

## Articles

# Walleye Age Estimation Using Otoliths and Dorsal Spines: Preparation Techniques and Sampling Guidelines Based on Sex and Total Length

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## Abstract

We used dorsal spines and otoliths from 735 Walleye *Sander vitreus* collected from 35 Wisconsin water bodies to evaluate whether 1) otolith and dorsal spine cross sections provided age estimates similar to simpler methods of preparation (e.g., whole otoliths and dorsal spines, cracked otoliths); and 2) between-reader precision and differences between spine and otolith ages varied in relation to total length (TL), sex, and growth rate. Ages estimated from structures prepared using simpler techniques were generally similar to ages estimated using thin sections of dorsal spines and otoliths, suggesting that, in some instances, much of the additional processing time and specialized equipment associated with thin sectioning could be avoided. Overall, between-reader precision was higher for sectioned otoliths (mean coefficient of variation [CV] = 3.28%; standard error [SE] = 0.33%) than for sectioned dorsal spines (mean CV = 9.20%; SE = 0.56%). When using sectioned otoliths for age assignment, between-reader precision did not vary between sexes or growth categories (i.e., fast, moderate, slow), but between-reader precision was higher for females than males when using sectioned dorsal spines. Dorsal spines were generally effective at replicating otolith ages for male Walleye <450 mm TL and female Walleye <600 mm TL, suggesting that dorsal spines can be used to estimate ages for male Walleye <450 mm TL and female Walleye <600 mm TL. If sex is unknown, we suggest dorsal spines be used to estimate ages for Walleye <450 mm TL, but that otoliths be used for fish >450 mm TL. Our results provide useful guidance on structure and preparation technique selection for Walleye age estimation, thereby allowing biologists to develop sampling guidelines that could be implemented using information that is always (TL) or often (sex) available at the time of fish collection.

**Keywords:** walleye; age; growth; otoliths; dorsal spines

Received: May 1, 2017; Accepted: August 4, 2017; Published Online Early: August 2017; Published: December 2017

Citation: Dembkowski DJ, Isermann DA, Koenigs RP. 2017. Walleye age estimation using otoliths and dorsal spines: preparation techniques and sampling guidelines based on sex and total length. *Journal of Fish and Wildlife Management* 8(2):474-486; e1944-687X. doi:10.3996/052017-JFWM-038

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## Introduction

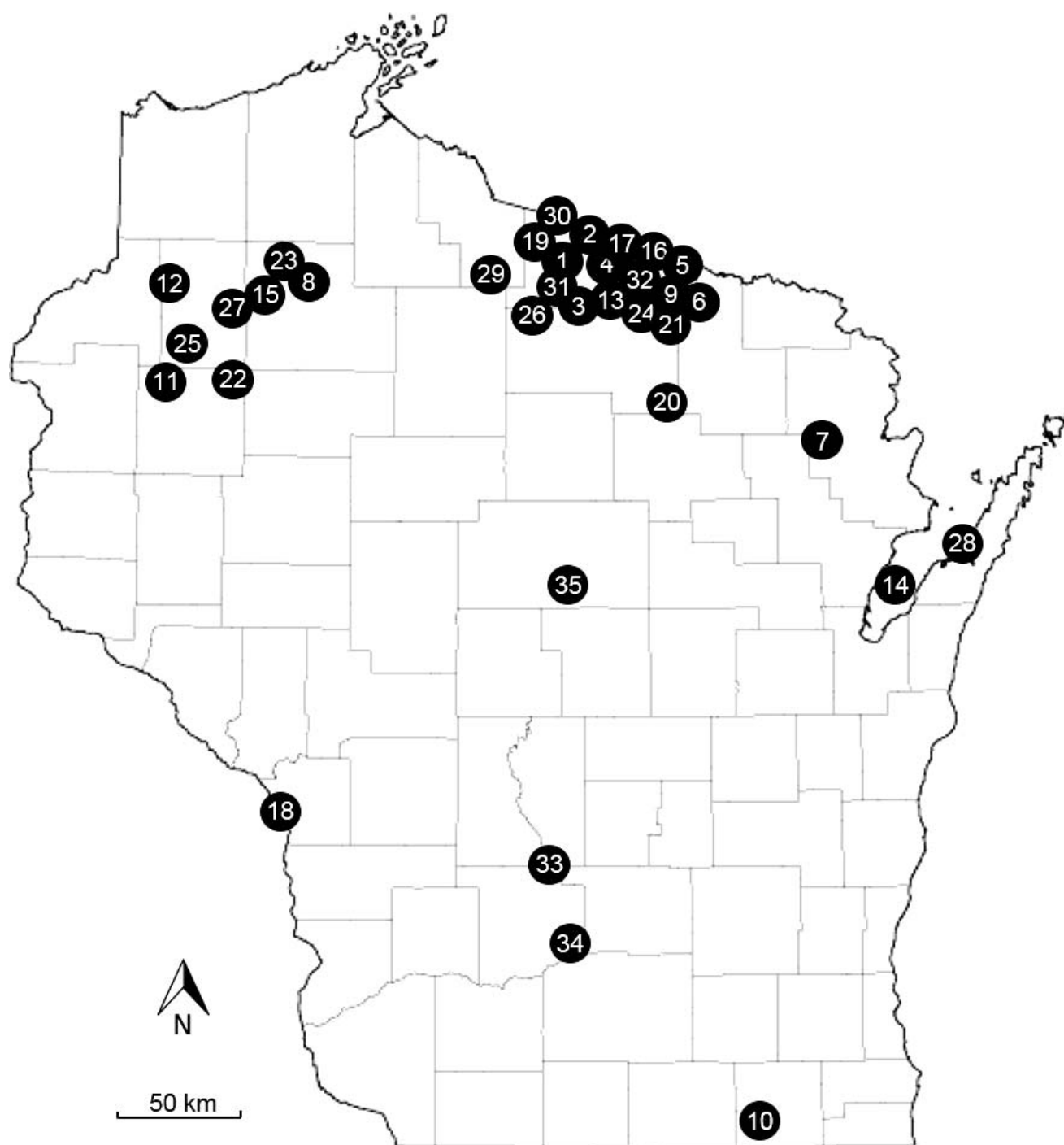
Walleye *Sander vitreus* support popular harvest-oriented fisheries across North America and are intensively managed using a variety of regulations, stocking strategies, and habitat manipulations (Baccante and Colby 1996; Isermann and Parsons 2011; Kerr 2011). Obtaining age estimates from Walleye is an important step in monitoring and managing these fisheries and, in many states and provinces, agency personnel will assign ages to hundreds of Walleye each year. Obtaining accurate and precise Walleye age estimates is essential to meaningful population assessment, because age data are used to estimate important dynamic parameters including growth, mortality, and recruitment (Koenigs et al. 2013). Although biologists may recognize otoliths as the preferred structure for estimating Walleye age (Erickson 1983; Kocovsky and Carline 2000; Maceina et al. 2007; Koenigs et al. 2013), in many states (e.g., Wisconsin, Iowa, Michigan), Walleye are not routinely sacrificed in standardized population assessments. Rather, dorsal spines are used as the primary structure to estimate Walleye ages.

