Methods

Sampling and Data Collection

Kiyi were collected during daylight at two stations on 5 Jun 2014 and at 21 stations from throughout Lake Superior between 7 Jul and 20 Jul 2014. Stations were categorized into five regions labeled as the Western Arm, Isle Royale, Northern Ontario, Southern Ontario, and Eastern Michigan (Figure 1). Fish were collected with the Research Vessel Kiyi (United States Geological Survey, Lake Superior Biological Station) using a Yankee bottom trawl with either a chain or rubber disk foot rope towed at approximately 3.5 km/h. Both nets had an 11.9 m head rope, 15.5 m foot rope, and a 2.2 m wing height with stretch mesh of 89 mm at the mouth, 64 mm for the trammel, and 13 mm at the cod-end. The June tows were cross-contour with a mean beginning depth of 26 m (range: 13-40), ending depth of 106 m (range: 68-144), and distance covered of 0.XX km (range: 0.XX-X.XX). The tows in July followed a depth contour, had a mean average depth of 186 m (range: 134-255), and a mean distance covered of 0.XX km (range: 0.XX-X.XX).

All Kiyi were immediately measured for total length (TL) to the nearest mm. A subsample that consisted of a maximum of five individuals per sex per 10 mm TL bin for fish between 160 and 279 mm and all Kiyi less than 159 mm and greater than 280 mm were immediately frozen. At a later date, the frozen fish were thawed at room temperature and TL, weight to the nearest gram, and sex (visually determined as female, male, or juvenile) were recorded and scales and saggital otoliths were removed and placed in a paper envelope to air dry. Scales were removed from directly above the lateral line as close to the anterior margin of the dorsal fin as possible from either side of the fish.

In the laboratory, otoliths were embedded in clear epoxy (Buehler EpoKwick™ Epoxy, 5:1 ratio Resin to Hardener) before a 0.5 mm thick section through the nucleus along the dorsoventral plane was obtained with a Buehler IsoMet™ Low Speed Saw. Otolith thin sections were lightly polished with 1000 grit sandpaper before viewing in mineral oil on a black background with reflected light applied at approximately a 45 degree angle to the section. A digital image of each thin section, or images for some sections where all fields of the section were not clear on one image, was captured with a Nikon DS-Fi2™ camera attached to a Nikon SMZ745T™ stereo microscope. Digital images of scales pressed into 5 mm thick acetate slides were taken with the a XXX dissecting microscope from a subsample of fish captured in the Eastern Michigan region.

Two readers who were blind to any biological information related to the fish identified annuli on otoliths from the digital images. The combination of a translucent band representing fast growth and an opaque band representing slow growth on the sectioned otolith was interpreted as one year of growth. Only completed translucent bands near the edge of the otolith were counted as annuli, as partial growth for the current year was present for some individuals. When the two readers disagreed on an age estimate, they further reviewed the otolith image in an attempt to achieve a consensus age estimate for analyses that required a single age estimate. One reader who was blind to biological information about the fish identified annuli on the scales from digital images. Annuli on scales were identified using “cutting-over” and “compaction” characteristics evident in the circuli (Quist *et al.,* 2012).

Total lengths of all Kiyi collected in similar samplings (i.e., similar gear, from locations throughout Lake Superior, and restricted to June and July) were available from 1992-2013. Length frequency distributions from these years were examined for evidence of strong year-classes that could be used to partially validate ages estimated in 2014.

Statistical Analyses

Differences in the length frequency distributions among regions were assessed with pairwise Kolmogorov-Smirnov tests (Neumann and Allen 2007; Ogle 2015) that used the bootstrap procedure implemented in ks.boot() from the Matching package (Sekhon 2011) in the RTM statistical environment v3.2.2 (R Development Core Team, 2015) to minimize the effect of the non-continuous length data on the test statistic (Abadie 2002). P-values from these multiple tests were corrected with the Holm (1979) method implemented in p.adjust(). Differences among regions in the sex ratio, after four juveniles were removed, in the subsample were assessed with a chi-square test.

Bias in otolith ages between two readers (e.g., one reader consistently had lower age estimates than the other reader) and between scale and otolith ages from the same reader were estimated with age-bias plots (Campana *et al.,* 1995) and the test of symmetry for the age-agreement table proposed by Evans and Hoenig (1998) and suggested for use by McBride (2015) as computed with ageBias() from the FSA package v0.8.1 (Ogle, 2015). If no significant bias between readers was detected, precision between readers was summarized as the percentage of fish for which the ages differed by zero or by one or fewer years and the average coefficient of variation (ACV; Chang, 1982; Kimura and Lyons, 1991) as computed with agePrecision() from the FSA package.

Differences in age-length keys (ALK; CITATION) derived from a multinomial distribution fit to the consensus otolith age estimates and 10 mm length categories were used to assess differences in age-length keys. The multinomial models were fit using multinom() from the nnet () package as described in Gerritsen et al. (2006) and Ogle (2015). Differences in ALKs between sexes within regions were assessed first. If no difference between sexes was found for all regions, then the sexes were pooled and differences in ALKs among regions were assessed. The p-values from these multiple comparison were adjusted with the Holm (1979) method.

