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1 **Correlated Biomarker Responses for DNA Damage and Innate Immunity Revealed in a Comparison**
2 **between Field and Laboratory Studies: Fathead Minnow Exposed to Tritium**

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4 E.D. Beaton¹, Isabelle Gosselin¹, Amy Festarini¹, Beatrice Gagnaire², Francesca Farrow¹, Isabelle Cavalié²,
5 Carmen Shultz¹, Sang Bog Kim¹, Stephanie Walsh¹, Heather Ikert¹, Hui Qun Chen¹, Christelle Adam-
6 Guillermin², Marilyne Stuart¹

7
8 ¹Canadian Nuclear Laboratories (formerly Atomic Energy of Canada Limited), Chalk River Laboratories,
9 286 Plant Road, Chalk River, ON K0J 1J0, Canada

10 ²Institut de Radioprotection et de Sureté Nucléaire (IRSN), PSE-ENV/SRTE/LECO, Cadarache, Saint-Paul-
11 lez-Durance 13115, France

12

13 **Abstract**

14 Tritium entering the aquatic environment contributes to the doses received by aquatic organisms.
15 Multiple stressors inherent in natural environments, however, confound estimates for radio-sensitivity
16 determined in controlled laboratory settings. To disentangle differences between field and laboratory
17 outcomes, a multivariate analysis of biomarkers is described for fathead minnows (*Pimephales*
18 *promelas*) exposed to tritium for 60 days and allowed to depurate for 60 days. In the laboratory study,
19 the biomarkers for DNA damage and innate immunity display a dose-response relationship to
20 internalized tritium. At the same tritium activity concentrations, the biomarkers for genotoxicity from
21 the field study were lower than those from the laboratory study. This finding does not support an
22 increase in genotoxic stress or sensitivity in the field-exposed fish, as was suggested by a meta-analysis
23 comparing field data for radiation exposure from the Chernobyl exclusion zone and data from laboratory
24 studies.

25 In the laboratory, we see strong correlations between tritium exposure (up to 180 kBq/L), DNA damage
26 (comet and micronuclei formation in gonad) and innate immune responses (phagocytosis and
27 fluorescence associated with lysosomal membrane integrity in spleen). In the field, we see a high
28 proportion of phagocytosis where the comet tail moment, but not the micronucleus frequency,
29 correlated with fluorescence associated with lysosomal membrane integrity in spleen.

30 The biomarkers for oxidative stress (catalase and superoxide dismutase) were specific to each study
31 location with higher liver catalase activity in the laboratory exposures and higher superoxide dismutase

32 in the field exposures. Indicators of overall health were not different between exposures, locations, or
33 weight, length and organ somatic indices.

34 This comparison highlights the relevance for using the DNA damages and innate immune system
35 response endpoints as biomarkers of chronic low-dose tritium exposure, and the need to obtain a better
36 mechanistic understanding of initiating events for the immune response.

37 **Introduction**

38 The principle of protecting the environment from anthropogenic sources of ionizing radiation recognizes
39 our responsibility to protect non-human biota from early mortality, reduced physical well-being, and
40 reduced fertility and fecundity. A recent critical examination of anthropocentric approaches to
41 environmental protection (Mothersill et al., 2018) acknowledged the common goals of radiobiology and
42 radioecology considering their different frames of reference (that of understanding the biological
43 mechanisms triggered by exposure to low doses or dose rates, and that of understanding the
44 consequences of radiation transfer within an ecosystem). Two points of discussion were (1)
45 disentangling the contributions of multiple environmental stressors on morbidity, mortality and
46 reproduction, and (2) the application of biomarkers to population level assessment of exposure and
47 fitness. This examination follows from a meta-analysis by Garnier-Laplace et al. (2013) that found that
48 the estimated dose rate for effects on non-human biota was about 8× lower for field studies (located
49 within the Chernobyl exclusion zone) compared to results from laboratory studies, suggesting that
50 multiple stressors are confounding estimates for radio sensitivity by lowering the apparent dose rate at
51 which radiation exposure effects may be seen (Garnier-Laplace et al., 2013).

52 Tritium is a naturally-occurring trace isotope of hydrogen that is produced when cosmic rays interact
53 with nitrogen atoms in the upper atmosphere. Aquatic concentrations of naturally-occurring tritium are
54 ~2 Bq/L or lower. It has a half-life of 12.3 years and decays to helium. Anthropogenic formation of
55 tritium occurs by the neutron activation of lithium, boron and hydrogen isotopes during, for example,
56 normal operation at nuclear power plants, at research reactors and at fuel processing facilities. Tritium
57 contamination in groundwater, due to planned and unplanned releases from such facilities (NRC, 2016;
58 Galeriu et al., 2005; CNSC, 2009), may impact organisms living in the local receiving water. Tritium
59 releases to the aquatic environment contribute to the doses received by aquatic organisms in the form
60 of the highly bioavailable tritiated water (HTO), and as bioavailable organically bound tritium (OBT).
61 Upon ingestion of bioavailable tritium, endogenous OBT will also be formed. Aquatic organisms
62 chronically exposed to tritium contaminated water, therefore, may display certain toxicological

63 endpoints like genotoxicity, cellular stress responses and developmental effects at dose rates from
64 tritium decay at or below 500 $\mu\text{Gy/hr}$ (Jha et al., 2006; Adam-Guillermin et al., 2012). A study of channel
65 catfish peripheral blood B-lymphoblast and fathead minnow testis cells exposed to tritium
66 concentrations of up to 100 kBq/L (Stuart et al., 2016) showed detectable DNA damage based on
67 formation of micronuclei and the comet assay. Exposure tests performed with teleost exposed to up to
68 1,000 \times higher tritium activity concentrations showed non-transient effects such as decreased gonad
69 organ weight, fewer spermatogonia and formation of OB_T (as tritiated nucleic acids and tritiated
70 phospholipids) (Hyodo-Taguchi and Egami, 1977; Ueno and Yusuke, 1978; Etoh and Hyodo-Taguchi,
71 1983; Hyodo-Tagushi and Etoh, 1993). At tritium activity concentrations much lower than
72 100,000 kBq/L, biomarker analyses from a field study (Gagnaire et al., 2017) and a laboratory study (in
73 preparation) showed exposure related changes for DNA damage in gonad tissue, micronucleus
74 frequency in blood cells, phagocytosis activity in spleen and lysosomal membrane integrity in spleen
75 from fathead minnows (*Pimephales promelas*). For the field study, the fish were raised within water
76 sources that varied in tritium contamination from background levels to levels that were 3.5 \times and 25 \times ,
77 respectively, higher than the current Canadian drinking water guideline for tritium of 7 kBq/L (Guidelines
78 for Canadian Drinking Water Quality – Summary Table, February 2017). The laboratory study emulated
79 these activity levels, but was not impacted by variable physicochemical characteristics at the different
80 field locations (conditions that may also account for some of the observed biomarker outcomes)
81 (Falfushynska et al., 2011; Omar et al., 2012; Conceição Vieira et al., 2012, Qu et al., 2014). Other
82 biomarkers tested included enzyme activities and fatty acid compositions within selected tissues. Both
83 studies consisted of a 60-day exposure period to each of the water sources followed by a 60-day
84 depuration period that, for the field study, started after moving the holding tanks to the
85 uncontaminated water source used as the reference site (Gagnaire et al., 2017) and, for the laboratory
86 study (in preparation), by discontinuing tritium additions to the aquaria.

87 In this analysis, we compare the common measured endpoints in the fathead minnow tissues from
88 these two studies. The fish were exposed to water containing increasing HTO activity concentrations
89 from background to up to 180 kBq/L. Each study also included feed containing tritiated amino acids to
90 gauge the influences of ingesting OB_T. After a 60-day exposure period, tritium activity was brought to
91 background levels for another 60 days (the depuration period). Throughout each study, both tritium
92 internalization and cellular endpoints for cell viability, genotoxicity, immune response and oxidative
93 stress were assessed. This comparison enabled us to gain insight into similarities and differences in

94 biological responses to chronic low-dose tritium exposure in a laboratory setting and in a natural
95 environment.

96 **Materials and Methods**

97 The locations of each exposure in the laboratory and field studies are shown in Figure S1. Also shown
98 are the average tritium activity concentrations at each exposure location and whether the tanks in the
99 field included feeding the fish tritiated feed (treatment group designated as “OBT”) or regular (non-
100 tritiated) feed (treatment group designated as “HTO”). For the laboratory study, we were able to test
101 fish fed OBT without the co-exposure of HTO. The laboratory aquarium in which control fish were
102 housed in water with background tritium and regular feed is designated as “T”. At the start of each
103 exposure, the fish density was 1 fish/L. Holding tanks in the field were 40 L, while aquaria in the
104 laboratory were 40 L and 20 L. Uptake of HTO was rapid, reaching equilibrium with the surrounding
105 medium within ~2 hrs. Uptake of OBT in the feed was much slower and was unlikely to be at
106 equilibrium by the end of the 60-day exposure period.

107 *Field*

108 The field study occurred between April and October, 2013. In the Chalk River Laboratories (CRL)
109 environment, groundwater plumes from waste management areas contain HTO; receiving waters are
110 therefore contaminated with tritium. Tritium on the aquatic vegetation, as OBT, is ~40% of the HTO
111 activity concentration. The source waters used for each exposure were chosen for their tritium content,
112 ranging from background (OR) to ~180 kBq/L (PC). One-month old fathead minnows, *Pimephales*
113 *promelas*, were received from the Toxicity Laboratory at the Ontario Ministry of the Environment
114 (Etobicoke, ON). These fish were a mixture of male and female juveniles. The fish were first
115 acclimatized for 20 days by housing them in the reference location in containers that were fed from the
116 reference source water, the Ottawa River (OR). In order of increasing tritium contamination, the other
117 source waters were from Maskinonge Lake (MAL), Perch Lake (PL), Duke Swamp (DSW) and Perch Creek
118 (PC). The locations of the test sites are shown in Figure S1. After the acclimatization period, fish in
119 tanks were transported to each of the test sites and the tritium exposures to above-background levels
120 started by continuous pumping source water at a rate of one tank volume per hour. For each treatment
121 group, ten fish were used for each time point. At each location, half of the tanks (OBT groups) also
122 received tritiated feed to a maximum activity concentration of up to 40,000 Bq/L (31,000 Bq/kg dry).
123 This activity concentration was chosen for OBT in feed as it represents the ~40% OBT of the HTO activity
124 of the aquatic vegetation found in the source water having the highest tritium contamination, PC. The

125 tritium exposure period was for 60 days, after which time the tritium activity concentrations (HTO and
126 OBT) were reduced to background by moving the fish at the contaminated sites to the references site
127 (i.e., OR), providing only regular feed to the fish, and then continuing the exposure to this reference
128 water and feed for an additional 60 days. The measured tritium content at each site is listed in Table 1.
129 Throughout the study, the fish were fed daily by adding an amount of commercial feed to achieve up to
130 1% of the fish body weight. Water quality parameters for pH, temperature and nitrogen compounds
131 were regularly measured. The temperature varied with the ambient air temperature, with an overall
132 average of $17.4^{\circ}\text{C} \pm 4.5^{\circ}\text{C}$. The water pH also varied over the course of the study, ranging from pH 5.5 to
133 pH 7.5. Ten individual fish were randomly sampled from each exposure group for analysis of physical
134 and biological markers at Day 15, 30, 60, 75, 90 and 120. The data obtained for Day 60 and Day 120
135 were used for the comparisons with the laboratory study.

136 *Laboratory*

137 The laboratory study occurred between September and January, 2015. Approximately five-month old
138 fathead minnows, *Pimephales promelas*, were received from Aquatics Research Organisms (Hampton,
139 NH), which was the original supplier to the Ontario Ministry of the Environment's Toxicity Laboratory
140 (Etobicoke, ON), who supplied the fish for the field study. Older fish were obtained for this study to
141 ensure that we were testing only males. The aquaria used for tritium exposures and the aquaria used
142 for the control were housed separately inside rooms that were maintained at $21 \pm 1^{\circ}\text{C}$. Each 40 L or 20 L
143 aquarium was equipped with circulation and filtration systems.

144 The fish were kept in reconstituted water prepared by adding 11.0 g of calcium chloride (CaCl_2), 8.4 g of
145 sodium bicarbonate (NaHCO_3), 3.0 g of magnesium sulfate (MgSO_4), 0.4 g of potassium chloride (KCl)
146 and 0.1 g of sodium bromide (NaBr) to 100 L of deionized (reverse osmosis) water. The tank water was
147 changed weekly and the fish were fed daily by adding an amount of commercial feed to achieve up to
148 1% of the fish body weight. Water quality parameters for pH, nitrogen compounds, temperature and
149 hardness were regularly measured.

150 Prior to being exposed to tritium, the fish were acclimated to the tank environment for 21 days, after
151 which tritium was added to cover an activity concentration range from background up to a maximum of
152 180,000 Bq/L. One tank, referred to as 25KOBT, was treated with an average of 25 KBq/L tritium as HTO
153 and also received tritiated feed to an activity concentration of 27,000 Bq/L (21,000 Bq/kg dry). This
154 activity concentration was chosen as it represents OBT at equilibrium with the HTO activity concentration.
155 A second tank, referred to as OBT, received background reference water and tritiated feed, also to an

156 activity concentration of 27,000 Bq/L (21,000 Bq/kg dry). The remaining aquaria are referred to as
157 T (control), 12K, 25K and 180K in reference to the added HTO tritium activity concentrations.

158 The tritium exposure period was for 60 days, after which time the tritium activity concentrations were
159 reduced to background values for an additional 60-day depuration period. Ten individual fish were
160 randomly sampled from each exposure group for analysis of physical and biological markers at multiple
161 time points. The data obtained at 60 days and 120 days were used for the comparison with the field
162 study.

