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Reproductive health of yellow perch (*Perca flavescens*) from a biological mercury hotspot in Nova Scotia, Canada

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HIGHLIGHTS

- It is not clear whether mercury (Hg) affects reproduction in wild fishes.
- We examined reproductive health indicators in wild male and female yellow perch.
- Indicators were gonadosomatic index, germ cell development, and plasma estradiol.
- Negative relationships were not found between these endpoints and perch Hg levels.
- Results indicated that Hg does not adversely affect these reproductive measures in perch.

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ABSTRACT

Methylmercury (MeHg) exposure is known to adversely affect the reproductive health of laboratory fish, but its impacts on the sexual development of wild fishes are not well studied. Kejimkujik National Park and National Historic Site (KNPNHS) region of Nova Scotia, Canada, has been identified as a biological mercury (Hg) hotspot. To determine whether Hg was adversely affecting the reproductive health of wild yellow perch (*Perca flavescens*), sexually mature male and female perch were collected from 12 lakes within KNPNHS (mean muscle total Hg: 0.28–0.54 μg/g ww). Gonadosomatic index and germ cell development of male and female perch were measured, as well as the plasma 17β-estradiol concentrations of females. These endpoints were compared between lakes, and were related to Hg concentrations measured in perch muscle and liver tissues. Our results indicate that the reproductive health of male and female perch was not adversely impacted by Hg, although a positive relationship existed between the proportions of primary spermatocytes in male testes and muscle total Hg concentrations. Perch were sampled at an early stage of recrudescence, and the tissue Hg concentrations in these perch were generally lower than those in laboratory studies reporting impacts on reproductive health, both of which may explain the absence of effects. Based on the measured endpoints, it appears that reproduction in perch was not affected at Hg concentrations known to affect fish eating wildlife.

1. Introduction

Mercury (Hg) can enter remote aquatic ecosystems through geological weathering as well as long range atmospheric transport and deposition (Jackson, 1997), and can be transformed into methyl Hg (MeHg) which both bioaccumulates in biota and biomagnifies through the food chain (Cabana and Rasmussen, 1994). MeHg is a more toxic form of Hg which wild fish are exposed to mainly through their diet (Hall et al., 1997). It is the predominant form of Hg in whole wild fish (Wyn et al., 2009) and can accumulate in the tissues of fish to potentially harmful concentrations. At the MeHg concentrations to which freshwater fish are currently exposed in North America, Sandheinrich and Wiener (2011) have concluded that the mostly likely adverse effects are sublethal tissue damage and depressed reproduction, similar to the findings of Depew et al. (2012).

MeHg exposure has been shown to adversely impact the reproductive health of laboratory fish (e.g. Drevnick and Sandheinrich, 2003; Drevnick et al., 2006; Friedmann et al., 1996a; Hammerschmidt et al., 2002), but its effects on the reproduction of wild fishes are still largely unknown. In the few studies that are available on wild fishes, female white sturgeon (*Acipenser transmontanus*) and largemouth bass (*Micropterus salmoides*) showed negative relationships between plasma 17β-estradiol concentrations and tissue Hg concentrations

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(Adams et al., 1999; Webb et al., 2006), as well as between GSI and Hg concentrations in white sturgeon (Webb et al., 2006). Similarly in wild male white sturgeon, negative relationships have been found between measures of GSI or plasma testosterone concentrations and tissue Hg concentrations (Webb et al., 2006). In contrast, concentrations of 11keto-testosterone were both positively (Friedmann et al., 2002) and negatively (Webb et al., 2006) related to tissue Hg concentrations in male fish. Recently, a protective dietary threshold for the effects of MeHg on fish reproduction was estimated to be 0.04 μg g^{-1} ww, using the highest ranked NOAEL (no observed adverse effect level) calculated from published laboratory studies (wet weight concentration equivalent to ~0.16 μg g^{-1} dry weight, using the 75% moisture content assumed by the authors) (Depew et al., 2012). Although it is difficult to determine the dietary MeHg intake of wild fishes, invertebrates consumed by yellow perch within Kejimkujik National Park and National Historic Site (KNPNHS), Nova Scotia, Canada, contain <0.54 μg g^{-1} of MeHg (dry weight, mean value within lakes) (Clayden, 2011). According to this proposed threshold, therefore, the reproductive health of KNPNHS yellow perch may be impaired by MeHg exposure.

The objective of this study was to determine whether the reproductive health of wild yellow perch within KNPNHS was adversely affected by MeHg exposure. This region has been identified as a biological Hg "hotspot" based on the Hg concentrations present in yellow perch and common loons (*Gavia immer*) (Evers et al., 2007). A recent study within KNPNHS has also shown an increased prevalence of macrophage aggregates (an indicator of oxidative stress and cellular damage) with increasing Hg concentrations in female yellow perch. This indicates that these fish may be experiencing increased tissue or cellular damage as a result of their Hg exposure (Batchelar et al., 2013). Therefore, this is an ideal site to study the effects of MeHg exposure on the reproductive health of wild fish. To achieve our objective, indicators of reproductive health (GSI, ovarian and testicular germ cell development, and plasma sex steroid concentrations) were measured in sexually mature male and female perch collected from 12 lakes within the park. GSI and germ cell development are both indicators of gonad development, with the latter providing more detailed information about development through the use of histology. Additionally, decreases in sex steroid concentrations may be associated with decreases in fish reproductive success (Drevnick and Sandheinrich, 2003). In order to determine whether these measurements were negatively affected by this contaminant, the endpoints were related to the muscle total Hg (THg) and liver Hg (methyl, total and inorganic) concentrations of the perch.

2. Methods

2.1. Study site and sample collection

KNPNHS is used mainly as a site for tourism and recreation, and is situated in southwestern Nova Scotia, Canada. The park is not impacted by industry or point source pollution (O'Driscoll et al., 2005), and its lakes are generally shallow, oligotrophic, and acidic. For physical and chemical characteristics of the lakes, see Supplementary data (Table S1). The total phosphorous (TP) and total nitrogen (TN) data for lakes used in this study were collected by Environment Canada.

