

Contaminants in Loon Eggs in New Hampshire



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Executive Summary

The Loon Preservation Committee (LPC) tested 81 Common Loon eggs from 24 lakes in New Hampshire for per- and polyfluoroalkyl substances (PFAS), polybrominated diphenyl ethers (BDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides, including DDT and chlordane. All contaminant classes were present in all loon eggs tested. Although the effects of these contaminants on loons are unknown, up to 60% of eggs tested exceeded levels documented to cause negative health or reproductive effects in other bird species in one or more contaminant classes. Lakes with notably elevated levels of contaminants included Arlington Mill Reservoir (PFAS), Canobie Lake (PFAS), Lake Francis (PCBs), Merrymeeting Lake (PCBs), and Squam Lake (BDEs, PCBs). Individual and cumulative effects of these contaminants on some lakes could, in conjunction with other co-occurring stressors, negatively affect loon health and/or reproductive success and also potentially affect other lake wildlife and human health.

LPC recognizes that state agency funding for testing of contaminants is limited and that contaminants testing is both complex and expensive. However, we believe long-term systematic testing of high trophic-level lake wildlife is needed to address this issue and gain a better understanding of the extent, levels, and potential impact of contaminants in New Hampshire's lakes. Given the levels of contaminants seen in some loon eggs tested and the importance of loons as indicators of the health of aquatic ecosystems, LPC recommends: 1) the creation and support of a state agency program to assess contaminants in high trophic-level aquatic wildlife; 2) follow-up research to identify sources and potential mitigation measures at contaminated sites; 3) re-evaluation of the state's soil remediation guidelines to determine their adequacy to protect ecological endpoints; 4) including ecological factors as potential triggers for further investigation or mitigation at contaminated sites; and 5) establishing criteria to list lakes as impaired for wildlife under the state 303(d) Consolidated Assessment and Listing Methodology program.

Background

The Loon Preservation Committee (LPC) tested 81 Common Loon eggs from 24 lakes in New Hampshire for a range of legacy and emerging contaminants. The genesis of this testing was an investigation into unprecedented declines in loon survival and reproductive success on Squam Lake; therefore, a majority of tested eggs (53) are from Squam. Given concerning levels of some contaminants in Squam loon eggs, LPC expanded testing to eggs from elsewhere in New Hampshire to provide a comparison to Squam eggs and to measure contaminant burdens in eggs from other lakes. This report summarizes results of LPC's testing of loon egg contaminants, which are deposited into eggs primarily as a result of dietary uptake in loon territories in the weeks preceding egg laying (C. Custer et al. 2010; C. Custer, U.S. Geological Survey, pers. com.; R. Letcher, Environment and Climate Change Canada, pers. com.; LPC, unpubl. data).

Levels of contaminants found in loon eggs are therefore indicators of levels of contaminants in lake ecosystems (Strong 1990, Evers 2006) and of potential effects on loons, other lake wildlife, and human health.

Methods

Sample Collection

The Loon Preservation Committee collects inviable loon eggs from failed nests for research purposes under permits from United States Fish and Wildlife Service and New Hampshire Department of Fish and Game. Upon collection, eggs were weighed on a digital scale (precision 0.1 g), measured with calipers (length and width, precision 0.1 mm), placed in a sealed plastic bag, and stored at -18° C. The majority of collected eggs were double-bagged to prevent moisture loss during storage. Eggs collected from Squam Lake since 2014 were wrapped in aluminum foil before being double-bagged for freezing. Beginning in 2019, LPC staff wore nitrile gloves while collecting and processing eggs and stored eggs in Ziploc™ brand bags.

Due to the Common Loon's status as a state-threatened species in New Hampshire and LPC's efforts to protect and recover the species in the state, only inviable eggs from failed nests were collected and tested. This non-random sampling of eggs introduces a bias into reported contaminant levels; however, these eggs nonetheless provide valuable geographic and temporal data on contaminant levels in loons and the potential effects of these contaminants (Gómez-Ramírez et al. 2012).

Contaminant Analysis and Reporting

Most (n = 75) eggs were packed intact in dry ice and shipped to laboratories, where they were homogenized. Six samples had been previously homogenized according to the methods in Evers et al. (2003). LPC tests eggs for organochlorine pesticides (e.g., chlordane, DDT and its breakdown products DDE and DDD), BDEs (flame retardants), PCBs (industrial insulators), and PFAS (stain repellants, also used in Teflon™ and fire-fighting foam). Eggs tested from 2013-2019 (n = 62) were tested at SGS AXYS Analytical Services Ltd. (Sidney, British Columbia, Canada) for all contaminants listed above. Prior to 2013, exploratory analyses and collaborations resulted in eggs being tested at various laboratories, including Wadsworth Center, New York State Department of Environmental Health (Albany, NY; n = 10), TDI-Brooks International, Inc. (College Station, TX; n = 5), and Geochemical and Environmental Research Group (GERG) of Texas A&M University (College Station, TX; n = 4). Ten eggs that had been tested for contaminants prior to 2013 at laboratories listed above were tested at SGS AXYS for PCBs and 4 were tested for PFAS. For details on analytical methods, see Appendix A.

Because eggs were tested at different laboratories prior to 2013, not all contaminants or congeners (i.e., types of chemicals within a given class of contaminants) were tested consistently. We have limited our reporting to the contaminants and congeners that were tested uniformly across all eggs (Table 1). For total BDEs and total PFAS contaminants, we report the sum of the congeners listed in Table 1. For PCBs, we present results for 66 eggs as the sum of

the 120 congeners in Table 1 (“total PCBs”). For 9 eggs tested at Wadsworth Center or GERG, we report total PCBs as recorded by the laboratory, as individual congeners were not tested.

Table 1: Contaminants and congeners tested in Common Loon eggs by Loon Preservation Committee.

Contaminant class	Contaminants/congeners tested
BDEs	BDE 47, 85, 99, 100, 153, 154
Organochlorine pesticides	Hexachlorobenzene, aldrin, heptachlor, heptachlor epoxide, endrin, dieldrin, α - and γ -chlordane, oxychlordane, <i>trans</i> - and <i>cis</i> -nonachlor, 2,4'- and 4,4'-DDT, 2,4'- and 4,4'-DDE, 2,4'- and 4,4'-DDD
PCBs	4-10, 15-19, 22, 24-28, 31, 32, 37, 40, 42-49, 51-53, 56, 57, 59-61, 63, 66, 67, 69, 70, 73-76, 80, 82, 84-86, 89-93, 95, 97-99, 101, 102, 110, 113, 120, 124, 128-130, 132, 133, 135-139, 141, 144, 146, 149, 151, 153, 161, 163, 164, 166, 168, 170-173, 176-180, 182, 183, 185, 187, 191, 193-203, 205-209
PFAS	PFOS, PFOSA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFBS

Seventeen of the Squam eggs were collected in different years from 7 banded, individually identifiable females. Numbers of eggs for each female ranged from 2-4. We treated these eggs as independent samples, based on evidence that nutrients and contaminants deposited in eggs are primarily from dietary uptake in the weeks preceding egg laying (C. Custer et al. 2010; C. Custer, pers. com.; R. Letcher, pers. com.; LPC, unpubl. data). For cases in which two eggs from the same nest were tested for certain contaminants (n = 6 eggs from 3 nests), we report the geometric mean of contaminants tested for both eggs.

Data Analysis: Adjustment for Moisture Loss

Data are reported on a wet weight (ww) basis to avoid influences relating to embryo development, putrefaction, or loss of egg content (Peakall and Gilman 1979, Helander et al. 2002, Hernández et al. 2018). To adjust contaminant levels for moisture loss, we calculated the volume of each egg and then calculated an adjustment factor to apply to contaminant results received from laboratories. We calculated volume for eggs for which morphometric data were available (n = 61) by using the following equation and correction value for Common Loon eggs from Anderson et al. (1970) and Pollentier et al. (2007):

$$V = k\pi L \left(\frac{W}{2}\right)^2 / 1000$$

where V is the calculated volume of the egg, k is a correction value specific to Common Loon eggs equaling 0.63, L is the length of the egg, and W is the width of the egg at the widest point. We then calculated an adjustment factor to correct contaminant values for moisture loss using the equation

$$A = \frac{m_t}{1.042V}$$

where A is the adjustment factor, m_t is the total mass of the egg, and 1.042 is the calculated density of Common Loon eggs (Evers et al. 2003). We multiplied contaminant values by A to correct for moisture loss (Stickel et al. 1973, Evers et al. 2003).

We calculated A for one additional egg based on volume measured from water displacement but lacking other morphometric data. Of these 62 values of A , 3 were outliers. Two eggs had low values of A (0.73 and 0.79), but we used these values to correct contaminant results as one egg was overincubated by ~79 days and the other egg was collected late in the season (September 7). In both cases, the eggs weighed less than expected and likely experienced substantial moisture loss, as reflected by A . The third outlier had a high value of A (1.26), which could not be readily explained by available data. We did not apply any adjustments to eggs where $A > 1$ ($n = 33$), since there was no evidence of moisture loss in those eggs. Adjusting for moisture loss, according to the above formula, would have increased apparent contaminant levels of these eggs, so this decision was conservative with respect to contaminant levels. We applied the calculated adjustment factor to 29 eggs where $A < 1$.

For 18 eggs lacking data to calculate A , the mean A from 54 eggs collected prior to 2019 (excluding the 3 outliers) was applied, as collection data did not indicate that these eggs were on the nest for an unusual amount of time prior to collection. One additional egg was not collected until ~5 weeks post-nest failure. In this case, we averaged the value of A for 3 other eggs with approximately similar collection times and applied it to the contaminant results for this egg.

Results and Discussion

Loon Preservation Committee tested 81 loon eggs from throughout New Hampshire. Fifty-three of those were from Squam Lake, covering the years 1993-2019, and 28 eggs were from other New Hampshire lakes spanning the years 1997-2019 (Appendix B). In addition to reporting on contaminant levels, we also report on contaminant profiles (i.e., the proportion of different congeners within a given contaminant class). Different congeners could reflect different potential sources of contaminants (Chen et al. 2010, Su et al. 2017), different bioaccumulation or biotransformation patterns (Chen et al. 2010, Su et al. 2017), and, although in many cases the literature is limited on this, different toxicities (Chen et al. 2010, Tartu et al. 2014).

Temporal trends are only presented for eggs from Squam Lake, given the limited data from lakes other than Squam. Results from Squam Lake eggs are presented in 3 time periods: 1993-2004, when numbers of paired adults and productivity were fairly stable; 2005-2007, the critical years of initial decline in Squam's loon population, including the unprecedented single-year loss of 44% of paired adults on the lake in 2005 and subsequent declining productivity; and 2008-2019, when the number of paired adults stabilized but productivity remained low (Table 2).

Table 2: Contaminant levels in Common Loon eggs from New Hampshire lakes and from Squam Lake, NH (geometric mean \pm standard error [range]). All results are reported in nanograms/gram wet weight. ND = Non-detect. If >50% of data is less than the reporting limit, only the maximum result (“Max”) is reported (Schmutz et al. 2009).