163 **Preparation of Tritiated Food**

164 The same material lots and batches were used for the field and laboratory studies. Silver Cup extruded,
165 sinking, 1.0 mm pellet fish feed was ordered from Martin Mills Inc. (Elmira, ON). The OBT-spiked feed
166 was prepared by adding three radio-labelled amino acids: lysine, leucine and arginine. The tritiated
167 amino acids (Perkin Elmer) were purchased as 1 mL volumes, each containing 37 MBq of tritium. These
168 amino acids were diluted to a concentration of 245 KBq/L, and then pre-determined quantities of the
169 diluted amino acids were further diluted and emulsified by mixing with vegetable oil, water and soy
170 lecithin to the final desired concentration (listed in Table 1). This was done inside of a 600 mL flask
171 warmed to 50°C on a stir plate and mixed for approximately 20 min to make a homogenous emulsion.
172 Non-labeled control food was also mixed with oil, water and soy lecithin to eliminate the potential for
173 the variable of feed palatability, and to ensure equivalent food quality was added to each tank and
174 aquarium. The emulsion was then added to the commercial feed pellets and thoroughly mixed inside of
175 a 1 L Nalgene bottle before dispensing.

176 **Tritium Measurements**

177 *HTO in source waters and aquaria*

178 HTO activity concentrations within the tanks or the aquaria were measured weekly throughout the
179 exposure and depuration periods. Water samples were collected from each tank and aquarium in 15 mL
180 centrifuge tubes that had been rinsed with sample water 3× beforehand. From each 15 mL volume, a
181 1 mL aliquot was pipetted and transferred into a labelled 20 mL polyethylene vial and mixed with 5 mL
182 of Ultima Gold XR scintillation cocktail. A standard of 242 Bq/mg tritium was used to determine counter
183 efficiency. A blank sample was also prepared that contained 1 mL of deionized water and 5 mL of Ultima
184 Gold XR scintillation cocktail. The samples were mixed and counted for 15 min on a Tri-Carb 2700 TR
185 Liquid Scintillation Analyzer. The detection limit was approximately 50 Bq/L.

186 *Tissue free water tritium and OBT in fish*

187 On Days 0, 15, 30, 60, 75, 90 and 120, ten minnows per treatment group were randomly collected for
188 analysis of tissue free water tritium (TFWT) and OBT activity concentrations. Two to four carcasses were
189 also pooled and analyzed at Days 0, 60 and 120. The resulting tritium values were corrected for dilution
190 and background. The resulting dose rates from TFWT and OBT were calculated as described in Gagnaire
191 et al. (2017).

192 TFWT was vacuum extracted from frozen fish and collected on a refrigerated circulator trap (-35°C) using
193 a freeze-drying system. OBT was determined by combustion as described by Kim et al. (2015). For the
194 fish samples, 2 g each of homogenized and dried carcass were mixed with 2 g of polyethylene beads.
195 The resulting tritium activity concentrations were determined by first adding tritium free water to the
196 volumes to a total volume of 10 mL, then mixing this volume with 10 mL of Ultima Gold XR scintillation
197 cocktail and counting on a Tri-Carb 3110 TR Liquid Scintillation Analyzer. The minimum detectable
198 activity (MDA) for TFWT was approximately 40 Bq/L, with an estimated uncertainty of 15%, and for OBT
199 was approximately 50 Bq/L, with an estimated uncertainty less than 50%.

200 *OBT in feed*

201 OBT concentration from duplicate samples of fish feed was determined by combustion as described by
202 Kim et al. (2015). The resulting combustion water was distilled and a 10 mL volume was mixed with
203 10 mL of Ultima Gold XR scintillation cocktail. The OBT activity concentration determined using a Wallac
204 QUANTALUS™ 1220 LSC with a count time of 360 minutes and a MDA of approximately 5 Bq/L.

205 **Biological Parameters**

206 *Fish dissection and sample collection*

207 At the designated time points, the sampled fish were euthanized via rapid cooling followed by
208 decapitation in accordance with the animal care protocol, approved by the CRL Animal Care Committee.
209 Each fish was weighed and dissected for various endpoint measurements. Spleen, liver and gonads
210 were weighed to calculate the Spleno-Somatic Index (SSI), Hepato-Somatic Index (HSI) and Gonado-
211 Somatic Index (GSI), respectively as:

$$\text{Organ Somatic Index} = \frac{100 \times \text{organ weight (g)}}{\text{fish weight (g)}}$$

212 Fulton's condition factor (K) was calculated as (fish weight (g) / (fish length (cm))) (Amara et al., 2009).

$$\text{Fulton's Condition Factor, } K = \frac{100 \times \text{fish weight (g)}}{\text{fish length (cm)}^3}$$

213 A small amount of blood was collected from each fish, smeared on a microscope slide and air dried
214 before processing for counting micronuclei (MN). More blood was collected and mixed with
215 cryopreservation buffer (8.55 g sucrose, 1.176 g sodium citrate tribasic dihydrate and 5 mL of dimethyl
216 sulfoxide (DMSO) in 100 mL of phosphate buffer saline solution (PBS)), and stored at -80°C for future
217 analyses.

218 The brain was extracted, weighed and placed in homogenization buffer consisting of PBS mixed with
219 20% glycerol and 1 M phenylmethylsulfonyl fluoride (PMSF). The brain tissues were stored at -80°C for
220 later protein analyses.

221 The spleen was removed, weighed and gently pressed through a sterilized $40\ \mu\text{m}$ Cell Strainer (Becton
222 Dickinson©) in modified L15 medium and kept at 4°C until analysis the following day. Spleen cell
223 phagocytosis activity and lysosomal membrane stability were then assessed by flow cytometry, as
224 described in the next section, within 24 h of collection.

225 The liver was removed, weighed and then divided into two fractions: one fraction was placed in
226 homogenization buffer and stored at -80°C for later enzymatic analyses, and the other fraction was
227 gently pressed through a sterilized $40\ \mu\text{m}$ Cell Strainer (Becton Dickinson©) then further sub-divided
228 (one sub-fraction was stored at -20°C and used for fatty acid composition analysis, and the other
229 sub-fraction was placed at -80°C).

230 Gonad tissues were removed, weighed and then pressed through a sterile $70\ \mu\text{m}$ cell strainer in
231 cryopreservation buffer (field study, stored at -80°C until analysis) or PBS (laboratory study, stored
232 overnight at 4°C and then analyzed). The pressed sample was used to measure cell viability and DNA
233 damage by the comet assay. Pieces of the gonad tissues were also archived by preserving them in
234 RNALater™ (Life Technologies) at 4°C overnight, then at -20°C .

235 *Flow cytometry analyses for immunity endpoints*

236 Analyses were carried out on the splenic suspensions in a 96-well plate using the MPL module of a Cell
237 Lab Quanta flow cytometer (Beckman Coulter). For each sample, at least 10,000 cells were counted. All
238 assays were performed using $100\ \mu\text{L}$ of 3×10^6 cells/mL in modified L15 buffer cell suspension buffer
239 mixed with $200\ \mu\text{L}$ of phosphate buffered saline. The total and propidium iodide-positive spleen cell
240 distributions were detected simultaneously as previously described (Bado-Nilles et al., 2014).

241 Phagocytosis activity was assessed using fluorescent beads (FluoSpheres® carboxylate-modified
242 microspheres, 1.0 µm, yellow-green fluorescent (505/515), Invitrogen). The splenic cells were incubated
243 with diluted beads (ratio beads:cells 130:1) for 1 hr at room temperature and then analyzed for uptake
244 of fluorescence. Only the events showing a fluorescence intensity of at least three beads were
245 considered positive for phagocytic activity. Results were expressed as the percentage of positive cells
246 for phagocytosis activity. Lysosomal membrane integrity (LMI) was measured (following excitation with
247 a 488 nm laser) as mean fluorescence intensity (MFI) of red fluorescence after incubating spleen cells
248 with acridine orange (10 µM, Sigma) (Bado-Nilles et al., 2013).

249 *Comet assay for DNA damage*

250 The comet assay was performed as previously described in Festarini et al. (2016). Images of at least
251 100 cells per fish were captured at 400× magnification using an Axio Observer.Z1 fluorescent
252 microscope system (Carl Zeiss Canada Ltd.), equipped with a fluorescein filter and an Exi Aqua colour
253 bioimaging microscopy camera (QImaging). For each cell, the CometN application of the Northern
254 Eclipse Version 8.0 image analysis software (Empix Imaging, Inc.) was used to measure comet length,
255 comet moment and tail moment.

256 *Micronucleus frequency for DNA damage*

257 The blood smears were stained with either a Giemsa solution (10% Giemsa in Gurr Buffer, during the
258 laboratory study) for 15-20 minutes or an acridine orange solution (50 µg/mL, during the field study) for
259 15 to 30 seconds, then the slides were rinsed twice with deionized water. Slides were viewed at
260 400× magnification using a Leica DMLS fluorescence microscope. At least 1,100 cells per slide were
261 scored and the micronucleus frequency (MN per 1,000 cells) was calculated using the following
262 equation:

$$MN \text{ frequency} = 1000 \left[\frac{1 \times n_{1MN} + 2 \times n_{2MN} + 3 \times n_{3 \geq MN}}{n_{total}} \right]$$

263 where n_{1MN} refers to the number of cells with a micronucleus, n_{2MN} refers to the number of cells with
264 two micronuclei, $n_{3 \geq MN}$ refers to the number of cells with three or more micronuclei, and n_{total} refers to
265 the total number of cells counted on the slide.

266 *Enzymatic assays*

267 Brain, liver and muscle were each homogenized in an ice cold potassium phosphate buffer (0.1 M,
268 pH 7.5) supplemented with 20% glycerol and 0.2 mM PMSF to inhibit proteolysis. Total protein

269 concentrations were determined by the Bradford (1976) method using bovine serum albumin as the
270 protein standard. The tissue homogenates were centrifuged at 10,000 g, 4°C for 15 min and the
271 resulting supernatant fraction was used for biomarker measurements. Hepatic biomarkers tested were
272 glutathione peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD), and brain biomarkers
273 tested were GPX and SOD. These were measured as described by Paglia and Valentine (1967), Babo and
274 Vasseur (1992) and Paoletti et al. (1986), respectively. All of these assays were adapted to 96-well
275 microplates. Results are expressed as enzyme activity units per mg of total protein, U/mg protein.

276 *Fatty acids*

277 The fatty acids were extracted to isolate lipids using the methods of Bligh and Dyer (1959) by Lipid
278 Analytical, University of Guelph, Guelph, Ontario.

279 **Statistical Analysis**

280 Multivariate analyses comparing the laboratory study (in preparation) and the field study (Gagnaire et
281 al., 2017) were performed using the R software (version 3.3.3) (R Core Team, 2017). Non-metric
282 dimensional scaling (NMDS) and analysis of similarity (ANOSIM) were performed using the 'vegan'
283 package, version 2.4-3. The number of dimensions was chosen to achieve a scaling stress of
284 approximately 5% or lower. The distance metric was Euclidian. Only those parameters that were
285 measured for both studies were included in the analysis. Pairwise comparisons between the field and
286 laboratory datasets were done by the t-test. Functions within the package, ggplot (version 2.2.1) and
287 ggcorrplot (version 0.1.1) were used to plot and to calculate linear correlations between variables.

288 **Results**

289 The two phases of each study are referred as the exposure phase (or Day 60) and the depuration phase
290 (or Day 120). We compared the experimental results for genotoxicity, immune response and oxidative
291 stress in tissues of fathead minnows exposed to tritium. The measured tritium values in food and water,
292 and estimated dose rates from each study, are listed in Table 1. Estimated dose rates ranged from 2.1
293 $\times 10^{-4}$ to 6.5×10^{-1} $\mu\text{Gy/hr}$. The laboratory study aimed to emulate the field conditions in terms of the
294 tritium activity concentrations (as tritiated water and as tritiated food). The range of tritium activities in
295 the laboratory study were wider than what was measured for tritium activity at the field sites. Figure 1
296 shows the estimated total dose rates from the 60 day exposures to HTO and OBT, as $\mu\text{Gy/hr}$, relative to
297 the average HTO activity concentration in the tanks and aquaria. The asterisks in the plot refer to the
298 estimated dose rates in the laboratory study when OBT was added to the feed either alone or in

299 combination with 25 kBq/L HTO. This plot shows that the estimated dose rates in the field, based on
300 internal tritium, appear to be higher for fish exposed in the field than for fish exposed in the laboratory.
301 This plot also shows that the OBT added with the feed did not contribute additional dose.

302 *Physicochemical conditions within tanks in the field and aquaria in the laboratory*

303 Water temperatures were more varied over the field study than over the laboratory study. The changes
304 in temperature over time are shown in Figure S2, and the average values over the exposure and
305 depuration phases are listed in Table 2. Over the exposure phase, the average temperatures were
306 similar between exposure locations, at approximately 20°C, but after Day 60 and to the end of the
307 study, the ambient air temperature in the field declined to an average of about 13°C at each exposure
308 location. By the end of the study, water temperatures in the field were below 10°C.

309 Water pH values were also more varied over the field study than over the laboratory study. The changes
310 in pH over time for both studies are shown in Figure S3. The average values by phase are listed in
311 Table 3. The pH values in the field were lower than in the laboratory, averaging about pH 6.6 in the field
312 compared to pH 7.7 in the laboratory. The trend of a rising pH between April and October, 2013 in the
313 field may reflect the lower carbon dioxide solubility at lower water temperatures.