Sexually mature male and female yellow perch were sampled in late September of 2009 and 2010 from 12 lakes within KNPNHS. Six of these lakes were sampled in 2009, nine were sampled in 2010, and three were sampled in both years. Yellow perch migrate to spawn; therefore fall is the recommended sampling time for this species due to concerns about their mobility just prior to spawning in the spring (Barrett and Munkittrick, 2010). In the present study, sampling was conducted in late September to eliminate the concern of capturing non-resident fish. Concentrations of THg in yellow perch vary among lakes within KNPNHS (Drysdale et al., 2005; Wyn et al., 2010), and lakes were selected to cover a range of yellow perch THg concentrations. Sampling was performed as previously described (Batchelar et al., 2013), and perch were dissected according to protocols approved by the University of New Brunswick Animal Care Committee. For each perch, the total weight (±0.1 g) and fork length (±1 mm) were recorded. Due to low blood volumes in small perch, blood samples were only collected from the larger perch (>~10 cm) and were stored on wet ice in heparinized vacutainers (Becton Dickson) or micro-capillary tubes (Fisher Brand) for 6–8 h before being centrifuged at approximately 1500 *g* for 4 min. Plasma was then transferred to cryovials using a Pasteur pipette and flash frozen in liquid nitrogen before being stored at −80 °C. Liver and dorsal muscle tissues were collected from each perch and frozen for Hg analysis. Whole gonad tissues were dissected, weighed (±0.001 g), and preserved in 10% phosphate buffered formalin at room temperature for histological analyses. For aging purposes, scales were removed from directly behind the pectoral fin of each perch, and age was estimated according to the method described by Devries and Frie (1996). Gonadosomatic (GSI) values were calculated as [100 * (gonad weight (g) / total weight (g))].

2.2. Mercury analysis

THg concentrations were measured in freeze-dried muscle tissue using a Direct Mercury Analyzer (Milestone DMA-80). THg concentrations in the muscle of these perch are comprised primarily (96.5%) of MeHg (Batchelar et al., 2013). The mean % recovery (±SE) of standard reference materials (SRMs) TORT-2 and DORM-2 (Lobster hepatopancreas and Dogfish muscle, National Research Council of Canada (NRCC)) was 107.8% ± 1.0 (n = 63), and 91.7% ± 0.8 (n = 44), respectively, and the recovery of a 15 ng liquid Hg standard was 85.6% ± 0.8 (n = 65). The mean blank concentration was 0.0087 μg g^{-1} Hg (n = 97) based on a sample weight of 10 mg, and the relative percent difference (RPD) between sample duplicates was 2.6% ± 0.5 (n = 42). The coefficient of variation (CV) of an intra-laboratory standard (yellow perch muscle sample) was 3.2% (n = 63).

In freeze-dried liver samples, MeHg and inorganic Hg (HgII) concentrations were determined using USEPA Method 1630 (USEPA, 2001) and a Brooks Rand Model II system. THg concentrations were calculated as the sum of all measured Hg within a sample. Prior to the analysis, samples were digested in 25% KOH/MeOH solution, shaken for 1 h, and then heated to 95 °C for 1 h, similar to the methods used by Cai and Bayona (1995). The mean % recoveries (±SE) of DORM-2 were 115.5 ± 4.4 (MeHg) and 167.9 ± 6.6 (HgII) (n = 4), and of DOLT-4 (Dogfish liver, NRCC) were 105.2 ± 0.16 (MeHg) and 96.8 ± 1.6 (HgII) (n = 31). The RPD of samples duplicated through the digestion process was 11.8% ± 2.7 (MeHg) (n = 24) and 16.2% ± 4.5 (Hg(II)) (n = 24), and the CV of samples analyzed in triplicate was 5.9% ± 1.1 (MeHg) (n = 13) and 6.1% ±0.9 (Hg(II)) (n = 14). The limits of detection were 0.0002 and 0.0013 μg g^{-1} for MeHg and Hg(II) respectively (3× the SD of method blanks). All Hg concentrations are presented here as wet weight concentrations, calculated using the moisture content of each sample.

2.3. Germ cell development

Although ovary tissues were collected in both years, only those collected in 2009 were prepared for analysis, while all testis tissues were processed. Tissues were prepared using methods similar to those described by Drevnick et al. (2008), and all samples were given new, random sample numbers before processing to prevent bias. Tissues were dehydrated, embedded in paraffin, sectioned (8 μm), mounted on slides, and stained with hematoxylin and eosin. For staining, slides were randomly assigned to batches, and all batches were treated using the same staining protocols and dyes. One stained slide was prepared per sample, and each slide contained multiple tissue sections (~5 to 10). A Leica DM 2500 microscope equipped with a camera (Leica DFC 290) was used to photograph ovary tissues at 70× and testis tissues at 280× magnification.

Each prepared ovary sample was photographed between 5 and 11 times, ensuring that the same area of tissue was not photographed twice. Within these photographs, the first 100 oocytes with a visible nucleus were selected. The developmental stages of these oocytes were then determined to be either chromatin nucleolar (stage 1), perinucleolar (stage 2), or cortical alveolar (stage 3) (Fig. S1) as outlined by USEPA (2006), and the proportions of oocytes at each stage were calculated. Oocytes in more advanced developmental stages were not found since perch spawn in May and samples were collected in September, and atretic oocytes were not detected in any of the samples.

For each testis sample, photographs were taken of 3 representative areas within the tissue. Each photograph was overlaid with a grid of 25 μm × 25 μm numbered squares using Adobe Photoshop CS5 (Adobe) software, and 20 (of 63) squares were selected using a random number generator. The developmental stage of cells located at the bottom right hand corner of each selected square (at the intersection of the grid lines) was determined. Spermatogenic cells were classified as primary spermatocytes (stage 1), secondary spermatocytes (stage 2), spermatids (stage 3), and spermatozoa (stage 4) (Fig. S1) as described by USEPA (2006). This process was completed for each of the 3 photographs taken per sample, and the proportion of squares containing each cell type (out of 20) was calculated for each photograph. Average proportions from the 3 photographs were used for data analysis.

2.4. Sex steroid analysis

Plasma sex steroids were extracted and quantified using methods similar to Drevnick and Sandheinrich (2003). For the extraction, plasma (20 μl), ether (3 ml), and distilled water (980 μl) were combined in a glass tube, vortexed for 60 s, and the ether layer was transferred to a clean tube using a Pasteur pipette. This process was repeated twice more for each sample, although 1 ml of ether was used, and samples were vortexed for 30 s. For the third and final extraction step, the water layer was frozen using a dry ice-acetone bath and the ether layer was decanted off. The ether was evaporated overnight in a water bath (40 °C), and samples were reconstituted with enzyme immunoassay buffer (Cayman Chemical). A 17β-estradiol calibration standard (Cayman Chemical) was used to spike perch plasma aliquots prior to extraction, and its recovery was 67.5% ± 10.4 (±SE, n = 5). Due to low plasma volumes in males, the extraction and analyses were limited to samples from female perch.