	Statewide lakes	NH lakes excluding Squam	Squam Lake	Squam 1993-2004	Squam 2005-2007	Squam 2008-2019
Organochlorine pesticides	n = 80	n = 27	n = 53	n = 15	n = 7 [†]	n = 31
Total chlordane	42.6 \pm 3.0 (10.4, 131.1)	41.5 \pm 5.4 (14.1, 118.1)	43.2 \pm 3.7 (10.4, 131.1 [‡])	54.9 \pm 8.2 (17.9, 131.1 [‡])	65.2 \pm 13.9 (43.0, 111.1)	36.1 \pm 2.8 (10.4, 89.1)
Sum DDT isomers	2.1 \pm 0.6 (ND, 31.7)	1.4 \pm 0.5 (0.2, 12.1)	2.6 \pm 0.8 (ND, 31.7)	1.3 \pm 1.4 (ND, 21.8)	11.2 \pm 5.1 (2.7, 31.7)	2.9 \pm 0.4 (0.5, 8.8)
Sum DDE isomers	392.0 \pm 21.9 (131.0, 961.6)	354.4 \pm 39.1 (147.5, 961.7)	413.5 \pm 25.9 (131.0, 874.9)	479.1 \pm 52.9 (163.8, 808.1)	446.8 \pm 108.9 (232.4, 874.9)	380.3 \pm 27.6 (131.0, 798.2)
Sum DDD isomers	8.9 \pm 1.2 (0.5, 52.6)	5.3 \pm 1.0 (0.5, 21.5)	11.7 \pm 1.6 (0.6, 52.6)	15.5 \pm 4.1 (2.6, 52.6)	19.1 \pm 1.9 (12.5, 23.9)	9.4 \pm 1.3 (0.6, 31.1)
Hexachlorobenzene	7.9 \pm 0.6 (2.8, 28.1)	10.1 \pm 1.4 (3.1, 28.1)	6.9 \pm 0.5 (2.8, 23.2)	5.7 \pm 0.7 (2.8, 13.6)	7.6 \pm 3.5 (3.3, 23.2)	7.5 \pm 0.4 (3.5, 15.8)
Heptachlor	Max = 7.1	ND	Max = 7.1	ND	0.5 \pm 1.4 (ND, 7.1)	ND
Heptachlor epoxide	2.8 \pm 0.2 (0.5, 10.7)	2.7 \pm 0.3 (0.9, 7.6)	2.9 \pm 0.3 (0.5, 10.7)	3.1 \pm 0.8 (0.5, 10.7)	3.8 \pm 1.4 (1.1, 9.0)	2.6 \pm 0.2 (0.9, 5.5)
Aldrin	ND	ND	ND	ND	ND	ND
Dieldrin	11.6 \pm 1.3 (2.1, 67.3)	9.1 \pm 1.5 (2.1, 30.3)	13.2 \pm 1.8 (2.7, 67.3)	15.3 \pm 4.1 (ND, 57.3)	18.1 \pm 10.8 (3.6, 67.3)	11.7 \pm 1.2 (2.9, 35.5)
Endrin	Max = 10.1	ND	Max = 10.1	ND	1.7 \pm 1.9 (ND, 10.1)	ND
PCBs	n = 75*	n = 25*	n = 50*	n = 15	n = 7 [†]	n = 28*
Total PCBs	1651.2 \pm 235.8 (353.7, 10,732.5)	1617.8 \pm 398.7 (431.4, 9525.0)	1668.8 \pm 290.4 (353.7, 10,732.5)	1707.0 \pm 282.1 (730.4, 5143.6)	3009.9 \pm 524.9 (2185.7, 5057.7)	1484.0 \pm 463.1 (353.7, 10,732.5)
BDEs	n = 80	n = 27	n = 53	n = 15	n = 7 [†]	n = 31
Sum 47, 85, 99, 100, 153, 154	79.3 \pm 8.1 (14.2, 336.5)	51.6 \pm 10.2 (14.2, 222.6)	99.6 \pm 10.4 (19.0, 336.52)	104.8 \pm 17.0 (30.6, 246.3)	221.0 \pm 40.8 (113.2, 336.5)	85.4 \pm 10.2 (19.0, 249.5)
PFAS	n = 76	n = 28	n = 48	n = 15	n = 5	n = 28
Total PFAS	372.3 \pm 35.0 (58.4, 1587.4)	329.4 \pm 89.3 (58.4, 1587.4)	398.9 \pm 22.7 (172.5, 960.9)	445.5 \pm 38.8 (303.4, 805.8)	563.0 \pm 112.5 (294.9, 960.9)	353.6 \pm 18.4 (172.5, 570.8)
PFOS	184.0 \pm 27.5 (36.3, 1310.0)	177.0 \pm 70.8 (36.3, 1310.0)	188.1 \pm 15.5 (66.5, 537.0)	270.3 \pm 26.9 (176.0, 537.0)	277.2 \pm 60.6 (136.6, 497.0)	144.5 \pm 9.6 (66.5, 279.0)
PFUnA	90.9 \pm 6.8 (7.6, 293.0)	64.6 \pm 12.7 (7.6, 267.0)	110.1 \pm 7.5 (59.7, 293.0)	97.9 \pm 9.3 (59.7, 173.0)	164.9 \pm 36.4 (79.8, 293.0)	110.1 \pm 8.6 (59.7, 293.0)

*PCB data for 2019 were not received in time for inclusion in this report.

[†]Seven individual eggs were tested, but this number includes 2 clutches in which both eggs were tested. These results were averaged for each clutch prior to inclusion in the dataset, see Methods.

[‡]This total includes a result for oxychlordane that was flagged by the laboratory as an estimated maximum value, see Results.

Squam Lake and Contaminants, 2005-2007

Squam Lake experienced an increase in mean levels of BDEs and PCBs during the 2005-2007 period compared with preceding and succeeding years, and a decrease in total chlordane subsequent to 2007 (Table 3). The change in some contaminant levels correlated with the period of declines in survival and reproductive success of the Squam loon population. These declines likely resulted from multiple co-occurring stressors facing Squam's loon population during these years, and contaminants may have been a contributing factor (LPC 2020, Siegel 2020).

The increases in contaminant levels that occurred during the 2005-2007 period may have resulted from an earlier increase in streamflow (runoff) that could have transported sediments and associated contaminants into Squam. LPC found a correlation between mean runoff and PCB, DDT, and chlordane levels in loon eggs two years subsequently (LPC 2017). The two-year lag likely reflects time for contaminants to magnify through trophic levels of the food web and reach loons. Predictions of more frequent and intense precipitation events associated with climate change in New England may lead to increased future runoff (Campbell et al. 2011), potentially flushing more contaminants into lake ecosystems.

Table 3: Results of Welch's *t*-test comparing contaminant levels between time periods on Squam Lake. Data were log transformed to meet assumptions of normality. Values indicate *P* values, and statistically significant ($P < 0.05$) results are in bold.

Contaminant	Contaminant levels between 1993-2004 vs. 2005-2007	Contaminant levels between 2005-2007 vs. 2008-2019
Total PFAS	0.32	0.08
BDEs	0.01	0.004
PCBs	0.02	0.005*
Sum DDE isomers	0.79	0.52
Total chlordane	0.50	0.03

*PCB data include results through 2018.

PFAS

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals that have been used since the 1950s as stain repellants and surfactants and have been found to persist in the environment and bioaccumulate in wildlife (Newsted et al. 2005, Verreault et al. 2005, T. Custer et al. 2010). Two sub-groups of PFAS are the PFCAs (perfluoroalkyl carboxylates) and PFSAs (perfluoroalkyl sulfonates). Types of PFCAs include PFOA, PFNA, PFDA, PFUnA, PFDoA, PFHpA, and PFTrDA. PFOS is a PFSA, and PFOSA is a perfluorosulfonamide and a precursor of PFOS. PFOS is generally the dominant type of PFAS found in wildlife tissues (Newsted et al. 2005).

PFAS Levels

One hundred percent of loon eggs tested across New Hampshire contained PFOS, PFUnA, PFDA, and PFDoA. PFNA, PFOA and PFOSA were detected in 96%, 58%, and 54% of eggs respectively, while PFHpA and PFBS were detected in <4% of eggs. The statewide geometric mean for total PFAS was 372.3 ng/g ww (range: 58.4-1587.4 ng/g ww) and for PFOS was 184.0 ng/g ww (range: 36.3-1310.0 ng/g ww). The geometric mean concentration of total PFAS in 11 eggs tested from Maine lakes was 281.5 ng/g ww (range: 94.6-568.8 ng/g ww; BRI, unpubl. data)—24% lower than the New Hampshire statewide geometric mean.

Custer et al. (2013) suggested that Great Blue Herons whose eggs contained PFOS >1,000 ng/g ww, a level associated with neurological and immune system effects in chickens (Peden-Adams et al. 2009), may have been exposed to “PFC hotspots” through their feeding territories (Custer et al. 2013, pg. 1081). In our study, PFOS exceeded 1,000 ng/g ww in loon eggs from Canobie Lake and Arlington Mill Reservoir, and total PFAS contamination exceeded 1,000 ng/g ww on Lake Winnepesaukee (2007, 2014, and 2018; Table 4). Given that loons feed in their territories throughout the period prior to egg-laying (Gingras and Paszkowski 1999), it is possible that these loons may have been exposed to similar “PFC hotspots” in their territories.

Table 4: Total PFAS and PFOS concentrations in eggs (ng/g ww) for which total PFAS exceeded 1,000 ng/g ww. Two eggs from the same clutch were tested from Canobie Lake in 2016, and results from each egg are presented below.

Lake	Year	PFOS	Total PFAS	% PFOS
Canobie Lake (a)	2016	1400.0	1624.4	86.2
Arlington Mill Reservoir	2019	1310.0	1587.4	82.5
Canobie Lake (b)	2016	1170.0	1370.7	85.4
Lake Winnepesaukee (Black Cove)	2018	905.0	1275.7	70.9
Lake Winnepesaukee (Breezy Island)	2014	834.0	1363.5	61.2
Lake Winnepesaukee (Spectacle Island)	2007	545.0	1004.2	54.3

The greater number and timespan of eggs collected from Squam Lake allows for a temporal examination of PFAS levels. Levels of total PFAS increased on average 26.4% from the 1993-2004 period to 2005-2007 before declining in the 2008-2019 period to levels similar to the geometric mean of eggs elsewhere in the state (Fig. 1). PFOS (the dominant PFAS congener in most loon eggs) increased by 2.5% on average from the 1993-2004 period to 2005-2007, but mean PFOS levels overall declined 46.6% from the 1993-2004 period to 2008-2019. In contrast, mean levels of PFUnA increased much more substantially (68.4%) than PFOS from the 1993-2004 period to 2005-2007. Although the mean of PFUnA subsequently declined from its 2005-2007 levels, mean levels in 2008-2019 were 11.4% higher than the period prior to 2005. This is in keeping with a trend towards declining levels of PFOS in bird eggs following its phase-out in 2002 and increasing PFCAs (Custer et al. 2013, Eriksson et al. 2016).

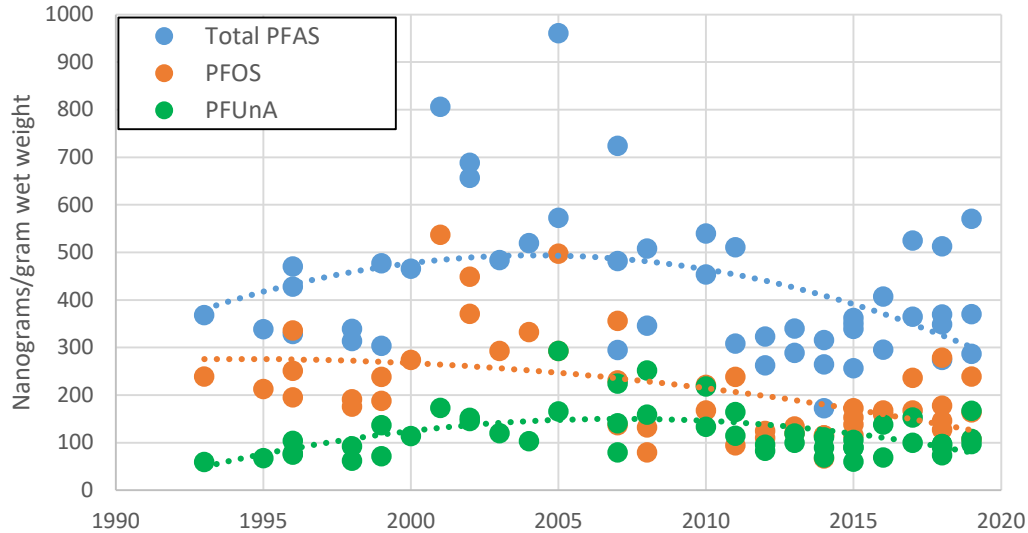


Fig. 1: Temporal trends of PFAS contaminants on Squam Lake 1993-2019. While PFOS has declined over time, mean levels of PFUnA have increased from the pre-2004 period to the post-2007 period.

PFAS Contaminant Profiles

The greater sample size and timespan of eggs collected from Squam Lake also allows for investigation of temporal trends in the PFAS contaminant profile. In keeping with the decline of PFOS in Squam loon eggs, the contaminant profile of total PFAS on the lake shifted. Proportions of PFOS declined from an average of 61% in 1993-2004 to 42% in 2008-2019, while PFUnA increased from an average of 22% to 32% during the same time periods (proportions of other PFCAs also increased; Fig. 2).

Excluding Canobie, Arlington Mill, and Winnepesaukee (Black Cove) (discussed below), the average proportions of PFAS congeners in eggs from lakes other than Squam are more similar to the 1993-2004 period on Squam, with a higher average proportion of PFOS (52%) and a lower average proportion of PFUnA (23%) compared with more recent years on Squam (Fig. 2). Compared with eggs from all lakes in New Hampshire, the 11 Maine eggs had a lower average proportion of PFOS (33% in ME vs. 51% in NH) and a higher average proportion of PFUnA (32% in ME vs. 26% in NH).