314 *Fathead minnow growth and fatty acid metabolism*

315 The growth characteristics of the fathead minnows are shown in Figure 4 as boxplots for fresh weight
316 and standard length. A plot of the weight-to-length ratios are shown in Figure S4. The fish used in the
317 field study were younger than the fish used in the laboratory study. Furthermore, the one-month
318 juveniles used in the field study were a mixture of males and females. These fish were also smaller than
319 the initially five-month old male fish used in the laboratory study. Age-related differences in weight and
320 lengths between the two studies can be seen in Figure 2. The change in weight and length between
321 exposures groups was not significant. The only notable difference between the field and laboratory
322 length-to-weight ratios were for the fish tested at the end of the depuration period of the laboratory
323 study. These fish were leaner at the last time point, when these fish were about nine-months old
324 (Figure S4). This observation was seen across each treatment group and is unlikely to be an effect of
325 tritium exposure.

326 To gauge differences in food sources, we compared the liver fatty acid compositions at the end of the
327 exposure phase (Day 60) and at the end of the depuration phase (Day 120). We find that the
328 metabolism of specific fatty acids differed between the two studies. For example, the C18:1 trans fatty

329 acid (vaccenic acid, octadec-11-enoic acid) in the laboratory study was not significantly correlated with
330 any of the other liver fatty acids (Figure 3B, Male Liver Day 60 and Day 120). In the field study, this same
331 fatty acid was negatively correlated (purple) with the omega-3 and omega-6 fatty acids, and positively
332 correlated (red) with the saturated fatty acids.

333 Non-metric multidimensional scaling of the fatty acids by class (unsaturated, monounsaturated and
334 polyunsaturated), split into omega-3 and omega-6 polyunsaturated fatty acids, show that the field
335 exposed fish had liver compositions higher in saturated and monounsaturated fatty acids and the
336 laboratory exposed fish had liver compositions that were higher in omega-3 and omega-6
337 polyunsaturated fatty acids.

338 *Multivariate analyses of biomarkers*

339 Biomarkers of exposure to toxicants provide a gauge for potential effects that may enable prediction of
340 overall consequences to a population. Both the laboratory study and the field study measured markers
341 for DNA damages, immune system and enzymatic activities in tritium exposed fathead minnows. These
342 were: comet assay in gonad, micronucleus frequency in blood, lysosomal membrane integrity and
343 phagocytosis activity in spleen cells, activity of enzymes linked to oxidative stress, and catalase and SOD
344 activities in liver, muscle and brain tissues. The results of these comparisons are shown in Figure 4 and
345 Figure 5.

346 *Non-metric multidimensional scaling using biomarkers for genotoxicity and lysosomal membrane* 347 *integrity*

348 We find that the biomarker responses differed between the two studies. Non-metric multidimensional
349 scaling of individual fish responses for biomarkers of genotoxicity and innate immunity showed clear
350 separation between the studies. Scaling was integrated over three dimensions, resulting in a stress of
351 3.9% (Figure 4A, NMDs plot). Analysis of similarities, ANOSIM, shows that the fish from each study
352 responded differently (R statistic of 0.513, significance 0.001, Figure 4A, NMDS plot). The fish from the
353 field study were more like other fish from the field study, and fish from the laboratory study were more
354 like other fish from the laboratory study. Responses based on these biomarkers were not significantly
355 different between exposure and depuration (R statistic of 0.01, significance 0.149, Figure 4A, NMDS
356 plot). The analyses of similarity are corroborated by multiple pairwise analyses within and between the
357 two studies (Table S1).

358 *Correlations between biomarkers for genotoxicity and lysosomal membrane integrity*

359 By plotting the correlations between the measures for relative lysosomal membrane integrity with the
360 measures for DNA damages and phagocytosis activity (Figure 4B), we find that, for both studies, the
361 lysosomal membrane integrity in spleen cells correlated with gonad DNA damages by the comet assay
362 ($R^2 = 0.83$, each, Figure 4B, top left plot), but only the results from the laboratory study were correlated
363 with DNA damages as measured by the micronucleus assay ($R^2 = 0.89$ vs 0.3 , Figure 4B, top right plot).
364 Temperature variations over the course of the field study do not appear to have influenced the
365 measurement of micronuclei (Figure 4 B top left inset and top right inset). The number of micronuclei
366 were higher in the laboratory exposed fish.

367 The values for fish exposed to OBT in feed (Figure 4B, large triangle all plots) were not included in the
368 correlations because, although the estimated dose rate from OBT was on a par with the fish from the
369 field study (Figure 1, asterisk), the DNA damage responses by these fish were like that of the control fish.
370 This relationship can be seen in the plot comparing results for micronucleus frequency and the comet
371 assay (Figure 4B, bottom right plot).

372 *Correlations between phagocytosis activity and lysosomal membrane integrity*

373 We find that the relative lysosomal membrane integrity in spleen cells correlated with spleen cell
374 phagocytosis activity in the laboratory study, but not in the field study ($R^2 = 0.98$ vs 0.22 , Figure 4,
375 bottom left plot). The phagocytosis activity in spleen for field exposed fish appeared to be at maximum
376 capacity for each exposure at around 31% (average). The inset plot shows that phagocytosis activity was
377 higher during the warmer exposure part of the field study, at about 40% activity (see the average
378 temperature values listed in Table 2 and shown in Figure S2). The inset also shows that phagocytosis
379 activity for these fish was lower during the cooler depuration part of the study, at about 20% activity.
380 Even at this lower rate, the cellular innate immunity in the field exposed fish is more highly activated
381 than that in the laboratory.

382 *Correlations between genotoxicity biomarkers*

383 Study locations display differences in the correlations between the comet and micronucleus frequency.
384 Figure 4B, bottom right plot, shows the relationship between DNA damage measured by the comet
385 assay and DNA damage measured by the micronucleus assay. For the laboratory exposed fish, these
386 measures are well correlated ($R^2 = 0.9$), but for the field exposed fish, DNA damage measured by the
387 comet assay is not well correlated with DNA damage measured by micronucleus frequency ($R^2 = 0.32$).
388 The higher proportion of activated spleen cells for fish in the field may help explain these differences.

389 *Non-metric multidimensional scaling using biomarkers for enzyme activity*

390 We find that the biomarker responses for enzymatic activities differed between the two studies.

391 Non-metric multidimensional scaling of individual fish responses for catalase (CAT), superoxide
392 dismutase (SOD) and acetylcholine esterase (AChE) activities in liver, brain and muscle of individual fish
393 showed separation between the laboratory study and the field study (Figure 5A), but the separation is
394 not as clear as the separation seen for the genotoxicity biomarkers (Figure 4A). Scaling was integrated
395 over four dimensions, resulting in stress of 5.4%. The analysis of similarity (ANOSIM) shows that the
396 location and phase of the studies are significant for these markers (p-value = 0.001), with similarity
397 coefficients of 0.084 and 0.39, respectively.

398 A comparison of catalase and superoxide dismutase activities between field and laboratory tritium
399 exposures (Figure 5B) show that liver catalase activity was higher in the laboratory exposures, but that
400 liver superoxide dismutase was higher in the field exposures. Multiple pairwise analyses within and
401 between the two studies (Table S2) show that these differences were significant across each exposure
402 group between the laboratory and the field. The differences in oxidative stress response in liver may be
403 associated with differences in field and laboratory conditions.

404 **Discussion**

405 We compared multiple biological markers in tissues of fathead minnows exposed to tritium in both field
406 and laboratory settings. Tritium activity concentrations ranged from background values of 0.002 and
407 0.06 kBq/L to up to 25× the Canadian drinking water guideline of 7 kBq/L. The initiating event for the
408 effects of radiation exposure is damage to macromolecules. The highest dose rate estimated from
409 internalized tritium is 0.14 µGy/hr (field) and 0.65 µGy/hr (laboratory). Aquatic organisms exposed to
410 much higher dose rates from HTO and tritiated amino acid uptake display DNA damage and markers for
411 cytotoxicity (Jha et al., 2005; Jha et al., 2006; Jaeschke et al., 2011; Hagger et al., 2005; Suyama et al.,
412 1981). At the much lower dose rate in our study, we also found that for both the field and laboratory
413 exposures, the measures for DNA damage and fluorescence associated with lysosomal processes
414 increased with increased tritium activity concentrations. The proportion of activated splenic cells,
415 however, was different for the two studies. In the field exposed fish, the markers for phagocytosis and
416 lysosomal activities were not correlated to the tritium exposure groups, while in the laboratory exposed
417 fish, these markers were correlated to the tritium exposure groups.

418 The range of values for DNA damage, as measured by the comet assay, were similar for both field and
419 laboratory (Figure 4B, top left plot), but the field exposed fish spanned a narrower range of comet tail
420 moment values. DNA damage, as measured by micronucleus frequency, was lower for the field exposed
421 fish compared to the laboratory study (Figure 4B, top right plot). These results contrast with the
422 outcome of a meta-analysis comparing estimated population dose rate effects on markers for morbidity
423 for biota living within the Chernobyl exclusion zone. This meta-analysis found an apparently higher
424 radio-sensitivity in field exposed biota compared with similar markers for morbidity from laboratory
425 data (Garnier-Laplace et al., 2013). However, in our comparison of fathead minnows grown in tritiated
426 environments, the markers for DNA damage do not indicate higher stress or sensitivity in the field
427 exposed fish.

428 Given that a goal of environmental protection is to gauge the risk to populations using the dose
429 response relationships of individuals within a population, the biomarkers employed would ideally
430 display a change with an increase in the exposure of interest. In our comparison, the estimated dose
431 rates in the field exposed fish were associated with lower average aqueous tritium activity
432 concentrations (Figure 1). This finding suggests that tritium accumulation in fathead minnows may
433 reflect multiple exposure pathways, namely the internalized tritium was not derived solely from the
434 aqueous tritium concentration in the water (which is easy to measure and forms the basis of dose
435 modeling), but that the internalized tritium was also derived from other forms of tritium that the fish
436 consumed or retained when feeding. Although the fish in both studies were given feed from the same
437 lot and, thus, the tritium activity concentration was the only variable, the possibility for access to
438 products of primary production and insects in the field means that field exposed fish had likely
439 consumed algae and other material that was not accessible to the laboratory exposed fish. The form of
440 the tritium consumed and the form of the tritium retained are, therefore, important considerations for
441 predicting any corresponding population effects. Differences in feeding opportunity is supported by the
442 measured differences in liver fatty acid compositions between the laboratory and field studies
443 (Figure 3).

444 Aquatic organisms equilibrate rapidly with the ambient water HTO concentrations (Blaylock et al., 1986;
445 Melintescu and Galeriu., 2011). The exchange of tritium from HTO with the hydrogen in biological
446 molecules (the exchangeable OBT) takes longer to equilibrate (Melintescu et al., 2011; Smith et al.,
447 2006). Longer still is time for formation of non-exchangeable OBT from ingested HTO (Kim et al., 2015)
448 and the non-exchangeable form of OBT also has a longer biological half life (Kim et al., 2013; Kim et al.,

2015). OBT obtained from the environment, rather than as HTO, may be bioavailable if it is ingested as part of the food chain. To account for multiple possible exposure pathways, minnows from both of the exposure studies were also exposed to tritiated food in the form of tritiated amino acids, and minnows from the field exposure, we posit, were also exposed to tritiated natural food sources such as from photosynthetic algae and insects. For example, algae growing within tritium contaminated water have extractable lipid fractions accounting for 45 and 58% of the tissue bound tritium and an extractable protein fraction accounting for 34% of the tissue bound tritium (IAEA, 1981). Fish feeding on these algae would digest a proportion of these tritiated macromolecules and use them in anabolic biochemical processes such as the biosynthesis of proteins, membranes and nucleic acid structures. In this scenario, the distribution of tissue bound tritium would be different than if these fish fed on only tritiated amino acids.

We also compared markers for the cellular immune response in tritium exposed minnows by measuring both the proportion of phagocytic splenic cells and the fluorescence associated with lysosomal membrane integrity of splenic cells. Since fish lack bone marrow and lymph tissues (Press and Evensen, 1999), hematopoietic cells come from the spleen and kidney (including the immune cells that function in tissue homeostasis after damage and as part of the host defence against pathogens) (Riera Romo et al., 2016; Sunyer, 2013), the difference between them being the recognized patterns for self and non-self, respectively (Tanekhy, 2016; Li et al., 2016; Matzinger, 2002).

In laboratory exposed fish, but not the field exposed fish, we observe a possible role of cell damage relative to the decay of tritium atoms since the percentage of phagocytosing splenic cells increased with exposure to increased tritium activity concentrations (Figure 4B, bottom left plot). For the field exposed fish, the lack of change in the proportion of phagocytosing splenic cells with tritium exposure may be because of the consistently high proportion of activated splenic cells (including in fish grown within the reference water source (Figure 4B, bottom left plot)) and due, we assume, to potentially unmeasured field stressors that laboratory grown fish would not experience.

The lower DNA damages and the higher proportion of activated splenic cells in field exposed fish suggest that the choice of biomarkers may help disentangle the contributions of multiple stressors on comparable low dose tritium exposures, and perhaps explain some of the differences between field and laboratory outcomes. The correlations between the genotoxicity and immunity biomarkers were high in the laboratory exposed fish, with R^2 values of 0.83 or higher (Figure 4B, all plots). The same correlations for the field study were not as good, with an R^2 values of 0.83 for the comet assay and lysosomal

480 membrane integrity. Otherwise, the comparisons between biomarkers were with R^2 values of 0.3 or
481 lower. The interrelationships between the genotoxicity markers and immunity markers from multiple
482 tissues suggest that the higher DNA damages seen in the laboratory exposures may be related to the
483 overall lower proportion of activated splenic cells in these fish, a process that would dynamically remove
484 the endogenous debris created when cells are damaged or tissues are infected by pathogens. Given
485 that challenges to innate immunity may be observable as improved fitness to subsequent challenges
486 (Wang et al., 2016; Yengkhom et al., 2018), we believe that the relationship between the markers for
487 DNA damage and the markers for immune response is worth further exploration.

