution Study reported exclusively on fish tagged in northern Lake Michigan and northern Lake Huron. Although the results may not be applicable to stocks in the southern main basin of Lake Huron, results do show site-specific fidelity to spawning grounds as well as mixture of stocks throughout Lake Michigan and Lake Huron outside of spawning season [R-33].

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Estimates of fishing mortality and natural mortality for Lake Whitefish stocks in northern Lake Michigan and northern Lake Huron were also reported [R-32]. Results may not be applicable to stocks in the southern main basin of Lake Huron.

The recovery of tags from Douglas Point fish in this study was not sufficient to judge spatial distributions.

Reports

Scientific publications resulting from the Lake Whitefish Distribution Study are listed in Table 1.

Bruce Power reports relevant to the Lake Whitefish Distribution Study are given in Table 2. These reports are reviewed in Section 10.0.

Final results have not been published at this time.

Table 1 Scientific reports produced as a result of the Lake Whitefish Distribution Study

[R-36]	Ebener M.P., Mohr L., Woldt A., Johnson J., Crawford S., 2003. Lake Huron Lake Whitefish distribution study. A proposal submitted to the U.S. Fish and Wildlife Restoration Act via Great Lakes Fishery Commission.	Technical report
n/a	Ebener, M. 2009. United States Fish and Wildlife Service Great Lakes Restoration Act: 2008 Project Progress Report, Agreement Number 301813J229 - Lake Huron Lake Whitefish Distribution Study.	Technical report
[R-34]	Ebener M.P., Brenden T.O., Wright G.M., Jones M.L., Faisal M., 2010. Spatial and temporal distributions of Lake Whitefish spawning stocks in Northern Michigan and Huron, 2003-2008. Journal of Great Lakes Research 36, 38-51.	Publication
[R-33]	Ebener M.P., Brenden T.O., Wright G.M., Jones M.L., Faisal M., 2010. Erratum to "Spatial and temporal distributions of Lake Whitefish spawning stocks in Northern Michigan and Huron, 2003-2008" [J. Great Laes Res. 36 (Supplement 1) (2010) 38-51. Journal of Great Lakes Research 36, 803.	Publication (erratum)
[R-32]	Ebener M.P., Brenden T.O., Jones M.L., 2010. Estimates of fishing and natural mortality rates for four Lake Whitefish stocks in Northern Lakes Huron and Michigan. Journal of Great Lakes Research 36, 110-120.	Publication
n/a	Ebener M.P., 2012. Lake Huron Lake Whitefish Distribution Study Final Report [draft]. December 28, 2012.	Technical report

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Table 2 Bruce Power reports relevant to the Lake Whitefish Distribution Study				
[R-15]	Bruce Power, 2006. Whitefish inv NK21-07015.01-P. June 22, 2006	-	summary.	Technical report
[] _ · · · · · · · · · · · · · · · · · ·			wer. Technical report	
[R-16]Bruce Power, 2007. Whitefish investigations 2006 summary.TechnicaB-REP-00531-00017. No date.report			Technical report	
[R-17]Bruce Power, 2008. Whitefish investigations 2007 Summary.TechnicB-REP-00531-00022. June 23, 2008.report			Technical report	
[R-18]Bruce Power, 2009. Whitefish investigations 2008 Summary.TechnicB-REP-00531-00027. July 9, 2009.report			Technical report	
[R-19]Bruce Power, 2010. Whitefish investigations 2009 summary.TechnicalB-REP-00531-00035. July 14, 2010.report			Technical report	
[R-20] Bruce Power, 2011. Whitefish investigations 2010 summary. Technical			Technical	

3.1.3 Summary

Given the limited recovery of tags from fish collected at Douglas Point, it is not possible to draw conclusions from this study regarding Lake Whitefish in the immediate vicinity of Bruce Power.

3.2 Saugeen Ojibway Nation-Bruce Power Collaborative Whitefish Agreement

3.2.1 Scope Development

B-REP-00531-00040. July 15, 2011.

Bruce Power and Saugeen Ojibway Nation agreed to sponsor independent research focused on 5 key research questions with respect to potential impacts of Bruce Power operations on Lake Whitefish [R-69] [R-26]:

- 1. **Population discrimination:** How many whitefish populations exist in Lake Huron, and is there any likelihood that these populations will be impacted by Bruce Power operations based on spatiotemporal distribution?
- 2. **Population modeling:** What is the most appropriate mathematical model(s) that can describe Lake Huron whitefish population dynamics and estimate any potential effects of Bruce Power?
- 3. **Entrainment effects:** Are there any significant effects (stress response/mortality) of Bruce Power entrainment on local or lake-wide whitefish populations?
- 4. **Thermal effects:** Are there any effects (negative or beneficial) of the Bruce Power thermal plumes from cooling water discharges on the whitefish population(s) in terms of stress response or mortality?

report

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5. **Combined stressors:** Are there any combined effects (negative or beneficial) of Bruce Power radiological and conventional emissions on the whitefish population(s) in terms of stress response or mortality?

Saugeen Ojibway Nation partnered with the University of Guelph to carry out a four-year research program addressing key research questions 1, 2, and 3 (2011-2015) [R-69]. Bruce Power agreed to provide Saugeen Ojibway Nation with funding of up to \$956,000 over four years for this research program.

3.2.2 Accomplishments

In 2012, the University of Guelph group collected more than 1000 samples of adult spawning Lake Whitefish across Lake Huron, the North Channel, and Georgian Bay.

In 2013, the University of Guelph group conducted weekly water sampling at five transects near Douglas Point (June-July). An additional field season was planned for 2014.

A total of four students participated in the research process. One Masters student and one Doctoral student have completed their degrees under this research program.

Population Discrimination

Tissue samples from spawning adult Lake Whitefish were provided to University of Regina for stable isotope analysis, as well as the United States Geological Survey for genetic analysis.

By 2015, the University of Guelph group planned to integrate all documented information on the distribution of Lake Whitefish in Lake Huron. Results have not been received by Bruce Power at this time.

Population Modeling

The University of Guelph group has reviewed the available scientific literature for estimates of Lake Whitefish mortality and life history parameters.

These estimates were to be used in conjunction with population models under development in order to determine the potential impact of Bruce Power operations on Lake Whitefish in Lake Huron.

Entrainment Effects

The University of Guelph group conducted weekly water sampling at five transects near Douglas Point (June-July, 2013). An Acoustic Doppler Current Profiler (ADCP) was used to determine water velocity near the Bruce B intake structure.

An additional plankton field sampling program was completed in the spring and summer of 2014. Laboratory observations of Lake Whitefish larval buoyancy have been completed.

Plankton (which includes larval Lake Whitefish) abundance in water samples was found to vary based on depth of sampling and dissolved oxygen concentration. Plankton tows were found to contain larval Lake Whitefish the week after ice-out, suggesting possible hatching while still under ice cover [R-85].

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A molecular probe was been developed for DNA-based identification of Lake Whitefish [R-85]. Based on lake-wide sampling, DNA barcode analysis identified a single species of Lake Whitefish in Lake Huron [R-85] [R-86].

By 2014, the University of Guelph researchers planned to integrate their research findings to report on Bruce Power entrainment mortality rates. Further results have not been received by Bruce Power at this time.

Reports

Interim scientific reports produced by the University of Guelph group are listed in Table 3. Reports of a primarily administrative nature (i.e., annual reports) have not been included.

 Table 3

 Scientific reports produced by the BP-SON Lake Whitefish partnership

[R-28]	Crawford S., 2013. Metadata for 2012 sampling of Lake Huron Lake Whitefish (<i>coregonus clupeaformis</i>). November 4, 2013.	Technical report
[R-88]	Overdyk, L., Crawford S., 2013. Metadata for 2013 plankton ecology sampling at Douglas Point, Lake Huron. December 11, 2013.	Technical report
[R-3]	Angevaare J., Gillis D., 2013. Estimates of life history and mortality parameters of Lake Whitefish (<i>coregonus clupeaformis</i>). December 20, 2013.	Technical report
[R-29]	Crawford S., 2014. Compilation of documented information/knowledge regarding population distribution of Lake Whitefish (<i>coregonus clupeaformis</i>) in Lake Huron (draft). February 3, 2014.	Technical report
[R-87]	Overdyk L, Braid H., Naaum A., Hanner R., Crawford S., 2014. Real-time PCR identification of Lake Whitefish (<i>coregonus clupeaformis</i>) in the Great Lakes. March 25, 2014.	Technical report
[R-2]	Angevaare J., Gillis D., Cox R., 2014. Report on application and evaluation of population models. June 14, 2014.	Technical report
[R-1]	Angevaare J., 2014. Efficient Bayesian inference for Conditionally Autoregressive Models. M.Sc. thesis, University of Guelph. April 2014.	M.Sc. thesis
[R-86]	Overdyk, L., Braid, H., Crawford, s., Hanner R., 2015. Extending DNA barcoding coverage for Lake Whitefish (<i>coregonus clupeaformis</i>) across the three major basins of Lake Huron. DNA Barcodes 3:59-65.	Publication
[R-85]	Overdyk, L. Ecological and genetic factors in the distribution and abundance of larval Lake Whitefish (<i>coregonus clupeaformis</i>) at Douglas Point, Lake Huron. PhD thesis, University of Guelph, Guelph, ON. July 2015.	PhD thesis

Each report and thesis is reviewed briefly in Section 10.2.

3.2.3 Summary

The limited scientific information available from these reports does not permit conclusions to be drawn regarding the population structure of Lake Whitefish in Lake Huron.

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3.3 McMaster University Whitefish NSERC Collaborative Research and Development Program

3.3.1 Scope Development

Bruce Power partnered with McMaster University and the University of Regina to carry out a four-year (2011-2015) research program addressing key research questions 4 and 5 [R-26]. Bruce Power agreed to provide McMaster University with funding of up to \$920,000 over four years for this research program.

Additionally, Bruce Power partnered with the University of Regina to assess Lake and Round Whitefish population structure and to characterize the heat shock response in Lake and Round Whitefish embryos. Bruce Power agreed to provide funding of up to \$600,000 over four years for this research program, which was intended to supplement the Environmental Assessment Follow-up Program.

Subsequently, McMaster University developed a broader research program (2012-2018) that combined the two research programs above and additionally addressed thermal and combined stress effects on Round Whitefish.

In addition to the funding already committed by Bruce Power, the Natural Sciences and Engineering Research Council of Canada (NSERC) committed funding of up to \$1,040,000 over five years (2013-2017). This funding was obtained through a competitive NSERC research grant competition (Collaborative Research and Development, or CRD), which included a thorough, independent, scientific peer-review process. McMaster University's success in obtaining external federal funding demonstrates the scientific rigor of the research and academic credibility of the research program.

With respect to combined research program ("Effects of thermal, chemical, and radiological emissions on whitefish"), researchers at McMaster University and the University of Regina planned:

- To determine the impact of chronic and transient thermal stress during embryogenesis on (i) the heat shock response in embryos and larvae, (ii) embryonic development, and (iii) larval phenotypes in Lake and Round Whitefish (*thermal effects*).
- To incubate Lake and Round Whitefish embryos within the Bruce Power thermal plume, and to determine the abundance of predators and prey in nursery grounds for early and late hatching (*thermal effects*).
- To determine the impact of chemical, radiological, and thermal stress (separately and in combination) during embryogenesis on survival and development of Lake and Round Whitefish (*combined stressors*).
- To determine the effects of chemical, radiological, and thermal stress (separately and in combination) during embryogenesis at the cellular and genetic level (*combined stressors*).
- To determine the genetic population structure of Lake and Round Whitefish with genetic microsatellite and mitochondrial DNA markers as well as single nucleotide polymorphisms (*population discrimination*).

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• To assess the movement, diet, and habitat use of breeding adults of Lake and Round Whitefish through stable isotope analysis (*population discrimination*).

3.3.2 Accomplishments

Adult Lake and Round Whitefish were collected with gillnetting near Douglas Point in 2010 and 2011. More limited gillnetting was conducted near Douglas Point in 2012 for temporal comparison. Adult Lake Whitefish were collected near the Fishing Islands for *in vitro* fertilization in 2012 and 2013. Unsuccessful attempts were made to collect Round Whitefish near Douglas Point in 2013 for *in vitro* fertilization. An additional 74 Lake Whitefish were collected in 2015 near Port Elgin for *in vitro* fertilization. Seven Round Whitefish were collected near Tobermory in 2015 and yielded 6,000 embryos, all of which died after transport to McMaster. Additional embryos were obtained from the MNRF for 16 Round Whitefish to complete 2015 experiments.

Laboratory incubation experiments were conducted with embryos obtained via *in vitro* fertilization. Table 4 details the annual Whitefish embryos incubations undertaken by the overall research program.

Year	Group	Lake Whitefish	Round Whitefish
2011	McMaster	60,000	0
2011	Regina	5,000*	0
2012	McMaster	90,000	0
2012	Regina	90,000	0
2013	McMaster	150,000	0
2013	Regina	100,000	0
2014	McMaster	10,000	1,500
2014	Regina	10,000	11,000
2015	McMaster	50,000	14,500 [†]
2015	Regina	7,000	9,350 [†]
2016	Regina	0	4,500

Table 4Whitefish Embryo Incubations, McMaster and Regina 2011-2016

* Lake Whitefish from a lake in Saskatchewan

[†] Approximately 18,000 of these are from the MNRF

In early 2015, there were technical difficulties with the Lake and Round Whitefish embryo fertilizations and incubations that significantly reduced the number of embryos available. Approximately 6,000 Round Whitefish embryos died shortly after transport to McMaster and an additional 50% of the embryos sent to Regina died shortly after transport.

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Both the Regina and McMaster groups found that of the Round Whitefish embryos that hatched, only 30% survived to transition to independent feeding although the reasons for this are not yet clear. Approximately 600 juvenile Round Whitefish were reared post-hatch. In December 2015, incubation of 4,500 Round Whitefish embryos began.

This research collaboration has supported the training of three post-doctoral fellows, three PhDs and eight MSc students. An additional three MSc and two PhD students are in the process of completing their studies.

3.3.3 Effects of Temperature on Lake and Round Whitefish

Lake Whitefish

Measurement of stress response

Stress responses in Lake Whitefish embryos and young of the year (YOY) juveniles were characterized by measuring Heat Shock Protein (HSP) levels. HSPs are a measure of stress response at the cellular level. They are responsible for protein folding and translocation, steroid and receptor binding and cell development. These HSPs help organisms maintain homeostasis when subjected to environmental stressors.

In order to quantify the cellular stress response in Lake Whitefish, Heat Shock Protein (HSP) gene sequences were required. The University of Regina group obtained full length gene sequences for several heat shock proteins (hsp47, hsp70, hsp90 α , hsp90 β , and hsc70). A real-time PCR assay was developed to quantify gene expression for these genes as well as for control genes (β -Actin and gapdh). A quantitative assay for protein expression was developed for hsp70.

Measurement of growth, development and mortality effects

The McMaster University group developed a recirculating incubation system in order to study temperature effects during embryogenesis. This included novel incubation strategies utilizing Petri dishes and multiwell plates [R-78].

As a standard for measurement of changes related to varying temperature regimes, baseline growth, hatch timing and mortality of Lake Whitefish embryos was recorded and correction factors for the effect of preservatives were determined [R-95].

Lake Whitefish embryos were then exposed to constant or variable temperatures. Growth, mortality, and hatch timing were recorded [R-78] [R-95].

Cellular response to temperature change in Lake Whitefish embryos

The University of Regina group has measured the age at which a temperature change can induce a heat shock response in Lake Whitefish embryos, as well as the magnitude of the temperature change required, the rate and duration of the heat shock response, and the recovery period for the heat shock response.

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The University of Regina group has conducted experiments in which Lake Whitefish embryos were exposed to weekly heat shocks. Embryos were snap-frozen for subsequent analysis of gene and protein expression. Embryos were more resistant to HSP expression than 2-month post-hatch YOY but once HSP expression was induced in embryos, it lasted beyond the last 48-hour sampling period [R-98].

The potential for thermotolerance in Lake Whitefish embryos was investigated through the use of regular transient heat shocks (6, 9 or 12°C) throughout development. This was followed by a high-level 12, 15 or 18°C heat shock at 64 or 82 days post-fertilization delivered 6 or 18 hours after the last transient heat shock. Embryos exposed to frequent transient heat shocks throughout development had lower hsp70 levels in general than controls incubated at 3°C. Older embryos and those a longer recovery period before the high-level heat shock had a lower hsp70 levels [R-94].

Changes in the cellular heat shock response in Lake Whitefish throughout embryonic development are being explored. Current data indicates that embryos as young as 21 days post fertilization are able to produce a heat shock response.

Growth, development and mortality effects of temperature changes on Lake Whitefish embryos

The McMaster group exposed Lake Whitefish embryos to constant or variable temperatures of 2, 5 or 8°C [R-41] [R-81]. Growth, mortality, and hatch timing were recorded for embryos reared at constant or variable temperatures. Embryos were preserved for morphometric analysis. Increased temperatures, particularly during the sensitive period of gastrulation, resulted in decreased survival and an earlier hatch. Larvae incubated at higher temperatures had smaller bodies and larger yolk reserves [R-81]. Survival and hatch timing results were generally similar to those indicated by Griffiths (1979) [R-56].

The McMaster University group changed the rearing temperature of Lake Whitefish embryos at specific milestones during embryogenesis. The whole-organism oxygen consumption and heart rate of embryonic and post-hatch Lake Whitefish was measured [R-41] [R-81]. Physiological changes in heart rate and oxygen consumption were recorded at several points in embryonic development and at hatch. Oxygen consumption and heart rate were consistently elevated when measured at 5°C or 8°C compared to 2°C. There was some evidence that incubation through to the beginning of organ development (organogenesis) at higher temperatures (5 or 8°C) resulted in continued higher oxygen consumption when hatched at 2°C. Other physiological changes were dependent on the size of the temperature change [R-41]. Although the data suggested phenotypic plasticity (i.e., changes in appearance, physiology and development in response to change in the environment), results did not consistently depend on magnitude of temperature shift [R-41].

The McMaster group completed lipid analysis for these embryos.

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The McMaster group examined the effect of weekly 1-hour heat shock of +3°C above control temperatures of 2°C and 5°C on Lake Whitefish embryo morphology and survival. They also considered the effect of a constant 8°C incubation temperature. There was a decrease in time to hatch with increasing incubation temperature (148 days at 2°C, 92 days at 5°C and 50 days at 8°C). Weekly heat shocks did not affect the time to hatch. There was no change in survival to the pre-hatch stage with increased incubation temperature or heat shocks (~50% cumulative survival). The 8°C and 5°C incubation groups had significantly smaller embryos and larger yolk sacks but there was no difference in morphology for heat shock groups compared to the constant temperature controls [R-81].

The McMaster group examined the effect of a gradual seasonal changes in temperature $(8^{\circ}C \rightarrow 2^{\circ}C \rightarrow 6^{\circ}C)$ with and without regular $3^{\circ}C$ 1-hour temperature spikes during the $2^{\circ}C$ period on Lake Whitefish embryo survival, growth, development and heart rate. Regular temperature spikes were applied three times weekly. They found no change in survival of embryos in the seasonal temperature change and/or temperature spike groups. Compared to the $2^{\circ}C$ control group, the embryos exposed to seasonal changes with temperature spikes had an elevated heart rate and exhibited rapid growth during the seasonal incline. The majority of hatch in the seasonal temperature change group occurred at $6^{\circ}C$ [R-72].

The Regina group examined the effect of thermal stress on embryonic (fin flutter, vitelline circulation stage) Lake Whitefish survival. Temperature regimes of 3, 6, or 9°C above the control for 0.25 to 4 hours with a two-hour recovery period were compared to constant control temperatures of 2°C. The Lake Whitefish embryos were able to mount a heat stress response that allowed them to survive a 4-hour 9 °C heat stress [R-97].

The Regina group also investigated the potential for thermotolerance in Lake Whitefish embryos was investigated through the use of regular transient heat shocks (6, 9 or 12°C) throughout development. This was followed by a high-level 12, 15 or 18°C heat shock at 64 or 82 days post-fertilization delivered 6 or 18 hours after the last transient heat shock. Mean age at hatch was not impacted by these heat shocks. A small difference in mean body length and eye diameter was noted only in embryos exposed to high-level heat shocks. Survival of embryos exposed to transient heat shocks and 1-hour high-level heat shocks was similar to controls. Embryos exposed to 4-hour high-level heat shocks had significantly decreased survival [R-94].

The McMaster group examined hatch timing, development stage-at-hatch and size-at-hatch among Lake Whitefish embryos under variable temperature regimes. Embryos were obtained in 2013 and 2014 and incubated at 0.5, 2, 5 and 8°C. Variable regimes consisted of 2 or 8°C for the first third of development, then transfer to increasing $(\rightarrow 5^{\circ}C \rightarrow 8^{\circ}C)$ or decreasing temperatures $(\rightarrow 5^{\circ}C \rightarrow 2^{\circ}C)$ for the other two-thirds of development. They also incubated embryos at 2°C for 81, 93, 106, 121, 138 and 151 days followed by a transfer to 5°C or 8°C. For the variable regimes across thirds of development, median hatch occurred much later (168-174 days) and lasted longer (21 to 28 days) for the decreasing regime than for the increasing regime (102-104 days and occurring over 1 to 2 days) despite similar mean temperatures. Fin indentation and length were greater under the increasing variable regime. The work showed that Lake Whitefish hatch at a more advanced stage of development when raised at lower temperatures. The term heterograde hatching was proposed to describe a situation where Lake Whitefish hatched at a more advanced stage of development when incubated at lower temperatures. This may be a way of ensuring that hatching is synchronized to the availability of zooplankton related to spring breakup [R-79].

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Models of growth, development and mortality effects of temperature changes on Lake Whitefish embryos:

The McMaster group developed a method to determine an equivalent or effective temperature from a variable thermal regime. The effective temperature is derived from a time series of temperature data to allow comparisons of growth between different variable thermal regimes to that of an equivalent constant temperature regime. This method is valid under several criteria based on the temperature dependence of growth in the species under consideration [R-79].

A new Lake Whitefish developmental model was developed that incorporated heterograde hatching. Prior models of Lake Whitefish development under variable thermal regimes (Price, 1940 and Brooke, 1975) are compared with the longer time to 50% hatch in the embryos raised by the McMaster group. Variable temperature regime outcomes were compared to existing models and to the proposed heterograde hatching model. The use of the heterograde hatching model had the lowest error in accurately predicting embryo hatch under variable thermal regimes. In comparison, the Griffiths model overestimated and the Brooke model underestimated development time. The model was further validated using data from [R-8] [R-81] and [R-90] [R-79].

Cellular response to temperature change in Lake Whitefish juveniles

The Regina group conducted experiments where the heat shock response was observed in Lake Whitefish juveniles (age-1 and age-2) reared at different acclimation temperatures.

Lake Whitefish 2-month post-hatch YOY were exposed to heat shocks to determine the heat-shock response in juveniles compared to embryos. Heat shock response was quicker in juveniles than in embryos but levels of HSPs started decreasing by 4 to 8 hours post-heat shock [R-98].

The potential for thermotolerance development in Lake Whitefish YOY was investigated. YOY Lake Whitefish were acclimatized to 8, 13 or 19°C conditions in 2014 and then exposed to one of three different heat shock temperatures. In 2015, sample analysis of liver, gill and muscle was completed for HSP expression (hsp70, hsc70, hsp47, hsp90, beta actin and gapdh).

Changes in the cellular heat shock response in Lake Whitefish during juvenile development are being examined.

<u>Growth, development and mortality effects of temperature changes on Lake Whitefish</u> juveniles

The Regina group effect of thermal stress on 2-month post-hatch YOY Lake Whitefish was examined. Temperature increases of 3, 6, or 9° C for 0.25 to 4 hours with a two-hour recovery period were compared to constant 14 °C control temperatures. The Lake Whitefish YOY were able to mount a heat stress response that allowed them to survive a 4-hour 9°C heat stress [R-97].

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The effect of rearing temperature for Lake Whitefish on growth, metabolism behaviour, thermal tolerance and swim performance at hatch, 6 months and 1 year post-hatch will be examined in 2017.

Whole organismal responses to temperature in Lake Whitefish embryos and juveniles

In situ (Lake Huron) incubation experiments were conducted in 2011, 2012, and 2013 close to the thermal discharge from Bruce A and Bruce B and in two control locations. Following 2011 and 2012 deployment, live embryos were retrieved in the spring and preserved for subsequent morphometric analysis. Following the 2013 deployment, 200 live larvae were retrieved and preserved for subsequent morphometric analysis (extensive ice cover prevented retrieval prior to hatch). Embryos incubated near the thermal discharge were 10% ahead of controls in time-to-hatch. No increase in embryonic mortality or development abnormalities was noted with the increased rate of development [R-103] [R-104].

The average water temperature near the thermal discharge was between 0.5 and 3.7°C. The temperature in the thermal discharge areas was an average of 3°C warmer than the surrounding waters and also more variable with a greater range and rate of change than surrounding waters. Discharge temperature spikes rarely exceed 8°C. Only twice did the temperature exceed 10°C for between 5 and 30 minutes [R-104].

The McMaster group has collaborated with Laurentian University to design and build a flume at the Living with Lakes facility. The flume is being used to examine the effect of temperature changes in the water column on Lake Whitefish embryos incubating at various depths in the cobble substrate. Analysis of the growth and development of embryos incubated in the simulated flume is currently underway.

Summary of Significant Effects

Table 5 provides a summary of the significant effects of temperature changes on Lake Whitefish from peer-reviewed publications.

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Table 5 Significant Published Thermal Effect Research Findings for Lake Whitefish

Life Stage	Temperature Regime	Significant effects	Ref
Cellular respo	onse to changes in temperature		
Embryo (fin-flutter, vitelline circulation stage)	Control: 2°C Heat shock: +3, 6 or 9°C for 0.25,0.5, 1, 2, 3 or 4 hours Recovery: 2 hours	Heat shock=↑ <i>hsp70</i> mRNA level Robust HSR allows survival of a 4-hour +9°C heat shock	
	Control: 2°C Heat shock: +3, 6 or 9°C for 2 hours Recovery: 1, 2, 4, 8,12, 16, 24, 36 or 48 hours	Duration and level of heat shock <i>hsp70</i> elevation increased through to 48 hours	[R-97]
Juvenile	Control: 14°C Heat shock: +3, 6 or 9°C for 0.25,0.5, 1,2,3 or 4 hours Recovery: 2 hours	Heat shock=↑ <i>hsp70, hsp90α</i> and <i>hsp47</i> mRNA level Robust HSR allows survival of a 4-hour +9°C heat shock	-
(Young of the Year)	Control: 14°C Heat shock: +3, 6 or 9°C for 2 hours Recovery: 1, 2, 4, 8,12, 16, 24, 36 or 48 hours	Duration of heat shock <i>hsp70</i> elevation peaked at 2 hours post-shock and dropped rapidly.	

Growth, development and survival responses to temperature change

,		-	
	Constant temperatures of 2, 5 or 8°C and shifts between them at the end of gastrulation and/or organogenesis	↑incubation temp in gastrulation= ↑oxygen consumption at end of gastrulation	
		†incubation temp in gastrulation/organogenesis= ↑oxygen consumption and heart rate	
Embryo		†incubation temp in gastrulation/organogenesis/to hatch= ↑oxygen consumption and heart rate	[R-41]
		Temperature regimes from earlier development periods are reflected in oxygen consumption and heart rate of embryos compared to constant temperature controls. Incubation temperature to end of gastrulation reflected in embryos with final incubation temperature of 8°C	

Rev 00	Significar ↑incubatio ↓time to pr 5°C, 50d a ↓yolk-free ↑yolk dry r ↓eye diam ↓body leng	n temp= re-hatch (148d at 2°C, s at 8°C) dry mass mass neter		84 of 108 Ref
	↑incubatio ↓time to pr 5°C, 50d a ↓yolk-free ↑yolk dry r ↓eye diam ↓body leng	n temp= re-hatch (148d at 2°C, s at 8°C) dry mass mass neter	92d at	Ref
	↑incubatio ↓time to pr 5°C, 50d a ↓yolk-free ↑yolk dry r ↓eye diam ↓body leng	n temp= re-hatch (148d at 2°C, s at 8°C) dry mass mass neter	92d at	Ref
	↓time to pr 5°C, 50d a ↓yolk-free ↑yolk dry r ↓eye diam ↓body leng	re-hatch (148d at 2°C, s at 8°C) dry mass nass neter	92d at	
		yo mass, ly length	[R-71]	
or 8°C end of sis	HS had hig fincubatio time to 50 days at 5° hatch win either 2 or survival (at 8°C) hatchling 2°C, 0.61n fyolk dry r yolk conv cost of de hatched at temperatu Fin-flutter developme greatest in incubation effect on e	gher cumulative surviva n temp= 0% hatch (164 days at C, 63 days at 8°C) ndow (66% less at 8°C 5°C) 55% at 2°C, 38% at 5° yolk-free dry mass (1.2 ng at 8°C) mass version efficiency evelopment (except reg t 8°C but incubated at of res fertilization-organoo to hatch a critical period ent where temperature npact on energy cost (i temperature shows grown embryo morphology and	al. 2°C, 94 versus C, 17% 21mg at 21mg at gimes colder genesis) d in has .e., final eatest	[R-81]
		↑yolk dry r ↓yolk conv ↑cost of de hatched a temperatu Fin-flutter developm greatest in incubation effect on e developm	hatched at 8°C but incubated at a temperatures fertilization-organo Fin-flutter to hatch a critical perio development where temperature greatest impact on energy cost (i incubation temperature shows gr effect on embryo morphology and development cost).	 ↑yolk dry mass ↓yolk conversion efficiency ↑cost of development (except regimes hatched at 8°C but incubated at colder temperatures fertilization-organogenesis) Fin-flutter to hatch a critical period in development where temperature has greatest impact on energy cost (i.e., final incubation temperature shows greatest effect on embryo morphology and

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Life Stage	Temperature Regime		Signific	ant effects		Ref
	<i>In-situ</i> incubation chambers over winters	3	Maximu above 8	m temperature = 10.7°C °C).	(Rarely	
	(Discharge area versus Referenc	e area)	Dischar referend	ge area up to 3°C warme ce area.	er than	
				on near thermal discharg	je=	
Embryo			↑embryo body length ↓embryo yolk area		[R-104]	
			No diffe	rence in embryo eye diar mental abnormalities	neter or	
			days in dischar	dvance (10 days in Year Year 3 – chamber directl ge outflow in Year 3, loca t to flow in Years 1 & 2)	y in	

Round Whitefish

<u>Growth, development and mortality effects of temperature changes on Round Whitefish</u> <u>embryos</u>

10% ahead in percent time-to-hatch

The McMaster group examined the effect of a gradual seasonal changes in temperature $(8^{\circ}C \rightarrow 2^{\circ}C \rightarrow 6^{\circ}C)$ with and without regular 3°C 1-hour temperature spikes during the 2°C period on Round Whitefish embryo survival, growth, development, heart rate and oxygen consumption. Regular temperature spikes were applied three times weekly. Additional temperature regimes included a more rapid warming for the seasonal incline and regular 3°C 1-hour temperature spikes throughout development. Embryo mortality was elevated in the seasonal change group that experienced rapid warming and in the constant 8°C group. Seasonal temperature changes increased the development rate and shortened hatch timing. Embryos incubated at lower temperatures had increased body mass and decreased yolk mass. Exposure to regular temperature spikes had little effect on development stage onset [R-72].

The effect of rearing temperature for Round Whitefish juveniles on growth, metabolism behaviour, thermal tolerance and swim performance at hatch, 6 months and 1 year of age will be examined in 2016-2017.

Molecular and cellular responses to temperature in Round Whitefish embryos and YOY

Full-length gene sequence measurement for HSPs (hsp47, hsp70, hsp90 α , hsp90 β , and hsc70) has been completed for Round Whitefish embryos.

Heat shock induction experiments were conducted in 2015 using YOY Round Whitefish at 4 temperatures (10, 16, 19 and 22°C) for varying durations (1-4 hours) and recovery times (0 to 2 hours). A novel real-time assay was developed for detecting two HSPs (hsp90 α and hsp90 β) and over 8000 DNA HSP expression measurements have been conducted (hsc70, hsp47, hsp47, hsp90a+b, beta actin, and gapdh).

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	Reports							
	A bibliography of scientific publications and theses is provided in Table 6. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to the ecology of Lake or Round Whitefish in Lake Huron have not been listed.							
	Each publication and thesis is revie	ewed in Section 2	10.3.1.					
Scie	Table 6 Scientific reports on thermal effects produced as a result of the NSERC CRD partnership							
	Baseline	growth and deve	lopment					
[R-82]	O'Bryhim, J., Somers, C., Lance, Taylor, E.B., 2013. Development microsatellite markers for the mou cross amplification in the Round V Illumina shotgun sequencing. Co	and characteriza Intain whitefish, p Vhitefish, P. cylii	tion of twenty two novel prosopium williamsoni ar ndraceum, using paired	nd end				
[R-95]	Sreetharan S., Thome C., Mitz C., Eme J., Mueller C.A., Hulley E.N., Manzon Publication R.G., Somers C.M., Boreham D.R., Wilson J.Y.,2015. Embryonic development of Lake (coregonus clupeaformis): a staging series, analysis of growth and impacts of fixation. Journal of Fish Biology, 87, 3:539-558.							
[R-78]	[R-78] Mitz, C., Thome, C., Cybulski, M.E., Laframboise, L., Somers, C.M., Manzon, Publicatio R.G., Wilson, J.Y., Boreham, D.R., 2014. A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture. Journal of North American Aquaculture 76, 179-184.							
	Cellular respor	nse to changes ir	n temperature					
[R-97]	Stefanovic D.I., Manzon L.A., McE Wilson J.Y., Manzon R.G, 2016. in embryonic and young of the yea Biochemistry and Physiology, Par	Thermal stress a ar juvenile Lake \	nd the heat shock respo					
[R-98]	Stefanovic D.I., 2015. Thermal st embryonic and young of the year j clupeaformis). MSc thesis Univer	iuvenile Lake Wh	nitefish (coregonus	MSc thesis				
[R-94]	Sessions, K. Plasticity of the heat thermotolerance during embryonic clupeaformis). MSC thesis Univer	development of	Lake Whitefish (corego					
	Growth, development and su	ırvival responses	to changes in temperat	ure				
[R-41]	Eme J., Mueller C.A., Manzon R.C 2015. Critical windows in embryo temperatures alter heart rate and (coregonus clupeaformis) embryo and Physiology, Part A 179, 71-81	nic development oxygen consump s and hatchlings.	shifting incubation shifting incubation of Lake Whitefish					
[R-71]	Lee AH, Eme J, Mueller CA, Manz JY, 2016. The effects of cumulation morphology and survival of Lake \ Journal of Thermal Biology 57:11-	ve acute heat sh Whitefish (corego	ock exposures on					

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[R-81]	[R-81] Mueller C.A., Eme J., Manzon R.G., Somers C.M., Boreham D.R., Wilson, J.Y., Publication accepted. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in Lake Whitefish (coregonus clupeaformis). Journal of Comparative Physiology B 185, 3:315-331.						
[R-104]	D4] Thome,C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R. & Wilson, Publication J.Y. Incubation of Lake Whitefish (coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development. Canadian Journal of Fisheries and Aquatic Sciences 73, 1213-1221.						
[R-103]	Thome, C. The effects of thermal, chemical and radiological stressors on PhD thesis embryonic development in Lake Whitefish (coregonus clupeaformis). PhD thesis thesis McMaster University, Hamilton, Ontario. 2015						
[R-72]	Lim, Michael. Thermal, morpholine, and radiation stressor effects on the MSc the embryonic development of Lake (coregonus clupeaformis) and Round Whitefish (prosopium cylindraceum). MSc thesis, McMaster University, Hamilton, ON, September 2016.						
[R-79]	Mitz, C., 2016. The cost of horme constrained environment: Embryc (coregonus clupeaformis). PhD th July 2016.	onic development	t of the Lake Whitefish	PhD thesis			

3.3.4 Effects of Combined Stressors on Lake and Round Whitefish

Whole organismal responses to combined stressors in embryos and juveniles

A custom irradiator was built at McMaster University to study the effects of chronic radiation on embryonic development.

Lake Whitefish embryos were exposed to constant or variable temperature, as well as one of acute or chronic gamma radiation or chemical stress (morpholine or sodium hypochlorite). Doses of chronic radiation of 0.06-4.4 mGy/day resulted in an earlier hatch. High dose of acute radiation increase larval mortality. Morpholine concentrations of 1 or 10 mg/L resulted in increased larval size at hatch. There were no significant effects of morpholine at 100 mg/l. From 500-1000mg/L there was a decreased larval size, earlier hatch and increased embryo mortality. Mortality effects on embryos in this study occurred at doses significantly greater than those in the thermal discharge channels at Bruce Power [R-103].

The McMaster group explored the potential protective effects of heat shocks against radiation exposure, the lethal heat shock dose and the potential for low dose radiation to be protective of acute heat shock in terms of mortality and morphology among Lake Whitefish. A heat shock of 2-hours duration (temperature increase of 3°C or 9°C) administered six hours prior to irradiation decreased embryo mortality by up to 25%. The same heat shock had no protective effect against subsequent morpholine exposure [R-103].

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The McMaster group also examined the effects of exposing Round Whitefish embryos to constant and variable temperature regimes, radiation and morpholine concentrations. Analysis of this work is in progress.

In collaboration McMaster and Laurentian University are raising Lake Whitefish embryos in the underground SNOLAB to examine the effects of reduced background radiation. Analysis of the 2015 results from the first round of embryo incubations is in progress.

The McMaster group examined how growth stimulated by exposure of Lake Whitefish embryos to ¹³⁷Cs affected metabolic efficiency. Embryos incubated at 5°C for 40 days, then at 2°C for the remainder of development were exposed to a dose of gamma radiation every 14 days in doses ranging from 14mGy to 8 Gy per dose. Measurements were taken at 14 and 42 days after the final dose. Irradiated embryos were 8 to 10% larger than controls and had no significant change in YCE [R-79].

The McMaster group examined the effect of chronic morpholine and low-dose radiation exposure on the survival, growth, development, heart rate and oxygen consumption of Round Whitefish embryos. Round Whitefish embryos were exposed to chronic levels of morpholine (10, 100, 500 and 1000 mg/L), pH controls (8, 9 and 10) or low-doses of radiation (0, 0.10, 0.16 or 0.30 mGy/day of¹³⁷Cs gamma rays) throughout development. There was increased mortality to hatch in the embryos exposed to morpholine \geq 500 mg/L. Post-hatch mortality decreased by >20% in the irradiated embryos (0.10 to 0.30mGy/day) with little effect on growth or hatch timing. Embryos exposed to 500mg/L morpholine had delayed development stage onset, shortened hatch windows and close to 100% mortality at 30 days post-hatch. There were no consistent effects on growth and development of Round Whitefish at morpholine levels of 0-100 mg/L. Round Whitefish were found to be more susceptible to morpholine and possibly less susceptible to low-dose radiation than Lake Whitefish [R-72].

Molecular and cellular responses to combined stressors in embryos and juveniles

Heat shock response and recovery is being examined in Round Whitefish embryos and YOY. Sample processing and statistical analysis is in progress.

The McMaster University group has optimized the micronucleus assay with cells in culture to determine the effects of ionizing radiation on an established fish cell line (rainbow trout cell line RTG-2). Attempts to transfer this work to Lake Whitefish cell lines were unsuccessful due to technical problems with maintaining Lake Whitefish cell lines and will be repeated in 2016-2017.

The effect of exposure to morpholine, temperature changes and/or ionizing radiation on micronuclei formation has been explored using the rainbow trout cell line.

Reports

A bibliography of scientific publications and theses is provided in Table 7. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to the ecology of Lake or Round Whitefish in Lake Huron have not been listed.

Each publication and thesis is reviewed in Section 10.3.2.

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WHITEF	WHITEFISH RESEARCH REVIEW					
Table 7 Scientific reports on combined stressors produced as a result of the NSERC CRD partnership						
[R-103]	R-103] Thome, C. The effects of thermal, chemical and radiological stressors on Phi embryonic development in Lake Whitefish (<i>coregonus clupeaformis</i>). PhD thesis McMaster University, Hamilton, Ontario. 2015					
[R-72]						

[R-79]Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically
constrained environment: Embryonic development of the Lake Whitefish
(coregonus clupeaformis). PhD thesis, McMaster University, Hamilton, ON,
July 2016.PhD thesis

3.3.5 Population Discrimination of Lake and Round Whitefish

Lake and Round Whitefish population structure

Hamilton, ON, September 2016.

The University of Regina group attempted to utilize a mitochondrial DNA control region to examine population structure in Lake Whitefish. No variation in the mitochondrial DNA sequence was observed, consistent with a single population or significant genetic mixing between populations. Mitochondrial DNA analysis was not continued for Lake Whitefish, but was continued for Round Whitefish.

New microsatellite markers have been developed for Round Whitefish [R-82]. The University of Regina group has genotyped 390 Round Whitefish at 11 microsatellite loci. Mitochondrial DNA sequencing at 2 loci has been completed in 124 Round Whitefish. Population structure analysis was accomplished for 390 Round Whitefish. Phylogenetic analysis (i.e., analysis of the genetic relationships between individual fish) is being finalized for 112 Round Whitefish.

The University of Regina is utilizing single-nucleotide polymorphisms (SNPs) to genotype Lake and Round Whitefish. The impact of DNA degradation on the characterization of SNPs in juvenile Lake Whitefish was investigated. Degradation had a minimal impact on genotyping identification until the samples had been held more than 48 hours at room temperature [R-55]. The DNA from 190 Round Whitefish has been sequenced at approximately 24,000 loci for each fish. Round Whitefish population structure analysis is complete. The DNA from 148 Lake Whitefish has been extracted and prepared and is waiting for sequencing at approximately 25,000 to 50,000 loci per fish.

The McMaster group has collaborated with Laurentian University to examine DNA barcoding of larval fish and eggs. They have also developed a real time PCR assay for detection of Lake Whitefish, Deepwater Sculpin and Round Whitefish using DNA barcoding.

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Lake and Round Whitefish movements and habitat use

Muscle tissue samples from nearly 32 spawning aggregations of adult Lake Whitefish (1474 fish) (collected in 2012, in collaboration with the University of Guelph research group) and 8 summer diet assemblages (220 fish) (collected in 2014) have been analyzed for carbon and nitrogen stable isotopes ratios. Changes in stable isotope ratios in fish tissue reflect differences in the isotopic baselines of specific lake locations, the use of different habitats and specific prey choices. The population of Lake Whitefish in Lake Huron could be divided into six ecological sub-populations based on local resource use (i.e., prey, habit and location). In the area local to Bruce Power, the Main Basin of Lake Huron could be divided into Southwestern and Northeastern (including the Bruce Power site) ecological groups with heavy population mixing within each of these groups. No small distinct ecological groups were identified within the area near the Bruce Power site (i.e., Scougall Bank and McRae Point) [R-38] [R-40].

Samples of liver, muscle and bones from Lake (n=60-182 annually) and Round (n=149-188 annually) Whitefish collected near Douglas Point from 2010 to 2012 have been analyzed for carbon and nitrogen stable isotope ratios. Additional tissue samples were also collected from summer feeding groups of Lake (n=31) and Round (n=29) Whitefish in Lake Huron (2014). Lake Whitefish used more diverse resources than Round Whitefish. Niche overlap occurred only in liver (representing the spawning period) while niche segregation was highest in the juvenile life stage. Spawning aggregations were composed of individuals with a variety of feeding sources and from a variety of locations for both Lake and Round Whitefish [R-39].

Genetic and stable carbon and nitrogen isotope ratios were used to examine the ecological and genetic structure of Lake (n=208-336) and Round (n=319-327) Whitefish in the immediate vicinity of the Bruce Power site. Whitefish from six potentially affected areas near the thermal effluent discharges at Bruce A and B and from two reference areas were gill netted from October to December of 2010 and 2011. Genetic analysis indicated that fish caught close to Bruce Power were part of a larger population going past the study area boundary. For one potentially affected sampling area for Lake Whitefish (i.e., McPherson Bay) and three potentially affected sampling areas for Round Whitefish (i.e., McPherson Bay, Gunn Point and Inverhuron Bay), the niche size was 1.3 to 2.8 times larger than the niche size in the reference areas. The larger niche sizes may be due to desirable habitat and greater mixing of fish from multiple feeding locations or to the lack of spawning in this area (i.e., that Lake or Round Whitefish captured there were simply moving through the area).There was significant overlap in resource use (over 72%) for Round and Lake Whitefish between reference areas and potentially affected sampling areas [R-54].

A genetic analysis of 414 Round Whitefish from 16 locations across North America (6 in the Western Region, 10 in the Eastern Region) and one site in eastern Russia was completed by the University of Regina group. The genetic analysis compared results from microsatellites (n=390), mitochondrial DNA sequencing (n=124) and next RAD SNP loci sequencing (n=190). For the Eastern population, the Lake Huron population appears to be a source population for Lakes Michigan, Superior and northern Georgian Bay as well as Lake Nipigon through Lake Superior. Sampling was not wide-spread enough within Lake Huron to draw conclusions regarding population structure within the lake, although microsatellite analysis suggested the possibility of a distinct Round Whitefish population in Southern Georgian Bay [R-80].

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Reports

A bibliography of scientific publications and theses is provided in Table 8. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to the ecology of Lake or Round Whitefish in Lake Huron have not been listed.

Each publication and thesis is reviewed in Section 10.3.3.