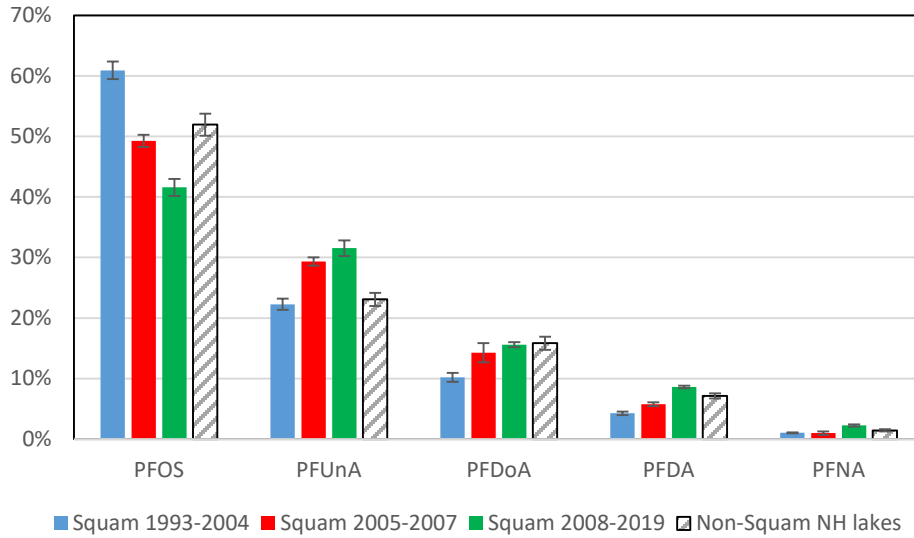


Fig. 2: Average proportions of PFOS and PFCAs (PFUnA, PFDoA, PFDA, and PFNA) in loon eggs on Squam Lake over time and other lakes in New Hampshire. On Squam, PFOS has shown an overall declining trend, while proportions of PFCAs have increased. Outliers were not included in the averages for non-Squam lakes and are discussed separately in the text. Error bars are standard error.

The contaminant profiles for eggs from Canobie Lake, Arlington Mill, and Winnipisaukee (Black Cove) were skewed in comparison with typical PFAS profiles in New Hampshire, being composed mostly of PFOS (>70%; Table 4). Additionally, the contaminant profile within the PFCAs is different from the average of eggs elsewhere in the state, with lesser proportions of PFUnA and PFDoA and a greater proportion of PFDA and PFNA (Fig. 3). This different profile may assist in identifying a source or sources of PFAS contaminants in these eggs.

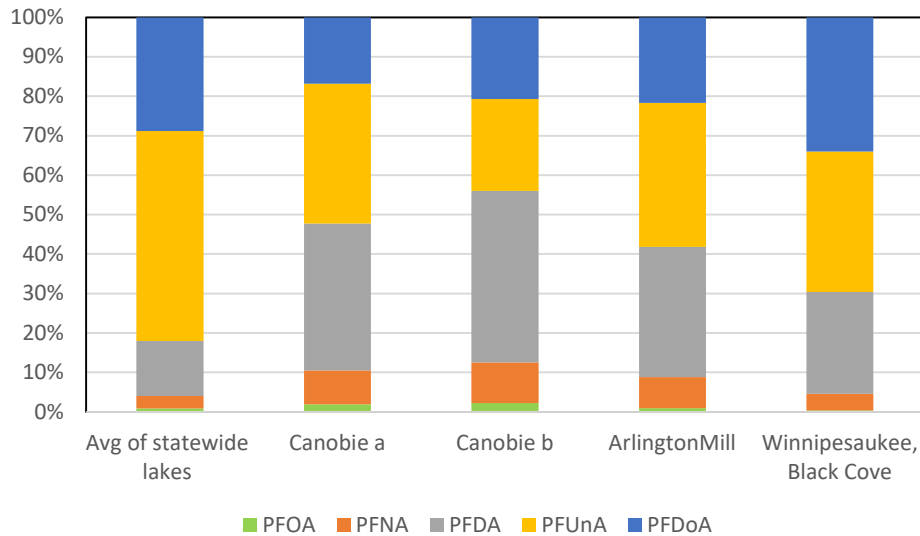


Fig. 3: Profile of PFCA contaminants in eggs with high levels of PFAS/PFOS contamination compared with the average profile of eggs from other lakes in the state. The profiles of eggs from Canobie Lake and Arlington Mill Reservoir show increased proportions of PFDA and PFNA and reduced proportions of PFUnA and PFDoA compared with other eggs. While the Winnepesaukee egg generally follows this same pattern, it has a slightly higher proportion of PFDoA and similar proportions of PFNA compared with statewide eggs.

Five eggs sent to SGS AXYS in 2019 were tested for 20 PFAS congeners in addition to the 13 PFAS congeners previously tested. Three of these eggs were from Squam Lake, 1 was from Arlington Mill Reservoir (Salem), and 1 was from Big Diamond Pond (Stewartstown). PFTrDA, a type of PFCA not previously tested in LPC’s eggs, was the dominant PFAS substance in the contaminant profile for two of the Squam eggs (Table 5). This is likely in keeping with trends towards declining PFOS and increasing PFCAs on Squam and in other studies (Custer et al. 2013, Eriksson et al. 2016). Nonetheless, it was surprising given that PFOS is commonly reported as the dominant PFAS congener in wildlife tissues. While PFTrDA has not, to our knowledge, been reported as the dominant PFCA in other freshwater piscivorous birds (Eriksson et al. 2016, Su et al. 2017) or in Common Loon liver samples (Martin et al. 2004), it has been reported as the dominant PFCA in some seabirds (Braune et al. 2013). The Big Diamond Pond egg reflects trends at lakes other than Squam in New Hampshire with continuing higher proportions of PFOS, although the proportion of PFTrDA is similar to the Squam eggs. The unusual profile of the Arlington Mill egg was discussed earlier. As LPC continues to test eggs for this wider range of PFAS contaminants, the presence and levels of PFTrDA, which has been correlated with decreased corticosterones in Black-legged Kittiwakes (Tartu et al. 2014) and to cause endocrine disruption in fish (Jo et al., 2014), merits further investigation.

Table 5: Percent of total PFAS of selected congeners in eggs tested from the 2019 breeding season for an expanded range of PFAS congeners, including PFTrDA. Values are percentages.

	Squam Kimball Isl.	Squam Mink Isl.	Squam Yard Isls.	Big Diamond Pond	Arlington Mill Res.
PFOS	24.3	27.7	30.6	39.2	75.9
PFTrDA	29.3	28.8	24.4	26.6	4.8
PFUnA	21.9	19.4	20.2	8.3	5.9

BDEs

Polybrominated diphenyl ethers (BDEs) are flame retardants used in electronics, fabrics, thermoplastics, and polyurethane foams (Chen et al. 2010). BDEs are persistent in the environment and bioaccumulate in food webs (de Wit 2002, Henny et al. 2009). Although certain classes of BDEs were phased out in North America in 2004 (Chen et al. 2010), others continue to be manufactured and used.

The congeners reported here (BDE-47, 85, 99, 100, 153, 154) comprised on average $92.6\% \pm 0.7\%$ (standard error) of the total BDE in eggs tested for a consistent set of 46 BDE congeners. BDE-47, 99, 100, 153, and 154 were detected in 100% of loon eggs tested, and BDE-85 was detected in 23% of eggs. The mean BDE levels in the state were 79.3 ng/g ww (range: 14.2-336.5). The highest levels were recorded from Squam Lake (336.5 ng/g ww, Kimball Island) and Lake Winnepesaukee (222.6 ng/ ww, Black Cove). Overall levels of BDE in eggs from Squam Lake (mean: 99.6 ng/g ww) were higher than in eggs from elsewhere in the state (mean of non-Squam eggs: 51.6 ng/g ww), and levels of BDEs peaked on Squam Lake between 2005-2007 (Fig. 4; Table 3).

Studies have shown that piscivorous birds show a different BDE congener pattern than birds from terrestrial ecosystems due to differing bioaccumulation and biotransformation patterns (Chen et al. 2010). As would be expected in piscivorous birds, the average congener profile in New Hampshire's loon eggs was dominated by BDE-47 (36%), followed by BDE-100 (24%), BDE-99 (19%), BDE-154 (11%), BDE-153 (8%), and BDE-85 (0.3%). Despite the increase in contaminant levels in the 2005-2007 period on Squam, there was no substantial difference in the contaminant profile during these years.

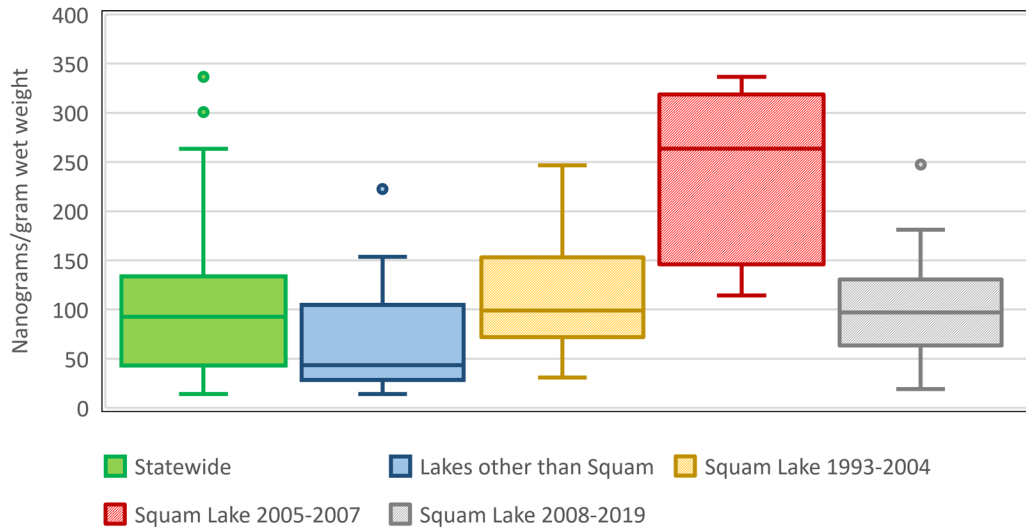


Figure 4: BDE levels (sum of BDE-47, 85, 99, 100, 153, 154) in loon eggs from across New Hampshire, lakes excluding Squam, and Squam Lake.

Total PCBs

Polychlorinated biphenyls (PCBs), consisting of 209 possible congeners, are ubiquitous environmental contaminants that were used as insulators and coolants in transformers prior to their ban on manufacture in the United States in 1979 (Hoffman et al. 1996). PCBs were detected in all loon eggs LPC tested. The most commonly-detected PCB congeners (61.5% of all of the PCB congeners reported here) were present in over 85% of eggs (see Table 6 for percent detection of congeners tested in loon eggs). Of PCB congeners reported here, only PCB-57 was not detected in loon eggs in New Hampshire. The statewide mean of total PCBs was 1,651 ng/g ww (range: 354-10,733 ng/g ww; Fig. 5).

Table 6: Detection of PCB congeners in New Hampshire Common Loon eggs. Slash marks indicate co-eluting congeners (i.e., congeners that are not clearly separated during testing).

Detection	PCB Congeners
>85% of eggs	PCB-28, 31, 43/49, 47/48/75, 52/73, 56/60, 61/74, 63, 66/80, 70/76, 85/120, 89/90/101, 91, 92, 93/95, 99, 110, 128, 130, 133, 135/144, 137, 138/163/164, 139/149, 141, 146, 151, 153, 166, 170/190, 171, 172/192, 176, 177, 178, 179, 180, 182/187, 183, 185, 191, 193, 194, 195, 196/203, 197, 198, 199, 200, 201, 202, 205, 206, 207, 208, 209
50-85% of eggs	PCB-26, 42/59, 44, 82, 84, 86/97, 113, 124, 129, 132/168, 136
25-49% of eggs	PCB-4/10, 16/32, 17, 18, 22, 37, 40, 45, 98/102, 173
<25% of eggs	PCB-5/8, 6, 7/9, 15, 19, 24/27, 25, 46, 51, 53, 57, 67, 69, 161

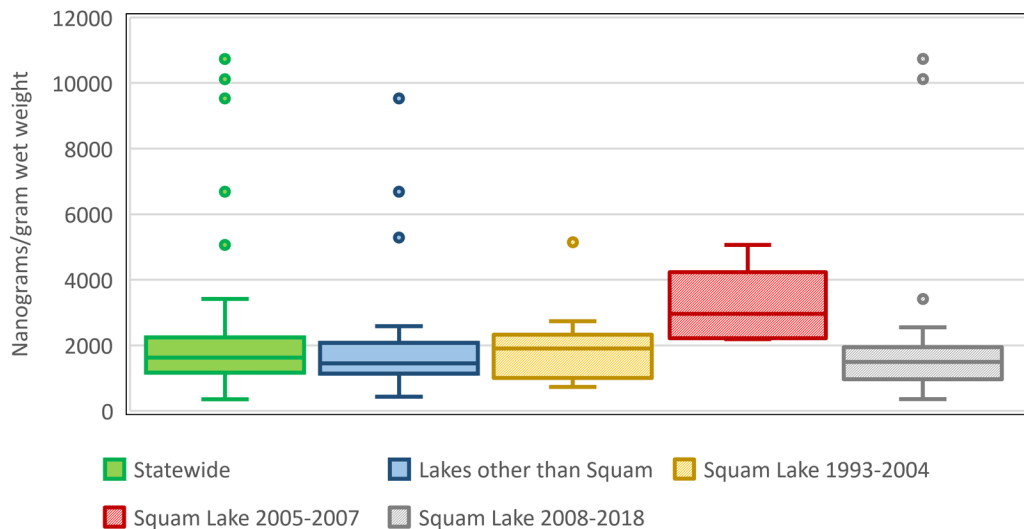


Figure 5: Total PCBs in loon eggs from across New Hampshire, lakes excluding Squam Lake, and Squam Lake.