488 **Conclusions**

489 Multivariate analysis of biomarkers for tritium exposure show that data obtained from the field are
490 distinct from data obtained from a laboratory study that aimed to emulate the field conditions for
491 chronic low dose tritium exposure.

492 The fatty acids composition of fish liver suggests that there were differences in feeding opportunities
493 between the two studies. Ingestion of organic tritium from natural food sources may explain the
494 differences in internalized tritium between the laboratory and field.

495 The biomarkers that display a dose-response relationship to internalized tritium are the markers for
496 genotoxicity (comet and micronuclei formation) and innate immunity (phagocytosis and lysosomal
497 membrane integrity) in both the laboratory and the field exposed fish. The genotoxicity biomarkers,
498 when viewed on their own, do not support an increase in genotoxicity in the field exposed fish. When
499 viewed in combination with the biomarkers for immunity, we see strong correlations between the
500 tritium exposures, DNA damage and innate immune responses. A high proportion of activated immune
501 cells in the field exposed fish appear to mask the correlations between micronucleus frequency and
502 lysosomal membrane integrity; even so, this host defence mechanism may protect the whole organism.
503 Indicators of overall health were not different between exposures or locations.

504 The biomarkers for oxidative stress (catalase and superoxide dismutase) were specific to each study
505 location, but may reflect ROS that is part of the innate immune response. The remaining biomarkers did
506 not correlate with tritium exposure.

507 This comparison highlights a potential for using biomarkers from the innate immune system to help
508 elucidate the impact of chronic low dose tritium exposure.

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- 668
- 669

670 **List of Tables**

671 Table 1. Tritium activity concentrations in water and feed. Values are the average concentrations
 672 measured over the 60-day exposure period. The number of samples analyzed per exposure condition
 673 was between 19 and 25 samples. The Ontario water quality guideline for tritium is 7×10^3 Bq/L
 674 (Guidelines for Canadian Drinking Water Quality – Summary Table, February 2017).

675 Table 2. Average water temperatures ($^{\circ}\text{C}$) at each study location.

676 Table 3. Average water pH at each study location.

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678 **Table 1. Tritium activity concentrations in water and feed. Values are the average concentrations**
 679 **measured over the 60-day exposure period. The number of samples analyzed per exposure condition**
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Location*	Aquarium Condition	Condition details	Average Tritium in water kBq/L $\times 10^3$	Range kBq/L $\times 10^3$	OBT in Feed kBq/L $\times 10^3 \pm$ sd	Estimated Dose Rate† $\mu\text{Gy/hr}$ at D60
L	Control (T)	Background	0.056	0 – 0.2	++	2.3×10^{-4}
L	OBT	tritiated feed	0.06	0 – 0.2	27	2.4×10^{-3}
L	12K	tritiated water	10.8	5.7 – 18.5	++	4.5×10^{-2}
L	25K	tritiated water	22.2	5.3 – 45.1	++	9.0×10^{-2}
L	25KOBT	tritiated feed and tritiated water	21.2	10.7 – 40.1	27	9.4×10^{-2}
L	180K	tritiated water	150.7	77.5 – 301.2	++	6.5×10^{-1}
F	OR**	Ottawa River	0.002	0.9 – 2	++	2.1×10^{-4}
F	MAL	Maskinonge Lake	0.3	0.2 – 0.5	++	2.8×10^{-3}
F	MAL + OBT		0.3	0.2 – 0.5	0.6 ± 0.1	2.7×10^{-3}
F	PL	Perch Lake	1.1	0.9 – 1.5	++	9.8×10^{-3}
F	PL + OBT		1.1	0.9 – 1.5	3.8 ± 0.3	6.0×10^{-3}
F	DSW	Duke Swamp	6.2	5.1 – 7.8	++	5.5×10^{-2}
F	DSW + OBT		6.2	5.1 – 7.8	10 ± 1	4.7×10^{-2}
F	PC	Perch Creek	12.4	6.6 – 22.4	++	1.3×10^{-1}
F	PC + OBT		12.4	6.6 – 22.4	63 ± 7	1.5×10^{-1}

682 * L - laboratory study location

683 * F - field study location

684 ** reference site

685 † estimated dose rates refer to total dose rates from internal HTO and OBT

686 †† below the minimum detectable activity

687 OBT organically bound tritium, added as part of the feed

688

689

690 **Table 2. Average water temperatures (°C) at each study location.**

Location	Mean (sd)	
	Exposure Phase	Depuration Phase
Laboratory	19.89 (0.64)	19.79 (0.56)
Field, average of mean values at each location	20.00 (1.34)	13.24 (0.10)
Ottawa River	20.21 (3.33)	13.28 (2.85)
Maskinonge Lake	21.96 (3.52)	13.43 (2.80)
Perch Lake	20.29 (3.75)	13.22 (2.71)
Duke Swamp	19.02 (3.77)	13.29 (2.60)
Perch Creek	18.51 (3.61)	13.16 (2.87)

691

692 **Table 3. Average water pH at each study location.**

Location	Mean (sd)	
	Exposure Phase	Depuration Phase
Laboratory	7.68 (0.14)	7.91 (0.15)
Field, average of mean values at each location	6.73 (0.22)	7.05 (0.17)
Ottawa River	6.56 (0.58)	6.91 (0.53)
Maskinonge Lake	7.11 (0.38)	7.21 (0.34)
Perch Lake	6.61 (0.18)	7.05 (0.38)
Duke Swamp	6.77 (0.18)	7.22 (0.36)
Perch Creek	6.6 (0.27)	6.86 (0.34)

693

694

695 **List of Figures**

696 Figure 1. Higher estimated dose rates (Log $\mu\text{Gy/hr}$) in the field (red circles) are associated with lower
697 average aqueous tritium activity concentrations (Log Bq/L), relative to the average measured HTO
698 activity concentration in the tanks and aquaria in the laboratory (blue triangles). The asterisks refer to
699 the estimated dose rates in the laboratory study when OBT was added to the feed either alone or in
700 combination with 25 kBq/L HTO.

701 Figure 2. Boxplot data showing the range of values for fish weights and lengths for each exposure
702 condition and at multiple time points: Day 0, 15, 30, 60, 75, 90 and 120. Labels for each exposure are
703 the same as those listed in Table 1 (red plots: the initially one-month old, un-sexed juveniles used in the
704 field; green plots: the initially five-month old males used the laboratory). Ottawa River (OR) is the
705 reference source water in the field and T refers to control in the laboratory study that had no added
706 tritium. Circles represent statistical outliers.

707 Figure 3. (A) Non-metric, multi-dimensional scaling of the liver fatty acid composition of individual fish
708 color-coded by either location (field or laboratory) or phase of the study (Day 60 or Day 120). (B)
709 Significant positive (red), significant negative (purple) and non-significant (white) linear correlations
710 between fatty acid compositions in laboratory and field exposed fish liver. Significance level was set to a
711 p-value<0.01.

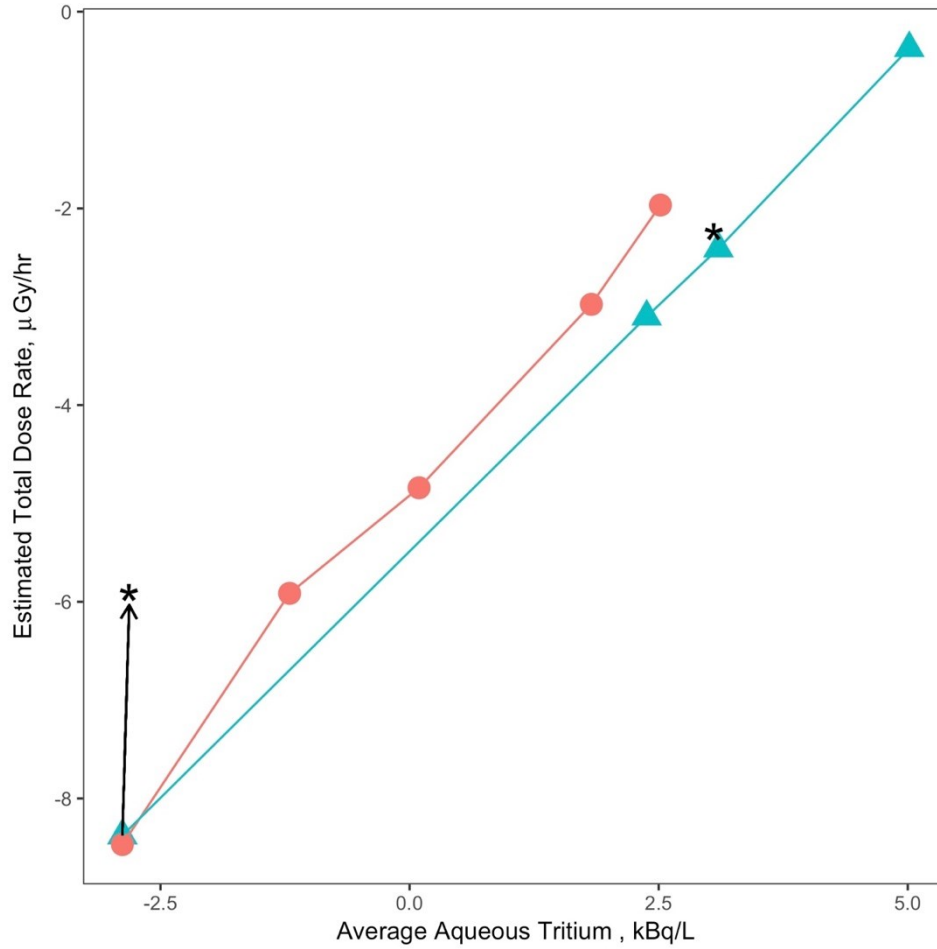
712 Figure 4. (A) Non-metric, multi-dimensional scaling of biomarker responses. Individual fish are color-
713 coded by either location (field or laboratory) or phase of the study (Day 60 or Day 120). The ANOSIM R
714 statistic for similarity and the significance values are shown. Biomarkers were DNA damage (comet and
715 MN counts) and immunity (fluorescence associated with lysosomal membrane integrity (AO) and
716 phagocytosis activity (phago)). (B) Linear correlations for the DNA damage (comet and MN counts),
717 phagocytosis activity relative to the lysosomal membrane integrity for the field data (black circles) and
718 the laboratory data (red triangles), respectively. The insets show the influence of field temperature over
719 the warmer exposure phase and cooler depuration phase. Fish exposed to OBT in feed in the laboratory
720 study (large red triangle) were not included in the correlations.

721 Figure 5. Non-metric, multi-dimensional scaling of fish responses (A) and the overall mean values by
722 study location and phase (B) for liver, muscle and brain enzymes. The ANOSIM R-statistic (A) and
723 significance values for all fish (B) are shown. The asterisks indicate significant differences in the average
724 responses, p-value>0.001, ns: s not significant.

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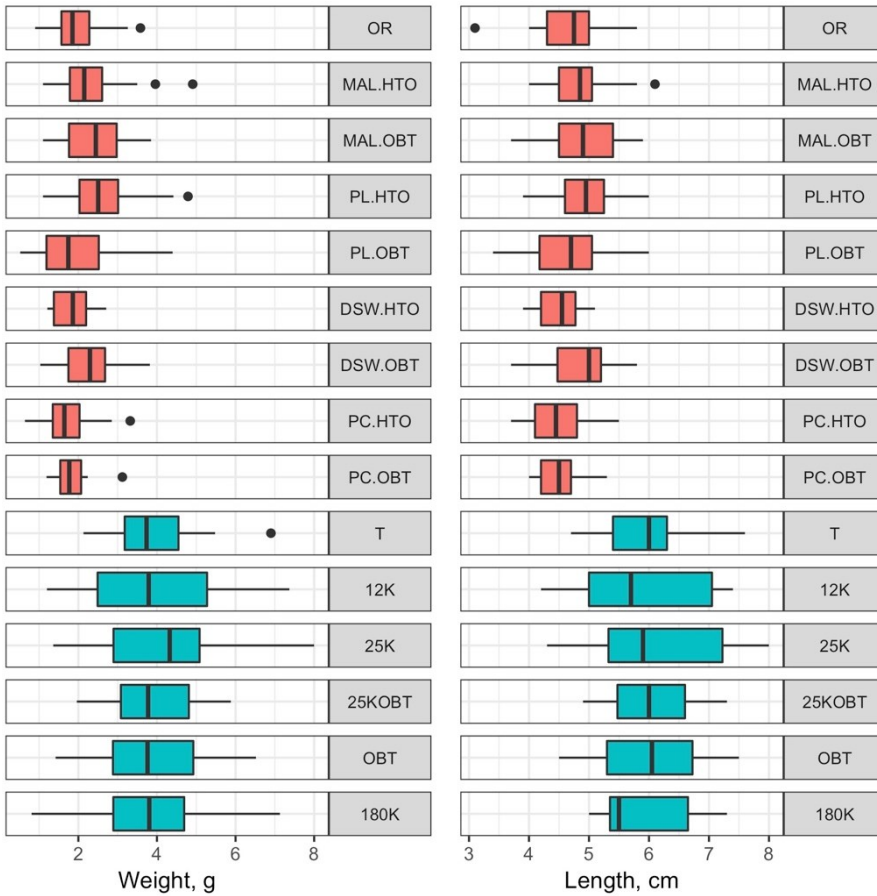
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731 **activity concentration in the tanks and aquaria in the laboratory (blue triangles). The asterisks refer**
732 **to the estimated dose rates in the laboratory study when OBT was added to the feed either alone or**
733 **in combination with 25 kBq/L HTO.**

