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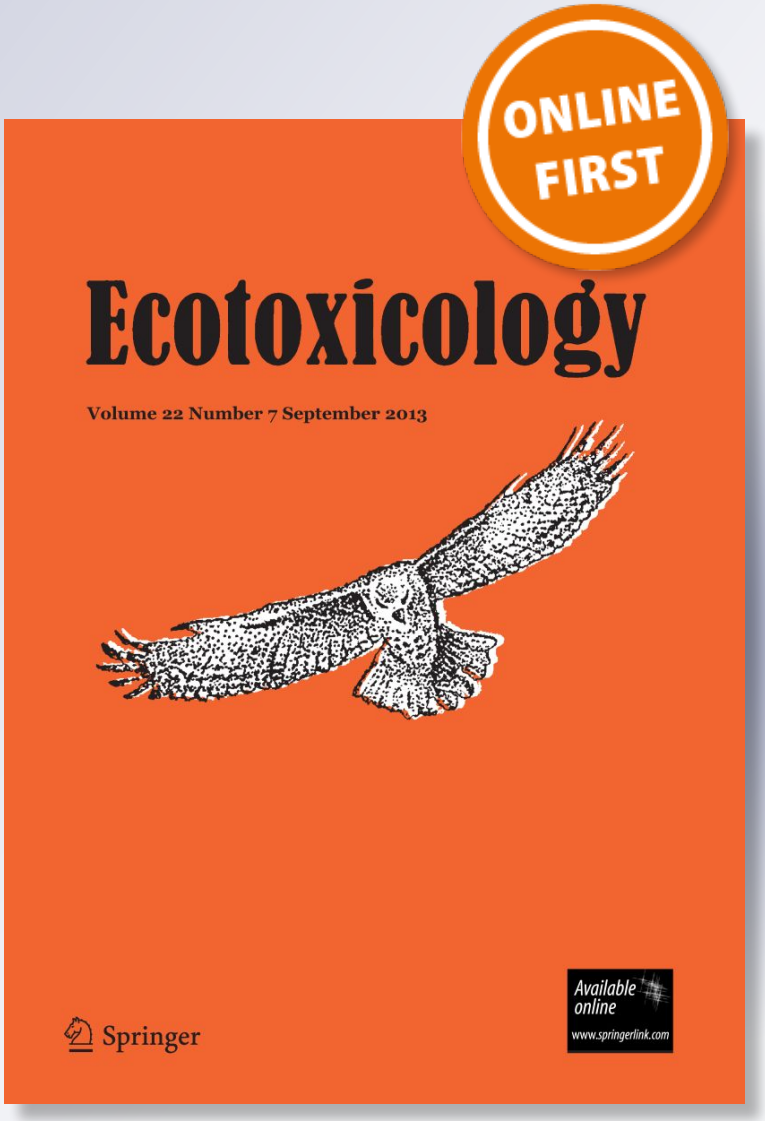

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Determining optimal sampling strategies for monitoring mercury and reproductive success in common loons in the Adirondacks of New York

Yang Yang[1] · Ruth D. Yanai[1] · Nina Schoch[2,3] · Valerie L. Buxton[2,4] · Kara E. Gonzales[5] · David C. Evers[6] · Gregory G. Lampman[7]

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Abstract

The common loon (*Gavia immer*), a top predator in the freshwater food web, has been recognized as an important bioindicator of aquatic mercury (Hg) pollution. Because capturing loons can be difficult, statistical approaches are needed to evaluate the efficiency of Hg monitoring. Using data from 1998 to 2016 collected in New York's Adirondack Park, we calculated the power to detect temporal changes in loon Hg concentrations and fledging success as a function of sampling intensity. There is a tradeoff between the number of lakes per year and the number of years needed to detect a particular rate of change. For example, a 5% year^{-1} change in Hg concentration could be detected with a sampling effort of either 15 lakes per year for 10 years, or 5 lakes per year for 15 years, given two loons sampled per lake per year. A 2% year^{-1} change in fledging success could be detected with a sampling effort of either 40 lakes per year for 15 years, or 30 lakes per year for 20 years. We found that more acidic lakes required greater sampling intensity than less acidic lakes for monitoring Hg concentrations but not for fledging success. Power analysis provides a means to optimize the sampling designs for monitoring loon Hg concentrations and reproductive success. This approach is applicable to other monitoring schemes where cost is an issue.