Eggs from Squam Lake, Lake Francis, and Merrymeeting Lake had the highest levels of PCBs in our dataset (Figure 6). On Squam, the most notable levels of total PCBs occurred in 2013 (10,733 ng/g ww) and 2016 (10,119 ng/g ww). Including all 209 PCB congeners rather than the more restricted subset tested for all eggs (see Methods), total PCB levels in these eggs were >12,300 ng/g ww. These two eggs were from the same banded female in Moultonborough Bay. Two eggs tested from Lake Francis in 2014 and 2018 also had elevated levels of total PCBs (6,680 and 9,525 ng/g ww respectively). These eggs were from two separate territories on the lake and are presumed to be two separate females, although the loons were not banded to allow individual identification. An egg from Merrymeeting Lake also showed elevated levels of PCBs (5,285 ng/g ww).

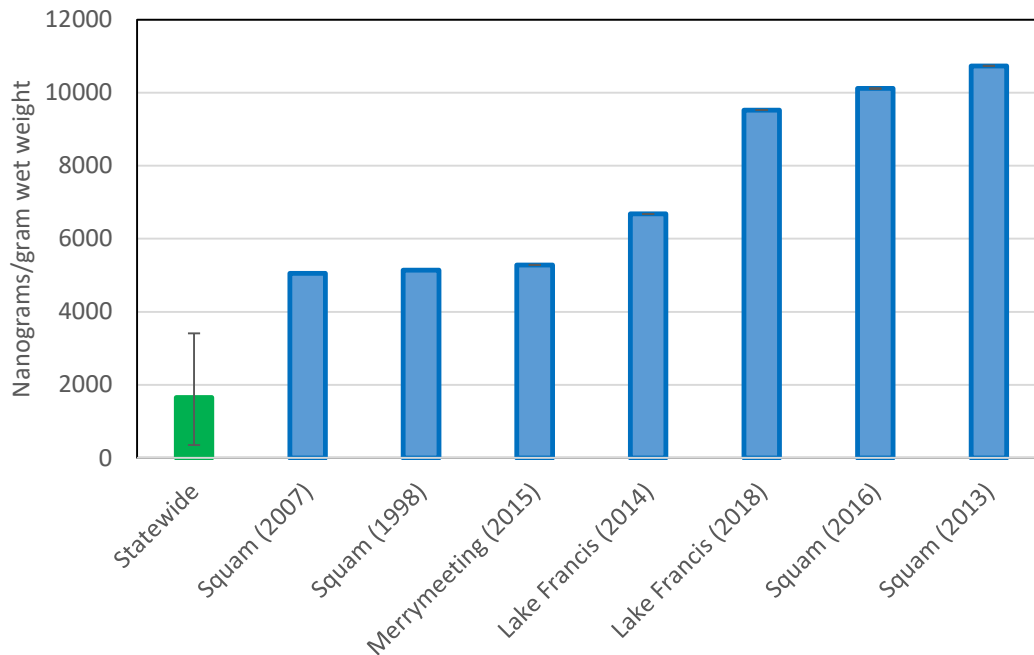


Figure 6: Statewide mean levels of total PCBs compared with lakes having the highest levels of total PCBs in loon eggs. The statewide bar includes an indicator of the range of PCB levels in New Hampshire loon eggs, excluding the eggs represented by the other bars.

On Squam Lake, the geometric mean of total PCBs was elevated in 2005-2007 compared with the other time periods (Fig. 5; Table 3). This may be consistent with an apparent influx of several contaminants into Squam during these years. In addition to the 2013/2016 eggs from the Moultonborough Bay female discussed above, Moultonborough Bay also had the highest level of total PCBs in eggs during the 2005-2007 period (5,058 ng/g ww), although eggs with considerably lower levels were recorded from Moultonborough Bay in 1995, 2000, and 2008 ($\leq 2,731$ ng/g ww). Sediments in the tributary flowing into Moultonborough Bay indicated background levels of PCB contamination (Vogel 2017), indicating the need for further testing to determine a source or sources of PCB levels detected in loon eggs in 2007, 2013, and 2016. An egg from Moon Island on Squam in 1998 likewise had elevated levels of PCBs compared with both the statewide and Squam means.

The levels of total PCBs reported here are considerably lower than most of those reported in loon eggs collected from the Lakes Region of New Hampshire in the mid-1970s (statewide mean 93% lower than mean reported in Sutcliffe 1978). However, the levels documented in the Moultonborough Bay female in 2013/2016 are similar to (and exceed, if all 209 congeners are counted) two of the eggs collected from Squam Lake in 1976 and are more than three times levels in a Squam egg from 1975 (Sutcliffe 1978). With the exception of the 2013/2016 Moultonborough Bay eggs, these present-day lower levels are both expected and positive; but levels are nonetheless concerning, and PCB levels in fish resulted in the issuance of more

restrictive fish consumption guidelines for Squam Lake in 2020 (NHDES 2020c). The statewide geometric mean of New Hampshire eggs was 1.9 times greater than the geometric mean of eleven loon eggs tested from Maine (848.0 ng/g ww; range: 141.7-2709.4 ng/g ww; BRI, unpubl. data).

The average PCB congener profile of New Hampshire loon eggs was dominated by PCB-153 (22%), co-eluting congeners PCB-138/163/164 (16%), PCB-180 (9%), and PCB-182/187 (7%) but differed among lakes and individual eggs, particularly eggs with elevated PCB levels (Table 7). Detection of 4 PCB congeners differed between Squam eggs and eggs from other lakes in the state. PCB-18, 26, and 40 were detected in 50-55% of eggs from Squam Lake but only in 32-36% of eggs from other lakes. Similarly, PCB-69 was detected in 26% of Squam eggs but only 9% of eggs from lakes elsewhere in New Hampshire. Whether these differences in congener profiles and detection may be useful in identifying sources of PCBs is unclear.

Table 7: Profile of selected PCB congeners in statewide eggs in New Hampshire compared with eggs from two locations (Moultonborough Bay on Squam Lake and Lake Francis) with elevated PCB levels. Values are percent of the total PCB profile. Values of all individual eggs are greater than 2 standard errors from the statewide mean.

PCB congener	Statewide (avg)	Moultonborough Bay, Squam Lake (2013)	Moultonborough Bay, Squam Lake (2016)	Lake Francis (2014)	Lake Francis (2018)
138/163/164*	15.5	17.5	12.9	8.3	8.4
153*	22.4	28.1	18.7	15.3	17.0
180*	9.4	5.0	2.8	19.5	19.3
182/187*	6.9	4.0	2.8	9.5	9.7
43/49	0.5	1.5	5.8	0.1	0.0
89/90/101	2.0	3.9	6.0	1.1	0.8
99	3.7	6.9	7.1	1.0	0.7

* Individual or co-eluting congeners that are found in the highest proportions in the average statewide profile.

Total PCBs is a poor measure of toxicity of these contaminants to wildlife (Schmutz et al. 2009). Using toxic equivalency factors for dioxin-like PCBs provides a better assessment of wildlife impacts, and LPC is currently analyzing results of dioxin-like PCBs on a subset of eggs. Preliminary analyses indicate elevated levels of dioxin-like PCBs in the 2015 egg from Merrymeeting Lake and the 2013 egg from Moultonborough Bay on Squam Lake (the 2016 Moultonborough Bay egg was not tested for dioxin-like PCBs). Despite its elevated levels of total PCBs, the 2018 egg from Lake Francis had low levels of dioxin-like PCBs (the 2014 Lake Francis egg was not tested for dioxin-like PCBs).

Organochlorine Pesticides

Organochlorine pesticides were widely used in agricultural applications from the end of World War II until their ban, primarily in the 1970s (Blus 1996, Wiemeyer 1996). In addition to the toxicity of the technical formulations of the pesticides, metabolites and breakdown products of

the pesticides are also persistent, bioaccumulative, and toxic. Metabolites of dichlorodiphenyltrichloroethane (DDT) include dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), and metabolites of chlordane include oxychlordane and heptachlor epoxide (Blus 1996, Wiemeyer 1996).

Organochlorine pesticides were found in 100% of tested loon eggs. Statewide, hexachlorobenzene, heptachlor epoxide, dieldrin, α -chlordane, oxychlordane, *trans*-nonachlor, *cis*-nonachlor, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, and 4,4'-DDT were detected in $\geq 85\%$ of loon eggs (Table 8). The majority of the averaged profile of organochlorine pesticides (85.0%) consisted of DDT, composed primarily of one of DDT's breakdown products, 4,4'-DDE (Table 8). Total chlordane accounted for 9.5% of the average contaminant profile. The statewide mean of the DDE isomers was 392.0 ng/g ww (range: 131.0-961.6) and of total chlordane was 42.6 ng/g ww (range: 10.4-131.1). (We note that, for the egg with the highest level of total chlordane, the result for oxychlordane was flagged by the laboratory as an estimated maximum concentration. Although we report it here, this result must be treated with caution. Oxychlordane accounted for 46.8% of the total chlordane in the egg in question, compared with an average of 25.9% for other eggs).

Table 8: Detection and average contaminant profile of organochlorine pesticides (OC) in Common Loon eggs in New Hampshire.

Analyte	% detection	Avg. % of total OC pesticide profile
Hexachlorobenzene	100	2.0
Heptachlor	6.3	0.1
Aldrin	1.3	0.0
Heptachlor epoxide	100	0.7
Dieldrin	97.5	2.8
Endrin	8.8	0.1
Chlordane		
γ -chlordane	58.8	0.0
α -chlordane	96.3	0.3
Oxychlordane	100	2.5
<i>Trans</i> -nonachlor	100	5.0
<i>Cis</i> -nonachlor	100	1.7
Sum of chlordane isomers		9.5
DDT		
2,4'-DDE	45.0	0.0
4,4'-DDE	100	81.7
2,4'-DDD	85.0	0.2
4,4'-DDD	100	2.2
2,4'-DDT	80.0	0.3
4,4'-DDT	96.3	0.6
Sum of DDT isomers		85.0

The egg with the highest level of DDE isomers in our dataset was recorded on Lake Sunapee in 2014 (961.7 ng/g ww). The DDE:PCB ratio of the Sunapee egg was 0.56 compared with a statewide average of 0.28. High DDE:PCB ratios may suggest greater influence of agricultural vs. industrial inputs of contaminants into a system (Cunha et al. 2012), potentially pointing to pathways for the higher DDE levels in the Sunapee egg. The egg with the highest DDE:PCB ratio was from Highland Lake (0.87), where DDE levels were 534.2 ng/g ww. As with PCBs, levels of DDE in our dataset were considerably lower than levels in loon eggs collected from the Lakes Region in the mid-1970s (statewide mean 93% lower than mean reported in Sutcliffe 1978).

Levels of DDE on Squam Lake declined from a geometric mean of 479 ng/g ww for the 1993-2004 period to 380 ng/g ww in 2008-2019, although elevated levels of DDE were recorded in individual eggs in 2013 (798.2 ng/g ww) and 2019 (786.3 ng/g ww). Eggs from these nests were in Moultonborough Bay and the Yard Islands on Squam, respectively. Water flows east to west on Squam, and both of these locations are east of Bennett Brook, the area of the most contaminated sediments for total DDT that have been documented to date in the Squam watershed (Vogel 2017, May 2020). It is unclear whether the levels of DDT in these eggs were associated with the other documented site for elevated DDT in sediments near Kent Island (Vogel 2017), which is upflow of these locations, or whether there are additional source(s) of DDT at the eastern end of the lake that are currently unknown.

In general, DDT isomers account for a minimal component of the overall profile of total DDT in eggs (average = 0.9%), as expected, given that use of DDT was banned in 1972 and DDE and DDD are the metabolized products of DDT (Blus 1996). However, one egg from each clutch from Mink Island and Piper Cove on Squam Lake in 2007 contained elevated proportions of DDT isomers (15.2% and 12.0% respectively), suggesting recent release of DDT into the environment, likely within the preceding decade. The second eggs from both of these clutches were also tested and contained 3.4% and 2.9% DDT isomers respectively.

These two clutches from 2007 likewise contained the only results for heptachlor and endrin in our dataset that were above minimal levels. Heptachlor levels were 3.0 and 3.7 ng/g ww for the two eggs at Mink Island and 2.7 and 11.6 ng/g ww at Piper Cove (maximum level in other eggs = 0.04 ng/g ww). Endrin measured at 4.0 and 8.8 ng/g ww at Mink Island and 5.2 and 15.0 ng/g ww at Piper Cove (maximum level in other eggs = 0.13 ng/g ww). The only other egg that tested for endrin above minimal levels was also collected in 2007 on Squam Lake at Moultonborough Bay, which contained 1.4 ng/g ww. The presence of these contaminants in 2007, along with the increased proportion of DDT isomers from these clutches, may suggest a pulse of contamination through the Squam system at this time.