Dorsal spines are easy to remove and do not require sacrificing fish, but the accuracy of spine-based Walleye age estimates has only been thoroughly evaluated once using known-age Walleye from the Lake Winnebago system, Wisconsin (Koenigs et al. 2015). Previous studies have demonstrated that ages from dorsal spines generally agree with otolith ages for younger Walleye, but that they underestimate otolith age for older fish (Logsdon 2007; Koenigs et al. 2015). In addition, dorsal spines often provide lower among-reader precision compared to otolith-based age estimates for Walleye (Erickson 1983; Kocovsky and Carline 2000; Isermann et al. 2003). Agreement between otoliths and dorsal spine ages could vary in relation to the growth rate and longevity of fish in a population because annuli are often difficult to detect on old or slow-growing fish (Marwitz and Hubert 1995; Kocovsky and Carline 2000). Moreover, Walleye exhibit sex-specific differences in maturation schedule, growth, longevity, and size structure (e.g., Lester et al. 2000; Henderson et al. 2003). Determining whether agreement between otolith and dorsal spine age assignments varies in relation to factors such as length, sex, and growth would allow biologists to decide whether their choice of calcified structure could potentially result in different conclusions regarding the status of Walleye populations (Koenigs et al. 2013) and could also reduce the number of fish that are sacrificed for otolith removal in regions where fish sacrifice is a concern. In addition, with the exception of Koenigs et al. (2013), most previous studies provided guidance on otolith-spine age agreement for Walleye in relation to otolith age (Erickson 1983; Kocovsky and Carline 2000; Logsdon 2007; Koenigs et al. 2015). However, fish age is not known at the time of collection, and guidance on structure selection related to Walleye length and sex may be more relevant to biologists because these

metrics can be measured at time of capture. Although sex cannot always be identified, in many states (e.g., Iowa, Michigan, Minnesota, Wisconsin), structures for Walleye age estimation are collected during spring assessments when sex can be determined by extrusion of gametes from spawning fish. Consequently, defining sex- and length-based trends in otolith-spine age agreement may allow biologists to develop sampling guidelines that could be implemented using information that is always (length) or often (sex) available at the time of collection.

The method used to prepare calcified structures for enumeration of annuli represents another important aspect of the age estimation process (Quist et al. 2012). Two primary methods have been used to obtain age estimates from Walleye or Sauger *Sander canadensis* dorsal spines. The first method requires polishing the base of each dorsal spine with wetted sandpaper or some other polishing agent and then using a fiber optic light to illuminate the base of the spine under a dissecting microscope (i.e., no sectioning required; e.g., Logsdon 2007; Williamson and Dirnsberger 2010). The second method requires that transverse sections from the base of the spine are obtained using some form of saw (Borkholder and Edwards 2001; Isermann et al. 2003), and in some cases spines are embedded in epoxy before they are sectioned (Borkholder and Edwards 2001; Williamson and Dirnsberger 2010). Similarly, Walleye ages have been estimated from whole otoliths (Isermann et al. 2003), otoliths broken in half (Kocovsky and Carline 2000), or from transverse cross sections through the core of otoliths embedded in epoxy (Belanger and Hogler 1982; Koenigs et al. 2015). Thin sections of both spines and otoliths are often polished to some degree and then viewed under a dissecting microscope to enumerate annuli. Sectioning structures with a saw adds time to the age-estimation process (Isermann et al. 2003; Vandergoot et al. 2008) that could be avoided if simpler methods provide similar age estimates. For example, Isermann et al. (2003) reported that the mean time required to remove, prepare, and view whole Walleye otoliths was 96 s, whereas the mean time required for sectioned otoliths was 208 s. Similarly, Vandergoot et al. (2008) reported that mean processing and reading time for Yellow Perch *Perca flavescens* sectioned anal fin spines (204 s) was significantly greater than for cracked otoliths (96 s). Determining the consistency of Walleye ages assigned from the same calcified structure by using different preparation techniques would allow biologists to decide whether the additional processing time associated with sectioning is warranted. Consequently, our objectives were to evaluate whether 1) otolith and dorsal spine cross sections provided Walleye age estimates similar to simpler methods of preparation (e.g., whole otoliths and dorsal spines, cracked otoliths); and 2) between-reader precision and differences between spine and otolith ages varied in relation to Walleye total length (TL), sex, and growth rate.





**Figure 1.** Map of Wisconsin depicting locations of 35 water bodies sampled for Walleye *Sander vitreus* during 2011–2014. Numbered locations correspond to map identification numbers in Table 1.

## Methods

### Sampling

During 2011–2014, 735 Walleye were collected from 35 bodies of water in Wisconsin (Figure 1; Table 1) using a variety of sampling gears. With the exception of samples collected from Sturgeon Bay and Green Bay, which were tournament mortalities collected in July 2013 and May 2014, respectively, Walleye (TL range = 130–797

mm; Figure 2; Data S1, *Supplemental Material*) were collected during standard assessments conducted in March–May when most biologists in Wisconsin collect structures for Walleye age estimation. We attempted to obtain samples of Walleye that encompassed the full TL range of Walleye encountered during sampling on each body of water (Table 1). The majority of Walleye from lakes in northern Wisconsin were collected by the Great

**Table 1.** Names; sample size (*N*); and minimum, mean, and maximum total length (TL) of Walleye *Sander vitreus* collected from 35 water bodies in Wisconsin during 2011–2014. Map identification numbers correspond to numbered locations in Figure 1.