Keywords Power analysis · Sampling guidance · Mercury · Fledging success · Bioindicator · Common loon · Lake acidity · Adirondack Park

Introduction

Mercury (Hg), a neurotoxic pollutant, can be methylated and then bioaccumulated in aquatic food webs (Chan et al. 2003; Chen et al. 2008). The common loon (*Gavia immer*), a top predator in the freshwater food web, has been recognized as an important bioindicator of aquatic Hg pollution in North America (Evers et al. 1998). High Hg levels in loons can produce behavioral changes resulting in reduced reproductive success, especially at blood Hg levels over 3.0 mg kg^{-1} (Barr 1986; Burgess and Meyer 2008; Evers et al. 2008). To provide insight on the risk posed by environmental Hg loads and the efficacy

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✉ Yang Yang
yangy48@gmail.com

[1] Department of Forest and Natural Resources Management, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA

[2] Adirondack Center for Loon Conservation, PO Box 195 Ray Brook, NY 12977, USA

[3] Formerly of Biodiversity Research Institute, 276 Canco Rd., Portland, ME 04103, USA

[4] Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

[5] California Department of Transportation, 111 Grand Ave, Caltrans, Oakland, CA 94612, USA

[6] Biodiversity Research Institute, 276 Canco Rd., Portland, ME 04103, USA

[7] New York State Energy Research and Development Authority, 17 Columbia Circle, Albany, NY 12203, USA

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of pollution reduction regulations, long-term monitoring programs have been established to detect temporal trends in loon Hg in Maine and New Hampshire (Evers et al. 2008), Wisconsin (Fevold et al. 2003, Meyer et al. 2011), and New York (Schoch et al. 2019). Loon reproductive success has been monitored in many areas including Ontario (Weeber 1999), New Brunswick and Nova Scotia (Burgess and Meyer 2008), Wisconsin (Burgess and Meyer 2008), and New York (Schoch et al. 2014). Guidance on the sampling intensity necessary to detect these temporal changes is lacking but would be extremely valuable, given the logistical difficulties associated with capturing and observing loons repeatedly over long periods.

Power analysis can be used to inform sampling schemes and guide efficient environmental monitoring (Levine et al. 2014). Previous studies have used power analysis to determine the number of lakes sampled per year and number of sampling years needed to detect temporal changes in loon blood Hg (Levine et al. 2014), breeding success (Weeber 1999), and survival rate (Mitro et al. 2008). However, power analysis has not been used to explore the sample sizes needed to detect trends in fledging success, which is considered a better indicator of overall loon productivity (Evers 2004). Because loon productivity is influenced by lake acidity, sample size requirements may vary based on lake pH. Lakes with lower pH have higher methylation rates of Hg (Miskimmin et al. 1992; Barkay et al. 1997, Kelly et al. 2003) and thus tend to have higher and broader ranges of Hg concentrations in loons (Meyer et al. 1998; Yu et al. 2011; Schoch et al. 2014) and greater rates of loon reproductive failure (Champoux et al. 2006; Burgess and Meyer 2008; Alvo 2009). Consequently, increased sampling may be required to detect changes in lakes with lower pH because of their larger variation in loon Hg concentrations and reproductive success. However, the sampling intensity needed for lakes of differing acidity has not yet been explored.