Table 8 Scientific reports on population structure produced as a result of the NSERC CRD partnership

[R-82]	O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the mountain whitefish, <i>prosopium williamsoni</i> and cross amplification in the Round Whitefish, p. <i>cylindraceum</i> , using paired end Illumina shotgun sequencing. Conservation Genetics Resources 5, 89 91.	Publication
[R-37]	Eberts, R., 2013. Dietary niche partitioning by two sympatric whitefish species in Lake Huron: stable isotope analysis at three temporal scales. B.Sc. (Hon.) thesis. University of Regina, Saskatchewan. April 2013.	B.Sc. thesis
[R-38]	Eberts, R., 2015. Resource use and ecological population structure of Lake Whitefish (<i>coregonus clupeaformis</i>) spawning aggregations in Lake Huron. MSc thesis. University of Regina, Saskatchewan. December 2015.	MSc thesis
[R-54]	Graham CF, Eberts RL, Morgan TD, Boreham DR, Lance, SL, Manzon RG, Martino JA, Rogers SM, Wilson JY, Somers CM, 2016, Fine-scale ecological and genetic population structure of two Whitefish (<i>Coregoninae</i>) species in the vicinity of industrial thermal emissions. Plos ONE. DOI: 10.1371/journal.pone.0146656.	Publication
[R-55]	Graham, CF, Glenn, TC, McArthur, AG, Boreham, DR, Kieran, T, Lance, S, Manzon RG, Martino, JA, Pierson, T, Rogers, SM, Wilson JY, Somers CM, 2015. Impacts of Degraded DNA on Restriction Enzyme DNA Sequencing (RADSeq). Molecular Ecology Resources 6:1304-15.	Publication
[R-39]	Eberts, R., Wissel, B., Boreham, D., Manzon, R., Wilson, J. and Somers, C., 2015. Consistent differential resource use by sympatric Lake (<i>coregonus clupeaformis</i>) and Round Whitefish (<i>prosopium cylindraceum</i>) in Lake Huron: a multi-time scale isotopic niche analysis. <i>Canadian Journal of Fisheries and Aquatic Sciences</i> . Published on the web 08 Dec 2015, 10.1139/cjfas-2015-0324.	Publication
[R-40]	Eberts, R., Wissel, B., Simpson, G, Crawford, S., Stott, W., Hanner, R., Manzon, R., Wilson, J., Boreham, D. and Somers, C., 2017. Isotopic structure of Lake Whitefish in Lake Huron: Evidence for regional and local populations based on resource use. North American Journal of Fisheries Management 37, 133-148.	Publication
[R-80]	Morgan, T., 2016. Genetic population structure of the Round Whitefish (<i>prosopium cylindraceum</i>) in North America: multiple glacial refugia and regional subdivision. MSc thesis, University of Regina, November 2016.	MSc thesis

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3.3.6 Summary

University of Regina and University of Guelph researchers collaborated with respect to collecting and sampling nearly 2000 Lake and Round Whitefish from spawning grounds around the main basin of Lake Huron as well as the North Channel and Georgian Bay from 2010 to 2014.

University of Regina researchers used genetic analysis to examine the relationships between spawning fish, and stable isotope analysis to examine the movement and resource use of adult fish at different locations relative to Bruce Power. They completed both fine-scale (near Bruce Power) and lake-wide assessments.

Research examining Lake and Round Whitefish genetics on a fine scale in the immediate vicinity of Bruce Power found no evidence for the presence of distinct genetic groups of Lake or Round Whitefish within the vicinity of Bruce Power.

Similarly, stable isotope analysis showed no evidence for distinct Lake or Round Whitefish feeding in this vicinity, as there was high resource use overlap between fish at different sampling areas. However, their research did show that Lake and Round Whitefish occupy separate ecological niches (i.e., different locations, habit and food sources), based on different isotope ratios. The period where Lake and Round Whitefish showed the most overlap in stable isotope ratios was during the spawning period.

From a lake-wide perspective, stable isotope analysis showed that six ecological groups of Lake Whitefish were present in Lake Huron. Lake Whitefish near Bruce Power were found to be part of a larger mixed group which encompasses the Northeastern Main Basin of Lake Huron. The results from the Lake Whitefish ecological analysis are consistent with the previous, more limited genetic analysis suggesting weak structure within the eastern main basin.

Also from a lake-wide perspective, a genetic analysis of the geographic distribution of Round Whitefish was completed. The genetic analysis found that Lake Huron is a source population for Round Whitefish for Lakes Michigan, Superior and northern Georgian Bay as well as Lake Nipigon through Lake Superior. Sampling was not wide-spread enough within Lake Huron to draw conclusions regarding population structure within the lake.

The research results conclusively demonstrate that spawning-condition Lake Whitefish near the Bruce Power site are members of a larger genetic and ecological group. There is no evidence of any distinct genetic or ecological population of Lake or Round Whitefish utilizing spawning grounds near Bruce Power.

Bruce Power has funded extensive research into the impacts of thermal and combined thermal, radiological and chemical stressors on developing Lake and Round Whitefish embryos. This has included examining stressor effects on cellular responses and growth, development and survival.

New incubation strategies were developed to study the effects of stressors on Whitefish. Heat shock protein gene sequences were identified to aid in the identification of cellular stress responses in Round and Lake Whitefish.

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Lake Whitefish embryos were incubated at several different temperatures and were exposed to regular heat shocks throughout development in multiple experiments by researchers at McMaster and Regina Universities. They found that embryos could mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. Lake Whitefish embryos were slower than 2-month post-hatch YOY to show a heat shock response but once they showed a response, it continued over at least 2 days following the heat shock.

Lake Whitefish incubated at higher constant temperatures showed increased heart rate and oxygen consumption. Incubation at higher constant temperatures earlier in incubation had a lasting effect even when the temperatures later in incubation were cooler. Higher constant temperatures also resulted in a shorter incubation period, higher yolk mass and lower body lengths and mass. Higher constant incubation temperatures also decreased the hatch window, survival and increased oxygen needs. Regular weekly heat shocks had no effect on time-to-hatch, survival, yolk mass, body mass, length or head width.

Incubation of Lake Whitefish embryos in chambers on the lake bottom in the Bruce Power discharge areas resulted in an average 10% advance in development. Embryos incubated in the discharge area were slightly longer with a smaller yolk area but showed no increase in developmental abnormalities. These chambers were deployed on the lake bottom, above the cobble substrate, whereas normal Lake Whitefish incubation tends to occur down within the cobble. There is the potential for the cobble substrate to provide a thermal buffer which may reduce the temperature effects observed in the *In situ* study. This is currently being investigated using the thermal flume at Laurentian University.

Round Whitefish embryos experienced no increase in mortality or changes in development with exposure to intermittent heat shocks.

Final publication of work surrounding the effects of different thermal regimes, the potential for the development of thermal tolerance and the effects of combined stressor in Lake and Round Whitefish is in progress.

There remain several uncertainties in the outcomes of Lake and Round Whitefish, particularly with respect to behaviour and fitness. It is not known whether increased incubation temperatures impact the cellular and whole organism stress responses. It is also not know how increased incubation temperatures impact swim performance and foraging capability in larval and juvenile Whitefish.

3.4 McMaster/Mitacs Accelerate Cluster

3.4.1 Scope

Bruce Power partnered with McMaster University and Mitacs to carry out a two-year research program to address additional potential effects of Bruce Power operations on aquatic biota. Four post-doctoral fellows were recruited for short-term research projects, including:

- Impacts of DNA degradation on single nucleotide polymorphism.
- Development of a chronic irradiator for whitefish embryos and optimization of DNA damage assays.

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• Effect of permanent changes in rearing temperature on growth, development, and juvenile phenotypes.

The research projects were integrated into the overall McMaster/Regina /NSERC collaboration to further complement the scope of research.

3.4.2 Summary

Scientific results from these research projects were integrated into the discussion in Sections 3.3 and 10.3.

3.5 COG Thermal Effects Benchmarks

3.5.1 Scope

CANDU Owners Group (COG) has sponsored a research and development work package intended to verify or review thermal effects benchmarks for indicator aquatic species, including Lake and Round Whitefish.

Laboratory research includes embryo incubation under realistic temperature conditions (i.e., variable temperatures per actual lake conditions).

3.5.2 Accomplishments

SENES Consultants Ltd. established egg incubation experiments at the Wesleyville Hatchery and Aquatic Research Facility (WHARF), utilizing water drawn from Lake Ontario. Incubation temperatures (and to a certain extent water quality) mimicked ambient or natural conditions.

In 2011, Lake and/or Round Whitefish eggs were collected from Lake Ontario, Lake Huron, and Lake Simcoe. Experimental treatments focused on constantly varying temperatures, cycling from ambient to a maximum ΔT (with respect to ambient) and back at a rate of 0.5°C/h. Thus, experimental groups with varying temperatures were exposed to constantly changing temperatures.

In 2012, Lake and/or Round Whitefish eggs were collected from Lake Ontario and Lake Huron. Experimental treatments replicated measured temperatures near nuclear power station plumes, with data collected in the winter of 2011-2012 (prior to four-unit operation at Bruce A).

Patrick reported an advanced (median) hatch of 3.5 weeks for Lake Whitefish eggs incubated at a base temperature corresponding to natural, variable Lake Ontario conditions with the addition of a continuously-varying cycle temperature (0.5° C/h; maximum temperature increase of 0°C to 5°C) [R-90].

Reports

An interim COG technical report [R-89] has been summarized in the form of a scientific publication [R-90], listed in Table 9 and reviewed in Section 10.4.

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Table 9 Scientific reports produced as a result of the COG thermal effects research						
[R-89] Patrick, P.H., Chen, E., Parks, J., Powell, J., 2013. Effects of fixed and fluctuating temperature on early hatch of Round and Lake Whitefish eggs. CANDU Owners Group Inc. TN 12 3049. March 2013.				Technical report		
[R-90]						

[R-92] Patrick P., Mason E., Parks J., Powell J., Garisto N., Janes A., 2014. Effects Technical of fixed and fluctuating temperature on mortality and hatch of Round Whitefish report and Lake Whitefish eggs. CANDU Owners Group Inc. COG-13-3025. October 2014.

Lake Whitefish eggs. North American Journal of Fisheries Management 33,

3.5.3 Summary

1091 1099.

Considering all experiments together, Patrick et al. concluded that generally increased temperatures resulted in advanced hatch [R-92]. Nonetheless, experiments in 2012-2013 that attempted to simulate realistic plume scenarios consistently resulted in *delayed* hatch. No explanation for this inconsistency was advanced. Additionally, the width of the hatch window did not vary consistently between treatment groups.

Patrick, et al., developed models of survival and hatch timing based on average temperatures experienced [R-92]. A more fine-grained model based on developmental blocks was not preferred due to a lack of developmental synchrony.

Patrick, et al., did not specify a thermal effects benchmark for Lake Whitefish, but suggested a benchmark for Round Whitefish survival of 3.7°C. This benchmark was shown to be protective of Lake Whitefish as well, with respect to direct mortality effects of either fixed or fluctuating temperature increases greater than ambient. However, the effects of advanced hatch were not assessed in terms of a thermal benchmark.

3.6 Laurentian/Mitacs Accelerate

3.6.1 Scope

Bruce Power has partnered with Laurentian University, University of Laval, and Mitacs to carry out a five-year research program to address scientific knowledge gaps with respect to:

- Determining appropriate thresholds for population-level effects
- Assessing the movement and speciation of isotopes in the environment
- Understanding the effects of very low dose radiation on humans and biota

Laurentian University and University of Laval are in the process of recruiting up to three post-doctoral fellows and six graduate students to conduct this research. The incubation of Lake Whitefish eggs in a simulated thermal plume was started in 2015-2016.

Mitacs approved this scientific proposal. The research is being conducted from 2015-2019. Master Created: 31Mar2017 10:53

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4.0 LAKE WHITEFISH POPULATION AND HABITAT SURVEYS

This section summarizes efforts to identify and quantify Lake Whitefish populations and habit in the vicinity of Bruce Power since 2000. These efforts have consisted of larval tows, substrate mapping, egg sampling and larval traps and index netting. Figure 2 shows the potential Whitefish spawning habitat and the locations covered by the population surveys completed near Bruce Power.

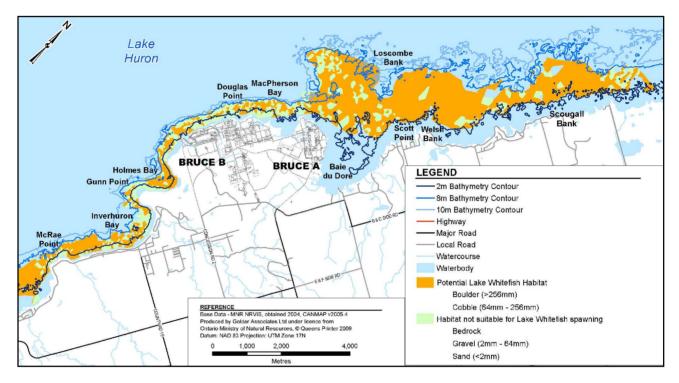


Figure 2 Potential Whitefish Habitat

4.1 Larval Tows

4.1.1 Baie du Dore and Inverhuron Bay (2000-2001)

Preliminary surface water tows were conducted during daytime tows (120 μ m mesh and 50 cm diameter net) in May 6, 2000 in Baie du Doré and Inverhuron Bay as part of the WINGS study [R-60]. Lake Whitefish were recovered at only one of seven sites (in Baie du Doré), for a mean Lake Whitefish density of 0.041 larvae/m³.

As part of the WINGS study, larval tows were conducted in 2001 in bays near Bruce Power (Baie du Doré, Holmes Bay and Inverhuron Bay) as well as a reference site (Howdenvale Bay, near the Fishing Islands) [R-60]. The mean larval density of Lake Whitefish in shallow bays near Bruce Power in 2001 was substantially greater than that observed in the source water near Bruce A in 2013 (0.01 larvae/m³ at Baie du Doré and Holmes Bay and 0.018 larvae/m³ at Inverhuron Bay, compared to 0.0001 larvae/m³ in source water trawls in 2013). The mean larval density at the reference site in 2001 was also substantially greater at 0.056 larvae/m³.

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Collection via seines and dip nets was also attempted but shown to be less feasible than plankton tows [R-60].

4.1.2 Baie du Dore, Inverhuron Bay & Shoals (2004)

In 2004, surface and substrate larval tows were performed near Bruce Power (Scougall Bank, Welsh Bank, Baie du Doré, Loscombe Bank, Gunn Point and Inverhuron Bay) and at a reference site (Howdenvale Bay, near the Fishing Islands) [R-96]. For tows at 1 m below the surface, the CPUE at both Scougall Bank and Welsh Bank was 0.013 whitefish larva/m³. No larvae were captured when the bongo nets were towed at deeper depths at these locations. Additionally, no larvae were captured at Loscombe Bank, Gunn Point or Inverhuron Bay. For comparison, the CPUE at the reference sites (0.113 larvae/m³ at Howdenvale Bay) was ten times greater than that the CPUE for near-surface tows at Scougall Bank and Welsh Bank.

4.1.3 Bruce A Intake & Shoals (2006)

Larval tows were conducted in 2006 at Scougall Bank, Welsh Bank, Loscombe Bank and near the Bruce A intake as well as at three shoals near the Fishing Islands (near Howdenvale Bay) [R-46]. Lake Whitefish larvae were captured only at Scougall Bank, and none were captured at other potentially affected sites (0.0086 larvae/m³ at Scougall Bank and 0.0017 larvae/m³ for all Bruce Power sites combined). CPUE was similar at the Fishing Islands sites (combined) when compared to the Bruce Power sites (combined): 0.0022 larvae/m³ versus 0.0017 larvae/m³.

4.1.4 MacPherson Bay (2007)

Larval tows were completed between MacPherson Bay and Bruce B in order to ensure that larval traps were being fished during the Lake Whitefish hatching period. 52 tows were conducted from April 10-May 30, 2007 resulting in the catch of 6 Lake Whitefish larvae. Larval densities were not reported [R-45].

4.1.5 Bruce A Intake (2013-2014)

A total of 5 source water sampling surveys (25 transects) were completed in May and June of 2013 and 2014, as reported in [R-24].

In 2013, Lake Whitefish was infrequently encountered (6 larval fish; CPUE of 0.0001 larvae/m³). Of the 6 Lake Whitefish that were captured in 2013 source water larval tows, 2 were alive and apparently healthy (AAH), 3 were alive but stressed (AS), and one was recently dead (RD). In 2014, Lake Whitefish were not captured [R-24].

4.2 Substrate Mapping

4.2.1 Substrate Mapping (2007)

Bottom substrate mapping was performed in MacPherson Bay [R-45]. A coarser map was completed in 2009 [R-47] [R-48], but this later map extended over the entire Bruce Power site shoreline.

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4.2.2 Substrate Mapping (2009)

Bottom substrate mapping was performed in MacPherson Bay [R-45]. A coarser map was completed in 2009 [R-47] [R-48], but this later map extended over the entire Bruce Power site shoreline.

Substrate mapping, conducted in 2009, determined that potential Lake Whitefish spawning habitat comprised roughly 72% of the area potentially affected by the Bruce A thermal plume between the 2 m and 8 m contours [R-47]. This proportion is slightly less than the proportion of potential Lake Whitefish spawning habitat in the reference areas. Scott Point, the mouth of Baie du Doré, Loscombe Bank and Welsh Bank were identified as potential spawning sites, while Gunn Point was shown to have limited potential spawning habitat. The results, which were consistent with previous surveys [R-83], were reported in the 2009 EA FUP annual report [R-48].

4.3 Egg Sampling and Larval Traps

4.3.1 North of Bruce A (2006)

Due to the possibility that newly-hatched larvae would be transported away from Bruce Power, emergent larval tarps were north of Bruce A (Scougall Bank, Welsh Bank and Loscombe Bank) and at two sites near the Fishing Islands in 2006 [R-46]. One Lake Whitefish larva was captured at a Bruce Power site (Gunn Point), while 14 were captured the Fishing Islands. In terms of catch-per-unit-area, the Fishing Islands sites had a substantially greater larval density of 0.071 larvae/m³ compared to 0.0065 larvae/m³ at Bruce Power (or 0.024 larvae/m³ at Gunn Point only).

The emergent trap study provides context indicating approximately three to ten times more emerging larvae at the reference site compared to Bruce Power.

4.3.2 MacPherson Bay (2007)

In spring of 2007, 17 larval traps (629 trap-nights between April 24 and May 9, 2007) were deployed on cobble substrate adjacent to MacPherson Bay at depths of 3-8 m. No larval coregonids were captured [R-45].

4.3.3 Airlift Sampling (2009)

Airlift egg sampling was conducted in 2009 in order to quantify egg deposition within the potentially affected and reference areas [R-47]. No Lake Whitefish eggs were collected. It was concluded that airlift sampling, deployment of egg bags, and use of towed sleds would not be practical for future sampling. With no direct evidence of egg deposition, it was recommended that presence and numbers of spawning adults could be used as a surrogate for spawning activity and egg deposition. Results were reported in the 2009 EA FUP annual report [R-48].

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4.4 Index Netting

Index netting was completed intermittently between 1979 and 2011 in the vicinity of Bruce Power. Sampling methods and equipment have varied considerably between years, making comparisons between years challenging.

4.4.1 Annual Gill Netting (1979-1989)

Standardized gill netting was carried out annually from May to November between 1979 and 1989, except in 1982. From 1987 to 1989, Round Whitefish contributed 1.6% to 2.5% of the total May to November gill netting catches. The highest Round Whitefish CPUE values of 0.8 to 1.5 fish per 24 hour net set were obtained in October and November from 1979 to 1989. Round Whitefish were found to use the entire near-shore area when they were present [R-7].

Lake Whitefish were reported to scarce in gill netting catches, with only nine caught from 1979 to 1986. From 1987 to 1989, the catch remained small at 12 Lake Whitefish over three years [R-7].

Round Whitefish have been captured in ripe and/or spent condition near Bruce B in 1987 and 1988 from late-November to early December. Benthic sled sampling in Inverturon Bay in 1986 found high concentrations of Round Whitefish [R-7].

4.4.2 WINGS (2000-2001)

Gillnets were deployed from May 2-6 and November 8-9, 2000 at eight sites near Douglas Point, corresponding to hydroacoustic survey transects. Although effort was limited, the greatest abundance was observed nearest Gunn Point/Inverhuron Bay in both the gillnetting data (Table 10 & Table 11) and hydroacoustic data (not discussed here).

Trapnets and gillnets were set November 15, November 23, and November 29, 2001 at two locations north and west of Bruce A (Table 10 & Table 11). Trapnets were not effective (but Lake Whitefish may have been in deeper water). The final sampling date (November 29, 2001) included only gillnet sampling. CPUE was greatest for Lake Whitefish on this sampling date.

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Location	Year	Method (Depth)	Length (m)	Time (h) ¹	LW	LW/km	LW/h
Welsh Bank	2000	S GN (12m)	274	16	1	3.6	<0.1
		GN (12m)	274	16	9	32.8	0.5
		GN (28m)	548	32	26	47.4	0.8
Scott Point	2001	TN	-	16	1	-	<0.1
Loscombe Bank	2000	S GN	274	16	0	0	0
		GN (12m)	548	32	6	10.9	0.2
		GN (28m)	274	16	12	43.8	0.7
	2001	GN (12m)	297	16	29	97.6	1.8
Douglas Point	2000	S GN	274	16	3	10.9	0.2
		GN (12m)	274	16	9	32.8	0.6
		GN (28m)	274	16	0	0	0
	2001	GN (12m)	274	16	0	0	0
		TN	-	64	2	-	<0.1

Table 10 Lake Whitefish catch-per-unit-effort (CPUE), WINGS 2000-2001

¹WINGS report indicates set time varied from 15.25-21 hours but uses 16 hours in calculate CUE

S: Spring 2000 sampling dates

GN: Gillnet

TN: Trap Net

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Table 11

Mean Lake Whitefish CPUE (2000-2001)						
Location	CPUE (LW/km)	CPUE (LW/h)				
Welsh Bank	27.9	0.45				
Scott Point		<0.1				
Loscombe Bank	38.0	0.7				
Douglas Point	10.9	0.2				

4.4.3 Fish Community Assessment (2007)

Fish community assessment was conducted with experimental gillnets and Nordic nets deployed at six near-shore locations at MacPherson Bay and Baie du Doré in June, July/August, and October, 2007. (Locations are shown in Figure 2.5.3.2-3 of [R-45]; Table E-5 groups these 6 locations into 5 "offshore" and "near-shore" locations.)

Offshore index netting was conducted with the OMNR (locations not shown in the EA study report).

4.4.4 Annual Spawning Assessment (2003-2011)

Trap nets and gillnets were used at Loscombe Bank and Gunn Point in 2003-2006, as part of participation in the lake-wide mark/recapture study [R-43] [R-15] [R-16]. Results of the trap and gill netting surveys are described in Table 12 and Table 13. Mean CPUE is highly variable and higher than that described from 2007-2011 (Table 14). The elevated CPUE for Loscombe Bank and Gunn Point is likely related to the relatively long set times and short gill nets used.

Location	Year	Length (m)	# Net lifts	Time (h)	LW	LW/km	LW/h	LW/lift
Loscombe Bank	2004	44.4	12	192	57	1,283.8	0.3	4.7
	2006	N/A	4	111.6	30		0.3	7.5
Gunn Point	2004	44.4	12	192	166	3,772.7	0.9	13.8
	2005		3	72	56		0.8	18.6
	2006		14	759.3	73		0.1	5.2
Sarnia	2004	532.8	144	264	906	1,700.4	3.4	6.3
	2005		79	168	418		2.5	5.3

Table 12 Lake Whitefish catch-per-unit-effort (CPUE), Trap Nets 2004-2006

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Table 13

Lake Whitefish catch-per-unit-effort (CPUE), Gill Nets 2003-2006

Location	Year L	_ength (m)	Time (h)	LW	LW/km	LW/h
Loscombe Bank*	2003	2742	-	297	108.3	-
	2006	200	48.1	29	145	0.6
Gunn Point	2003	1828	-	163	89.2	-
	2005	-	48	7	-	0.1
	2006	1000	169.6	49	49	0.3
Stony Point	2003	457	-	3	6.6	-
Kettle Point*	2003	2285	-	238	104.2	-

*Includes both deep (25-30') and mid-depth (15-20') gillnet sets

Table 14 Mean Lake Whitefish catch-per-unit-effort (CPUE), available years 2003-2006

Location	CPUE (LW/km) ¹	CPUE (LW/h) ²
Loscombe Bank	126.6	0.3
Gunn Point	69.1	0.6
Stony Point	6.6	-
Kettle Point	104.2	-
Sarnia	-	2.9

¹Gill net results only

² Trap net results only

Gillnets only were deployed at Loscombe Bank and Gunn Point in 2007-2008 [R-17] [R-18]. Gillnetting to document the presence and spawning condition of Lake Whitefish at several sites north and south of Douglas Point was conducted in 2009, 2010 and 2011 [R-48] [R-49] [R-50]. A limited gillnetting program was undertaken in 2012, primarily for the purpose of sample collection. No comparable gillnetting was conducted from 2013 to 2015.

A comparison of catch per unit effort (CPUE) from 2007-2011 is shown in Table 15 (updated from Figure 50 and Table 51 in [R-21]) and Figure 3. CPUE was variable, ranging from 1.7 to 104.5 fish per km gillnet.

Considering the total CPUE over 2009-2011 (Table 16), Scougall Bank, Welsh Bank, and McRae Point (reference areas) have ranked first, second and third, respectively, of the eight sites, while Loscombe Bank ranks fifth of eight sites.

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	Lake whitensh catch-per-unit-effort (CPUE), 2007-2011								
L	ocation	Year	Length (m)	Time (h)	LW	LW/km	LW/h		
1	Scougall Bank	2009	2285	109.0	103	45.1	0.9		
	(Northern reference	2010	2850	128.5	171	60.0	1.3		
	area)	2011	2375	104.1	58	24.4	0.6		
2	Welsh Bank	2009	1828	86.0	191	104.5	2.2		
		2010	2850	131.0	50	17.5	0.4		
		2011	2850	124.1	18	6.3	0.1		
3	Scott Point	2009	2285	110.0	92	40.3	0.8		
		2010	2850	131.0	85	29.8	0.6		
		2011	2375	101.6	19	8.0	0.2		
4	Loscombe Bank	2007	7312	388.5	161	22.0	0.4		
		2008	5941	291.0	51	8.6	0.2		
		2009	5027	267.0	118	23.5	0.4		
		2010	3800	181.5	19	5.0	0.1		
		2011	2850	132.8	25	8.8	0.2		
5	MacPherson Bay	2009	4570	223.0	17	3.7	0.1		
		2010	2850	131.0	10	3.5	0.1		
		2011	2375	105.6	4	1.7	0.0		
6	Gunn Point	2007	7312	388.5	205	28.0	0.5		
		2008	5941	289.5	140	23.6	0.5		
		2009	5484	324.0	54	9.8	0.2		
		2010	3325	150.5	17	5.1	0.1		
		2011	2850	132.1	16	5.6	0.1		
7	Inverhuron Bay	2009	3199	157.0	69	21.6	0.4		
		2010	2375	110.5	13	5.5	0.1		
		2011	2375	116.6	8	3.4	0.1		
8	McRae Point	2009	1828	96.0	78	42.7	0.8		
	(Southern reference	2010	1900	89.5	78	41.1	0.9		
	area)	2011	2850	134.0	35	12.3	0.3		
				·					

Table 15 Lake Whitefish catch-per-unit-effort (CPUE), 2007-2011

Trends for CPUE of ripe male and female Lake Whitefish were slightly different (Figure 3 & Table 17). CPUE effort for ripe female Lake Whitefish (averaged over 2009-2011) was generally between 2-3 fish/km gillnet (less at MacPherson Bay and Gunn Point), with Loscombe Bank ranking third of eight sites. CPUE effort for ripe male Lake Whitefish varied greatly (1-25 fish/km gillnet), with Loscombe Bank ranking fifth of eight sites.

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Table 16
Mean Lake Whitefish CPUE (2009-2011)

	Location	CPUE (LW/km)	CPUE (LW/h)	
1	Scougall Bank (Northern unaffected area)	44.2	0.9	
2	Welsh Bank	34.4	0.9	
3	Scott Point	26.1	0.5	
4	Loscombe Bank	13.9	0.3	
5	MacPherson Bay	3.2	<0.1	
6	Gunn Point	7.5	0.3	
7	Inverhuron Bay	11.3	0.2	
8	McRae Point (Southern unaffected area)	29.0	0.7	

Table 17						
Lake Whitefish by sex and condition, 2009-2011						

			Female			Male			
			Mature	Ripe	Spent	Mature	Ripe	Spent	Total
1	Scougall Bank	2009	2	5	1	18	62	2	90
	(Northern unaffected area)	2010	16	15	5	20	93	8	157
		2011	6	0	4	3	36	9	58
2	Welsh Bank	2009	3	16	1	4	79	1	104
		2010	1	5	0	10	24	0	40
		2011	4	1	3	0	10	0	18
3	Scott Point	2009	2	8	0	4	78	0	92
		2010	3	7	2	1	66	7	86
		2011	1	1	5	2	9	1	19
4	Loscombe Bank	2009	2	24	3	3	77	6	115
		2010	3	4	1	3	6	2	19
		2011	3	1	6	4	9	2	25
5	MacPherson Bay	2009	3	5	2	0	7	0	17
		2010	1	2	0	2	5	0	10
		2011	1	0	1	0	1	1	4
6	Gunn Point	2009	6	13	3	7	21	4	54
		2010	3	2	2	1	7	1	16
		2011	3	0	0	4	8	1	16
7	Inverhuron Bay	2009	7	17	2	7	29	4	66
		2010	0	2	0	1	10	0	13
		2011	2	0	3	0	1	2	8
8	McRae Point	2009	7	11	1	8	48	3	78
	(Southern unaffected area)	2010	4	2	2	7	42	21	78
		2011	5	2	1	1	24	2	35

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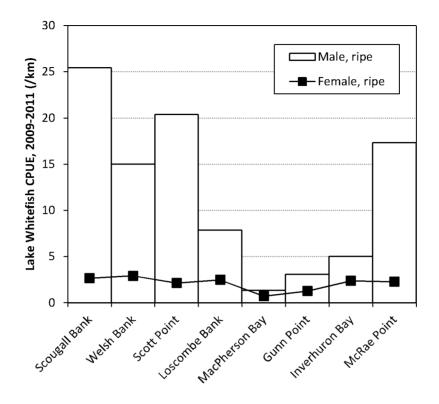


Figure 3 Mean Lake Whitefish CPUE, ripe males and females (2009-2011)

There remain a number of unknowns. It is not known whether Lake Whitefish spawn at discrete locations (in other words, whether the observed CPUE is a reliable proxy for use of spawning habitat), whether Lake Whitefish show fidelity to specific locations, or whether spawning groups consist of many or few individuals. Additionally, the frequent storms in November pose a challenge for gillnetting and may prevent observations from targeting the peak of spawning. Finally, ambient environmental conditions could affect spawning success, egg transport, and hatching success.

It is clear that Lake Whitefish in spawning condition are present at sites in the potentially affected areas (Areas 2-7). However, it is not clear to what extent the observed CPUE reflects variation in habitat use across the potentially affected area. If CPUE is a reasonable proxy for habitat use, then Loscombe Bank is not likely a preferred location for Lake Whitefish spawning compared to other sites in the reference and potentially affected areas.

5.0 TEMPERATURE MONITORING

Substrate temperature monitoring and impacts on Lake Whitefish habitat are discussed in the 2013 EA FUP Report [R-22] and in a technical memo [R-107].

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6.0 IMPINGEMENT AND ENTRAINMENT MONITORING

6.1 Impingement Monitoring

Impingement monitoring of Lake and Round Whitefish in 2013 and 2014 was part of the integrated Impingement and Entrainment analysis completed for the 2015 EA FUP Report [R-53] [R-24]. As part of this report, historical Lake Whitefish impingement was reported.

Lake Whitefish impingement at Bruce A and Bruce B from 2013 to 2015 ranged from 54 to 84 fish annually (Table 18)

Year	Bruce A	Bruce B	Total
2013	22	51	73
2014	13	41	54
2015	23	61	84

Table 18Impinged Lake Whitefish, 2013-2015

Impingement density of Lake Whitefish was generally lower in 2014 than in 2013 (Table 19).

Table 19Average Impingement Densities of Lake Whitefish at Bruce A

Year	Lake Whitefish per million m ³				
	Mean	Standard Deviation			
2013	0.010	0.031			
2014	<0.001	<0.001			

Overall, no consistent inter-annual pattern of species-specific impingement at Bruce A was observed (Figure 4).

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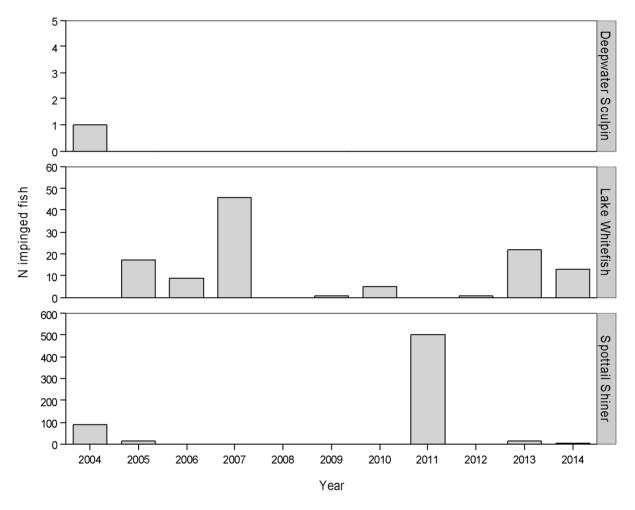


Figure 4 Comparison of VEC Species Impingement 2004-2014 at Bruce A, plotted by year Note: y-axis are different

6.2 Entrainment Sampling

6.2.1 Bruce A (1977)

An entrainment study was completed in 1977 at Bruce A with Units 1 and 2 in operation [R-31]. Sampling occurred in the intake and immediate discharge outflow on 23 occasions from April to November. Sampling lasted for 10 minutes on each occasion. Total sampling volume was not reported.

No Lake or Round Whitefish were identified during entrainment sampling. Rainbow Smelt (*Osmerus Mordax*), Alewife (*Alosa Pseudoharengus*) and Deepwater Sculpin (*Myoxocephalus Quadricornus*) made up 96.1% of the larvae sampled.

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6.2.2 Bruce A (1985-1986)

Entrainment sampling was again conducted from April-September 1985 and March-September 1986 with all four Bruce A units in operation.[R-74] A total of 88 sampling event occurred in each year with each event consisting of 28m³ of water. Sampling was conducted using the pump-in-water system.

There were 4,554 eggs and 3,204 larvae collected over the two years of the study. Rainbow Smelt, Burbot (*Lota Lota*), Alewife and Deepwater Sculpin accounted for 90 percent of the eggs and larvae collected in the intake-forebay during 1985 and 1986. No Lake Whitefish eggs or larvae were collected. No Round Whitefish eggs were collected. There were 2 Round Whitefish larvae collected in 1985 and 22 in 1986, accounting for 0.2% and 1.1% of the entrained larval fish in each year.

6.2.3 Bruce B (1988-1989)

Entrainment sampling was conducted in from April to August 1988 (52 events) and May to June 1989 (20 events) with all four Bruce A units in operation [R-68]. Two sets of 15 minute samples were taken each day using a pump-in-water system.

Overall, 525 larvae and 234 eggs were collected. Burbot, Rainbow Smelt and Deepwater Sculpin accounted for over 90% of the entrained fish each year. In 1988, one Lake Whitefish larvae (0.2%) and eight Round Whitefish (1.5%) larvae were collected. In 1989, 11 Round Whitefish (2.2%) larvae were collected and no Lake Whitefish larvae were collected. No Lake or Round Whitefish eggs were identified. Maximum weekly density of Round Whitefish larvae ranged from four to six larvae per 1000m³. No density estimated for Lake Whitefish were provided given the low entrainment catch.

6.2.4 Bruce A (2001)

An entrainment study was conducted in 2001 at Bruce A [R-12].

Approximately 2,800,000 L of water was pumped for entrainment sampling (pump-in-net method at the upwelling end of the Bruce A intake channel) from April 26-August 29, 2001. Overall, 56 larvae and 15 eggs were collected. No Lake or Round Whitefish larvae were identified. However, only 11 larvae could be identified. Most samples appeared to be long dead prior to collection.

Additionally, under low-flow conditions (all 4 units at Bruce A were in lay-up), a lack of turbulence at the upwelling end of the Bruce A intake channel may not have resulted in sufficient mixing of organisms within the water column.

6.2.5 Bruce B (2001)

An entrainment study was conducted in 2001 at Bruce B [R-91], which resulted in six entrained Lake Whitefish larvae.

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Five Lake Whitefish larvae were entrained on June 12, 2001, for a density of approximately 0.0055/m³, and one Lake Whitefish larvae was entrained on May 1, 2001 for a density of approximately 0.0015/m³. Although exact sampling volumes are not provided in the report, an approximate total volume can be estimated as 26,640 m³, corresponding to Lake Whitefish larval densities of 0.0002 over the sampling period (April 24-August 30, 2001).

6.2.6 Bruce A (2004)

An entrainment study was conducted in 2004 at Bruce A [R-43], with two units in operation.

Entrainment monitoring was conducted weekly (26 events) from late March through August, 2004. No Lake or Round Whitefish larvae were identified. The data supported the U34 EA Study Report prediction that entrainment would have no significant adverse effect on Lake or Round Whitefish.

6.2.7 Bruce A (2013-2014)

A two-year entrainment study was conducted from 2013 through 2014, with four units in operation, as reported in the 2014 EA follow-up monitoring report [R-23].

Data analysis of entrainment data was completed by an independent contractor [R-53] and reported in the 2015 EA FUP Report [R-24].

A total of 41 day entrainment surveys and 41 night entrainment surveys (82 total surveys, including 220 samples) were completed between March and December of 2013 [R-51]. A total of 40 day entrainment surveys and 39 night entrainment surveys (79 total surveys, including 239 samples) were completed between April and December of 2014 [R-52].

Water flow measurements were available for 205 of 220 total samples in 2013 (1.27 \pm 0.22 m/s, resulting in a mean sample volume of 1230 \pm 680 m³), with a minimum total sampling volume of 252,250 m³. Water flow measurements were available for 225 of 239 total samples in 2014 (1.13 \pm 0.22 m/s, resulting in a mean sample volume of 980 \pm 248 m³), with a minimum total sampling volume of 220,500 m³. For comparison, in 2004 approximately 3100 m³ of water was sampled with the pump-in-net method. The total volume sampled during the 2013-2014 Operations Phase entrainment monitoring is 70 to 80 times greater than the volume sampled in 2004.

Captured Lake Whitefish and Unidentified Coregonids, are listed in Table 20.

Table 20Counts of Entrained Lake Whitefish and Unidentified Coregonids at Bruce A, 2013-2014

Year Species		Live/recently dead			Long-dead	Total
rear	Species	Juveniles	Larvae	Total	Larvae	TOLAT
2013	Unidentified Coregonids	0	12	12	6	18
2013	Lake Whitefish	0	8	8	1	9
2014	Lake Whitefish	0	3	3	0	3

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These values differ slightly from the values reported in the 2013 and 2014 EA FUP reports as a result of the comprehensive QA/QC procedures applied to the data prior to examining the population level effects of entrainment [R-24].

Table 21 presents the annual average Lake Whitefish entrainment density for the two years of monitoring.

Table 21Yearly Averages of Entrained Lake Whitefish Larvae and Juvenile Density

			Fish Densit	y (fish/10,000 m³)
Year	Species	Live/Dead Status	Mean	Standard Deviation
2013	Lake Whitefish	Live/RD (SD)	0.49	2.38
	Lake Whitefish	Long-dead (SD)	0.03	0.28
2014	Lake Whitefish	Live/RD (SD)	0.19	0.96

Notes:Results are based on daily entrainment samples; values in parentheses are standard deviations.RD:Recently Dead

6.3 Impingement & Entrainment Analysis

Impingement and entrainment data from 2013 and 2014 were combined to estimate the population level effect of Bruce Power on Lake Whitefish populations. Two models were used to estimate this impact, the Adult Equivalent Model (EAM) and the Forgone Fishery Yield Model (FFYM). All model calculations followed USEPA 316(b) methodology [R-108]. Full descriptions of the processes and specifications used for these models can be found in [R-53].

The EAM was used to determine the representative number of adult fish lost to entrainment and impingement. The EAM expresses impinged and entrained fish as the equivalent number of fish that would have survived to an older year-class.

The FFYM estimates the amount of fish that is not harvested due to entrainment/impingement of fish

The total numbers of entrained and impinged Lake Whitefish, broken down by life stages were multiplied by the cumulative survival rates, to yield the number of age-at-equivalency Lake Whitefish lost to the fishery as a result of Bruce A operations in 2013 and 2014. Since multiple age classes of fish were entrained and impinged, depending on species, each age class had a different cumulative survival rate to age-at-equivalency.

Table 22 presents estimates of the number of Lake Whitefish from larval to adult life stages entrained and impinged annually at Bruce Power at actual flows.

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Table 22

Estimated Number of Lake Whitefish Entrained and Impinged by Bruce A at Actual Flows

Year Species	Entrainment	Impingement
2013 Lake Whitefish	50,000 (497,000)	22
2014 Lake Whitefish	21,000 (206,000)	13

Note: Numbers in parentheses are upper 95% credibility limits, based on Bayesian model of entrainment.

Under actual flows scenario at Bruce A, annual numbers of lost equivalent adult Lake Whitefish ranged from 185 in 2014 to 445 in 2013 (Table 23).

Table 23Estimated Number of Adult-Equivalent LakeWhitefish Entrained and Impinged by Bruce A at Actual Flows

Year	Species	Entrainment	Impingement	Total
2013	Lake Whitefish	446 (4,425)	16	462 (4,441)
2014	Lake Whitefish	185 (1,838)	5	190 (1,843)

Notes: Numbers in parentheses are upper 95% credibility limits, based on Bayesian model of entrainment. Units are the number of individual fish at age-of-equivalency.

Estimates of Lake Whitefish foregone fishery yield at Bruce A ranged from 179 kg in 2014 to 432 kg in 2013 (Table 24) [R-53]. The addition of unidentified coregonids to Lake Whitefish counts increased the FFYM results to 1,079 kg in 2013. Since no unidentified coregonids were collected in 2014, the FFYM value of combined Lake Whitefish and unidentified coregonids was identical to that of Lake Whitefish only.

Table 24Summary Foregone Fishery Yield (kg) based on Entrainment and Impingementof Lake Whitefish and Unidentified Coregonids at Bruce A under Actual Flows

Year	Species	Entrainment	Impingement	Total
2013	Lake Whitefish	432 (4,272)	25	457 (4,298)
2014	Lake Whitefish	179 (1,775)	7	186 (1,781)

Notes: Numbers in parentheses are upper 95% credibility limits, based on Bayesian model of entrainment.

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A total of 191,155 kg and 149,275 kg of Lake Whitefish were harvested in 2013 and 2014 in Zone 1 of Lake Huron [R-76]. Using the Lake Huron 2013/2014 Commercial Harvest report, the foregone production of commercially-harvested species was converted into percentages of Zone 1 quota (Table 25). In 2013, the impingement and entrainment loss of Lake Whitefish due to FFYM were estimated to equal 0.16% of the annual Zone 1 quota, which translated to a lost production value of \$1,917. When unidentified coregonid fish were added, the loss increased to 0.37%, with a corresponding cost of \$4,643. In 2014, estimated FFYM losses were lower than in 2013, with 0.06% of the annual Zone 1 quota and a cost of \$928.

 Table 25

 Foregone Fishery Yield at Bruce A and Lake Huron Zone 1 Lake Whitefish Catch

Year	Species	Annual Zone 1 Valu	Catch Value of Value Harvest	Percent of Annual Zone 1 Quota		Lost Production Value (\$)				
	·	Quota (kg) ¹	(\$)	(^{\$/kg)} Imping.	Entrain.	Total	Imping.	Entrain.	Total	
2013	Lake Whitefish	292.287	722.040	3.78	<0.01	0.15	0.16	111	1,805	1,917
2013		292,207	722,040	5.70	<u><u></u></u>	(1.46)	(1.47)		(18,003)	(18,115)
2014	Lake Whitefish	292,287	642,283	4.30	<0.01	0.06	0.06	30	898	928
2014	Lake Whitehsh				<0.01	1 (0.61) (0.61)	30	(8,952)	(8,982)	

Notes: Numbers in parentheses are calculated from upper 95% credibility limits, based on Bayesian model of entrainment. ¹= Data from MNRF (2014) for Zone 1 [R-76]. Imping.= Impingement Entrain.=Entrainment. Results are for maximum design scenario only.

When actual impingement data and estimated entrainment data from Bruce B were included, the total estimated impingement and entrainment of Lake Whitefish at Bruce A and B combined was 988 kg (95% UCL 9,117 kg) in 2013 and 443 kg (95% UCL 4,067 kg) in 2014. This represents 0.33% (95% UCL 3.1%) of the annual MNRF Zone 1 quota in 2013 and 0.15% (95% UCL 1.39%) of the annual MNRF Zone 1 quota in 2014.

7.0 OTHER

7.1 WINGS

7.1.1 Scope

As part of the Bruce Used Fuel Dry Storage Environmental Assessment, the Chippewas of Nawash expressed concern regarding potential radiological risk due to consumption of Lake Whitefish. OPG (later in partnership with Bruce Power) sponsored a study to examine the potential for Bruce Power operations to affect populations of whitefish [R-4] [R-5].

