PCB Trends in Snapping Turtle Eggs from Lyons Creek East

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Background

Sediment in Lyons Creek has a long history of polychlorinated biphenyl (PCB) contamination that was associated with industrial sources and spills in the headwaters of the creek from the 1940s to the 1960s. Construction of the Welland Ship Canal Bypass in the early 1970s bisected the creek and subsequently these areas of contaminated sediment. Following cleanup of this area on the west side of the canal, PCB contamination was subsequently identified in sediment on the east side, where water is actively pumped to maintain water flow in the creek. This portion of the creek, known as Lyons Creek East (LCE), flows 20 kilometres from the canal through ecologically significant wetlands to the Welland River, where it drains into the Niagara River. In 2008, several sediment remediation options were evaluated in LCE to reduce and manage PCB exposure to biota. Monitored Natural Recovery (MoNR) was selected as the preferred remediation strategy. However, recent data from long-term monitoring studies of water, sediment, and biota from 2005–2022 suggest that natural recovery of some upstream zones of LCE has not been progressing. PCB concentrations in sediment, water, invertebrates, and young-of-year fish in these zones remain elevated. In addition, PCB concentrations in surface sediment have not consistently decreased, nor met the PCB target concentration, and remain toxic to invertebrates. Following this, an active process is currently underway to evaluate sediment management options in these upstream LCE zones. Ongoing monitoring of sediment, water, and biota will contribute to this evaluation process. Natural recovery is occurring or has been achieved at LCE zones further downstream.

Among biota to be assessed for PCBs in LCE is the common snapping turtle (*Chelydra serpentina serpentina*). This species has been frequently used as a bio-indicator of local contaminants and their effects on wildlife health in Great Lakes Areas of Concern (Bishop *et al.* 1991, 1998; de Solla *et al.* 2007, 2008). The snapping turtle is a long-lived omnivorous reptile that commonly inhabits most wetlands in the lower Great Lakes. As a top predator, it can also accumulate relatively high levels of contaminants such as PCBs, which are primarily acquired through dietary sources (largely fish, but also molluscs, crustaceans, and insects). Contaminant concentrations in eggs are reflective of maternal contaminant body burdens and increased concentrations of some compounds in eggs have been associated with poor developmental success (e.g., Bishop *et al.* 1991, 1998; Pagano *et al.* 1999). In Ontario, the snapping turtle's home range can vary from a few hectares to a few dozen hectares (Pettit *et al.* 1995; Paterson *et al.* 2012). Several extrinsic (e.g., size and type of available habitat, population density) and intrinsic factors (e.g., body size and energetic demands) are important determinants of turtle movement. In LCE where turtles appear to be abundant, it is likely that their home range is relatively smaller due to the creek's high productivity and abundant food supply, particularly in some parts of the creek (S.R. de Solla, *pers. comm.*). Female turtles also tend to show high nest site fidelity often returning to the same site in subsequent years to nest (Loncke and Obbard 1977). This provides additional support for the use of this species as a good bio-indicator of local contaminant exposure in LCE.

This report provides an overview of PCB trends in snapping turtle eggs collected from sites along LCE from 2018 to 2022. These data were also compared to PCB concentrations in eggs collected from this area in 2002 and 2010 to provide an assessment of temporal changes in PCB exposure in turtles. This study is part of a broader assessment of PCBs in water, sediment, invertebrates, and fish that will inform the evaluation of sediment remediation options for the most PCB-contaminated LCE zones.

Assessment Approach

For purposes of assessment, areas of LCE were sub-divided into seven zones that correspond with those delineated by the Ministry of the Environment and Conservation Protection (MECP) for sediment sampling. Zones 1–3 extend downstream from the canal to Highway 140. Zones 4–7 extend further downstream with boundaries that were largely delineated by roadways that crossed the creek and ended at Crowland Avenue (**Figure 1**). Where possible, turtle eggs were collected from each zone and analyzed for PCBs. For each zone, status criteria associated with PCB exposure and the long-term health of LCE turtles were assessed.

This approach is based on a tiered status criteria system from worst (red) to best (green) conditions. Ideally, the goal of this strategy is to meet the status associated with the green condition. Status criteria and conditions (identified as a colour code) are as follows:

RED: Sum PCBs in eggs from Lyons Creek East are above concentrations associated with reproductive effects in birds, i.e., 8–25 µg/g.

YELLOW: Mean PCB concentrations are significantly higher in eggs from Lyons Creek East compared to the reference site (Long Point, Lake Erie) and mean PCB concentrations in liver (estimated) exceed the no adverse effect concentration (NOAEC) associated with hepatic expression of *cyp1a,* a gene involved in metabolism and detoxification of PCBs in wildlife.

GREEN: Mean PCB concentrations in liver (estimated) do not exceed the NOAEC associated with hepatic expression of the *cyp1a* gene suggesting that concentrations of PCBs are not sufficiently high to elicit an increase in metabolism and detoxification response.

Further details and rationale for the status criteria associated with each of these conditions are as follows. With respect to the red condition, in contrast to birds, egg PCB threshold concentrations that are associated with adverse reproductive effects in reptiles remain largely unknown. As such, egg PCB thresholds identified for birds were used as a surrogate for turtles, since both are amniotes and lay eggs.

In addition to comparisons of egg PCB concentrations between LCE turtles and reference site turtles (yellow condition), an assessment of an enzymatic detoxification response to PCB exposure in LCE turtles was conducted (yellow and green conditions). Specifically, this was achieved by estimating hepatic (liver) concentrations in LCE turtles that would be expected to elicit hepatic expression of *cyp1a*, a gene involved in metabolism and detoxification of PCBs. Experimental dosing of juvenile turtles with PCBs found that there was no induction of this gene at a liver PCB concentration (wet weight) of 1.21 µg/g (Colson *et al.* 2021); this is considered the no adverse effect concentration (NOAEC) in turtles in this assessment. Induction of this gene was reported at a liver PCB concentration of 3.54 µg/g. On a lipid weight basis, this corresponds to 28.81 µg/g (NOAEC) and 84.29 µg/g for induction of this gene (based on a mean percent lipid content of 4.2%; Pagano *et al.* 1999). Since PCBs were not quantified in turtle liver in this study, PCB concentrations in liver were estimated using the relationship found between egg and liver tissues (lipid weight basis) of gravid female snapping turtles (Pagano *et al.* 1999). Estimated liver PCB concentrations in LCE turtles were then compared on a zone-by-zone basis against the NOAEC concentration to assess whether these concentrations might be expected to elicit hepatic induction of

Figure 1. Collection locations of snapping turtle egg clutches (53) along Lyons Creek East from 2018–2022. Study zones 1–7 correspond to designated MECP sediment collection zones. Note that symbols may be overlapping and therefore represent the collection location of multiple clutches. An additional 6 clutches were collected at sites beyond zone 7 in 2018 and 2019; collection locations of these clutches are designated as in zone 8 and are not shown here.

the *cyp1a* gene. This determination is based on the following assumptions: 1) that distribution of PCBs among tissues in the animal is in steady state equilibrium at the time of collection; 2) that induction levels of the gene are the same in juveniles and gravid females, and; 3) the percentage lipid content in liver of juveniles and females are the same (or similar). It should be noted that exceedance of the NOAEC suggests a *possible* detoxification response versus the concentration at which induction of this gene was reported (roughly three times higher than the NOAEC). It is expected that the actual concentration associated with induction of this gene would fall somewhere within the range of these two concentrations.