Specific ages were assigned to all Kiyi captured in 2014 using region-specific (pooled sexes) ALKs and the method described by Isermann and Knight (2005) as implemented in the alkIndivAge() function in the FSA package. Age-length keys based on observed consensus otolith ages rather than on the multinomial model fit were used so as not to average out any distinct year-classes that may be present in the ALKs. Regional differences in the distribution of ages assigned with the ALKs were assessed with a chi-square test.

All statistical tests used α=0.05 to determine significance.

Results

A total of 938 Kiyi were sampled in 2014. These fish were between 108 and 266 mm TL with a mean (SD) TL of 197 (19.3) mm. The length frequency of Kiyi from the Northern Ontario region differed significantly from the length frequencies of Kiyi captured from the Isle Royale, Southern Ontario, and Eastern Michigan regions (p<0.008), but not from the Western Arm region (p=0.084). The length frequencies of Kiyi did not differ between any of the other regions (p>0.084). The Northern region had fewer longer fish. In the subsample of 335 fish, four were juveniles and 60.1% of non-juvenile fish were female. The sex ratio did not differ between regions (p=0.8755).

The examination of length frequencies from 2001 through 2014 revealed distinct modes near 80-100 mm in 2004, 2006, and 2010 (Figure 2). These modes correspond to age-1 fish as Kiyi hatch at approximately 20 mm in the early spring (CITATION). Thus, these cohorts corresponded to ages 11, 9, and 5, respectively, in 2014. There is little evidence for the 2005 cohort in 2007 which suggests few age-9 fish may exist in 2014.

Ages were estimated by two readers from 267 thin-sectioned otoliths. Of these otoliths, 22 (6.6%) were deemed unreadable (cracked or cloudy image) and were removed from further consideration. Ages estimated from the two readers perfectly agreed for 72.3% of the fish, agreed within one year for 97.0% of the fish, had a between-reader ACV of 2.8%, and showed no significant systematic bias (p=0.470; Figure 3). However, the mean estimated age for the second reader was slightly greater when the first reader estimated an age of 5 (95% CI: 5.14-5.37; p<0.001) and slightly lower when the first reader estimated an age of 12 (95% CI: 11.09-11.79; p=0.031). Mean scale age for each otolith age was less than the otolith age for all observed otolith ages with adequate sample sizes (Figure 4).

Kiyi for which a consensus otolith age estimate was obtained were used to generate ALKs. Four Kiyi less than 140 mm TL (all of the juvenile fish) were excluded from all ALK analyses because of sample size considerations. An additional seven fish that were estimated to be age-7 or older were also removed from the analysis that compared ALKs among regions because of sample size considerations. Age-length keys did not differ significantly between sexes within any region (p>0.109) or among regions when sexes were pooled (p=0.104). Despite this finding, to minimize the loss of any regional differences in the relationship between age and length, region-specific observed age-length keys were generated and used to assign ages by region to the 979 sampled Kiyi that were longer than 140 mm.

The age distribution was bimodal in each region (Figure 5) with an upper mode centered at age-11 in all five regions and a lower mode that consisted of nearly equal numbers of age-5 and age-6 fish in all regions except for Eastern Michigan where there were nearly twice as many age-5 as age-6 fish. The age distribution, after age-4 and 5 fish were pooled and age-11 and older fish were pooled within each region for sample size reasons, differed significantly among regions (p<0.001). Variability around the age-11 mode and the relative frequency of intermediate aged (ages 7-9) fish appear to explain much of the difference in age distribution among regions.

**Figure Captions**

Figure 1. Locations of 2014 Kiyi collections in Lake Superior with regions identified.

Figure 2. Relative within-year frequency of total length for all Kiyi captured in Lake Superior from May-July 2001-2014. Note that each plot has been scaled such that the mode has a height equal to 1.

Figure 3. Difference in estimated otolith ages for Lake Superior Kiyi from two readers at estimated otolith ages for the first reader (i.e., a modified age-bias plot), with mean (short horizontal lines) and 95% confidence intervals (vertical lines). Darker points represent more individuals and gray confidence intervals represent estimated otolith ages for the first reader where the mean estimated otolith age for the second reader differed significantly. The horizontal dashed line represents ages that agreed. Sample sizes for each estimated otolith age of the first reader are shown above the x-axis.

Figure 4. Difference in estimated scale and otolith ages for Lake Superior Kiyi from one reader at estimated otolith ages for the reader (i.e., a modified age-bias plot), with mean (short horizontal lines) and 95% confidence intervals (vertical lines). Darker points represent more individuals and gray confidence intervals represent estimated otolith ages for the first reader where the mean estimated otolith age for the second reader differed significantly. The horizontal dashed line represents ages that agreed. Sample sizes for each estimated otolith age of the first reader are shown above the x-axis.

Figure 5. Frequency of ages assigned with regional age-length keys for all Kiyi captured in Lake Superior from June-July 2014. Note that each plot has a different scale for the y-axis.