Map no.	Water body name	<i>N</i>	Minimum TL (mm)	Mean TL (mm)	Maximum TL (mm)
1	Annabelle	2	396	429	462
2	Big	10	345	434	584
3	Big Arbor Vitae	12	376	476	711
4	Big Gibson	2	338	411	483
5	Big Sand	10	333	435	582
6	Butternut	10	310	448	673
7	Caldron Falls	20	394	531	665
8	Chippewa	12	343	412	490
9	Cranberry	10	290	430	630
10	Delavan	55	190	416	620
11	Duck	10	363	427	561
12	Dunn	2	432	524	615
13	Eagle	10	312	425	561
14	Green Bay	50	381	586	779
15	Lac Courte Oreilles	10	318	463	610
16	Lac Vieux Desert	67	130	370	619
17	Mamie	12	310	471	648
18	Mississippi River	22	173	327	455
19	Oxbow	11	325	440	640
20	Pelican	12	330	453	615
21	Planting Ground	10	330	474	615
22	Red Cedar	5	356	370	401
23	Round	11	366	476	579
24	Scattering Rice	4	318	470	612
25	Shell	7	325	398	536
26	Squirrel	2	495	522	549
27	Stone	12	330	464	617
28	Sturgeon Bay	171	354	614	797
29	Turtle Flambeau Flowage	2	312	417	584
30	Tenderfoot	11	445	557	668
31	Trout	9	338	459	645
32	Twin	10	323	461	617
33	Wisconsin River–Kilbourn	26	343	508	668
34	Wisconsin River–Prairie du Sac	41	201	379	701
35	Wausau	65	226	435	739

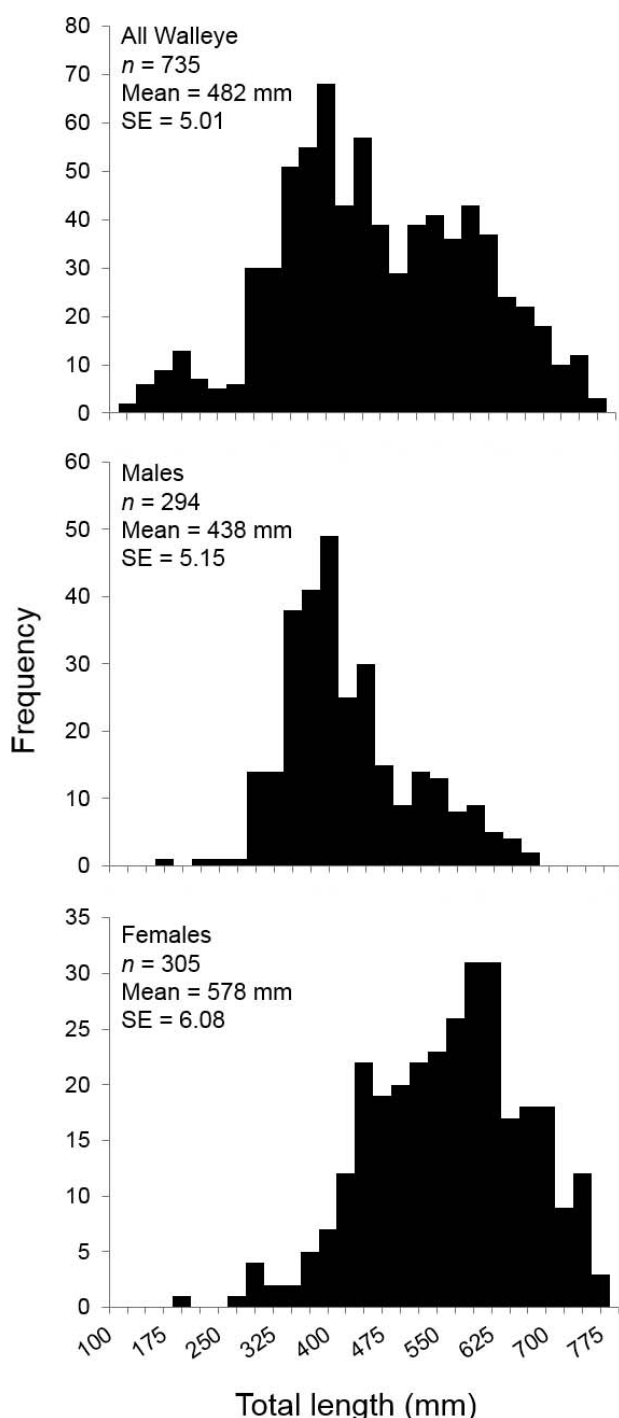
Lakes Indian Fish and Wildlife Commission in late March and early April 2012 as part of contaminant sampling and sample sizes were  $\leq 12$  fish per lake (Table 1). All Walleye were measured to the nearest millimeter, sex and maturation status were determined by visual inspection of gonads (when possible), and sagittal otoliths and the second dorsal spine were removed following methods described in several previous studies (e.g., Borkholder and Edwards 2001; Isermann et al. 2003; Logsdon 2007). Sagittal otoliths were removed, wiped clean, and stored in plastic vials. A pair of side cutters was used to remove the second dorsal spine at a point just above the flesh; spines were stored in coin envelopes. All structures were allowed to dry for  $\geq 2$  wk before additional processing.

### Objective 1

We used structures from 200 randomly selected Walleye to evaluate whether age assignments differed between methods used to prepare dorsal spines and otoliths. We also included all additional fish with sectioned otolith age estimates  $\geq 12$  ( $N = 36$ ) to increase the number of older fish in the sample, as we expected the potential for age assignment discrepancies to be

highest for relatively old Walleye. We compared side-illumination of whole spines and thin sections for estimating Walleye age from dorsal spines. The base of each dorsal spine was polished with wetted 1,000 grit sandpaper, and the spine (polished base facing up) was placed in a dish filled with plumber's putty. Dorsal spines were viewed under a dissecting microscope at  $\times 30$ – $60$  magnification. A drop of immersion oil was added to the base of the spine and annuli were illuminated by moving a fiber optic light (terminal aperture = 0.81 mm) around the edge of the spine. Annuli were enumerated by two experienced readers with more than 10 y of previous experience in assigning ages to freshwater fish (ages assigned to  $> 5,000$  fish) for state and provincial resource agencies using scales, otoliths, and dorsal spines. Specifically, the two experienced readers had previously assigned ages to  $\geq 1,000$  Walleye using these structures. For both structures and all preparation methods, only visible annuli were included in age assignments (i.e., readers did not count the edge of the structure as an annulus).

After ages were assigned to dorsal spines using the fiber optic light method, spines were embedded in



**Figure 2.** Total length (TL) distributions (25-mm TL bins) and mean TL for all Walleye *Sander vitreus*, males, and females collected from 35 Wisconsin water bodies during 2011–2014.

epoxy resin (Buehler Epo-Kwick®; Buehler, Ltd., Lake Bluff, IL) and a 1.5-mm transverse section was obtained from each spine using a low-speed saw (Buehler Isomet™ 1000; Buehler, Ltd.). Dorsal spine sections were mounted to a glass microscope slide with cyanoacrylic cement and viewed under a dissecting microscope at  $\times 40$ – $90$  magnification with transmitted light. A drop of

immersion oil was used to improve image clarity, and annuli were enumerated independently by the same two experienced readers.