Biodiversity Research Institute, the Adirondack Center for Loon Conservation, and their collaborators have been monitoring Hg concentrations and reproductive success of common loons in New York's Adirondack Park since 1998. We used power analysis on this long-term dataset to determine the number of loons sampled per lake, the number of lakes sampled per year, and the number of sampling years necessary to detect trends in loon Hg concentrations. We also explored different combinations of the number of lakes per year and the number of years necessary to detect trends in fledging success. Finally, we examined how sampling intensity might vary based on lake pH. This study aimed to provide guidance on the optimal sampling design of monitoring programs to assess the impacts of mercury exposure in loons to their Hg concentrations and reproductive success.

Methods

Study area and lake selection

The study site is the Adirondack Park of New York State, USA (43°59' N, 74°14' W), an area of 2.4 million ha that received 20 $\mu g\ m^{-2}\ year^{-1}$ of atmospheric Hg deposition before 2002 (Miller et al. 2005) and 17 $\mu g\ m^{-2}\ year^{-1}$ from 2009–2011 (Yu et al. 2013).

The Adirondack Park contains approximately 2800 lakes, of which 831 are large enough to provide breeding habitat for loons. Study lakes were chosen based on accessibility, observations of loons with chicks, and prior water quality research. A total of 116 lakes were monitored from 1998 to 2016.

Collection of common loon tissue samples and Hg analysis

From 1998 to 2016, loon Hg concentrations were monitored from blood samples and whole eggs collected from study lakes. Adult and juvenile loons were captured annually during a one to two week period in July or August using night-lighting and playback techniques (Evers 2001). Birds were banded with a USGS aluminum band and a unique color combination of up to three plastic bands to enable subsequent identification. Blood samples were collected from the tibiotarsal vein. Abandoned loon eggs were collected when field staff confirmed the adult loons were no longer incubating them or they were determined to be nonviable (i.e., emitted a strong odor). Because most lakes support only one nesting pair of loons, the number of samples collected per lake rarely exceeded one male, one female, and 1–2 juveniles or eggs.

Loon blood and egg samples were submitted to the Animal Health Diagnostic Laboratory, University of Pennsylvania, New Bolton, PA; the Trace Element Research Laboratory, Texas A&M, College Station, Texas; and the Biodiversity Research Institute in Portland, Maine, for analysis. Blood was analyzed as whole blood. Egg samples were freeze dried in a Labconco Lyph Lock 12 freezer dryer and powdered in a Spex Mixer Mill. Only total Hg was measured, as >95% of blood Hg in loons is in the methyl form (Wolfe et al. 2007). Total Hg was determined following U.S. Environmental Protection Agency (2007) Method 7473 using thermal decomposition and atomic absorption spectroscopy with an automated direct Hg analyzer (DMA 80, Milestone Incorporated, USA).

Monitoring reproductive success

Only banded loons sampled for Hg were included in this analysis of reproductive success. These loons were observed weekly on the territories where they were originally captured, using 10 × 40 binoculars from a canoe or kayak on 37–96 lakes for an 11- to 15-week period from late May until mid-August or early September of each year from 2001 to 2016. If a loon was not found in the territory it had occupied in previous years, then territories in close proximity were also checked throughout the field season to determine if the loon had returned to the area but had changed territories. For each loon, we recorded fledging success (number of chicks that survived past 6 weeks; chicks 6 weeks old and older are likely to survive to the actual fledging age of 11 weeks; Evers 2004). In this study, fledging success per territorial pair was used as an indicator of reproductive success.

Power analysis to detect trends in loon Hg concentrations and fledging success

Concentrations of Hg naturally differ among eggs and blood from males, females, and juveniles. We used the relationships identified by Evers et al. (2011) and adapted by Schoch et al. (2011) to convert all Hg concentrations to female loon units (FLU). These conversions allowed us to combine Hg levels for all loon age groups and eggs in a single analysis, which improved the sample size and thus the statistical power. The dataset for loon Hg represented 760 different loons (or eggs) from 116 lakes. Concentrations of Hg in juveniles older than 6 weeks were poorly correlated with Hg concentrations in female adults, so they were not included in this study.