17β-estradiol was quantified in the extracted samples using Cayman Chemical enzyme immunoassay kits. Assays were conducted according to kit procedures (Cayman Chemical Company, 2010) with the exception that the recommended sample, standard, and reagent volumes were halved (e.g. 25 μl) as a result of low sample volumes. Samples were analyzed in duplicate, reanalyzed if the CV between duplicates exceeded 20%, and were omitted from the data analysis (n = 10) if the CV exceeded 20% after reanalysis. The mean CV of intra-assay samples (n > 6 per assay) was 7.8% ± 2.3 (±SE), and the CV of interassay samples was 24.2% (n = 40). Results were adjusted to 100% recovery using the mean extraction recovery of 67.5%.

2.5. Statistical analyses

Only male and female perch considered to be sexually mature (GSI > 1) were included in the data analyses. In addition to the use of a 1% GSI cutoff value, plots of gonad weight data versus total weight data were visually inspected, as recommended by Environment Canada (2010), to ensure that sexually immature perch were excluded from the statistical analyses. Length, weight, age, GSI, 17β-estradiol, and tissue Hg concentration data were log transformed, and germ cell stage data were arcsine square root transformed so assumptions of the analyses were met in most cases. Analyses were performed using Systat 11 (Systat Software), and values of $p < 0.05$ were considered statistically significant. All results are presented here as mean values ± standard error. In each of the lakes that were sampled during both 2009 and 2010, male and female GSI, 17β-estradiol, and muscle THg concentrations did not differ among sampling years (two sample t-tests; males and females analyzed separately within each lake). These data were therefore combined within sex in each of the lakes, and sample sizes <3 were combined without analysis.

Analysis of variance (ANOVA) was used to determine differences in GSI, length, weight, age, and muscle THg, liver THg, and 17β-estradiol concentrations among lakes and within sex. Tukey's post-hoc test was used to determine pairwise differences. The statistical power of these tests to detect within-sex differences in length, weight, age, and tissue Hg concentrations among lakes ranged from 0.65 to 0.99 (calculated for a balanced ANOVA using the lowest sample size as a common value, and using the largest difference between means as the effect size). The power to detect a 25% difference in GSI, an effect size recommended for a variety of biological monitoring endpoints (Munkittrick et al., 2009), was 0.88 for females and 0.12 for males (also using the smallest sample size value). For 17β-estradiol, the results from two lakes were omitted from the ANOVA due to their small sample sizes (n = 2). Another ANOVA was conducted in which 17β-estradiol concentrations were compared among lakes which had been pooled into "low", "medium", and "high" Hg groups according to their mean perch muscle THg concentrations (Group 1: US, BVS, MTN, BDE; Group 2: CBR, BDW, GFT, HMK; Group 3: PK, GRG, KJI, NC (see Table 1 for definition of the abbreviations)).

Simple linear regressions were used to relate muscle and liver Hg concentrations to the health endpoints GSI, germ cell development stage, and plasma 17β-estradiol concentrations, and to relate lake water characteristics (pH, total phosphorous (TP), and total nitrogen (TN)) to mean values of these endpoints for each lake. These regressions were performed for males and females separately using data pooled from all the lakes. Similar to Wyn et al. (2010), length standardized THg concentrations were calculated for female perch by applying a length of 12 cm to linear regression equations produced from regressions of tissue Hg concentrations and perch length for each lake. For all regressions, studentized residuals with an absolute value >3 were considered outliers and were omitted from the analyses (Rousseeuw and Leroy, 2003). Female plasma 17β-estradiol concentrations were positively related to length as well as tissue Hg concentrations (see Section 3.4) which may have confounded the relationships between 17β-estradiol and tissue Hg concentrations. Residuals of the relationship between 17β-estradiol and length were therefore related to tissue Hg concentrations to determine these relationships independent of perch length.

3. Results

3.1. Tissue mercury concentrations

Total Hg concentrations in muscle from male and female perch ranged from 0.13 to 2.13 μg g^{-1} ww, and in females the mean concentrations varied approximately 2 fold among lakes (Table 1). Mean liver THg concentrations of females were more variable among lakes, with a >10 fold difference between the highest and lowest mean concentrations (Table 1). Liver THg concentrations of individuals (both sexes) also ranged widely, from 0.13 to 6.04 μg g^{-1} ww. Muscle THg concentrations were positively related to the length ($F_{1,262} = 133.880$, $p < 0.001$, $r^2 = 0.338$), weight ($F_{1,263} = 123.307$, $p < 0.001$, $r^2 = 0.319$), age ($F_{1,251} = 105.217$, $p < 0.001$, $r^2 = 0.295$) and liver THg concentrations ($F_{1,201} = 164.476$, $p < 0.001$, $r^2 = 0.450$) of all perch. Liver THg concentrations of all perch were positively related to length ($F_{1,176} = 51.606$, $p < 0.001$, $r^2 = 0.227$), and perch weight increased with the age of the fish ($F_{1,250} = 679.399$, $p < 0.001$, $r^2 = 0.731$). Lengths and weights of both male and female perch, however, differed among lakes (Table 1), and length standardized muscle THg concentrations of female perch varied approximately 1.5 times among lakes while those in liver tissue varied >5 fold (Table 1).

Table 1
Summary statistics for sexually mature male and female yellow perch from 12 lakes within Kejimkujik National Park and National Historic Site, Nova Scotia (mean ± SE (n)); Upper Silver (US), Beaverskin (BVS), Mountain (MTN), Big Dam East (BDE), Cobrielle (CBR), Big Dam West (BDW), Grafton (GFT), Hilchemakaar (HMK), Peskowesk (PK), George (GRG), Kejimkujik (KJI) and North Cranberry (NC). The gonadosomatic index (GSI) is a measure of perch gonad weight relative to whole body weight. Length standardized total Hg concentrations were not calculated for male perch due to small sample sizes. Values within sex that do not share the same uppercase letter are significantly different ($p < 0.05$).