Egg Collections and Methods

Searches for freshly-laid clutches of snapping turtle eggs were conducted along LCE from the Welland Canal and moving downstream and eastward along the creek in 2018, 2019, 2021, and 2022. Five eggs were randomly selected from each clutch for contaminant analysis. All remaining eggs in the clutch were then reburied to allow for successful hatching, with one exception; in 2018, the remaining eggs were collected and assessed for hatching success in the laboratory. Throughout the entire study period, eggs were collected from a total of 59 clutches in June of 2018 (18 clutches), 2019 (20 clutches), 2021 (17 clutches), and 2022 (4 clutches). Of the 59 clutches collected, 53 clutches were collected in zones 1 and 3–7; no eggs were found in zone 2 (**Figure 1**). Eggs from the remaining six clutches were collected further downstream in 2018 and 2019 and beyond zone 7. Specifically, five clutches were collected along LCE between Crowland Avenue and Montrose Avenue and one clutch was collected beyond Montrose Avenue and approximately 700 metres from the Queen Elizabeth Way (highway); for this purpose, the collection location of these six clutches is designated as in zone 8. Eggs from twenty clutches were also collected in 2018 (10 clutches) and 2019 (10 clutches) from a reference site at Long Point on Lake Erie and analyzed for PCBs.

Chemical analyses of snapping turtle eggs for PCBs were conducted by gas chromatograph coupled with a mass selective detector at the National Wildlife Research Centre (NWRC) in Ottawa. Sum PCBs were based on the sum concentrations of 35 individual or co-eluting PCB congeners and are reported on a wet weight basis. Method Detection Limits (MDLs) for PCB congeners ranged from 0.01–3.7 ng/g.

For temporal assessments, PCB concentrations were compared between turtle eggs collected from LCE in 2002 and 2010 (early period) to those in collections conducted in the current study from 2018–2022. In 2002, eggs were collected from a total of 9 clutches in zones 4, 5, and 6. In 2010, eggs were collected from 15 clutches in zones 3–6 and zone 8. Chemical analyses were conducted at NWRC for collections in 2002 and at SGS AXYS Analytical Services Ltd. in Sidney, British Columbia in 2010. Sum PCB concentrations for this assessment were based on the sum concentration of 30 individual or co-eluting congeners that were common to all chemical analyses.

Student's t-tests were conducted to statistically compare sum PCB concentrations between eggs from each zone individually and the reference site with egg collection years grouped together. On a zone-by-zone basis, sum PCB concentrations were also compared (where possible) between egg collections in 2002 and 2010 and those in 2018–2022 using Student's t-tests. Grouping data in this way would increase sample sizes and thereby statistical power for comparisons between the early period and the current period. Data were log-transformed (log_{10}) to meet conditions of equal variance and normality

for parametric analysis and if data failed these assumptions, comparisons were made using a Mann Whitney test. PCB congener patterns in eggs were examined using Principal Component Analysis using varimax normalized rotation on untransformed concentrations for the most prevalent 30 PCB congeners. Since PCBs are lipophilic compounds, differences in lipid content in eggs may influence PCB concentrations in eggs. As such, differences in percent lipid content were first statistically assessed prior to further statistical testing. If this difference was significant, sum PCB concentrations were assessed on a lipid weight basis and if not, sum PCB concentrations were assessed on a wet weight basis. For samples that were below MDLs, replacement values were calculated for individual PCB congeners using maximum likelihood (de Solla *et al*. 2012). Regression analysis was used to examine the relationship between sum PCB concentrations in eggs and clutch collection location along LCE (water's edge) starting at Canal Road which runs parallel to the Welland Canal. All results were considered significant at $p<0.05$.

Results

1. Spatial Trends

On a broad geographic scale, sum PCB concentrations in eggs decreased moving downstream from the Welland Canal and along LCE, i.e., from zones 1– 8, from 2018–2022 (**Figure 2**). The highest mean sum PCB concentration was in eggs from zone 3, although mean concentrations in zones 3–5 were largely very similar. Mean concentrations showed a relative decrease in eggs from zone 7 and then were markedly lower in eggs from zone 8. Mean PCB concentrations were significantly higher in eggs from all LCE zones compared to those in eggs from the reference site at Long Point (**Figure 2**). The maximum PCB concentration was 31,500 ng/g on a lipid weight basis in an egg clutch from zone 4 and 1,596 ng/g on a wet weight basis in an egg clutch from zone 1. Sum PCB concentrations in eggs declined with distance from Canal Road ($r^2=0.43$, $p<0.00001$; **Figure 3**). A similar declining trend with distance was found in egg collections conducted along LCE in 2010 (de Solla *et al.* 2007). Within each zone, sum PCB concentrations ranged widely among individual clutches; PCB trends in eggs on a wet weight basis are provided in the Appendix (**Figure A1**).

Several factors contribute to the variability in egg sum PCB concentrations within zones. For example, it is important to note that egg collection locations may not necessarily reflect precisely where turtles have spent most of their time feeding. Turtles can travel 1–2 kilometres to their nesting site as they move to areas that are more suitable for nesting (usually on sand or gravel banks). Many egg collection locations were near roads (**Figure 1**) which also correspond to zone borders. Variation in feeding preferences among females (larger fish vs smaller fish) and prey types will also influence the degree of contaminant exposure.

Figure 2. Mean (±SE) concentrations of sum PCBs (ng/g, lipid weight) in snapping turtle eggs from sites along Lyons Creek East, on a zone-by-zone basis, from 2018–2022 (see text for further details). The mean sum PCB concentration in eggs from the reference site at Long Point in 2018 and 2019 is also shown. The asterisk indicates a significant difference in mean concentrations between each individual zone and the reference site. Numbers in bars indicate the number of clutches analyzed for PCBs in each zone or site.