We used resampling to provide input to the power analysis for detecting changes in FLU using the entire dataset. A sampling scheme was defined by the number of loons (male, female, juveniles, or eggs) sampled per lake (1–3), the number of lakes sampled per year (5–25), and the number of years (10–25). We sampled the FLU dataset 5000 times with replacement for each combination of the aforementioned factors. To describe change over time, we used the average FLU across lakes for each year sampled, with the average for each lake based on different numbers of loons. The coefficient of variation over time was computed for each random sample of the dataset. The power required to detect a linear change of 5% year^{-1} in FLU associated with different numbers of loons, lakes, or years sampled was obtained using the powertrend function (Gerrodette 1987) in the 'fishmethods' package (Nelson 2015) in R Studio®. We chose a temporal change of 5% year^{-1} because this was the rate observed in our dataset (Schoch et al. 2019). We used a two-tailed test and a significance level (α) of 0.05.

We applied a similar method to our data on fledging success. For the 42 lakes with more than one territory, we used the average fledging success. We reported the power needed to detect linear changes in fledging success of 1–3% year^{-1} based on the number of lakes sampled per year (30–50) and number of years sampled (5–20).

Because we expected optimal sampling strategies for monitoring loon Hg concentrations or fledging success to vary by lake acidity, we conducted a separate power analysis on data from two classes of lakes (pH < 6.5 and ≥ 6.5) using lake pH values available from the Adirondack Lakes Survey Corporation (http://www.adirondacklakessurvey.org/historic.php). We chose a cutoff point of pH 6.5 because lakes with pH < 6.5 in the Adirondacks contained yellow perch with Hg levels ≥300 ng g^{-1} (Simonin et al. 2008). Yellow perch are a common prey item for loons and fish Hg levels ≥300 ng g^{-1} have been associated with reduced loon productivity (Barr 1986). Not all the lakes in the study have been monitored for lake pH. The dataset that included lake pH contained 624 loons from 98 lakes for Hg concentrations and 200 loons from 77 lakes for fledging success.

Results

Current sampling intensity

Eggs provided the most observations of loon Hg concentrations (12 ± 6 SD per year) to the dataset, followed by adult males (8 ± 5) and females (7 ± 4) (Fig. 1). Juvenile loons had the lowest annual sampling intensity (4 ± 3 for juveniles <4 weeks old and 3 ± 2 for juveniles from 4 to 6 weeks old; Fig. 1c). Combining all these data, an average of 23 ± 6 lakes was sampled each year (Fig. 1d), and an average of 2 ± 1 loons (eggs or birds) were sampled per lake per year.

An average of 48 lakes with banded loons were monitored annually to determine the fledging success of the birds from 2001 to 2016, with a minimum of 29 lakes monitored in 2001 and a maximum of 57 lakes monitored in 2014.

Power analysis to detect trends in loon Hg concentrations

The sampling intensity required to detect a particular change in FLU depends on three levels of sampling: loons per lake, lakes per year, and number of years. Increasing the effort in one variable decreases the intensity required in another. For example, using the entire dataset, if only one loon Hg sample were collected per lake, at least 10 lakes per year for 15 years would be required to detect a 5% year^{-1}