The study consisted of a three-year post-doctoral fellowship (J. Holmes) at the University of Guelph, advised by a Core Group of representatives from OPG (D. Wismer; later also J. Verburg from Bruce Power), Nawash (S. Crawford), and the University of Guelph (D. Noakes). Results were reviewed by a broader Scientific Advisory Group (G. Bird, CNSC; D. Bodaly, Freshwater Institute; S. Crawford, Nawash; R.H. Green, UWO; K. Orr, OPG; K.E. Smokorowski, DFO; G. Van Der Kraak, UG; J. Verburg, Bruce Power; D. Wismer, OPG; B. Henderson, OMNR; D. McLeish, OMNR) prior to finalization.

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7.1.2 Accomplishments

The WINGS group produced two substantive literature reviews [R-63] [R-64], one interim progress report [R-59] and poster [R-62], detailed field monitoring results [R-60] and Access database, and a final recommendations report [R-61] with associated summary [R-6]. Additionally, the WINGS group provided a curated set of technical reports relevant to the ecology of whitefish in Lake Huron. These reports are listed in Table 26 and briefly reviewed in Section 10.5.

Table 26Reports produced as a result of the WINGS study

[R-64]	Holmes JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Lake Whitefish and Round Whitefish biology: a review of ecological factors affecting growth, survival, and reproduction. July 15, 2002.	Technical report
[R-63]	Holmes JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Aquatic effects of nuclear generating stations on fishes. July 15, 2002.	Technical report
[R-60]	Holmes JA, 2002. WINGS field program, May 2000 – Nov 2001: objectives, methods, results and interpretation. August 31, 2002.	Technical report
[R-73]	MacPherson A, Holmes JA, Muir AM, Noakes DLG, 2010. Assessing feeding competition between Lake Whitefish (<i>coregonus clupeaformis</i>) and Round Whitefish (<i>prosopium cylindraceum</i>). <i>Current Zoology</i> 56, 109-117.	Publication

7.2 FISHES

7.2.1 Scope

As part of the Bruce Used Fuel Dry Storage Environmental Assessment, the Chippewas of Nawash expressed concern regarding potential radiological risk due to consumption of Lake Whitefish. OPG (later in partnership with Bruce Power) sponsored a study to sample Lake Whitefish for radionuclide concentrations.

Note that this study was not intended to focus on the ecology of Lake Whitefish in Lake Huron.

7.2.2 Accomplishments

A dietary survey was conducted, as well as detailed radionuclide analysis. Results were provided in a technical report and a plain-language summary (see Table 27).

A bibliography of final reports is provided in Table 27. Each report is reviewed briefly in Section 10.6.

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Table 27 Reports produced as a result of the FISHES study						
[R-105]	[R-105] Thorburn, M., 2002. Radionuclide sampling of Lake Whitefish and lake trout from Chippewas of Nawash Fishing Grounds (south-west of Georgian Bay and Fishing Islands, Lake Huron) and the vicinity of the Bruce Nuclear Power Development Site (Lake Huron).					
[R-106]						

[R-13] Bruce Power, 2002. Measurement of radionuclides in fish and lake water for Technical Chippewas of Nawash Dietary Studies. B-REP-03443.6-00001. report

7.3 U34 Restart EA

FISHES study. October 2002.

An impingement and entrainment study was conducted in 2001 at Bruce A [R-12].

Impingement monitoring was conducted from April 11-August 29, 2001. No Lake or Round Whitefish were observed during impingement monitoring.

The U34 Restart EA FUP work plan included elements for field monitoring of impingement and entrainment. A Technical Working Group (TWG) was formed to develop the follow-up work plan for whitefish (discussed below).

Monitoring results for impingement and entrainment were reported in the 2004 U34 Restart EA FUP report [R-43].

24-hour impingement samples were collected 28 times from late March through August, 2004. One adult Lake Whitefish was recorded as impinged in November of 2004, while no adult Round Whitefish were recorded as impinged. The data supported the prediction that impingement would not have a significant adverse effect on Lake or Round Whitefish populations.

7.4 Environmental Risk Assessment

The Environmental Risk Assessment (ERA) currently in progress will use relevant research described in this report as supplementary information for risk assessment.

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8.0 CONCLUSIONS

Lake Whitefish are the most important commercially-harvested species in the Canadian waters of Lake Huron. Given their commercial importance, Bruce Power has completed significant work to measure potential interactions with Lake Whitefish. These interactions include the possibility of impingement and entrainment in the cooling water intake and through the return of warmed waters to Lake Huron.

Round Whitefish were selected as a second species of interest because they share habit and have similar life histories as Lake Whitefish. Preliminary evidence indicated they may be more thermally sensitive than Lake Whitefish. Round Whitefish were also historically more common than Lake Whitefish near the Bruce Power site [R-11].

The potential for interaction of Bruce Power site cooling water intake and discharge with Lake and Round Whitefish has been recognized since the late 1990s. These potential interactions include the entrainment and impingement of Whitefish and possible effects from the return of warmer water to Lake Huron. Since that time, two Environmental Assessment [R-12] [R-14] have concluded that the potential for harm to Lake and Round Whitefish populations is low. However, uncertainties remained in the scientific understanding of Lake and Round Whitefish, and in the assessment of potential impacts.

Since 2011, Bruce Power has expended significant

Box A – Key Research Questions

Population discrimination: How many whitefish populations exist in Lake Huron, and is there any likelihood that these populations will be impacted by Bruce Power operations based on spatiotemporal distribution?

Population modeling: What is the most appropriate mathematical model(s) that can describe Lake Huron whitefish population dynamics and estimate any potential effects of Bruce Power?

Entrainment effects: Are there any significant effects (stress response/mortality) of Bruce Power entrainment on local or lake-wide whitefish populations?

Thermal effects: Are there any effects (negative or beneficial) of the Bruce Power thermal plumes from cooling water discharges on the whitefish population(s) in terms of stress response or mortality?

Combined stressors: Are there any combined effects (negative or beneficial) of Bruce Power radiological and conventional emissions on the whitefish population(s) in terms of stress response or mortality?

effort to improve the scientific understanding of Lake and Round Whitefish, particularly with respect to potential impacts of Bruce Power site operations. In partnership with Saugeen Ojibway Nation, Bruce Power-funded independent, multi-year university-based research programs to address key research questions with respect to Lake and Round Whitefish (Box A) [R-26] [R-69].

The University of Guelph research program was funded entirely by Bruce Power. The McMaster/Regina University research program was funded by Bruce Power, with matching funds obtained through a rigorous competitive peer-reviewed NSERC collaborative research and development grant. Under the terms of the funding contracts, researchers were free to publish their findings in the peer-reviewed scientific literature.

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Over 5 field seasons, researchers have collected nearly 2000 adult Lake and Round Whitefish and raised over 550,000 Lake Whitefish and more than 40,000 Round Whitefish embryos and larvae. The program at the University of Guelph has generated two theses and one peer-reviewed publication. The joint program between McMaster and Regina Universities has generated seven theses and 13 peer-reviewed publications

This independent university-based research and development has endeavored to identify Whitefish populations in Lake Huron and model these populations. Laboratory and *in situ* studies have been completed to identify both thermal and combined radiological, chemical and thermal stressor effects on developing Lake Whitefish embryos and YOY.

The Whitefish research programs have supported the training of 3 post-doctoral fellows, 4 PhDs and 9 MSc students. An additional 3 MSc and 2 PhD students are in the process of completing their studies. The training of highly qualified personnel and the development of expertise in Lake Huron Whitefish has added valuable resources to tackling the myriad of ecological challenges facing the Great Lakes.

This report summarizes the extensive published scientific results that derive from these university-based research programs up to November 2016. Additionally, we have placed these results into their proper context, with reference to historic or parallel monitoring programs conducted by Bruce Power and/or independent consultants.

8.1 **Population Discrimination**

Prior to the initiation of the research program, little was known with respect to the population structuring of Lake Whitefish in Lake Huron, and almost nothing was known with respect to Round Whitefish in Lake Huron. Some evidence of limited population structuring along the Bruce peninsula had been observed previously through genetic analysis, while interim results from a lake-wide mark/recapture study did not shed additional light on the nature of whitefish population structuring in the vicinity of the Bruce Power site.

While extensive spawning grounds were known to be used by Lake Whitefish along the coast of the Bruce Peninsula well to the north of the Bruce Power site, there was almost no direct evidence of Lake Whitefish spawning near the Bruce Power site itself. However, annual gillnetting studies demonstrated the presence of ripe Lake and Round Whitefish, indicating at least the possibility of spawning near the Bruce Power site. It was assumed that, if spawning activity took place, the Lake and Round Whitefish individuals spawning near the Bruce Power site were members of a much larger population.

The research program was intended to challenge this assumption. University of Regina and University of Guelph researchers collaborated with respect to collecting and sampling some of nearly 2000 Lake and Round Whitefish from spawning grounds around the main basin of Lake Huron as well as the North Channel and Georgian Bay from 2010 to 2014.

University of Regina researchers used genetic analysis to examine the relationships between spawning fish, and stable isotope analysis to examine the movement and resource use of adult Lake and Round Whitefish at different locations relative to Bruce Power. They completed both fine-scale (near Bruce Power) and lake-wide assessments.

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The fine-scale assessment found no evidence for the presence of distinct genetic groups of Lake or Round Whitefish within the vicinity of Bruce Power. Similarly, stable isotope analysis showed no evidence for distinct Lake or Round Whitefish feeding behavior in this vicinity, as there was high resource use overlap between fish at different sampling areas. However, their research did show that Lake and Round Whitefish occupy separate ecological niches, defined as differential resource use. The most overlap between species occurred during the spawning period.

The lake-wide assessment found that for Round Whitefish, Lake Huron was determined to be a genetic source population for Lakes Michigan, Superior and northern Georgian Bay as well as Lake Nipigon through Lake Superior. Sampling was not wide-spread enough within Lake Huron to draw conclusions regarding population structure within the lake.

Also on a lake-wide scale, stable isotope analysis showed that six ecological groups of Lake Whitefish were present within Lake Huron and Georgian Bay. In the area local to Bruce Power, the Main Basin of Lake Huron could be divided into Southwestern and Northeastern (including the Bruce Power site) ecological groups with heavy population mixing within each of these groups. No small distinct ecological groups were identified within the area near the Bruce Power site (i.e., Scougall Bank and McRae Point). These results are consistent with previous genetic analysis suggesting weak structuring within the eastern main basin.

The research results conclusively demonstrate that spawning-condition Lake Whitefish near the Bruce Power site are members of a larger genetic and ecological group. There is no evidence of any distinct genetic or ecological group of Lake or Round Whitefish utilizing spawning grounds near Bruce Power.

8.2 Population Modeling

University of Guelph led the research program with respect to population modeling. The University of Guelph research team reviewed the available scientific literature for estimates of Lake Whitefish mortality and life history parameters. Initial technical reports were provided that led to two theses and one publication with the involvement of two students.

The primary result from the University of Guelph research program is the development of chemical probes for the identification of Lake Whitefish (the DNA "barcoding" technique). Identification of larval Lake Whitefish through the use of a key-based technique is challenging, while identification of eggs is virtually impossible. The DNA-based probes may allow for more accurate quantification of impacts in the future, through accurate and rapid identification of the species for collected larvae and embryos.

The research team was not able to identify population structure using the DNA barcoding technique.

Initial efforts were made to determine appropriate mortality and life-history parameters, and to develop appropriate models for spatial data. However, the key research question (what is the most appropriate mathematical model to describe Lake Huron whitefish population dynamics, and to estimate the potential effects of Bruce Power?) was not answered through this research program.

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Nonetheless, we have made significant advances in understanding potential impacts on Lake Whitefish since 2011. First, the overall research program has definitively shown no distinct ecological or genetic group of Lake or Round Whitefish in the immediate vicinity of the Bruce Power site. Second, extensive monitoring conducted by Bruce Power demonstrated minimal Lake Whitefish losses from impingement and entrainment: less than 0.5% of the annual Zone 1 quota from impingement and entrainment (see 8.3).

8.3 Effects of Impingement and Entrainment

The EA FUP included an extensive analysis of the effects of entrainment and impingement on Lake Whitefish. An environmental consulting firm conducted entrainment monitoring for two years at the Bruce A intake, supplemented with larval tows in the vicinity of the intake, and performed quality assurance checks on the routine Bruce Power impingement monitoring program. Two years of data was analyzed to estimate the cumulative impact of impingement and entrainment of Lake Whitefish at Bruce Power relative to the commercial fishing quota.

Overall, the results demonstrated a minimal effect of impingement and entrainment relative to the commercial fishing quota. The total effect of impingement and entrainment for Bruce Power site operations (including Bruce A and Bruce B generating stations) is the annual loss of less than 0.5% of the commercial fishing quota (0.33% of quota in 2013 and 0.15% of quota in 2014). In other words, the impact of Bruce Power site operations is very small and the combined effect of the commercial fishing harvest (191,155kg in 2013 and 149,275kg in 2014) and Bruce Power site operations (988kg in 2013 and 443kg in 2014) is well below the commercial fishing quota of 292,287kg [R-24] [R-76]. The quota is established to maintain a sustainable fishery in Lake Huron and is meant to be protective of the long term health of the population.

8.4 Thermal effects

8.4.1 Survival and Development of Lake Whitefish Embryos

Prior to the initiation of the research program, the effects of warmer temperatures on incubating Lake and Round Whitefish embryos was not fully understood. Several published studies indicated that warmer temperatures decreased time-to-hatch and increased the probability of embryos mortality, with the specific details dependent on incubation techniques and/or source of Lake Whitefish embryos. However, a detailed study indicated that Lake Whitefish embryos nonetheless were able to withstand cyclic heat shocks of up to 7° C for 6-h each day throughout development, with virtually no impact on survival, although hatch could occur up to several weeks earlier than when incubated at 0°C.

No scientific information was available with respect to the impact of warmer temperatures on whitefish cellular responses. Additionally, it was not known whether an earlier hatch would be beneficial or harmful to Lake Whitefish; whitefish that hatch early may have access to more or less food, or may be more or less susceptible to predation.

Bruce Power has funded extensive research into the impacts of thermal and combined thermal, radiological and chemical stressors on developing Lake and Round Whitefish embryos. This has included examining stressor effects on cellular responses and growth, development and survival.

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From 2011 to 2016, the McMaster University and University of Regina research teams incubated over 550,000 Lake Whitefish embryos and over 40,000 Round Whitefish embryos in ongoing efforts to define thermal and combined stressor effects on Whitefish. Novel incubation and irradiation methods were developed to support this research. Heat shock protein gene sequences were identified to aid in the identification of cellular stress responses in Round and Lake Whitefish.

Key results from this research indicate that Lake Whitefish embryos and Young of the Year (YOY) mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. This level of heat shock exceeds the temperature changes seen in the temperature loggers placed in the *in situ* incubation near the discharge channel.

Lake Whitefish embryos were slower than YOY to show a heat shock response but once they showed a response, it continued over at least 2 days following the heat shock. This response suggests that embryos and YOY are likely able protect themselves from subsequent exposure to heat, and may provide an explanation at the biological level for the observation that survival is not affected by intermittent exposure to heat.

Lake Whitefish hatch earlier and have reduced survival with increasing constant temperatures. Infrequent exposure of embryos and YOY to warmer water has little effect on survival or development. Lake Whitefish embryos and hatchlings incubated at higher constant temperatures showed increased heart rate and oxygen consumption. Incubation at higher constant temperatures earlier in incubation had a very small lasting effect even when the temperatures later in incubation were cooler. Higher constant temperatures during incubation also resulted in a shorter incubation period, higher yolk area and mass and lower body lengths and mass at hatching. Higher constant incubation temperatures also decreased the hatch window, survival and increased oxygen needs. Regular weekly heat shocks had no effect on time-to-hatch, survival, yolk mass, body mass, length or head width.

Incubation of Lake Whitefish embryos in chambers on the lake bottom in the Bruce Power discharge areas resulted in an average 10% advance in development. Embryos incubated in the discharge area were slightly longer with a smaller yolk area but showed no increase in developmental abnormalities. These chambers were deployed on the lake bottom, above the cobble substrate, whereas normal Lake Whitefish incubation tends to occur down within the cobble. There is the potential for the cobble substrate to provide a thermal buffer which may reduce the temperature effects observed in the *in situ* study. This is currently being investigated using a simulated thermal flume at Laurentian University.

Embryos hatching earlier in warmer temperature conditions have larger yolk sacs and smaller bodies. Embryos incubated at higher temperatures may be less developed and have greater capacity to maintain themselves without external food sources than embryos hatching after long incubation at cooler temperatures. The possibility of a mismatch between plankton availability and hatch timing produced by exposure to warmer temperature may be reduced because these embryos may be hatching at an earlier stage of development with greater yolk reserves. The exact mechanism that triggers embryo hatch remains unknown but may be related to a change in the movement of embryos, temperature changes and/or light exposure. In addition, hatch windows (i.e., the time from first to last hatch) increase with cooler incubation temperatures.

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It is still not known whether Lake Whitefish use spawning grounds near Bruce Power. Although direct evidence of spawning has almost never been observed, the potential for spawning has been inferred from catches of ripe adult Lake Whitefish and the potential suitability of the substrate [R-47]. Nonetheless, the research program results support the general understanding that the direct survival impact of the Bruce Power site thermal discharge on Lake Whitefish embryos, YOY and juveniles, if present near the Bruce Power site, is likely to be limited.

8.4.2 Survival and Development of Lake Whitefish Young of the Year

Bruce Power has also funded research into the impacts of thermal stressors on Lake Whitefish YOY. This has included examining effects on cellular responses and growth, development and survival.

Lake Whitefish YOY were incubated at several different temperatures and were exposed to regular heat shocks throughout development in multiple experiments by researchers at McMaster and Regina Universities. They found that YOY could mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. Lake Whitefish YOY quickly mounted a cellular heat shock response and it dropped much more rapidly than in embryos, peaking at 2-hours after the heat shock. Subtle differences in the physiology of YOY exposed to heat shocks during embryonic development were observed. Although these differences are not expected to impact survival of YOY, the link between heat exposure during embryogenesis and post-hatch effects is not yet fully clear.

8.4.3 Survival and Development of Round Whitefish Embryos

Prior work indicated that Round Whitefish embryos were able to withstand cyclic heat shocks of up to 5.5°C for 6-h each day throughout development, with virtually no impact on survival, although hatch could occur up to several weeks earlier than when incubated at a constant 1.7°C.

Bruce Power-funded research showed increased mortality in Round Whitefish embryos exposed to rapid warming near the end of development or incubated at a constant 8°C. Seasonal temperature changes increased the development rate and shortened hatch timing. Embryos incubated at lower temperatures had increased body mass and decreased yolk mass. Exposure to regular temperature spikes had little effect on development stage onset

8.4.4 Thermal effects summary

The outflow from Bruce Power site facilities may generate a sinking thermal plume in the winter, with temperatures reaching a maximum of 4°C warmer than the lake bottom temperature. Results from the *in situ* incubation of Lake Whitefish support this range of temperature change. Our research program has demonstrated that Lake Whitefish are capable of surviving a 4-hour, 9°C heat shock and that Round Whitefish can survive regular 1-hour 3°C heat shocks and incubation at a constant 5°C. In light of these results, no impact on embryo survival is expected in the range of temperature changes related to a sinking thermal plume near the discharge area.

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8.5 Combined stressors

Prior to the research program, it was not known whether multiple external stressors could affect whitefish embryos synergistically or antagonistically. Assessment of potential impacts on Lake Whitefish considered each stressor separately, without consideration for the possibility of interactions between cellular or organism response to multiple stressors.

During the Bruce Power-funded program, Lake Whitefish embryos were exposed to constant or variable temperature, as well as one of acute or chronic gamma radiation or chemical stress (morpholine or sodium hypochlorite). Doses of chronic radiation of 0.06-4.4 mGy/day resulted in an earlier hatch. High doses of acute radiation increase embryo mortality.

Morpholine concentrations of 1 or 10 mg/L resulted in increased larval size at hatch. There were no significant effects of morpholine at 100mg/l. From 500-1000mg/L there was a decreased larval size, earlier hatch and increased embryo mortality.

Lake Whitefish embryos were then exposed to a combination of thermal, chemical and radiological stressors. A heat shock administered prior to irradiation had a protective effect on Lake Whitefish embryos. The same heat shock administered prior to exposure to morpholine had no protective effect. A heat shock of 2-hours duration (temperature increase of 3°C or 9°C) administered six hours prior to irradiation decreased embryo mortality by up to 25%.

Compared to Lake Whitefish, Round Whitefish are likely more sensitive to morpholine exposure, with developmental and mortality effects starting at 500mg/l. No consistent effects are seen at levels between 0 and 100 mg/L. Conversely, Round Whitefish are likely more resistant to low dose radiation than Lake Whitefish, with a 20% reduction in post-hatch mortality for Round Whitefish embryos.

The relationship between radiation and morpholine exposure and Lake and Round Whitefish embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and are different for Lake and Round Whitefish. Lake Whitefish are likely more sensitive to radiation exposure whereas Round Whitefish appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for Lake Whitefish.

The Bruce Power ECA limit for morpholine is 2.5 mg/L measured at the cooling condenser water duct. Any morpholine released from the cooling condenser duct would be rapidly diluted in the discharge channel. Morpholine monitoring at Bruce Power has a Minimum Detectable Level (MDL) of 0.02 mg/L. Monthly monitoring from January 2011 to November 2016 ranged from the MDL to 1 mg/L, with 90% of monitoring results less than the MDL. Mortality effects on embryos in this study occurred at morpholine doses at least 200 times greater than the ECA limit at Bruce Power. No measurable effects on Lake or Round Whitefish embryos were seen at doses up to 40 times greater than the ECA limit at Bruce Power.

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The International Commission on Radiological Protection lists natural background levels of radiation exposure for fish up to 0.01 mGy/day. Dose rates of 0.1 to 1 mGy/day are considered low dose rates for fish [R-66]. Fish are thought to be more resistant to radiation exposure than humans and dose recommendations for aquatic organisms have been set at 3.5 Gy/yr, compared to the human limit of 0.05 Gy/yr [R-65].

Results from the combined stressor research showed that effects from morpholine and gamma radiation were only seen at levels substantially higher than those seen operationally at the Bruce Power site.

8.6 Summary

Bruce Power has funded extensive independent university-based research and development surrounding potential interactions with Whitefish. This independent, university-based research has added to a limited knowledge base regarding population discrimination and the effect of thermal and combined radiological, chemical and thermal stressors on Lake and Round Whitefish. Results of this research are publically available for use by industrial, scientific and regulatory groups. The research has also supported the training of highly qualified personnel with expertise in Lake Huron Whitefish.

Key research results are summarized in Box B for each the identified research questions.

It is unclear how the changes in Lake and Round Whitefish embryo size and



Population discrimination: No local genetic or ecological populations of Lake Whitefish (LWF) or Round Whitefish (RWF) have been identified in the area near Bruce Power. The LWF and RWF in the area surrounding Bruce Power belong to genetic and ecological groups that encompass large parts of Lake Huron.

Population modeling: DNA barcode identification developed for LWF.

Entrainment effects: The impact of Bruce site operations on LWF has been shown to be less than 0.5% of the quota set for a sustainable fishery.

Thermal effects: Intermittent temperature increases throughout LWF development have no impact on embryo mortality. LWF and RWF embryos exposed to regular heat shocks throughout development do experience a small advance in hatch and some changes in size and metabolism.

LWF embryos are able to mount a response to intermittent heat shocks that ensure survival of a 4-hour 9°C heat shock. RWF showed no change in survival with regular 1-hour 9°C heat shocks from a constant 5°C base. These intermittent heat shocks exceed the temperature changes recorded by the temperature loggers from *in situ* incubation chambers near the discharge channels. Temperature changes of the magnitude seen in the vicinity of the thermal discharge have been shown to have no significant effect on the mortality or stress response of LWF embryos.

Combined stressors: The relationship between radiation and morpholine exposure and LWF and RWF embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and different for LWF and RWF. LWF are more sensitive to radiation exposure whereas RWF appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for LWF.

metabolism in response to heat shocks impact the fitness, behaviour and survival of Lake and Round Whitefish at 6 months and 1 year of age. Bruce Power expects to fund further research to explore these uncertainties in Lake and Round Whitefish outcomes.

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Future research plans to examine the potential protective insulating effect of the cobble substrate for incubating embryos. There also remain several uncertainties in the outcomes of Lake and Round Whitefish embryos, YOY and juveniles exposed to thermal stressors, particularly with respect to behaviour and fitness. It is not known whether increased incubation temperatures and/or heat shock exposure impacts the hormonal stress responses (i.e., blood sugar, adrenaline, electrolyte levels and metabolic rate) of larval and juvenile Whitefish. It is also not know how increased incubation temperatures and/or heat shock exposure impacts swim performance, foraging capability and behaviour in larval and juvenile Whitefish. Warmer incubation temperatures may lead to hatching at an earlier development stage where the larvae are potentially less able to capture prey or avoid predators and where their shorter lengths impose greater energy demands due to viscosity.

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10.0 REVIEW OF REPORTS, PUBLICATIONS AND PRESENTATIONS

10.1 Lake Huron Lake Whitefish Distribution Study

Interim scientific publications resulting from the Lake Whitefish Distribution Study are listed in Table 28.

Bruce Power reports relevant to the Lake Whitefish Distribution Study are given inTable 28. These reports are reviewed below.

Table 28 Interim scientific reports produced as a result of the Lake Whitefish Distribution Study

[R-36]	Ebener M.P., Mohr L., Woldt A., Johnson J., Crawford S., 2003. Lake Huron Lake Whitefish distribution study. A proposal submitted to the U.S. Fish and Wildlife Restoration Act via Great Lakes Fishery Commission.	Technical report
n/a	Ebener, M. 2009. United States Fish and Wildlife Service Great Lakes Restoration Act: 2008 Project Progress Report, Agreement Number 301813J229 - Lake Huron Lake Whitefish Distribution Study.	Technical report
[R-34]	Ebener M.P., Brenden T.O., Wright G.M., Jones M.L., Faisal M., 2010. Spatial and temporal distributions of Lake Whitefish spawning stocks in Northern Michigan and Huron, 2003-2008. <i>Journal of Great Lakes Research</i> 36, 38-51.	Publication

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WHITEF	WHITEFISH RESEARCH REVIEW					
[R-33]	[R-33]Ebener M.P., Brenden T.O., Wright G.M., Jones M.L., Faisal M., 2010. Erratum to "Spatial and temporal distributions of Lake Whitefish spawning stocks in Northern Michigan and Huron, 2003-2008" [J. Great Lakes Res. 36 (Supplement 1) (2010) 38-51. Journal of Great Lakes Research 36, 803.Publicat (erratum)					
[R-32]	Ebener M.P., Brenden T.O., Jone mortality rates for four Lake White Michigan. <i>Journal of Great Lakes</i>	fish stocks in No	rthern Lakes Huron and			
n/a	Ebener M.P., 2012. Lake Huron I [draft]. December 28, 2012.	₋ake Whitefish Di	istribution Study Final R	eport Technical report		

Ebener, M. 2009. United States Fish and Wildlife Service Great Lakes Restoration Act: 2008 Project Progress Report, Agreement Number 301813J229 - Lake Huron Lake Whitefish Distribution Study.

This report was not available for review.

Ebener M.P., Brenden T.O., Wright G.M., Jones M.L., Faisal M., 2010. Spatial and temporal distributions of Lake Whitefish spawning stocks in Northern Michigan and Huron, 2003-2008. Journal of Great Lakes Research 36, 38-51.

Ebener, et al., reported preliminary results from the Lake Whitefish Distribution Study, focused exclusively on tags made in northern Lake Michigan and northern Lake Huron. Although the results may not be applicable to stocks in the southern main basin of Lake Huron, results do show site-specific fidelity to spawning grounds as well as mixture of stocks throughout Lake Michigan and Lake Huron outside of spawning season [R-34].

Ebener M.P., Brenden T.O., Jones M.L., 2010. Estimates of fishing and natural mortality rates for four Lake Whitefish stocks in Northern Lakes Huron and Michigan. Journal of Great Lakes Research 36, 110-120.

Ebener, et al., reported estimates of fishing mortality and natural mortality for Lake Whitefish stocks in northern Lake Michigan and northern Lake Huron. Results may not be applicable to stocks in the southern main basin of Lake Huron [R-32].

Ebener M.P., 2012. Lake Huron Lake Whitefish Distribution Study Final Report [draft]. December 28, 2012.

Recovery of tags from Douglas Point fish was not sufficient to judge spatial distributions.

Note, however, that data in the draft final report is not consistent with respect to data regarding recoveries in the whitefish investigations summaries (as given in Table 2).

10.2 Saugeen Ojibway Nation-Bruce Power Collaborative Whitefish Agreement

Interim scientific reports produced by the University of Guelph group are listed in Table 29. Reports of a primarily administrative nature (i.e., annual reports) have not been included.

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WHITER	TISH RESEARCH REVIEW		•	•
	Scientific reports produced	Table 29 by the BP-SON	Lake Whitefish partne	rship
[R-28]	Crawford S., 2013. Metadata for 2 (coregonus clupeaformis). Noven		f Lake Huron Lake Whit	efish Technical report
[R-88]	Overdyk, L., Crawford S., 2013. N at Douglas Point, Lake Huron. De			pling Technical report
[R-3] Angevaare J., Gillis D., 2013. Estimates of life history and mortality parameters of Lake Whitefish (<i>coregonus clupeaformis</i>). December 20, 2013.			Technical 013. report	
[R-29]	R-29] Crawford S., 2014. Compilation of documented information/knowledge regarding population distribution of Lake Whitefish (<i>coregonus clupeaformis</i>) in Lake Huron (draft). February 3, 2014.			Technical <i>is</i>) in report
[R-87] Overdyk L, Braid H., Naaum A., Hanner R., Crawford S., 2014. Real-time PCR identification of Lake Whitefish (<i>coregonus clupeaformis</i>) in the Great Lakes. March 25, 2014.				
[R-2]	Angevaare J., Gillis D., Cox R., 20 population models. June 14, 2014		pplication and evaluatio	on of Technical report
[R-1]	Angevaare J., 2014. Efficient Bay Autoregressive Models. M.Sc. th			M.Sc. thesis
[R-86]	Overdyk, L., Braid, H., Crawford, S barcoding coverage for Lake Whit three major basins of Lake Huron.	efish (<i>coregonus</i>	s <i>clupeaformis</i>) across th	Publicatior ne
[R-85]	Overdyk, L. Ecological and genet of larval Lake Whitefish (<i>coregonu</i> Huron. PhD thesis, University of (ıs clupeaformis)	at Douglas Point, Lake	nce PhD thesis

Crawford S., 2013. Metadata for 2012 sampling of Lake Huron Lake Whitefish (coregonus clupeaformis). November 4, 2013.

34 samples of Lake Whitefish were collected from 29 total locations in the Lake Huron main basin (17 locations), North Channel (4 locations), and Georgian Bay (8 locations). University of Guelph contributed 1133 Lake Whitefish from 23 samples while the University of Regina and United States Geological Survey contributed the remainder.

Tissues were subsampled and provided to the University of Regina for genetic and stable isotope analysis [R-28].

No scientific results or analysis were included in the report.

Overdyk, L., Crawford S., 2013. Metadata for 2013 plankton ecology sampling at Douglas Point, Lake Huron. December 11, 2013.

Water samples were collected once per week for five weeks at selected locations along five transects established in the vicinity of Bruce B [R-88].

No scientific results or analysis were included in the report.

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Angevaare J., Gillis D., 2013. Estimates of life history and mortality parameters of Lake Whitefish (coregonus clupeaformis). December 20, 2013.

This report was intended to summarize known growth, maturation, and mortality model parameters for Lake Whitefish in Lake Huron.

A database of parameter estimates was prepared based on a literature review. An anonymous scientific peer-review confirmed that the database may be a useful resource for understanding the life history of Lake Whitefish in Lake Huron (although the database neglects gender as a relevant variable for some of the compiled parameters).

Limited analysis was performed with respect to growth, maturation, and mortality parameters relevant to the Main Basin East location.

Of note, mean annual total mortality (for Main Basin East populations) was estimated to be 0.37 per [R-25] with 95% confidence interval (0.29, 0.45). (However, this estimate disagrees with [R-25], which provides a pooled parameter estimate of mortality for Main Basin East populations of 0.32.) Additionally, Angevaare & Gillis estimate annual natural mortality to be 0.3.

Angevaare & Gillis perform a Monte Carlo simulation in which human-induced mortality (assumed to be due to fishing and Bruce Power operations) was estimated to lie between 0.0004 and 0.54, with a mean value of approximately 0.15 (actual value was not listed in the report).

The result of this simulation is clearly inconsistent with the parameters used as input (i.e., total mortality of 0.37, and natural mortality of 0.3). A bias was inadvertently introduced into the simulation through the assumption of normality and independence of the total and natural mortality parameters [R-3].

Crawford S., 2014. Compilation of documented information/knowledge regarding population distribution of Lake Whitefish (coregonus clupeaformis) in Lake Huron (draft). February 3, 2014.

This report is a verbatim compilation of extracts of approximately 180 primary sources, including peer-reviewed scientific literature and technical reports (gray literature), intended to document all the knowledge regarding distributions of populations of Lake Whitefish in Lake Huron [R-29].

No scientific analysis or synthesis was attempted.

Overdyk L, Braid H., Naaum A., Hanner R., Crawford S., 2014. Real-time PCR identification of Lake Whitefish (coregonus clupeaformis) in the Great Lakes. March 25, 2014.

Overdyk, et al., reported the development of a PCR primer suitable for differentiation of Lake Whitefish from related fish species based on the DNA barcoding technique. Differentiation with an appropriate cut-off value was proposed as potentially being adequate for identification of Lake Whitefish [R-87].

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Angevaare J, Gillis D., Cox R., 2014. Report on application and evaluation of population models. June 14, 2014.

Angevaare, et al., performed a literature review to identify the most appropriate models for assessing Lake Whitefish mortality. The report provides a description of stock-based models, structured stock-based models, and individual based models. No recommendations were made regarding the appropriate model for population structure of Lake Whitefish in Lake Huron [R-2].

Angevaare J., 2014. Efficient Bayesian inference for Conditionally Autoregressive Models. *M.Sc. thesis, University of Guelph. April 2014.*

Angevaare simulated spatially-correlated data in order to investigate the relative performance of two algorithms for modelling spatial data. Lake Whitefish CPUE data was used as a test case, but explicit analysis of population structure was not performed [R-1].

Overdyk, L., Braid, H., Crawford, s., Hanner R 2015. Extending DNA barcoding coverage for Lake Whitefish (coregonus clupeaformis) across the three major basins of Lake Huron. DNA Barcodes 3:59-65.

Overdyk et al. (2015) performed DNA barcode sequencing on 208 Lake Whitefish. DNA barcodes are species-specific differences in short standard mitochondrial 5' cytochrome c oxidase subunit I (COI) gene sequences. The study identified 12 unique barcodes (or haplotypes) in Lake Whitefish. The barcode sequencing found nine unique haplotypes, seven found only in Lake Huron in 148 Lake Whitefish from 28 sites across Lake Huron. Haplotype A dominated both the Lake Huron and other site samples. There was no evidence for cryptic lineages (i.e., no disconnected haplotype networks). This was consistent with a single species across Lake Huron and other sites. The rare haplotypes in Lake Huron were distributed around the lake. The article also notes that DNA barcoding may lack sensitivity in identifying population structures [R-86].

Overdyk, L. Ecological and genetic factors in the distribution and abundance of larval Lake Whitefish (coregonus clupeaformis) at Douglas Point, Lake Huron. PhD thesis, University of Guelph, Guelph, ON. July 2015.

Aim 1: Investigate effect of conditions at Douglas Point on zooplankton distribution and abundance.

Sampling of zooplankton at various depths in five transects near the Bruce B cooling water intake and discharge and in control locations showed a high abundance both offshore and deeper in the water column. Abundance of zooplankton was mostly determined by the time and depth of sampling and dissolved oxygen rather than other environmental variables (temperature, chlorophyll, pH, phosphorus, turbidity and specific conductivity). Zooplankton was hypothesized to have migrated to deeper waters during daytime hours.

Aim 2: Determine the consistency of visual species identification in comparison to DNA barcoding of larval fish in plankton tows in Stokes Bay.

DNA barcoding identified 51 of 55 larval fish collected in Stokes Bay, Lake Huron. These larval fish sample consisted of a random sample of five fish from each week of sampling

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between April 21 and July 8, 2011. There was a 94% and 71.5% concordance between visual identification done by four independent Identifiers and DNA barcode identification at the family (chi-squared p=0.1351) and species (chi-squared p=0.004) levels respectively. The 27 DNA barcoding identified Lake Whitefish showed a species level 90.7% consistency between DNA barcoding and visual identification.

Aim 3: Explore haplotype variation among Lake Whitefish in Lake Huron and the potential for cryptic diversity.

This research is reviewed in Overdyk et al. (2015) above.[R-86]

Aim 4: Assess the potential to develop a real-time polymerase chain reaction (qPCR) genetic primer/probe assay to identify Lake Whitefish using DNA barcoding to increase accurate larval identification.

The study aimed to identify larval Lake Whitefish using a real-time polymerase chain reaction (PCR). PCR is a DNA identification technique that has the potential to be applied in the field even with very small amounts of DNA. DNA barcoding was used as the basis for the PCR development. The PCR was tested using samples of both Lake Whitefish and other fish found in the Great Lakes, including mixing of larval fish samples. The efficiency of the process developed (i.e., the chance of successfully replicating only Lake Whitefish DNA) was 90.77%. There were no false negatives in the testing. Some amplification was seen with three other fish sampled but this may have been due to DNA contamination from batch storage. The cost of PCR is substantially less than that of DNA barcoding or visual identification.

Aim 5: Assess the uncertainty that exists surrounding environmental conditions impacting larval Lake Whitefish near Douglas Point.

The research examines plankton tow transects from Inverhuron Bay and Holmes Bay (n=8 in the embayment, 6 shallow at 2m, 2 mid-embayment, n=5 outside the embayment) and water pump sampling from near-shore waters (n=9 locations), Plankton tows from Baie du Dore were also examined (n=2 at 2-3m south side, n=2 at 3m on north side within the embayment, n=2 deeper ones near the embayment and n=1 near-shore north of the embayment). These tows occurred over six weeks from May 5 to June 21, 2014. Time/location of sampling and environmental conditions (wind direction and speed, temperature dissolved oxygen and pH) were examined in terms of their effect on larval fish density in general and on Lake Whitefish larvae density.

Lake Whitefish density was low in 10 of the 13 transect samples (less than 10 individuals per 1000m³. Only 3 samples had high densities, Week 1 near-shore outside Holmes Bay near Bruce B intake (21.1 individuals per 1000m³ and Week 3 near-shore inside Inverturon Bay (25.7 and 52.2 individuals per 1000m³).

Northeasterly wind direction and week 2 of sampling were found to be associated with overall larval fish density.

With larvae retrieved the week immediately following ice-out, this study suggests the possibility of embryos hatching while still under embayment ice cover [R-85].

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10.3 McMaster University Whitefish NSERC Collaborative Research and Development Program

10.3.1 Effects of Temperature on Lake and Round Whitefish

A bibliography of scientific publications and theses is provided in Table 30. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to the effects of temperature on Lake or Round Whitefish in Lake Huron have not been listed.

Table 30Scientific reports produced as a result of theNSERC CRD partnership on the effects of temperature

Baseline growth and development

[R-82]	O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the Mountain Whitefish, prosopium williamsoni and cross amplification in the Round Whitefish, P. cylindraceum, using paired end Illumina shotgun sequencing. Conservation Genetics Resources 5, 89 91.	Publication
[R-95]	Sreetharan S., Thome C., Mitz C., Eme J., Mueller C.A., Hulley E.N., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y.,2015 Embryonic development of Lake Whitefish (coregonus clupeaformis): a staging series, analysis of growth and impacts of fixation. Journal of Fish Biology, 87, 3:539-558.	Publication
[R-78]	Mitz, C., Thome, C., Cybulski, M.E., Laframboise, L., Somers, C.M., Manzon, R.G., Wilson, J.Y., Boreham, D.R., 2014. A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture. Journal of North American Aquaculture 76, 179-184.	Publication
	Cellular response to changes in temperature	
[R-97]	Stefanovic D.I., Manzon L.A., McDougall C.S., Boreham D.R., Somers C.M., Wilson J.Y., Manzon R.G, 2016. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish. Comparative Biochemistry and Physiology, Part A 193:1-10	Publication
[R-98]	Stefanovic D.I., 2015. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish (coregonus clupeaformis). MSc thesis University of Regina, Saskatchewan. 2015	MSc thesis
[R-94]	Sessions, K. Plasticity of the heat shock response and development of thermotolerance during embryonic development of Lake Whitefish (coregonus clupeaformis). MSC thesis University of Regina, Saskatchewan. July 2015.	MSc Thesis
	Growth, development and survival responses to changes in temperature	
[R-41]	Eme J., Mueller C.A., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y., 2015. Critical windows in embryonic development: shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (coregonus clupeaformis) embryos and hatchlings. Comparative Biochemistry and Physiology, Part A 179, 71-81.	Publication

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[R-71]	Lee AH, Eme J, Mueller CA, Manz JY, 2016. The effects of cumulati morphology and survival of Lake V Journal of Thermal Biology 57:11-	ve acute heat sh Whitefish (corego	ock exposures on	
[R-81] Mueller C.A., Eme J., Manzon R.G., Somers C.M., Boreham D.R., Wilson, J.Y., 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in Lake Whitefish (coregonus clupeaformis). Journal of Comparative Physiology B 185, 3:315-331.				
[R-104]	-104] Thome,C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R. & Wilson, J.Y. Incubation of Lake Whitefish (coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development. Published on the web 5 January 2016, 10.1139/cjfas-2015-0286.			oling
[R-103]	R-103] Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (coregonus clupeaformis). PhD thesis McMaster University, Hamilton, Ontario. 2015			PhD thesis
[R-72]	Lim, Michael. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (coregonus clupeaformis) and Round Whitefish (prosopium cylindraceum). MSc thesis, McMaster University, Hamilton, ON, September 2016.			
[R-79]	Mitz, C., 2016. The cost of horme constrained environment: Embryo (coregonus clupeaformis). PhD th July 2016.	onic developmen	t of the Lake Whitefish	PhD thesis N,

Sreetharan S., Thome C., Mitz C., Eme J., Mueller C.A., Hulley E.N., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y., submitted. Embryonic development of Lake Whitefish (coregonus clupeaformis): a staging series, analysis of growth and impacts of fixation. Journal of Fish Biology.

Sreetharan, et al. provided a reference staging series for qualitative morphological staging of Lake Whitefish embryos. Embryo size and mass were measured, and correction factors were determined to account for the effects of preservatives [R-95].

Mitz, C., Thome, C., Cybulski, M.E., Laframboise, L., Somers, C.M., Manzon, R.G., Wilson, J.Y., Boreham, D.R., 2014. A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture. Journal of North American Aquaculture 76, 179-184.

Mitz et al. (2014) describes the development of the recirculating incubation system in order to study temperature effects during embryogenesis. This included novel incubation strategies utilizing Petri dishes and multiwell plates [R-78].

Stefanovic D.I., Manzon L.A., McDougall C.S., Boreham D.R., Somers C.M., Wilson J.Y., Manzon R.G, 2016. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish. Comparative Biochemistry and Physiology, Part A 193:1-10.

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Stefanovic et al. (2016) examined the effect of thermal stress on embryonic (fin flutter, vitelline				

circulation stage) and young of the year juvenile Lake Whitefish. They subjected the fish to heat shock stress of three different temperatures (3, 6, or 9°C above control) for six lengths of time (0.25, 0.5, 1, 2, 3, or 4 hours) with two hour recovery periods (at 2 and 14°C respectively for embryonic and juvenile fish) prior to sampling of embryos. The control temperatures were 2°C for embryos and 14°C for juveniles. Heat stress response is a cellular response to stressors that protect proteins and increase fish tolerance for increased water temperatures and is measured by heat stress response proteins (hsp) mRNA levels. The study examined the level of hsp mRNA levels of three specific hsp (hsp90 α , hsp70 and hsp47). All fish mounted a heat stress response that allowed them to survive a 4-hour 9°C heat stress [R-97].

Stefanovic D.I., 2015. Thermal stress and the heat shock response in embryonic and young of the year juvenile Lake Whitefish (coregonus clupeaformis), MSc thesis University of Regina, Saskatchewan, 2015.

The major findings of this thesis were covered in [R-98] above.

Sessions, K. Plasticity of the heat shock response and development of thermotolerance during embryonic development of Lake Whitefish (coregonus clupeaformis). MSC thesis University of Regina, Saskatchewan, July 2015.

Sessions (2015) examined the development of thermotolerance in Lake Whitefish embryos. Embryos were exposed to repeat transient 1-hour heat shocks of +3, 6 9 or 12°C above the control temperature of 3°C every 3 or 6 days. These embryos were then given recovery periods of 6 or 18 hours prior to the application of high level heat shocks. High level heat shocks consisted of exposing groups of 75 embryos from each transient heat shock group and a control group to high-level 12, 15 or 18°C 1- or 4-hour heat shocks at either 64 or 82 days post-fertilization. HSP levels were measured 2 hours after the high-level heat shock and percent survival, time to hatch and morphometrics were recorded.