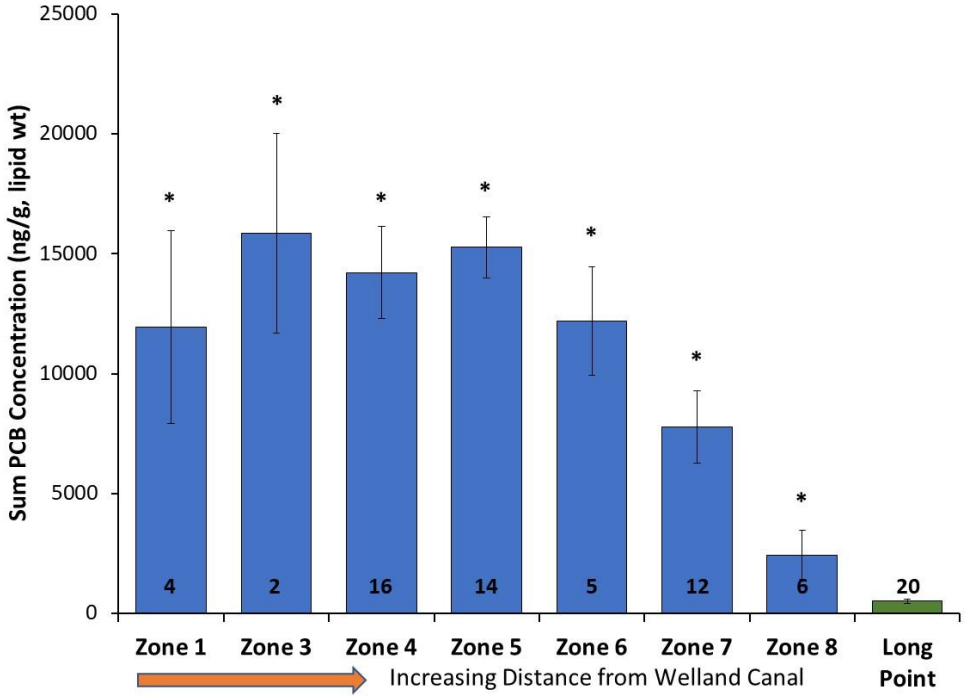
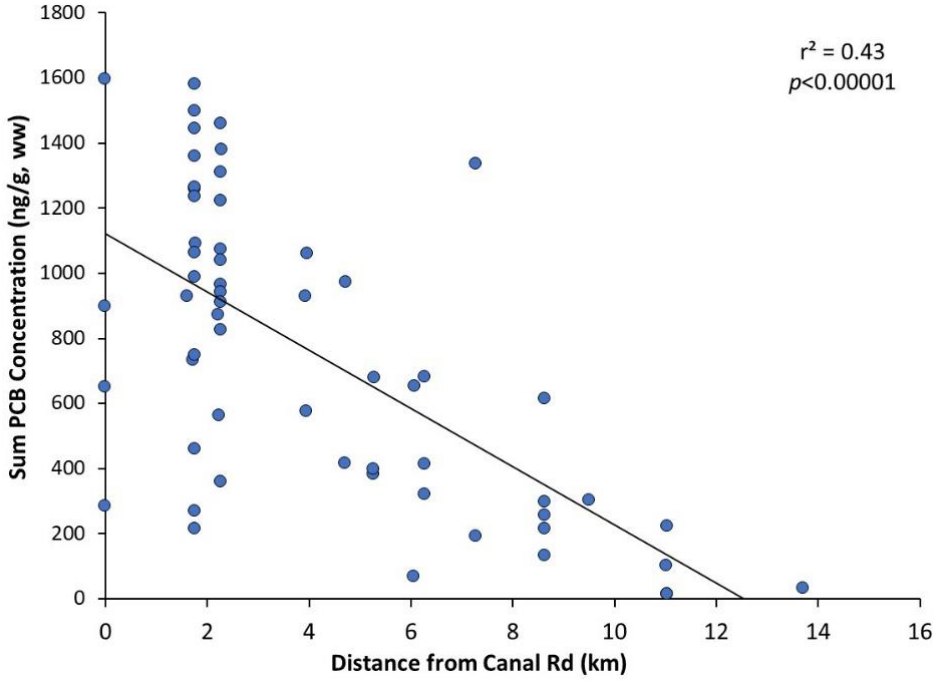


Figure 3. Sum PCB concentrations in eggs (ng/g, wet weight) collected from 2018–2022 in relation to distance of egg clutch collection location along Lyons Creek East from Canal Road (in kilometres).



With regard to PCB congener patterns, four lower chlorinated PCB congeners (PCB 118, PCB 105, PCB 99, and PCB 74) that are associated with Aroclor 1254 dominated the PCB profile in eggs from LCE (shown as positive scores for factor 1; **Figure 4**). Moving downstream, the more highly chlorinated congeners (e.g., PCB 180 and PCB 153) became increasingly important (shown as negative scores for factor 1; polynomial curve, r^2=0.37, p<0.00001). This group of congeners is commonly associated with background Great Lakes PCB profiles typically associated with Aroclor 1260. This same overall pattern was also found in LCE egg collections in 2010 (de Solla *et al.* 2007). In contrast, eggs from Long Point (Lake Erie) had relatively lower factor 1 scores suggestive of an Aroclor 1260 source and similar to patterns in turtle eggs collected from other Great Lakes sites (de Solla *et al.* 2007); see **Figure A2** for further details of factor loadings and congener patterns in LCE and Long Point eggs. These results provide further evidence that turtles continue to be exposed to a historical and distinctive PCB source, particularly in the upper reaches of LCE.

Compared to other Great Lakes sites, including Areas of Concern, sum PCB concentrations were generally higher in turtle eggs from LCE compared to sites on the St. Clair River, St. Lawrence River, and Detroit River but were relatively lower than those in eggs from Hamilton Harbour (**Figure 5**). Eggs from LCE also had consistently higher sum PCBs than eggs from Long Point across multiple years as well as other reference sites at Tiny Marsh (near Wasaga Beach) and Algonquin Provincial Park in the early 2000s.

Figure 4. Factor 1 loadings in snapping turtle eggs from sites along Lyons Creek East, on a zone-by-zone basis, from 2018–2022.