Lake	Fork length (cm)	Weight (g)	Age	GSI	17ß-estradiol (pg/ml)	Tissue total Hg (μg/g)		12 cm length standardized tissue total Hg	
						Muscle	Liver	Muscle	Liver
Females									
US	11.4 ± 0.8 (18)AB	20.8 ± 5.1 (18)AB	1.9 ± 0.3 (18)CD	2.11 ± 0.10 (18)ABC	985.1 ± 331.5 (7)ABC	0.27 ± 0.02 (18)C	0.25 ± 0.03 (15)E	n/a[a]	0.24
BVS	11.6 ± 0.9 (13)AB	20.5 ± 6.2 (13)AB	1.8 ± 0.3 (13)BCD	2.45 ± 0.24 (13)A	2017.4 ± 1570.0 (2)[b]	0.36 ± 0.05 (13)BC	0.39 ± 0.10 (12)DE	0.35	0.34
MTN	10.6 ± 0.7 (14)B	14.8 ± 3.5 (14)B	1.6 ± 0.3 (14) D	1.90 ± 0.11 (14)ABC	766.8 ± 530.3 (2)[b]	0.38 ± 0.05 (14)BC	0.60 ± 0.08 (12)BCD	0.44	0.64
BDE	12.7 ± 0.6 (25) AB	24.3 ± 3.8 (26)AB	2.8 ± 0.3 (25)ABCD	1.73 ± 0.06 (26)C	450.4 ± 94.8 (15)C	0.41 ± 0.04 (26)BC	0.48 ± 0.07 (23)DE	0.36	n/a[a]
CBR	13.0 ± 0.5 (14)AB	24.9 ± 3.4 (14)AB	2.9 ± 0.4 (14)ABCD	1.89 ± 0.12 (14)ABC	604.7 ± 204.2 (11)BC	0.45 ± 0.04 (14)ABC	0.46 ± 0.05 (12)CDE	n/a[a]	n/a[a]
BDW	13.6 ± 1.0 (11)AB	38.3 ± 8.7 (11)AB	4.0 ± 0.6 (11)AB	2.37 ± 0.18 (11)A	2213.7 ± 517.7 (5)A	0.46 ± 0.05 (11)ABC	1.17 ± 0.27 (11)ABC	0.39	n/a[a]
GFT	14.0 ± 1.3 (13)AB	38.5 ± 9.9 (13)AB	3.2 ± 0.6 (13)ABCD	2.29 ± 0.13 (13)AB	2265.2 ± 756.4 (5)A	0.51 ± 0.09 (13)AB	0.58 ± 0.08 (11)BCDE	0.40	0.52
HMK	14.4 ± 1.0 (18)AB	39.8 ± 10.3 (18)AB	4.2 ± 0.5 (18)A	2.30 ± 0.11 (18)A	615.4 ± 120.3 (11)BC	0.54 ± 0.10 (18)AB	0.73 ± 0.23 (17)BCD	0.38	0.39
PK	12.6 ± 0.6 (27)AB	26.1 ± 3.8 (27)AB	3.6 ± 0.3 (26)AB	1.89 ± 0.06 (27)ABC	931.2 ± 188.5 (8)ABC	0.60 ± 0.05 (27)AB	1.20 ± 0.16 (19)AB	0.52	n/a[a]
GRG	14.6 ± 1.2 (11)AB	46.0 ± 14.8 (11)A	3.4 ± 0.6 (11)ABCD	1.68 ± 0.08 (11)BC	401.2 ± 151.8 (4)BC	0.61 ± 0.16 (11)AB	1.87 ± 0.42 (11)A	0.35	n/a[a]
KJI	13.8 ± 1.6 (11)AB	44.5 ± 21.0 (11)AB	3.7 ± 0.7 (11)ABC	2.26 ± 0.18 (11)ABC	1523.9 ± 421.6 (6)AB	0.63 ± 0.16 (11)AB	2.52 ± 0.68 (11)A	0.43	1.25
NC	14.2 ± 0.7 (24)A	36.3 ± 5.5 (24)AB	4.3 ± 0.4 (23)A	1.97 ± 0.10 (24)ABC	949.1 ± 200.5 (11)ABC	0.63 ± 0.05 (24)A	0.97 ± 0.13 (20)ABC	0.50	0.64
Males									
US	9.6 ± 0.6 (5)AB	9.9 ± 1.8 (5)AB	1.2 ± 0.4 (5)	4.45 ± 1.14 (5)AB		0.37 ± 0.04 (5)ABC	0.30 ± 0.16 (2)		
BVS	10.1 ± 0.8 (7)A	12.3 ± 4.0 (7)A	2.0 ± 0.5 (7)	3.22 ± 0.56 (7)ABC		0.43 ± 0.05 (7)ABC	0.53 ± 0.28 (4)		
MTN	8.7 ± 0.5 (4)AB	6.8 ± 1.3 (4)AB	1.3 ± 0.3 (4)	1.24 ± 0.15 (4)C		0.32 ± 0.07 (4)ABC	0.47 ± 0.03 (2)		
BDE	8.4 ± 0.2 (5)AB	6.6 ± 0.4 (5)AB	1.0 ± 0.3 (5)	2.10 ± 0.49 (5)ABC		0.29 ± 0.05 (5)BC	n/a		
CBR	9.0 ± 0.4 (6)AB	7.7 ± 1.3 (6)AB	1.0 ± 0.3 (6)	3.17 ± 0.76 (6)ABC		0.40 ± 0.06 (6)ABC	1.18 ± 0.43 (2)		
BDW	9.0 ± 0.4 (3)AB	8.5 ± 0.9 (3)AB	1.3 ± 0.3 (3)	1.80 ± 0.18 (3)ABC		0.28 ± 0.02 (3)ABC	0.71 ± 0.10 (2)		
GFT	7.8 ± 0.5 (3)AB	5.8 ± 1.5 (3)AB	0.3 ± 0.3 (3)	2.57 ± 0.76 (3)ABC		0.20 ± 0.05 (3)C	0.34 (1)		
HMK	8.2 ± 0.2 (7)B	5.5 ± 0.4 (7)B	1.1 ± 0.1 (7)	4.08 ± 0.53 (7)AB		0.35 ± 0.05 (7)ABC	0.43 ± 0.10 (6)		
PK	8.0 ± 0.2 (10)B	6.1 ± 0.5 (11)AB	1.0 ± 0.1 (11)	3.41 ± 0.56 (11)ABC		0.50 ± 0.05 (10)AB	0.87 ± 0.20 (4)		
GRG	8.4 ± 0.2 (3)AB	6.3 ± 0.7 (3)AB	0.7 ± 0.3 (3)	1.93 ± 0.64 (3)ABC		0.27 ± 0.05 (3)ABC	n/a		
KJI	9.2 ± 0.2 (5)AB	8.6 ± 0.3 (5)AB	2.0 ± 0.0 (5)	4.62 ± 0.66 (5)A		0.37 ± 0.07 (5)ABC	0.96 ± 0.29 (5)		
NC	9.0 ± 0.1 (8)AB	6.6 ± 0.3 (8)AB	1.5 ± 0.2 (8)	1.61 ± 0.14 (8)BC		0.60 ± 0.09 (8)A	0.75 (1)		