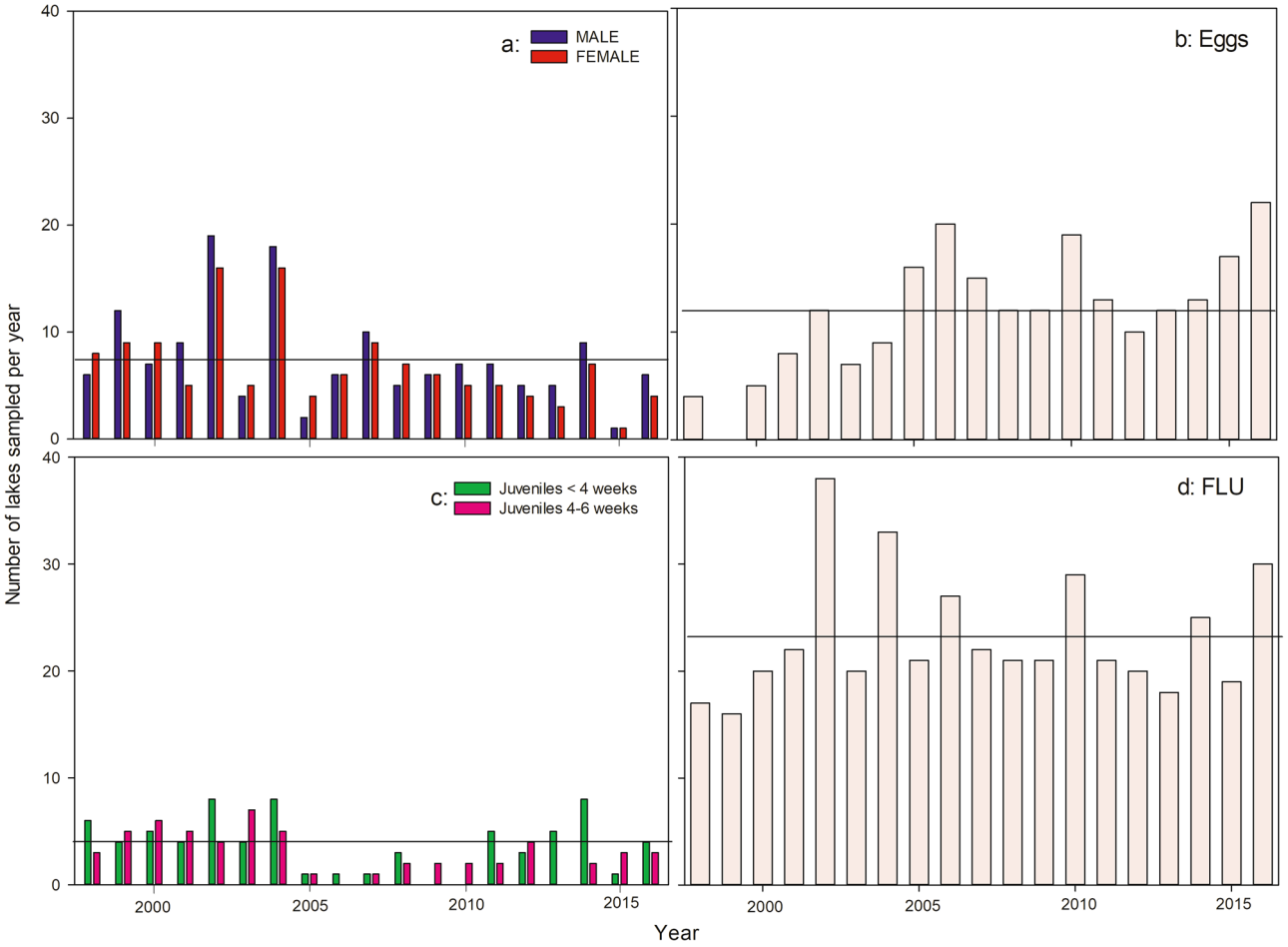

Fig. 1 Number of lakes sampled from 1998 to 2016 for **a** male and female loons, **b** eggs, **c** juvenile loons, and **d**, converted FLU. Solid lines indicate the average number of lakes sampled across years for each group

change in FLU (Fig. 2). If the number of loon Hg samples were increased to 3 per lake, then sampling 5 lakes per year for 15 years or sampling 10 lakes for 10 years would be required to detect the same magnitude of change (Fig. 2). If the sampling period were 20 years or more, with only 1 loon Hg sample collected per lake and 5 lakes sampled per year, we would still have sufficient power to detect a change of 5% year^{-1} (Fig. 2). Note that, in this study, we used a two-tailed test for the power analysis to detect a change either for upward or downward trends. A one-tailed test should be used to test for a temporal trend in only one direction. For a given effect size, alpha, and power, a smaller sample size would be required for a one-tailed test than for a two-tailed test.