Contaminant Levels in Eggs Compared with Lowest Observed Effects Levels

Effects of the contaminants we studied on Common Loons are unknown, as few studies have been conducted on organic pollutants in the species. Dosing data are largely lacking in loons due to the difficulty of keeping the species alive in captivity and resultant “practical, financial, and ethical constraints” on laboratory studies (Hendriks 2013, p. 3546; Hoondert et al. 2018, p.

3716), while field studies on organic contaminant impacts on loons have been limited (Fox et al. 1980). Our discussion below of effects levels should be interpreted with caution given different and poorly-known species sensitivities and possible physiological or behavioral effects at lower concentrations (Letcher et al. 2010, Su et al. 2017). Additionally, effects of contaminants are likely compounded, even at low levels, by changing environmental conditions or in populations that are stressed or facing multiple stressors (Letcher et al. 2010, Bustnes et al. 2015). A systems dynamics analysis indicated likely reproductive effects of observed contaminant levels on Squam Lake (Siegel 2020). However, similar to Su et al. (2017), we cannot state with certainty whether contaminant levels observed in New Hampshire's loon eggs are resulting in adverse effects, or whether the synergistic effects of these contaminants are contributing to effects beyond those of individual contaminants (Letcher et al. 2010, Huber et al. 2015).

PFAS/PFOS

While laboratory dosing studies have often set high threshold levels for reproductive impairment from PFOS (>5,000 ng/g ww; studies summarized in Custer et al. 2014, Su et al. 2017), a field study investigating Tree Swallows found reduced hatching success at 150 ng/g ww (Custer et al. 2012, Custer et al. 2014). The authors suggest reasons for this difference, including differences between the amount of injected contaminant vs. what may be biologically incorporated into the developing egg, the use of artificial incubation in laboratory studies that do not account for behavioral effects of a contaminant, single-chemical treatments vs. the mixture of PFAS in environmental exposure, or that Tree Swallows may be particularly sensitive to PFAS (Custer et al. 2014). In our study, PFOS levels in 60% of statewide eggs, 69% of Squam eggs, and 41% of eggs from lakes other than Squam exceeded 150 ng/g ww. PFOS levels at Canobie Lake and Arlington Mill Reservoir were >850% of this level.

BDEs

Total BDE levels of 86.1 ng/g ww were associated with a decrease in spleen somatic index and other measures of immune system function in nestling American Kestrels (Fernie et al. 2005), while levels of 289 ng/g ww were associated with reduced egg size and eggshell weight and delayed egg-laying (Fernie et al. 2009). Impaired pipping and hatching success in American Kestrels was associated with BDE levels of 1800 ng/g ww (McKernan et al. 2009). The Canadian Government's Federal Environmental Quality Guideline (FEQG) threshold for the subset of BDEs reported here is 29 ng/g ww for possible ecological hazard (Environment Canada 2013). Statewide, 51% of eggs tested exceeded the 86 ng/g ww level for impaired immune function, including 65% of Squam eggs and 26% of eggs from other lakes. An additional 6% of Squam eggs and 4% of eggs from other lakes approached this level (>95% of level). Ninety-four percent of Squam eggs and 74% of eggs from other lakes exceeded the Canadian FEQG threshold. Two eggs from Squam Lake exceeded the 289 ng/g threshold for total BDEs associated with reduced egg size and eggshell weight and delayed egg-laying. Five additional eggs from Squam ranged from 84%-90% of this value.

Total PCBs

Total PCB levels of 7-9 ppm in Double-crested Cormorant eggs were associated with 25% reduced hatching success, although the association was statistically stronger with dioxin-like compound equivalency levels of 150-250 ppt (Hoffmann et al. 1996). Three of the eggs in LPC's current dataset exceeded the 7-9 ppm level of total PCBs: the two eggs from the Moultonborough Bay female on Squam Lake (2013 and 2016) and the 2018 egg from Lake Francis.

Another way to investigate potential effects of contaminant levels on species is through effects concentrations, which are the levels at which 10% or 50% of test subjects exhibited measurable impacts on the health, survival, or reproductive effect being studied. In calculating 10% and 50% effects concentrations for ecological risk assessment associated with total PCBs, Hoondert et al. (2018) calculated a 50% effects concentration (EC_{50}) for Osprey of 8.5 mg/kg ww and an EC_{50} of 4.62 mg/kg for Snowy Egrets. The 2 eggs from Moultonborough Bay on Squam and the 2018 egg from Lake Francis exceeded the EC_{50} for Osprey, and 2 additional eggs from Squam, the 2014 egg from Lake Francis, and the 2015 egg from Merrymeeting Lake also exceeded the EC_{50} for Snowy Egrets. The EC_{10} for Bald Eagles of 1.52 mg/kg (Hoondert et al. 2018) was similar to the average PCB levels in loon eggs for New Hampshire lakes, and 17% of Squam eggs tested exceeded the EC_{10} for Osprey (2.72 mg/kg; Hoondert et al. 2018). Only the same three eggs from lakes other than Squam (Lake Francis 2014 and 2018 and Merrymeeting 2015) exceeded this EC_{10} .

Meaningful discussion of the toxicity of PCBs should take into account levels of dioxin-like PCBs in conjunction with levels of dioxins and furans (Schmutz et al. 2009; cf. Custer et al. 1999), and LPC's current investigations into dioxin-like PCBs in a subset of eggs will provide a better measure of the potential toxicity of PCBs in loon eggs. Further research is needed to understand whether low levels of dioxin-like PCBs found in eggs from Lake Francis may be linked to the higher levels of breeding success achieved by loons there compared with loons from Squam Lake or Merrymeeting Lake.

Organochlorine Pesticides

DDE levels of 500-1000 ng/g ww have been associated with reduced survival of young Black-crowned Night-Herons (Findholt et al. 1985, Connell et al. 2003). Thirty-three percent of statewide eggs, 35% of Squam eggs, and 30% of eggs from lakes other than Squam exceeded the lowest observed effects level of 500 ng/g ww. An additional 6% of Squam eggs and 7% of eggs from other lakes approached this level (>95% of level). None of the eggs tested exceeded 1000 ng/g ww. Lowest observed effects levels for DDE for other species are >2500 ng/g ww (summarized by Custer et al. 1999). Fox et al. (1980) found a mean DDE value of 5.80 ppm in Common Loon eggs but did not find any relationship between contaminant levels and productivity. This level is almost 15 times higher than the statewide mean of DDE in eggs in our study.

Levels of total chlordane in New Hampshire eggs were below no observed adverse effect levels of 200 ng/g ww (Eisler 1990, Gómez-Ramírez et al. 2012). The highest egg was from Squam

Lake at 131 ng/g ww, although, as indicated above, levels of oxychlordanes for this egg were estimated maximum levels.

Levels of other organochlorine pesticides in New Hampshire eggs were well below effects levels (effects levels: heptachlor/heptachlor epoxide = 1.5 ppm [Wiemeyer 1996]; hexachlorobenzene = 6.2 ppm [Wiemeyer 1996]; dieldrin = 1 ppm [Peakall 1996]; endrin = 0.27 ppm [Fleming et al. 1982, Peakall 1996]).

Preliminary analyses of possible effects of contaminants on loon productivity

Comparisons of contaminant concentrations and loon reproductive success must be interpreted with caution but suggest that further research may be warranted to investigate productivity impacts of PFOS, total PFAS, BDEs, PCBs, and chlordanes on New Hampshire's loons. A systems dynamics analysis identified contaminants as likely contributors to reduced breeding success of Squam loons (Siegel 2020); however, additional research is needed to measure the extent to which the levels of contaminants we found, alone or in concert with other co-occurring stressors, could significantly impact loons. Further testing of dioxin-like PCBs will be important to better assess toxicity and potential effects of PCBs on loon productivity. LPC will be conducting analyses to investigate relationships between contaminant levels, egg morphometrics, eggshell thickness, and productivity parameters.

Total Contaminant Egg Burden

The total contaminant burdens for the contaminants we tested in eggs throughout New Hampshire ranged from 794-12,070 ng/g ww, with a geometric mean of 2,796 ng/g. On average, PCBs comprised 64.2% of the overall contaminant profile, DDE 15.2%, total PFAS 14.5%, BDE 3.1%, total chlordanes 1.7%, and DDD and DDT isomers, heptachlor epoxide, dieldrin, and HCB <1%. On Squam, the mean egg burden peaked between 2005-2007 at 4,516 ng/g (Fig. 7). Mean contaminant profiles were slightly different on Squam between 2005-2007 than the statewide averages, with PCBs increasing to 69.0% and BDEs increasing to 5.1% of the overall profile. This change in the contaminant profile on Squam was driven by increases in the mean levels of PCBs by 80.3% and of BDEs by 121.9% between 2005-2007.

Figure 7: Total egg contaminant burdens in loon eggs from across New Hampshire, lakes excluding Squam, and Squam Lake.

Eggs with particularly high levels of a given contaminant class had skewed contaminant profiles, as would be expected. PCBs made up 89-91% of the contaminant profile in the eggs from Moultonborough Bay on Squam in 2013 and 2016 and 95-96% of the profile in the eggs from Lake Francis. Similarly, total PFAS made up 44% of the contaminant profile in eggs from Canobie Lake and 34% of the contaminant profile in the egg from Black Cove on Winnepesaukee. Total PFAS also accounted for 50% of the contaminant profile in an egg from Heron Cove on Squam in 2017. Although total PFAS levels in this egg were higher than average on Squam in the 2008-2019 period, total PCB levels in this egg were low (354 ng/g ww), accounting for the skewed contaminant profile. We are unable to report the overall percentage of PFAS in the Arlington Mill egg, as PCB results were not received in time for incorporation into this report. DDE made up 39% of the contaminant profile in the egg from Highland Lake in 2018, the egg with the highest DDE:PCB ratio in the dataset.

In addition to summing total contaminant levels, egg contaminant burdens may also be considered in terms of effects levels. An effects profile provides a better representation of contaminants that may be present in small amounts but have a high degree of toxicity. While it is useful to examine egg contaminant burden from this perspective, effects profiles must be interpreted with caution because 1) effects levels for the contaminants reported here for loons are unknown; and 2) effects of contaminants are likely not strictly additive: some may act synergistically or negate the effects of another contaminant (Letcher et al. 2010, Huber et al. 2015). Therefore, effects profiles should not be construed as suggesting a total contaminant effect level but may be useful for comparing relative estimated effects of one contaminant class versus another within a given egg. They may also be useful for comparing the relative toxicity

of contaminants and estimations of the relative overall egg contaminant burdens between eggs, within the limitations discussed above.

Given these caveats, a stacked graph of lowest observed effects levels illustrates the differing relative contribution from each contaminant class within eggs and between eggs (Fig. 8). The greater contribution of BDEs to the potential overall toxicity in Squam eggs vs. statewide eggs is apparent in this graph, as well as the greater influence of total PCBs in the Moultonborough Bay egg and PFOS in the Canobie Lake egg. The toxicity of PCBs is likely underestimated in this graph for all eggs, given that data on dioxin-like PCBs are still being analyzed and not included in this graph. Lowest observed effects levels are based on values discussed in detail above (PFOS = 150 ng/g ww; BDE = 86 ng/g ww; DDE = 500 ng/g ww; total chlordane = 200 ng/g ww; total PCBs = 8000 ng/g ww).

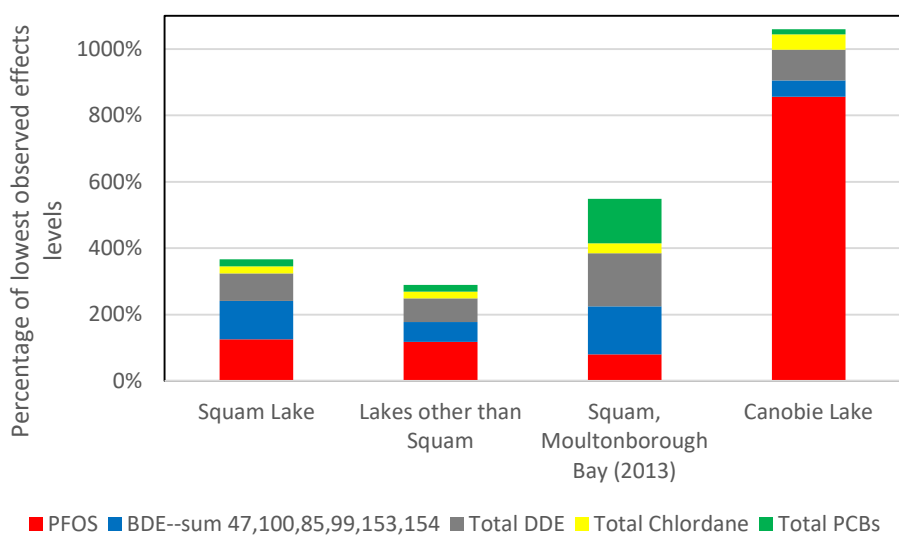


Figure 8: Stacked lowest observed effects levels as an illustration of total egg contaminant burden for Squam vs. lakes other than Squam and for two individual eggs. This graph must be interpreted according to the caveats discussed in the text. The bars for Squam Lake and lakes other than Squam are geometric means. The different estimated contributions of toxicity from each contaminant class within the eggs and between eggs are apparent from this graph.