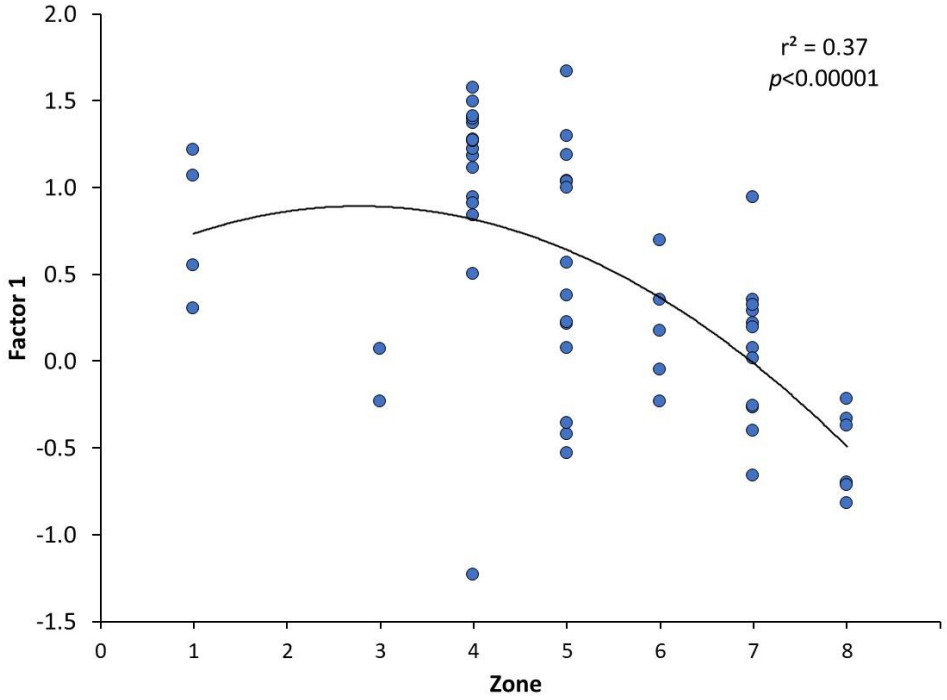
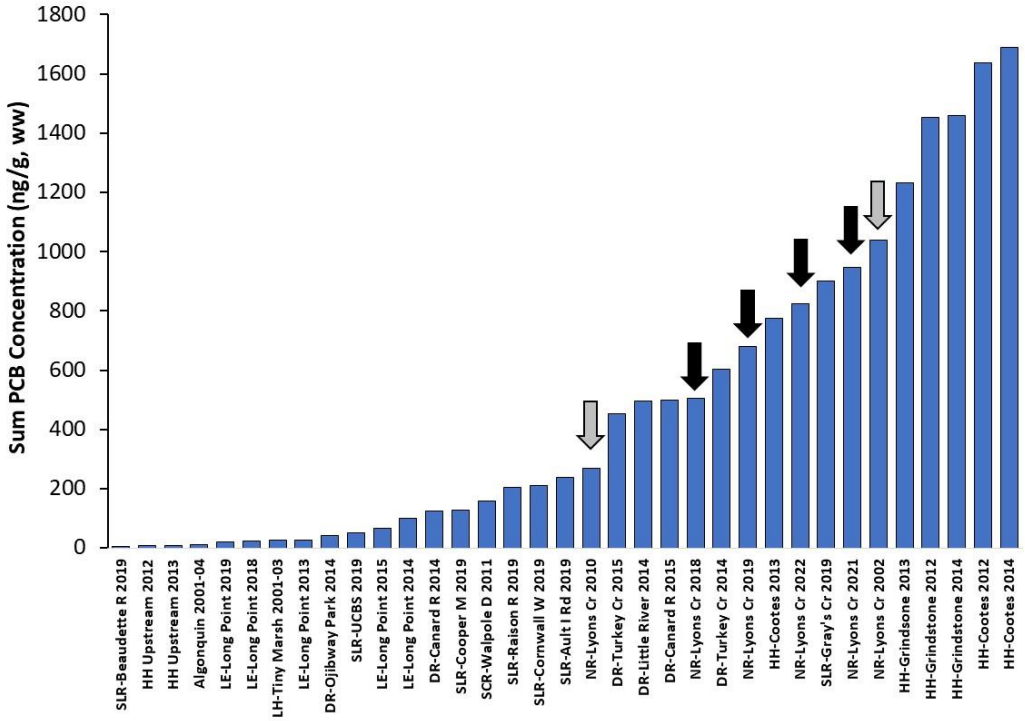
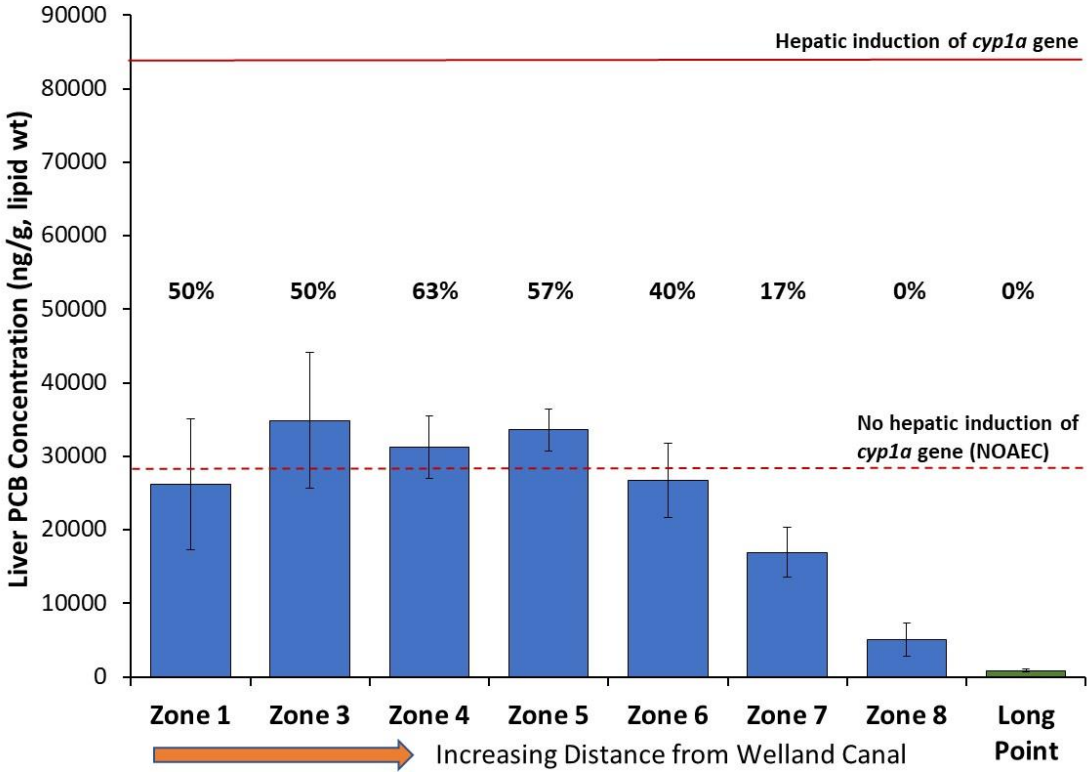


Figure 5. Mean sum PCB concentrations (ng/g, wet weight) in snapping turtle eggs from sites along Lyons Creek East in 2002 and 2010 (gray arrows), and in current collections from 2018–2022 (black arrows) and other sites in the Great Lakes basin including other AOCs (St. Clair River (SCR), Detroit River (DR), St. Lawrence River (SLR), and Hamilton Harbour (HH)) from 2011–2019 and Tiny Marsh and Algonquin Provincial Park from 2001–2004.



PCBs were also estimated in liver of LCE turtles to assess induction of the *cyp1a* gene associated with PCB detoxification and metabolism. Mean estimated sum PCB concentrations in liver of females nesting in LCE zones 1–6 were close to the NOAEC associated with no hepatic expression of the *cyp1a* gene (equal to 28,810 ng/g; **Figure 6**). When assessed against this target concentration specifically, mean estimated sum PCB concentrations in females nesting in LCE zones 3, 4, and 5 exceeded the NOAEC while those in zones 1 and 6–8 did not exceed this concentration. Percentages of clutches that exceeded the NOAEC in zones 1–6 were similar and ranged from 40%–63%; see the Appendix for estimated PCB concentrations in individual clutches of LCE eggs from each zone (**Figure A3**). At zone 7, 17% (2 of 12 clutches) exceeded the NOAEC while at zone 8 and the reference site, no clutches (0%) exceeded this concentration. No turtles exceeded the concentration associated with hepatic induction of this gene (equal to 84,290 ng/g).