We compared three preparation methods for estimating Walleye ages from sagittal otoliths: whole view, breaking otoliths in half along a transverse plane through the focus (i.e., cracking), and obtaining a thin transverse section through the focus using a low-speed saw. Whole view otoliths were placed in a black dish, submerged in water, and viewed under a dissecting microscope at  $\times 10$ – $30$  magnification using reflected light. One otolith was broken in half by hand along a transverse plane through the focus. The surfaces of the otolith halves exposed by the break were polished with wetted 1,000 grit sandpaper and placed in a dish of plumber's putty so that the polished surfaces were facing up. A drop of immersion oil was added to the polished surfaces, and the otolith halves were viewed under a dissecting microscope ( $\times 30$ – $60$  magnification) using the same fiber optic light to illuminate annuli. The second otolith from each fish was embedded in epoxy resin and a 1.2-mm transverse section through the focus was obtained from each otolith using a low-speed saw. Otolith sections were mounted to a glass microscope slide with cyanoacrylic cement and viewed under a dissecting microscope at  $\times 20$ – $80$  magnification with transmitted light. A drop of immersion oil was used to improve image clarity. For all three preparation methods, annuli were enumerated independently by the same two experienced readers without knowledge of fish sex or length.

We constructed age-bias plots (Campana et al. 1995) for each reader to evaluate whether age assignments were similar among different methods used to prepare each structure. For dorsal spines, age-bias plots were constructed by plotting mean whole dorsal spine age and 95% confidence intervals for all fish assigned a specific sectioned dorsal spine age. For otoliths, age-bias plots were constructed by plotting mean whole or cracked otolith age and 95% confidence intervals for all fish assigned a specific sectioned otolith age. Age assignments were considered significantly different between preparation techniques if 95% confidence intervals did not include the corresponding sectioned dorsal spine or otolith age (i.e., did not bind a 1:1 agreement line).

## Objective 2

Sectioned dorsal spines and otoliths from all Walleye were read independently by the two experienced individuals as described previously, and only visible annuli were included in age assignments. To evaluate whether age assignments differed between readers, age-bias plots were constructed by plotting mean spine or otolith ages assigned by reader 2 with 95% confidence intervals for all fish assigned a specific spine or otolith age by reader 1. Age assignments were considered significantly different between readers if 95% confidence



intervals around mean ages assigned by reader 2 did not include the age assigned by reader 1. To evaluate whether age assignments differed between structures, age-bias plots were constructed by plotting mean sectioned dorsal spine age and 95% confidence intervals for all fish assigned a specific sectioned otolith age. Age assignments were considered significantly different between structures if the 95% confidence intervals around mean spine age assignments did not include the specified otolith age. Age-bias plots were constructed for each sex and for all fish combined. We calculated complete and  $\pm 1$  y agreement rates and mean coefficient of variation ( $CV = [\text{standard deviation (SD)} / \text{mean age}] \times 100$ ) to compare between-reader precision between structures.

We used analysis of variance (ANOVA) to evaluate whether between-reader precision varied between structures and in relation to sex and growth rate. Growth of individual Walleye was ranked as slow, moderate, or fast using the 33rd and 67th percentiles calculated for distributions of TL-at-age estimated from 46,847 Walleye collected in Wisconsin Department of Natural Resources surveys; growth rankings were not sex specific. We calculated the 33rd and 67th percentiles for each distribution using the following formula:

$$x = \mu_i + Z\sigma_i,$$

where  $x$  is the 33rd or the 67th percentile,  $\mu_i$  is mean TL at age  $i$ ,  $Z$  is value from the standard normal distribution associated with the percentile  $x$ , and  $\sigma_i$  is SD for mean TL at age  $i$ . Walleye with TLs less than or equal to the 33rd percentile for a specific age were considered slow growing, fish with TLs that were greater than the 33rd percentile but less than or equal to the 67th percentile were considered to have moderate growth, and fish with TLs greater than the 67th percentile for a specific age were considered fast growing (Data S1). Growth rankings were assigned using sectioned otolith ages (i.e., visible annuli + 1 y) estimated by the most experienced reader (reader 1).

Age-difference plots (Campana et al. 1995) were used to evaluate differences between sectioned dorsal spine and otolith age assignments for individual fish in relation to TL (categorized using 25-mm bins). Mean differences between dorsal spine and otolith age assignments and 95% confidence intervals were plotted as a function of individual 25-mm TL groups. For a given TL group, dorsal spine and otolith age assignments were considered significantly different if the 95% confidence interval around the mean difference did not include 0. Mean differences  $>0$  would suggest that dorsal spines overestimate ages relative to otoliths, whereas mean differences  $<0$  would suggest that dorsal spines underestimate ages relative to otoliths. Age-difference plots were constructed for each reader and by sex or growth rate if ANOVA indicated that between-reader precision differed in relation to these two additional factors. All statistical tests were performed using the

Statistical Analysis System software package (SAS Institute, Inc. 2010) and decision probability was set at  $\alpha = 0.05$ .