Embryos exposed to transient heat shocks every 3 days had the lowest levels of hsp70 among heat shock treatment groups. Older embryos mounted a larger response with lower levels of HSP increase. Transient heat shock routines conferred greater protection to embryos in surviving a 4-hour 18°C high-level heat shock.

Transient heat shocks enabled embryos to have a quicker response to and tolerance for heat stress, particularly if the recovery period between the transient heat shock and high-level heat shock was long enough [R-94].

Eme J., Mueller C.A., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y., 2015. Critical windows in embryonic development: shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (coregonus clupeaformis) embryos and hatchlings. Comparative Biochemistry and Physiology, Part A 179, 71-81.

Eme, et al., investigated the effect of temperature on metabolic and cardiac phenotype of embryos. The incubation temperature of Lake Whitefish embryos was changed at either the end of gastrulation or primordial organogenesis. Temperature was increased or decreased (with respect to controls at constant temperature) through Price stage 320, extending through Griffiths' Block 1 (stages 1-9 per [R-9]) and into Griffiths' Block 2.

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Heart rate and oxygen consumption were measured throughout embryogenesis as well as 1 day post-hatch. Eme, et al., found that metabolic rate (oxygen consumption) depended on thermal history. Thermal history during gastrulation resulted in changes in metabolic rate when measured following primordial organogenesis and potentially following hatch, although not when measured at developmental stages in between. Thermal history during gastrulation and organogenesis resulted in changes in metabolic rate following hatch, but not at developmental stages prior to hatch. The greatest changes in metabolic rate were observed for embryos incubated at 5°C and 8°C through gastrulation and primordial organogenesis, followed by incubation at 2°C (63% increase and 20% decrease in oxygen consumption, respectively). Although the data suggests phenotypic plasticity, results did not consistently depend on magnitude of temperature shift [R-41].

Lee AH, Eme J, Mueller CA, Manzon EG, Somers CM, Boreham DR, Wilson JY, 2016. The effects of cumulative acute heat shock exposures on morphology and survival of Lake Whitefish (coregonus clupeaformis) embryos. Journal of Thermal Biology 57:11-20.

Lee et al. (2016) examined the effect of weekly 1-hour heat shock of $+3^{\circ}$ C above control temperatures of 2°C and 5°C on Lake Whitefish embryo morphology and survival. They also considered the effect of a constant 8°C incubation temperature. There was a decrease in time to hatch with increasing incubation temperature (148 days at 2°C, 92 days at 5°C and 50 days at 8°C).

Weekly heat shocks did not affect the time to hatch. There was no change in survival to the pre-hatch stage with increased incubation temperature or heat shocks (~50% cumulative survival). The constant 8°C and 5°C incubation groups had significantly smaller embryos and larger yolk sacks but there was no difference in morphology for heat shock groups compared to the constant temperature control.

Infrequent exposure to thermal effluent is not likely to affect Lake Whitefish embryo morphology and development. The effect of warmer incubation temperatures on the immediate pre-hatch/post-hatch period survival of embryos was not explored in this paper [R-71].

Mueller C.A., Eme J., Manzon R.G., Somers C.M., Boreham D.R., Wilson, J.Y., 2015. Embryonic critical windows: Changes in incubation temperature alter hatchling phenotype, survival and cost of development in Lake Whitefish (coregonus clupeaformis). Journal of Comparative Physiology B 185, 3:315-331.

Mueller, et al., examined the effect of temperature on yolk conversion efficiency and cost of development in Lake Whitefish embryos and larvae. Hatchling yolk-free dry mass decreased, and dry yolk mass increased, with increasing constant temperature. Yolk conversion efficiency decreased with increasing constant temperature. Total cost of development increased with increasing constant temperature. Changes in phenotypic characteristics were observed for temperature shifts, but the developmental period most sensitive to temperature change depended on the characteristic.

Overall, Mueller, et al., found increasing temperature resulted in earlier hatch, as well as larvae with smaller bodies and larger yolk reserves. The effect that increased temperature may have on fitness of Lake Whitefish larvae is not known.

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Survival and hatch timing results were similar to those indicated by [R-56]. For example, Mueller, et al., found that time to 50% hatch decreased with increasing constant incubation temperature, hatch window decreased at constant 8°C (compared to constant 5°C and 2°C), and survival decreased with increasing constant incubation temperature. Gastrulation was observed to be a critical window for survival [R-81].

Thome, C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R. & Wilson, J.Y. Incubation of Lake Whitefish (coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development. Published on the web 5 January 2016, 10.1139/cjfas-2015-0286.

Thome et al. (2016) deployed fertilized Lake Whitefish embryos in incubation chambers near the thermal discharge from Bruce A and Bruce B and in two control locations.

The average water temperature near the thermal discharge was between 0.5 and 3.7°C. The temperature in the thermal discharge areas was an average of 3°C warmer than the surrounding waters and also more variable with a greater range and rate of change than surrounding waters. Discharge temperature spikes rarely exceed 8°C. Only twice did the temperature exceed 10°C for between 5 and 30 minutes.

There were some difficulties with retrieval of the incubation chambers due to ice cover and damage over the winter. In several cases, all the embryos had hatched and the larval fish had swum free from the chambers by the time retrieval occurred. The differences in retrieval were accounted for in the analysis.

Embryos incubated near the thermal discharge were larger with smaller yolks than the reference embryos. Modelling predictions were validated with embryos incubated in the thermal discharge predicted to be greater than 10% ahead of controls. Although this increased rate of development was not enough to increase embryonic mortality or developmental abnormalities, it may impact larva survival[R-104].

Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (coregonus clupeaformis). PhD thesis McMaster University, Hamilton, Ontario 2015.

The first part of the thesis describes the work published in [R-104]. Subsequent work in the thesis does not deal directly with the effects of temperature changes on Round or Lake Whitefish.

Lim, Michael. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (coregonus clupeaformis) and Round Whitefish (prosopium cylindraceum). MSc thesis, McMaster University, Hamilton, ON, September 2016.

The effects of seasonal changes in incubation temperature combined with regular temperature spikes on Lake and Round Whitefish embryo survival, growth, development, heart and oxygen consumption was examined.

Lake Whitefish embryos were raised with seasonal temperature changes and/or temperature spikes in several groups:

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- a) Seasonal decline from 8°C to 2°C over 6 weeks, then incline back up to 6°C over 4 weeks.
- b) Repeating 3°C 1-hr spikes every 2-3 days starting at 1.5 months of age.
- c) Seasonal plus with spikes during the 2°C period.
- d) Constant temperatures of 2, 5 & 8°C.

For Lake Whitefish, survival to hatch, morphometrics and heart rate measured at 2°C were examined at the fin flutter, protruding mouth and pre-hatch stages and compared to the constant 2°C group. There was a 3-fold increase in mortality in the constant 8°C group. There was no change in survival in any of the variable temperature groups. Embryo heart rate was elevated in the seasonal temperature change with temperature spikes group. The seasonal change embryos had the largest change in body growth and yolk consumption from the protruding mouth to the pre-hatch stage. They also experienced rapid growth with the seasonal incline. The majority of hatches occurred at 6°C in seasonal change group.

Round Whitefish embryos were raised with seasonal temperature changes and/or temperature spikes in several groups:

- a) Seasonal decline from 8°C to 2°C over 6 weeks, then incline back up to 6°C over 4 weeks at 1 (S_A) per week.
- b) Seasonal decline from 8°C to 2°C over 6 weeks, then incline back up to 6°C over 2 weeks at 2°C (S_B) per week.
- c) Repeating 3°C 1-hr spikes every 2-3 days starting at 1.5 months of age over 8 weeks (SP_A).
- d) Repeating 3°C 1-hr spikes every 2-3 days throughout development (SP_B).
- e) Seasonal changes plus temperature spikes (S_A+ SP_A, S_A+ SP_B, S_B+ SP_A & S_B+ SP_B).
- f) Constant temperatures of 2, 2.5, 5 & 8°C.

Round Whitefish embryo mortality to hatch and at 30 days post-hatch, growth, development, heart rate and oxygen consumption rate were examined at the fully eyed, fin flutter, protruding mouth and pre-hatch stage and compared to the constant 2°C group. Mortality in 2015 was significantly higher than in 2014 for the 5°C and 2°C constant temperature groups, possibly due to the warmer temperature at spawning or the quality of the gametes. There was a 20% increase in post-hatch mortality in the rapid seasonal incline group (S_B) and a 15% increase in mortality in the constant 8°C group. Seasonal temperature changes increased the development rate and shortened hatch timing. Lower incubation temperatures increased body mass and decreased yolk mass. Exposure to temperature spikes had little effect on embryo development stage onset. Embryo heart rates decreased with lower incubation temperatures and increased development stage. Oxygen consumption was similar across all temperature groups [R-72].

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The effects of morpholine and low-dose radiation exposure on Round Whitefish are reviewed in Section 10.3.2.

Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (coregonus clupeaformis). PhD thesis, McMaster University, Hamilton, ON, July 2016.

The first part of this thesis covers the material in [R-78].

Mitz (2016) developed a method to determine an equivalent or effective temperature from a variable thermal regime. The effective temperature is derived from a time series of temperature data to allow comparisons of growth between different variable thermal regimes to that of an equivalent constant temperature regime. There is an Excel spreadsheet presented that can be used to apply this method to time series temperature data. This method is valid under several criteria based on the temperature dependence of growth in the species under consideration.

Mitz (2016) examined hatch timing, development stage-at-hatch and size-at-hatch among Lake Whitefish embryos under variable temperature regimes. Embryos were obtained in 2013 and 2014 and incubated at 0.5, 2, 5 and 8°C. Variable regimes consisted of 2 or 8°C for the first third of development, then transfer to higher (\rightarrow 5°C \rightarrow 8°C) or lower temperature (\rightarrow 5°C \rightarrow 2°C) for the other thirds of development. They also incubated embryos at 2°C for 81, 93, 106, 121, 138 and 151 days followed by a transfer to 5°C or 8°C. The Fin Definition Ratio (FDR) and Yolk Conversion Efficiency (YCE) were used to measure developmental stage.

Larvae raised at a constant 2°C were significantly longer and heavier than those exposed to constant 5°C or 8°C temperatures. There was a decreased FDR for embryos raised at lower temperatures. For the variable regimes across thirds of development, median hatch occurred much later (168-174 days) and lasted longer (21 to 28 days) for the decreasing regime than for the increasing regime (102-104 days and occurring over 1 to 2 days) despite similar mean temperatures Fin indentation and length were greater under the increasing variable regime.

The work showed that Lake Whitefish hatch at a more advanced stage of development when raised at lower temperatures.

The thesis proposed the term heterograde hatching to describe a situation where Lake Whitefish hatched at a more advanced stage of development when incubated at lower temperatures (Figure 5). This may be a way of ensuring that development stage at hatch matches the available of zooplankton related to spring breakup.

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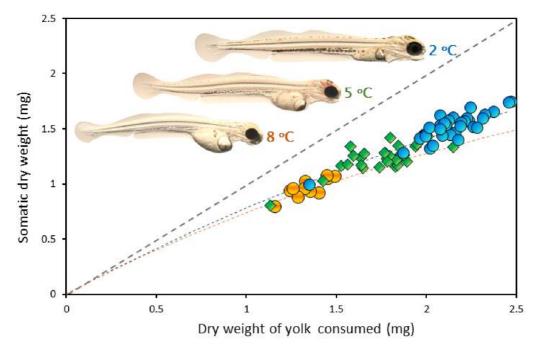


Figure 5 Observed conversion of yolk dry weight to somatic dry weight for embryos hatching at different constant incubation temperatures (• 2°C, • 5°C, • 8°C) [R-79]

A new Lake Whitefish developmental model was developed that incorporated heterograde hatching. Prior models of Lake Whitefish development under variable thermal regimes (Price, 1940 and Brooke, 1975) are compared with the longer time to 50% hatch in the embryos raised during thesis work. Embryos were obtained in 2013 and 2014 and incubated at 0.5, 2, 5 and 8°C. Variable regimes consisted of 2 or 8°C for the first third of development, then transfer to higher (\rightarrow 5°C \rightarrow 8°C) or lower temperature (\rightarrow 5°C \rightarrow 2°C) for the other thirds of development. They also incubated embryos at 2°C for 81, 93, 106, 121, 138 and 151 days followed by a transfer to 5°C or 8°C. There were also temperatures cycles that varied after every 6 days for 1 day and after every five days for 2 days throughout development between 0, 2, 5 and 8°C. Incubation times for constant temperatures were greater than for Brooke (1975) and much greater than for Price (1940).

Variable temperature regime outcomes were compared to existing models and to the proposed heterograde hatching model. The use of the heterograde hatching model had the lowest error in accurately predicting embryo hatch under constant and variable thermal regimes. In comparison, the Griffiths overestimated and the Brooke model underestimated development time. The model was further validated using data from [R-8] [R-81] [R-90] [R-79].

Work in this thesis related to exposure of Whitefish embryos to radiation is covered in Section 10.3.2.

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10.3.2 Effects of Combined Stressors on Lake and Round Whitefish

A bibliography of scientific publications and theses is provided in Table 31. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to the effects of combined stressors on Lake or Round Whitefish in Lake Huron have not been listed.

Table 31 Combined stressor reports produced as a result of the NSERC CRD partnership

Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (<i>coregonus clupeaformis</i>). PhD thesis McMaster University, Hamilton, Ontario. 2015	PhD thesis
Lim, Michael. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (<i>coregonus clupeaformis</i>) and Round Whitefish (<i>prosopium cylindraceum</i>). MSc thesis, McMaster University, Hamilton, ON, September 2016.	MSc thesis
Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (<i>coregonus clupeaformis</i>). PhD thesis, McMaster University, Hamilton, ON, July 2016.	PhD thesis
	 embryonic development in Lake Whitefish (<i>coregonus clupeaformis</i>). PhD thesis McMaster University, Hamilton, Ontario. 2015 Lim, Michael. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (<i>coregonus clupeaformis</i>) and Round Whitefish (<i>prosopium cylindraceum</i>). MSc thesis, McMaster University, Hamilton, ON, September 2016. Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (<i>coregonus clupeaformis</i>). PhD thesis, McMaster University, Hamilton, ON,

Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (coregonus clupeaformis). PhD thesis McMaster University, Hamilton, Ontario. 2015.

The first part of the thesis describes the work published in [R-104].

The second part examines acute and chronic exposure to chemical (morpholine and sodium hydrochlorite) and radiological effects in Lake Whitefish embryos. Acute exposures were done over 96 hours on days 1, 7, 15 and 30 after fertilization to target sensitive developmental periods.

Embryos became increasingly resistant to the effects of low dose ionizing radiation as they approach hatch. Acute exposure of embryos consisted of acute doses of ¹³⁷Cs gamma rays at five sensitive periods in doses ranging from 0.008 to 15.5 Gy. Chronic dosing was at rates between 0.06 and 4.4 mGy/day with a total dose between 10 to 664 mGys. There was a high resistance to acute doses with $LD_{50/hatch}$ of 5.0 ± 0.7 Gy post-fertilization up to $LD_{50/hatch}$ of 14.2 ± 0.1 Gy in later developmental stages. Chronic doses of ionizing radiation resulted in a significantly earlier hatch and up to a 60% increase in embryo size.

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Conversely, embryos were most susceptible to mortality from morpholine exposure closest to hatch. Chronic morpholine exposure resulted in a lower LC_{50} (219 ± 54 mg/L) when the exposure began at fertilization than when the exposure began at the eyed stage (LC_{50} 674 ± 12 mg/L). Higher morpholine concentrations resulted in earlier mortality of embryos. Chronic exposure resulted in 30% earlier hatching and up to a 10% smaller body length at hatch. Yolk conversion efficiency was reduced with morpholine exposures over 1000mg/L. Acute exposure lasting 96 hours showed minimal effects when tested up to 1000 mg/L, except when exposure began on Day 1. For those embryos with acute exposures beginning on Day 1, the effects were similar to chronic morpholine exposure at the 500mg/L and 1000mg/L level. No increase in developmental abnormalities with morpholine exposure was noted.

Minimal impact was seen with embryo incubation in sodium hypochlorite. Acute exposure up to 0.1 mg/L showed no effect on size, hatch dynamics or survival. Only with chronic exposure from fertilization was any effect on survival seen, with total residual chlorine LC_{50} 0.097 ± 0.007 mg/L.

Incubation of Lake Whitefish in water with artificially increased pH levels to simulate the effect of morpholine on pH independent of the effect of the chemical itself. Up to a pH of 10, embryos were larger at hatch. At a pH of 10, embryos were smaller at hatch. No change in survival or hatch timing was noted.

The combination of radiological (ionizing radiation) and chemical stressors (mild heat shock or morpholine exposure) produced variable mortality effects. Placing embryos in 100 mg/L morpholine for 96 hours in combination with acute ionizing radiation exposure increased embryo mortality. No effect was observed for the same event at morpholine concentrations of 10 mg/L. A heat shock of 2-hours duration (temperature increase of 3°C or 9°C) administered six hours prior to irradiation decreased embryo mortality by up to 25%. All of the mortality effects on embryos in this study occurred at doses significantly greater than those in the thermal discharge channels at Bruce Power. Relevant doses of chronic radiation (0,008mGy/day) resulted in an earlier hatch and morpholine concentrations of 1 or 10 mg/L increase larval size at hatch. Both of these changes could potentially affect larval survival [R-103].

Lim, Michael. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (coregonus clupeaformis) and Round Whitefish (prosopium cylindraceum). MSc thesis, McMaster University, Hamilton, ON, September 2016.

The effects of seasonal temperature changes with or without temperature spikes on Lake and Round Whitefish are reviewed in Section 10.3.1.

The effects of exposure to morpholine and low-dose radiation were examined in Round Whitefish. Round Whitefish embryos were exposed to chronic levels of morpholine (10, 100, 500 and 1000 mg/L), pH controls (8, 9 and 10) or low-doses of radiation (0, 0.10, 0.16 or 0.30 mGy/day of ¹³⁷Cs gamma rays) throughout development. Mortality to hatch and at 30 days post-hatch, growth, development, heart rate and oxygen consumption rate were examined at the fully eyed, fin flutter, protruding mouth and pre-hatch stages.

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There was increased mortality to hatch in Round Whitefish exposed to morpholine levels ≥500mg/L. There was also decreased post-hatch mortality of >20% for irradiated Round Whitefish embryos. The pH controls had no change in mortality. Exposure to 500 mg/L of morpholine delayed development stage onset, shortened hatch windows and resulted in close to 100% mortality at 30 days post-hatch for the embryos. There was no consistent effect on embryos at 0-100 mg/L.

The findings suggest that Round Whitefish embryos may be more susceptible to morpholine and possibly less susceptible to low-dose radiation than Lake Whitefish embryos [R-72].

Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (coregonus clupeaformis). PhD thesis, McMaster University, Hamilton, ON, July 2016.

Mitz (2016) examined how growth stimulated by exposure of Lake Whitefish embryos to ¹³⁷Cs affected metabolic efficiency. Embryos incubated at 5°C for 40 days, then at 2°C for the remainder of development were exposed to a dose of gamma radiation every 14 days in doses ranging from 14mGy to 8 Gy per dose. Measurements were taken at 14 and 42 days after the final dose.

Embryos groups that received doses of 3.8 and 7.6 Gy per irradiation had high mortality (>50%), whereas all other groups had low (<5%) mortality. Embryos groups exposed to between 0.9 Gy and 1.9 Gy (i.e., less than 7.6 Gy total dose) per dose were heavier than control embryos by 10%. No significant difference was seen for exposures between 14 mGy and 0.9 Gy. Between 1.9 and 7.6 Gy per dose, embryos were significantly lighter than controls [R-79].

10.3.3 Population Discrimination of Lake Whitefish

A bibliography of scientific publications and theses is provided in Table 32. Interim scientific reports, reports of a primarily administrative nature, and scientific publications not specifically related to population discrimination of Lake or Round Whitefish in Lake Huron have not been listed.

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WHITEF	TISH RESEARCH REVIEW					
Ро	Table 32 Population discrimination reports produced as a result of the NSERC CRD partnership					
[R-82] O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the Mountain Whitefish, <i>prosopium williamsoni</i> and cross amplification in the Round Whitefish, <i>P. cylindraceum</i> , using paired end Illumina shotgun sequencing. Conservation Genetics Resources 5, 89 91.						
[R-37] Eberts, R., 2013. Dietary niche partitioning by two sympatric whitefish species B.Sc. in Lake Huron: stable isotope analysis at three temporal scales. B.Sc. (Hon.) thesis thesis. University of Regina, Saskatchewan. April 2013.						
[R-38]Eberts, R., 2015. Resource use and ecological population structure of LakeMSc theseWhitefish (coregonus clupeaformis) spawning aggregations in Lake Huron.MSc thesis. University of Regina, Saskatchewan. December 2015.MSc thesis.						
[R-54] Graham CF, Eberts RL, Morgan TD, Boreham DR, Lance, SL, Manzon RG, Martino JA, Rogers SM, Wilson JY, Somers CM, 2016, Fine-scale ecological and genetic population structure of two Whitefish (<i>Coregoninae</i>) species in the vicinity of industrial thermal emissions. Plos ONE. DOI: 10.1371/journal.pone.0146656.				cal		
[R-55] Graham, CF, Glenn, TC, McArthur, AG, Boreham, DR, Kieran, T, Lance, S, Manzon RG, Martino, JA, Pierson, T, Rogers, SM, Wilson JY, Somers CM, 2015. Impacts of Degraded DNA on Restriction Enzyme DNA Sequencing (RADSeq). Molecular Ecology Resources 6:1304-15.				1		
[R-39]						
[R-40]						
[R-80]	Morgan, T., 2016. Genetic popula (<i>prosopium cylindraceum</i>) in North regional subdivision. MSc thesis, I	n America: multip	ble glacial refugia and	MSc thesis		

O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the mountain whitefish, prosopium williamsoni and cross amplification in the Round WhitefishRound Whitefish, P. cylindraceum, using paired end Illumina shotgun sequencing. Conservation Genetics Resources 5, 89 91.

O'Bryhim, et al., reported the development of new microsatellite markers for Round Whitefish, which has not previously been studied as part of population structure research [R-82].

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Eberts, R., 2013. Dietary niche partitioning by two sympatric whitefish species in Lake Huron: stable isotope analysis at three temporal scales. B.Sc.(Hon.) thesis. University of Regina, Saskatchewan. April 2013.

Eberts analyzed muscle, liver, and opercular bone for ¹³C and ¹⁵N from approximately 50 individuals each of Lake and Round Whitefish collected during spawning near Douglas Point in 2011. Results indicated that Lake Whitefish occupied a more pelagic niche than Round Whitefish at each temporal scale (summer growing period, end of summer growing period, and juvenile versus adult). Less dietary niche segregation was observed near the end of the growing period. Juveniles and adults of each species had similar dietary niches. Individuals of both species had both benthic- and pelagic-oriented shifts in ¹³C, suggesting complex prey sources and high inter-individual variation in diet for both species [R-37].

Eberts,R., 2015. *Resource use and ecological population structure of Lake Whitefish (coregonus clupeaformis) spawning aggregations in Lake Huron. MSc thesis. University of Regina, Saskatchewan. December* 2015.

Eberts (2015) aimed to establish a greater understanding of the ecological spawning structure of Lake Whitefish in Lake Huron through the use of a short-term food web based tracers. δ 13C and δ 15N isotope analysis was used to examine resource use before spawning across Lake Huron. Among the 32 spawning aggregations (1474 fish) examined in the fall of 2012 and the 8 summer diet assemblages (220 fish) in the summer of 2014, large variation in isotopes and heterogeneity in resource use identity and diversity were found. The δ 13C value of the summer fish explained 82% of the variation in the δ 13C value at the nearest spawning location. This indicated that aggregations in different parts of the lake use different local food webs.

The population of Lake Whitefish in Lake Huron could be divided into six ecological sub populations based on local resource use. Current fisheries policies using 25 management zones may be using undersized management zones.

In the area local to Bruce Power, the Main Basin of Lake Huron could be divided into Southwestern and Northeastern (including the Bruce Power site) ecological groups. Both the Southwestern and Northeastern ecological groups had heavy population mixing within each group. No small distinct ecological groups were identified within the area near the Bruce Power site (i.e., Scougall Bank and McRae Point) [R-38].

Graham CF, Eberts RL, Morgan TD, Boreham DR, Lance, SL, Manzon RG, Martino JA, Rogers SM, Wilson JY, Somers CM, 2016, Fine-scale ecological and genetic population structure of two Whitefish (Coregoninae) species in the vicinity of industrial thermal emissions. Plos ONE. DOI: 10.1371/journal.pone.0146656.

Graham et al. (2016) examined ecological and genetic population structures of Lake and Round Whitefish in the area surrounding the thermal effluent discharges from Bruce A and Bruce B. Eight sampling areas were used, six sampling areas near the cooling water discharges and two adjacent reference areas to the north and south of the Bruce Power site. Gill nets were set for 19 to 24 hours on 13 dates in 2010 and 15 dates in 2011 between October and December.

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The study used DNA microsatellite analysis to examine genotype diversity in 208 Lake Whitefish and 327 Round Whitefish. Genetic analysis indicated that fish caught close to Bruce Power were part of a larger population going past the study area boundary.

The study also used $\delta 13C$ and $\delta 15N$ stable isotopes to examine isotopic niche size in 336 Lake Whitefish and 319 Round Whitefish. Isotopic niche size reflects the diversity of resource use by individual fish. There was significant overlap in resource use (over 72%) for Round and Lake Whitefish between adjacent reference areas and near sampling areas. For one near sampling area for Lake Whitefish (MacPherson Bay) and three near sampling areas for Round Whitefish (McPherson Bay, Gunn Point and Inverhuron Bay), the niche size was 1.3 to 2.8 times larger than the niche size in the reference areas. This suggests that fish in the near sampling areas relied on a more diverse diet and habitat with higher levels of fish mixing [R-54].

Graham, CF, Glenn, TC, McArthur, AG, Boreham, DR, Kieran, T, Lance, S, Manzon RG, Martino, JA, Pierson, T, Rogers, SM, Wilson JY, Somers CM, 2015. Impacts of Degraded DNA on Restriction Enzyme DNA Sequencing (RADSeq). Molecular Ecology Resources 6:1304-15.

Graham et al. (2015) examined DNA sample degradation in samples from juvenile Lake Whitefish held at room temperature and sampled at 0, 12, 48 and 96 hours post-euthanasia.

DNA samples at 12 and 48 hours performed well in the modified double digest RADSeq approach. Using the 96 hour group resulted in highly degraded DNA and poor performance in terms of RADtags per individual, number of variable sites and percentage of identical RADtags retained. Changes in restriction enzymes, modified fold-coverage values and additional read-length trimming did not change the results. RADSeq is a robust approach except for instances of highly degraded DNA [R-55].

Eberts, R., Wissel, B., Boreham, D., Manzon, R., Wilson, J. and Somers, C., 2015. Consistent differential resource use by sympatric Lake (coregonus clupeaformis) and Round Whitefish (prosopium cylindraceum) in Lake Huron: a multi-time scale isotopic niche analysis. Canadian Journal of Fisheries and Aquatic Sciences. Published on the web 08 Dec 2015, 10.1139/cjfas-2015-0324.

Eberts et al. (2015) examined long-term resource use and niche overlap in Round and Lake Whitefish. Samples of liver, muscle and bones from Lake (n=60-182 annually) and Round (n=149-188 annually) Whitefish collected near Douglas Point from 2010 to 2012 were analyzed for carbon and nitrogen stable isotope ratios. The study used multi-year (2010-2012) and multi-tissue (liver, muscle and bone layers) isotopic niche analysis from adult (age 5+) Lake (LW) and Round (RW) Whitefish sampled from eight locations within 20km of Douglas Point in 2010 (n=182 LW & 188 RW), 2011 (n=148 LW & 149 RW) and 2012 (n=60LW & 156 RW). Additional tissue samples were also collected from summer feeding groups of Lake (n=31) and Round (n=29) Whitefish in Lake Huron (2014).

Lake Whitefish used more diverse, 13C-depleted (mean $\delta 13C = -21.9\%$) and 15N-enriched (mean $\delta 15N = +9.3\%$) resources than Round Whitefish (mean: $\delta 13C = -18.2\%$; $\delta 15N = +8.3\%$). Niche overlap occurred only in liver (representing the spawning period) while niche segregation was highest in juvenile life stages.

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Spawning aggregations were composed of individuals with a variety of feeding sources and locations for both Lake and Round Whitefish. Round Whitefish had less niche overlap than Lake Whitefish. Life-long niche partitioning before age 2 and differential resource use in adulthood are important to both species [R-39].

Eberts, R., Wissel, B., Simpson, G, Crawford, S., Stott, W., Hanner, R., Manzon, R., Wilson, J., Boreham, D. and Somers, C.,2017. Isotopic structure of Lake Whitefish in Lake Huron: Evidence for regional and local populations based on resource use. North American Journal of Fisheries Management 37, 133-148.

This paper presents the lake-wide findings described in [R-40] above.

Morgan, T., 2016. Genetic population structure of the Round Whitefish (prosopium cylindraceum) in North America: multiple glacial refugia and regional subdivision. MSc thesis, University of Regina, November 2016.

Round Whitefish (n=414) tissue samples were collected from 16 locations across North America (6 in the Western Region, 10 in the Eastern Region) and one site in eastern Russia. Genetic analysis using microsatellites (n=390), mitochondrial DNA sequencing (n=124) and next RAD SNP loci sequencing (n=190) was completed for the Round Whitefish collected. Mitochondrial DNA is inherited from maternal sources only and because of this becomes less variable more quickly within an isolated population. Mitochondrial DNA represents a shorter time scale of DNA analysis compared to nextRAD sequencing.

Analysis of the genetic populations of Round Whitefish in Alaska, the Yukon and Northwest Territories, Labrador and the Laurentian Great Lakes was completed. This analysis used both a population and phylogeographic (i.e., analysis of historical events that may be responsible for current population distributions) genetic analysis approach.

Separate genetic populations exist in Western and Eastern North America at both the mitochondrial and next RAD level.

The Western populations are connected via river basin flow. Five mitochondrial halotypes were found in the Western region (Alaska, Yukon and Northwest Territories). The Russian site was distinct from the other Western sites in the analysis of nextRAD sequencing. Microsatellite analysis showed three distinct population in the Great Lakes, Simpson Lake (Yukon) and Labrador. Separate population identified through microsatellite analysis within the Western population included North Alaska, Bennett Lake (Yukon) and Simpson Lake (Yukon).

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For the Eastern population, the Lake Huron population appears to be a source population for Lakes Michigan, Superior and northern Georgian Bay as well as Lake Nipigon through Lake Superior. The Lake Ontario population is distinct from other Great Lakes populations. There were six distinct halotypes in the Great Lakes and Labrador sites. The Labrador site was distinct from the other Eastern sites in the analysis of nextRAD sequencing. Also in the nextRAD sequencing analysis, Lake Nipigon and Lake Superior formed their own group as did Lake Ontario. There were separate genetic populations within Lake Huron, Lake Michigan and Georgian Bay. Separate population identified through microsatellite analysis within the Great Lakes populations included Lake Ontario and South Georgian Bay. Sampling was not wide-spread enough within Lake Huron to draw conclusions regarding population structure within the lake.[R-80]

10.4 COG Thermal Effects Benchmarks

Table 33 Scientific reports produced as a result of the COG thermal effects research

[R-89]	Patrick, P.H., Chen, E., Parks, J., Powell, J., 2013. Effects of fixed and fluctuating temperature on early hatch of Round and Lake Whitefish eggs. CANDU Owners Group Inc. TN 12 3049. March 2013.	Technical report
[R-90]	Patrick, P.H., Chen, E., Parks, J., Powell, J., Poulton, J.S., Fietsch, C. L., 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs. <i>North American Journal of Fisheries Management</i> 33, 1091 1099.	Publication
[R-92]	Patrick P., Mason E., Parks J., Powell J., Garisto N., Janes A., 2014. Effects of fixed and fluctuating temperature on mortality and hatch of Round Whitefish and Lake Whitefish eggs. CANDU Owners Group Inc. COG-13-3025. October 2014.	Technical report

Patrick, P.H., Chen, E., Parks, J., Powell, J., 2013. Effects of fixed and fluctuating temperature on early hatch of Round and Lake Whitefish eggs. CANDU Owners Group Inc. TN 12 3049. March 2013.

This COG technical report was summarized in the form of a publication [R-90], which is reviewed below.

The technical report includes some discussion of mortality, as well as a comparison to data obtained from incubation of Lake Simcoe embryos [R-89].

Patrick, P.H., Chen, E., Parks, J., Powell, J., Poulton, J.S., Fietsch, C.L., 2013. Effects of fixed and fluctuating temperature on hatch of Round Whitefish and Lake Whitefish eggs. North American Journal of Fisheries Management 33, 1091-1099.

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Patrick, et al., incubated Lake Whitefish (Lake Huron) and Round Whitefish (Lake Ontario) embryos in filtered and disinfected lake water with fixed (Round Whitefish only) and variable temperature increments above baseline. Note that baseline or ambient temperatures (actual Lake Ontario temperatures) were not fixed as in prior laboratory incubation studies. Embryos incubated at variable temperatures experienced continuously variable temperature, with a temperature increment (greater than ambient) that varied at a rate of 0.5°C/h. A complete cycle consisted of temperature beginning at ambient, increasing to ambient plus the maximum temperature increment (up to 5°C), and returning to ambient.

Experimental conditions contrast with those utilized previously by Griffiths [R-56]. In the latter study, abrupt temperature changes were made twice daily.

Patrick, et al., found that greater fixed temperature increments resulted in earlier hatch. Incubation of Round Whitefish at ambient temperature resulted in 50% hatch at 103 days, compared to 62 days for a fixed 5°C greater than ambient. (No comparable data was obtained for Lake Whitefish.)

Patrick, et al. found that greater variable temperature increments resulted in earlier hatch. Incubation of Lake Whitefish at ambient temperature resulted in 50% hatch at 111 days, compared to 76 days for incubation at a variable temperature increase of 0-5°C at 0.5°C/h. Incubation of Round Whitefish at ambient temperature resulted in 50% hatch at 103 days. compared to 70 days for incubation at a variable temperature increment of 0-5°C at 0.5°C/h.

Additionally, Patrick, et al., reported a general trend towards greater hatching windows with increasing temperatures, whether fixed or variable temperature increments. However, this relationship seems weak, considering the reported hatching windows and large variance. Finally, hatching at lower temperatures may have been triggered by the relatively rapid warm up of ambient lake temperatures.

Mortality generally increased with increasing temperature increments. from nearly 0% to 13% for Round Whitefish (all treatment groups) and from 0% to 4% for Lake Whitefish (all treatment groups; variable temperature increment only) [R-89]. Almost no deformities were reported (<0.1% for all treatment groups) [R-89].

Survival was very high, when compared with Griffiths' results [R-56]. Griffiths found a maximum survival of greater than 75%, while Patrick, et al., observed survival at nearly 100%. The difference may be attributable to differences in handling of eggs, particularly during the more sensitive earlier stages. The decrease in survival is qualitatively similar between Patrick, et al., and Griffiths. For example, Griffiths found survival to be greater than 70% for groups incubated at 7°C (base temperature) or less. Lower survival was found at greater temperatures, but this range of temperature was not explored by Patrick, et al.

Mortality was greatest near hatch [R-89], in contrast to results obtained by Griffiths, who found that mortality was greatest in the earliest developmental period [R-56].

Patrick P., Mason E., Parks J., Powell J., Garisto N., Janes A., 2014. Effects of fixed and fluctuating temperature on mortality and hatch of Round Whitefish and Lake Whitefish eggs. CANDU Owners Group Inc. COG-13-3025. October 2014.

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The technical report summarizes two years of experimental data, including data previously reported (and reviewed above).

In 2012-2013 (the second study year), experimental treatments focused on approximate simulations of in-field thermal plume exposure, as determined by over-winter substrate temperature data collected the previous year. While some variable temperature experiments were repeated in 2012-2013, the focus of this review is on the plume simulations, as well as overall conclusions following two years of experiments.

For the Bruce Power plume simulation, data was obtained near Loscombe Bank (2011-2012), as well as at two reference locations located over 10 km north and south of Loscombe Bank. The "plume" ΔT was determined by subtracting temperature at Loscombe Bank from the reference temperature. This ΔT (Lake Huron; 2011-2012) was then added to the "ambient" temperature (Lake Ontario; 2012-2013) at the experimental facility. The intent was to simulate the observed (2011-2012) temperature fluctuations.

Beginning three weeks of incubation at ambient temperatures, experimental "plume" temperatures were applied, initially based on daily mean temperatures and subsequently on half-daily mean temperatures. However, the correlation between the applied temperature fluctuations in the laboratory and the intended temperature fluctuations is very poor (Figure A.5 of [R-92]). The experimental treatment included generally had consistently greater temperatures (1-2°C) than observed in the field, and included larger temperature spikes.

Hatching was delayed in the simulated plume experiments. For the Bruce Power "plume", median hatch occurred at 140 dpf, while for controls, median hatch occurred at 106 dpf. However, the experimental groups experienced, on average, greater temperatures than the control groups, so an earlier hatch would have been expected. The authors alluded to negative temperature feedback affecting the control groups, but the hatch timing for the 2012-2013 experiments was essentially the same as for 2011-2012 (111 days in 2011-2012).

The effect of the thermal plume on mortality and hatch timing is difficult to interpret even from the experiment intended to simulate that effect. First, plume simulation data was collected in 2011-2012 in a location that would be affected by Bruce A with only 2 units in operation. (Full four-unit operation was not achieved until October 2012) At best the experiments simulate the effect of two-unit operation.

Both mortality and hatch timing may have been impacted by the experimental conditions. Incubation at Lake Ontario 2012-2013 "ambient" temperature during the early part of development resulted in embryos experiencing generally lower temperatures (as Lake Huron 2011-2012 "ambient" temperature was lower). In the later parts of development, temperatures were generally greater than would have been observed in the in-field location.

A model was developed for prediction of survival of Lake Whitefish. However, the weak correlation between the model and mean temperature suggests that the model may not be appropriate. On the other hand, survival in all groups was quite high, suggesting that temperature along may not have a strong impact on Lake Whitefish survival.

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A model was developed for prediction of days to median hatch (based on degree-days):

 $\gamma = 1129 / (T + 7.5)$

with T as mean temperature in °C. Two years of experimental data was included to fit the model, with the exception of the plume simulations. The model provided reasonable correlation between observed and calculated days to median hatch, with no more than 10 days difference between observed and predicted values. A comparison of days to median hatch and average temperature suggests that the model may over-predict near 2° and 6°C.

Unfortunately, as the model does not apply to the plume scenarios, this model may not be applicable to actual in-field data.

Patrick, et al., did not propose a quantitative benchmark for Lake Whitefish. However, for Round Whitefish, a benchmark of $\Delta T = 3.7^{\circ}C$ was proposed, which was equivalent to 90% survival as determined by a fit of survival to average temperature greater than ambient. For Lake Whitefish, all experimental groups showed survival at or greater than 94%, including the groups experiencing variable temperature increases of up to 5°C greater than ambient. A similar curve fit would suggest a potential benchmark of 5.1°C, but this is an extrapolation well beyond the data available to Patrick, et al [R-92].

10.5 WINGS

Table 34Reports produced as a result of the WINGS study

[R-64]	Holmes, JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Lake Whitefish and Round Whitefish biology: a review of ecological factors affecting growth, survival, and reproduction. July 15, 2002.	Technical report
[R-63]	Holmes, JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Aquatic effects of nuclear generating stations on fishes. July 15, 2002.	Technical report
[R-60]	Holmes JA, 2002. WINGS field program, May 2000 – Nov 2001: objectives, methods, results and interpretation. August 31, 2002.	Technical report
[R-73]	MacPherson A., Jolmes JA, Muir AM, Noakes DLG, 2010. Assessing feeding competition between Lake Whitefish (<i>coregonus clupeaformis</i>) and Round Whitefish (<i>prosopium cylindraceum</i>). <i>Current Zoology</i> 56, 109-117.	Publication

To the best of our knowledge, the only peer-reviewed scientific publication resulting from the WINGS study is the following:

MacPherson A., Jolmes JA, Muir AM, Noakes DLG, 2010. Assessing feeding competition between Lake Whitefish (*coregonus clupeaformis*) and Round Whitefish (*prosopium cylindraceum*). *Current Zoology* 56, 109-117.

Holmes, JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Lake Whitefish and Round Whitefish biology: a review of ecological factors affecting growth, survival, and reproduction. July 15, 2002.

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The objective of this report was to compile a literature review of the biology of Lake Whitefish and Round Whitefish in North America and to identify ecological factors important to growth, survival, and reproduction of both species.

Holmes et al. emphasize that "considerable attention" has been given to the incubation period (fertilization to hatching). However, hatching is not an "ecologically significant event" in that habitat and mode of feeding are unchanged before and after hatch.

Briefly (note that review encompasses a range of lakes, not just Lake Huron), Lake Whitefish spawning occurs with water temperatures ranging from 4-12°C (after fall turnover). Round Whitefish spawn later, with cooler waters. The timing and duration of spawning is inferred indirectly: from observations of spawning aggregations, from collection of ripe and spent adults, and from collection of fertilized and unfertilized eggs.

Lake Whitefish development decreases exponentially with increasing (constant) temperature. Mortality and abnormalities increase greatly at temperatures greater than 8°C. However, the review noted that reports differ with respect to hatching time, survival, and abnormalities at constant temperature, with those differences potentially due to experimental differences or genetic differences. The review noted that potential hatch triggers have not been identified.

Survival of Lake Whitefish embryos is negatively correlated with sedimentation and positively correlated with the formation of ice (winter severity).

Lake Whitefish larvae reside in nursery areas for 1-2 months, before dispersing into deepwater water. The literature is not consistent on the type of habitat (gentle slopes and reed beds versus steep rocky habitat). It may be that Lake Whitefish larvae prefer shallow, warm, protected waters with only secondary consideration given to substrate type. Additionally, the tendency of Lake Whitefish larvae to aggregate is also unknown. The potential impact of prey availability, predation, and feeding competition is unclear for Lake Whitefish in the Great Lakes [R-64].

Holmes, JA, Noakes DLG, Crawford SC, Wismer DA, 2002. Aquatic effects of nuclear generating stations on fishes. July 15, 2002.

The intent of this review was "to identify important mechanisms of impact and to identify life history periods in which the vulnerability of fishes is sufficiently high that population level responses (growth, survival, reproductive) [were] detectable".

The following types of impacts were reviewed:

- Physical (such as offshore displacement)
- Impingement and entrainment
- Thermal
- Toxic chemical exposure
- Radionuclide exposure

Additionally, the review also considered the potential existence of compensatory mechanisms in fish populations.

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The review covers published scientific literature and unpublished technical reports (gray literature), including EPRI reports. Impingement and entrainment estimates at Bruce B are reviewed, including an estimate of seasonal entrainment patterns at Bruce B in 1988.

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The contaminants of concern that were reviewed included: ammonia, chlorine, copper, dioxins/furans, hydrazine, iron, morpholine, nitrogen oxides, oil/grease, sulphur dioxide, total suspended solids, and zinc.

The review of radionuclides was somewhat more limited.

Review of compensation mechanisms was even more limited, but noted that compensation mechanisms "have been well documented in laboratory and field studies of fish populations" [R-63].

Recommendations from this report are listed in Section 1.4 of this report.

10.6 FISHES

Table 35Reports produced as a result of the FISHES study

[R-105]	Thorburn, M., 2002. Radionuclide sampling of Lake Whitefish and lake trout from Chippewas of Nawash Fishing Grounds (south-west of Georgian Bay and Fishing Islands, Lake Huron) and the vicinity of the Bruce Nuclear Power Development Site (Lake Huron).	Technical report
[R-106]	Thorburn, M., & Berti, P., 2002. The Chippewas of Nawash: Is the community exposed to radioactive chemicals and other contaminants from eating locally harvested fish? A community guide to understanding the results of the Nawash FISHES study. October 2002.	Summary report
[R-13]	Bruce Power, 2002. Measurement of radionuclides in fish and lake water for Chippewas of Nawash Dietary Studies. B-REP-03443.6-00001.	Technical report

Thorburn, M., 2002. Radionuclide sampling of Lake Whitefish and lake trout from Chippewas of Nawash Fishing Grounds (south-west of Georgian Bay and Fishing Islands, Lake Huron) and the vicinity of the Bruce Nuclear Power Development Site (Lake Huron).

Lake Whitefish and Lake Trout were collected from 2000-2002 at traditional fishing grounds (Fishing Islands; Georgian Bay), near Douglas Point, and at reference sites (De Tour, MI; Whitefish Bay, Lake Superior). Samples were analyzed for concentrations of gamma emitters, tritium (TFWT and OBT), ¹⁴C, ³²P, ⁹⁰Sr, ¹³⁷Cs, and gross α .

Gillnets were set near Douglas Point on November 9, 2000 and June 20, 2001. CPUE was not reported but may be derived from the Appendices (if data is complete). Additional data may be available through the Chippewas of Nawash Fisheries Assessment program.

A detailed analysis of ingested dose was not performed. However, radionuclide levels were shown to be generally very low (comparable to background).

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To our knowledge, a detailed comparison of dose to members of the Chippewas of Nawash community with respect to critical groups identified through the site-specific survey (<10 km from Bruce Power) has not been performed [R-105].

Thorburn, M., & Berti, P., 2002. The Chippewas of Nawash: Is the community exposed to radioactive chemicals and other contaminants from eating locally harvested fish? A community guide to understanding the results of the Nawash FISHES study. October 2002.