Management Implications

The contaminant levels reported here indicate the importance of loons, as a long-lived species at the top of aquatic food webs, as indicators of contaminant levels and the health of aquatic ecosystems (Strong 1990, Evers 2006). We encourage ongoing and new collaborations between state and federal agencies, non-profit organizations, lake associations, and academia to investigate contaminants and the potential threat they may pose to the integrity of aquatic ecosystems and wildlife and human health in New Hampshire. We recognize that funding for state environmental and wildlife agencies is limited, as is funding for all state agencies, and that contaminants testing is both complex and expensive. However, we believe the results presented

in this report indicate the need for long-term systematic testing of high trophic-level lake wildlife to address this issue and gain a better understanding of the extent, levels, and potential impact of contaminants in the state's lakes. Such a program would seem to fall within the purview of NH Department of Environmental Services (NHDES). Therefore, given our findings to date, the Loon Preservation Committee recommends the following actions:

1. **Create and fund a state agency program to assess bioaccumulative contaminants in high trophic-level aquatic wildlife.** New Hampshire is not unusual in having widespread background levels of persistent organic pollutants with isolated areas of elevated contaminant levels; however, the expense of comprehensive contaminant testing has limited LPC's testing to eggs from 24 of 212 lakes with a known presence of territorial loons. This low percentage (11.3%) of potential lakes does not provide an adequate sample to determine the number and location of contaminated areas. The increased frequency and severity of rain events predicted by climate change models (Campbell et al. 2011) may increase potential runoff of sediments and associated contaminants into lakes. Systematic long-term testing of fish and/or other high trophic-level species, in addition to LPC's testing of loon eggs, would help our understanding of the distribution of these contaminants in space and time and the evaluation of potential risks to wildlife and human health.

The importance of fish testing was clearly demonstrated at Squam Lake. In response to contaminant levels reported by LPC in loon eggs, crayfish, and sediments at Squam Lake and its tributaries, NH Department of Environmental Services conducted a follow-up investigation of PCB and PFAS levels in fish from Squam Lake. This testing resulted in more stringent fish consumption guidelines to protect human health (NHDES 2020c) and the addition of the Squam Lakes to the 303(d) list of impaired water bodies for fish consumption (NHDES 2020b). Results reported here point to the need for further fish testing to evaluate human health risks on lakes such as Lake Francis and Merrymeeting (for PCBs) and Canobie Lake, Arlington Mill Reservoir, and parts of Lake Winnepesaukee (for PFAS).

2. **Respond to any findings of concerning levels of contaminants with follow-up research to identify sources and potential for mitigation of sources, if warranted and feasible.** Contaminants in loon eggs, fish, and other high trophic-level species can serve as indicators of the health of aquatic ecosystems and watersheds, as well as risks to loons, other wildlife, and people. LPC's intensive research into contaminants in the Squam watershed revealed three areas of sediments with concerning levels of contaminants (the outflow from Kusumpe Pond for PCBs [including dioxin-like PCBs], dioxins, and furans; and Bennett Brook and opposite Kent Island on Bean Road for DDT [Vogel 2017]). Crayfish sampled by LPC and Plymouth State University in the Kusumpe outflow indicated that these crayfish were substantially elevated for PCBs (35 times higher than background levels; LPC 2015; LPC unpubl. data). Follow-up investigations conducted by Plymouth State University in Bennett Brook revealed crayfish with 4,4'-DDE levels up to ten times higher than background levels in the Squam watershed (Bennett Brook

mean: 5.03 µg/kg ww, background mean: 0.83 µg/kg ww [May 2020]; background mean: 0.467 ng/g ww [LPC unpubl. data]). Additional sources of contaminants may exist in the Squam watershed that have not yet been detected.

Investigations are needed to understand the extent of contamination and the feasibility of mitigation in the Squam Lake, Lake Francis, Merrymeeting Lake, Canobie Lake, and Arlington Mill Reservoir watersheds. We also recommend sampling to identify potential sources of contaminants at lakes where loon eggs indicate concerning levels of contaminants (Appendix B). Investigations to date to identify contaminant sources in the Squam watershed have been privately funded or funded through academia, as programs and funding do not exist at the state level to address instances of non-point source contamination from legacy contaminants.

3. **Re-evaluate New Hampshire’s soil remediation guidelines to determine whether they are adequately protective of ecological as well as human endpoints.** New Hampshire lacks sediment mitigation guidelines. A brief survey of New Hampshire’s soil mitigation guidelines indicated that New Hampshire’s standards were significantly higher than those of some other states. The lack of sediment guidelines could hamper investigations to measure and mitigate potential sources of contaminants that may be impacting wildlife.
4. **Include ecological inputs as potential triggers for further investigation or mitigation at contaminated sites.** States like New York (W. Ottaway, NY State Dept. of Environmental Conservation, pers. com.) and California (B. Faulkner, CA Dept. of Toxic Substances Control, pers. com.) use ecological indicators as justification for further investigation and potential mitigation of contaminated sites. In California, the presence of contaminants in bird eggs is considered strong evidence of exposure, with exceedance of lowest observed effects levels indicative of potential impacts, which would likely lead to a recommendation for further evaluation and/or remediation (B. Faulkner, pers. com.).

In the case of Squam, the presence of contaminants in three biotic matrices (loon eggs, fish, and crayfish) indicate current exposure and uptake into the food web. Both in regards to ecological harm and the potential risk to human health, given the new and more stringent fish consumption guidelines, further investigation in the Squam watershed is warranted to identify potential additional sources of contamination into the lake and to ascertain the best options for mitigation at known sites.

5. **Establish criteria for listing lakes that are impaired for wildlife under Section 303(d) of the Clean Water Act.** The draft *2020 Section 305(b) and 303(d) Consolidated Assessment and Listing Methodology* (NHDES 2020a) states that the indicators and assessment criteria for “determining use (i.e., wildlife) support are under development” (NHDES 2020a, p. 106). We recommend the prompt establishment of these criteria so lakes can be assessed for wildlife use and listed, if necessary. We suggest that levels of

chemical contaminants that may be harmful for wildlife should be considered among the criteria, and that Arlington Mill Reservoir, Canobie Lake, Merrymeeting Lake, and Squam Lake, all of which have concerning levels of one or more contaminants and impaired loon breeding, should be considered for listing as impaired for wildlife to identify a need for further research and action.

LPC will continue, as available funds allow, to monitor contaminants in loon eggs across the state and report to NHDES and New Hampshire Department of Fish and Game (NHF&G) on eggs with levels of contaminants that indicate contaminated ecosystems and could pose risks to loons, other wildlife, and/or human health. We will also be conducting further research to investigate potential impacts of contaminants on loon productivity as we continue to work with NHF&G and NHDES for the health of New Hampshire's loons and aquatic ecosystems.

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Literature cited

- Anderson, D.W., H.G. Lumsden, and J.J. Hickey. 1970. Geographical variation in the eggshells of Common Loons. *Canadian Field-Naturalist* 84:351-356.
- Blus, L.J. 1996. DDT, DDD, and DDE in birds. In *Environmental contaminants in wildlife: Interpreting tissue concentrations*, ed. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood. Boca Raton, FL.
- Braune, B.M., and R.J. Letcher. 2013. Perfluorinated sulfonate and carboxylate compounds in eggs of seabirds breeding in the Canadian Arctic: Temporal trends (1975-2011) and interspecies comparison. *Environmental Science and Technology* 47:616-624.
- Bustnes, J.O., S. Bourgeon, E.H.K. Leat, E. Magnúsdóttir, H. Strøm, S.A. Hanssen, A. Petersen, K. Olafsdóttir, K. Borgå, G.W. Gabrielsen, and R.W. Furness. 2015. Multiple stressors in a top predator seabird: Potential ecological consequences of environmental contaminants, population health and breeding conditions. *PLoS One* 10:e0131769. doi: 10.1371/journal.pone.0131769.
- Campbell, J.L., C.T. Driscoll, A. Pourmokhtarian, and K. Hayhoe. 2011. Streamflow responses to past and projected future changes in climate at the Hubbard Brook Experimental Forest, New Hampshire, United States. *Water Resources Research* 47. doi: 10.1029/2010WR009438.
- Chen, D., R.C. Hale, B.D. Watts, M.J. La Guardia, E. Harvey, and E.K. Mojica. 2010. Species-specific accumulation of polybrominated diphenyl ether flame retardants in birds of prey from the Chesapeake Bay region, USA. *Environmental Pollution* 158:1883-1889.
- Connell, D.W., C.N. Fung, T.B. Minh, S. Tanabe, P.K.S. Lam, B.S.F. Wong, M.H.W. Lam, L.C. Wong, R.S.S. Wu, and B.J. Richardson. 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: Evidence from Organochlorine compounds in eggs. *Water Research* 37:459-467.
- Cunha, L.S.T., J.P.M. Torres, J. Muñoz-Arnanz, and B. Jiménez. 2012. Evaluation of the possible adverse effects of legacy persistent organic pollutants (POPs) on the brown booby (*Sula leucogaster*) along the Brazilian coast. *Chemosphere* 87:1039-1044.
- Custer, C.M., T.W. Custer, P.M. Dummer, M.A. Etterson, W.E. Thogmartin, Q. Wu, K. Kannan, A. Trowbridge, and P.C. McKann. 2014. Exposure and effects of perfluoroalkyl substances in Tree Swallows nesting in Minnesota and Wisconsin, USA. *Archives of Environmental Contamination and Toxicology* 66:120-138.

- Custer, C.M., T.W. Custer, H.L.Schoenfuss, B.H. Poganski, and L. Solem. 2012. Exposure and Effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reproductive Toxicology* 33(556-562).
- Custer, C.M., B.R. Gray, and T.W. Custer. 2010. Effects of egg order on organic and inorganic element concentrations and egg characteristics in Tree Swallows, *Tachycineta bicolor*. *Environmental Toxicology and Chemistry* 29:909-921.
- Custer, T.W., C.M. Custer, R.K. Hines, S. Gutreuter, K.L. Stromborg, P.D. Allen, and M.J. Melancon. 1999. Organochlorine contaminants and reproductive success of Double-crested Cormorants from Green Bay, Wisconsin, USA. *Environmental Toxicology and Chemistry* 18:1209-1217.
- Custer, T.W., P.M. Dummer, C.M. Custer, Q. Wu, K. Kannan, and A. Trowbridge. 2013. Perfluorinated compound concentrations in Great Blue Heron eggs near St. Paul, - Minnesota, USA, in 1993 and 2010-2011. *Environmental Toxicology and Chemistry* 32:1077-1083.
- Custer, T.W., K. Kannan, L. Tao, S.H. Yun, and A. Trowbridge. 2010. Perfluorinated compounds and polybrominated diphenyl ethers in Great Blue Heron eggs from three colonies on the Mississippi River, Minnesota. *Waterbirds* 33:86-95.
- de Wit, C. 2002. An overview of brominated flame retardants in the environment. *Chemosphere* 46:583-624.
- Eisler, R. 1990. Chlordane hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service, Biological Report 85(1.21).
- Environment Canada. 2013. Federal Environmental Quality Guidelines: Polybrominated diphenyl ethers (PBDEs).
<http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=05DF7A37-1>
- Eriksson, U., A. Roos, Y. Lind, K. Hope, A. Ekblad, and A. Kärman. 2016. Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (*Pandion haliaetus*), tawny owl (*Strix aluco*), and common kestrel (*Falco tinnunculus*). *Environmental Research* 149:40-47.
- Evers, D.C. 2006. Loons as biosentinels of aquatic integrity. *Environmental bioindicators* 1:18-21.
- Evers, D.C., K.M. Taylor, A. Major, R.J. Taylor, R.H. Poppenga, and A.M. Scheuhammer. 2003. Common Loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* 12:69-81.