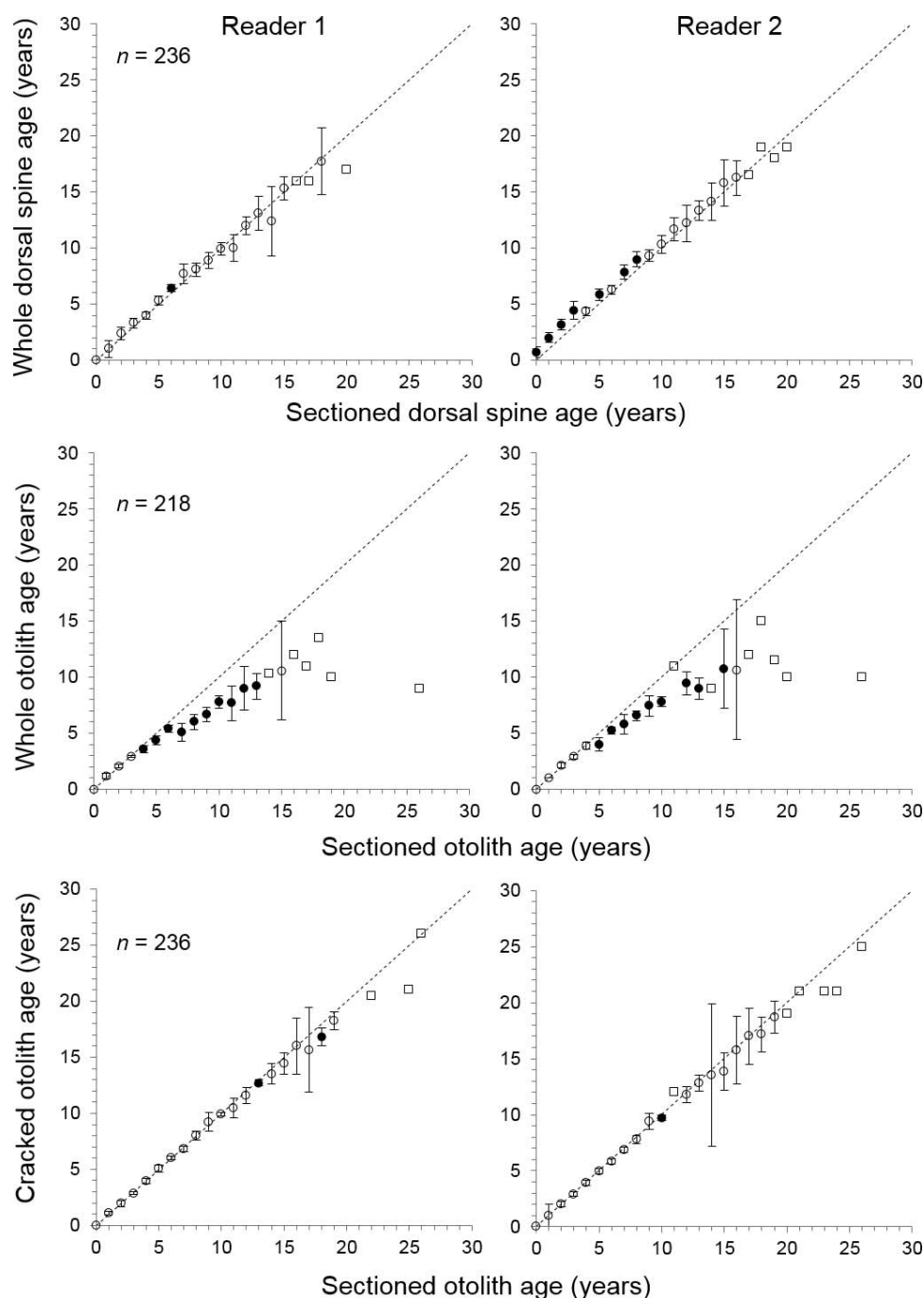
## Results

### Objective 1

We obtained 236 paired age estimates from whole and sectioned dorsal spines, 218 paired age estimates from whole and sectioned otoliths, and 236 paired age estimates from cracked and sectioned otoliths. Differences in sample size among groups were largely due to lack of a whole otolith for some fish. On average, ages estimated by reader 1 did not differ between methods used to view dorsal spines (Figure 3). Whole dorsal spine age was significantly greater than sectioned spine age at age 6, but this discrepancy was  $<0.5$  y. Reader 2 tended to assign higher whole dorsal spine ages to fish with sectioned spine ages  $\leq 8$ ; average discrepancies were  $\geq 1$  y at sectioned spine ages of 2–4 (mean difference = 1.0–1.4 y) and 8 (mean difference = 1.0 y). Trends in age assignment between whole and sectioned otoliths were similar between readers, with whole otoliths providing significantly lower age estimates than sectioned otoliths beginning at age 4 (reader 1) or age 5 (reader 2; Figure 3). Regardless of reader, average discrepancies between whole and sectioned otolith age assignments were always  $\geq 1$  y for all Walleye with sectioned otolith age estimates  $\geq 7$  (Figure 3). Mean ages assigned from cracked otoliths were generally similar to sectioned otolith ages for both readers. Mean ages estimated from cracked otoliths were significantly lower than sectioned otolith age in three instances, but average discrepancies were always  $<1$  y (Figure 3).

### Objective 2

We obtained paired age estimates from sectioned dorsal spines and sectioned otoliths for 294 males, 305 females, and 136 Walleye of unknown sex. Age estimates for reader 1 (most experienced) varied from 0 to 26 for sectioned otoliths (Figure 4) and from 0 to 20 for sectioned dorsal spines; estimates from reader 2 encompassed a similar range of ages (Figure 5). Complete agreement rate between reader age assignments was 76% (553 of 735) for otoliths and 54% (398 of 735) for dorsal spines; agreement rates within 1 y were 99% for otoliths (729 of 735) and 90% for dorsal spines (655 of 735). Dorsal spine age estimates from reader 2 were significantly different than estimates from reader 1 for fish that reader 1 assigned ages 2–5 (mean difference = 0.21–0.68 y), age 8 (mean difference = 0.36 y), and ages 10–12 (mean difference = 0.28–0.69 y; Figure 5). Otolith age estimates from reader 2 were significantly different than estimates from reader 1 for fish that reader 1 assigned age 1 (mean difference = 0.17 y), age 7 (mean difference = 0.22 y), and ages 10 and 11 (mean difference = 0.2–1.1 y; Figure 5). Because a few observed differences

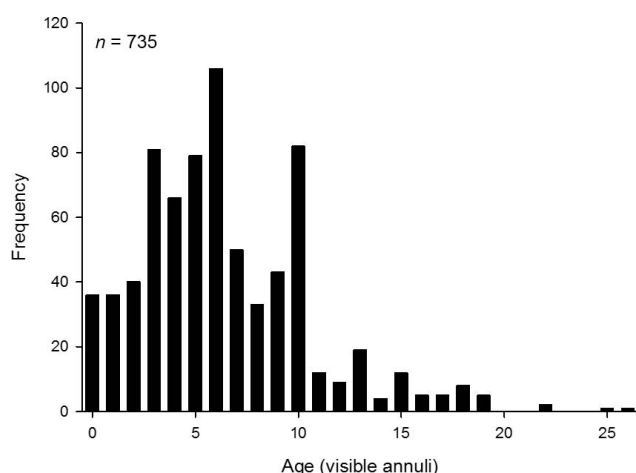


**Figure 3.** Age-bias plots comparing ages estimated using different methods of preparation for dorsal spines (top panel) and otoliths (bottom two panels) for two readers for Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014. The dashed line represents a 1:1 relationship in age assignments between preparation methods. Filled circles represent significant differences in age estimates between preparation methods, open circles indicate no significant difference, and open boxes represent ages with three or fewer observations. Error bars represent 95% confidence intervals.

in age assignments were  $\geq 0.5$  y, we did not combine reader age assignments in subsequent analyses.