This report is a plain-language summary of the technical report, which concluded "it is very safe to eat fish from traditional waters." [R-106]

Bruce Power, 2002. Measurement of radionuclides in fish and lake water for Chippewas of Nawash Dietary Studies. B-REP-03443.6-00001.

Supplementary technical information and detailed results are provided in this internal report [R-13].

Enclosure 2

University Research Summary June 2017

(Enclosure 1 of Letter, F. Saunders to M. Leblanc, "Supplement to the Application for the Renewal of the Power Reactor Operating Licence: University Research Summary", June 30, 2017, NK21-CORR-00531-13587 / NK29-CORR-00531-00531-14219)



UNIVERSITY RESEARCH SUMMARY

June 2017

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UNIVERSITY RESEARCH SUMMARY

Highlights

The Integration Department at Bruce Power provides oversight for the coordination and development of multiple relevant academic research programs. From 2013 to 2017, we facilitated the funding and progress of independent academic research in several areas.

Bruce Power supports academic research with over \$1 million annually in direct research funding. Current research programs explore the importance to Bruce Power of effects of low-dose radiation and interactions with the environment. We also support programs exploring Aboriginal-specific health issues.

Bruce Power support allows researchers to succeed in applying for competitive, peerreviewed funding from federal and provincial agencies. The receipt of these matching funding grants demonstrates the scientific rigor of the academic research that Bruce Power is supporting. Research findings continue to be published in peer reviewed academic journals and are available to all interested parties.

This report presents research progress for our diverse programs from 2013 to April 2017.

BP-CHER

Established in late 2016, the BP-CHER (Bruce Power Research Centre for Health, Environment and Radiation) at the Northern Ontario School of Medicine in Sudbury, Ontario will support academic research collaborations.

The goals of the Centre are: 1) to sponsor and promote research on the effects of radiation at low doses and dose rate 2) to support education and understanding of the effects of radiation at low doses and dose rates and 3) to contribute to guideline development for the use of radiation in industry, science and regulation.

Senior scientists have begun BP-CHER sponsored work reviewing risk estimation and epidemiology related to the Linear No Threshold model of biological response to low-dose radiation.

In 2017-18, the Integration Department expects to work toward the 5-year goals of BP-CHER by developing a national presence, a sustainable network of researchers and a credible public outreach program.

The biological microbeam program will explore the use of a biological microbeam to examine radiobiological effects on cells. Identification of markers of DNA damage and the development of the microbeam are underway. Starting in the fall of 2016, the program is funding a research management position to coordinate and manage external university-based scientific research programs in low-dose radiation, aquatic studies and biological microbeam projects.

Environment

EA FUP

The 2015 Environmental Assessment Follow-Up Program (EA FUP) included an extensive analysis of the effects of Impingement and Entrainment (I & E) on Lake Whitefish. In addition to a detailed I & E report, a sensitivity analysis of the population levels effects models and a

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plain language summary of the results were obtained by the Integration department. The final 2015 EA FUP was submitted to the CNSC in November 2016 by the Integration Department.[R-2]

Whitefish

Bruce Power has been working with independent university researchers from 2011 to the present to understand more about Lake and Round Whitefish in Lake Huron. The research focused on how site operations might affect Lake and Round Whitefish. Bruce Power worked with the Saugeen Ojibway Nation to develop key research questions about Lake and Round Whitefish.[R-4][R-13]

The researchers collected over 2,000 adult Lake and Round Whitefish in the last 5 seasons. They have raised over 1.3 million Lake Whitefish and more than 60,000 Round Whitefish embryos in the laboratory.

The University of Guelph program generated two theses and one peer-reviewed publication. The joint program between McMaster and Regina Universities generated 7 theses and 13 peer-reviewed publications to date.

The Whitefish research programs supported the training of four post-doctoral fellows, four PhDs and nine MSc students.

Population Discrimination

Researchers at the University of Regina wanted to understand the populations of Lake and Round Whitefish in Lake Huron. They looked at genetic differences between fish. They also looked at differences in stable isotopes of carbon and nitrogen. Stable isotopes are carbon and nitrogen atoms that are slightly different from normal carbon and nitrogen. These isotopes occur in different quantities depending on the location in Lake Huron. Researchers can measure isotopes in fish tissue to learn about where the fish lives and what it eats. For this project, researchers looked at the local area close to Bruce Power and the entire lake.

In the local area close to Bruce Power, researchers compared the Lake and Round Whitefish that live in an area near Bruce Power to those living just outside that area. This was to determine if there existed a unique population of Lake or Round Whitefish near Bruce Power that could be impacted by site operations. The researchers found that there were no distinct genetic groups of Lake or Round Whitefish in the local area close to Bruce Power. The isotope study also did not show any significant isotope differences in the Lake or Round Whitefish in the local area close to Bruce Power.

Isotope studies also examine ecological niches. Ecological niches are a combination of the location of the fish and the food they consume. The Lake and Round Whitefish in the study lived and ate in mostly separate ecological niches. Lake and Round Whitefish shared more of their niches in the spawning period than in any other life period.[R-5][R-7]

For the entire lake, the isotope study found six ecological groups of Lake Whitefish in Lake Huron and Georgian Bay. Near Bruce Power, the main basin divided into Southwestern and Northeastern ecological groups. The Northeastern ecological group included Bruce Power. There was a heavy mixing of populations in each ecological group. There were no small,

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distinct ecological groups identified near Bruce Power (i.e. Scougall Bank and McRae Point).[R-6]

Researchers found that Lake Huron is the genetic source population for Round Whitefish for Lakes Michigan, Superior and northern Georgian Bay as well as for Lake Nipigon through Lake Superior. There were not enough samples within Lake Huron to determine the Round Whitefish population structure in Lake Huron.[R-18]

Population Modeling

The University of Guelph program developed genetic techniques to identify Lake Whitefish. They were not able to determine the population structure using these genetic techniques.[R-20]

Effects of Impingement and Entrainment

Bruce Power has a very small impact on Lake Whitefish populations through impingement and entrainment. Impingement and entrainment are where lake water enters the cooling water system and brings in fish and other aquatic life. Lake Whitefish losses to impingement and entrainment for Bruce Power operations in 2013 and 2014 were less than 0.5% of the annual commercial fishing quota each year. The commercial fishing quota for Zone 1 was 292,287kg in 2013 and 2014. In 2013, these losses represented 0.33% of quota. In 2014, these losses represented 0.15% of quota. The quota maintains a sustainable fishery in Lake Huron and is protective of the long-term health of the population.[R-2]

Thermal Effects

Before the research program, we did not fully understand how warmer temperature affected incubating Lake and Round Whitefish embryos. Researchers exposed Lake Whitefish to periodic increases in water temperature, known as heat shocks, and compared them to Lake Whitefish not exposed to these increases in water temperature. To do these experiments, the researchers had to develop new ways to incubate fish embryos.[R-16] They also had to identify new markers of cell stress, called heat shock proteins, for Lake and Round Whitefish.

Researchers looked at the growth of Lake Whitefish embryos exposed to warm water. The first set of experiments looked at Lake Whitefish embryos raised in water that was constantly warmer than normal. The second set of experiments looked at Lake Whitefish embryos exposed to periodic increases in water temperature as they developed.

Lake Whitefish embryos that developed in water that was constantly warmer than normal hatch earlier and have reduced survival.[R-19]

Lake Whitefish embryos exposed to periodic increases in water temperature as they develop did not show any changes in development or survival. There were some small changes in size and metabolism in these embryos.[R-14][R-17]

Researchers incubated Lake Whitefish embryos in chambers on top of the lake bottom in the Bruce Power discharge areas. These embryos had an average 10% advance in development compared to embryos placed away from Bruce Power outside of the discharge area. They also had small size differences compared to embryos incubated far outside the discharge area.[R-22]

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The research shows that Lake Whitefish embryos and young of the year juveniles can survive a 4-hour, 9°C heat shock.[R-21] This temperature change is larger than the temperature changes measured in incubation chambers for Lake Whitefish placed near the discharge channel.[R-22] The research program results support the limited impact of the thermal discharge on Lake Whitefish survival.

Researchers studied the effect of warmer temperatures and periodic increases in water temperature on Round Whitefish. They found that Round Whitefish had lower survival when incubated at constantly warmer temperatures (8°C). They also had lower survival when the temperature increased quickly at the end of their development.

Round Whitefish embryos exposed to 1-hour 3°C increases in water temperature throughout incubation had no changes in survival or development.[R-15]

Combined Stressors

Before the research program, we did not understand how stress from a combination of radiation, temperature increases and chemicals (i.e. morpholine) would affect Lake Whitefish. The research looks at how stress from these three sources would affect Lake Whitefish.

Low doses of chronic radiation (0.06-4.4 mGy/day) made the embryos hatch earlier. High doses of acute radiation decreased embryo survival.[R-23]

Low doses of morpholine (1 or 10 mg/L) made the larvae larger when they hatched. There were no effects at a moderate dose (100mg/L). Higher doses (500-1000mg/L) cause smaller size of the larvae at hatch, an earlier hatch and decreased survival.[R-23]

Researchers exposed Lake Whitefish embryos to increases in water temperature 2 hours before exposure to radiation or morpholine. The increase in water temperature protected them from some of the effects of the radiation exposure but not from the effects of the morpholine.[R-23]

In similar experiments, Round Whitefish were more sensitive to morpholine but less sensitive to radiation than Lake Whitefish.[R-15]

The Bruce Power ECA limit for morpholine is 2.5 mg/L measured at the cooling condenser water duct. Any morpholine released from the cooling condenser duct is rapidly diluted in the discharge channel.

Morpholine monitoring at Bruce Power has a Minimum Detectable Level (MDL) of 0.02 mg/L. Monthly monitoring from January 2011 to November 2016 ranged from the MDL to 1 mg/L, with 90% of monitoring results less than the MDL.

Mortality effects on embryos in this study occurred at morpholine doses at least 200 times greater than the ECA limit at Bruce Power. No measurable effects on Lake or Round Whitefish embryos occurred at doses up to 40 times greater than the ECA limit at Bruce Power.

The International Commission on Radiological Protection lists natural background levels of radiation exposure for fish up to 0.01 mGy/day. Dose rates of 0.1 to 1 mGy/day are considered low dose rates for fish.[R-12] Fish are more resistant to radiation exposure than

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humans and dose recommendations for aquatic organisms have been set at 3.5 Gy/yr, compared to the public dose limit of 0.001 Sv/yr (equivalent to 0.001 Gy/yr for x- and gamma rays).[R-8][R-9]

The combined stressor research results demonstrate that effects from morpholine and gamma radiation occurred only at levels substantially higher than at the Bruce Power site.

Summary

Bruce Power has been working with independent university researchers from 2011 to the present to understand more about Lake and Round Whitefish in Lake Huron. The key research results are summarized in Table 1 for each of the identified research questions. The Whitefish Research Review [R-3] summarizes the extensive research efforts in this area over the last five years.

Table 1 Key Whitefish Research Results, 2013-2017

Population discrimination		
Population modeling	Genetic techniques developed for Lake Whitefish identification.	
Entrainment effects	The impact of Bruce site operations impingement and entrainment was less than 0.5% of the Zone 1 Lake Whitefish fishing quota.	
Thermal effects	Intermittent temperature increases throughout Lake or Round Whitefish development have no impact on embryo mortality. Lake and Round Whitefish embryos exposed to regular heat shocks throughout development do experience a small advance in hatch and some changes in size and metabolism. Lake Whitefish embryos are able to mount a response to intermittent heat shocks that ensure survival of a 4-hour 9°C heat shock. This intermittent heat shock exceeds the temperature changes recorded by the temperature loggers from <i>in situ</i> incubation chambers near the discharge channels. Temperature changes of the magnitude seen in the vicinity of the thermal discharge have been shown to have no significant effect on mortality or stress response of Lake Whitefish embryos.	
Combined stressors	The relationship between radiation and morpholine exposure and Lake and Round Whitefish embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and different for Lake and Round Whitefish. Lake Whitefish are more sensitive to radiation exposure whereas Round Whitefish appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for Lake Whitefish	

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Whitefish DNA Sequencing

This research program supports genetic "barcode" identification of larval fish and eggs collected during entrainment monitoring. The project aimed to compare the effectiveness of genetic and visual identification of fish larvae.

Larval fishes and eggs were collected from Bruce Power in 2013 and 2014. An independent contractor visually identified the larval fish. The larval fish and eggs were sent to Laurentian University for genetic identification. There was a high failure rate for genetic egg identification.

Techniques were developed to rapidly identify 4 species of interest: Lake Whitefish (*Coregonus clupeaformis*), Round Whitefish (*Prospium cylindraceum*), Deepwater Sculpin (*Myoxocephalus thompsonii*) and Spottail Shiner (*Notropis hudsonius*).

This project examined 657 both visually and genetically identified larval fish. Visual and genetic identifications did not match in 27 cases. There were 37 fish that could not be visually identified. There were 26 fish that could not be genetically identified. There was no pattern as to which species of fish had identifications that did not match or that were identified by only one method.

The genetic "barcode" techniques designed for Lake Whitefish did not differentiate Lake Whitefish from other species in the Clupeaformis genus (e.g. Lake Herring). There are not enough differences in the Lake Whitefish "barcode" to use that genetic technique.

A genetic "barcode" set was developed for Round Whitefish that is able to differentiate Round Whitefish from other closely related species of fish. More testing is needed before it can be used. Genetic "barcodes" were also designed for both Deepwater Sculpin and Spottail Shiner.

In 2017, genetic "barcode" sets were designed for the four new target species (Smallmouth Bass (*Micropterus dolomieu*), Brook Trout (*Salvelinus fontinalis*), Yellow Perch (*Perca flavescens*) and Rainbow Smelt (*Osmerus mordax*)).

All eight genetic "barcode" sets developed to date are currently being validated. This will support the development of an expanded genetic library of Lake Huron species.

Thermal Study

The Thermal Study research program will improve understanding and technology for substrate temperature monitoring.

During normal development, the small fertilized Lake Whitefish embryos will sink down into the cobble substrate. The cobble may act as insulation that would reduce the impacts observed in the above substrate incubation chambers. Researchers examined the insulating effect of the cobble using a simulated thermal discharge, or thermal flume.

During the winter of 2015 - 2016, embryos were raised in incubations chambers at four sites in the simulated thermal flume. A data logger was placed next to each chamber.

The cobble substrate reduced the temperature fluctuations following an acute heat shock. The two locations within the cobble had a lower peak temperature and the temperature increased

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at a slower rate. The "deep cobble" location was the most stable, and only increased by 0.2° C after a 12° C heat shock.

During embryo development, the average temperatures within the cobble and in the open flowing water were the same. However, the temperature changes were smaller within the cobble. A fungal buildup on the "flume front" chamber resulted in almost total embryo mortality, so no size or weight data were collected from that chamber.

The embryos incubating within the cobble, compared to the "flume end", were of normal length and weight, suggesting that they were developing normally. The "flume end" embryos were longer and larger, suggesting they were developing faster than normal. These differences in development were expected based on the smaller temperature fluctuations seen in the cobble.

During the winter of 2016 - 17, the thermal flume experiment was repeated. Chambers were loaded with more embryos and an additional chamber was added to the fume to achieve larger sample sizes. Temperature data was recorded during development and embryos will be analyzed for survival, development rate and size.

Effects of Radiation

Ultra-low dose

The ultra-low dose research program will explore the effect of ultra-low levels of background radiation on Lake Whitefish embryo survival and development. Single-cell models will also be grown in typical ambient radiation and under ultra-low dose Sudbury Neutrino Observatory Laboratory (SNOLab) conditions and evaluated for mutation frequency, chromosomal aberrations and differentiation. The effect of irradiation on single cell models and Lake Whitefish embryos under ambient and ultra-low-dose conditions will be determined.

Phase 1 of this project, which was completed in 2015 - 2016, involved incubation of Lake Whitefish embryos in SNOLab. The main goal of this year was to determine if embryos could be successfully incubated underground from fertilization until hatch. In 2016-17, Lake Whitefish embryos were incubated in refrigerated incubators underground in SNOLab and in the control laboratory at the Living with Lakes Centre.

Embryos raised underground were longer and weighed more on average than the surface embryos. The yolks of the underground embryos were smaller compared to the surface embryos.

In 2017, 3,200 embryos were once again reared in refrigerated incubators underground in SNOLAB and in the control laboratory at the Living with Lakes Centre. A subset of embryos was preserved at 40% development (December 30) for morphology. A refrigeration unit compressor failure led to all remaining embryos being preserved for analysis at 60% development. There were no significant differences in survival between the two environments at 60% development. Morphology of the preserved embryos is underway.

In 2017, researchers are designing a low-radon whitefish incubation chamber that will reduce radon levels below natural background and achieve much lower radiation levels underground compared to surface. Once the low-radon chamber is designed, the lake whitefish experiment will be repeated.

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Work has also been progressing in the NOSM laboratory with the cell culture systems and genetic techniques to be used underground. Researchers are working with a design team at SNOLAB to build a low-radon glovebox for culturing cells. Similar to with Lake Whitefish, the low-radon glovebox will enable researchers to culture cells in an ultra-low background radiation environment. Glove-box design plans are completed and construction is under way. It is expected that the glove-box will be ready for underground SNOLab cell cultures in 2017.

Radiation Hormesis

The radiation hormesis research program aimed to measure the effect of ionizing radiation on behavioral, physiological and molecular biomarkers in a cricket model for radiation research. In addition, the protective effects of antioxidants were explored. Funding for this research is being matched by a Mitacs grant.

Four planned rounds of cricket irradiations are complete and measurements of growth, maturation, longevity, fecundity, motor and cognitive status and cellular function is in progress. Initial results show changes to female fertility, immune function and gut microbiome with a single irradiation in early life. In 2017, the final round of irradiation and measurements will be completed, along with data analysis and publication of results.

Fetal Programming

The fetal programming research explores the effects of radiation exposure to a developing fetus (fetal programming) in mice on cardiovascular and metabolic disease outcomes. The research will also examine the effect of low-dose radiation in reducing the effect of induced fetal programming. Bruce Power funding for this research is matched by a competitive, peer-reviewed NSERC grant.

This research is important for the medical community to help characterize risks associated with radiation exposure during pregnancy. Sources of radiation exposure include both diagnostic imaging exposure and occupational exposure. The research is relevant to Bruce Power in adding to the knowledge base used to set dose limits and determine regulations surrounding occupational radiation exposure in pregnancy.

Pregnant mice were exposed to 0 to 1000 mGy of ¹³⁷Cs gamma radiation and their offspring were monitored for growth, metabolic and cardiovascular outcomes. Preliminary results show female mice irradiated at higher doses (100-1000 mGy) during gestation had lower body weights, heavier livers, higher blood pressure and changes in glucose metabolism. Male mice irradiated at lower doses (5-50 mGy) during gestation had higher body weights. Mice irradiated at low doses (5 mGy) during gestation are showing signs of improved glucose metabolism while mice irradiated at a high dose (1000 mGy) are showing some preliminary signs of worsened glucose metabolism.

Analysis of tissues and lifespan outcomes from Phase I mice will continue in 2017. New experiments in 2017 will continue to examine the effects of fetal exposure to ionizing radiation in the last trimester on adult cardiovascular outcomes.

Genetic Models of Risk

The genetic models of risk research program aims to characterize the adverse biological effects of low-dose radon exposures in cellular and animal models. In addition, animal

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experiments are underway to examine connections between low dose radiation exposure and both Acute Lung Injury (ALI) and immune modulation.

Since Oct 2015, the ALI model has been developed to establish baseline radiation responses in healthy Sprague-Dawley rats. In these experiments, rats are irradiated with 0, 20 or 200 mGy of X-rays. The effects of these low, clinically relevant doses are evaluated 30 min, 4h, and 24h later to examine the acute stages of the immunological response in the lungs and the spleen, an important immune organ. Changes within this time frame can be associated with long term consequences of exposure to ionizing radiation, such as fibrosis of lung tissue.

In 2016, researchers completed these single-dose healthy animal experiments, then extended into multi-dose response experiments, in which rats were irradiated daily to mimic multiple exposures, such as the daily chest X-rays required for many intensive care patients. Researchers will then translate these baseline findings to models of ALI, including endotoxins and mechanical ventilation induced lung injury. These experiments will determine whether low doses of X-rays change the immune response, reducing inflammation and damage caused during ALI, while still mounting an effective response.

In 2017, this research program will continue to examine clinically and environmentally relevant doses of x-ray and inhaled radon radiation on pulmonary immune response. The effect of radiation doses during acute disease will be a focus in 2017. Parallel experimental programs will be established at Flinders University and at NOSM laboratories. These programs will examine radon and x-ray effects on cell lines at doses ranging from absolute zero (SNOLab) to those mimicking diagnostic, environmental and occupational exposures.

Radiation Tools

The radiation tools research programs aims to develop new radiation detectors to measure the dose and impact of realistic low-dose radiation exposure. Funding is being provided jointly by the Candu Owners Group and Bruce Power as part of a larger NSERC CRD project. The Bruce Power funding is being used for proton microbeam detector development.

In 2015/2016, a new proton microbeam detector was successfully designed. Proton microbeam test measurements began in 2016 and continue into 2017.

Naturally Occurring Radioactive Material

The Naturally Occurring Radioactive Materials (NORMs) project examined radiation decay in a watershed contaminated by uranium tailings.

Lens of the Eye

The lens of the eye program aims to investigate the development of cataracts following exposure to ionizing radiation. A review of current evidence for the proposed new 20mSv per year dose limit to the lens of the eye has been accepted for publication. A research proposal is under development with three streams of research. Stream 1 will be an epidemiological study of nuclear energy workers in Ontario. Stream 2 will be an epidemiological study of medical workers and patients exposed to diagnostic imaging. Stream 3 will be an animal study examining chronic radiation exposure in a cataract prone mouse model.

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Stream 2 is being initiated, with preliminary data access efforts in progress and research ethics board approval pending. Part 1 of the analysis will examine the association between patient exposure to multiple head CT scans and subsequent cataract formation.

Environment, Radiation & Health

Environment, Radiation & Health Part 1 – Environmental Impacts

Part 1 of the Environment, Radiation and Health program is supporting the salaries of students and researchers working on projects described elsewhere in this report. Scientific progress and outcomes and future plans for each of these projects are described in the relevant sections. Bruce Power funding for this research support is being matched by a Mitacs grant.

Environment, Radiation & Health Part 2 – Effects of Radiation

Part 2 of the Environment, Radiation and Health program is exploring the effects of adaptive immunity in prostate cancer patients who have received radiation therapy. This work is awaiting medical ethics board approval. Researchers have started to develop blood sample assays while awaiting ethics approval.

Part 2 also includes a review of the Linear No Threshold (LNT) radiation science model using a literature review and a re-analysis of existing data. A systematic literature review of 198 studies published in the last 5 years on low dose radiation exposure in mammals is in progress. Information has been extracted for each study on the specific radiation exposure, the model and the key findings. The studies have been organized by endpoint and tissue type in 16 categories. The preliminary findings suggest that there needs to be a "paradigm shift" in the understanding of the biological response to low dose radiation and that LNT is not an accurate model for low dose exposures. The LNT review is also using existing data on inhaled beta-gamma emitting radionuclides to complete three studies on dose-rate exposure. Bruce Power funding for this research is being matched by a Mitacs grant. Both LNT review projects will continue in 2017.

<u>Health</u>

Aboriginal health

The goal of this project was to examine immunity to *Haemophilus influenza type a* (HiA) and *Streptococcus pneumoniae* among Saugeen First Nation members. Bruce Power funding for this research is being matched by a Mitacs grant.

Blood sample collection and antibody analysis is complete. The next milestone will be preparation of a research manuscript for submission in 2017. The manuscript will be shared with participating communities.

Conclusion

Since 2013, the Integration Department has facilitated the funding and progress of independent academic research into interactions between Bruce Power and the environment. Supported research is examining the measurement and effects of low-dose radiation on

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human and animal models. We also support programs exploring Aboriginal-specific health issues.

Many Bruce Power-funded independent research programs at external universities have also been supported by peer-reviewed funding agencies such as Mitacs and NSERC. The support of these external funding agencies speaks to the credibility and strength of the research programs.

We expect that researchers will continue to make progress in 2017 and Bruce Power looks forward to seeing the results of their research. Research results will be incorporated into relevant regulatory and business initiatives.

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1.0 INTRODUCTION

The Integration Department at Bruce Power provides oversight for the coordination and development of relevant academic research programs. The Integration Department is led by Dr. Doug Boreham, the Bruce Power Chair in Radiation and Health at the Northern Ontario School of Medicine and Department Manager of the Integration Department.

Bruce Power supports academic research with over \$1 million annually in direct research funding. Current research programs explore the importance to Bruce Power of effects of low-dose radiation and interactions with the environment. We also support programs exploring Aboriginal-specific health issues.

Bruce Power support allows researchers to succeed in applying for competitive, peerreviewed funding from federal and provincial agencies. The receipt of these matching funding grants demonstrates the scientific rigor of the academic research that Bruce Power is supporting. Research findings continue to be published in peer reviewed academic journals and are available to all interested parties.

This report presents the research progress for our diverse programs and provides an update on research plans for 2017. Figure 1 presents the research program structure for 2017.

We expect that researchers will continue to make excellent progress in 2017 and Bruce Power looks forward to seeing the results of their research. Research results will be incorporated into relevant regulatory and business initiatives.

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Integration Research Programs

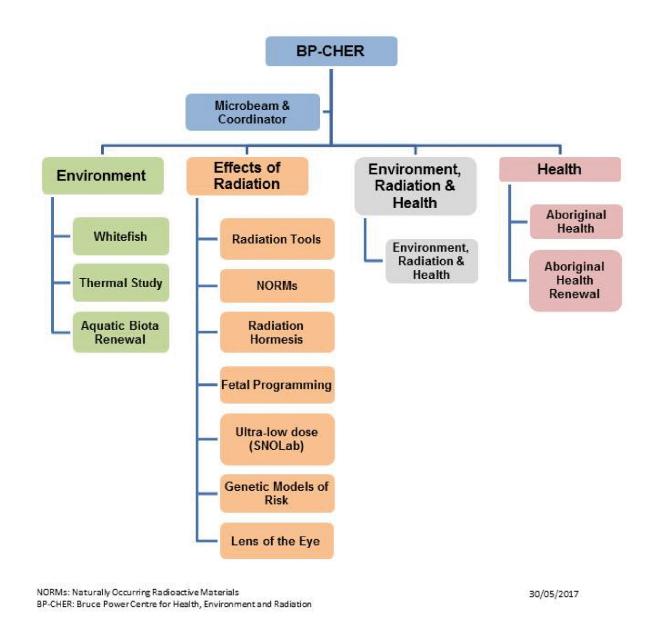


Figure 1 Integration Research Program Organization, 2017

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2.0 BP-CHER

2.1 BP-CHER (2016-2020)

Based at the Northern Ontario School of Medicine in Sudbury, Ontario, BP-CHER (Bruce Power Research Centre for Health, Environment and Radiation) was established in late 2016.

The goals of the Centre are:

- 1. To sponsor and promote research on the effects of radiation at low doses and dose rates
- 2. To support education and understanding of the effects of radiation at low doses and dose rates
- 3. To contribute to guideline development for the use of radiation in industry and science

2.1.1 2016 Research Activities and Results

In November 2016, funding was announced for the Northern Ontario School of Medicine/Bruce Power Research Centre for Health, Environment and Radiation (BP-CHER). Funding consisted of a \$5 million investment over the next five years.

The \$1 million in annual funding for five years will be used to continue research that has taken place during the first four years of the existence of the Bruce Power Chair in Radiation and Health, specifically:

- The impact of low-dose radiation on health.
- The environmental impacts of radiation and how they impact health.
- The effects of radiation and diagnostic imaging on fetal programming.
- The effect of radiation on specific species of fish.
- The impact of radiation on Indigenous communities.

The establishment of BP-CHER will allow the Bruce Power Integration Department to consolidate and better coordinate research collaborations.

2.1.2 2017 Research Activities

One senior research scientist (Dr. Anthony G. Brooks), along with two Bruce Power funded Post-Doctoral Fellows, are reviewing risk estimation and the epidemiology of the Linear No Threshold (LNT) concept for biological responses to low dose radiation exposure. These contributions will be included in a special toxicology journal issue dedicated to the subject matter.

Progress towards the purchase of a Precision irradiator for laboratory experiments at NOSM has continued. Three quotes for irradiators were obtained and a model selected for

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procurement. Engineering assessment of the installation location is complete and floor reenforcement plans are in progress.

In order to fulfill commitments made during the 2016 funding announcement, electrical engineering consultations have started for the installation of an electric charging station at NOSM and quotes are being solicited for the purchase of an electric car for research use.

2.1.3 2017 Research Plan

The Integration department expects to work towards the 5-year goals of BP-CHER, including:

- Developing a sustainable network of researchers: BP-CHER will use partner funding to direct research to priority areas, and will leverage partner funding to the maximum extent possible.
- Developing a national presence: BP-CHER will support the development of sustainable policies for the radioprotection community, including industry, medical professionals, regulators, and radioprotection professionals.
- Developing credible public outreach programs: BP-CHER members will lead outreach programs targeted at educating the future public and regulatory community.

2.2 Biological microbeam (2015-2019)

The biological microbeam program is intended to explore the use of a biological microbeam to examine radiobiological effects on cells. In addition, the program funds a research management position to coordinate and manage external university-based scientific research programs in low-dose radiation, aquatic studies and biological microbeam projects.

2.2.1 2016 Research Activities and Results

The microbeam laboratory has been assessed with respect to available and needed equipment and required supplies. The McMaster Biosafety inspector has audited the lab space and provided recommendations to bring the lab up to current university codes. The majority of the recommendations have been implemented; the remainder are close to completion. Experimental protocols are under development that will use *in vitro* cell cultures to optimize the proton irradiations, assess the accuracy of the computer targeting system and establish dosimetry for the microbeam. Once targeting and dosimetry are completed, biomarkers of DNA damage, cell death and cellular transformation frequency will be used to assess the biological effects of proton irradiation. Parallel studies will be performed with gamma radiation from McMaster's 137-Cs source to use as a comparator to the proton irradiations.

The laboratory has developed biomarkers of DNA damage and repair. They implemented high-throughput gene expression methodology using real time qPCR technology to rapidly detect gene expression changes for large sample sets. This technique employed stringent data quality parameters coupled with advanced statistical models to identify gene targets with significant differences among data sets. They have successfully performed over 15,000 qPCR reactions and analyzed over 50 genes in multiple tissues. Results from these analyses have allowed them to identify key molecular genes involved in radiation-induced damage in multiple tissues including brain, kidney and adrenal tissues, and immune system function.

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The laboratory is currently in a position to profile gene expression changes for any sample set with any gene of interest. They have gained experience in identifying key genes through literature databases, followed by gene primer design, primer validation, and tissue extraction methods. The laboratory hopes to build on this knowledge base to identify novel molecular and cellular pathways involved in radiation-induced tissue damage.

The research manager at NOSM was on-boarded in the fall of 2016.

2.2.2 2017 Research Plan

Further work on microbeam development at McMaster in 2017 is reported under the Radiation Tools program (Section 4.5). Progress on biomarkers of DNA damage and repair in 2017 is reported under the Ultra-Low Dose program (Section 4.1). The research manager at NOSM will continue to provide coordination and management of BP-CHER related activities in 2017.

3.0 ENVIRONMENT

3.1 EA FUP (2015-2016)

The 2015 EA FUP included an extensive analysis of the effects of entrainment and impingement on Lake Whitefish.[R-2] An independent contractor conducted entrainment monitoring for two years at the Bruce A intake, supplemented with larval tows in the vicinity of the intake, and performed quality assurance checks on the routine Bruce Power impingement monitoring program.

Two years of data was analyzed to estimate the cumulative impact of impingement and entrainment of Deepwater Sculpin, Spottail Shiner and Lake Whitefish at Bruce Power relative to the commercial fishing quota and estimated species biomass. A detailed I & E report, including a sensitivity analysis of the population levels effects models and a plain language summary of the results, was obtained by the Integration Department.

The final 2015 EA FUP was submitted to the CNSC in November 2016 by the Integration department.

3.1.1 2017 Research Plan

In 2017, the Integration Department will continue to retain the services of the independent contractor. This will support response to regulator reviews and the presentation of I & E information to stakeholders and Aboriginal groups as needed.

3.2 Whitefish (2013-2017)

The 5-year Whitefish research program is in progress. The goals of the program are to examine the effect of thermal and combined thermal, chemical and radiological stress on Lake and Round Whitefish embryos, larvae and juveniles. The research also aims to gather information regarding the ecological and genetic population structure of Lake and Round Whitefish in Lake Huron.

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3.2.1 2011-2016 Research Activities and Results

Research efforts related to this project have been extensive and are summarized in the 2016 Whitefish Research Review[R-3]. A short summary is presented below.

Since 2011, Bruce Power has expended significant effort to improve the scientific understanding of Lake and Round Whitefish, particularly with respect to potential negative impacts of Bruce site operations. In partnership with Saugeen Ojibway Nation, Bruce Power funded independent, multi-year university-based research programs to address key research questions with respect to Lake and Round Whitefish [R-4][R-13]

- **Population discrimination:** how many whitefish populations exist in Lake Huron, and is there any likelihood that these populations will be impacted by Bruce Power operations based on spatio-temporal distribution?
- **Population modeling:** what is the most appropriate mathematical model(s) that can describe Lake Huron whitefish population dynamics and estimate any potential effects of Bruce Power?
- Entrainment effects: are there any significant effects (stress response/mortality) of Bruce Power entrainment on local or lake-wide whitefish populations?
- **Thermal effects:** are there any effects (negative or beneficial) of the Bruce Power thermal plumes from cooling water discharges on the whitefish population(s) in terms of stress response or mortality?
- **Combined stressors:** are there any combined effects (negative or beneficial) of Bruce Power radiological and conventional emissions on the whitefish population(s) in terms of stress response or mortality?

The University of Guelph research program was funded entirely by Bruce Power. The McMaster/Regina University research program was funded by Bruce Power, with matching funds obtained through a rigorous competitive peer-reviewed NSERC collaborative research and development grant. Under the terms of the funding contracts, researchers were free to publish their findings in the peer-reviewed scientific literature.

Over 5 field seasons, researchers have collected nearly 2000 adult Lake and Round Whitefish and raised over 1.3 million Lake Whitefish and more than 60,000 Round Whitefish embryos and larvae. The program at the University of Guelph has generated two theses and one peerreviewed publication. The joint program between McMaster and Regina Universities has generated seven theses and 13 peer-reviewed publications.

This independent university-based research and development has endeavored to identify Whitefish populations in Lake Huron and model these populations. Laboratory and *in situ* studies have been completed to identify both thermal and combined radiological, chemical and thermal stressor effects on developing Lake Whitefish embryos and juveniles.

The Whitefish research programs have supported the training of four post-doctoral fellows, four PhDs and nine MSc students. An additional four MSc and two PhD students are in the process of completing their studies. The training of highly qualified personnel and the

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development of expertise in Lake Huron Whitefish has added valuable resources to tackling the myriad of ecological challenges facing the Great Lakes.

3.2.2 Population Discrimination

Prior to the initiation of the research program, little was known with respect to the population structuring of Lake Whitefish in Lake Huron, and almost nothing was known with respect to Round Whitefish in Lake Huron. Some evidence of limited population structuring along the Bruce peninsula had been observed previously through genetic analysis, while interim results from a lake-wide mark/recapture study did not shed additional light on the nature of whitefish population structuring in the vicinity of the Bruce site.

While extensive spawning grounds were known to be utilized by Lake Whitefish along the coast of the Bruce Peninsula well to the north of the Bruce site, there was almost no direct evidence of Lake Whitefish spawning near the Bruce site itself. However, annual gillnetting studies demonstrated the presence of ripe Lake and Round Whitefish, indicating at least the possibility of spawning near the Bruce site. It was assumed that, if spawning activity took place, the Lake and Round Whitefish individuals spawning near the Bruce site were members of a much larger population.

The research program was intended to challenge this assumption. University of Regina and University of Guelph researchers collaborated with respect to collecting and sampling some of nearly 2000 Lake and Round Whitefish from spawning grounds around the main basin of Lake Huron as well as the North Channel and Georgian Bay from 2010 to 2014.

University of Regina researchers used genetic analysis to examine the relationships between spawning fish, and stable isotope analysis to examine the movement and resource use of adult fish at different locations relative to Bruce Power. They completed both fine-scale (near Bruce Power) and lake-wide assessments.

The fine-scale assessment found no evidence for the presence of distinct genetic groups of Lake or Round Whitefish within the vicinity of Bruce Power. Similarly, stable isotope analysis showed no evidence for distinct Lake or Round Whitefish feeding behavior in this vicinity, as there was high resource use overlap between fish at different sampling areas. However, their research did show that Lake and Round Whitefish occupy separate ecological niches (based on different isotope ratios) with the most overlap occurring during the spawning period.[R-5][R-7]

The lake-wide assessment found that Lake Whitefish near Bruce Power were part of a larger mixed group which encompasses the Northeastern Main Basin of Lake Huron. These results are consistent with the previous, more limited genetic analysis suggesting weak structuring within the eastern main basin. Additionally, stable isotope analysis showed that six ecological groups of Lake Whitefish were present in Lake Huron. In the area local to Bruce Power, the Main Basin of Lake Huron could be divided into Southwestern and Northeastern (including the Bruce Power site) ecological groups with heavy population mixing within each of these groups. No small distinct ecological groups were identified within the area near the Bruce Power site (i.e. Scougall Bank and McRae Point).[R-6]

A genetic analysis of the geographic distribution of Round Whitefish in North America was also completed. The genetic analysis found that Lake Huron is a source population for Round Whitefish for Lakes Michigan, Superior and northern Georgian Bay as well as Lake Nipigon

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through Lake Superior. Sampling was not widespread enough within Lake Huron to draw conclusions regarding population structure within the lake.[R-18]

The research results conclusively demonstrate that spawning-condition Lake Whitefish near the Bruce site are members of a larger genetic and ecological group. There is no evidence of any distinct population of Lake or Round Whitefish utilizing spawning grounds near Bruce Power.

3.2.3 Population Modeling

University of Guelph led the research program with respect to population modeling. The University of Guelph research team reviewed the available scientific literature for estimates of Lake Whitefish mortality and life history parameters. Initial technical reports were provided that led to two theses and one publication with the involvement of two students.

The primary result from the University of Guelph research program is the development of chemical probes for the identification of Lake Whitefish (the DNA "barcoding" technique). Identification of larval Lake Whitefish using a key-based technique is challenging, while identification of eggs is virtually impossible. The DNA-based probes may allow for more accurate quantification of impacts in the future, through accurate and rapid identification of the species for collected larvae and embryos. [R-20]

The research team was not able to identify population structure using the DNA barcoding technique.

Initial efforts were made to determine appropriate mortality and life-history parameters, and to develop appropriate models for spatial data. However, the key research question (what is the most appropriate mathematical model to describe Lake Huron whitefish population dynamics and to estimate the potential effects of Bruce Power) was not answered through this research program.

Nonetheless, researchers have made significant advances in understanding potential impacts on Lake Whitefish since 2011. First, the overall research program has definitively shown no distinct population of Lake or Round Whitefish in the immediate vicinity of the Bruce Power site. Second, extensive monitoring conducted by Bruce Power demonstrated minimal Lake Whitefish losses from impingement and entrainment: less than 0.5% of the Zone 1 quota from impingement and entrainment.[R-2]

3.2.4 Thermal effects

3.2.4.1 Survival and Development of Lake and Round Whitefish Embryos

Prior to the initiation of the research program, the effects of warmer temperatures on incubating Lake and Round Whitefish embryos was not fully understood. As the outflow from Bruce site facilities could generate a sinking thermal plume in the winter (with temperature up to 4° C warmer than the lake bottom temperature), it was expected that Lake Whitefish embryos, if present, would not be significantly impacted by Bruce site operations.[R-1] No scientific information was available with respect to the impact of warmer temperatures on whitefish cellular responses. Additionally, it was not known whether an earlier hatch would be beneficial or harmful to Lake Whitefish; whitefish that hatch early may have access to more or less food, and may be more or less susceptible to predation.

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Bruce Power has funded extensive research into the impacts of thermal and combined thermal, radiological and chemical stressors on developing Lake and Round Whitefish embryos. This has included examining stressor effects on cellular responses and growth, development and survival.

From 2011 to 2017, the McMaster University and University of Regina research teams incubated over 1.3 million Lake Whitefish embryos and over 60,000 Round Whitefish embryos in ongoing efforts to define thermal and combined stressor effects on Whitefish. Novel incubation and irradiation methods were developed to support this research. Heat shock protein gene sequences were identified to aid in the identification of cellular stress responses in Round and Lake Whitefish.

Key results from this research indicate that Lake Whitefish embryos mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. This level of heat shock exceeds the temperature changes seen in the temperature loggers placed in the *in situ* incubation near the discharge channel. Lake Whitefish embryos were slower than juveniles to show a heat shock response but once they showed a response, it continued over at least 2 days following the heat shock. This response suggests that embryos are able protect themselves from subsequent exposure to heat, and may provide an explanation at the biological level for the observation that survival is not affected by intermittent exposure to heat.[R-21][R-22]

Lake Whitefish hatch earlier and have reduced survival with increasing constant temperatures. Lake Whitefish incubated at higher constant temperatures showed increased heart rate and oxygen consumption. Incubation at higher constant temperatures earlier in incubation had a lasting effect even when the temperatures later in incubation were cooler. Higher constant temperatures also resulted in a shorter incubation period, higher yolk area and mass and lower body lengths and mass. Higher constant incubation temperatures also decreased the hatch window, survival and increased oxygen needs.[R-19]

Infrequent exposure of Lake Whitefish embryos and juveniles to warmer water has little effect on survival or development. Regular weekly heat shocks had no effect on time-to-hatch, survival, yolk mass, body mass, length or head width.[R-14]

In situ incubation of Lake Whitefish near the discharge area resulted in an average 10% advance in development. Embryos incubated near the discharge area were slightly longer with a smaller yolk area but showed no increase in developmental abnormalities. These chambers were deployed on the lake bottom, above the cobble substrate, whereas normal lake whitefish incubation tends to occur down within the cobble.[R-22] There is the potential for the cobble substrate to provide a thermal buffer which may reduce the temperature effects observed in the *in situ* study. This is currently being investigated using a simulated thermal flume at Laurentian University (see Section 3.4).

Embryos hatching earlier in warmer temperature conditions have larger yolk sacs and smaller bodies. Embryos incubated at higher temperatures may be less developed and have greater capacity to maintain themselves without external food sources than embryos hatching after long incubation at cooler temperatures. The possibility of a mismatch between plankton availability and hatch timing produced by exposure to warmer temperature may be reduced because these embryos may be hatching at an earlier stage of development. The exact mechanism that triggers embryo hatch remains unknown but may be related to a change in the movement of embryos, temperature changes and/or light exposure. In addition, hatch

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windows (i.e. the time from first to last hatch) increase with cooler incubation temperatures. This may be related to the potential for lower plankton availability at cooler temperatures.[R-17]

Researchers studied the effect of warmer temperatures and periodic increases in water temperature on Round Whitefish. They found that Round Whitefish had lower survival when incubated at constantly warmer temperatures (8°C). They also had lower survival when the temperature increased quickly at the end of their development.[R-15]

Round Whitefish embryos exposed to infrequent increases in water temperature throughout incubation had no changes in survival or development. [R-15]

3.2.4.2 Survival and Development of Lake Whitefish Larvae and Juveniles

Bruce Power has also funded substantial research into the impact of thermal stressors on developing Lake and Round Whitefish larvae and juveniles. This has included examining effects on cellular responses and growth, development and survival.

Lake Whitefish juveniles were incubated at several different temperatures and were exposed to regular heat shocks throughout development in multiple experiments by researchers at McMaster and Regina Universities. They found that juveniles could mount a cellular response to heat shocks that allowed them to survive a 4-hour, 9°C heat shock. Lake Whitefish juveniles quickly mounted a cellular heat shock response and it dropped much more rapidly than in embryos, peaking at 2-hours after the heat shock. Subtle differences in the physiology of juveniles exposed to heat shocks were observed.[R-21] Although these differences are not expected to impact survival of juveniles, the link between heat exposure during embryogenesis and post-hatch effects is not yet fully clear.

3.2.4.3 Thermal effects summary

There remain several uncertainties in the outcome of Lake and Round Whitefish larvae, particularly with respect to behaviour and fitness. It is not known whether increased incubation temperatures and/or heat shock exposure impacts the hormonal stress responses (i.e. blood sugar, adrenaline, electrolyte levels and metabolic rate) of larval and juvenile Whitefish. It is also not known how increased incubation temperatures and/or heat shock exposure impact swim performance, foraging capability and behaviour in larvae and juvenile Whitefish.

Although direct evidence of spawning near Bruce Power has almost never been observed, the potential for spawning has been inferred from catches of ripe adult Lake Whitefish. Nonetheless, the research program results to date support the general understanding that the impacts of Bruce site thermal discharge on Lake and Round Whitefish embryos, if present near the Bruce site, is likely to be limited.

3.2.5 Combined stressors

Prior to the research program, it was not known whether multiple external stressors could affect whitefish embryos synergistically or antagonistically. Assessment of potential impacts on Lake Whitefish considered each stressor separately, without consideration for the possibility of interactions between cellular or organism response to multiple stressors.