Monitoring intensities needed to detect changes in loon Hg concentrations were affected by lake acidity. The sample size required to detect a 5% change in FLU was smaller in lakes with pH > 6.5 than lakes with pH < 6.5 (Fig. 3). For example, if monitoring two loons per lake over 10 years, it would require 15 of the more acidic lakes but only 10 of the less acidic lakes to have the power to detect a change of 5% year^{-1}.

Power analysis to detect trends in loon fledging success

Sampling effort for detecting changes in loon fledging success involves tradeoffs between the numbers of lakes sampled and the number of years sampled. For example, to detect a 2% year^{-1} change in fledging success requires sampling at least 40 lakes per year for 15 years or 30 lakes per year for 20 years (Fig. 4). However, if the actual change is only 1% year^{-1}, at least 50 lakes per year for 20 years would be required to detect the change (Fig. 4). The sample size required to detect a temporal change in loon fledging success was similar for lakes with pH < 6.5 and lakes with pH ≥ 6.5 (Appendix A).

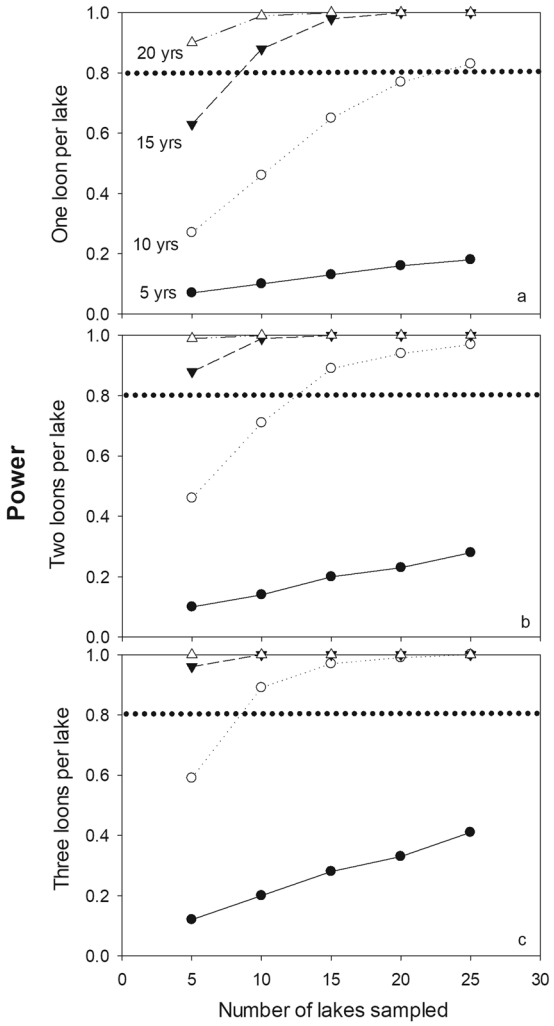

Fig. 2 Power to detect a change of 5% year^{-1} in loon Hg concentration (FLU), with alpha = 0.05, based on **a** one loon sample per lake, **b** two loon samples per lake, and **c** three loon samples per lake. The loon samples collected from each lake can include abandoned eggs or blood from males, females, or juveniles. Sampling designs with power > 0.8, indicated by the dotted line, are considered statistically significant

Discussion

Power analysis is a useful tool for guiding sampling intensity. For future monitoring of loon Hg concentrations in the Adirondacks, our results suggest that the number of lakes sampled annually could be decreased from the current average of 23 to as few as 10 lakes per year, if the rate of change remains at 5% year^{-1}. Detecting a smaller rate of change, as might be anticipated as Hg deposition continues to decline, would require greater sampling intensity. Increasing the number of loons sampled per lake would decrease the number of lakes and years required to detect the same rate of change. However, it may not be realistic to increase loon sampling intensity per lake, because most of the lakes are not large enough to support more than one nesting pair. Of the 441 cases in our dataset (lakes and years), 52% had 1 loon sample, 33% had 2 loon samples, and only 15% had 3 or more (including eggs as well as birds). Thus, adjustments to loon sampling targets will be made in the number of lakes and the temporal sampling frequency, rather than in the number of samples collected per lake.