Dorsal spine ages from both readers tended to be higher than otolith ages for fish with otolith ages  $\leq 6$  (Figure 6). Conversely, dorsal spine ages from both readers tended to be lower than otolith ages for fish

assigned otolith ages  $> 7$  (Figure 6). Regardless of reader, average discrepancies between dorsal spine and otolith age assignments were always  $< 1$  y for Walleye with otolith age estimates  $\leq 7$ . Discrepancies in age assignments between structures tended to increase progressively as otolith age increased beyond age 7 (Figure 6),



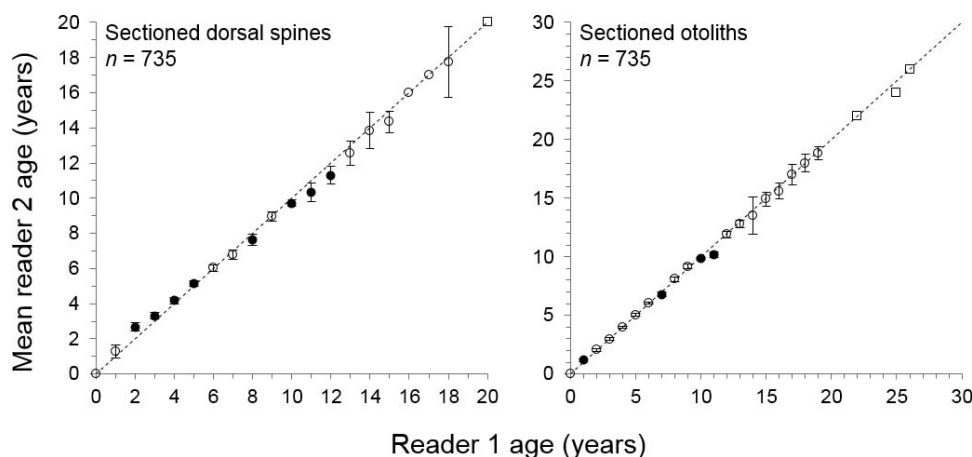
**Figure 4.** Age-frequency distribution based on ages estimated from reader 1 (the most experienced reader) by using sectioned otoliths for Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014.

and this trend was more apparent for males than females. For both readers, most of the significant differences (8 of 12) in age assignments between structures occurred for male Walleye with otolith age estimates  $>7$ , and average age discrepancies varied from 0.95 to 3.0 y (reader 1) and 0.66 to 2.4 y (reader 2; Figure 6). The majority (seven of nine) of significant differences in age assignments between structures for female Walleye also occurred for fish with otolith ages  $>7$ , and average age discrepancies varied from 0.44 to 2.4 y (reader 1) and 0.23 to 2.1 y (reader 2).

Between-reader precision was significantly higher ( $df = 1,734$ ;  $t = -8.45$ ;  $P < 0.01$ ) for otoliths (mean CV = 3.28%; standard error [SE] = 0.33%) than dorsal spines (mean CV = 9.20%; SE = 0.56%; Table 2). When using otoliths for age assignment, between-reader precision did not vary

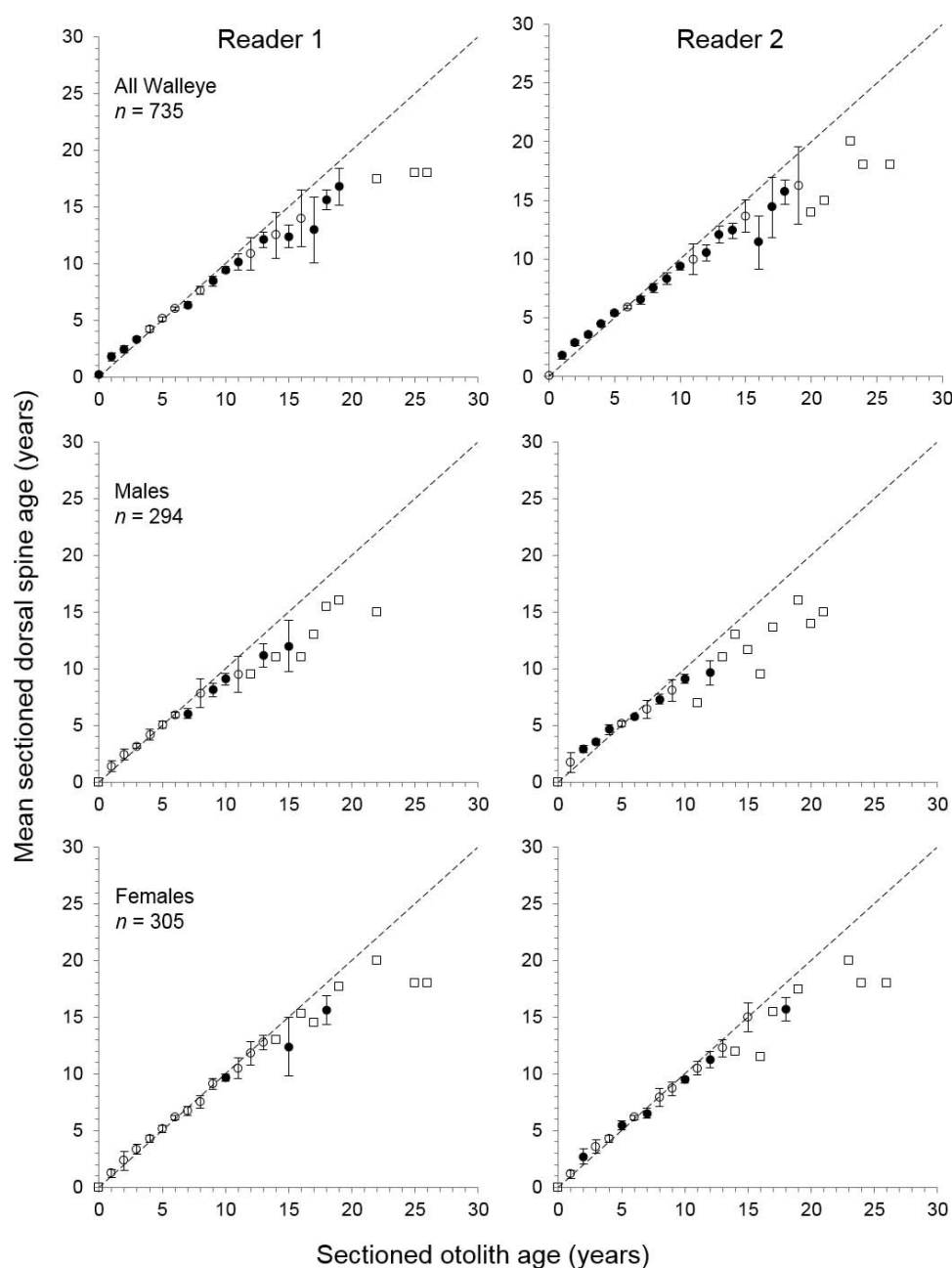
in relation to sex ( $df = 1,589$ ;  $F = 0.47$ ;  $P = 0.49$ ), growth rate ( $df = 2,589$ ;  $F = 0.04$ ;  $P = 0.92$ ), or the interaction between the two ( $df = 2,589$ ;  $F = 0.23$ ;  $P = 0.78$ ; Table 2). When using dorsal spines for age assignment, between-reader precision was significantly higher for female Walleye than for male Walleye ( $df = 1,589$ ;  $F = 8.71$ ;  $P < 0.01$ ), but precision did not vary in relation to growth rate ( $df = 2,589$ ;  $F = 0.85$ ;  $P = 0.43$ ) or the interaction between sex and growth rate ( $df = 2,589$ ;  $F = 1.73$ ;  $P = 0.18$ ; Table 2).