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Lake Whitefish embryos were exposed to constant or variable temperature, as well as one of acute or chronic gamma radiation or chemical stress (morpholine or sodium hypochlorite). Doses of chronic radiation of 0.06-4.4 mGy/day resulted in an earlier hatch. High doses of acute radiation increase embryo mortality.[R-23]

Morpholine concentrations of 1 or 10 mg/L resulted in increased larval size at hatch. There were no significant effects of morpholine at 100mg/l. From 500 to 1000mg/L there was a decreased larval size, earlier hatch and increased embryo mortality. Mortality effects on embryos in this study occurred at gamma radiation and morpholine doses at least 10 times greater than those in the thermal discharge channels at Bruce Power.[R-23]

Lake Whitefish embryos were exposed to a combination of thermal, chemical and radiological stressors. A heat shock administered prior to exposure to morpholine had no protective effect. The same heat shock administered prior to irradiation had a protective effect on Lake Whitefish embryos. In this experiment, a heat shock of 2-hours duration (temperature increase of 3°C or 9 °C) administered six hours prior to irradiation decreased embryo mortality by up to 25%.[R-23]

Round Whitefish exhibited a greater sensitivity to morpholine exposure than Lake Whitefish, with developmental and mortality effects starting at 500mg/l. No consistent effects were seen at levels between 0 and 100 mg/L. Conversely, Round Whitefish were more resistant to low dose radiation than Lake Whitefish, with a 20% reduction in post-hatch mortality for irradiated Round Whitefish embryos.[R-15]

The relationship between radiation and morpholine exposure and Lake and Round Whitefish embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and are different for Lake and Round Whitefish. Lake Whitefish were more sensitive to radiation exposure whereas Round Whitefish appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for Lake Whitefish.

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3.2.6 Summary

Bruce Power has funded extensive independent university-based research and development surrounding potential interactions with Whitefish. This independent, university-based research has added to a limited knowledge base regarding population discrimination and the effect of thermal and combined radiological, chemical and thermal stressors on Lake and Round Whitefish. Results of this research are publically available for use by industrial, scientific and regulatory groups. The research has also supported the training of highly gualified personnel with expertise in Lake Huron Whitefish.

Key research results are summarized in Box A for each of the identified research questions.

It is unclear how changes in Lake Whitefish embryo size and metabolism in response to heat shocks impact the fitness, behaviour and survival of Lake Whitefish larvae and juveniles. Bruce Power expects to fund further research to explore these uncertainties in larval and juvenile Lake Whitefish outcomes.

3.2.7 Outcomes

There have been 13 academic peer reviewed papers published to date as a result of this partnership, in addition to related academic theses (Table 2 and Table 3). There are approximately ten additional papers in press or in preparation at of the writing of this report.

Box A – Key Research Results

Population discrimination: No local genetic or ecological populations of Lake or Round Whitefish have been identified in the area near Bruce Power. The Lake and Round Whitefish in the area surrounding Bruce Power belong to genetic and ecological groups that encompass large parts of Lake Huron.

Population modeling: DNA barcodes were developed for Lake Whitefish.

Entrainment effects: The impact of Bruce site operations has been shown to be less than 0.5% of the Zone 1 quota.

Thermal effects: Intermittent temperature increases throughout Lake or Round Whitefish development have no impact on embryo mortality. Lake and Round Whitefish embryos exposed to regular heat shocks throughout development do experience a small advance in hatch and some changes in size and metabolism.

Lake Whitefish embryos are able to mount a response to intermittent heat shocks that ensure survival of a 4-hour 9°C heat shock. This intermittent heat shock exceeds the temperature changes recorded by the temperature loggers from *in situ* incubation chambers near the discharge channels. Temperature changes of the magnitude seen in the vicinity of the thermal discharge have been shown to have no significant effect on mortality or stress response of Lake Whitefish embryos.

Combined stressors: The relationship between radiation and morpholine exposure and Lake and Round Whitefish embryo outcomes is dose-dependent. High doses of radiation and morpholine are clearly detrimental to development with increased mortality rates. The effects of low doses of radiation or morpholine are more variable and different for Lake and Round Whitefish. Lake Whitefish are more sensitive to radiation exposure whereas Round Whitefish appear more sensitive to morpholine exposure. Prior exposure to heat shock is somewhat protective for future exposure to radiation for Lake Whitefish.

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Table 2 Scientific reports on thermal effects and combined stressors produced as a result of the NSERC CRD

partnership	
Baseline growth and development	
Sreetharan S., Thome C., Mitz C., Eme J., Mueller C.A., Hulley E.N., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y., 2015. Embryonic development of lake whitefish (Coregonus clupeaformis):a staging series, analysis of growth and impacts of fixation. <i>Journal of Fish Biology</i> , 87(3):539-558.	Publication
Mitz, C., Thome, C., Cybulski, M.E., Laframboise, L., Somers, C.M., Manzon, R.G., Wilson, J.Y., Boreham, D.R., 2014. A self-contained, controlled hatchery system for rearing Lake Whitefish embryos for experimental aquaculture. <i>Journal of North American Aquaculture</i> 76:179-184.	Publication
Cellular response to changes in temperature	
Stefanovic D.I., Manzon L.A., McDougall C.S., Boreham D.R., Somers C.M., Wilson J.Y., Manzon R.G, 2016. Thermal stress and the heat shock response in embryonic and young of the year juvenile lake whitefish. <i>Comparative Biochemistry and Physiology, Part A</i> 193:1-10.	Publication
Stefanovic D.I., 2015. Thermal stress and the heat shock response in embryonic and young of the year juvenile lake whitefish (Coregonus Clupeaformis). MSc thesis University of Regina, Saskatchewan. 2015.	MSc thesis
Sessions, K. Plasticity of the heat shock response and development of thermotolerance during embryonic development of Lake Whitefish (Coregonus clupeaformis). MSC thesis University of Regina, Saskatchewan. July 2015.	MSc Thesis
Growth, development and survival responses to changes in temperature	
Eme J., Mueller C.A., Manzon R.G., Somers C.M., Boreham D.R., Wilson J.Y., 2015. Critical windows in embryonic development: shifting incubation temperatures alter heart rate and oxygen consumption of Lake Whitefish (<i>Coregonus clupeaformis</i>) embryos and hatchlings. <i>Comparative Biochemistry and Physiology, Part A</i> 179, 71-81.	Publication
Lee A.H., Eme J., Mueller C.A., Manzon E.G., Somers C.M., Boreham D.R., Wilson J.Y., 2016. The effects of cumulative acute heat shock exposures on morphology and survival of Lake Whitefish (Coregonus clupeaformis) embryos. <i>Journal of Thermal Biology</i> 57:11-20.	Publication
Mueller C.A., Eme J., Manzon R.G., Somers C.M., Boreham D.R., Wilson, J.Y., accepted. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in Lake Whitefish (Coregonus clupeaformis). <i>Journal of Comparative Physiology B</i> 185(3):315-331.	Publication
Thome, C., Mitz, C., Somers, C.M., Manzon, R.G., Boreham, D.R., Wilson, J.Y, 2016. Incubation of Lake Whitefish (Coregonus clupeaformis) embryos in cooling water discharge and the impacts of fluctuating thermal regimes on development. <i>Canadian Journal of Fisheries and Aquatic Sciences</i> 73:1-9.	Publication
Thome, C. The effects of thermal, chemical and radiological stressors on embryonic development in Lake Whitefish (Coregonus clupeaformis). PhD thesis McMaster University, Hamilton, Ontario. 2015.	PhD thesis
Lim, M. Thermal, morpholine, and radiation stressor effects on the embryonic development of Lake (Coregonus Clupeaformis) and Round Whitefish (Prosopium Cylindraceum). MSc thesis, McMaster University, Hamilton, ON, September 2016.	MSc thesis
Mitz, C., 2016. The cost of hormesis: Trade-offs in an energetically constrained environment: Embryonic development of the Lake Whitefish (Coregonus Clupeaformis). PhD thesis, McMaster University, Hamilton, ON, July 2016.	PhD thesis
Mueller, C.A., Doyle, L., Eme, J., Manzon, R.G., Somers, C.M., Boreham, D.R., Wilson, J.Y., 2017. Lipid content and fatty acid profile during lake whitefish embryonic development at different incubation temperatures. <i>Comparative Biochemistry and Physiology, Part A</i> 203:201-209.	Publication

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Table 3	
Scientific reports on population structure produced as a result of the NSERC CRD partn	iership
O'Bryhim, J., Somers, C., Lance, S.L., Yau, M., Boreham, D.R., Jones, K.L., Taylor, E.B., 2013. Development and characterization of twenty two novel microsatellite markers for the mountain whitefish, Prosopium williamsoni and cross amplification in the round whitefish, P. cylindraceum, using paired end Illumina shotgun sequencing. <i>Conservation Genetics Resources</i> 5:89-91.	Publication
Eberts, R., 2013. Dietary niche partitioning by two sympatric whitefish species in Lake Huron: stable isotope analysis at three temporal scales. B.Sc. (Hon.) thesis. University of Regina, Saskatchewan. April 2013.	B.Sc. thesis
Eberts, R., 2015. Resource use and ecological population structure of Lake Whitefish (Coregonus clupeaformis) spawning aggregations in Lake Huron. MSc thesis. University of Regina, Saskatchewan. December 2015.	MSc thesis
Graham C.F., Eberts R.L., Morgan T.D., Boreham D.R., Lance, S.L., Manzon R.G., Martino J.A., Rogers S.M., Wilson JY, Somers CM, 2016. Fine-scale ecological and genetic population structure of two Whitefish (Coregoninae) species in the vicinity of industrial thermal emissions. <i>Plos ONE</i> 11(1). DOI: 10.1371/journal.pone.0146656.	Publication
Graham, C.F., Glenn, T.C., McArthur, A.G., Boreham, D.R., Kieran, T., Lance, S., Manzon R.G., Martino, J.A., Pierson, T., Rogers, S.M., Wilson J.Y., Somers C.M., 2015. Impacts of Degraded DNA on Restriction Enzyme DNA Sequencing (RADSeq). <i>Molecular Ecology Resources</i> 6:1304-15.	Publication
Eberts, R., Wissel, B., Boreham, D., Manzon, R., Wilson, J. and Somers, C., 2015. Consistent differential resource use by sympatric Lake (Coregonus clupeaformis) and Round Whitefish (Prosopium cylindraceum) in Lake Huron: a multi-time scale isotopic niche analysis. <i>Canadian Journal of Fisheries and Aquatic Sciences</i> 73(7):1072-1080	Publication
Morgan, T., 2016. Genetic population structure of the round whitefish (Prosopium cylindraceum) in North America: multiple glacial refugia and regional subdivision. MSc thesis. University of Regina, Saskatchewan. November 2016.	MSc thesis
Eberts, R.L., Wissel, B., Simpson, G.L., Crawford, S.S., Stott, W., Hanner R.H., Manzon, R.G., Wilson, J.Y., Boreham, D.R, Somers, C.M., 2017. Isotopic structure of Lake Whitefish in Lake Huron: Evidence for regional and local populations based on resource use. <i>North American Journal of Fisheries Management</i> 37(1):133-148.	Publication

3.2.8 2017 Research Plan

Plans for 2017 include the publication of papers related to thermal effects on Round Whitefish and the effects of combined stressors on Lake and Round Whitefish.

Lake Whitefish collection in November and December 2016 occurred at Stokes Bay and the Fishing Islands in Lake Huron. There were 52 males and 22 females collected at Stokes Bay and 17 males and 11 females collected at the Fishing Islands site. These fish collections generated approximately 790,000 embryos. Eight male and four female Round Whitefish were collected from Lake Ontario at Port Darlington and generated approximately 20,000 embryos.

The Lake Whitefish embryos collected from Lake Huron in November and December 2016 experienced very high embryo mortality on transfer to the University of Regina. As a result, approximately 132,000 Lake Whitefish embryos were collected from three lakes in Saskatchewan during the 2016 field season.

3.2.8.1 Population discrimination

The University of Regina is utilizing single-nucleotide polymorphisms (SNPs) to genotype Lake Whitefish. The DNA from 142 Lake Whitefish from six sites (five in Lake Huron and one outgroup) was extracted and sequenced for 25,000 and 50,000 loci per fish. Preliminary analysis shows some differentiation between Eastern and Western Lake Huron Lake Whitefish.

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The DNA from an additional 380 adult Lake Whitefish from 20 sites in Lake Huron and Lake Superiors has been extracted and sequenced for 20,000 loci per fish. The objective is to understand the genetics of Lake Whitefish on a larger geographic scale and the geographic subdivision within the Great Lakes.

Data analysis for both DNA projects will continue throughout 2017.

3.2.8.2 Thermal effects

Four thermal effects experiments are underway in 2017.

In one experiment, Lake Whitefish embryos were subjected to 1-hr 3°C daily heat shocks and then to a final more extreme heat shock of between 3°C and 27°C above the 3-4°C ambient temperature. Of the 19,200 Lake Whitefish embryos involved in this experiment, approximately 1200 Lake Whitefish fry are being reared at the University of Regina. Heat shock protein levels were measured in some of the embryos. This heat shock protein experiment is in the data analysis phase and will be published in 2017.

A second experiment involved exposure of 480 embryos to one of four different heat shock temperatures (0, 6, 9 or 12°C above control) at four times throughout embryogenesis. A third experiment involved 630 embryos subjected to one of three heat shock regimes (0, +6°C for 1 h every 6 days or +9°C for 1 h every 6 days) during embryogenesis. Both the second and third experiment involved the measurement of heat shock protein gene expression.

The fourth experiment in 2017 consisted of an assessment of Lake and Round Whitefish fitness following thermal stress during embryonic development. The experiment will assess the transition to independent feeding in the larval stage and ecologically relevant behaviour in the juvenile stage including predator avoidance and aggression. In this first year, the embryos were raised at constant temperature regimes of 0.5, 2, 5, or 8 °C. Hatched larvae are being fed daily and larval survival, gape size, feeding capacity and growth in length and weight are being monitored. Aggression and predator avoidance behavior testing will occur once the embryos have developed into juvenile fish. Preliminary results suggest increased embryonic and larval mortality at 5 and 8°C. Round Whitefish experience greater mortality at 8°C but reduced mortality at 5°C compared to Lake Whitefish. As of April 30, 2017, the larvae have not yet hatched from the 0.5 and 2°C temperature regimes.

3.2.8.3 Combined stressors

The protective effect of heat shock against future radiation stressors is being investigated using two different regimes (Figure 2).

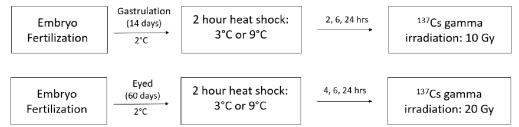


Figure 2 Combined stressor treatment regimes, 2017

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Preliminary results suggest that at 14 days (gastrulation stage), the 3°C heat shocks decreased mortality compared to the control group and the 9°C heat shock group. Radiation treatment increased mortality in all groups. The groups that had received the prior heat shocks saw reduced mortality with radiation treatment than the constant 2°C groups.

At 60 days (eyed stage), the 3°C heat shocks increased mortality compared to the control group and the 9°C heat shock group. Radiation treatment increased mortality in all groups. The groups that had received the 3°C prior heat shock saw reduced mortality with radiation treatment than the constant 2°C groups and the 9°C prior heat shock groups. Final hatching of embryos and data analysis are still pending for this experiment.

3.2.8.4 Future research planning

In 2017, the Integration Department is participating in defining and submitting a research plan for further investigating the effects of Bruce Power on aquatic biota. This research plan will address remaining uncertainties in Lake and Round Whitefish thermal effects and will also explore effects of Bruce Power on community composition.

3.3 Whitefish DNA Sequencing (2015-2016)

This research program was intended to support DNA identification of larval fish and eggs collected during entrainment monitoring.

3.3.1 2015-2016 Research Activities and Results

The research project aimed to compare the efficacy of genetic and morphological identification of fish larvae collected during impingement and entrainment monitoring. Larval fishes and eggs were collected during entrainment monitoring at Bruce Power in 2013 and 2014. Expert independent contractors visually identified the larval fish based on morphology.

Following their morphological identification, the larval fish were sent to Laurentian University, where their DNA was extracted and sequenced to genetically identify the fish. When fish were unable to be identified to the species, they were identified to their genus.

Upon the completion of DNA sequencing, the genetic sequences of each larval fish was used to identify the species of fish using the Basic Local Alignment Search Tool (BLAST). This database allows for the comparison of new genetic sequences with sequences that are associated with known specimens. Specimens that were not identified the first time through sequencing (either due to PCR failure or sequencing difficulties) underwent a second PCR reaction and subsequent sequencing.

Primers and probes were developed to rapidly identify four species of interest, namely: Lake Whitefish (*Coregonus clupeaformis*), Round Whitefish (*Prospium cylindraceum*), Deepwater Sculpin (*Myoxocephalus thompsonii*) and Spottail Shiner (*Notropis hudsonius*). The primers and probes were designed using the Cytochrome c Oxidase I (COI) "barcode" region of the genome. When the genetic "barcode" did not provide adequate interspecific differentiation, the Cytochrome B region of the genome was used for primer and probe design (i.e. for Lake Whitefish).

DNA was extracted from 156 eggs, representing 53.4% of the sample. Of the samples sent for sequencing, 94.5% were successfully identified (52 eggs identified) as Walleye (*Sander*

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vitreus). Relatively few eggs were extracted and sequenced due to the difficultly of extracting DNA from eggs. There were some varieties of eggs for which the DNA extraction process did not work (i.e. the proteinase K could not break down the outer layer of the egg). Due to the high failure rate of the DNA extraction process for eggs, larval fishes became the focus of the project.

There were 657 larval fish with dual visual and genetic identifications, including 7 Lake Whitefish, 144 Deepwater Sculpin. No Round Whitefish or Spottail Shiners had dual identifications. There were some discrepancies between morphological identifications and identifications based on genetic analysis. Of the 657 larval fish with dual identifications, 27 (4.13%) had discordant morphological and genetic identifications. For Lake Whitefish, five morphological identifications were made in error. These included four samples where the Lake Whitefish was identified as another fish species (false positives) and one sample where another fish species was identified as a Lake Whitefish (false negative). There were also discordant identifications for three Deepwater Sculpin and one Spottail Shiner. No Round Whitefish were identified.

Both the morphological and genetic techniques of identifying larval fish were unable to identify some of the fish. Morphologically, there were 37 (7.03%) fish that were unidentified for various reasons (e.g. the fish was mangled or missing part of its body). Genetically, there were 26 (3.98%) unidentified fishes (due to a lack of amplification during the PCR). There were no discernable patterns as to which species of fish were unidentified morphologically or genetically.

Genetically, it was not possible to differentiate between Lake Herring and Bloater; therefore, these fish were identified to the Coregonus genus. This phenomenon has also been noted in other research endeavors that attempted to use the mitochondrial "barcode" region to identify this genus of fish.

The primer and probe set that were designed for Lake Whitefish failed to differentiate Lake Whitefish from other species in the Clupeaformis genus (e.g. Lake Herring). There does not appear to be adequate differentiation in the COI gene to differentiate *C. clupeaformis*, therefore, other target genes are being investigated. One primer and probe set has been developed for Round Whitefish that is able to differentiate Round Whitefish from other closely related species of fish; however, further validation is required. Primers and probe sets have been designed for both Deepwater Sculpin and Spottail Shiner but these have not yet been tested.

Conclusions

Using the "barcode" region of the genome has allowed for the identification of 657 larval fishes and 52 eggs. Of the Valued Ecosystem Component (VEC) larval fish that were genetically identified, four were Lake Whitefish, 147 were Deepwater Sculpin, none were Round Whitefish and one was Spottail Shiner.

Genetic identification can distinguish Lake Whitefish from other Coregonus species. The genetic "barcode" technique is unable to distinguish Coregonus species from one another.

Since the beginning of 2016, four primer and probe sets were designed for the detection of Lake Whitefish, Round Whitefish, Deepwater Sculpin and Spottail Shiner. These primers and probes are designed to work in a multiplex fashion in a real-time PCR reaction for the

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detection of target species DNA. Unfortunately, due to technological constraints, only three of the target VEC species were able to be multiplexed (Lake Whitefish, Round Whitefish and Deepwater Sculpin). The primers and probes were validated and found to be reliable detectors for target DNA when a cut-off was used for the number of replication sequences.

3.3.2 2017 Research Plan

The goal for 2017 is to continue building the library of species-specific primer and probe sets for Lake Huron species.

In 2017, genetic "barcode" sets were designed for the four new target species (Smallmouth Bass (*Micropterus dolomieu*), Brook Trout (*Salvelinus fontinalis*), Yellow Perch (*Perca flavescens*) and Rainbow Smelt (*Osmerus mordax*)). All new primer and probe sets were designed utilizing the COI "barcode" gene sequence. Each species-specific probe was designed with a different fluorescence reporter.

All eight primer and probe sets are currently being validated. Once validation is complete, researchers will work to multiplex up to five of the target species at one time using qPCR.

3.4 Thermal Study (2016-2020)

The Thermal Study research program is intended to develop and improve technology for substrate temperature monitoring. This includes examining the accuracy of aerial thermal scans. The program intends to explore the effect of the substrate on temperature changes experienced by aquatic biota. Finally, the program examines the biological mechanisms affecting growth and metabolic efficiency for embryos exposed to heat and other stressors (multi-stressor effects) and endeavors to predict the effects of multi-year operation on potentially sensitive aquatic species.

3.4.1 2015-2016 Research Activities and Results

Background

Results from the in-situ incubation chamber study in Lake Huron suggested that temperatures on the lake bottom near the Bruce A and B discharge were warmer compared to ambient water temperature. Embryos incubated in these locations were significantly larger compared to those incubated at offsite control locations, indicating that development rate was advanced in front of Bruce Power. The incubation chambers were deployed on the lake bottom, above the cobble substrate. However, during natural development, the small fertilized lake whitefish embryos will sink down within the cobble substrate. The cobble may provide a thermal buffer that would reduce the impacts observed in the incubation chamber study. Substrate effects on embryonic development were examined using a thermal flume.

Methods

During the summer of 2015, a flume was constructed at the Living with Lakes Centre at Laurentian University. The flume was 3.66 m long with a continuous flow through of water taken directly from Ramsey Lake (Figure 1). Approximately 2.4m from the intake of the flume, a lower compartment was filled with cobble similar to what would be found in Lake Huron near Bruce Power. During the winter of 2015 - 2016, embryos were reared within the flume using

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the same incubation chambers as the in-situ Lake Huron study. Chambers were incubated at four sites within the flume (Figure 3). A data logger was placed next to each chamber.

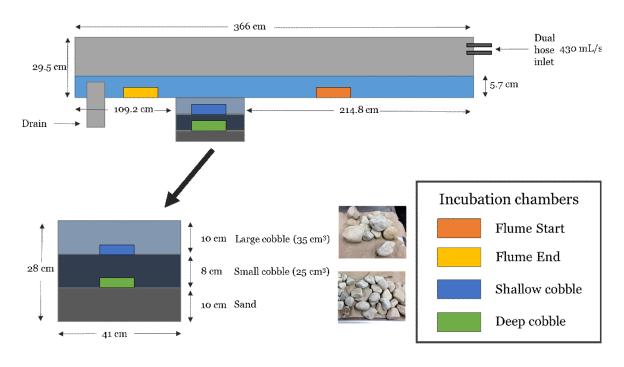


Figure 3 Thermal flume design and location of incubation chambers

Prior to loading embryos in the chambers, temperature fluctuations were examined after an acute heat shock. Twenty liters of heated water was added to the flume at 3, 6, 9 and 12°C above ambient lake temperature. At each of the four locations, data loggers were used to measure the peak temperature spike, the time to peak and the time for temperature to return to baseline.

Embryos were incubated in the flume from January 13 to February 17, 2016. Twice per week, a one hour heat shock was administered at 3°C above ambient lake temperature. After 36 days, embryos were removed from the flume, formalin fixed and analyzed for size and weight.

Outcomes

The cobble substrate reduced the temperature fluctuations following an acute heat shock. The two locations within the cobble had a lower peak temperature and the temperature increased at a slower rate. The "deep cobble" location was the most stable, and only increased by 0.2°C after a 12°C heat shock.

In 2015-16, the average temperatures during embryo development within the cobble and in the open flowing water were not significantly different. However, the temperature fluctuations were significantly less within the cobble. A fungal buildup on the "flume front" chamber

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resulted in almost total embryo mortality, so no size or weight data were collected from that chamber. The embryos incubating at the "flume end", compared to in the cobble substrate, were significantly larger in body length and weight, suggesting that they were advanced in their development. This advanced development would be expected based on the increased temperature fluctuations at the "flume end". Overall, these results suggest that embryos incubating within a cobble substrate will be less impacted by temperature fluctuations (such as those resulting from thermal discharges) compared to embryos incubating in the open water column.

3.4.2 2017 Research Activities and Results

During the winter of 2016 - 17, the thermal flume experiment was repeated. Embryos were collected in November and incubated in the flume throughout the winter development period. Chambers were loaded with more embryos (100 per chamber instead of 50) and an additional chamber was added to the fume to achieve larger sample sizes. Temperature data was recorded during development and embryos will be analyzed for survival, development rate and size.

The embryos were loaded in the flume in five locations on December 5, 2016. Temperature was monitored for 37 days during the natural cooling of Ramsey Lake. A second set of embryos was loaded on January 11, 2017 for 51 days. Twice per week, a one-hour heat shock was administered at 1.5°C above ambient lake temperature to simulate a thermal plume. All embryos were preserved and have been imaged for size and weight.

In 2016-17, there was once again no significant difference in average temperature within the flume. The average rate of change in temperature was significantly less within the cobble substrate locations.

3.4.3 2017 Research Plan

Publication of the simulated thermal flume results is expected in late 2017.

Research activities relating to the accuracy of thermal monitoring technology and aerial scans will be advanced in 2017. Also to begin in 2017 is work examining the biological mechanisms affecting growth and metabolic efficiency for embryos exposed to heat and other stressors (multi-stressor effects) and efforts to predict the effects of multi-year operation on potentially sensitive aquatic species.

4.0 EFFECTS OF RADIATION

4.1 Ultra-low dose (2016-2019)

The ultra-low dose research program is exploring the effect of ultra-low levels of background radiation on Lake Whitefish embryo survival and development. In the future, single-cell models will be grown in typical ambient radiation and under ultra-low dose Sudbury Neutrino Observatory Laboratory (SNOLab) conditions and evaluated for mutation frequency, chromosomal aberrations and differentiation. The effect of irradiation on single cell models and Lake Whitefish embryos under ambient and ultra-low-dose conditions will then be determined.

Background

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The laboratory established in the SNOLab will allow us to examine the biological effects of prolonged exposure to a sub-natural background radiation environment. The 2 km of overhead rock effectively shields out cosmic radiation. Because living organisms have evolved in the continual presence of natural background ionizing radiation, researchers believe it is essential for life and helps to maintain genomic stability. Prolonged exposure to sub-background radiation environments will therefore be detrimental to biological systems.

Phase 1 of the project, which was completed in 2015 - 2016, involved incubation of Lake Whitefish embryos in the SNOLab. The goal of the first year was to determine if embryos could be successfully incubated underground from fertilization until hatch. Lake Whitefish are a good model species of studying the impacts of sub-background exposure on growth and development because of their extended development period (4-6 months).

The impacts of low-dose ionizing radiation (above background) on Lake Whitefish embryogenesis have been previously examined. Embryos responded to small changes in radiation environments, where a chronic exposure as low as 0.06 mGy per day resulted in significant growth stimulation. This sensitivity to low-dose radiation suggests that Lake Whitefish may be impacted by development in a sub-background environment. In addition, Lake Whitefish develop slowly, close to 200 days depending on temperature, which is important since many of the previous sub-background experiments observed impacts only after prolonged incubation. Lastly, embryos can be easily staged and quantified for growth rate.

4.1.1 2015-2016 Research Activities and Results

Methods

From December 2, 2015 to April 29, 2016 Lake Whitefish embryos were reared in refrigerated incubators underground in SNOLAB and in our control laboratory at the Living with Lakes Centre. Embryos were incubated at 3°C or 5°C and temperature data was logged for the entirety of the experiment. Water was changed on each dish twice per week. At the time of water change, the number of dead or hatched embryos was recorded. At several critical points throughout development (approximately 40%, 60% and 80% development), a subset of embryos were formalin fixed from each refrigeration unit. Fixed embryos were evaluated morphometrically for embryo length, embryo weight, yolk area and yolk dry weight.

Results

Weekly embryonic survival and percent hatch were documented for the entirety of the experiment. No statistically significant difference was seen in the cumulative percent survival between the two radiation environments. Total embryonic mortality in both locations was approximately 70%. Percent mortality can vary between years, but the 70% mortality observed was within the range observed in previous years and was consistent with what was found in experiments run at McMaster University. A significant difference in the time to reach 50% mortality tends to occur at the same development stage and embryos at warmer temperatures will reach this stage sooner. No difference in the timing of mortality was observed between the two radiation environments.

In 2015-16, embryos incubating at 5°C reached the hatching stage earlier than embryos incubating at 3°C, which is known for Lake Whitefish. Near the end of development, the 3°C

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refrigeration unit within SNOLab failed and the embryos received a heat shock that triggered hatch. At that point, all the embryos from both the underground and surface 3°C fridge were formalin fixed. Therefore, a time to 50% hatch could not be accurately calculated at the 3°C temperature.

Although many differences were not significant, the general trend was that the embryos raised underground has smaller yolks and larger bodies when compared to the surface embryos.

4.1.2 2017 Research Activities and Results

From November 16, 2016 to January 25, 2017, 3,200 embryos were once again reared in refrigerated incubators underground in SNOLAB and in our control laboratory at the Living with Lakes Centre. A subset of embryos was preserved at 40% development (December 30) for morphology. A refrigeration unit compressor failed on January 24, 2017 and the temperature rose to 15°C. At that point, all remaining embryos were preserved for analysis.

In 2016-17, there was no significant difference in survival between the two environments at 60% development.

4.1.3 2017 Research Plan

Morphology of the preserved embryos will be completed in 2017.

Due to radiological decay in the surrounding rock, underground radon levels are higher than on the surface (approximately 130 Bq/m³ underground compared to 5 Bq/m³ on surface). A low-radon whitefish incubation chamber will be designed in 2017 to reduce radon levels below natural background and allow us to achieve much lower radiation levels underground compared to surface. However, for the 2015-16 and 2016-17 preliminary lake whitefish work, the high radon may mean that embryos incubated underground had a higher dose rate compared to those on the surface. It is possible that the increased radiation levels are acting in a stimulatory manner, which may explain the increased body lengths and weights observed. Once the low-radon chamber is designed, the lake whitefish experiment will be repeated.

Preliminary work has been progressing in the NOSM laboratory with the cell culture systems and endpoints to be used underground. This included the FADU (Fluorescence Analysis DNA Unwinding), comet and transformation genetic assays. Researchers are working with a design team at SNOLAB to build a low-radon glovebox that will be used for culturing cells. Similar to with Lake Whitefish, the low-radon glovebox will enable us to culture cells in an ultra-low background radiation environment. Glove-box design plans are completed and construction is under way. It is expected that the glove box will be ready for underground SNOLab cell cultures in 2017.

4.2 Radiation Hormesis (2015-2016)

The radiation hormesis research program aimed to quantify the effect of ionizing radiation on behavioral, physiological and molecular biomarkers in a primitive invertebrate model for radiation research. In addition, the protective effects of antioxidants were explored. Funding for this research was matched by a Mitacs grant.

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4.2.1 2015-2016 Research Activities and Results

All personnel were cleared and trained for access to the Taylor Radiation Source at McMaster University. A breeding colony for crickets (*A. domesticus*) was established. Incubators, holding enclosures and handling stations were purchased, constructed and installed (Figure 4). A feeding study comparing palatability of control diet versus diet enhanced with antioxidants was conducted. The maximal sub-lethal radiation exposure threshold for crickets was experimentally established.



Figure 4 Experimental cricket monitoring rooms funded by BP/Mitacs

Four rounds of early-life radiation exposures have been completed to date (Table 4). Combined, animals from these four rounds will be sufficient to satisfy all the proposed objectives.

Round	Dose range (Gy)	Animals/group	# Groups
1	0 - 10	60	8
2	0 - 7	120	6
3	0 - 4	90	5
4	0 - 25	60	14

Table 4 Radiation exposure regimens (0.25 Gy/min) for 2-week old crickets

The researchers measured the survival, growth, maturation, longevity, fecundity, motor and cognitive status and cellular function of the irradiated and control crickets. Development and validation of specific motor and cognitive outcomes is complete. Outcomes related to radiation

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effects on gut microbiome and immune function have been expanded and testing is underway to validate these outcomes prior to the final round of irradiations.

4.2.2 Outcomes

No palatability issues were found with the antioxidant enhanced diet. The maximum sublethal radiation exposure for juvenile *A. domesticus* was established at <10Gy at 0.5Gy/min and <15Gy at 0.25Gy/min.

Data has been collected for all rounds of radiation exposure for body size, growth rates, maturation, mean survival, longevity, feeding rates and locomotor function. Tissue collection (frozen samples) for molecular assays is ongoing. The visual learning approach was attempted but proved unsuitable given the volume of animals and timeline. Learning based on spatial and olfactory cues is proving successful.

Juvenile exposure to a single bout of low-dose radiation had reduced mortality and improved survival (Figure 5). There were limited reductions in body size at high dose rates (7-10Gy).

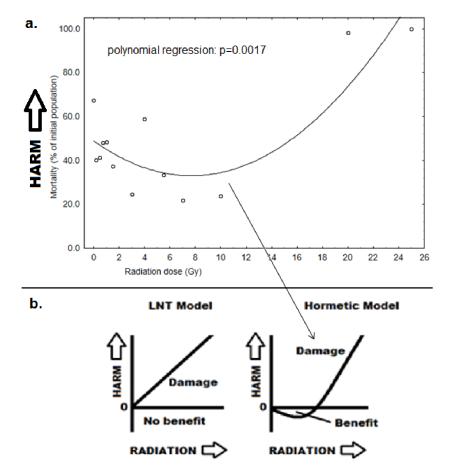


Figure 5. Juvenile cricket survival following radiation exposure.

a) Low-Dose radiation improved survivorship of crickets by nearly 50%. This distribution is a classic example of potential hormesis (Fig. b). b) Original Figure from MITACS grant application: supporting a "Hormetic" framework versus the "Linear No Threshold" Model

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From a subset of animals irradiated in Round 1 we found out that at higher doses (~7Gy) crickets survived, but the females developed no eggs. The fact that females that develop eggs will not lay them unless mated allows researchers to examine the impacts of radiation on reproductive effort, maternal survival, and maternal effects on offspring phenotypes. Despite noticeable inhibitory effects of even low dose radiation on development of the egg complement, the number of eggs laid over a given period was not affected at lower doses. Crickets irradiated in the low dose range (1 - 2 Gy) tended to produce higher quality offspring relative to controls (i.e., larger eggs, greater hatching success, and more robust hatchlings), whereas crickets irradiated in the higher dose range (4 - 5.5 Gy) produced offspring of poorer quality. These results suggest that offspring fitness was stimulated by low-dose radiation (i.e., hormesis). Changes in reproductive strategies in irradiated female crickets show that oxidative stress (i.e. early-life radiation exposure) strongly influences adult crickets later in life.

The immune system of crickets is similar to that of vertebrates. Following radiation exposure, immune functions of the crickets were measured. Low-dose radiation was found to significantly elevate immune system function. This suggested hormesis at doses below 1Gy.

Early radiation exposure may also be associated with reduced immune regulation in the form of "dysbiosis" of the gut microbial composition. Researchers are characterizing the gut microbiome after irradiation. This has implication for survival, longevity and general life history trade-offs.

4.2.3 2017 Research Plan

A final round of radiation exposure is planned for Spring 2017 to complete the olfactory learning protocol with the last group of crickets.

Molecular assays, data analysis and preparation of four manuscripts are ongoing.

Funding for this research ended in December 2016. Results and publications will continue to be finalized and reported.

4.3 Fetal Programming (2015-2020)

The fetal programming research explores the effects of radiation exposure during pregnancy (fetal programming) in mice on cardiovascular and metabolic disease endpoints. The effect of low-dose radiation in attenuating the effect of induced fetal programming will be explored. This research is important for the medical community to help characterize risks associated with radiation exposure during pregnancy. Sources of radiation exposure include both diagnostic imaging exposure and occupational exposure.

The research is relevant to Bruce Power in adding to the knowledge base used to set dose limits and determine regulations surrounding occupational radiation exposure in pregnancy.

Bruce Power funding for this research is matched by a peer-reviewed NSERC grant.

4.3.1 2015-2016 Research Activities and Results

The Animal Use Protocol was approved at both NOSM and McMaster University and mice were ordered. Phase I consisted of a full dose response study of the effects of prenatal ionizing radiation exposure on cardiovascular health and disease. Irradiation protocols were

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established and optimized. Animals exposed to prenatal irradiation at McMaster were transported to NOSM and testing of micro-PET imaging and assays was initiated.

This research requires that control mice be exposed to a minimal level of stress to avoid confounding of the relationship between irradiation and fetal programming. Initial testing of the irradiation protocol involved the use of an audible alarm that was shown to significantly increase stress hormone levels in the mice. Researchers worked with the CNSC to modify the irradiation protocol and silence the alarm during experimental irradiations.

Female mice were irradiated on Day 15 of pregnancy with 0, 5, 10, 50, 100, 300 and 1000 mGy of ¹³⁷Cs gamma radiation at 9.1 mGy/min. The mice pups had weekly blood pressures and weights completed until they reached 16 weeks. At 8 weeks, female pups irradiated *in utero* with doses of 100-1000mGy had significantly lower body weights and higher blood pressures than controls. At 8 weeks, male pups irradiated *in utero* at lower doses of 5-50 mGy had significantly higher body weights than controls.

A second cohort of mice was irradiated *in utero* on gestational day 17 at doses of 0, 5, 50, 100, 300 and 1000 mGy. Mice irradiated at 1000 mGy had an increase in liver weight and a decrease in interscapular brown adipose tissue weight (iBAT). iBAT presence and increased weight has been associated with longevity, improved glucose metabolism and better body weight control in animals in prior studies. A novel use of micro positron emission tomography (micro-PET) imaging of ¹⁸flurodeoxyglucose (¹⁸FDG) uptake is being used to study glucose metabolism. Mice irradiated at 5mGy had increased glucose uptake into iBAT.

4.3.2 2017 Research Activities and Results

At 16 weeks of age, the first cohort mice pups was sacrificed and tissue samples collected for measurement of molecular markers. The heart, interscapular brown adipose tissue (iBAT) and liver were weighed. Liver weights and expression of proteins involved with insulin resistance and glucose production in female mice exposed to 1000mGy *in-utero* were significant higher than those with no *in-utero* radiation exposure. There were no significant differences for male mice. Glucose uptake into iBAT in the second cohort of mice was significantly higher for mice exposed to 1000mGy *in-utero*.

New mice were obtained in early 2017 for further experiments.

4.3.3 Outcomes

To date this project has supported the training of six highly qualified personnel, including three MSc students, two PhD students and one post-doctoral fellow. One MSc student on this project is nearing completion. Preliminary results have been presented at two radiation research conferences and have been accepted for two further conferences in 2017.

Two publications are in-press:

• Sreetharan S, Thome C, Tharmalingam S, Jones DE, Kulesza AV, Khaper N, Lees SJ, Wilson JY, Boreham DR and Tai TC. Ionizing radiation exposure during pregnancy: effects on postnatal development and life. Radiation Research. (In Press)

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• Tharmalingam S, Sreetharan S, Kulesza AV, Boreham DR and Tai TC. Low dose ionizing radiation exposure, oxidative stress and epigenetic programming of health and disease. Radiation Research (In Press).

4.3.4 2017 Research Plan

Data analysis of 2016 mice blood pressure data is in progress. Long-term outcome measurements are required to determine the lifespan implications of in-utero exposures.

In 2017, a new round of mothers will be irradiated at doses of 0, 50, 300 or 1000 mGy following the same procedures as in 2016. These mice pups will be used for behavioral experiments and microPET and glucose tolerance measurements.

4.4 Genetic Models of Risk (2016-2019)

The genetic models of risk program explores the effects of low dose radiation in three ways. First, the biological effects of low-dose radon exposure are being explored in cellular and invertebrate models. Second, the effects of low-dose radiation on immune function are being examined. Finally, the association between low-dose radiation exposure and acute lung injury in both animal models and human hospital patients is being observed.

4.4.1 2015-2016 Research Activities and Results

Since Oct 2015, the Acute Lung Injury (ALI) model has been developed to establish baseline radiation responses in healthy Sprague-Dawley rats. The effects of low, clinically relevant radiation doses are evaluated to examine the acute stages of the immunological response in the lungs and the spleen, an important immune organ. Parameters measured include physiological and histological lung injury, immune cell activity, apoptosis, and gene and protein expression. Changes within these acute time frames can be associated with long-term consequences of exposure to ionizing radiation, such as fibrosis of lung tissue.

In 2016, researchers completed these single-dose healthy animal experiments, then extended into multi-dose response experiments, in which rats were irradiated daily to mimic multiple exposures, such as the daily chest X-rays required for many intensive care patients. Researchers will then translate these baseline findings to models of ALI, including endotoxin (LPS) and mechanical ventilation induced lung injury. These experiments will determine whether low doses of X-rays moderate the immune response, reducing inflammation and damage caused during ALI, while still mounting an effective response.

In April 2016, a site visit was completed by Bruce Power's Doug Boreham to sites at Flinders University and the Environment Protection Authority, specifically the laboratories, animal facilities, ICCU, X-Rad 320 and radon chamber.

2016 RESEARCH COMPLETED

Study #1: Immunological Effects

Aim: To conduct an *in vivo* assessment of the acute immunological effects of clinically relevant radiation doses in a rat model.

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Progress: Institutional ethics approval obtained. Rats (n=90) were irradiated using the X-RAD 320 (Precision X-Ray) with whole body single doses of 0 (sham), 2, 20 and 200 mGy before assessment at 30 min, 6 and 24 h post irradiation. Immunological assays were completed and statistical analysis of results completed. Irradiation of a high dose control group of six rats at 4Gy and subsequent assays were then completed.

Preliminary results were presented at the Australasian Radiation Protection Society Annual Scientific Meeting, Adelaide. Final results were presented at the Radiation Research Society Annual Meeting 2016, Hawaii. An abstract has been submitted to the American Thoracic Society 2017 international conference "*The Effect of low dose Diagnostic Level Radiation on the Lung and Spleen in an in vivo Model*".

Study #2: ICU Radiation Exposure

Aim: To conduct a retrospective audit of daily and cumulative radiation exposure over the course of hospital admission in ICCU patients.

Progress: Ethics approval was received. Patient records were audited retrospectively to assess the daily and cumulative diagnostic procedures prescribed from which radiation doses will be calculated. As length of stay and severity of acute illness are strongly associated with cumulative radiation dose the audit will be limited to patients who have been admitted to ICCU and have a \geq 5 day stay; for a total of approximately 500 patients.

Preliminary results were presented at the Australasian Radiation Protection Society Annual Scientific Meeting in Adelaide and at the Radiation Research Society Annual Meeting 2016, Hawaii "*Exposure levels of low dose radiation in an intensive and critical care unit*". Statistical analysis is complete and a draft paper is in progress.

Study #3: Lung Injury

Aim: To examine the effect of low dose radiation, comparable to diagnostic use within the ICCU, on the innate immune response immediately before and during acute lung injury. The study protocol will consist of three streams. Stream A will include single and multiple (once daily over four days) dose irradiations in 134 rates with the last irradiation concluding 24 hours before induction of acute lung injury. Stream B consists of single dose irradiation of 67 rats immediately after induction of acute lung injury. Stream C includes single and multiple (once daily over four days) dose irradiations of 67 rats with the final irradiation concluding 72 hours after the induction of acute lung injury.

Progress: Ethical approval obtained. Pilot study initiated of 25 rats to investigate the progression of the acute lung injury (30min, 4h, 12h, 24h, and 72h) and determine the optimal time for radiation exposure.

Study #4: Cell Lines

Aim: To understand the effect of both diagnostic (X-rays) and occupational (Radon) radiation on cell viability, proliferation, RNA and protein expression.

Progress: Irradiations began using the X-Rad 320 irradiator for diagnostic exposure (200mGy) of respiratory epithelial cell line A549.Preliminary work completed to establish a radon incubator system at Flinders University.

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Study #5: Radon Exposure

Aim: To examine the acute immunological effects of environmentally relevant doses of inhaled radon gas in a healthy rat model.

Progress: Preliminary authorizations for the use of radon chamber for animal experiments initiated.

4.4.2 2017 Research Plan

In 2017, this research program continues to examine clinically and environmentally relevant doses of x-ray (Study #3) and inhaled radon (Study #5) radiation on pulmonary immune response. These immune responses include oxidative stress, acute physiological and longer term tissue repair and fibrosis in rat models. The effect of radiation doses during acute disease is a focus in 2017.

Parallel *in vitro* experimental programs are being established at Flinders University and at NOSM laboratories. These programs will examine radon and x-ray effects on cell lines at doses ranging from absolute zero (SNOLAB) to those mimicking diagnostic, environmental and occupational exposures.

Study #1: Immunological Effects

In 2017, researchers will complete additional 4Gy irradiations and assays in eight rats. Following this, they plan to complete apoptosis for 104 rats and DNA sequencing of transcriptome in the lung and spleen. Final SMRT results will then be re-analyzed with the inclusion of 4Gy rats and a manuscript submitted. Final results will be presented ("*The Effect of Low Dose Diagnostic Level Radiation on the Lung and Spleen in an in vivo Model*") at the American Thoracic Society Annual Meeting 2017 in Washington, USA.