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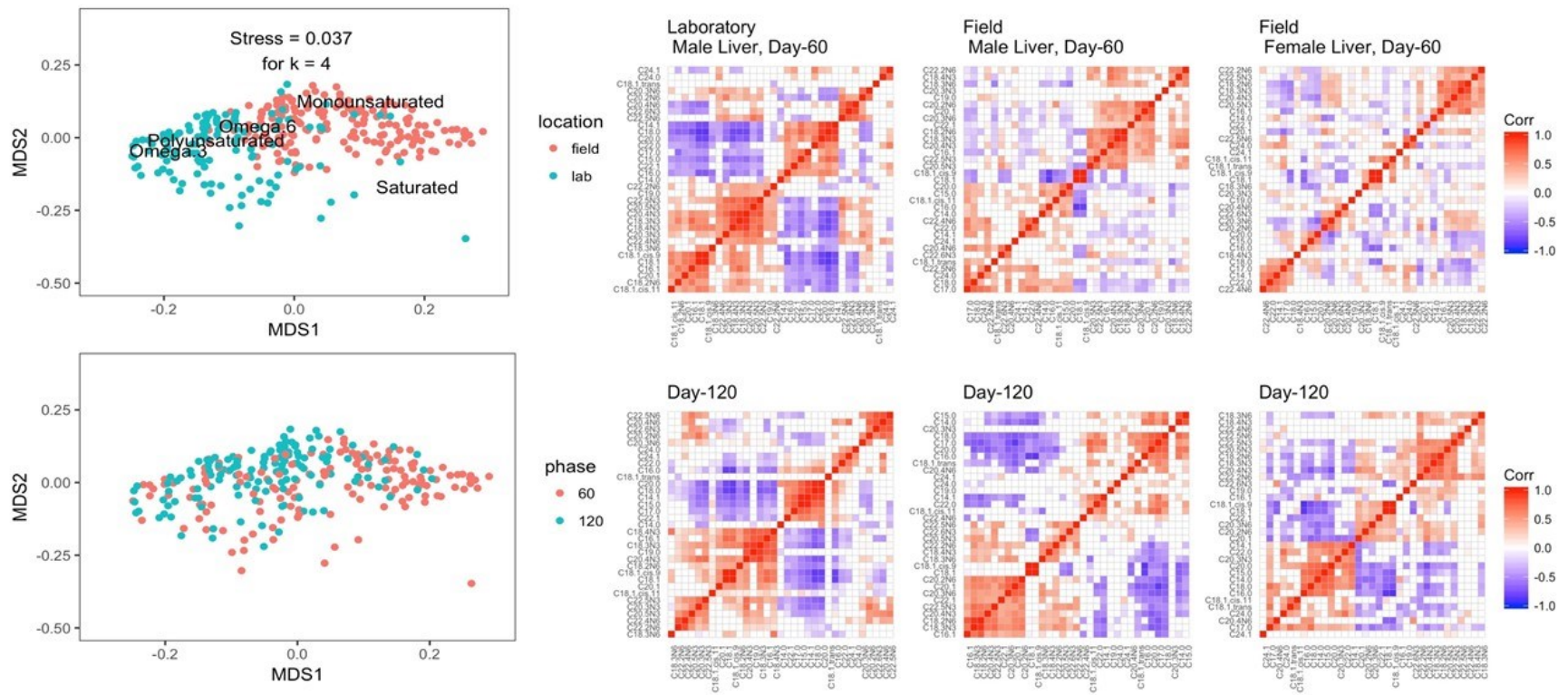


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738 **the same as those listed in Table 1 (red plots: the initially one-month old, un-sexed juveniles used in**
739 **the field; green plots: the initially five-month old males used the laboratory). Ottawa River (OR) is**
740 **the reference source water in the field and T refers to control in the laboratory study that had no**
741 **added tritium. Circles represent statistical outliers.**

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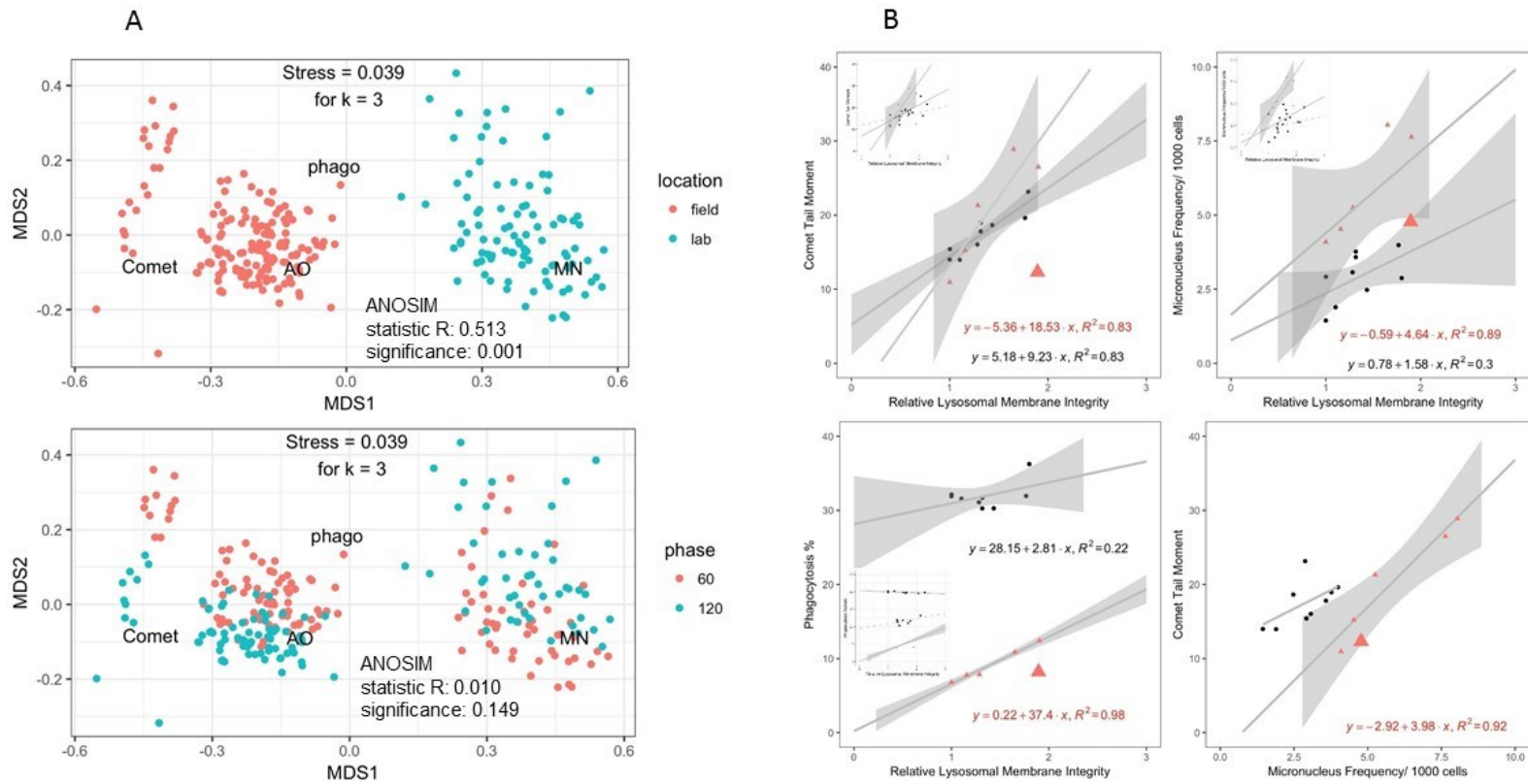
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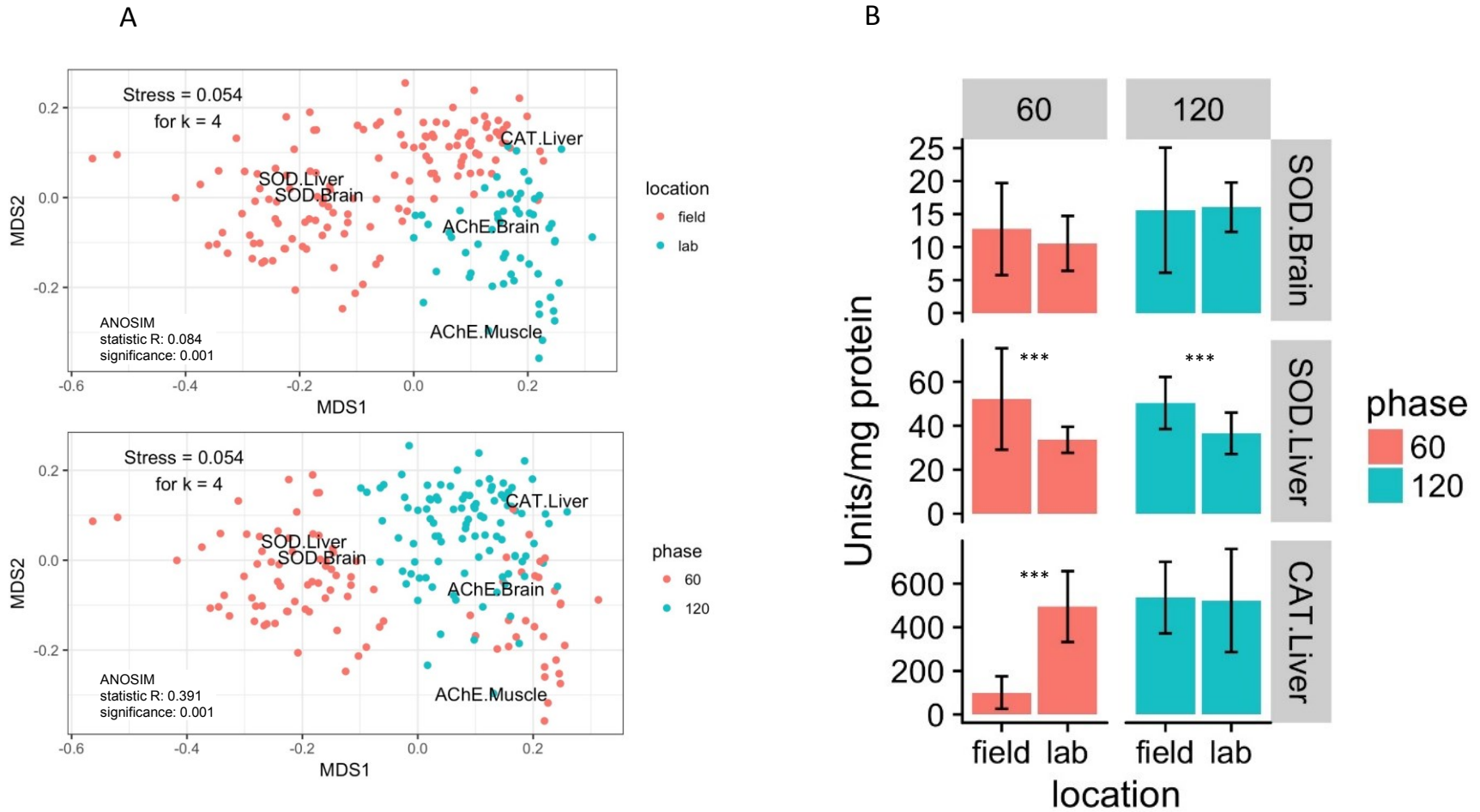
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746 **laboratory) or phase of the study (Day 60 or Day 120). (B) Significant positive (red), significant negative (purple) and non-significant (white)**
747 **linear correlations between fatty acid compositions in laboratory and field exposed fish liver. Significance level was set to a p-value<0.01.**

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 751 **laboratory) or phase of the study (Day 60 or Day 120). The ANOSIM R statistic for similarity and the significance values are shown.**
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 753 **phagocytosis activity (phago)). (B) Linear correlations for the DNA damage (comet and MN counts), phagocytosis activity relative to the**
 754 **lysosomal membrane integrity for the field data (black circles) and the laboratory data (red triangles), respectively. The insets show the**
 755 **influence of field temperature over the warmer exposure phase and cooler depuration phase. Fish exposed to OBT in feed in the laboratory**
 756 **study (large red triangle) were not included in the correlations.**



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 759 **muscle and brain enzymes. The ANOSIM R-statistic (A) and significance values for all fish (B) are shown. The asterisks indicate significant**
 760 **differences in the average responses, p-value>0.001, ns: s not significant.**

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762

SUPPLEMENTARY INFORMATION

763 **Table S1. Multiple pairwise comparisons for biomarkers: Comet, micronucleus frequency, phagocytosis activity, lysosomal membrane**
 764 **integrity. Adjustment of p-values by Benjamini-Hochberg.**