Because age assignments differed slightly between readers (Figure 5) and sex had a significant effect on between-reader precision when using dorsal spines for age assignment (Table 2), age differences plotted as a function of TL were constructed for each reader, for each sex, and for all fish combined. Differences between dorsal spine and otolith age estimates were generally similar between readers within each group of fish (i.e., all Walleye, males, and females; Figure 7). For both readers, the difference between dorsal spine and otolith age estimates tended to be  $<0$  for all Walleye  $\geq 450$  mm TL, but the majority (9 of 12) of significant differences in ages between structures occurred for fish  $\geq 600$  mm TL (Figure 7). Average discrepancies in age estimates between structures varied from 0.41 to 1.92 y for reader 1 and from 0.78 to 1.58 y for reader 2. For both readers, the difference between dorsal spine and otolith age estimates tended to be  $<0$  for male Walleye  $\geq 450$  mm TL, with 10 of the 11 significant differences in ages occurring for males  $\geq 450$  mm TL (Figure 7). Average age discrepancies for males varied from 0.60 to 2.75 y for reader 1 and from 0.50 to 3.0 y for reader 2. Regardless of reader, ages from otoliths and dorsal spines generally agreed for female Walleye  $<600$  mm TL. Dorsal spine ages tended to underestimate otolith age for females  $\geq 600$  mm TL, with six of the seven significant differences in ages occurring for females  $\geq 600$  mm TL. Average



**Figure 5.** Age-bias plots comparing ages estimated between two readers by using sectioned dorsal spines and otoliths for Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014. The dashed line represents a 1:1 relationship in age assignments between readers. Filled circles represent significant differences in age estimates between readers, open circles indicate no significant difference, and open boxes represent ages with three or fewer observations. Error bars represent 95% confidence intervals.





**Figure 6.** Age-bias plots comparing ages estimated using sectioned dorsal spines and sectioned otoliths for two readers for male, female, and all Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014. The dashed line represents a 1:1 relationship in age assignments between structures. Filled circles represent significant differences in age estimates between structures, and open circles indicate no significant difference. Error bars represent 95% confidence intervals. Open boxes represent ages with three or fewer observations.

discrepancies for female Walleye varied from 0.38 to 1.91 y for reader 1 and from 0.65 to 1.58 y for reader 2.

### Discussion

Although thin sections of otoliths and dorsal spines are often used for Walleye age estimation (Borkholder and Edwards 2001; Isermann et al. 2003; Koenigs et al. 2015), preparation has often included specialized equipment and additional processing time (e.g., Isermann et al. 2003; Vandergoot et al. 2008). For dorsal spines, our

results suggest that ages estimated from whole spines illuminated with a fiber optic light can replicate ages estimated from sectioned spines with less processing time and no need for a specialized cutting device. However, we did observe that both readers assigned higher whole dorsal spine ages to some Walleye that were assigned sectioned spine ages between 1 and 8 (mean differences  $\leq 1$  y), resulting in some significant differences in age estimates between the two preparation methods. Post-assessment discussion revealed that

**Table 2.** Sample size (*N*) and mean coefficient of variation (CV) with SE in parentheses for ages estimated independently by two experienced readers using sectioned otoliths and sectioned dorsal spines for Walleye *Sander vitreus* collected from 35 water bodies in Wisconsin during 2011–2014. Mean CVs (%) were calculated for each sex, for all Walleye combined, and for slow-, moderate-, and fast-growing individuals.

	<i>N</i>	Sectioned otoliths	Sectioned dorsal spines
All	735	3.28 (0.33)	9.20 (0.56)
Growth			
Slow	213	3.10 (0.36)	8.57 (0.88)
Moderate	177	3.49 (0.62)	8.09 (1.23)
Fast	345	3.28 (0.57)	8.47 (0.84)
Sex			
Female	305	2.88 (0.35)	5.60 (0.53)
Male	294	3.27 (0.43)	8.43 (0.68)