Stratifying lakes by acidity proved important to evaluating the relationship between sampling effort and detectable rates of change in loon Hg concentrations. Ideally, future efforts would allocate more sampling effort in more acidic lakes, where loon Hg concentrations are more variable. The Adirondack loon monitoring program has been sampling a greater number of high pH lakes (averaging 12 lakes annually) than low pH lakes (averaging 6 lakes annually). However, collecting loon tissue samples from the more acidic lakes is logistically challenging because loons are difficult to capture unless they have chicks, and loons hatch chicks on acidic lakes very rarely (Alvo 2009).

Monitoring loon fledging success requires more lakes than monitoring Hg concentrations, because the rate of change is small and reproductive success is quite variable. Loon reproductive success is influenced by a multitude of factors, such as water level fluctuation (Hake et al. 2005; Windels et al. 2013), human recreational activity (Ream 1976; Field and Gehring 2015; Buxton et al. 2019), and predation (Götmark 1992; Uher-Koch et al. 2015). The number of lakes sampled per year should be increased if the expected rate of change in fledging success is only 1% year^{-1}, but the current sampled intensity of 48 to 50 lakes is sufficient if the actual change is >2% year^{-1}. If factors that predict fledging success could be used to stratify lakes for analysis, as was the case for lake acidity and loon Hg, then sampling intensity might be reduced due to reduced variability in the dataset.

When monitoring ecological indicators over time, evaluating the efficacy of the current sampling design can help inform future allocations of cost and effort. Power analysis is useful for determining the sampling intensity needed to detect significant changes. Other factors are important to the design of environmental monitoring programs. For example, the costs or risks of accessing specific sampling units (lakes, in this case) may vary considerably. Choosing sites where other data are available may provide benefits (Levine et al. 2014), as we found in the case of stratifying by lake acidity. Monitoring programs should be revisited on a regular basis to account for changes in the patterns under study (Lovett et al. 2007). In addition, as more data become available, the ability to detect trends is generally improved (Levine et al. 2014).