	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
1	OR vs MAL.HTO	17	0.530	0.032	0.529	0.585		17	0.486	0.029	0.519	0.574	
2	OR vs MAL.OBT	19	0.756	0.040	0.403	0.470		19	0.114	0.006	0.855	0.872	
3	OR vs PL.HTO	18	8.408	0.331	0.007	0.011	.	17	0.042	0.003	0.988	0.988	
4	OR vs PL.OBT	17	6.456	0.287	0.020	0.028	.	18	1.031	0.057	0.344	0.405	
5	OR vs DSW.HTO	18	2.652	0.135	0.099	0.128		19	2.478	0.121	0.128	0.170	
6	OR vs DSW.OBT	17	5.398	0.252	0.039	0.053		19	5.338	0.229	0.019	0.031	.
7	OR vs PC.HTO	19	18.043	0.501	0.002	0.004	*	18	6.581	0.279	0.014	0.023	.
8	OR vs PC.OBT	19	20.390	0.531	0.002	0.004	*	12	9.069	0.452	0.013	0.022	.
9	OR vs T	19	24.565	0.577	0.001	0.002	*	17	59.914	0.789	0.001	0.002	*
10	OR vs 12K	16	10.856	0.420	0.002	0.004	*	16	36.395	0.708	0.001	0.002	*
11	OR vs OBT	19	6.278	0.259	0.003	0.005	*	16	35.147	0.701	0.001	0.002	*
12	OR vs 25K	17	10.366	0.393	0.001	0.002	*	17	64.977	0.802	0.001	0.002	*
13	OR vs 25KOBT	15	14.733	0.513	0.001	0.002	*	17	84.617	0.841	0.001	0.002	*
14	OR vs 180K	18	13.528	0.443	0.001	0.002	*	16	16.441	0.523	0.002	0.004	*
15	MAL.HTO vs MAL.OBT	17	0.249	0.015	0.798	0.810		17	0.370	0.023	0.584	0.639	
16	MAL.HTO vs PL.HTO	16	5.471	0.267	0.029	0.040	.	15	0.350	0.024	0.674	0.722	
17	MAL.HTO vs PL.OBT	15	3.833	0.215	0.059	0.079		16	0.656	0.042	0.447	0.510	
18	MAL.HTO vs DSW.HTO	16	0.951	0.060	0.366	0.432		17	2.443	0.132	0.136	0.179	
19	MAL.HTO vs DSW.OBT	15	2.976	0.175	0.093	0.122		17	5.688	0.262	0.020	0.032	.
20	MAL.HTO vs PC.HTO	17	14.690	0.479	0.004	0.007	*	16	7.568	0.335	0.011	0.019	.
21	MAL.HTO vs PC.OBT	17	17.724	0.526	0.002	0.004	*	10	27.610	0.754	0.009	0.016	.
22	MAL.HTO vs T	17	28.701	0.642	0.001	0.002	*	15	61.521	0.815	0.001	0.002	*
23	MAL.HTO vs 12K	14	14.288	0.524	0.001	0.002	*	14	34.973	0.729	0.001	0.002	*
24	MAL.HTO vs OBT	17	8.242	0.340	0.001	0.002	*	14	37.378	0.742	0.001	0.002	*
25	MAL.HTO vs 25K	15	14.076	0.501	0.002	0.004	*	15	66.581	0.826	0.001	0.002	*
26	MAL.HTO vs 25KOBT	13	20.300	0.628	0.001	0.002	*	15	102.860	0.880	0.001	0.002	*
27	MAL.HTO vs 180K	16	21.651	0.591	0.001	0.002	*	14	15.782	0.548	0.002	0.004	*
28	MAL.OBT vs PL.HTO	18	11.672	0.407	0.004	0.007	*	17	0.135	0.008	0.865	0.873	
29	MAL.OBT vs PL.OBT	17	8.861	0.356	0.009	0.014	.	18	1.342	0.073	0.246	0.300	
30	MAL.OBT vs DSW.HTO	18	2.230	0.116	0.128	0.164		19	3.660	0.169	0.069	0.102	
31	MAL.OBT vs DSW.OBT	17	6.558	0.291	0.019	0.027	.	19	7.514	0.295	0.012	0.021	.
32	MAL.OBT vs PC.HTO	19	27.363	0.603	0.001	0.002	*	18	9.584	0.361	0.008	0.015	.
33	MAL.OBT vs PC.OBT	19	36.484	0.670	0.001	0.002	*	12	21.233	0.659	0.008	0.015	.
34	MAL.OBT vs T	19	39.926	0.689	0.001	0.002	*	17	72.317	0.819	0.001	0.002	*
35	MAL.OBT vs 12K	16	19.895	0.570	0.001	0.002	*	16	41.983	0.737	0.001	0.002	*
36	MAL.OBT vs OBT	19	10.848	0.376	0.002	0.004	*	16	43.312	0.743	0.001	0.002	*
37	MAL.OBT vs 25K	17	19.319	0.547	0.001	0.002	*	17	78.377	0.830	0.002	0.004	*
38	MAL.OBT vs 25KOBT	15	30.157	0.683	0.001	0.002	*	17	114.765	0.878	0.001	0.002	*
39	MAL.OBT vs 180K	18	31.683	0.651	0.001	0.002	*	16	18.819	0.556	0.001	0.002	*

	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
40	PL.HTO vs PL.OBT	16	0.628	0.040	0.463	0.528		16	0.635	0.041	0.463	0.523	
41	PL.HTO vs DSW.HTO	17	1.425	0.082	0.238	0.287		17	1.569	0.089	0.218	0.273	
42	PL.HTO vs DSW.OBT	16	0.434	0.028	0.536	0.586		17	3.553	0.182	0.069	0.102	
43	PL.HTO vs PC.HTO	18	5.690	0.251	0.021	0.029	.	16	4.434	0.228	0.059	0.090	
44	PL.HTO vs PC.OBT	18	6.095	0.264	0.009	0.014	.	10	6.256	0.410	0.035	0.056	
45	PL.HTO vs T	18	53.609	0.759	0.001	0.002	*	15	47.337	0.772	0.001	0.002	*
46	PL.HTO vs 12K	15	32.403	0.698	0.001	0.002	*	14	28.694	0.688	0.001	0.002	*
47	PL.HTO vs OBT	18	19.930	0.540	0.001	0.002	*	14	27.882	0.682	0.001	0.002	*
48	PL.HTO vs 25K	16	33.535	0.691	0.001	0.002	*	15	51.233	0.785	0.001	0.002	*
49	PL.HTO vs 25KOBT	14	48.482	0.789	0.002	0.004	*	15	66.937	0.827	0.001	0.002	*
50	PL.HTO vs 180K	17	64.629	0.802	0.001	0.002	*	14	13.053	0.501	0.002	0.004	*
51	PL.OBT vs DSW.HTO	16	0.712	0.045	0.423	0.488		18	0.552	0.031	0.497	0.555	
52	PL.OBT vs DSW.OBT	15	0.091	0.006	0.931	0.931		18	3.127	0.155	0.096	0.136	
53	PL.OBT vs PC.HTO	17	10.081	0.387	0.008	0.013	.	17	4.559	0.222	0.045	0.069	
54	PL.OBT vs PC.OBT	17	14.422	0.474	0.001	0.002	*	11	13.585	0.576	0.005	0.011	.
55	PL.OBT vs T	17	49.855	0.757	0.001	0.002	*	16	66.853	0.817	0.001	0.002	*
56	PL.OBT vs 12K	14	29.904	0.697	0.001	0.002	*	15	38.641	0.734	0.001	0.002	*
57	PL.OBT vs OBT	17	17.275	0.519	0.001	0.002	*	15	41.272	0.747	0.001	0.002	*
58	PL.OBT vs 25K	15	30.698	0.687	0.001	0.002	*	16	72.163	0.828	0.001	0.002	*
59	PL.OBT vs 25KOBT	13	48.266	0.801	0.001	0.002	*	16	107.366	0.877	0.001	0.002	*
60	PL.OBT vs 180K	16	64.511	0.811	0.001	0.002	*	15	17.982	0.562	0.001	0.002	*
61	DSW.HTO vs DSW.OBT	16	0.464	0.030	0.556	0.602		19	1.410	0.073	0.250	0.302	
62	DSW.HTO vs PC.HTO	18	7.972	0.319	0.012	0.018	.	18	2.518	0.129	0.128	0.170	
63	DSW.HTO vs PC.OBT	18	8.617	0.336	0.006	0.010	*	12	9.974	0.476	0.006	0.012	.
64	DSW.HTO vs T	18	35.641	0.677	0.001	0.002	*	17	75.847	0.826	0.001	0.002	*
65	DSW.HTO vs 12K	15	19.183	0.578	0.001	0.002	*	16	44.045	0.746	0.001	0.002	*
66	DSW.HTO vs OBT	18	12.167	0.417	0.002	0.004	*	16	47.759	0.761	0.001	0.002	*
67	DSW.HTO vs 25K	16	19.424	0.564	0.001	0.002	*	17	81.487	0.836	0.001	0.002	*
68	DSW.HTO vs 25KOBT	14	25.753	0.665	0.001	0.002	*	17	121.280	0.883	0.001	0.002	*
69	DSW.HTO vs 180K	17	30.301	0.654	0.001	0.002	*	16	20.913	0.582	0.001	0.002	*
70	DSW.OBT vs PC.HTO	17	7.553	0.321	0.009	0.014	.	18	0.160	0.009	0.759	0.789	
71	DSW.OBT vs PC.OBT	17	8.897	0.357	0.006	0.010	*	12	2.790	0.202	0.098	0.137	
72	DSW.OBT vs T	17	43.995	0.733	0.001	0.002	*	17	71.819	0.818	0.001	0.002	*
73	DSW.OBT vs 12K	14	25.747	0.664	0.001	0.002	*	16	42.716	0.740	0.001	0.002	*
74	DSW.OBT vs OBT	17	15.557	0.493	0.001	0.002	*	16	46.489	0.756	0.001	0.002	*
75	DSW.OBT vs 25K	15	26.470	0.654	0.002	0.004	*	17	76.576	0.827	0.001	0.002	*
76	DSW.OBT vs 25KOBT	13	38.550	0.763	0.001	0.002	*	17	108.834	0.872	0.001	0.002	*
77	DSW.OBT vs 180K	16	49.908	0.769	0.001	0.002	*	16	21.329	0.587	0.001	0.002	*
78	PC.HTO vs PC.OBT	19	0.534	0.029	0.489	0.552		11	1.786	0.152	0.206	0.264	
79	PC.HTO vs T	19	61.896	0.775	0.001	0.002	*	16	65.769	0.814	0.001	0.002	*
80	PC.HTO vs 12K	16	39.850	0.727	0.001	0.002	*	15	38.934	0.736	0.001	0.002	*
81	PC.HTO vs OBT	19	27.800	0.607	0.001	0.002	*	15	43.090	0.755	0.001	0.002	*
82	PC.HTO vs 25K	17	42.461	0.726	0.002	0.004	*	16	70.074	0.824	0.001	0.002	*

	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
83	PC.HTO vs 25KOBT	15	54.399	0.795	0.001	0.002	*	16	101.123	0.871	0.001	0.002	*
84	PC.HTO vs 180K	18	77.165	0.819	0.001	0.002	*	15	19.641	0.584	0.001	0.002	*
85	PC.OBT vs T	19	73.468	0.803	0.001	0.002	*	10	26.221	0.744	0.008	0.015	.
86	PC.OBT vs 12K	16	48.496	0.764	0.001	0.002	*	9	14.525	0.645	0.011	0.019	.
87	PC.OBT vs OBT	19	30.883	0.632	0.001	0.002	*	9	18.101	0.694	0.007	0.014	.
88	PC.OBT vs 25K	17	51.372	0.763	0.001	0.002	*	10	27.746	0.755	0.009	0.016	.
89	PC.OBT vs 25KOBT	15	74.701	0.842	0.001	0.002	*	10	47.141	0.840	0.004	0.009	*
90	PC.OBT vs 180K	18	109.190	0.865	0.001	0.002	*	9	7.640	0.488	0.007	0.014	.
91	T vs 12K	16	1.706	0.102	0.183	0.229		14	0.381	0.028	0.673	0.722	
92	T vs OBT	19	3.981	0.181	0.019	0.027	.	14	2.536	0.163	0.092	0.132	
93	T vs 25K	17	3.093	0.162	0.085	0.113		15	0.392	0.027	0.716	0.759	
94	T vs 25KOBT	15	1.535	0.099	0.200	0.247		15	1.085	0.072	0.347	0.405	
95	T vs 180K	18	8.517	0.334	0.004	0.007	*	14	1.663	0.113	0.213	0.269	
96	12K vs OBT	16	0.529	0.034	0.591	0.621		13	2.507	0.173	0.109	0.149	
97	12K vs 25K	14	0.129	0.010	0.757	0.779		14	0.286	0.022	0.742	0.779	
98	12K vs 25KOBT	12	0.366	0.032	0.802	0.810		14	2.246	0.147	0.146	0.189	
99	12K vs 180K	15	1.310	0.086	0.237	0.287		13	1.420	0.106	0.225	0.278	
100	OBT vs 25K	17	0.364	0.022	0.695	0.723		14	4.231	0.246	0.036	0.056	
101	OBT vs 25KOBT	15	1.180	0.078	0.299	0.357		14	0.981	0.070	0.383	0.442	
102	OBT vs 180K	18	0.707	0.040	0.509	0.569		13	0.359	0.029	0.806	0.830	
103	25K vs 25KOBT	13	0.605	0.048	0.583	0.618		15	2.973	0.175	0.086	0.125	
104	25K vs 180K	16	0.599	0.038	0.572	0.613		14	2.354	0.153	0.103	0.142	
105	25KOBT vs 180K	14	2.372	0.154	0.140	0.177		14	1.266	0.089	0.277	0.331	