Study #2: ICU Radiation Exposure

In 2017, researchers plan to submit a manuscript of results for the first chart audit and to present the results "*Diagnostic Radiation in the ICCU: cumulative patient exposures*" at the American Thoracic Society Annual Meeting 2017 in Washington, USA. The chart audit will then be repeated in a similarly sized ICU at Health Sciences North Hospital, Sudbury, ON.

Study #3: Lung Injury

The pilot study and Stream C will be completed in 2017.

Study #4: Cell Lines

In 2017, researchers plan to complete X-Rad cell line experiments. CGL1 cell lines will be transported from Canada to Australia and established. Cell line radon will be initiated. A site visit by Prof Doug Boreham and Dr Chris Thome to Flinders University is planned to assist in the establishment of the radon incubator system. Cell line radon experiments will also be completed at NOSM and SNOLab.

Study #5: Radon Exposure

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In 2017, the radon chamber site animal welfare inspection will be completed. Ethical approval will be obtained. Male Sprague-Dawley rats (n=40) will be exposed to doses of inhaled radon gas equivalent to 0 Bq/m3 (sham), residential regulatory action level (200 Bq/m3; which equates to 3.4 mSv/yr) and the occupational regulatory action level (1000 Bq/m3; 17 mSv/yr). Australian underground uranium miners are, on average, exposed to 400 Bq/m3 inhaled radon annually. Tissue collection and assays will be completed.

4.5 Radiation Tools (2015-2019)

The radiation tools research programs aims to develop new radiation detectors to measure the dose and impact of realistic low-dose radiation exposure. Funding is being provided jointly by the Candu Owners Group and Bruce Power as part of a larger NSERC CRD project. The Bruce Power funding is being used for proton microbeam detector development.

4.5.1 2015-2016 Research Activities and Results

A microbeam is a vital probe in modern radiation biology experiments. However, instrumentation of a proton or alpha microbeam facility is extremely challenging and only a small number of laboratories offer these microbeams worldwide. The McMaster proton microbeam project began in 2005 and a dedicated proton collimation and control system has been built.

To conduct radiation biology experiments using a microbeam, the number of protons hitting a cell or another biological target must be accurately controlled and therefore, reliable operation of the proton counter is vital. To this end, the proton counter must have a high signal to noise ratio, excellent time stability and high throughput. In 2015/2016, a new proton microbeam detector using the Si semiconductor technology was successfully designed. The detector fabrication procedure has already been finalized and a thin Si detector was fabricated in August 2016. Test measurements were then carried out using alpha emitting radionuclides and the proton microbeam.

4.5.2 2017 Research Plan

Proton microbeam test measurements will continue in 2017.

4.6 Naturally Occurring Radioactive Material (2015-2016)

The Naturally Occurring Radioactive Materials (NORMs) project aimed to examine radiation decay in a watershed contaminated by uranium tailings.

4.6.1 2015-2016 Research Activities and Results

Naturally occurring radioactive materials (NORMs) were investigated by collecting water samples and sampling insects in a watershed once contaminated by uranium tailings. Research conducted 30 years ago in 1985 at Laurentian University showed significant contamination of Radium226, heavy metals, acidic pH, and low abundance of insects in the water. Research conducted in 2015 replicated the work done in 1985. Water samples were collected at nine sampling sites in early summer (July) and again in late summer (August) of 2015. The samples were analyzed for Radium-226 and heavy metals. pH measurements and water conductivity were assessed.

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At the same locations, twig bundles were anchored to the riverbed and left for two weeks to attract and capture water invertebrates. Bundles were also deployed at two different dates. Twig bundles were constructed using green alder twigs cut to roughly 20-25 cm and dried at 80oF for 24 hours. Concrete weights were mixed and set into plastic cups with a piece of hose cut to around 17cm with a hole drilled into the top. The twig bundles were fastened at each end using tie wraps and strung through the hole in the hose using nylon string. The first set of bundles were collected three weeks later on July 29 2015, placed into freezer lock bags, and covered with 86% ethanol. The twig bundles were placed into a cooler on ice and transported into the cold room in the lab on the same day. The invertebrates were removed from the twig bundles by gently using small paintbrushes. After all the twigs were washed clean of invertebrates, they were discarded and the invertebrates were removed from the ethanol using a 25mL pipette. The mixture of the invertebrates and dirt were then put through a BD Falcon 40 µm Nylon Cell Strainer and rinsed then placed into a 50mL centrifuge tube. The invertebrates were preserved in 86% Ethanol and Identified. The Invertebrates were identified using the Dorset Environmental Science Center Family classification website.

Radium-226 levels in the nine sample locations collected in 1985 were compared with those collected in 2015.

4.6.2 2017 Research Plan

In 2017, uranium measurement instruments were repaired. A technician has been hired and is being trained in instrument use.

Funding for this research ended in December 2016. Results and a publication will be finalized in 2017.

4.7 Lens of the Eye

The lens of the eye program aims to investigate the development of cataracts following exposure to ionizing radiation. Ionizing radiation exposure to the lens of the eye is a known cause of cataracts. Historically it was believed that the threshold dose for cataract formation was 5 Sv and annual equivalent dose limits to the lens were set at 150 mSv.[R-10] Recently, however, the International Commission on Radiological Protection (ICRP) and International Atomic Energy Agency (IAEA) have reduced their threshold dose estimate for deterministic effects to 0.5 Gy and are now recommending an occupational limit of 20 mSv per year, averaged over 5 years, up to a maximum of 50 mSv in a single year. [R-9][R-11]

A comprehensive review of current evidence for the proposed new 20mSv per year dose limit to the lens of the eye has been accepted for publication.

The research proposal will consist of three streams of research. Stream 1 is an epidemiological study of nuclear energy workers in Ontario. Stream 2 is an epidemiological study of medical workers and patients exposed to diagnostic imaging in Ontario. Stream 3 is an animal study examining chronic radiation exposure in a cataract prone mouse model.

4.7.1 2017 Research Plan

Stream 2 is being initiated, with preliminary data access efforts in progress and pending research ethics board approval. Part 1 of the analysis will examine the association between patient exposure to multiple head CT scans and subsequent cataract formation.

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5.0 ENVIRONMENT, RADIATION & HEALTH

5.1 Environment, Radiation & Health Part 1 – Environmental Impacts (2015-2019)

Part 1 of the Environment, Radiation and Health program is supporting the salaries of researchers working on projects described elsewhere in this report. Bruce Power funding for this research is being matched by a Mitacs grant.

5.1.1 2015-2016 Research Activities and Results

This program supports the salaries of researchers working on:

- Ultra-low dose radiation (three students) see Section 4.1
- DNA Barcoding (two students) see Section 3.3
- Radiation tools and microbeam refurbishment (one student) see Section 4.5

Scientific progress and outcomes and future plans for each of these projects are described in the referenced sections.

5.2 Environment, Radiation & Health Part 2 – Effects of Radiation (2015-2019)

Part 2 of the Environment, Radiation and Health program is exploring the effects of adaptive immunity in prostate cancer patients who have received radiation therapy. In addition, a review of the Linear No Threshold (LNT) radiation science model is in progress. Bruce Power funding for this research is being matched by a Mitacs grant.

5.2.1 2015-2016 Research Activities and Results

5.2.1.1 Adaptive immunity in prostate cancer patients

This study is currently awaiting medical ethics board approval.

5.2.1.2 LNT Review – Literature review

A systematic review of 198 studies published in the last 5 years on low dose radiation exposure in mammals is in progress. Information has been extracted for each study on the specific radiation exposure, the model and the key findings. The studies have been organized by endpoint and tissue type in 16 categories.

The preliminary findings suggest that there needs to be a "paradigm shift" in the understanding of the biological response to low dose radiation and that LNT is not an accurate model for low dose exposures. The recent published data collectively supports a model based on low dose radiation mediated adaptive response.

Preliminary conclusions of the work include:

 Cell-to-cell communication is an underlying mechanism that allows tissues and organs to respond as a whole and not as single cells, thereby providing evidence to refute the traditionally believed "hit theory".

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- Radiation induced genomic instability is not prevalent in the low dose range. The lack
 of genomic instability in A-bomb survivors and other systems helps to further support
 this idea.
- The importance of radiation-induced changes in reactive oxygen species (ROS) status and chronic inflammatory disease represents a new paradigm that moves away from the single hit, single mutation theory of cancer to whole tissue or organ response.
- Current studies point to low dose radiation induced epigenetic modifications (DNA methylation, histone modification, non-coding RNA regulation) which allow permanent cellular transformations without altering the underlying DNA nucleotide sequence. Epigenetic regulation provides another paradigm shift in radiation biology.
- Many studies suggest that low doses may be protective.

5.2.1.3 LNT review – Data re-analysis

Researchers have been working to investigate cancer risk due to inhaled beta-gamma emitting radionuclides in beagle dogs. From the late 1960s through the 1970's the Lovelace Respiratory Research Institute conducted lifespan studies on beagles that inhaled a single dose of ¹⁴⁴Ce, ⁹⁰Sr, ⁹⁰Y or ⁹¹Y imbedded in an insoluble fused aluminosilicate matrix. This existing data is being used to complete three studies.

Study #1 (*Dose rate*) is examining the role of dose-rate on cancer induction in the lung following inhalation of beta-gamma emitting radionuclides. Using the data collected from the Lovelace studies, Bruce Power-funded researchers are re-analyzing the dose-rates of dogs that developed lung cancer. Previous work calculated the dose rate using the cumulative dose at death and over the lifespan. Since each emitter has a different half-life, the dose rate used for previous work is believed to be incorrect. This study aims to calculate the correct dose rate and examine its association with lung cancer induction. Researchers plan to complete and submit one manuscript for this study by the end of 2017.

Study #2 (*Lung inflammation*) will investigate the role of inflammatory disease in radiation induced lung cancer in these dogs. Previous work using this data on the induction of cancer in these dogs found that higher doses lead to cancers, mainly lung cancer. However, the dogs that received a total dose >25 Gy had fewer incidences of cancer than the control dogs. Bruce Power-funded researchers are currently analyzing the dogs that received a cumulative dose of >25 Gy. The status of the lungs of the dogs that developed lung cancer at time of death is being investigated with focus on incidences of lung disease and inflammation. Researchers believe that the lung cancer induced by the beta-gamma emitters may have been due to chronic lung inflammation.

Study # 3 (*Liver cancer risk*) will use data from the Lovelace Institute on the lifespan studies of beagle dogs that inhaled a single dose of ¹⁴⁴CeCl₃ in fused aluminosilicate. Researchers will investigate liver cancer risk from internally deposited radioactive materials. Combining the data from these dogs with the data from the NCRP report on liver cancer risk from internally-deposited radionuclides will produce a liver cancer risk number with a focus on the low dose region.

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5.2.2 2017 Research Plan

5.2.2.1 Adaptive immunity in prostate cancer patients

Medical ethics board approval is expected in 2017 followed by study initiation. Researchers at McMaster University have begun developing assays for use during the study.

5.2.2.2 LNT Review

Data organization and analysis is in progress for the Study #2 (*Lung inflammation*). Two manuscripts have been prepared to date.

The first submitted manuscript focused on the role of dose-rate on cancer induction in the lung following inhalation of beta-gamma emitting radionuclides. A different dose calculation rate is proposed in this manuscript.

The second manuscript evaluated the role of cell killing, tissue disorganization and tissue damage on the induction of lung cancer following low dose rate radiation due to inhaled beta-gamma emitting radionuclides in beagle dogs. This manuscript is near completion and will be submitted for publication in 2017. An abstract with the data from this study has been submitted to the Radiation Research Society annual meeting to be presented in October.

Study #3 (Liver cancer risk) will be initiated in 2017.

The combination of the three studies will add to scientific knowledge of cancer risk at low doses of radiation.

6.0 HEALTH

6.1 Aboriginal health (2014-2016)

The goal of this project was to examine immunity to *Haemophilus influenza type a* (Hia) and *Streptococcus pneumoniae* (pneumococcal) bacteria among Saugeen First Nation members. Bruce Power funding for this research was matched by a Mitacs grant.

6.1.1 2014-2016 Research Activities and Results

Background

North American Indigenous people experience an increased burden of several infections compared to the general population. In particular, there is a high incidence of bacterial Hia and pneumococcal respiratory infections in young Canadian Indigenous children.

Socioeconomic and environmental factors are undoubtedly responsible for some of the high susceptibility to infections in certain populations. However, the dynamic interactions between the virulence factor of the pathogen and immunological mechanisms of the host ultimately determine whether an individual exposed to an infectious agent develops clinical disease and how severe the disease will be.

Specific Objectives

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Objective 1: Engage with the Saugeen First Nation (Bruce County) and develop partnerships between NOSM and this community to collect blood samples for laboratory testing.

Objective 2: Analyze antibody concentrations and functional activity against Hia and pneumococcus disease using serum bactericidal assay and enzyme-linked immunosorbent assay (ELISA) to test collected serum specimens.

Significance

The results of this study are important for understanding immunological factors underlying an increased susceptibility of Canadian First Nations to serious bacterial infections and may help to improve current immunization policy to prevent infections in susceptible populations. The data on serum concentrations of specific antibodies directed against antigens of *Haemophilus influenzae* and *Streptococcus pneumoniae* among First Nations of Northwestern Ontario will help to interpret the new data obtained from Saugeen First Nation in the context of different environmental conditions. This comparison will help to optimize immunization strategies in these higher risk communities and will have a positive impact on Public Health.

Research activities

Continuing engagement and collaboration with First Nations communities has been a key component of this research. The research strongly adheres to the principles outlined in "Ownership, Control, Access, and Possession (OCAP) or Self-Determination Applied to Research" and "Research Involving the First Nations, Inuit and Métis Peoples of Canada" as outlined in Chapter 9 of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. This project is community based participatory research that is guided by the needs of the community to ensure the fostering of relational and ethical collaborations.

Over the last 3 years, the researchers successfully established a collaborative partnership with the Saugeen First Nation (Bruce County) community. The community engagement process included meetings with Chief, health director, health team and community health representative as well as presentations and information sharing sessions familiarizing the community with the research project. In addition, a Community Research Assistant (CRA) position was established, and a person was hired to help with recruitment of study participants.

Because of the community engagement process, a collaborative research agreement was signed between Saugeen First Nation and the Northern Ontario School of Medicine. In accordance with this agreement, all adult Saugeen First Nation community members were invited to participate in the study. After obtaining written informed consent, blood samples were taken from 50 adult volunteers. These samples were processed for antibody analysis and bactericidal activity in 2016 using standard procedures.

In August of 2016, Dr. Eli Nix traveled back to the Saugeen First Nation community. This visit coincided with the annual community health fair. Dr. Nix was given space for a booth where he engaged community members who attended. As part of the research plan, the health director received an informal project update and a draw was held for a big screen television from among the 50 community members who had contributed blood specimens for the study.

The results of these analyses will be used to understand the natural immunity of the population, the potential spread of infection, and the need for protection of infants and young

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children. Analysis of naturally acquired antibodies against Hia and *Streptococcus pneumoniae* along with data on Hia carriage in First Nations communities will help to understand within-community risk factors for contracting infection.

Information gathered in this study will also be important for determining target populations for immunization and for the development of optimal immunization strategies.

6.1.2 2017 Research Plan

Funding for this project is complete. Publication of the findings in a peer-review journal is expected to occur in 2017.

Bruce Power expects to renew funding for related aboriginal health research in 2017.

7.0 CONCLUSION

Since 2013, the Integration Department has facilitated the funding and progress of independent academic research into interactions between Bruce Power and the environment and the measurement and effects of low-dose radiation on human and animal models. We have also supported programs exploring Aboriginal-specific health issues.

From 2013 to 2017, we facilitated the funding and progress of independent academic research in several areas. Bruce Power is planning to support relevant academic research with over \$1 million annually in direct research funding over the next 5 years. Current research programs explore the importance to Bruce Power of effects of low-dose radiation and interactions with the environment. We also support programs exploring Aboriginal-specific health issues.

Bruce Power support allows researchers to succeed in applying for competitive, peerreviewed funding from federal and provincial agencies. The receipt of these matching funding grants demonstrates the scientific rigor of the independent academic research that Bruce Power is supporting. Research findings continue to be published in peer reviewed academic journals and are available to all interested parties.

We expect that researchers will continue to make excellent progress in 2017 and the Integration Department at Bruce Power looks forward to seeing the results of their research. Research results will be incorporated into relevant regulatory and business initiatives.

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Enclosure 3

Bruce Power's Response to SON's April 23, 2018 Written Submissions

NK21-CORR-00531-14285 NK29-CORR-00531-14980 NK37-CORR-00531-02956

Bruce Power's Response to the SON's April 23, 2018 Written Submissions

<u>Overview</u>

On April 23, 2018, the Saugeen Ojibway Nation ("SON") filed a written submission with the Canadian Nuclear Safety Commission ("CNSC") regarding Bruce Power's application to renew its Nuclear Power Reactor Operating Licence for the Bruce Nuclear Generating Stations (the "Site") for 10 years and to undertake certain life extension activities, including Major Component Replacement ("MCR") for six reactors (the "Application"). This submission raises a number of concerns and suggests that the Crown has failed to meet the duty to consult and that Bruce Power has not met the statutory requirements under the *Nuclear Safety and Control Act* ("NSCA") for license approval.

Bruce Power respectfully disagrees with both assertions. With respect to the duty to consult, there has been significant and robust consultation with the SON about the Application by CNSC staff and Bruce Power. Bruce Power has made repeated efforts to engage the SON about the Application since 2015. It has undertaken consultation in good faith and meaningful measures to address the SON's concerns have been proposed by Bruce Power and CNSC staff, who have been separately engaging with the SON. This includes additional environmental study and monitoring programs, an alternative mitigation measures study, additional potential fisheries offset projects to benefit the SON fishery, and the opportunity to participate in and shape the scope of a 3-year climate change study that was recently announced by Bruce Power and the Council for the Great Lakes Region.

Bruce Power values its relationship with the SON and is working to strengthen this relationship. It has gone beyond what is legally required in engaging the SON on this Application. At law, any duty to consult owing would be at the low end of the spectrum given that the impacts of the Application on the SON's asserted and established Aboriginal or treaty rights are at most minimal and likely not appreciable. Moreover, even if a duty of deep consultation is owed (which it is not), it would be met based on the level of consultation that has taken place and the significant measures that have been proposed in response to the SON's concerns.

The SON have taken the position that a decision on MCR cannot be made until the proposed accommodation measures are implemented. There is no duty to accommodate in this case given that the impacts of the continued operation of the Site on SON Aboriginal and treaty rights are at most minimal and likely not appreciable. Even if accommodation measures were required, they do not need to be implemented before making a decision on MCR. Accommodation measures for energy and resource development projects are frequently implemented after a Crown decision is made and there have been numerous court decisions, including decisions of the Supreme Court of Canada that have determined that the duty to consult was met where the accommodation measures would be implemented in the future. The SON have also not taken advantage of all of the consultation opportunities afforded to them in this process, dating back to 2015, which could have been used to address their concerns earlier in the process. Despite this and Bruce Power's conclusions about impacts of the Application, the company is committed to ongoing engagement and to be as a responsive as reasonably possible to the issues raised.

With respect to the statutory requirements, the Commission has ample and reliable evidence before it to satisfy itself that Bruce Power will make adequate provision for the protection of the environment, which is the focus of concern of the SON. This has been demonstrated in this

Application and in previous processes where similar activities were predicted with rigorous follow-up programs.

Moreover, this is not just the position of Bruce Power. The CNSC staff have reviewed the Application and concluded that Bruce Power meets the statutory requirements under the Nuclear Safety and Control Act—ie. that, in respect of the Application, Bruce Power has and will continue to make adequate provision for the protection of the environment and the health of persons. Further, CNSC staff concluded that the potential risks to the environment are generally low to negligible.

It is important to underscore that this is not a greenfield project but a continuation of activities that have been underway since 2001. It is a facility that has been safely operating for decades and has undergone numerous environmental assessments and follow-up monitoring programs, which have confirmed that the Site has no significant adverse effects on the environment as predicted. This is also not the first life extension of the Site. Two of the eight reactors were previously refurbished and restarted in 2012, extending the life of the facility to 2043. The CNSC staff have independently reviewed Bruce Power's environmental performance each year over the past 17 years (since 2001) and determined that Bruce Power has continued to implement and maintain an effective environmental protection program at the Site that met CNSC requirements.

The SON raise numerous concerns about the ERA/PEA methodology and the data relied upon by Bruce Power. These are fundamentally disputes about the level of scientific certainty required to make decisions under the NSCA. While Bruce Power disagrees with the SON's views on these issues, it has (along with CNSC staff) proposed measures to further study and monitor areas of particular concern to the SON. This information can be incorporated into future ERAs and PEAs, which will be updated every five years or earlier if there are significant changes in operations or in the science on which the ERA is based. The next ERA and PEA will be submitted to the CNSC in 2022 prior to the restart of the first refurbished reactor in December 2023 under the current proposed schedule. As a lifecycle regulator, CNSC staff can direct Bruce Power to take further action in response to any updated information. Moreover, the predictive assessments made are based on actual performance of identical activities previously carried-out over many years.

In considering the SON's submissions, it is important to have regard to what the Commission is being asked to determine in this Application. The Commission is not making a decision on whether Bruce Power can operate the Site until 2064. It is being asked to determine whether Bruce Power can operate the Site until 2028, during which it will undertake MCR activities. These activities would extend the life of certain reactors but the ability to operate those reactors after 2028 will depend upon Bruce Power being able to obtain further operating licenses from the Commission, at which time it will review further assessments and determine whether Bruce Power will continue to make adequate protection for the environment and the health of persons. Bruce Power will also be required to obtain necessary permit renewals which will require additional ongoing review of impacts to fish from impingement, entrainment, and thermal effects, which is the overarching area of concern to the SON.

This regulatory framework, in addition to annual independent monitoring by the CNSC, provides for significant and robust oversight of Bruce Power's operations which will verify that Bruce Power is continuing to make adequate provision for the protection of the environment during the licensing period. For all of these reasons and the reasons set out below, Bruce Power has met the statutory requirements and any duty to consult owing has been fulfilled.

1. Any Duty to Consult Owing Has Been Fulfilled

As noted above, this is not a greenfield project or the first life extension. The ongoing operations and life extension activities will take place entirely within the existing facility footprint of the Site. Courts have held that brownfield projects, similar to the current Application, generally fall at the low end of the consultative spectrum if the duty to consult is triggered.¹ It is Bruce Power's position that any duty to consult owing in this case would be at the low end of the spectrum and that even if deep consultation was required it would be met on the facts of this case.

By way of background, in order to trigger the duty to consult, there must be a Crown decision that has an appreciable or discernible adverse impact on an asserted or established Aboriginal or treaty right. The duty is not triggered by speculative impacts and where there is an existing facility the focus is on the impacts of the specific decision at issue and whether it has the potential to cause a novel adverse impact on an asserted or established right.²

If the duty to consult is triggered, the level of consultation is proportionate to a preliminary assessment of the strength of the claim and the seriousness of the potential impact of the proposed government action on the asserted or established Aboriginal or treaty right at issue. The level of consultation required falls along a spectrum, ranging from mere notice at the low end to deep consultation at the high end.³

In their written submission, the SON takes issue with the determination of CNSC staff that any duty to consult owed in this case is at the low end of the spectrum. The SON suggest that the CNSC staff have erred in their interpretation of what constitutes a "novel adverse impact" for the purposes of triggering the duty to consult and determining the extent of consultation that would be required. Even if the Commission accepted the SON's interpretation of "novel", it would not change the level of consultation owed because the impacts (whether novel or not) are still at most minimal which gives rise to a duty to consult at the low end of the spectrum.

The SON have raised numerous environmental concerns with a specific focus on impacts to the aquatic environment. Bruce Power acknowledges that there is a minimal loss of fish every year due to impingement and entrainment. However, the SON have not explained how these impacts (or any other impact of the Site) is having or will have an appreciable impact on their asserted or established rights, including their established commercial fishing right for sustenance purposes.

Under their Substantive Commercial Fishing Agreement with the Ministry of Natural Resources and Forestry, the SON are required to collect data on the commercial fishing effort of all SON commercial fisherman, including any species caught, the location of all catches, and the length

² Rio Tinto Alcan Inc. v. Carrier Sekani Tribal Council, [2010] 2 SCR 650 (SCC) at paras. 46 & 49.

¹ Wabauskang First Nation v Minister of Northern Development and Mines et al, 2014 ONSC 4424, at paras 8, 226, 234 (duty to consult and accommodate must be considered in the context of a mine's location on an abandoned brownfield site that had already undergone considerable historical development and mining); *Louis v British Columbia (Minister of Energy, Mines, and Petroleum Resources)*, 2013 BCCA 412 at paras 84, 85, 88, 92 (building of a mill on virtually the same footprint as existing facilities to be demolished would trigger a minimal level of consultation); *Athabasca Regional Government v. Canada (Attorney General)*, 2010 FC 948 at paras 217–219, 221 (if the duty to consult was triggered at all in respect of a ten-year licence renewal for an existing uranium mine and mill, it would be at the lower end of the spectrum); *Brokenhead Ojibway First Nation v Canada (Attorney General)*, 2009 FC 484 at paras. 42-45 (the duty to consult was at the extreme low end of the spectrum where the three pipeline projects at issue had been built almost completely over existing rights-of-way and on privately owned and actively utilized land that was not likely to be available for land claims settlement).

³ Haida Nation v. British Columbia (Minister of Forests), [2004] 3 SCR 511 (SCC) ("Haida") at paras. 39 and 44.

of time that nets were in the water,⁴ The SON declined Bruce Power's request to share this data and the SON have not used this data or any other data to demonstrate that the Site is having a specific impact on their asserted or established rights. Given how long the Site has been operating and the absence of concrete information on impacts, it is reasonable to conclude that the impacts are at most minimal. This is further supported by the fact that:

- Impingement and entrainment losses of local commercial species (Lake Whitefish, Lake Trout, Walleye, and Yellow Perch) is very small and equates to ~1% of the SON's annual total catch limit for Lake Whitefish along in the Lake Huron Fisheries Management Zone;
- The SON's harvest has been consistently well below than their total catch limit for Lake Whitefish (65% of their total catch limit in 2014 and 51.1% of their total catch limit in 2014);
- The total catch limit for Lake Whitefish is jointly set by the SON and the Ministry of Natural Resources and Forestry ("MNRF") to ensure the sustainability of the resource; and
- To Bruce Power's knowledge, there have been no Total Catch Limits set for other species despite the SON's ability to request a TAC for another species to ensure the sustainability of the resource.⁵

Even if the Commission accepted the SON's position that a duty of deep consultation is owed in this case, it is Bruce Power's position that such a duty would be met given the significant and robust consultation that has taken place in this case. In particular:

- The SON have been receiving information about the Application since December 2015 and were provided a copy of the Application in July 2017;
- The SON did not start engaging with Bruce Power about the Application until December 2017 but since that time have had 9 meetings with Bruce Power including:
 - 6 meetings to discuss issues and questions relating to the Application and associated regulatory approvals, which have included several presentations, a tour of the facility to help better understand the impingement and entrainment process, and the provision of additional information in response to questions;
 - 3 meetings between the leadership of Bruce Power and the SON to discuss employment, training, and business opportunities from the Site and life extension investments and ways to enhance the SON's involvement in regulatory decisionmaking, environmental monitoring, and stewardship activities, which included some discussions relating to the Application.
- The SON have had at least 4 meetings with CNSC staff and exchanged detailed correspondence;
- Both Bruce Power and CNSC staff have engaged in good faith and demonstrated significant responsiveness by proposing a number of additional measures to address the SON's concerns, including additional environmental study and monitoring programs, an

⁴ The Substantive Commercial Fishing Agreement between the SON and the Minister of Natural Resources entered into on February 25, 2013 was amended and restated on February 20, 2018. The current and previous Section 5.3 provides that "SON agrees that SON commercial fishers shall report to SON their daily catch for all species and their fishing effort, including location of catch, gear types and the length of time nets were in the water in accordance with Schedule D." Under Section 5.4, the SON agrees to share this information with MNR in accordance with the process set out by the Governance Committee.

⁵ Section 4.6 provides that "In order to ensure the sustainability of the resource, the TAC Working group shall recommend to the Governance Committee no later than June 15 in each year an appropriate TAC for lake whitefish, and any other species for which it is of the view that TAC should be established, for Zones 1, 2, and 3

alternative mitigation measures study, additional potential fisheries offset projects to benefit the SON fishery, and the opportunity to participate in and shape the scope of a 3year climate change study that was recently announced by Bruce Power and the Council for the Great Lakes Region; and,

 The SON have had the opportunity to make detailed written submissions and will have the opportunity to make oral submissions to the Commission.

Further details on Bruce Power's engagement with the SON can be found in the SON Community Interest Report (the "SON CI") and the Supplementary SON Community Interest Report (the "Supplementary SON CI"), which Bruce Power has filed with the Commission.

In their written submission, the SON have taken the position that a decision on MCR cannot be made until specific accommodation measures outlined by the SON are agreed to and implemented. This position is inconsistent with the law on the duty to consult.

First, there is no stand-alone duty to accommodate and accommodation is not required in this case given that the duty is at most at the low end of the consultation spectrum. Moreover, even if it were required, the Supreme Court of Canada has confirmed that there is no duty to agree, the duty to consult does not provide a veto over government decision-making, and that "section 35 guarantees a process, not a particular result."⁶ Where accommodation is required, it involves "seeking compromise in an attempt to harmonize conflicting interests" and necessitates balancing Aboriginal concerns with other societal interests, as "balance and compromise are inherent in the notion of reconciliation".⁷

Second, the SON has not taken advantage of earlier consultation opportunities available to it which could have been used to address its concerns earlier in the process. These earlier opportunities were identified in a 5-year look-ahead provided in December, 2015 in response to previous concerns raised by the SON about having more notice and time for engagement relating to upcoming regulatory approvals. The SON did not start substantively engaging with Bruce Power on the Application until December 2017, almost two years after Bruce Power first started providing information to the SON and attempting to engage with it about the Application.

In *Ktunaxa*, the Supreme Court of Canada recently reaffirmed that consultation is "a two-way street" and Aboriginal groups also have obligations to engage in good faith and clearly set out their concerns and claims as early as possible, facilitate the process of consultation and accommodation, and to not frustrate reasonable good faith consultation attempts or take unreasonable positions.⁸

Third, in the event accommodation measures were in fact required which they are not in this case, accommodation measures for energy and resource development projects are frequently implemented after a Crown decision is made. There have been numerous court decisions that have determined that the duty to consult was met where the accommodation measures would be implemented in the future, including decisions of the Supreme Court of Canada.⁹ The SON's

⁶ Ktunaxa Nation v. British Columbia, [2017] 2 SCR 386 (SCC) ("Ktunaxa") at para. 79 and Haida at para. 42. See also Mikisew Cree First Nation v. Canada, [2005] 3 SCR 388 (SCC) at para. 66; Beckman v. Little Salmon/Carmacks, [2010] 3 S.C.R. 103 (SCC) at para. 14; and Yellowknives Dene First Nation v. Canada, [2015] F.C.J. No. 829 (FCA) at para. 56.

⁷ Haida at paras. 49-50.

⁸ Ktunaxa Nation at paras 79–80.

⁹ See, for example, *Chippewas of the Thames v. Enbridge Pipelines Inc.,* [2017] 1 SCR 1099 (SCC) paras. 51-57 and *Taku River Tlingit First Nation v. British Columbia,* [2004] SCC 74 (SCC) at paras. 45-46.

reliance in the *Gitxaala* decision in its submissions is misplaced as the decision is distinguishable. Unlike in *Gitxaala*, this is not the last opportunity for Crown consultation for the life of the facility.¹⁰ If the licence is approved, there will be further consultation by the Crown on federal and provincial permitting as well as additional Crown consultation for subsequent licence renewals. Moreover, the decision at issue in *Gitxaala* was an approval for a greenfield project that would lay over 1,000 km of new pipeline and significantly increase tanker traffic in the Douglas Channel in northem B.C.. It was not a licence renewal or refurbishment of an existing facility that had been safely operating for decades.

For all of the reasons above, it is Bruce Power's submission that any duty to consult owing in this case has been fulfilled.

2. Bruce Power Has Met the Statutory Requirements Under the NSCA

i. The Site Has Been the Subject of Numerous Environmental Assessments

In their submission, the SON have raised numerous concerns with the methodology of the Environmental Risk Assessment and the Predictive Effects Assessment and suggested that they cannot be relied upon by the Commission to determine whether Bruce Power will make adequate provision for the protection of the environment. Before addressing the SON's specific concerns, it is important to underscore that the ERA/PEA cannot be assessed in a silo without regard to Bruce Power's operating history and the prior environmental assessments that have been undertaken at the Site.

Since Bruce Power took over operations of the Site in 2001, a number of environmental assessment studies were conducted at key licensing and operational milestones under the *Canadian Environmental Assessment Act, 1992* and the NSCA. These have included:

- 2002 Environmental Assessment Study Report for the Bruce A Units 3 & 4 Restart, which concluded that no significant adverse effects were predicted and required follow-up monitoring which confirmed the results of the assessment;
- 2004 Environmental Assessment Study Report for the Bruce B New Fuel Project which assessed impacts assuming continued operations at the Site to 2043 and concluded that no significant adverse effects were predicted;
- 2005 Environmental Assessment Study Report for the Bruce A Refurbishment Project (Units 1 & 2 Restart) which assessed impacts assuming continued operations at the Site until 2043 and concluded that no significant adverse effects were predicted and required follow-up monitoring which confirmed the results of the assessment;
- 2008 Environmental Impact Statement for the Bruce New Nuclear Power Plant Project which assessed impacts assuming continued operations of the Site to 2084 including for a period of time where 12 reactors would be operating at the same time and concluded that no significant adverse effects were predicted (this project was eventually withdrawn from the regulatory review process).

¹⁰ In *Gitxaala*, the majority noted at paragraph 237 that the Governor in Council's decision was a high-level strategic decision that sets in motion risks to the applicant/appellant First Nations' Aboriginal rights and that further consultation, as contemplated by the Joint Review panel conditions would not involve the Crown and future decision-making lies with the National Energy Board. This was prior to the Supreme Court of Canada's decisions in *Clyde River* and *Chippewas of the Thames* clarifying that NEB decisions can trigger the duty to consult even though it is not strictly speaking the Crown or an agent of the Crown. Notably, there has never been a similar dispute regarding the CNSC as s. 8(2) of the NCSA stipulates that the Commission is for all its purposes an agent of the Crown.

- 2015 Environmental Risk Assessment which assessed impacts assuming continued operations of the Site to 2043 and determined any impacts to the environment were low to negligible (not significant); and
- 2017 Environmental Risk Assessment and Predictive Effects Assessment which assessed impacts assuming operations of the Site to 2064 and determined any impacts to the environment were low to negligible (not significant).

With the completion of each of the above environmental assessments, progressively more environmental data has been collected for the Site, and follow-up monitoring has continued to confirm that the assessment approach used in the environmental assessments were sufficiently conservative and effects were as predicted or lower. Furthermore, Bruce Power has continued to study how Bruce A and Bruce B interact with the environment, both to support regulatory applications and address stakeholder concern. These studies have generally increased in scope and nature over time from the original environmental assessments, commensurate with stakeholder expectations and industry practices.

In addition, environmental monitoring at Bruce Power has continued to collect environmental data as part of regular operations. Results of Bruce Power's environmental monitoring are reported annually to the CNSC in the Annual Environmental Monitoring Program Reports, which are also publically available on the Bruce Power external website and shared with stakeholders. The CNSC also completes independent environmental monitoring surrounding the Site. All of these studies demonstrate that the Site is operating as expected, and that risks to the environment are low to negligible.

To support licence renewal, Bruce Power prepared and submitted in 2017 an Environmental Risk Assessment (ERA) and Predictive Effects Assessment (PEA) as part of the Application. The 2017 Baseline ERA updates the ERA submitted in 2015, and a Screening Level Risk Assessment submitted prior to that, as well as all of the above-mentioned environmental assessments. The ERA is based on hundreds of environmental reports with environmental quality data and information related to habitats and human use of the area. These reports represent the culmination of decades of environmental monitoring at the Site. Furthermore, additional baseline data was collected in 2016 at numerous locations across the Site and surrounding area, over multiple seasons, with effort made to align the locations, updated habitat mapping, align with historic sampling locations to allow for identification of trends in time, if required.

Environmental monitoring at the Site will continue throughout future operations and will allow continual risk-based decision-making related to potential effects on the environment.

ii. The SON's Concerns Related to the ERA and PEA

In their submission, the SON assert that the Environmental Qualitative Risk Assessment ("**ERA**") and the Predictive Effects Assessment ("**PEA**") are deficient because they both rely on flawed data and methodologies.

Bruce Power disagrees with these assertions. Both the ERA and PEA rely on years of data collected through numerous studies and monitoring and analyze such data in accordance with best scientific practices for environmental risk assessments. Notwithstanding that all legal requirements have been met or exceeded for the ERA and PEA, Bruce Power remains committed to working with the SON to undertake further assessments and monitoring in an effort to address their concerns and narrow areas of disagreement over assessed impacts.

Environmental risk assessment is a systematic process used to quantify and characterize the risk posed by contaminants and stressors on the environment. The objective of an ERA is to evaluate the risk to humans and the environment from potential effects from the operation of a project or site (in this case the continued operation of Bruce A and Bruce B, including Major Component Replacement) and recommend further action or assessment based on the results. The ERA uses a tiered approach starting from a broad evaluation using protective generic parameters and a high degree of conservatism (i.e., precautionary approach, overestimated risk) and, in areas where potential risk is identified, progressively developing the assessment towards a more precise analysis (i.e., site-specific, realistic and more detailed parameters) with conservatism removed.

Bruce Power's ERA was developed using widely accepted procedures and best practices in the nuclear industry for pathway analysis, exposure and dose derivation, and risk characterization. A full list of sources is included in the Baseline ERA¹¹ and includes guidance from Health Canada ¹²¹³the United States Environmental Protection Agency^{14 1516}), the Canadian Council for Ministers of the Environment (CCME) ¹⁷¹⁸as well as various other agencies and literature. The guiding document for development of the Baseline ERA was the Canadian Standards Association (CSA) Standard N288.6-12 Environmental Risk Assessment at Class I Nuclear Facilities and Uranium Mines and Mills. This CSA Standard incorporates best practices used in Canada and internationally and was developed specifically to provide guidance for Class I Nuclear Facilities and uranium mines and mills on the preparation of ERAs for regulatory reviews.

When assessing effects in an ERA or EA, it is not possible to directly assess the risk for each individual species. Therefore, to focus the assessment, the ecosystem is divided into components (e.g., plants, invertebrates, birds, mammals, and fish). For birds, mammals and fish, a number of representative species were therefore selected. These ecological receptors, or Valued Ecosystem Components ("VECs") were selected for the ERA with consideration of their susceptibility to exposure to effects from the Site, their interest or significance to Indigenous communities or other stakeholders, their conservation status, their use in previous assessments at the site, and various other factors.

As part of the first tier of the assessment, the ERA considered all of the possible routes for exposure to these stressors. Environmental stressors include physical (e.g., fish impingement,

¹¹ Bruce Power. 2017. Bruce Power 2017 Environmental Quantitative Risk Assessment.

¹² Health Canada. 1994. Appendix B: Criteria for Classification of Carcinogenicity. In: Canadian Environmental Protection Act, Human Health Risk Assessment for Priority Substances. Ottawa, ON.

¹³ Health Canada. 2010. Federal contaminated Site Risk Assessment in Canada, Part I: Guidance on Human Health Preliminary Quantitative Risk Assessment (PQRA). Version 2.0. Contaminated Sites Division, Safe Environments Programme, Ottawa, Ontario.

¹⁴ U.S. EPA. 2000. Supplemental Guidance to RAGS: Region 4 Bulletins, Human Health Risk Assessment Bulletins. EPA Region 4, originally published November 1995, Website version last updated May 2000 (currently under revision). Available online from: http://www.epa.gov/region4/waste/ots/healtbul.htm. Website last accessed December 12, 2009 (no longer available). ¹⁵ U.S. EPA (United States Environmental Protection Agency). 2012. EPISuite version 4.11.

¹⁶ U.S. EPA (United States Environmental Protection Agency). 2013. Integrated Risk Information System (IRIS), last updated June 25, 2014. Available online from: http://www.epa.gov/iris/. Last accessed June 30, 2014.

CCME (Canadian Council of Ministers of the Envrionment). 1996. A Framework for Ecological Risk Assessment: General Guidance. Winnipeg, MB.

¹⁸ CCME. 1997. A Framework for Ecological Risk Assessment: Technical Appendices. Winnipeg, MB.

entrainment, temperature etc.), chemical (e.g., non-radiological contaminants) and radiological factors. In this first screening, exposures were compared to the most restrictive applicable noeffect benchmark available from federal, provincial/territorial guidelines and available literature. Potential exposures below these "no observable effects" levels thresholds would not be expected to affect an individual, and therefore have no potential effect at the population level. Where possible risks are estimated (i.e., near or exceeding no effect benchmarks), further assessment was completed to characterize the risk as part of the next tier of assessment.

In the second tier, "the risk characterization" step of the ERA, information from the exposure and toxicity assessments are combined to determine if an overall potential risk exists to the receptors. Risks were estimated quantitatively where possible, by comparing the estimated exposures to the derived benchmarks, which were set for each receptor and stressor (referred to as hazard quotients, or "**HQ**"). Hazard quotients of less than or equal to one are associated with negligible risk, as it means the calculated risk is less than accepted overall effects thresholds. Doses to receptors calculated as part of this step considered multiple pathways of exposure, including direct and indirect pathways such as ingestion of vegetation and/or prey.

Finally, for those stressors where risk cannot be discounted as part of the preliminary risk characterization, or where there is significant community interest, detailed quantitative risk assessment is performed to further characterize the effect, remove uncertainty and make recommendations for monitoring or mitigation, as appropriate. In the Baseline ERA, this was completed for potential effects on the aquatic environment from physical stressors, specifically impingement and entrainment ("**I&E**") and thermal. These studies are described further in sections below.

A predictive environmental risk assessment is used to help define potential effects before an activity is initiated in order to ensure that potential effects that could occur are acceptable. The PEA was prepared by Bruce Power to support the CNSC's completion of the environmental assessment under the NSCA. The PEA was prepared to determine and evaluate, using the substantial body of existing monitoring and assessment data, the effects of future site activities. The approach applied in the PEA was a modification to the tiered assessment process defined in CSA Standard N288.6-12, as described above. This assessment is based on actual performance of identical activities previously carried out over many years.

As a first tiered screening in the PEA, the interaction was considered for whether a) baseline is bounding of predictions (e.g., in the case of reductions), or b) where baseline may not be bounding, are risks below acceptable thresholds. For cases where baseline was not bounding, past environmental assessments and follow-up monitoring were reviewed to consider whether effects would be similar to those already experienced on Site and whether they were below thresholds. "Bounding" is used in the assessment when effects (in this case baseline or previous operations) are likely to be equal or higher in magnitude than those considered in the future. The PEA considered the future operation of the site until 2064, however, was most focused on activities within the next 10 years, which is the licensing period that is the subject of the Application.

Overall, the ERA and PEA conclude that the risk from existing and future physical stressors and from radiological and non-radiological releases to the environment from the Site are generally low to negligible. Potential environmental effects of future activities are anticipated to be similar to those of existing operations and/or those observed during refurbishment of Units 1 and 2.

Report of Marc Cadotte

In their submission, the SON enclosed a report from Professor Marc Cadotte who raises several criticisms of the ERA and PEA. Bruce Power's responses to these criticisms are set out below.

Professor Cadotte's Criticisms of the ERA

1. The report does not assess important sub-lethal and indirect effects on organisms, which can result in important ecological changes to the distribution and abundance of species and alter their interactions.

Bruce Power disagrees with the assertion that sub-lethal and indirect effects are not considered in the ERA. The ERA uses a tiered approach that starts with a broad evaluation using protective generic parameters and a high degree of conservatism (*i.e.*, precautionary approach, overestimated risk) and, in areas where potential risk is identified, the assessment is developed towards a more precise analysis (*i.e.*, site-specific, realistic and more detailed parameters). In the ERA, potential exposures were first compared to the most restrictive applicable federal or provincial/territorial guidelines, or literature value. Where possible, these were "no observable effect" levels, which are well below thresholds that would have a lethal effect. Potential exposures below no observable effects levels would not be expected to affect an individual, and would therefore not have a potential effect at the population level.

Where possible risks were estimated (i.e., near or exceeding benchmarks), further investigation was completed in the ERA. During this step of the assessment, for ecological receptors, risks were estimated qualitatively based on scientific judgement or quantitatively by comparing the estimated exposures taking into consideration the degree to which a fish or wildlife receptor could be directly or indirectly exposed to a stressor as a result of its foraging behaviour, feeding habits and habitat. The resultant dose or exposures was then compared to derived benchmarks considering both direct and indirect pathways of effect. Where risks are determined to be negligible (i.e., HQ<1), there is a high degree of confidence that no adverse effects would be expected. Again, HQs were set using benchmarks well below lethal levels of effect. Therefore, based on the above, sub-lethal effects were considered directly in the ERA, and all effects are assessed to be of low to negligible magnitude.

2. No consideration of interactive effects among stressors. For example, chemical exposure and seasonality or variation in precipitation.