[a] Perch length and total Hg concentrations in the tissue were not significantly related in these lakes, and length standardized concentrations were not calculated.
[b] Due to small sample sizes (n = 2), samples from these lakes were not included in the comparison among lakes using analysis of variance.

3.2. Gonadosomatic indices

The GSI of female perch ranged from 1.01 to 4.62%, and differed significantly among lakes (Table 1). These differences, however, did not correspond with the trend in muscle and liver THg concentrations among lakes. The highest and lowest mean GSI values occurred in lakes with the second lowest and third highest mean muscle THg concentrations, respectively (Table 1). In addition, female GSI was not related to muscle THg, liver THg, MeHg, or inorganic Hg concentrations (Table 2), fish length ($p = 0.408$) or age ($p = 0.770$). Similarly, no relationships existed between the mean GSI of female perch within each lake and the lake water characteristics pH, TN or TP ($p > 0.319$, data not shown).

Male GSI ranged from 1.04 to 8.18%, and differed significantly among lakes, varying >3 fold (Table 1). Similar to females, these differences did not correspond with the trend in muscle or liver THg concentrations among lakes (Table 1), and no significant relationships were found between male GSI and their muscle THg or liver Hg concentrations (THg, MeHg or inorganic Hg) (Table 2). In addition, relationships were not present between the mean GSI of male perch from each lake and the lake water characteristics pH, TN or TP ($p > 0.479$, data not shown).

3.3. Germ cell development

In female perch, the proportions of oocytes in each developmental stage were similar among lakes ($p > 0.609$). Oocytes in the second stage (perinucleolar) were the most prevalent in all lakes (57.5% ± 1.0), followed by those in stage 1 (chromatin nucleolar; 25.3% ± 1.0), while those in stage 3 (cortical alveolar) were the least common (17.2% ± 0.8). Proportions of oocytes in these three development stages were not related to muscle THg or liver Hg concentrations (Table 2), or female GSI (Stage 1: $F_{1,80} = 0.634$, $p = 0.428$, $r^2 = 0.008$; Stage 2: $F_{1,80} = 0.023$, $p = 0.880$, $r^2 < 0.001$; Stage 3: $F_{1,80} = 1.077$, $p = 0.303$, $r^2 = 0.013$). Similarly, the mean proportions of oocytes (all stages) from each lake were unrelated to the lake water variables pH, TN or TP ($p > 0.142$, data not shown). However, positive relationships existed between the proportions of stage 3 oocytes in individuals and plasma 17β-estradiol concentrations ($F_{1,50} = 8.873$, $p = 0.004$, $r^2 = 0.151$), age ($F_{1,78} = 12.947$, $p = 0.001$, $r^2 = 0.142$), and length ($F_{1,80} = 26.184$, $p < 0.001$, $r^2 = 0.247$). Negative relationships existed between the proportions of stage 2 oocytes and age ($F_{1,78} = 10.658$, $p = 0.002$, $r^2 = 0.120$) as well as length ($F_{1,80} = 10.363$, $p = 0.002$, $r^2 = 0.115$).

Table 2
Least squares linear regression relationships between tissue Hg concentrations (μg g^{-1} ww) and reproductive health variables gonadosomatic index (GSI), plasma 17β-estradiol concentrations (pg/ml), and the proportion of germ cells in each development stage in sexually mature female (F) and male (M) yellow perch. $p < 0.05$ was considered statistically significant and these values are present in bold font.

Dependent variable	Independent variable	Slope	Intercept	r^2	p	n
Females						
GSI	Muscle total Hg	0.012	0.300	0.001	0.715	200
	Liver total Hg	0.003	0.299	0.000	0.890	174
	Liver inorganic Hg	0.004	0.301	0.000	0.801	174
	Liver methyl Hg	0.009	0.303	0.001	0.750	174
Chromatin nucleolar oocytes (stage 1)	Muscle total Hg	0.017	0.525	0.001	0.747	82
	Liver total Hg	−0.010	0.515	0.001	0.841	69
	Liver inorganic Hg	−0.003	0.516	0.000	0.944	69
	Liver methyl Hg	−0.018	0.508	0.002	0.699	69
Perinuleolar oocytes (stage 2)	Muscle total Hg	−0.054	0.845	0.019	0.218	82
	Liver total Hg	−0.001	0.866	0.000	0.984	69
	Liver inorganic Hg	−0.010	0.857	0.001	0.771	69
	Liver methyl Hg	0.007	0.870	0.000	0.859	69
Cortical alveolar oocytes (stage 3)	Muscle total Hg	0.048	0.437	0.015	0.271	82
	Liver total Hg	0.007	0.420	0.000	0.858	69
	Liver inorganic Hg	0.010	0.426	0.001	0.766	69
	Liver methyl Hg	0.008	0.423	0.001	0.825	69
17β-estradiol (pg/ml)	Muscle total Hg	0.348	2.918	0.045	**0.049**	87
	Liver total Hg	0.325	2.884	0.089	**0.007**	81
	Liver inorganic Hg	0.288	3.002	0.103	**0.003**	81
	Liver methyl Hg	0.175	2.900	0.023	0.178	81
Males						
GSI	Muscle total Hg	0.025	0.411	0.000	0.883	66
	Liver total Hg	−0.151	0.502	0.034	0.339	29
	Liver inorganic Hg	−0.099	0.484	0.021	0.454	29
	Liver methyl Hg	−0.166	0.445	0.038	0.313	29
Primary spermatocytes (stage 1)	Muscle total Hg	0.405	0.838	0.106	**0.011**	60
	Liver total Hg	0.002	0.567	0.000	0.982	26[a]
	Liver inorganic Hg	−0.003	0.564	0.000	0.969	26[a]
	Liver methyl Hg	0.063	0.604	0.014	0.560	26[a]
Secondary spermatocytes (stage 2)	Muscle total Hg	−0.310	0.668	0.063	0.052	60
	Liver total Hg	0.027	0.825	0.002	0.824	27[b]
	Liver inorganic Hg	−0.013	0.810	0.001	0.897	27[b]
	Liver methyl Hg	0.024	0.832	0.002	0.845	27[b]
Spermatids (stage 3)	Muscle total Hg	−0.031	0.164	0.001	0.789	60
	Liver total Hg	−0.084	0.206	0.017	0.510	28
	Liver inorganic Hg	−0.020	0.217	0.001	0.854	28
	Liver methyl Hg	−0.152	0.139	0.051	0.247	28
Spermatozoa (stage 4)	Muscle total Hg	−0.075	0.105	0.010	0.456	60
	Liver total Hg	−0.043	0.190	0.007	0.674	28
	Liver inorganic Hg	0.007	0.206	0.000	0.935	28
	Liver methyl Hg	−0.126	0.127	0.056	0.226	28