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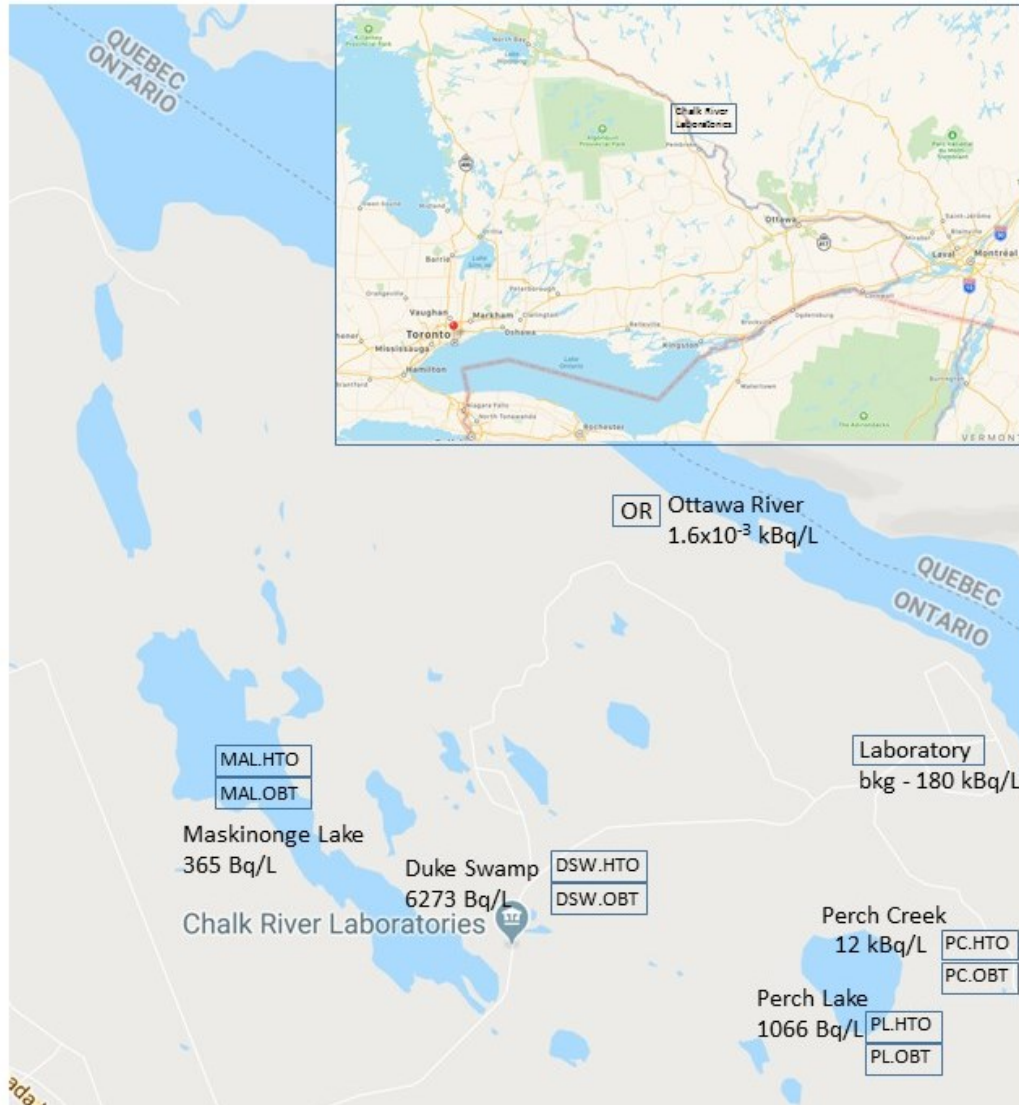
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767 **Table S2. Multiple pairwise comparisons for biomarkers: Superoxide dismutase and catalase. Adjustment of p-values by**
 768 **Benjamini-Hochberg.**

	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
1	OR vs MAL.HTO	12	0.034	0.003	0.986	0.986		15	3.554	0.202	0.071	0.373	
2	OR vs MAL.OBT	16	0.924	0.058	0.401	0.540		16	1.359	0.083	0.244	0.595	
3	OR vs PL.HTO	14	0.857	0.062	0.521	0.659		16	1.004	0.063	0.342	0.653	
4	OR vs PL.OBT	13	0.326	0.026	0.736	0.868		15	1.915	0.120	0.176	0.540	
5	OR vs DSW.HTO	13	0.143	0.012	0.923	0.986		15	4.501	0.243	0.041	0.272	
6	OR vs DSW.OBT	14	0.768	0.056	0.477	0.618		15	1.162	0.077	0.289	0.601	
7	OR vs PC.HTO	13	0.341	0.028	0.857	0.957		15	15.154	0.520	0.003	0.272	
8	OR vs PC.OBT	12	2.306	0.173	0.110	0.193		8	3.903	0.358	0.054	0.315	
9	OR vs T	12	25.527	0.699	0.002	0.006	*	13	2.823	0.190	0.110	0.444	
10	OR vs 12K	10	17.856	0.665	0.001	0.006	*	9	6.097	0.433	0.032	0.272	
11	OR vs OBT	12	29.989	0.732	0.001	0.006	*	10	1.867	0.172	0.185	0.540	
12	OR vs 25K	13	24.623	0.672	0.001	0.006	*	11	1.292	0.114	0.255	0.595	
13	OR vs 25KOBT	11	21.950	0.687	0.002	0.006	*	11	3.586	0.264	0.044	0.272	
14	OR vs 180K	13	20.753	0.634	0.001	0.006	*	11	1.851	0.156	0.180	0.540	
15	MAL.HTO vs MAL.OBT	15	1.306	0.085	0.265	0.428		18	1.363	0.074	0.252	0.595	
16	MAL.HTO vs PL.HTO	13	0.833	0.065	0.540	0.675		18	0.577	0.033	0.514	0.729	
17	MAL.HTO vs PL.OBT	12	0.108	0.010	0.901	0.985		17	0.611	0.037	0.454	0.706	
18	MAL.HTO vs DSW.HTO	12	0.082	0.007	0.972	0.986		17	0.157	0.010	0.797	0.853	
19	MAL.HTO vs DSW.OBT	13	0.887	0.069	0.378	0.524		17	1.201	0.070	0.292	0.601	
20	MAL.HTO vs PC.HTO	12	0.356	0.031	0.749	0.874		17	1.611	0.091	0.212	0.557	
21	MAL.HTO vs PC.OBT	11	2.635	0.209	0.087	0.155		10	0.204	0.022	0.766	0.838	
22	MAL.HTO vs T	11	25.348	0.717	0.005	0.011	.	15	0.367	0.026	0.582	0.736	
23	MAL.HTO vs 12K	9	17.913	0.691	0.007	0.014	.	11	6.116	0.380	0.029	0.272	
24	MAL.HTO vs OBT	11	29.816	0.749	0.002	0.006	*	12	0.315	0.028	0.640	0.781	
25	MAL.HTO vs 25K	12	24.095	0.687	0.001	0.006	*	13	0.594	0.047	0.450	0.706	
26	MAL.HTO vs 25KOBT	10	22.179	0.711	0.008	0.016	.	13	4.373	0.267	0.038	0.272	
27	MAL.HTO vs 180K	12	19.752	0.642	0.003	0.008	*	13	0.441	0.035	0.554	0.731	
28	MAL.OBT vs PL.HTO	17	3.966	0.199	0.016	0.030	.	19	0.539	0.029	0.495	0.722	
29	MAL.OBT vs PL.OBT	16	1.031	0.064	0.331	0.496		18	1.310	0.072	0.264	0.595	
30	MAL.OBT vs DSW.HTO	16	1.030	0.064	0.362	0.514		18	2.381	0.123	0.132	0.495	
31	MAL.OBT vs DSW.OBT	17	4.128	0.205	0.047	0.085		18	1.745	0.093	0.195	0.553	
32	MAL.OBT vs PC.HTO	16	0.782	0.050	0.465	0.610		18	5.919	0.258	0.015	0.272	
33	MAL.OBT vs PC.OBT	15	0.992	0.066	0.379	0.524		11	0.758	0.070	0.397	0.664	
34	MAL.OBT vs T	15	23.406	0.626	0.001	0.006	*	16	1.561	0.094	0.212	0.557	
35	MAL.OBT vs 12K	13	16.066	0.572	0.002	0.006	*	12	2.331	0.175	0.130	0.495	
36	MAL.OBT vs OBT	15	28.304	0.669	0.001	0.006	*	13	1.042	0.080	0.326	0.634	
37	MAL.OBT vs 25K	16	22.640	0.601	0.001	0.006	*	14	0.530	0.039	0.511	0.729	
38	MAL.OBT vs 25KOBT	14	19.689	0.602	0.003	0.008	*	14	3.207	0.198	0.068	0.373	
39	MAL.OBT vs 180K	16	19.016	0.559	0.001	0.006	*	14	1.022	0.073	0.325	0.634	

	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
40	PL.HTO vs PL.OBT	14	1.091	0.077	0.337	0.496		18	0.375	0.022	0.661	0.798	
41	PL.HTO vs DSW.HTO	14	1.577	0.108	0.185	0.308		18	0.835	0.047	0.405	0.664	
42	PL.HTO vs DSW.OBT	15	0.447	0.031	0.786	0.897		18	0.578	0.033	0.533	0.731	
43	PL.HTO vs PC.HTO	14	1.197	0.084	0.342	0.496		18	2.647	0.135	0.091	0.403	
44	PL.HTO vs PC.OBT	13	4.658	0.280	0.012	0.023	.	11	0.316	0.031	0.699	0.815	
45	PL.HTO vs T	13	24.265	0.669	0.001	0.006	*	16	0.495	0.032	0.541	0.731	
46	PL.HTO vs 12K	11	16.452	0.622	0.003	0.008	*	12	2.237	0.169	0.142	0.514	
47	PL.HTO vs OBT	13	26.932	0.692	0.001	0.006	*	13	0.230	0.019	0.733	0.819	
48	PL.HTO vs 25K	14	24.914	0.657	0.001	0.006	*	14	0.113	0.009	0.903	0.942	
49	PL.HTO vs 25KOBT	12	20.528	0.651	0.001	0.006	*	14	2.740	0.174	0.095	0.403	
50	PL.HTO vs 180K	14	21.825	0.627	0.001	0.006	*	14	0.225	0.017	0.724	0.817	
51	PL.OBT vs DSW.HTO	13	0.195	0.016	0.837	0.945		17	0.811	0.048	0.372	0.656	
52	PL.OBT vs DSW.OBT	14	1.148	0.081	0.277	0.441		17	0.344	0.021	0.624	0.778	
53	PL.OBT vs PC.HTO	13	0.378	0.031	0.710	0.857		17	3.763	0.190	0.043	0.272	
54	PL.OBT vs PC.OBT	12	1.933	0.149	0.161	0.276		10	0.326	0.035	0.804	0.853	
55	PL.OBT vs T	12	15.867	0.591	0.004	0.009	*	15	0.825	0.056	0.375	0.656	
56	PL.OBT vs 12K	10	10.703	0.543	0.005	0.011	.	11	6.073	0.378	0.029	0.272	
57	PL.OBT vs OBT	12	18.440	0.626	0.002	0.006	*	12	0.495	0.043	0.557	0.731	
58	PL.OBT vs 25K	13	15.971	0.571	0.002	0.006	*	13	0.496	0.040	0.537	0.731	
59	PL.OBT vs 25KOBT	11	13.387	0.572	0.005	0.011	.	13	4.378	0.267	0.035	0.272	
60	PL.OBT vs 180K	13	13.486	0.529	0.003	0.008	*	13	0.577	0.046	0.491	0.722	
61	DSW.HTO vs DSW.OBT	14	1.409	0.098	0.228	0.374		17	1.311	0.076	0.268	0.595	
62	DSW.HTO vs PC.HTO	13	0.338	0.027	0.774	0.893		17	0.847	0.050	0.396	0.664	
63	DSW.HTO vs PC.OBT	12	1.960	0.151	0.163	0.276		10	0.225	0.024	0.747	0.826	
64	DSW.HTO vs T	12	25.107	0.695	0.001	0.006	*	15	0.442	0.031	0.565	0.732	
65	DSW.HTO vs 12K	10	17.695	0.663	0.002	0.006	*	11	7.222	0.419	0.023	0.272	
66	DSW.HTO vs OBT	12	29.651	0.729	0.003	0.008	*	12	0.278	0.025	0.711	0.815	
67	DSW.HTO vs 25K	13	23.995	0.667	0.002	0.006	*	13	0.899	0.070	0.370	0.656	
68	DSW.HTO vs 25KOBT	11	21.768	0.685	0.002	0.006	*	13	4.789	0.285	0.027	0.272	
69	DSW.HTO vs 180K	13	19.417	0.618	0.002	0.006	*	13	0.469	0.038	0.580	0.736	
70	DSW.OBT vs PC.HTO	14	0.717	0.052	0.506	0.648		17	4.821	0.232	0.030	0.272	
71	DSW.OBT vs PC.OBT	13	4.752	0.284	0.040	0.074		10	0.656	0.068	0.475	0.713	
72	DSW.OBT vs T	13	30.399	0.717	0.001	0.006	*	15	0.995	0.066	0.310	0.626	
73	DSW.OBT vs 12K	11	21.126	0.679	0.003	0.008	*	11	6.152	0.381	0.020	0.272	
74	DSW.OBT vs OBT	13	35.167	0.746	0.002	0.006	*	12	0.595	0.051	0.458	0.706	
75	DSW.OBT vs 25K	14	29.640	0.695	0.001	0.006	*	13	0.550	0.044	0.464	0.706	
76	DSW.OBT vs 25KOBT	12	26.055	0.703	0.004	0.009	*	13	4.436	0.270	0.039	0.272	
77	DSW.OBT vs 180K	14	25.774	0.665	0.001	0.006	*	13	0.706	0.056	0.462	0.706	
78	PC.HTO vs PC.OBT	12	1.150	0.095	0.307	0.467		10	0.961	0.096	0.360	0.656	
79	PC.HTO vs T	12	13.006	0.542	0.004	0.009	*	15	1.370	0.089	0.279	0.598	
80	PC.HTO vs 12K	10	8.696	0.491	0.009	0.018	.	11	15.149	0.602	0.008	0.272	
81	PC.HTO vs OBT	12	15.526	0.585	0.004	0.009	*	12	1.034	0.086	0.366	0.656	
82	PC.HTO vs 25K	13	12.934	0.519	0.004	0.009	*	13	2.904	0.195	0.096	0.403	