- Fernie, K.J., G. Mayne, J.L. Shutt, C. Pekarik, K.A. Grasman, R.J. Letcher, and K. Drouillard. 2005. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. *Environmental Pollution* 138:485-493.
- Fernie, K.J., J.L. Shutt, R.J. Letcher, I.J. Ritchie, and D.M. Bird. 2009. Environmentally relevant Concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. *Environmental Science and Technology* 43:2124-2130.
- Findholt, S.L., and C.H. Trost. 1985. Organochlorine pollutants, eggshell thickness, and reproductive success of Black-crowned Night-herons in Idaho, 1979. *Colonial Waterbirds* 8:32-41.
- Fleming, W.J., M.A.R. McLane, and E. Cromartie. 1982. Endrin decreases screech owl productivity. *Journal of Wildlife Management* 46:462-468.
- Fox, G.A., K.S. Yonge, and S.G. Sealy. 1980. Breeding performance, pollutant burden and eggshell thinning in Common Loons *Gavia immer* nesting on a boreal forest lake. *Ornis Scandinavica* 11:243-248.
- Gingras, B.A. and C.A. Paszkowski. 1999. Breeding patterns of Common Loons on lakes with three different fish assemblages in north-central Alberta. *Canadian Journal of Zoology* 77:600-609.
- Gómez-Ramírez, P., E. Martínez-López, A.J. García-Fernández, A.J. Zweers, N. W. van den Brink. 2012. Organohalogen exposure in a Eurasian Eagle owl (*Bubo bubo*) population from Southeastern Spain: Temporal-spatial trends and risk assessment. *Chemosphere* 88:903-911.
- Helander, B., A. Olsson, A. Bignert, L. Asplund, and K. Litzén. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the White-tailed Sea Eagle (*Haliaeetus albicilla*) in Sweden. *Ambio* 31:386-403.
- Hendriks, A.J. 2013. How to deal with 100,000+ substances, sites, and species: overarching principles in environmental risk assessment. *Environmental Science and Technology* 47:3546-3547.
- Henny, C.J., J.L. Kaiser, R.A. Grove, B.L. Johnson, and R.J. Letcher. 2009. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of Ospreys in Oregon and Washington, USA. *Ecotoxicology* 18:802-813.

- Hernández, M., M.À. Colomer, M. Pizarro, and A. Margalida. 2018. Changes in eggshell thickness and ultrastructure in the Bearded Vulture (*Gypaetus barbatus*) Pyrenean population: A long-term analysis. *Science of the Total Environment* 624:713-721.
- Hoffman, D.J., C.P. Rice, and T.J. Kubiak. 1996. PCBs and dioxins in birds. In *Environmental contaminants in wildlife: Interpreting tissue concentrations*, ed. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood. Boca Raton, FL.
- Hoondert, R.P.J., J.P. Hilbers, A.J. Hendriks, and M.A.J. Huijbregts. 2018. Deriving field-based ecological risks for bird species. *Environmental Science and Technology* 52:3716-3726.
- Huber, S., N.A. Warner, T. Nygård, M. Remberger, M. Harju, H.T. Uggerud, L. Kaj, and L. Hanssen. 2015. A broad cocktail of environmental pollutants found in eggs of three Seabird species from remote colonies in Norway. *Environmental Toxicology and Chemistry* 34:1296-1308.
- Jo, A., Ji, K., and K. Choi. 2014. Endocrine disruption effects of long-term exposure to perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (PFTrDA) in zebrafish (*Danio rerio*) and related mechanisms. *Chemosphere* 108:360-366.
- Letcher, R.J., J.O. Bustnes, R.Dietz, B.M. Jenssen, E.H. Jørgensen, C. Sonne, J. Verreault, M.M. Vijayan, G.W. Gabrielsen. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment* 408:2995-3043.
- Loon Preservation Committee (LPC). 2015. The Squam Lake Loon Initiative Progress Report, November 2015. Moultonborough, NH.
- Loon Preservation Committee (LPC). 2017. The Squam Lake Loon Initiative Progress Report, November 2017. Moultonborough, NH.
- Loon Preservation Committee (LPC). 2020. The Squam Lake Loon Initiative Progress Report, November 2020. Moultonborough, NH.
- Martin, J.W., M.M. Smithwick, B.M. Braune, P.F. Hoekstra, D.C.G. Muir, and S.A. Mabury. 2004. Identification of long-chain perfluorinated acids in biota from the Canadian Arctic. *Environmental Science and Technology* 38:373-380.
- May, A. 2020. DDT contamination in Squam Lake, NH: A watershed approach. Thesis. Plymouth State University, Plymouth, NH.

- McKernan, M.A., B.A. Rattner, R.C. Hale, and M.A. Ottinger. 2009. Toxicity of polybrominated diphenyl ethers (DE-71) in Chicken (*Gallus gallus*), Mallard (*Anas platyrhynchos*), and American Kestrel (*Falco sparverius*) embryos and hatchlings. *Environmental Toxicology and Chemistry* 28:1007-1017.
- New Hampshire Department of Environmental Services (NHDES). 2020a. 2020 Section 305(b) and 303(d) consolidated assessment and listing methodology, draft. October 16, 2020. Concord, NH.
- New Hampshire Department of Environmental Services (NHDES). 2020b. Impairments added to the 2020 303(d) list of threatened or impaired waters, draft. October 16, 2020. Concord, NH.
- New Hampshire Department of Environmental Services (NHDES). 2020c. NHDES issues new fish consumption advisory for Squam Lake. High levels of polychlorinated biphenyls (PCBs) detected in fish tissue. March 27, 2020.
<https://www.des.nh.gov/news-and-media/nhdes-issues-new-fish-consumption-advisory-squam-lake-high-levels-polychlorinated>.
- Newsted, J.L., P.D. Jones, K. Coady, and J.P. Giesy. 2005. Avian toxicity reference values for perfluorooctane sulfonate. *Environmental Science and Technology* 39:9357-9362.
- Peakall, D.B. 1996. Dieldrin and other cyclodiene pesticides in wildlife. In *Environmental contaminants in wildlife: Interpreting tissue concentrations*, ed. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood. Boca Raton, FL.
- Peakall, D.B., and A.P. Gilman. 1979. Limitations of expressing organochlorine levels in eggs on a lipid-weight basis. *Bulletin of Environmental Contamination and Toxicology* 23:287-290.
- Peden-Adams, M.M., J.E. Stuckey, K.M. Gaworecki, J. Berger-Ritchie, K. Bryant, P.G. Jodice, T.R. Scott, J.B. Ferrario, B. Guan, C. Vigo, J.S. Boone, W.D. McGuinn, J.C. DeWitt, and D.E. Keil. 2009. Developmental toxicity in white leghorn chickens following *in ovo* exposure to perfluorooctane sulfonate (PFOS). *Reproductive Toxicology* 27:307-318.
- Pollentier, C.D., K.P. Kenow, and M.W. Meyer. 2007. Common Loon (*Gavia immer*) eggshell thickness and egg volume vary with acidity of nest lake in northern Wisconsin. *Waterbirds* 30:367-374.
- Schmutz, J.A., K.A. Trust, and A.C. Matz. 2009. Red-throated loons (*Gavia stellata*) breeding in Alaska, USA, are exposed to PCBs while on their Asian wintering grounds. *Environmental Pollution* 157:2386-2393.

- Siegel, L. 2020. Squam Lakes Loon Model (SLLM). Unpublished report.
- Stickel, L.F., S.N. Wiemeyer, and L.J. Blus. 1973. Pesticide residues in eggs of wild birds: adjustment for loss of moisture and lipid. *Bulletin of Environmental Contaminants and Toxicology* 9:193-196.
- Strong, P.I.V. 1990. The suitability of the Common Loon as an indicator species. *Wildlife Society Bulletin* 18:257-261.
- Su, G., R.J. Letcher, J.N. Moore, L.L. Williams, and K.A. Grasman. 2017. Contaminants of emerging concern in Caspian tern compared to herring gull eggs from Michigan colonies in the Great Lakes of North America. *Environmental Pollution* 222:154-164.
- Sutcliffe, S.A. 1978. Pesticide levels and shell thickness of Common Loon eggs in New Hampshire. *Wilson Bulletin* 90:637-640.
- Tartu, S., G.W. Gabrielsen, P. Blévin, H. Ellis, J.O. Bustens, D. Herzke, and O. Chastel. 2014. Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding Black-legged Kittiwakes. *Environmental Science and Technology* 48:13504-13510.
- Verreault, J., M. Houde, G.W. Gabrielsen, U. Berger, M. Haukås, R.J. Letcher, and D.C.G. Muir. 2005. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of Glaucous Gulls (*Larus hyperboreus*) from the Norwegian Arctic. *Environmental Science and Technology* 39:7439-7445.
- Vogel, H. 2017. Contaminated sediments in Squam Lake Tributaries, 2015-2016. Loon Preservation Committee, Moultonborough, NH.
- Wiemeyer, S.N. 1996. Other organochlorine pesticides in birds. In *Environmental contaminants in wildlife: Interpreting tissue concentrations*, ed. W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood. Boca Raton, FL.

Appendix A: Laboratory Analytical Methods

Analytical Methods at SGS AXYS Analytical Services Ltd. (Sidney, British Columbia, Canada)

For all analyses, ^{13}C -labelled surrogate standards were added to the samples prior to extraction. Blanks, spikes, and duplicates were analyzed for quality assurance and control for each batch (~5-15 samples).

For PCBs and organochlorine pesticides, samples were analyzed using AXYS method MLA-007. Samples were dried with sodium sulphate and Soxhlet extracted with dichloromethane. Tissue extracts were eluted using a gel permeation column to remove lipids, fractionated on a Florisil column, and ^{13}C -labelled recovery standards were added. Low polarity pesticides (E1) and PCBs were then analyzed using gas chromatography/low-resolution mass spectrometry connected to a DB-5 capillary chromatography column. The mass spectrometer was operated in the electron ionization mode using multiple ion detection. Polar pesticides (E2) were analyzed by gas chromatography/electron capture detector with a DB-5 capillary column. Minimum reporting limits for E1 pesticides ranged from 0.0039-5.85 ng/g ww, for E2 pesticides from 0.0034-1.26 ng/g ww, and for PCBs from 0.0023-71.7 ng/g ww. The average percent recovery for OC pesticides was 76% (range: 12%-510%; the 510% value was flagged as an estimated maximum value). The average percent recovery for PCBs was 75% (range: 26%-110%).

BDEs were analyzed using EPA method 1614 and AXYS Method MLA-033. Samples were extracted using a solvent, spiked with a cleanup standard, and cleaned on chromatographic columns. Analysis was performed using a DB-5HT capillary column (30 m, 0.25 mm internal diameter, 0.1 μm film thickness) coupled to a high-resolution mass spectrometer. Data were collected using voltage selected ion recording mode. Minimum reporting limits for BDEs ranged from 0.133-229 pg/g ww. The average percent recovery for BDEs was 97% (range: 49-189%).

PFAS contaminants were analyzed using AXYS Method MLA-043. Samples were extracted with methanolic potassium hydroxide solution. After dilution with water and clean-up by solid phase extraction, the sample was spiked with recovery standards and analyzed using a high-performance liquid chromatograph couple to a triple quadrupole mass spectrometer. The mass spectrometer was run in the Multiple Reaction Monitoring mode. Minimum reporting limits for perfluorinated organic compounds ranged from 0.398-42.5 ng/g ww. The average percent recovery for PFAS was 77% (range: 4%-140%).

Five eggs were analyzed at SGS AXYS in 2020 for PFAS using SGS AXYS Method MLA-110. After spiking the samples with surrogate standards, samples were extracted using methanolic potassium hydroxide solution, then with acetonitrile, and then again with methanolic potassium hydroxide solution. Supernatants from each treatment were combined and treated with ultra-pure carbon powder before being evaporated, diluted with water, and cleaned by solid phase extraction. The extract was analyzed using ultrahigh performance liquid chromatography with a reversed phase C18 column coupled to a triple quadrupole mass spectrometer (LC-MS/MS). The analysis was run in Multiple Reaction Monitoring in negative electrospray ionization mode. Minimum reporting limits for perfluorinated organic compounds ranged from 0.193-15 ng/g ww. The average percent recovery was 81% (range: 1%-194%).

Analytical Methods at Wadsworth Center, New York State Department of Environmental Health (Albany, NY)

For analysis of PCBs, BDEs, and OC pesticides, all samples were spiked with a surrogate standard and Soxhlet extracted using dichloromethane and hexane (1:3). The extract was then further spiked with ¹³C-labeled surrogates. For PCBs, BDEs, and organochlorine pesticides, sample extracts were purified using silicagel (Davisil 100-200 mesh, Aldrich, WI). Analytes were eluted with 150 mL of 20% dichloromethane in hexane and extracts were concentrated with sulfuric acid and a rotary evaporator.

For PCBs and BDEs, extracts were placed in a gas chromatograph (Hewlett-Packard 6890) joined to a mass-selective detector (Hewlett-Packard, series 5973), using a capillary column coated with RTX-5MS (30 m x 0.25 mm i.d. x 0.25 μm film thickness; Restek Corp, Bellefonte, PA) to separate the isomers. An Agilent Technologies 6890N gas chromatograph-electron capture detector (GC-ECD) was used to analyze hepta- through deca-BDEs. For organochlorine pesticides, HCH isomers were analyzed using the same GC-ECD, while DDT, chlordanes, and HCB were analyzed using the gas chromatograph (Hewlett-Packard 6890) joined to the mass-selective detector (Hewlett-Packard, series 5973). Pesticides were separated using a capillary column coated with DB-5 (30 m x 0.25 mm i.d. x 0.25 μm film thickness).