[a] Two outliers omitted from the analysis.
[b] One outlier omitted from the analysis.

In male perch, the proportion of spermatogenic cells in each development stage differed among lakes ($p < 0.006$). However, in all lakes (excluding Kejimkujik), the proportions of cells in the 1st and 2nd stages were more prevalent (13.8–71.7%) than those in the 3rd and 4th stages (0–15.4%). Proportions of stage 1 cells (primary spermatocytes) were positively related to muscle THg concentrations, explaining only 10.6% of the variability in the presence of these cells, but were not related to liver THg, MeHg, or inorganic Hg concentrations (Table 2). Similarly, the proportions of cells in the other stages of development (2, 3 or 4) were unrelated to muscle or liver Hg concentrations (Table 2). However, the mean proportions of stage 4 cells in males from each lake were positively related to aqueous concentrations of both TN and TP (TN: $F_{1,10} = 8.214$, $p = 0.017$, $r^2 = 0.451$; TP: $F_{1,10} = 6.412$, $p = 0.030$, $r^2 = 0.391$), while no relationships existed between lake pH and the mean proportions of cells in any stage ($p > 0.325$). It should be noted that the p-value (0.052) of the regression between stage 2 cells and muscle THg concentrations was very close to the level of significance set for this study. The relative abundance of secondary spermatocytes ($F_{1,58} = 10.877$, $p = 0.002$, $r^2 = 0.158$), spermatids ($F_{1,59} = 44.470$, $p = <0.001$, $r^2 = 0.430$), and spermatozoa ($F_{1,59} = 30.599$, $p < 0.001$, $r^2 = 0.342$) was related to male GSI; these relationships were positive in the case of spermatids and spermatozoa, and negative in the case of secondary spermatocytes. In contrast, no relationship existed between the proportions of primary spermatocytes and GSI ($F_{1,59} = 0.037$, $p = 0.848$, $r^2 = 0.001$), or between the proportions of cells in any of the stages and perch age or length ($p > 0.060$).

3.4. Sex steroids

Plasma 17β-estradiol concentrations of female perch varied >5 fold (Table 1) and differed significantly among lakes, although we did not find that lower mean 17β-estradiol concentrations generally occurred in perch from lakes with higher mean perch muscle THg concentrations, or vice versa (Table 1). Similarly, when these results were pooled into 3 groups of lakes with low, medium, and high mean perch muscle THg concentrations (4 lakes per group), no differences in 17β-estradiol concentrations existed among these groups ($F_{2,85} = 2.170$, $p = 0.120$). The 17β-estradiol concentrations of female perch from all lakes were positively related to their muscle THg, liver THg, and liver inorganic Hg concentrations (Table 2) although the r^2 values for these relationships were <0.103. The strongest of these relationships occurred with liver inorganic Hg. In addition, the mean 17β-estradiol concentrations from each lake were unrelated to the lake water characteristics pH, TP and TN ($p > 0.301$, data not shown).

Plasma 17β-estradiol concentrations were positively related to perch length ($F_{1,76} = 18.022$, $p < 0.001$, $r^2 = 0.192$), so residuals of this relationship were used to determine the relationships between 17β-estradiol concentrations and tissue Hg concentrations, independent of perch length. These residuals were not related to muscle THg ($F_{1,76} = 0.149$, $p = 0.701$, $r^2 = 0.002$), or liver concentrations of THg ($F_{1,70} = 1.504$, $p = 0.224$, $r^2 = 0.021$), MeHg ($F_{1,70} = 0.193$, $p = 0.662$, $r^2 = 0.003$), or inorganic Hg ($F_{1,70} = 1.504$, $p = 0.224$, $r^2 = 0.021$). Additionally, 17β-estradiol concentrations were also positively related to age ($F_{1,75} = 14.661$, $p < 0.001$, $r^2 = 0.164$) and GSI ($F_{1,76} = 7.839$, $p = 0.006$, $r^2 = 0.093$).

4. Discussion

To determine whether MeHg exposure was affecting sexually mature male and female yellow perch from KNPNHS, several indicators of reproductive health (GSI, 17β-estradiol concentrations, and germ cell development) were measured and related to their tissue Hg concentrations. We found that muscle THg concentrations were positively related to perch length, age, and weight, and that female GSI was positively related to plasma estradiol concentrations, however no relationships existed between female GSI and fish size or age. In addition, all of the measured health indicators were unrelated to the tissue Hg concentrations of both male and female perch with the exception of a positive relationship between the proportion of primary spermatocytes in males and muscle THg concentrations. Collectively, the results indicated that the reproductive health of KNPNHS perch during early gonadal recrudescence was not adversely impacted by Hg exposure. However, it remains unknown whether MeHg affects later stages of gonadal development or the spawning behavior and success of wild fish.