Figure 6. Estimated mean (±SE) concentrations of sum PCBs (ng/g, lipid weight) in snapping turtle liver from sites along Lyons Creek East, on a zone-by-zone basis, from 2018–2022. Liver PCB concentrations are based on the relationship between egg and liver PCB concentrations (see text for further details). Estimated liver PCB concentrations in turtles from Long Point in 2018 and 2019 are also provided. The liver PCB concentration associated with no hepatic induction of the *cyp1a* gene (NOAEC) is equal to 28,810 ng/g and for hepatic induction of the gene is 84,290 ng/g; these are shown by the dotted and solid red lines, respectively. The percentage of clutches that exceeded the NOAEC is shown for each zone or site.



2. Temporal Trends

To assess temporal trends, sum PCB concentrations in eggs collected in 2002 and 2010 (early period) were compared to those in collections from 2018–2022 (current period) on a zone-by-zone basis. This was conducted at zones 4, 5, 6, and 8 only where sample sizes were sufficient for statistical analysis. Overall, there were no significant differences in sum PCB concentrations between the early period and the current period at each of these four zones (**Figure 7**). With respect to direction of trends, mean PCB concentration in eggs from zone 4 were in a favourable downward direction between time periods whereas this was not evident in other zones. Sum PCB concentrations in all LCE eggs by zone and collection year, shown as mean concentrations or for single clutches of eggs, are provided in the Appendix (**Figures A4 and A5**).

While PCBs in eggs reflect recent dietary exposure in female turtles, it is important to note that a significant period of time, i.e., greater than 10 years, may be required to see a consistent decrease in sum PCBs in LCE turtle eggs. Weathering and degradation of PCBs in sediment will directly impact those congeners that are available to biota, e.g., invertebrates and fish, and moving up the food chain to turtles. Degradation half-lives of some tri- to hepta- chlorinated PCB congeners in sediment were estimated to range between 3 and 38 years (Sinkkonen and Paasivirta 2000). Four congeners, PCB 105, PCB 118, PCB 138, and PCB 153, that were prevalent in turtle eggs (representing on average a combined total of 56% of sum PCB concentrations), have half-lives that range from 7–20 years in sediment. This consideration may be particularly important in LCE zones where natural recovery does not appear to be progressing.

Figure 7. Temporal trends for mean (±SE) concentrations of sum PCBs in snapping turtle eggs collected in 2002 and 2010 (early period) and from 2018–2022 (current period) for zones 4, 5, 6, and 8 along Lyons Creek East. Concentrations are shown both as wet weight (ww) and lipid weight concentrations. Number in bars indicate the number of clutches analyzed for PCBs in each time period.

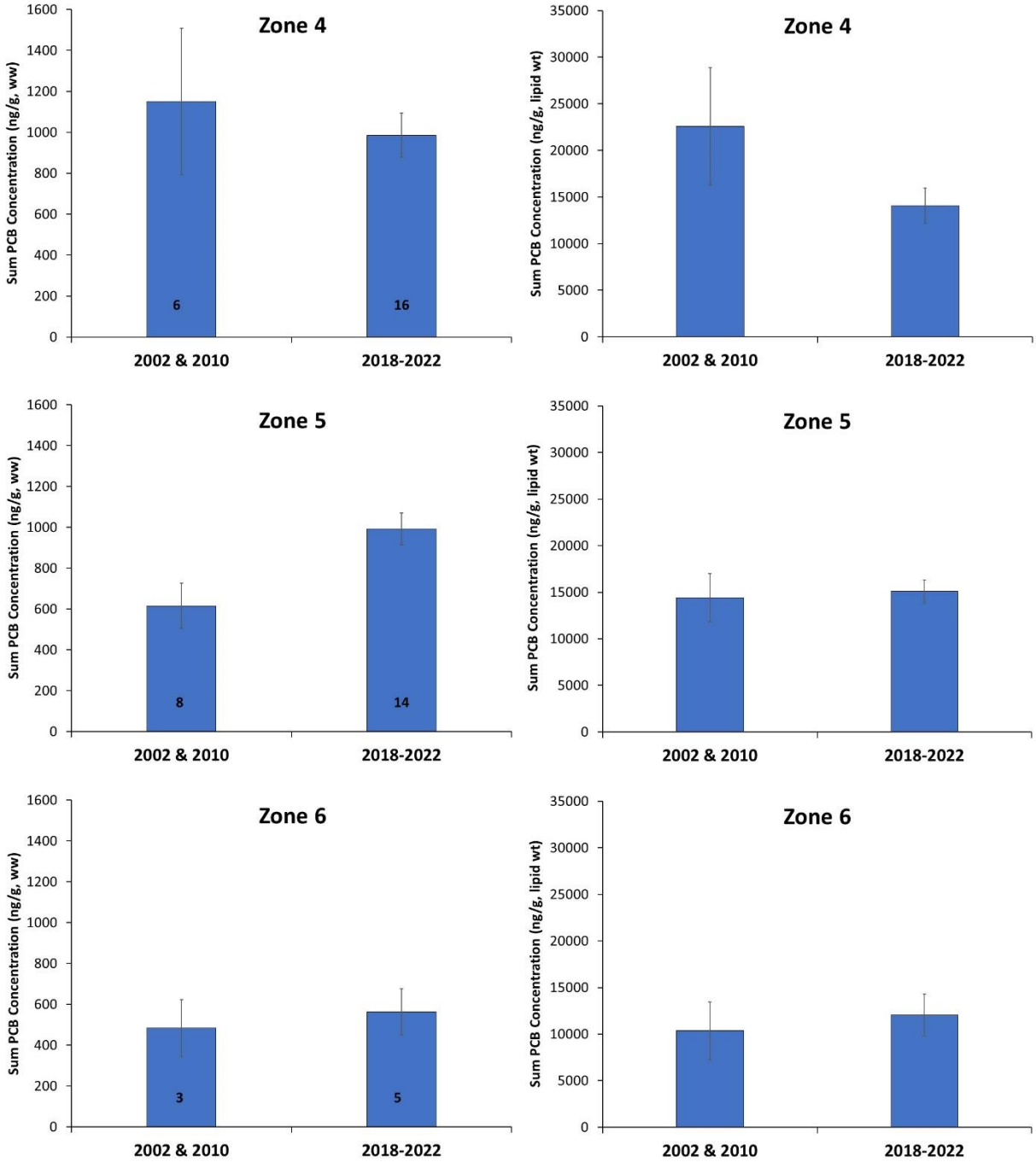
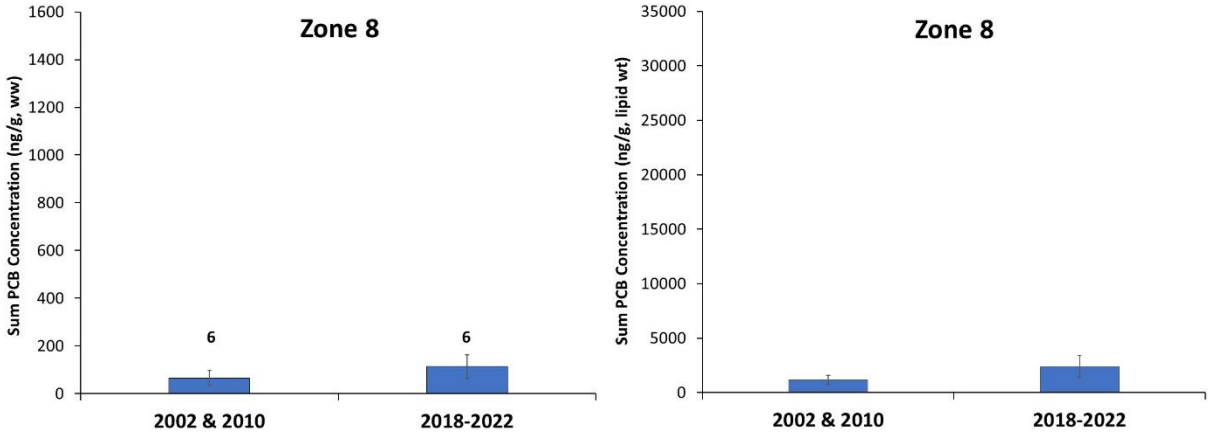