Mean recoveries of ¹³C-labeled PCB congeners were between 80-95% and, for PCB-spiked surrogates, were 72-90%. Recoveries of BDEs were between 80-120% and, for OC pesticides, between 85-110%. Limits of quantitation for OC pesticides were 50-280 pg/g ww and for BDE congeners were 10-2500 pg/g ww. Limits of quantitation were not reported by the laboratory for PCBs.

For PFAS, egg samples were spiked with ¹³C-labeled standards and mixed with 2 mL of 0.25 M sodium carbonate buffer and 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate solution. The sample was then extracted using methyl-tert-butyl ether. An Agilent 1100 series high-performance liquid chromatograph and Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS) were used for analysis. Extract was injected onto a 50 x 2 mm (5 μm) Keystone Betasil C18 column, and the MS/MS was used in electrospray negative ion mode. Multiple reaction monitoring was used to identify target compounds. Recoveries were within acceptable limits. Limits of quantitation for PFDS were 0.94 ng/g ww, for PFBS 1.12 ng/g ww, and for other PFAS compounds 0.28-0.6 ng/g ww.

QA/QC was performed using blanks and analysis of recoveries for spiked samples. Blanks were analyzed for every 10 samples.

Analytical Methods at Geochemical and Environmental Research Group (GERG; Texas A&M University, College Station, TX)

Tissue samples were homogenized and extracted according to the NOAA Status and Trends Method using a Teckmar Tissumizer and adding surrogate standards, sodium sulfate, and methylene chloride. Extracts were then purified using silica/alumina column chromatography. For OC pesticides and PCBs, capillary gas chromatography with electron capture detector was

used to perform the analyses. For BDEs, analyses were performed using a capillary gas chromatograph and mass spectrometer detector in SIM (selected ion monitoring) mode. Detection limits ranged from 0.27-0.98 ng/g ww for organochlorine pesticides; 5.3-8.7 ng/g ww for total PCBs; and 0.67-4.90 ng/g ww for BDEs. QA/QC was performed using blanks and were within acceptable limits.

Analytical Methods at TDI-Brooks International, Inc. (College Station, TX)

Samples were homogenized and extracted using a Dionex ASE200 Accelerated Solvent Extractor using dichloromethane. The extract was concentrated and then processed using silica gel/alumina chromatography columns and high performance liquid chromatography. For PCBs and OC pesticides, extracts were then analyzed using a Hewlett Packard model 5890 gas chromatography/electron capture detectors with two columns (capillary column: J&W DB-5 30m x 0.25 mm ID and 0.25 mm film thickness; confirmation column: J&W DB-17HT 30 m x 0.25 mm ID and 0.15 film thickness). For BDEs, extracts were analyzed using a Thermo Trace GC and DSQ-II MS operated in SIM mode and a capillary column (Agilent Technologies DB-XXLB 15 m x 0.25 mm ID and 0.10 mm film thickness). Detection limits ranged from 0.26-0.54 ng/g ww for organochlorine pesticides; 11.1-11.3 ng/g ww for total PCBs; and 0.33-0.86 ng/g ww for BDEs. QA/QC was performed using blanks and duplicates and were within acceptable limits.

Appendix B: Summary of contaminant results for Common Loon eggs tested in New Hampshire by Loon Preservation Committee. See “Methods” for congeners included in contaminant groupings. All results are in nanograms/gram wet weight. NT = Not tested; N/A = Data not available. Bolded cells indicate lakes and territories with contaminants that exceeded 100% of observed effects levels for other species based on levels used in Figure 8 and with the caveats noted elsewhere in this report (see “Contaminant Levels in Eggs Compared with Lowest Observed Effects Levels.”)

Lake	Town	Territory	Year	Total PFAS	Sum BDE	Total PCBs	Total DDE	Total Chlordane
Arlington Mill Reservoir	Salem	Arlington Mill	2019	1587.4	60.2	N/A	663.0	109.0
Bow Lake	Strafford	Blueberry	2015	219.1	104.8	1926.9	608.3	52.5
Canobie Lake	Windham	Canobie	2016	1497.6*	42.0	1254.7	463.0	92.3
Connecticut Lake, First	Pittsburg	Dam Island	2015	206.9	58.2	1262.4	210.3	41.5
Conway Lake	Conway	South End	2005	289.1	56.8	1312.9	489.2	39.2
Deering Reservoir	Deering	Deering	2014	197.3	84.6	2579.1	471.6	73.2
Diamond Pond, Big	Stewartstown	Big Diamond	2019	58.4	56.7	N/A	267.0	39.5
Grafton Pond	Grafton	North	2005	240.3	26.3	1168.9	187.3	36.5
Highland Lake	Andover	Highland	2018	150.1	14.2	613.4	534.2	20.4
Ivanhoe Lake	Wakefield	Ivanhoe	2005	214.6	130.0	1494.2	614.9	118.1
Kusumpe Pond	Sandwich	Kusumpe	1997	284.1	36.7	1123.0	202.0	43.5
Lake Francis	Clarksville	Dam North	2014	118.1	17.9	6679.8	178.1	17.9
Lake Francis	Clarksville	Deadwater Stream	2018	92.7	31.5	9525.0	178.8	22.0
Lake Sunapee	Sunapee	Sunapee	2014	606.2	147.7	1702.6	961.7	73.6
Little Squam	Ashland	Riverbend	2014	223.7	38.9	2224.9	294.9	39.9
Loon Pond	Hillsborough	Loon	2015	215.5	43.6	1155.5	439.2	32.6
Massabesic	Manchester	Birch Island	2007	862.8	58.1	1722.8	413.5	44.6
Merrymeeting Lake	New Durham	Merrymeeting	2015	303.7	130.7	5284.5	485.4	65.0
Pleasant Lake	Francestown	Pleasant	2017	226.8	17.5	866.6	212.0	21.5
Pleasant Lake	New London	Pleasant	2016	133.7	39.2	1904.3	515.0	37.4
Squam Lake	Sandwich	Five Finger	1999	477.3	82.3	1314.4	418.2	38.8
Squam Lake	Sandwich	Five Finger	2010	539.7	77.5	1787.5	493.6	37.2
Squam Lake	Holderness	Bowman	1998	339.1	123.1	5143.6	808.1	103.9
Squam Lake	Holderness	Great Island	2010	NT	145.9	1787.4	420.0	25.3
Squam Lake	Holderness	Great Island	2018	512.8	247.4	1788.1	505.0	56.8
Squam Lake	Center Harbor	Heron Cove	1993	367.9	55.1	1909.0	502.2	73.1

Lake	Town	Territory	Year	Total PFAS	Sum BDE	Total PCBs	Total DDE	Total Chlordane
Squam Lake	Center Harbor	Heron Cove	1998	313.5	153.1	1894.4	495.0	65.2
Squam Lake	Center Harbor	Heron Cove	2005	572.4	177.7	2185.7	552.2	48.1
Squam Lake	Center Harbor	Heron Cove	2010	453.9	133.1	1982.1	660.9	62.2
Squam Lake	Center Harbor	Heron Cove	2014	264.5	107.2	1498.7	457.5	52.7
Squam Lake	Center Harbor	Heron Cove	2015	362.0	96.8	1198.1	387.5	34.6
Squam Lake	Center Harbor	Heron Cove	2017	525.0	25.7	353.7	131.0	10.4
Squam Lake	Center Harbor	Kimball Island	2005	960.9	336.5	2952.8	364.0	43.0
Squam Lake	Center Harbor	Kimball Island	2008	345.9	71.7	1780.4	425.5	50.7
Squam Lake	Center Harbor	Kimball Island	2009	NT	63.2	1250.0	240.0	26.7
Squam Lake	Center Harbor	Kimball Island	2013	339.7	83.3	685.3	273.1	21.7
Squam Lake	Center Harbor	Kimball Island	2019	286.6	41.0	N/A	238.8	20.6
Squam Lake	Sandwich	Long Point	1996	428.0	112.6	1800.7	800.1	131.1 [†]
Squam Lake	Sandwich	Long Point	2011	510.8	136.6	2035.5	616.0	58.0
Squam Lake	Sandwich	Long Point	2015	339.5	91.0	1225.4	317.1	30.2
Squam Lake	Holderness	Mink Island	1996	328.7	71.6	995.6	538.2	75.4
Squam Lake	Holderness	Mink Island	2001	805.8	242.6	2156.5	358.0	37.7
Squam Lake	Holderness	Mink Island	2002	656.4	94.0	2314.6	625.8	60.6
Squam Lake [‡]	Holderness	Mink Island	2007	724.1	113.2	2231.4	232.4	53.1
Squam Lake	Holderness	Mink Island	2015	350.6	35.4	1587.8	317.0	27.8
Squam Lake	Holderness	Mink Island	2017	365.0	107.7	3413.2	489.0	43.2
Squam Lake	Holderness	Mink Island	2018	348.5	85.7	2488.7	380.8	42.7
Squam Lake	Holderness	Mink Island	2019	570.8	105.8	N/A	411.0	37.5
Squam Lake	Holderness	Moon Island	2012	323.0	124.2	1480.1	501.1	58.4
Squam Lake	Holderness	Moon Island	2014	315.6	97.0	1262.9	461.4	47.8
Squam Lake	Holderness	Moon Island	2018	273.7	163.6	1378.7	555.9	45.8
Squam Lake	Moultonborough	Moultonborough Bay	1995	338.2	205.8	2552.3	755.7	84.4
Squam Lake	Moultonborough	Moultonborough Bay	2000	465.5	136.1	2730.3	775.3	94.7
Squam Lake	Moultonborough	Moultonborough Bay	2007	294.9	300.6	5057.7	874.9	96.5
Squam Lake	Moultonborough	Moultonborough Bay	2008	507.9	25.4	660.7	300.9	30.1

Lake	Town	Territory	Year	Total PFAS	Sum BDE	Total PCBs	Total DDE	Total Chlordane
Squam Lake	Moultonborough	Moultonborough Bay	2013	289.1	124.6	10,732.5	798.2	60.8
Squam Lake	Moultonborough	Moultonborough Bay	2016	407.4	107.2	10,116.6	397.2	39.0
Squam Lake	Holderness	Perch Island	2011	308.1	106.1	619.6	313.9	22.1
Squam Lake	Holderness	Perch Island	2016	295.6	249.5	2539.7	590.3	58.4
Squam Lake	Holderness	Piper Cove	2002	688.5	246.3	2263.7	453.8	41.6
Squam Lake [‡]	Holderness	Piper Cove	2007	481.5	258.9	3391.8	435.4	111.1
Squam Lake	Sandwich	Rattlesnake Cove	2003	484.1	98.9	812.2	241.6	18.9
Squam Lake	Sandwich	Squaw Cove	1996	470.5	30.6	953.9	317.0	41.3
Squam Lake	Sandwich	Squaw Cove	1999	303.4	95.4	1558.5	594.1	62.1
Squam Lake	Sandwich	Squaw Cove	2012	262.7	44.2	886.6	307.1	30.1
Squam Lake	Sandwich	Squaw Cove	2014	172.5	19.0	441.5	135.0	11.5
Squam Lake	Center Harbor	Sturtevant Bay	2004	519.8	64.2	730.4	163.8	17.9
Squam Lake	Center Harbor	Sturtevant Bay	2010	NT	69.5	1215.7	267.7	27.1
Squam Lake	Center Harbor	Sturtevant Bay	2018	369.7	47.2	760.1	270.0	25.5
Squam Lake	Center Harbor	Yard Islands	2015	256.8	130.4	1624.1	355.4	57.5
Squam Lake	Center Harbor	Yard Islands	2019	370.3	181.1	N/A	786.3	89.1
Umbagog	Errol	Gull Island	2007	661.3	28.4	1115.0	163.4	14.1
White Oak Pond	Holderness	White Oak	2005	578.2	153.6	1668.5	297.0	27.0
Wicwas	Meredith	Wicwas	2018	190.7	41.7	2248.7	268.0	42.6
Winnepesaukee	Meredith	Black Cove	2018	1275.7	222.6	1445.0	680.0	74.6
Winnepesaukee	Meredith	Breezy Island	2014	1363.5	135.3	1345.6	568.1	84.2
Winnepesaukee	Moultonborough	Evergreen	2017	372.1	20.8	915.9	354.7	33.5
Winnepesaukee	Moultonborough	Spectacle Island	2007	1004.2	28.2	431.4	147.5	17.5

*Geometric mean of two eggs from a single clutch.

†Includes results of oxychlordane, flagged in laboratory report as estimated maximum value.

‡Geometric mean of two eggs from a single clutch for all contaminant classes except PFAS.