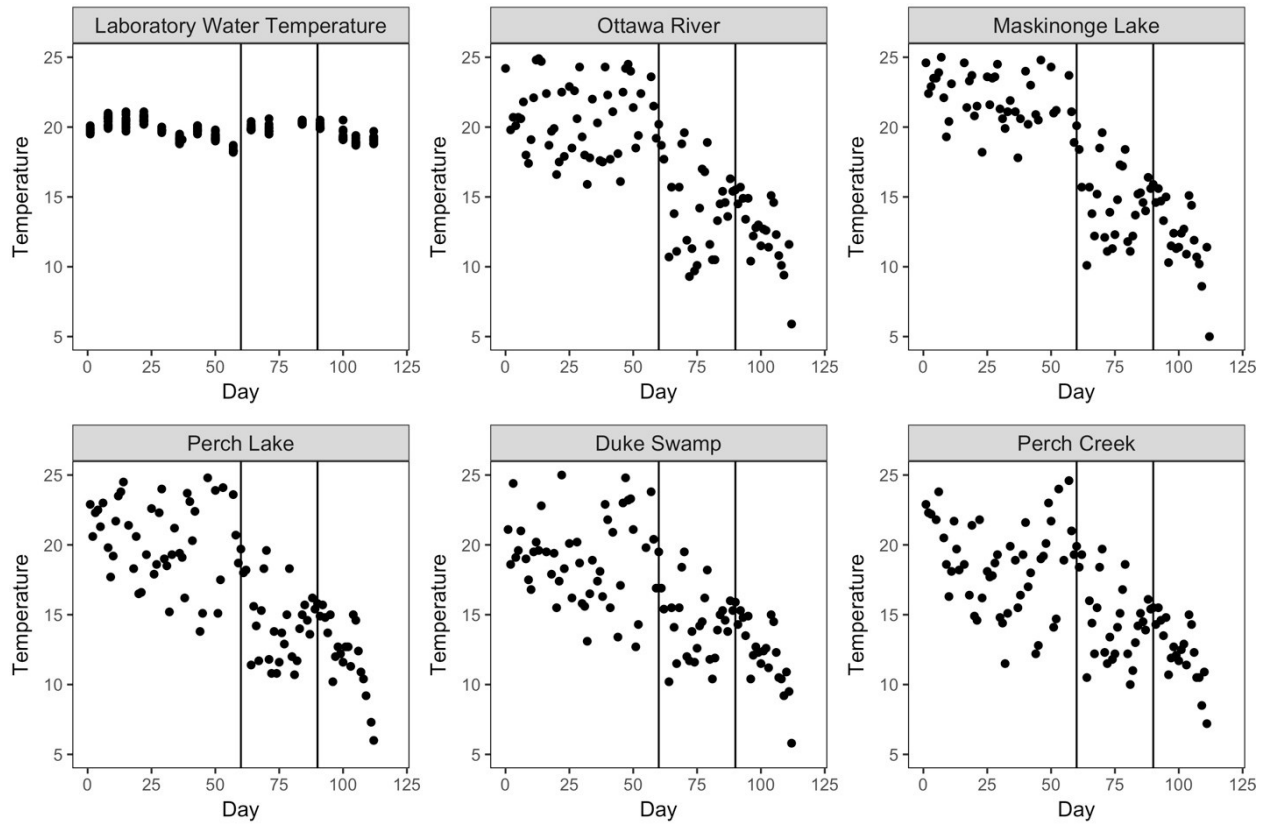
	pairs	Day 60						Day 120					
		total.DF	F.Model	R ²	p.value	p.adjusted	Significance	total.DF	F.Model	R ²	p.value	p.adjusted	Significance
83	PC.HTO vs 25KOBT	11	10.821	0.520	0.006	0.012	.	13	6.464	0.350	0.018	0.272	
84	PC.HTO vs 180K	13	11.214	0.483	0.004	0.009	*	13	1.457	0.108	0.243	0.595	
85	PC.OBT vs T	11	31.064	0.756	0.002	0.006	*	8	0.418	0.056	0.683	0.815	
86	PC.OBT vs 12K	9	24.402	0.753	0.002	0.006	*	4	3.280	0.522	0.200	0.553	
87	PC.OBT vs OBT	11	42.041	0.808	0.002	0.006	*	5	0.313	0.072	0.800	0.853	
88	PC.OBT vs 25K	12	25.016	0.695	0.002	0.006	*	6	0.505	0.092	0.714	0.815	
89	PC.OBT vs 25KOBT	10	28.330	0.759	0.002	0.006	*	6	1.184	0.192	0.403	0.664	
90	PC.OBT vs 180K	12	19.615	0.641	0.002	0.006	*	6	0.328	0.062	0.710	0.815	
91	T vs 12K	9	0.030	0.004	0.952	0.986		9	3.868	0.326	0.094	0.403	
92	T vs OBT	11	0.746	0.069	0.411	0.546		10	-0.013	-0.001	0.979	0.980	
93	T vs 25K	12	0.039	0.004	0.930	0.986		11	0.296	0.029	0.630	0.778	
94	T vs 25KOBT	10	0.034	0.004	0.949	0.986		11	2.857	0.222	0.084	0.403	
95	T vs 180K	12	0.210	0.019	0.699	0.857		11	0.045	0.004	0.932	0.950	
96	12K vs OBT	9	0.820	0.093	0.395	0.539		6	2.310	0.316	0.151	0.529	
97	12K vs 25K	10	0.012	0.001	0.980	0.986		7	2.114	0.261	0.183	0.540	
98	12K vs 25KOBT	8	-0.023	-0.003	0.985	0.986		7	0.557	0.085	0.547	0.731	
99	12K vs 180K	10	0.113	0.012	0.873	0.965		7	2.402	0.286	0.166	0.540	
100	OBT vs 25K	12	1.031	0.086	0.345	0.496		8	0.094	0.013	0.906	0.942	
101	OBT vs 25KOBT	10	1.209	0.118	0.296	0.464		8	1.562	0.182	0.242	0.595	
102	OBT vs 180K	12	1.180	0.097	0.301	0.465		8	0.011	0.002	0.980	0.980	
103	25K vs 25KOBT	11	0.040	0.004	0.932	0.986		9	1.729	0.178	0.184	0.540	
104	25K vs 180K	13	0.159	0.013	0.710	0.857		9	0.122	0.015	0.918	0.945	
105	25KOBT vs 180K	11	0.190	0.019	0.722	0.861		9	1.787	0.183	0.272	0.595	



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771 **Figure S1. Location of Chalk River Laboratories within Ontario, Canada, and the locations of where**
772 **fathead minnows were exposed to tritium in the field and in the laboratory. Average tritium activity**
773 **concentrations are listed in Bq/L. bck =background levels; ~2 Bq/L.**

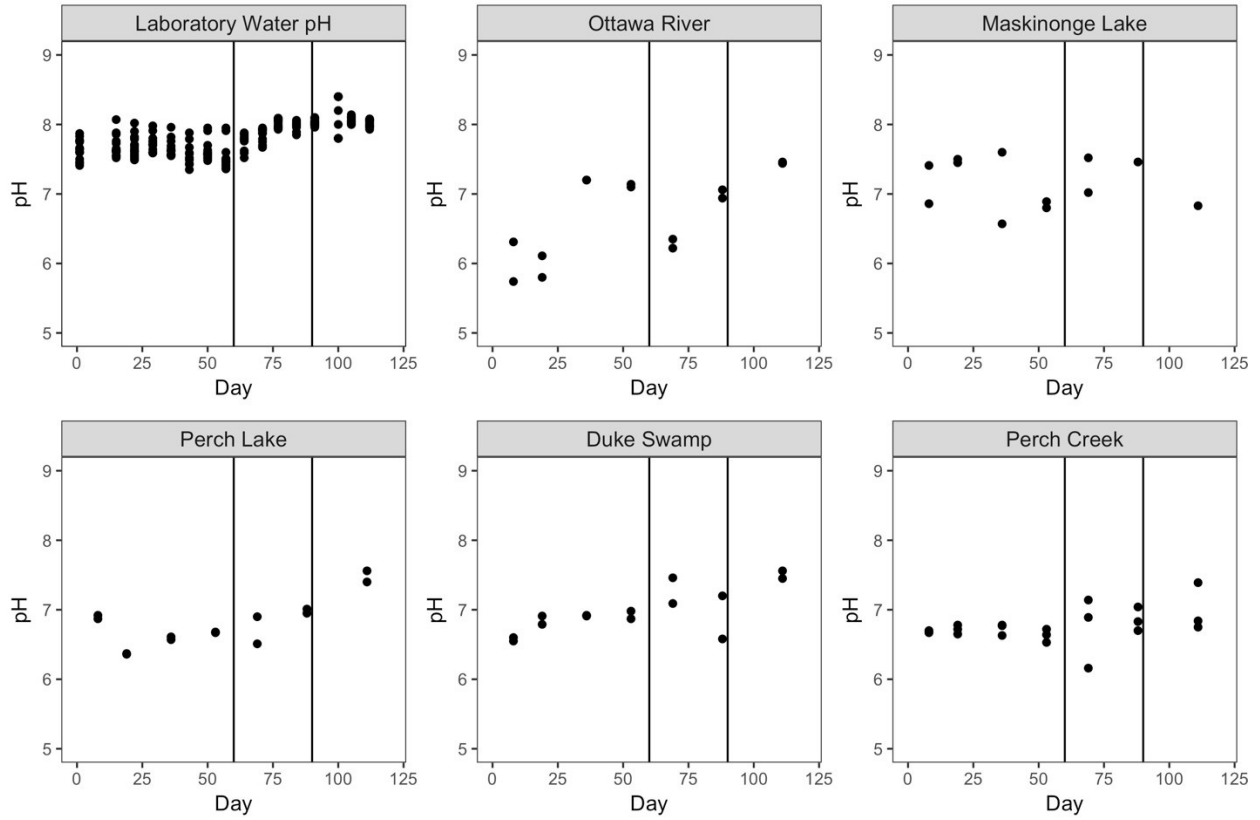
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776 **Figure S2. Water temperature values (°C) measured throughout the exposure and depuration phases**
777 **during the laboratory study and at each exposure location of the field study. The vertical lines mark**
778 **Day 60 and Day 90.**

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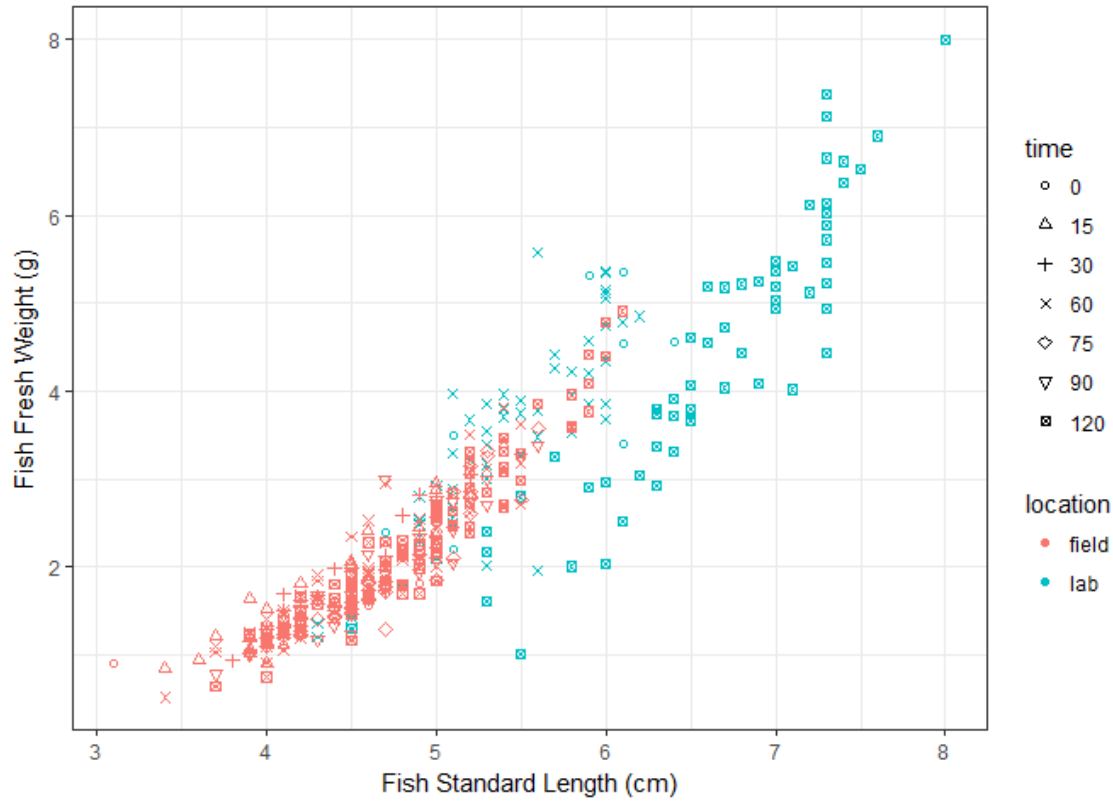


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781 **Figure S3. Water pH values measured throughout the exposure and depuration phases during the**
782 **laboratory study and at each exposure location during the field study. The vertical lines mark Day 60**
783 **and Day 90.**

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787 **Figure S4. Ratio of fathead minnow length and weight values from the start and end of the field and**
788 **laboratory studies.**

789