Figure 7 (continued).



Assessment for Tracking Lyons Creek East Turtles

Assessments of PCB concentrations in eggs were conducted on a zone-by-zone basis against the three status criteria and conditions associated with the health of LCE turtles. This was achieved for six of seven MECP zones since no assessment was possible for zone 2 where no eggs were found. Results based on 2018–2022 collections of eggs indicate the following:

RED: Sum PCBs in eggs from Lyons Creek East are above concentrations associated with reproductive effects in birds, i.e., 8–25 µg/g.

Using egg PCB thresholds identified for birds as a surrogate for turtles in this assessment, the maximum sum PCB concentration (1.6 µg/g) in the single clutch from zone 1 was well below egg PCB concentrations in the range of 8 to 25 µg/g that were associated with decreased hatching success for cormorants and terns (Hoffman *et al*. 1996). Therefore, these results suggest that current PCB concentrations in eggs from all six LCE zones would not be expected to adversely impact hatching success of turtles. This finding is consistent with that following artificial incubation of eggs collected from LCE zones 3–7 in 2018. Mean hatching success in these clutches was high and not significantly different between eggs from LCE and the Long Point reference site (equal to 92% and 98%, respectively; ECCC unpublished). Similarly, there was no significant difference in rates of deformed hatchlings between the two sites in 2018. In similar studies conducted in 2002–2004, no significant differences in hatching success and rates of deformed hatchlings were found between eggs from LCE and Great Lakes reference sites (de Solla *et al.* 2008). This provides additional support that current PCB concentration in turtle eggs from LCE were below concentrations associated with adverse reproductive and developmental effects.

YELLOW: Mean PCB concentrations are significantly higher in eggs from Lyons Creek East compared to the reference site (Long Point, Lake Erie) and mean PCB concentrations in liver (estimated) exceed the no adverse effect concentration (NOAEC) associated with hepatic expression of *cyp1a,* a gene involved in metabolism and detoxification of PCBs in wildlife.

Mean sum PCB concentrations were significantly higher in eggs from all six LCE zones compared to those from the Long Point reference site. However, mean PCB concentrations in liver (estimated) exceeded the NOAEC associated with hepatic expression of *cyp1a* in turtles from zones 3, 4, and 5 only. Therefore, both parts of this status criterion (yellow condition) were met at these three zones only.

GREEN: Mean PCB concentrations in liver (estimated) do not exceed the NOAEC associated with hepatic expression of the *cyp1a* gene suggesting that concentrations of PCBs are not sufficiently high to elicit an increase in metabolism and detoxification response.

Mean sum PCB concentrations in liver (estimated) did not exceed the NOEAC associated with hepatic expression of *cyp1a* in turtles from zones 1, 6, and 7. Therefore, these zones met the status criterion for this condition, i.e., that PCBs in liver were not sufficiently elevated to elicit an enzymatic detoxification response to exposure.

Following these results, conditions based on status criteria for each zone are shown in Table 1.

Table 1. Condition based on status criteria for collections of snapping turtle eggs from zones 1–7 along Lyons Creek East from 2018–2022. Zone 2 could not be assessed since no eggs were found.

Condition Based on Status Criteria	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Zone 7
Snapping Turtles							

Conclusions

On a broad scale, sum PCB concentrations in eggs decreased moving downstream from the Welland Canal and along LCE from 2018 to 2022. This spatial pattern is generally consistent with proximity to the historic local PCB source on LCE and demonstrates that snapping turtle eggs are good bioindicators of local sediment contaminant conditions. PCB concentrations in eggs from zones 1, 6, and 7 in LCE have reached the desired target (based on the no adverse effect concentration [NOAEC] threshold) indicating that turtles from these zones showed relatively low PCB exposure (green condition). Turtles from the remaining zones showed relatively higher PCB exposure and thereby met the status criterion associated with the yellow condition. Eggs from all zones had sum PCB concentrations that were well below those associated with reproductive effects in birds which supports the results of an artificial incubation study of LCE turtle eggs conducted in 2018. Concentrations of sum PCBs in eggs did not significantly change between the early period (2002 and 2010) and the current period (2018–2022) at LCE zones where this could be reliably assessed. These results suggest that there were no significant changes in PCB exposure in turtles foraging in the upper reaches of LCE and at downstream sites between these two time periods.

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Appendix

Figure A1. Sum PCB concentrations (ng/g, wet weight) in snapping turtle eggs from sites in Lyons Creek East, on a zone-by-zone basis, from 2018–2022.