4.1. Gonadosomatic indices

Oocytes mature within the gonads of female fish in preparation for spawning, and GSI is a measure of this development (ovaries in this case). In the present study, the GSI of female perch differed among lakes but these did not correspond with the among-lake differences in their muscle or liver Hg concentrations. Rather, the differences in GSI were likely a result of variability among the lakes in abiotic and biotic factors (i.e. temperature (Lam, 1983)) which are known to influence fish gonad development. Similarly, using linear regression we found that the GSI of individual female perch within KNPNHS was unrelated to the Hg concentrations present in their muscle or liver tissues. This suggests that the development of their ovaries was not affected by Hg exposure, and is similar to the results of Webb et al. (2006) and Friedmann et al. (1996b); both found that female GSI was unrelated to the tissue Hg concentrations of wild female fish (white sturgeon (0.171 ± 0.013 μg g^{-1} ww in muscle (males and females)) and northern pike (*Esox lucius*, 0.303 ± 0.006 μg g^{-1} ww in muscle) respectively). However, the germ cell scoring used by Webb et al. (2006) indicates that both immature females and those in early recrudescence may have been included in these analyses. In the laboratory, the effects of MeHg on GSI are variable. For instance, the GSI of female zebrafish (*Danio rerio*) was unchanged following exposure to dietary MeHg (0.89 or 3.4 μg Hg g^{-1}) (Susnik, 2010), as was the GSI of juvenile female walleye (*Sander vitreus*, 0.25–2.37 μg Hg g^{-1} ww in whole body) (Friedmann et al., 1996a). In contrast, the GSI of female fathead minnows (*Pimephales promelas*) fed a MeHg contaminated diet (3.84 ± 0.15 μg Hg g^{-1} ww in whole body of exposed fish) was lower than control fish (Drevnick and Sandheinrich, 2003), and the GSI of this species was also inversely related to body THg concentrations (0.05–5.28 μg g^{-1} ww in whole body, assuming 80% moisture) (Hammerschmidt et al., 2002) following dietary MeHg exposure. The muscle THg concentrations of KNPNHS female perch (0.13–2.13 μg g^{-1} ww) were lower than those in fish from the two laboratory studies reporting decreases in GSI, as were the GSI values of KNPNHS perch ($2.03\% \pm 0.04$), which may account for the absence of an effect in this study. Specifically, the GSI of fathead minnows in the first of the two studies ranged from 3.0 to 16.4% (Hammerschmidt et al., 2002), while the mean GSI of control females was 6.46% in the study by Drevnick and Sandheinrich (2003), and females in both studies had spawned or were at sexual maturity when collected. In KNPNHS perch, all of the examined ovaries were in early stages of development (contained primarily chromatin nucleolar or perinucleolar oocytes) and lacked vitellogenic oocytes. It is not known whether Hg affects later stages of oocyte development as our study focused only on earlier stages of gonadal recrudescence of these female perch.

As for females, the GSI of male perch from KNPNHS was also unrelated to Hg concentrations in the muscle and liver tissues of these fish. This finding concurs with some but not all of the previous studies. Relative to other male yellow perch collected in the fall (mean GSI: 5.03 ± 2.64 (±SE)) (McMaster et al., 2002), KNPNHS males were at an earlier stage of recrudescence (mean GSI: 2.99 ± 0.21 (±SE)). Given the elevated tissue Hg concentrations in KNPNHS perch, it is possible that Hg exposure is responsible for this difference, although this requires further investigation. The impacts of MeHg on gonad growth in males are unclear in the current literature (Crump and Trudeau, 2009).

For instance, the GSI of juvenile male walleye decreased following MeHg exposure (0.25–2.37 μg Hg g^{-1} ww, whole body) (Friedmann et al., 1996a), and was also negatively related to the THg concentrations of testes from wild immature white sturgeon (0.171 ± 0.013 μg g^{-1} ww, muscle (males and females)) (Webb et al., 2006). However, the GSI of male fathead minnows (~0.06–~4.77 μg Hg g^{-1} ww, whole body) was unaffected by dietary MeHg exposure (Drevnick and Sandheinrich, 2003). In wild male fish, GSI was also unrelated to muscle THg concentrations in northern pike (0.347 ± 0.06 μg Hg g^{-1} ww, muscle) (Friedmann et al., 1996b) and did not differ among sampling sites in largemouth bass with muscle Hg concentrations up to 5.42 μg Hg g^{-1} (ww, muscle) (Friedmann et al., 2002). Our findings support these latter studies, both laboratory and field, which report no effect of Hg on male GSI.

4.2. Germ cell development

Histology has been suggested as more sensitive than GSI for detecting the effects of Hg on fish gonad development, particularly in males (Crump and Trudeau, 2009). In female fish, oocytes mature from primary growth stages (1 and 2) to the cortical alveolar stage (3) and vitellogenesis, and finally to maturation and ovulation (Tyler and Sumpter, 1996). Oocytes in the gonads of female perch from this study were present in three stages of development (1, 2, and 3), and their proportions were not related to Hg concentrations in the muscle or liver tissues of these perch, indicating oogenesis in these perch was unaffected by Hg exposure. This finding is in agreement with the absence of a relationship between GSI and tissue Hg concentrations in these fish. In contrast, oocyte development of female murrel (*Channa punctatus*) and catfish (*Clarias batrachus*) was inhibited following exposure to waterborne inorganic Hg (10 mg l^{-1}) (Ram and Sathyanesan, 1983), and MeHg (0.04 mg l^{-1}) (Kirubagaran and Joy, 1988), respectively, and their ovaries were void of the mature vitellogenic ooctyes prevalent in control fish. The aqueous exposure concentrations in these laboratory studies, however, were $>10^4$ fold higher than those present in KNPNHS lakes (<5 ng l^{-1} for both THg and MeHg (Clayden, 2011)), and do not provide a good comparison. Therefore, to more accurately understand the effects of Hg on oocyte development in wild fish it would be valuable to conduct laboratory studies which use dietary and aqueous Hg exposures at environmentally-relevant concentrations.