The ERA considers the potential for combined and/or interactive effects among stressors. As described in the response above, exposures are first screened for their potential to exceed thresholds and benchmarks established to be protective of both individuals, where available, and populations. Where no risk is identified through an individual pathway by screening against these benchmarks, there is no potential for them to act additively with other stressors. Where potential risk is identified in more than one pathway, they are considered together as part of the Tier 2 non-radiological and radiological assessment.

Further, the ERA is also supported by the results of recent combined stressor research (presented in Section 5.3.1.5 of the ERA), which demonstrates that levels of stressors required to result in an additive or synergistic effect are substantially higher than those experienced at the Site. Current available research indicates that combined stressors showed an effect (e.g., morpholine and gamma radiation; thermal and morpholine) at levels substantially higher than those observed during monitoring at the Site.

For parameters more susceptible to seasonal variations (e.g., water quality), samples were collected over multiple seasons and over multiple years. Monitoring was also specifically conducted where possible in 2016 during a Station Containment Outage, where units were taken offline, and significant additional workforce was on site, to capture operational variability. This reflects a similar scenario to what would be experienced during MCR. Of the data available during the screening, both the maximum and average values were compared with applicable thresholds, to bound variability. As noted above, all risks were assessed to be low to negligible. Similarly, all interactive effects between stressors were also assessed to be low to negligible.

To the extent there are incremental changes in operational and environmental conditions, this will be captured through ongoing environmental monitoring and the ERA update every five years. The ERA is a living document and new information and changing conditions are addressed through the requirement to regularly update the ERA and through an adaptive management approach.

3. The report assumes constant supply rates and organismal uptake. It is reasonable to assume that contaminant build-up and release over time with rainfall or thaw events.

Exposures and dose assessment calculations were completed using applicable methods referred to in CSA N288.6-12, including those described by the Canadian Council of Ministers of the Environment (CCME). The exposure assessment for ecological receptors includes the process of estimating the degree to which a fish or wildlife receptor could be directly or indirectly affected, through specifying various inputs around species behaviour, habits and habitat. These inputs were derived from literature, scientific studies, and industry best practices, using measured data where applicable.

Where there was uncertainty, conservative assumptions were applied. For example, it was assumed in the ERA that biota were exposed to the maximum radionuclide concentrations in air, soil, water or sediment 100% of the time. This is a very conservative assumption for migratory species such as mammals and fish, given the mobile nature of these species and that their home range size is much larger than the spatial area where the maximum radionuclide concentrations are observed.

As described above, monitoring data reviewed and used as part of the ERA included data collected over multiple seasons and multiple years, including under different operating conditions. For example, as part of 2016 environmental monitoring, sediment quality and soil quality samples were collected at a number of locations within the Site and in the surrounding area. These samples reflect any accumulation over time in these locations as a result of past and existing operations at the site and in the surrounding area. Both maximum and average concentrations were used in the ERA. Given the conservative assumptions in the ERA, variation in uptake by organisms is accounted for in the bounding approach taken.

To the extent there are incremental changes in operational and environmental conditions, this will be captured through ongoing environmental monitoring and the ERA update every five years and can be addressed through adaptive management.

Professor Cadotte's Criticisms of the PEA

1. There is a general lack of data and analysis which would normally be required to support the conclusions of the PEA

The PEA is a predictive environmental risk assessment for future operations of the Site, including Major Component Replacement (MCR). The ERA forms the basis for forward-looking predictions within the PEA. As described above, the ERA and PEA are a culmination of decades of monitoring data at the Site. Furthermore, the operational conditions to be experienced during MCR activities, including construction activities, are consistent with those encompassed during Bruce A Units 1&2 refurbishment and restart, as well as during recent site outages. Monitoring data encompasses these various operating conditions, including EA follow-up monitoring collected for activities at Units 1&2. This collective body of data supports the PEA and provides confidence in the conclusions of the PEA.

2. The report does not assess important sub-lethal and indirect effects on organisms, which can result in important ecological changes to the distribution and abundance of species and alter their interactions.

The PEA considered direct and indirect effects at sub-lethal levels. As noted above, the PEA relies on the substantial analysis completed as part of the ERA and follow-up monitoring. Bruce Power disagrees with the assertion that sub-lethal and indirect effects are not considered in the ERA. Benchmarks used to screen effects are established at levels well below where lethal effects would be observed, using where available, "no observable effects" levels to determine whether there was a potential for risk. Where there is no potential risk via a pathway, there cannot be further additive or combined indirect risks with other pathways. Where potential risk was identified, these were considered for their combined risk on receptors. All potential risks to the environment in the ERA and PEA were determined to be low to negligible.

3. The report does not consider interactive effects among stressors, and especially with other long-term stressors like climate change and exotic species invasion.

As described in the above responses, the ERA and PEA do consider interactive effects among stressors. With respect to changing climate, the PEA considers continued operations to 2064, with focus on the next 10 years (the subject licensing period), where no significant changes in climate are anticipated. Although not presented in the PEA, since taking over operations of the site, Bruce Power has considered climate change in relation to long term business operations planning, as well as in response to concerns raised by Indigenous and non-indigenous community members. The consideration has evolved with the current state of the science and best practices at the time of the consideration. To the extent baseline conditions change over-time, these are reflected into future regulatory approvals required to operate the facility.

The effect of climate change on the environment and operations will continue to be monitored as part of Bruce Power's environmental planning and incorporated into future ERA and PEA updates, as applicable. Moreover, ongoing monitoring commitments related to existing approvals, such as the Thermal ECA amendment at Bruce A, will continue to provide the pre-emptive information necessary to identify whether specific adaptive management steps may be required. Where the potential for significant adverse effects is identified, suitable operational mitigation measures will be pre-emptively identified and implemented.

Further, Bruce Power has recently established a partnership with the Council of the Great Lakes Region on a three-year climate change study that will help assess the potential impact of climate change in the longer term at the ecological and socio-economic level. The SON have been invited to participate in this study and to help shape its scope.

4. The report does not reply on the analyses and modelling approaches required to make informed predictions and does not provide confidence intervals (i.e. upper and lower bounds) of the likely impacts of stressors.

As outlined above, the PEA is a predictive assessment for the MCR, and as the operational conditions to be experienced are consistent with those encompassed by recent monitoring data the PEA builds on the site ERA. Therefore, the PEA has applied a tiered screening approach to identify the upper bound condition to base conclusions on. The outcome of the assessment demonstrated that existing conditions or operational conditions observed during Bruce A Unit 1&2 refurbishments and restart represent the bounding condition and predictive modelling was not necessary.

For the derivation of exposure/dose in the screening level assessment, it is appropriate to use the upper confidence limits or the maximum values as the first tier of assessment. The assessment is recognized as conservative, using upper-bound release rates, nearest exposure points and maximum measured concentrations to predict exposure/dose. If the first tier of assessment, using conservative assumptions, demonstrates no risk (as was the case in the PEA), no further assessment is warranted. Further tiers of assessment are warranted where there remains potential risk, and the predictions become more realistic, or multiple lines of reasoning are used, to put further bounds on the predictions provided. For example, this is the case within the ERA, where both maximum and average conditions are used in some cases to provide further context for the assessment. Uncertainties in the assessment, and how they have been accounted for, are described within both the ERA and PEA, where applicable.

iii. Impingement & Entrainment

The SON submit that the impingement and entrainment data and analysis that Bruce Power and CNSC staff rely upon is flawed and that no credible conclusions relating to impacts from impingement and entrainment can be drawn from this data.

Bruce Power disagrees with these assertions. The Impingement and Entrainment ("I&E") analyses rely on years of data collected through numerous studies and modelling in accordance with best scientific practices and with input from I&E experts. Bruce Power acknowledges that the SON has concerns about past I&E monitoring which it feels have not been adequately addressed. Bruce Power has indicated that it wants to work collaboratively with the SON in developing a new I&E monitoring plan for the *Fisheries Act*. Bruce Power is hopeful that this new plan along with other measures in the Joint Environmental Monitoring Program it has proposed to the SON will help to narrow areas of disagreement and build SON confidence in the I&E data and Bruce Power's assessment. However, Bruce Power remains of the view that this data is robust and reliable and the conclusions that have been drawn based on this data are sound.

I&E Studies and Monitoring

By way of background, the potential effects of I&E have been considered as part of all of the environmental assessments completed by Bruce Power to date. The Bruce A Refurbishment

Project EA Study Report (2005) predicted that the restart of Units 1 and 2 would approximately double the velocity of water entering the intake structures, potentially affecting I&E rates. As such, a two year I&E study was proposed as part of the EA follow-up monitoring program to understand how operation of Bruce Power might affect Lake Huron fish species.

At hearings in October 2009, SON provided a submission to the Commission expressing their concerns regarding I&E monitoring, and the Commission directed CNSC and Bruce Power to work with SON to address these concerns. In May 2011, the collaborative Lake Whitefish research program was finalized, which was jointly led by SON and Bruce Power, and included researchers from University of Guelph, McMaster University and University of Regina. The draft I&E Monitoring Plan, which outlined the proposed I&E monitoring approach to be used for the EA Follow-up Monitoring Program and the Fisheries Act application, was circulated by Bruce Power to regulatory agencies and the SON in July 2011. The I&E Monitoring Plan followed guidance from Section 316(b) of the United States Environmental Protection Agency (USEPA 2002), as well as other public and peer reviewed guidance on I&E studies.

SON Comments on I&E Monitoring Plan

The SON provided Bruce Power with 296 comments on the I&E Monitoring Plan in October 2011. SON indicated that they have unresolved concerns with the 296 comments in relation to the I&E monitoring plan for the EA FUMP, which concluded last year. Bruce Power prepared full dispositions to all the 296 comments, which were provided to both the CNSC and SON. This disposition table is attached as Appendix B to the Supplementary SON CI. Further, the comments were a priority agenda item at the subsequent EA Follow-Up Monitoring workshop and were incorporated into EA Follow-up Reports and the final I&E monitoring results.

Bruce Power remains confident that its approach to I&E monitoring is scientifically defensible and the conclusions made about I&E losses are reliable and sound, notwithstanding the fact that the SON has ongoing concerns in several areas including timely communication of results, definition and characterization of effects, appropriateness of monitoring and effects assessment methodologies, cumulative effects, and uncertainty arising from the SON's views about the potential unreliability of I&E effects estimates. The program and methods were based on consultation with I&E experts, CNSC, ECCC, and followed or exceeded scientifically published and widely accepted guidance and best practices for conducting I&E monitoring, and calculating I&E losses.

Thresholds for I&E effects were determined through consultation with CNSC and ECCC staff at the 2014 I&E Workshop, in advance of calculating I&E losses. Since this time, various iterations of I&E modelling have been completed incorporating feedback from external experts and CNSC. Statistical analyses closely followed the I&E Plan; however, improvements and alternate analyses were used in some instances following comments received, and to further reduce uncertainty.

To put I&E losses into context within the Lake Huron commercial fishery, a Foregone Fishery Yield Model (FFYM) was used to calculate the biomass (kg) of future fishery production that was lost due to I&E. The commercial value of the lost production was then compared to the harvest value of commercial fishery. For 2013 and 2014, the loss of Lake Whitefish to I&E was less than 1% of the commercial harvest in Zone 1. In comparison to I&E sampling conducted in U.S. in the early 2000s under the Phase II 316(b) Rule, I&E rates per megawatt or per unit flow at Bruce Power would be considered low (EPRI 2011b).

Bruce Power has proposed projects to offset its impacts from impingement and entrainment in its *Fisheries Act* Authorization. The SON have raised issues with these proposed measures but they have not to date proposed alternative projects, despite Bruce Power's repeated request to them to do so for the past two years. In an effort to advance dialogue on this issue, Bruce Power proposed several additional projects that were identified by Saugeen First Nation community members. Bruce Power is eager to work with the SON in identifying and implementing alternative measures that would benefit the SON fishery.

Future I&E Studies and Monitoring

Future I&E monitoring and assessment is being developed to follow guidance provided in CSA Standard N288.9-18, which will be published in late May 2018. This CSA Standard incorporates best practices for I&E monitoring used in Canada and internationally. Further, fish losses because of Bruce Power operations will be offset through offset projects proposed in the Fisheries Act Authorization application.

Bruce Power will be consulting the SON on a new I&E monitoring plan as part of the Fisheries Act Authorization and intends to work through the concerns that the SON believe are still outstanding in an effort to minimize areas of disagreement going forward and build greater SON confidence in the data and related assessments. On May 10, 2018, Bruce Power and the SON EO met to further discuss the development of a Joint Environmental Monitoring Program, which Bruce Power proposed at the May 2, 2018 Leadership meeting. During the meeting on May 10th, Bruce Power and the SON-EO agreed to action items for the development of this program and that it will focus on monitoring and assessment impacts from I&E, thermal, and climate change. This is further discussed in Section 2.9 of the Supplementary SON CI. Bruce Power looks forward to working with the SON on the development and implementation of this program.

I&E Mitigation Measures

In their written submission, the SON assert that a decision should not be made on MCR until Bruce Power undertakes a further alternative mitigation measures assessment for I&E and thermal effects. It is Bruce Power's position that this study does not need to be undertaken prior to a decision on MCR as the minimal impacts identified in the Application and previous environmental assessments do not warrant further mitigation measures at this time.

By way of background, there are already a number of mitigation measures incorporated into Bruce A and Bruce B. These have been considered as part of previous EAs and within the ERA as "in-design" or inherent mitigation measures. The design and use of mitigation technologies have been implemented to minimize impacts of I&E to the greatest extent possible. Both Bruce A and B are equipped with velocity caps which are considered the best technology available for mitigating impingement. The intake locations for both Bruce A and B are also located offshore where less fish are present and Bruce B is equipped with a chain rope barrier to act as a deterrent to fish. Bruce A does not have a chain rope barrier because the velocity cap was not engineered to withstand the additional weight of the chain. There is also no indication that a chain rope barrier at Bruce A would make a material difference as the intake of fish is more impacted by the patterns of the lake current, fish community, and wave action.

The low rate of I&E can be attributed in part to these mitigation measures already in place. Additional mitigation has been considered as part of past EAs and the *Fisheries Act* Authorization, but the effectiveness of additional mitigation measures and their need is unwarranted given the relatively low levels of I&E at Bruce A and Bruce B. In most cases, additional mitigation measures will require significant cost and/or research to implement, with little anticipated improvement in I&E reduction. There are limited entrainment mitigation technologies available and entrainment accounts for >80% of the total annual age-1 equivalent biomass lost at Bruce A and Bruce B.

Further measures to reduce impingement have been previously evaluated including the installation of lights, sounds, bubble air curtains, or a chain rope barrier (Bruce A only) at the velocity cap. The overall efficacy of these fish behaviour modifying technologies to reduce impingement is low as the response is often species- and site-specific (e.g., some fish are deterred by strobe lights while others are attracted). The relocation of the intakes to waters even further offshore or the implementation of flow reduction technologies have been considered to further reduce impingement and entrainment. Costs to implement these strategies are significant but the outcome is highly uncertain and could be coupled with additional negative effects associated with reduced cooling capacity (additional waste heat) and impacts to the lake bottom.

The detailed quantitative risk assessment for I&E effects indicated that Bruce Power's impact to the Lake Huron fishery is very low; therefore, mitigation at Bruce Power is being effectively implemented and further mitigation measures are not warranted at this time. Bruce Power continues to monitor I&E effects to inform the ERA, and the need for adaptive management is and will be regularly evaluated.

The Precautionary Principle

The SON submit that the regulatory requirements for the CNSC to fulfill its mandate under the NSCA in a manner that respects the precautionary principle have not been met, particularly due to Bruce Power's failure to consider available mitigation measures for impingement/entrainment or thermal effects or provide a mitigation measures plan.

The SON disregards the fact that Bruce Power has previously considered alternative mitigation measures for impingement, entrainment, and thermal effects in prior environmental assessments and in relevant permitting applications. The SON's submissions on the precautionary principle also do not take into account the role of adaptive management.

The Supreme Court of Canada has referred to the precautionary principle as an "emerging international law principle" that "recognizes that since there are inherent limits in being able to determine and predict environmental impacts with scientific certainty, environmental policies must anticipate and prevent environmental degradation."¹⁹ While the principle has been cited in numerous Canadian cases, its application has depended on the particular facts of each case. In a report filed by the SON, Professor Tollefson of the University of Victoria refers to the application of the precautionary principle in *Taseko Mines v. Canada*²⁰. This case involved a new open-pit mining project that a Review Panel had concluded presented significant potential adverse environmental effects. Also, the Review Panel had sought further information in respect of such environmental effects and potential mitigation measures, and the proponent refused to provide such information. This situation, and the specific application of the precautionary principle, is different from Bruce Power's Application which is <u>not</u> a new project with unknown environmental impacts. The environmental impacts of Bruce Power's continued operation are well-understood and the impacts of such operations are clearly-set out in the ERA and PEA.

¹⁹ Castonguay Blasting Ltd. v. Ontario (Environment) 2013 SCC 52, at para. 20.

²⁰ 2017 FC 1099.

Further, Bruce Power is committed to (and mandated to by the CNSC's licensing regime) continuing to study the impacts of its operations and to providing further information to the CNSC as requested.

In his report, Professor Tollefson suggests adopting a test for the application of the precautionary principle that is based in large part on the 2006 Australian court decision in *Telstra Corporation Ltd. v. Hornsby Shire Council²¹* ("*Telstra*"). This test and application of the precautionary principle has not been adopted by the Canadian courts, which have made more recent pronouncements regarding this principle and its application in Canadian law. Further and as stated recent by the B.C. Environmental Appeal Board which specifically referred to *Telstra*, decisions from other commonwealth jurisdictions are not binding on Canadian courts and tribunals.²²

Finally, REGDOC-2.9.1 refers to both the *precautionary principle* **and** the *principle* of adaptive management. The Federal Court, in Canadian Parks and Wilderness Society v. Canada (*Minister of Canadian Heritage*)²³, referred to the importance of both of these principles in maintaining ecological integrity:

The concept of "adaptive management" responds to the difficulty, or impossibility, of predicting all the environmental consequences of a project on the basis of existing knowledge. It counters the potentially paralysing effects of the precautionary principle on otherwise socially and economically useful projects. The precautionary principle states that a project should not be undertaken if it may have serious adverse environmental consequences, even it if it is not possible to prove with any degree of certainty that these consequences will in fact materialise. Adaptive management techniques and the precautionary principle are important tools for maintaining ecological integrity.²⁴

Bruce Power has undertaken numerous and extensive studies regarding the environmental impacts of the Site, including impacts related to thermal effluent, impingement and entrainment. Such studies have repeatedly shown that the operation of the Site does not result in significant adverse environmental impacts, and the Application demonstrates that MCR and operating license renewal will not result in significant adverse environmental impacts. The precautionary principle has been followed in the past and in the current Application, as Bruce Power has gone above and beyond regulatory requirements to ensure ecological integrity. To the extent that any new or unanticipated impacts result, the concept of adaptive management will ensure that measures are taken to eliminate or mitigate such impacts, as required by the CNSC's ongoing regulatory and environmental oversight of the Bruce Power nuclear facilities.

In response to the SON's concerns, Bruce Power will be required to undertake a further assessment of feasible mitigation measures for thermal effluent and impingement/entrainment by December 31, 2019. CNSC staff does not believe that additional mitigation measures are necessary at this time but are asking Bruce Power to undertake this study in the event the level of risk changes in the future. This information can be incorporated into the next ERA which will be submitted to the CNSC in 2022, which is prior to the restart of the first refurbished reactor.

²¹ [2006] NSWLEC 133.

 ²² Toews v. British Columbia (Director, Environmental Management Act) 2015 CarswellBC 3804, [2015] B.C.E.A. No. 25)

²³ [2003] F.C.J. No. 703.

²⁴ *Ibid.* at para. 24.

Given the current level of impact and the ability to deploy additional mitigation measures if the situation changes, the SON's request to defer MCR until this study is completed should be denied.

iv. Thermal Impacts

The SON assert that the assessment of thermal effects in the ERA was inadequate and state that the data used by Bruce Power in the ERA may not reflect the conditions created by the thermal plume or the conditions present in the receiving environment. The SON assert that a finding of "low to negligible impact" cannot be made based on present data.

Bruce Power disagrees with the SON's assertions. The assessment of thermal effects in the ERA was based on numerous studies and monitoring results, as further outlined below, and the conclusion that such effects are low to negligible is fully supported by the data collected to date. Bruce Power is continually striving to refine the models that are used in evaluating the thermal plume, and is committed to collecting additional data to assist in defining with greater accuracy the extent of the thermal plume such as during the winter months. Bruce Power is also committed to working with the SON to further enhance thermal monitoring through the Joint Environmental Monitoring Program that it has proposed and the thermal effluent study and analysis program proposed by CNSC staff

Thermal Studies and Monitoring

By way of background, water drawn from Lake Huron is pumped through the Condenser Cooling Water ("**CCW**") system through which waste heat is transferred and discharged to the Bruce A and Bruce B discharge channels. Discharges from the Bruce A channel are directed into relatively shallow waters that generally remain above 8 m depths for a distance of approximately 2 km. Discharges from the Bruce B channel are directed into generally deeper waters than at Bruce A that drop below 8 m depths within 400 m of the discharge channel. These warmer discharges have the potential to affect aquatic biota and habitat and are referred to as the "thermal plume" or "thermal discharge".

Bruce Power has undertaken extensive study of the thermal effects of its operations since assuming responsibility for the operations of the Site in 2001. Bruce Power has monitored, and continues to monitor, the thermal effects of the cooling water discharges for both Bruce Power A and B plants at a number of locations in the aquatic environment surrounding the Site. This data, combined with a number of numeric modelling investigations, has facilitated a significant knowledge base from which to evaluate the various interactions between CCW intakes and discharges and the surrounding environment.

Much of this knowledge has been documented in numerous study reports conducted for the Site since 2001, focusing on the range of physical, thermal, chemical and biological conditions within the receiving environment. Studies completed to date in relation to thermal effects evaluation include: WINGS research collaboration, larval Lake Whitefish sampling, mark-recapture fish studies, fish community assessments, nearshore hydrodynamic monitoring, Smallmouth Bass nesting monitoring, creel surveys, airlift egg sampling, gas bubble trauma surveys, and annual lake temperature monitoring. The number and diversity of surveys demonstrates the extent to which Bruce Power has gone to develop its knowledge of operational and environmental interactions related to the thermal plume.

Review of the monitoring and modelling results developed to document operational effects on lake temperatures have been conducted with MOECC and SON on an annual basis since 2013 to afford interested parties the opportunity to understand thermal effects and highlight any areas of concern which may warrant further modelling investigation. Modelling completed includes a wide range of environmental and operational settings that have the potential to affect the thermal regime of the nearshore environment around Bruce Power. This includes conditions such as meteorological (i.e., ambient air temperatures, solar radiation), lake temperature (i.e., range of hourly and monthly average temperatures), lake level (high and low lake levels based on historic trends) and operational intensity (i.e., 2-unit and 4-unit operation).

Given the commercial and cultural importance of Lake Whitefish to the SON and their commercial fishery, Bruce Power has been working with independent university researchers since 2011 to understand more about Lake Whitefish and Round Whitefish in Lake Huron, and in particular how Site operations might affect these species. With respect to thermal effects, from 2011 to 2016, the McMaster University and University of Regina research team incubated over 550,000 Lake Whitefish embryos and over 40,000 Round Whitefish embryos in ongoing efforts to define thermal and combined stressor effects. Overall, results provide evidence that the temperature fluctuations measured over winter near the thermal effluent are well within the range for Lake Whitefish and will have no effect on survival. The research program results support the conclusion of limited to no impact of the thermal discharge on Lake Whitefish survival.

There are a variety of thermal criteria reported in the literature for each fish species and life stage. These benchmarks consist of acute and chronic temperature limitations, lab and field based studies and lethal and sub-lethal endpoints. There is variation in how the temperatures were determined and the availability of thermal data varies for each species. Monitoring data was used to compare observed temperatures in the lake with the various benchmarks. Data was also compared at reference locations to get an indication of whether observations may be a result of natural variation. Given the conservative benchmarks, multiple years of data collection and the resulting hazard quotients, it is concluded that thermal effluent causes little to no risk to fish and this conclusion is backed-up by robust studies and monitoring. As part of future updates to the ERA, further analyses will be done on Lake Whitefish to incorporate updated benchmarks from recent research and to further support the finding of little to no risk.

Further, the discharge temperatures from Bruce A and B are regulated through Environmental Compliance Approvals ("ECAs") issued by the Ontario Ministry of Environment and Climate Change ("MOECC"). The ECAs for Bruce A and Bruce B stipulate both maximum temperature increases between the intake and discharge, as well as maximum discharge temperatures for Bruce A tied to the time of year. The maximum temperatures stipulated in the ECA are based on levels that are protective of the receiving environment. These limits have been the subject of recent study as part of an ECA amendment application. Through this (or any future) amendment process, the MOECC considers the impacts of the discharges on the receiving environment prior to making a decision and Bruce Power will be required to ensure that water is discharged at temperatures that are protective of the receiving environment.

Report of Michael Nichols

In their submission, the SON enclosed a report from Michael Nichols who raises several criticisms related to the consideration of thermal impacts in the ERA. Bruce Power's responses to these criticisms are set-out below.

Dr. Nichols' Criticisms of Temperature Data Presentation

1. The definition of ambient temperature is not clear

In the 2017 ERA thermal assessment, sites 1 and 29 were used as reference sites (also known as ambient sites) for comparison to potential exposure sites as they are situated to the far north and south of the facility, respectively, and are known to be outside the thermal plume during the summer months from modelling work. Temperature from these sites represents the ambient temperature of the water.

2. There is no description in the ERA under Cold Weather Conditions of the buoyant plume, vertical extent of the plume, direction, or distance until it decreases to 4°C

Bruce Power disagrees with the assertion that there is no thermal assessment during cold weather conditions. During the winter months, the 2017 ERA thermal risk assessment used measured temperatures at lake bottom and assessed the risk based on thermal conditions at relevant habitat sites.

3. There is no information provided on the direction, vertical and horizontal extent, and variation with time of thermal plumes that may affect temperature differences at specific sites when compared to "ambient" sites 1 and 29

The rationale for site selection was provided, which included areas most impacted by the plume and which experienced the warmest temperatures. Measured temperatures were used to flag a potential elevated risk. In most cases, the elevated temperatures were related to natural lake conditions and not from thermal discharge.

4. There is no description or indication as to the depth of the temperature monitoring point for the Bruce A Discharge

The depth of the monitor in the Bruce A discharge channel is not needed as the temperatures throughout the water column near the monitoring location are generally uniform as this location is well mixed due to the turbulence of the flow in the discharge channel. The depth varies with lake levels, however it is located not far off the bottom of the channel.

5. The predictive tool used to estimate temperatures presented in Table 75 of the 2016 Environmental Monitoring Report [10] for location 31-1 is not described (nor is it accurate)

The author has confused the data included in Table 75 of the 2016 Environmental Monitoring Report. The purpose of the table was to show that the daily variation predicted by the model (RMA-10) is consistent with the observed variation at a monitoring site and that there is little risk of acute temperature effects associated with a daily average temperature criterion.

6. There is no presentation of temperature data by day, week, or month which may indicate variation in the path of the thermal plume over time. No indication of the range temperatures is provided for each site during cold weather conditions.

Bruce Power disagrees with the assertion that temperatures and ranges for various temporal scales during the cold weather conditions were not included in the ERA. Where there was an HQ>1 in the thermal assessment, the temperatures at all assessment sites and reference sites

were examined for: (1) the entire time period on a daily basis for the percent of time assessment and (2) the day on an hourly basis for the Max HQ assessment.

Dr. Nichols' Criticisms of Risk Evaluation

1. There is no monitoring of hatch success and larval development of Whitefish as the Phase III of the objective of the EA FUMP was eliminated

Corresponding monitoring of hatch success and larval development was assessed as part of the EA FUMP. The approach was phased and the execution of a subsequent phase was dependent on the result of the prior phase. Adaptive management was used to develop a more conservative assessment. Hatch advance was considered and was found to be within a short timeframe and thus the conclusion remains that there is little to no risk to Lake Whitefish.

2. BP infers from the three seasons of temperature monitoring and comparison to laboratory-based studies that there is "no significant risk to survival" for Lake Whitefish eggs. No data are provided regarding the actual spawning times and locations for Lake and Round Whitefish relative to thermal plumes that exist during cold weather conditions (i.e. the known spawning period).

Extensive monitoring for the presence and location of spawning Lake Whitefish has been conducted since 1979 as shown in the Lake Whitefish Research Summary submitted as part of the Application. From this work, it is known when Lake Whitefish start to spawn: November 15th (as an early date) and December 1st (as a realistic date) to begin the consideration of the presence of whitefish embryos. Loscombe Bank (the location closest to the Bruce A discharge) was identified.

3. Table 37 "Delta T Thermal Criteria for Fish Species (°C)" (page 209) limits the scope for considering Delta T to sites 11, 9, 5 for Lake and Round whitefish. The tabulated results indicate the Delta T criteria of 3.7°C is exceeded 30% of the time at site 9B.

Thermal monitoring sites 11, 9 and 5 were selected for the preliminary thermal assessment (PQRA) as they are in the areas that are most impacted by the plume and located on shoals that could be potential spawning habitat for Lake Whitefish. The locations are relevant to the species and have the highest temperatures, so that the assessment of risk considered the maximum possible conditions which are not necessarily reflective of the conditions in the lake. Further, not all exceedances can be attributed to the thermal plume; natural conditions in the lake may also result in temperature exceedances. The PQRA used all available monitoring locations at the appropriate depth for the presence of Whitefish eggs. The conclusion remains that there is little to no risk to Lake Whitefish as a result of thermal discharge.

4. The Risk Assessment HQ are only indications of potential thermal impact and not indications of the presence or absence of Valued Ecosystem Components.

It is true that the use of HQs only indicate the potential thermal impact and not the presence or absence of VECs in the local area. The species selected for the 2017 ERA thermal assessment were chosen to represent a range of species, based on known presence in the area (bass nesting surveys, Creel surveys, I&E) and/or interest to Indigenous groups and other stakeholders. Much work has been done on collecting Lake Whitefish eggs, larvae and adults in the nearshore; there are few Lake Whitefish utilizing the area for spawning.

Other Criticisms from the SON regarding the 2017 ERA Thermal Risk Assessment

1. During the egg/incubation life stage, the Emerald Shiner was in exceedance of its HQ 66% of the time between July and August and Bruce Power did not determine there was the potential for significant effect.

The environmental risk assessment took a conservative approach with site selection and maintained conservatism in its approach hence the identification of the exceedance. If the assessment was more realistic, the egg stage of Emerald Shiner would not have been assessed further at this location as they are not known to spawn here. Thus, the assessment of Emerald Shiner eggs in the discharge channels was done to be conservative.

2. The spawning stage of Yellow Perch had an HQ value of 5.4 (intra-deltaT) and 7.04-6.87 (inter-deltaT), which seems to have more than a trivial potential for causing adverse effects but no additional data was presented.

The delta T benchmark for Yellow Perch spawning stage was 2-6°C and the lower value was used in the assessment to be conservative. Analysis of the daily temperature at all assessment and reference sites showed that the fluctuation was short lived and other sites also experienced this large daily range. The abrupt change was due to natural lake variation in the nearshore. If the upper value of the benchmark was used (i.e. 6°C), the HQ value would be lower.

3. The Smallmouth Bass experienced HQ>1 exceedance during the egg/intubation stage but no additional analysis was presented.

For Smallmouth Bass eggs, the benchmark was a range (5-10°C) and the lower value was used to be conservative. The exceedance occurred in the Bruce B discharge and looking at all the other assessment and reference sites for that date showed that another site experienced the same temperature increase at the same time. This could only be related to the abrupt changes in the lake. If the upper benchmark value was used (i.e. 10°C), the HQ value would be lower.

4. There is a need to better understand thermal dynamics in the winter months, specifically in relation to Lake Whitefish

Bruce Power has monitored lake conditions in the winter months for several years, with a focus on habitat potentially used by Lake Whitefish eggs for incubation. These conditions do not pose a risk to Lake Whitefish survival as temperatures are below the acute and chronic thresholds. Furthermore, there has been significant effort in determining the abundance of Lake Whitefish in the local area. In over 30 sampling campaigns, only a limited number were found near Bruce Power compared to other locations. The area near Bruce Power is not prime spawning habitat for Lake Whitefish.

Thermal Mitigation Measures

The impacts of thermal discharge are minimized to the greatest extent possible using a combination of design strategies and mitigation technologies. The location of Bruce Power, situated on the Douglas Point headland, was strategically chosen because of its high energy zone with access to cold, deep water. The headland juts into Lake Huron providing a natural feature for dispersion of thermal effluent and the shoreline location itself is naturally low in diversity of fish species due to high wave action and winter ice movement. The placement and

orientation of the intake and outfall structures at each station effectively minimize the physical (flow and temperature) and ecological (fish response) changes to the water body.

Bruce Power has previously evaluated additional mitigation strategies to further reduce thermal impact on the environment, including in previous ECA Applications. There are a number of possibilities that have been considered that include alterations to the current system or a complete replacement of the current system to an alternative technology. Of these options, some would decrease the size of the plume but at the expense of increasing the temperature (concentrating the heat), while others would lower the temperature output to the water body. With the objective of lowering the effluent temperature, options include altering the intake or outfall structure, changing operational mechanisms such as flow rate or water recirculation and using external cooling systems such as cooling basins or towers. These mitigation strategies are accompanied by potential negative impacts to humans and/or the environment, feasibility due to complexity or space limitations on Site, and the uncertainty in how effective the technology would be in lowering the discharge temperature. With these considerations in mind, and the outcome of years of monitoring data and environmental assessments that indicate no unreasonable risk to local aquatic biota from thermal discharge, it is concluded that additional cooling strategies are not required or merited.

Bruce Power looks forward to working with the SON on the alternative mitigation measures study that has been proposed by the CNSC. However, Bruce Power disagrees with the SON that this assessment needs to be carried out prior to a decision on MCR for the reasons articulated previously.

v. Climate Change

In their written submission, the SON have raised concerns about the consideration of future impacts of climate change on Site operations. They have also filed a report from Professor Philip Byer who has concluded that the conclusions of the PEA cannot be relied upon because it did not consider the impacts of climate change. Bruce Power disagrees with these criticisms, which do not take into account the licence period at issue (2018-2028), the slow evolution of impacts from climate change and the ability to incorporate these changes into future ERAs/PEAs (updated every five years), and the role of environmental monitoring and adaptive management. Bruce Power acknowledges the need for further study in this area to monitor impacts and assess future impacts beyond the proposed licencing period. The company is looking forward to working with the SON on joint monitoring and through the 3-year climate change study that Bruce Power has announced with the Council of the Great Lakes Region

Bruce Power has long considered climate change in relation to long term business operations planning, as well as in response to concerns raised by Indigenous and non-Indigenous community members. This approach to considering climate change has evolved with the current state of the science and best practices at the time. In accordance with Federal-Provincial-Territorial Committee on Climate Change and Environmental Assessment (FPTCCCEA, 2003) guidance, as part of previous assessments, climate change is considered from two perspectives:

- how the project affects climate change (through the emission of greenhouse gases or GHGs); and
- how a changing climate affects the project (through changes in the expected weather patterns and extreme events).

As part of previous assessments conducted since 2004, Bruce Power has considered the GHG emissions emitted by both the construction and operation of the proposed project under assessment. As nuclear is a far less carbon intensive power source than fossil-fuel based sources, the emissions of GHGs are negligible in comparison to the Ontario power sector total emissions. Even with the consideration of short-term construction emissions, the emission of GHGs are very small or negligible in comparison to the Ontario power sector total emissions. As noted in the assessments, there are long-term benefits of reduced GHG emissions during operation due to the displacement of higher carbon intensity fossil fuel derived power.

Bruce Power has also long considered the vulnerability of its operations, and the surrounding environment, to climate change. This started with the New Fuel Project EA, and currently, Bruce Power is incorporating climate change considerations as part of the ERA and PEA process to support long-term planning, through understanding of conditions and including this when evaluating the outcome of the assessment.

The PEA considers continued operations to 2064, with focus on the proposed licencing period (the next 10 years), where no significant changes in climate are anticipated. Bruce Power will not be able to operate after 2028 unless it obtains a further operating licence from the Commission. In general, recent current climate models and trends (between 1981 and 2010) would suggest that current climate is likely to become warmer and drier over time although the impacts of climate change have and are expected to continue to evolve slowly. It should be noted that the observed rate of decadal change is much less than the seasonal variability currently experienced at the Site. This implies that the changing climate under current conditions.

Further work to assess the temperature changes associated with combined operational and atmospheric effects under projected climate change conditions is currently being carried out and will continue to be refined with the support of ongoing physical and biological effects monitoring data. Bruce Power is committed to meeting regulatory requirements in place, including limits to maximum discharge temperatures which will continue to limit Bruce Power's thermal impact and operations. Should changing temperatures cause Bruce Power to exceed those limits, Bruce Power will be required to deploy additional mitigation measures or initiate a partial shut-down of operations.

In addition, Bruce Power has recently established a partnership with the Council of the Great Lakes Region on a three-year climate change study that will help assess the potential impacts of climate change in the longer term at the ecological and socio-economic level. The SON have been invited to participate in this study and help shape its scope.

In addition, as part of the proposed Joint Environmental Monitoring Program, Bruce Power and the SON EO have agreed to undertake further initiatives to enhance monitoring of impacts from climate change. This will include hosting an annual climate change and fisheries workshop with the SON every year for the next 10 years to gather community information to understand what is being observed by SON community members and will include the development of a survey for SON members. Bruce Power looks forward to working with the SON to enhance its monitoring and future assessments of climate change, which can be incorporated into future ERA/PEAs, including the next one in 2022.

vi. Cumulative Effects

In their written submission, the SON raise concerns about the adequacy of the cumulative effects assessment in the PEA. Bruce Power disagrees with the SON's criticisms about the cumulative effects assessment, the findings of which were consistent with previous cumulative effects assessments at the Site concluding that there are likely no significant adverse cumulative effects from Site operations.

The potential for cumulative effects was considered in previous environmental assessments conducted for Bruce Power projects/activities at the Site since 2001. For the purposes of the EAs under the *Canadian Environmental Assessment Act, 1992*, cumulative effects were defined as those incremental environmental effects associated with each Bruce Power project, added or combined with, effects associated with other projects and activities both at the Site and offsite (Bruce Power 2002, 2004, 2005, 2008). These assessments demonstrated that there was likely no significant adverse cumulative effects on the environment, including for the previous life extension of the Site to 2043. Furthermore, as part of the Bruce New Nuclear Power Plant Project Environmental Impact Statement, a cumulative effects assessment was completed, considering continued operation of Bruce A and Bruce B along with a new generating station, which was proposed to operate past 2080. The assessment concluded no significant adverse cumulative effects for all VECs considered, including for a period of time where up to 12 reactors would be operating on Site instead of the current 8.

Other projects and activities on the Site have the greatest potential to act cumulatively with the effects of Bruce Power's operations. This is reflected in the projects included in past cumulative effects assessments. Furthermore, the cumulative interaction of the operation of Bruce Power facilities with the operation of these other existing facilities at the Site (e.g., OPG's Western Waste Management Facility [WWMF]) has been considered implicitly in the ERA and the PEA through inclusion of baseline monitoring and emissions data. It is not possible to isolate potential effects associated with these facilities when reviewing monitored data as a whole, therefore the cumulative influence of past and existing projects is encompassed in the baseline monitoring data subject to the ERA. The PEA also included discussion of the potential cumulative effect of reasonably foreseeable projects and activities on the site, specifically the DGR Project. No adverse cumulative effects are likely.

The tiered nature of the ERA takes into consideration combined effects. As described in the response above, exposures are first screened for their potential to exceed thresholds and benchmarks established to be protective of individuals. Where no risk is identified through an individual pathway through screening against these benchmarks, there is no potential for them to act additively with other stressors. This is further supported in the ERA by the results of recent combined stressor research, which demonstrates that levels of stressors required to result in an additive or synergistic effects are substantially higher than those experienced at the Site. Current available research, indicates that combined stressors showed an effect (e.g., morpholine and gamma radiation; heat shock and morpholine) at levels substantially higher than those seen operationally at the Site.

With respect to combined physical stressors on the aquatic environment, it is predicted that the expected effects are negligible given the limited spatial extent of effects to the aquatic environment because of fish loss due to impingement and entrainment and thermal inputs are localized to the area in the immediate vicinity of the Site. Research examining Lake Whitefish and Round Whitefish genetics in the immediate vicinity of the Site found no evidence for the presence of distinct genetic groups compared to the larger lake population, and as such fish

losses can be quantified in terms of the greater Lake Huron fish community. As a result of the effectiveness of the mitigation measures in place at the Site, which are reflective of current best practices, and the little to no risk to the aquatic environment related to I&E and thermal effects on their own, it is predicted that the overall cumulative effect on the aquatic environment is negligible.

vii. Waste Issues

The submission from SON raises concerns about the management of waste generated from the facility during operations and MCR. As an environmentally-focused organization, Bruce Power does everything it reasonably can to minimize its environmental footprint including the minimization of waste. Through its operation, Bruce Power generates relatively small volumes of radioactive waste that need to be safely managed onsite, which are eventually transported to Ontario Power Generation (OPG).

Under its lease arrangement with OPG dating back to 2001, all waste and the eventual decommissioning of the facility at the end of operational life is the responsibility of OPG as the owner of the facility. Under this arrangement, Bruce Power is responsible for the full cost of these activities as it relates to its operations.

Every very five years, fees for waste (inclusive of all forms) is reset to ensure the full life-cycle cost is recovered by OPG. The same is the case with decommissioning. All of these costs are reset through an independent process led by the provincial government known as the Ontario Nuclear Funds Agreement (ONFA).

This delineation of responsibility was the construct put in place by the Province in the late 1990's when the Site was leased to a private-operator to allow the organization to focus on operations and potential life extension.

In addition to funding the cost of waste generated, Bruce Power is responsible for the safe management of these products in its operation and has an extensive program to reduce volumes. CNSC Staff through their regulatory oversight activities have concluded "*Bruce Power's waste management programs exceeded expectations in all specific areas for managing radioactive waste. Bruce Power has minimized the production of radioactive wastes through various plans, programs and procedures as well as minimizing impacts from such wastes on workers and the environment*". (Source: Page 115, March, 2018 CMD)

The company has been successful at minimizing waste volumes through an extensive multilayered approach starting with reduction of material that enters the facility (such as packaging), segregation at source, decontamination of material for free-release, use of launderable clothing where practical versus disposable, staff awareness and incineration where applicable. This approach to reducing waste volumes is estimated to have diverted approximately 95% of the low level waste products generated from being sent to OPG for long-term management.

The SON submission also notes that waste volumes will be generated during the MCR Project. All of the minimization practices applied as noted above will be fully implemented during the MCR. With respect to waste products generated from component replacement activities, a series of minimization activities will be applied to this work. Reactor components including feeders and pressure tubes will be cut into smaller pieces to optimize the space in waste containers and other components as appropriate will be sent for metal melt before they are sent to OPG for long-term management. In addition to Bruce Power's waste activities being the subject of ongoing regulatory oversight, OPG's Western Waste Management Facility is subject to regular CNSC reviews and oversight. It must obtain separate operating licences from the CNSC, the last of which was obtained in April 2017.

viii. Conclusion

Bruce Power is committed to continuing to build and maintain a positive relationship with the SON and ensuring that the SON's asserted and established rights are not impacted by the Site.

Bruce Power and the CNSC staff have made significant efforts to engage with the SON about the Application, which go well beyond what would be required to meet any duty to consult owing in this case. Bruce Power has heard the concerns raised by the SON and both Bruce Power and the CNSC staff have proposed measures that would help address their concerns, including various environmental study and monitoring measures, additional fisheries offset projects, a study on alternative mitigation measures, and the opportunity to participate in and help shape the scope of a 3-year climate change study that is being conducted in partnership with Bruce Power and the Council for the Great Lakes Region.

Contrary to the SON's submissions, Bruce Power has satisfied the statutory test for this Application. Bruce Power has safely operated this Site for the past 17 years and the impacts of the Site are well understood after numerous environmental assessments and monitoring programs which have confirmed no significant adverse impacts to the environment. The Application demonstrates that impacts to the environment from the continued operation and life extension will be low to negligible and monitoring measures will be put in place to confirm the continued accuracy of this assessment. CNSC staff will conduct independent monitoring and adaptive management measures will be deployed if necessary. The ERA/PEA will be updated in 2022 before the restart of the first refurbished reactor and Bruce Power will be required to obtain further operating licences from the Commission to operate after 2028, which will result in ongoing review and oversight by the Commission.

Bruce Power is very grateful for the input that it has received from the SON and the time that they have taken to engage in this process. Bruce Power looks forward to continuing to work with the SON on the various measures that have been proposed to address the SON's concerns in order to ensure that SON rights and interests are not impacted by the Site. Bruce Power also looks forward to working with the SON to advance other shared priorities, including initiatives that will increase SON employment, training, and business opportunities relating to the Site and with Bruce Power's suppliers.

Letter, F. Saunders to M. Leblanc, "Application for the Renewal of the Power Reactor Operating Licence: Supplemental Material", May 16, 2018, NK21-CORR-00531-14285 / NK29-CORR-00531-14980 / NK37-CORR-00531-02956 (excluding Enclosures 4, 5, and 6).

Please note that Enclosures 4, 5, and 6 have not been included in this document as these enclosures may <u>NOT</u> be released to the public.