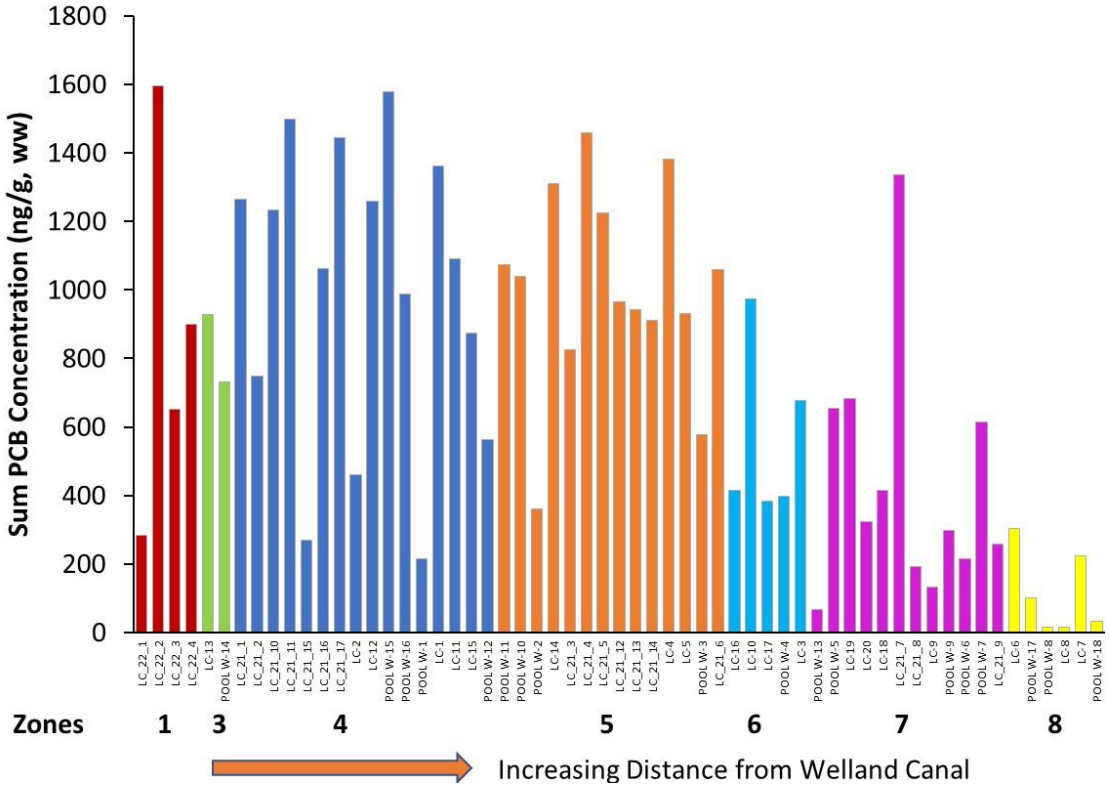
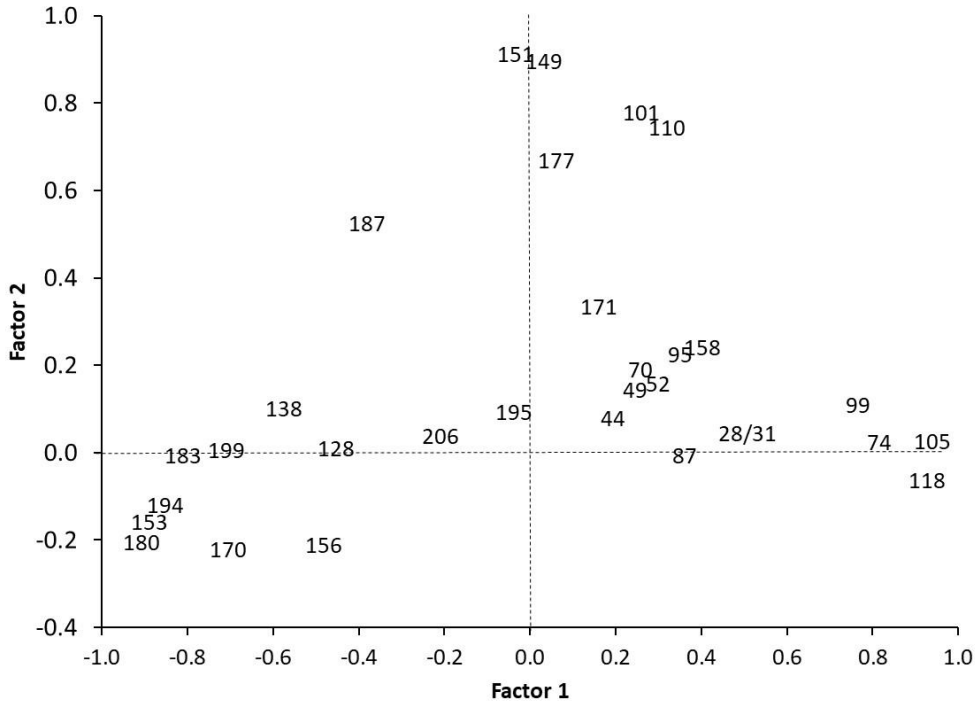


Figure A2. Factor loadings of PCB congener patterns in turtle eggs from Lyons Creek East and Long Point from 2018–2022 (a). High loadings of factor 1 are indicative of Aroclor 1254 and low loadings are indicative of Aroclor 1260. Factor scores of PCB congeners in eggs from LCE and Long Point are shown in (b).

a)



b)

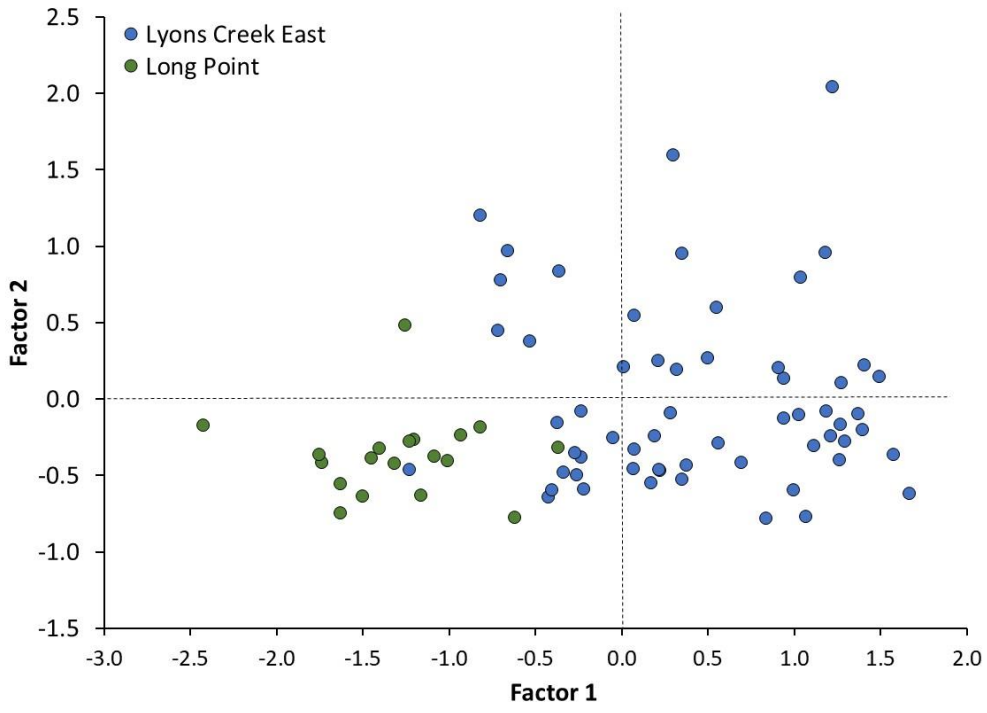


Figure A3. Estimated sum PCB concentrations (ng/g, lipid weight) in snapping turtle liver from sites in Lyons Creek East, on a zone-by-zone basis, from 2018–2022.