To our knowledge, this is the first study to examine whether wild fish with higher Hg have delayed sperm cell development. In contrast to some previous lab studies, the proportion of primary spermatocytes was positively related to muscle THg concentrations of male perch from KNPNHS. This would suggest a consequent decrease in the proportions of other spermatogenic cells with increasing muscle THg, however this was not the case. The proportions of cells in stages 2, 3, and 4 were unrelated to tissue Hg concentrations in these perch, suggesting no overall effect of Hg on spermatogenesis of male perch from these lakes. However, spermatogenesis of catfish was inhibited following laboratory exposures to 40 μg l^{-1} of MeHg (Kirubagaran and Joy, 1992). It is interesting that mean proportions of mature spermatozoa (stage 4) in male perch were positively related to aqueous concentrations of both TP and TN in KNPNHS lakes. It is possible that the spermatogenic cells of male perch from higher-nutrient lakes matured more rapidly than those from lower-nutrient lakes, due to a greater availability of food. In the laboratory, the testes of goldfish (*Carassius auratus*) contained primarily stage 1 and 2 cells when fish were fed a limited diet, but reached full maturity within a month of being fed an increased diet (Clemens and Reed, 1967).

4.3. Sex steroids

17β-estradiol is the primary reproductive hormone in female fish, and decreases in the plasma concentrations of this hormone may be indicative of altered reproductive success (Drevnick and Sandheinrich, 2003). Our findings indicate that plasma 17β-estradiol concentrations in female perch were not related to Hg exposure. The concentrations of 17β-estradiol in these perch (117.7–5103.8 pg ml^{-1}) were similar to those in other yellow perch populations, including both wild (~800–~3200 pg ml^{-1}, sampled in fall) (van den Heuvel et al., 1999) and laboratory-reared (~1000–~1500 pg ml^{-1}, 2 and 3 year olds) (Ciereszko et al., 1997) fish. Additionally, the plasma 17β-estradiol concentrations of female perch from KNPNHS were positively related ($r^2 < 0.103$) to muscle THg, liver THg and liver inorganic Hg concentrations. This was a result of the confounding positive relationship between 17β-estradiol and female perch length, as the residuals of the relationship between 17β-estradiol and length were unrelated to tissue Hg concentrations.

Our finding that 17β-estradiol concentrations of female KNPNHS perch were unaffected by Hg is in contrast to the majority of results in previous studies. Plasma 17β-estradiol was negatively related to whole body THg concentrations of fathead minnows exposed to dietary MeHg in the laboratory (~0.07–~5.07 μg g^{-1} ww) (Drevnick and Sandheinrich, 2003), to muscle THg concentrations of wild largemouth bass (Adams et al., 1999), and to liver THg concentrations of wild immature female white sturgeon (0.140 ± 0.023 μg g^{-1} ww, both sexes) (Webb et al., 2006). Also, 17β-estradiol concentrations decreased in largemouth bass fed MeHg contaminated feed in the laboratory (3.12 and 6.25 mg MeHg/kg, dry weight) (Fynn-Aikins et al., 1998). In contrast, Friedmann et al. (1996b) reported no relationship between 17β-estradiol concentrations and muscle Hg content (0.139–0.623 μg g^{-1} ww) in wild northern pike. The tissue Hg concentrations of fish in the studies reporting relationships exceed those measured in female perch from the present study, and likely explain the absence of a negative relationship herein. For instance, the maximum whole body THg concentration of fathead minnows measured by Drevnick and Sandheinrich (2003) is >2 fold greater than that in the muscle of KNPNHS female perch (2.13 μg g^{-1} ww). Also, MeHg exposure concentrations used by Fynn-Aikins et al. (1998) are $>10^3$ times higher than those that are present in yellow perch prey, aquatic invertebrates (<0.54 μg g^{-1}; dry weight, mean value within lakes) (Clayden, 2011). In the liver, the range of THg concentrations in the present study (0.13–6.04 μg g^{-1} ww) exceeds those reported by Webb et al. (2006), however differences in fish maturity (sexually immature vs. mature) may account for the difference in results. The muscle THg concentrations measured in fish from the study reporting no relationship (Friedmann et al., 1996b), however, were comparable to the lower muscle THg concentrations present in KNPNHS perch (perch THg range: 0.13 to 2.13 μg g^{-1} ww). It appears therefore that the Hg concentrations of female perch within KNPNHS were below those that would impact their plasma 17β-estradiol concentrations.

Despite this, the THg concentrations present in these perch are high enough to pose a reproductive risk to common loons within the park (Burgess et al., 1998; Nocera and Taylor, 1998) and these concentrations are thought to be increasing (Wyn et al., 2010). In addition, a previous study has demonstrated that Hg exposure may be adversely affecting the general health of KNPNHS perch at the cellular level, as evidenced by an increase in the prevalence of macrophage aggregates (indicators of oxidative stress and cellular damage) in perch with higher tissue Hg concentrations (Batchelar et al., 2013). If Hg concentrations increase in perch, both perch and loons will be put at a greater risk of adverse reproductive effects. Perch are known to be tolerant of metal contamination (Couture and Pyle, 2008), so it is possible that the elevated Hg in this region is affecting other more sensitive fish species in KNPNHS. For yellow perch within this region, results of the present study serve as a baseline for monitoring any changes in their reproductive health over time.

4.4. Conclusions

Based on the current data, our findings demonstrate that Hg did not adversely impact the reproductive health of wild KNPNHS yellow

perch at an early stage of reproductive development (early vitellogenesis in the case of females). Despite this, the THg concentrations present in the muscle tissue of many of these perch, as well as the concentrations present in their prey, exceed the estimated whole body Hg (0.2 μg g^{-1} ww (~0.33 μg/g ww in muscle)) and dietary MeHg thresholds for adverse effects to the general and reproductive health of fish (Beckvar et al., 2005; Depew et al., 2012). Perch in this study were sampled at an early stage of gonadal recrudescence and it is possible that effects of Hg on reproduction occur at a later stage of sexual maturation. In addition, yellow perch are known to be a metal-tolerant species, whereas species used to estimate the above thresholds may be more sensitive to the reproductive effects of MeHg. These thresholds are also based solely on the findings of laboratory studies which may not accurately predict effects in wild fish. Given that this is a field-based study, the impact of other stressors on the measured endpoints must also be considered. Although the KNPNHS area is fairly pristine, it is possible that other natural or anthropogenic influences may be affecting the reproductive health of these perch in a manner that we were unable to measure or predict within the scope of this study. Finally, it is possible that the reproductive health of KNPNHS perch may be affected by Hg in a manner not assessed in this study (e.g. delayed spawning, decreased number of spawning pairs, or altered spawning behavior), or that other fish species within the park may be affected. Further investigation is needed to increase our currently limited understanding of the reproductive effects of Hg on wild fish populations.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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