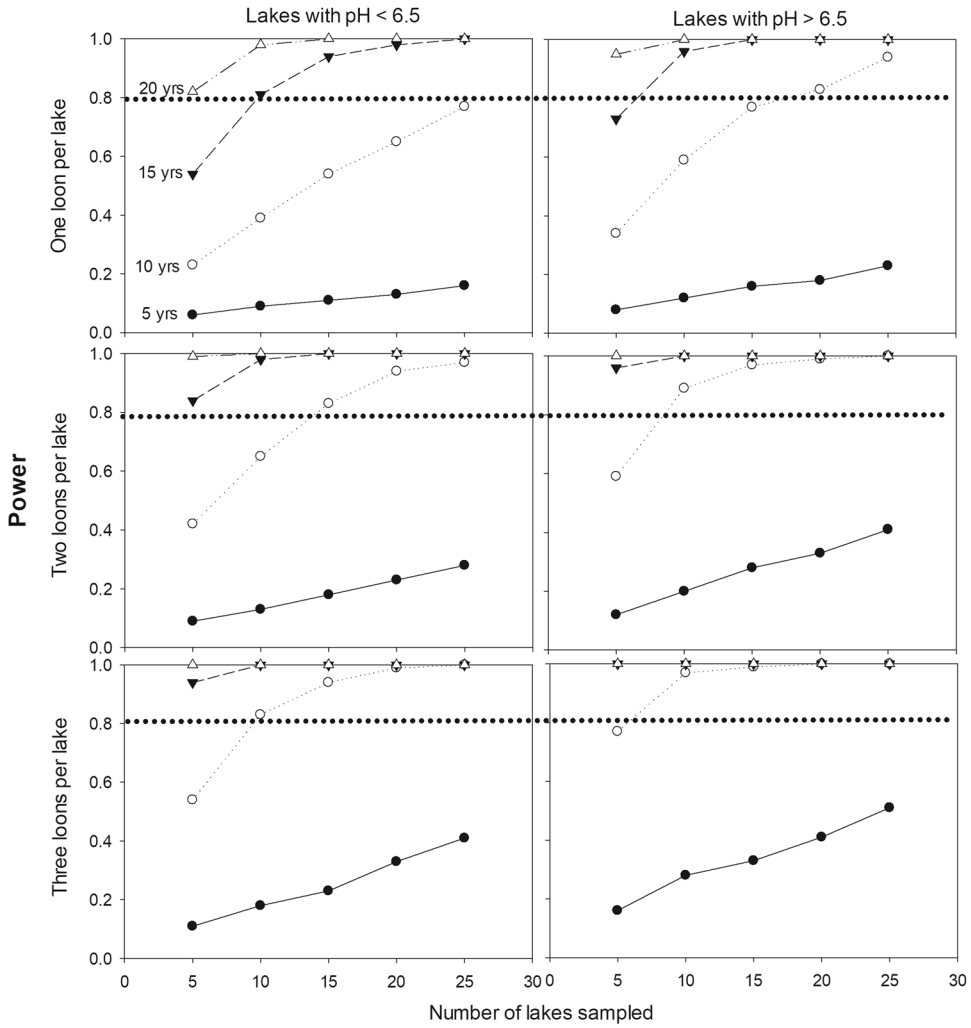

Fig. 3 Power to detect a change of 5% year^{-1} in loon Hg concentrations (FLU), with alpha = 0.05, with different combinations of number of loon samples per lake, number of lakes and number of years for lakes with pH < 6.5 and with pH > 6.5. The loon samples collected in each lake can include Hg concentrations in abandoned eggs and in blood from males, females, or juveniles. Sampling designs with power > 0.8, indicated by the dotted line, are considered statistically significant

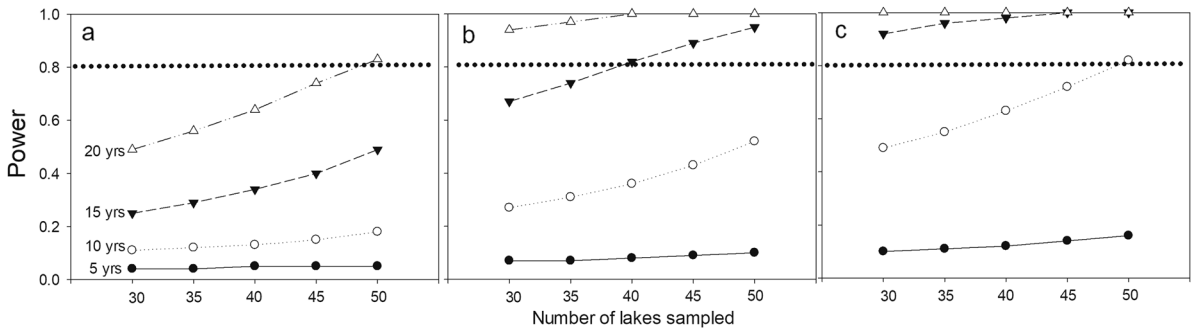

Fig. 4 Power to detect linear changes of **a:** 1% year^{-1}, **b:** 2% year^{-1}, **c:** 3% year^{-1} in loon fledging success, with alpha = 0.05, with different combinations of number of lakes per year and number of years. Sampling designs with power > 0.8, indicated by the dotted line, are considered statistically significant

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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