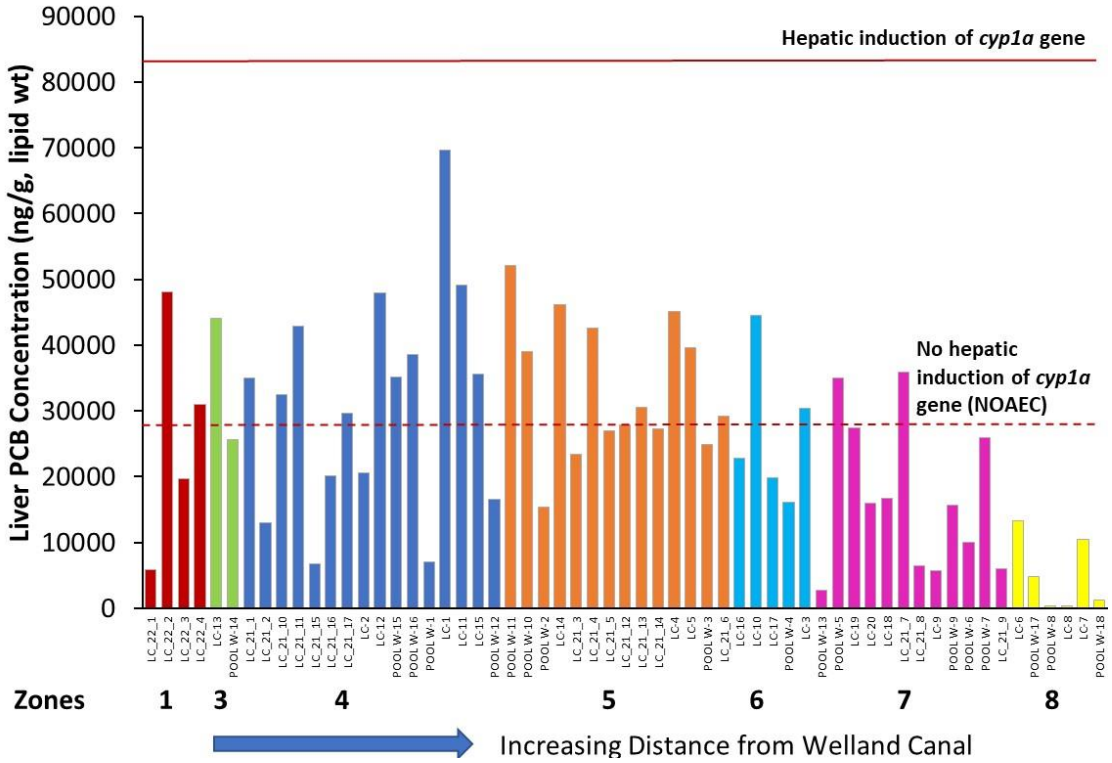


Figure A4. Mean (±SE) concentrations of sum PCBs (ng/g, wet weight) in snapping turtle eggs collected in 2002, 2010, 2018, 2019, 2021, and 2022 on a zone-by-zone basis in Lyons Creek East. Number in bars indicate the number of clutches analyzed for PCBs in each year.

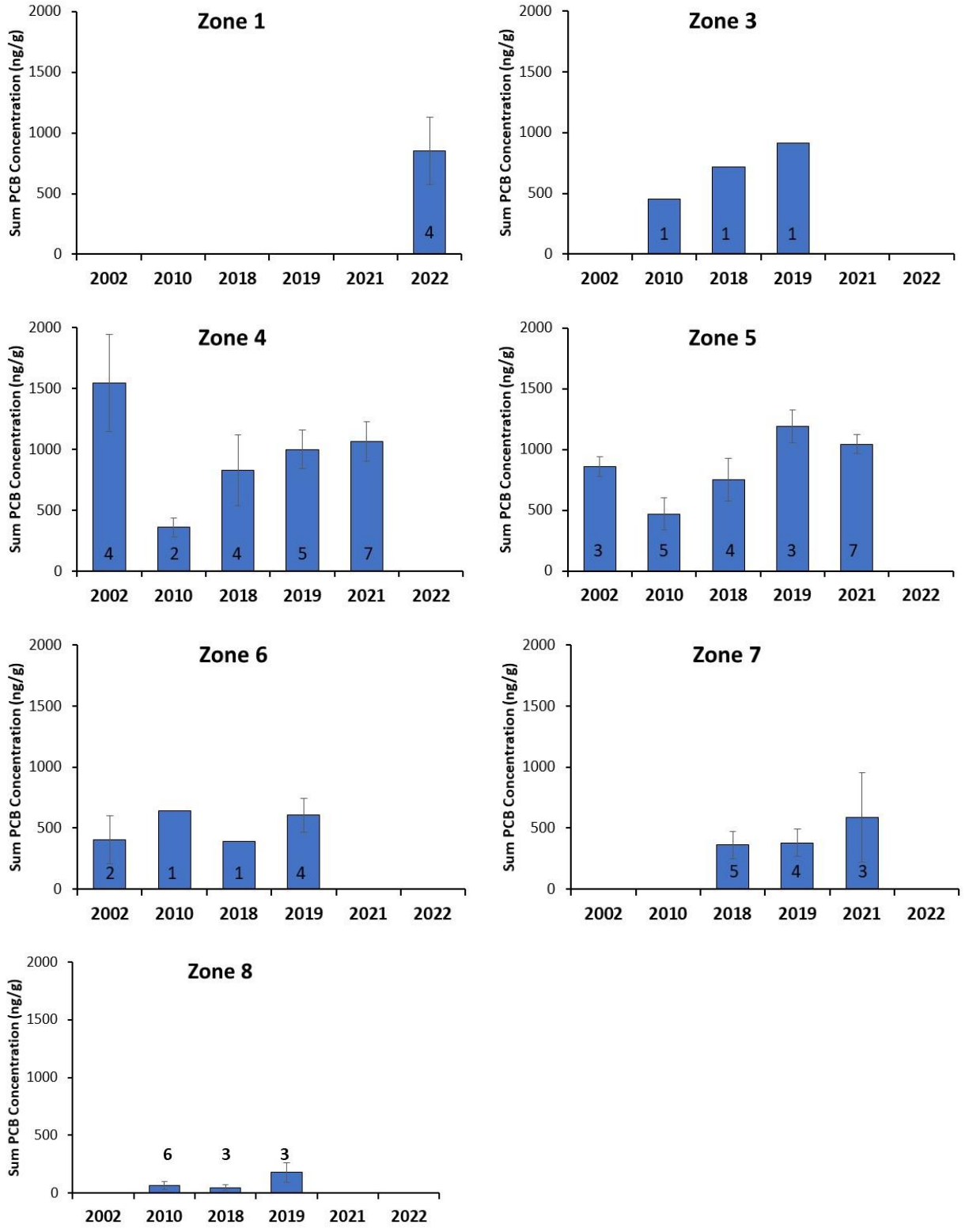


Figure A5. Mean (±SE) concentrations of sum PCBs (ng/g, lipid weight) in snapping turtle eggs collected in 2002, 2010, 2018, 2019, 2021, and 2022 on a zone-by-zone basis in Lyons Creek East.