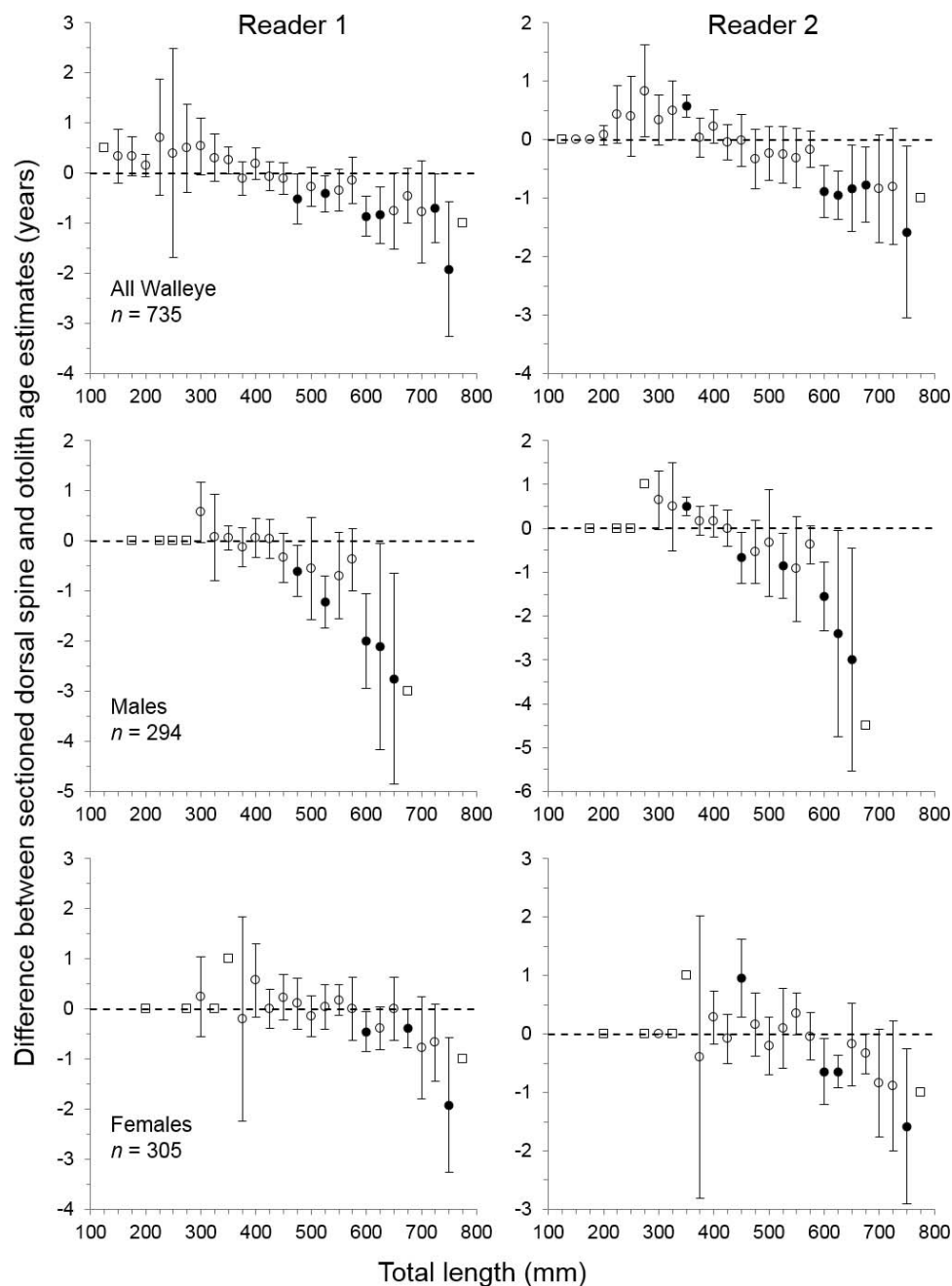
this occurred because readers detected an annulus on the outside edge of whole dorsal spines from some fish by using the fiber optic light, but they did not detect this annulus when viewing sectioned spines or otoliths. Or, readers were detecting the newest annulus on whole dorsal spines of some Walleye. Although we know of no paper specifically documenting the timing of annuli appearance in dorsal spines of percids, Graff et al. (2014) suggested that new annuli in Walleye otoliths appeared in July for fish collected from Lake Sharpe, South Dakota, whereas Blackwell and Kaufman (2012) reported that new annuli became visible on Yellow Perch otoliths in June or July. Furthermore, most of the Walleye in our study (55–63% depending on reader) with whole dorsal spine ages greater than ages from sectioned spines were either collected from the southern portion of Wisconsin where annulus appearance may occur earlier in the year or were mortalities obtained from Walleye tournaments that occurred in June or July. Consequently, identifying the timing of annulus appearance in Walleye dorsal spines relative to otoliths would be useful in defining age assignment guidelines.

Biologists generally do not use whole otoliths to estimate ages for all Walleye in a sample, but to save time, whole otoliths could be used to replicate sectioned otolith ages for fish where four or fewer annuli are visible in whole view. Otolith cracking or sectioning should occur where more than four annuli are visible in whole view or for any otolith that is difficult to interpret in whole view. Merely cracking otoliths in half and using a fiber optic light saves time compared to sectioning and was effective for replicating ages estimated from sectioned otoliths. Although our results suggest that simpler preparation techniques (i.e., whole dorsal spines and cracked otoliths) can yield similar age estimates to sectioned structures, choice of preparation techniques should depend on study objectives rather than just processing time (Koenigs et al. 2013). For example, if study objectives involve back-calculation of length-at-age, thin sections of otoliths or dorsal spines likely provide more consistent images and better planes for

accurate measurements than do cracked otoliths or whole spines (Quist et al. 2012). In such instances, the benefits of better images and measurements likely outweigh the costs associated with additional preparation time.

As noted in several previous studies (Marwitz and Hubert 1995; Kocovsky and Carline 2000; Isermann et al. 2003; Logsdon 2007; Koenigs et al. 2015), Walleye ages estimated using sectioned otoliths were more precise than those estimated using sectioned dorsal spines. We anticipated that between-reader precision would be lower for slow-growing individuals due to crowding of annuli and associated difficulties in annulus detection (Quist et al. 2012), but between-reader precision for either structure was not directly related to our growth categories. However, it should be noted that growth categories were defined without regard to sex, and differences in between-reader precision could potentially exist among sex-specific growth categories. For Walleye with ages estimated using dorsal spines, precision was lower among males than females. Kocovsky and Carline (2000) attributed differences in precision between sexes to sexually dimorphic growth and differences in age at sexual maturity. The difference in precision observed between dorsal-spine age estimates for male and female Walleye may be related to differences in timing of maturation between sexes. Male Walleye typically reach sexual maturity at an earlier age than females (Lester et al. 2000), somatic growth slows substantially after maturation, and slower growth results in crowding of annuli (Quist et al. 2012). Hence, crowding of annuli may begin earlier in life for male Walleye than for females, making it more difficult to discern annuli in dorsal spines of male Walleye. Despite variation among structures and sexes, the levels of between-reader precision we observed for both structures were similar to values reported in related studies (e.g., Isermann et al. 2003; Logsdon 2007; Koenigs et al. 2015) and were generally within the 5 and 10% levels of precision recommended by Campana (2001) as benchmarks in fish age estimation studies.

Similar to previous studies (e.g., Kocovsky and Carline 2000; Isermann et al. 2003), we found that ages from sectioned dorsal spines tend to overestimate otolith ages for Walleye assigned otolith ages  $\leq 6$ , but the average differences were generally small ( $<0.5$  y; e.g., Figure 5). Furthermore, Koenigs et al. (2015) showed that dorsal spines slightly overestimated known age of Walleye for fish at ages 2–5. This bias is consistent with misidentification of the innermost annulus on some, but not all, dorsal spine sections, which was previously noted by Isermann et al. (2003). If this problem was systematic, discrepancies in age assignments between structures might be resolved with specific decision rules or correction factors. However, our experience estimating ages for thousands of Walleye across North America suggest that problems identifying the first annulus on dorsal spines vary among populations, individual fish,



**Figure 7.** Age-difference plots depicting the mean difference between sectioned dorsal spine and otolith age assignments in relation to total length (25-mm total length bins) for two readers, each sex, and all Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014. The dashed line represents no difference between dorsal spine and otolith age assignments. Filled circles represent length bins where differences in ages between dorsal spines and otoliths were significantly different from 0, open circles indicate no significant difference, and open boxes represent length bins with three or fewer observations. Error bars represent 95% confidence intervals.

and individual readers and thus cannot be easily resolved.

We also observed that dorsal spine ages underestimate otolith ages for Walleye assigned otolith ages  $>7$ , which has been reported in several previous studies (Erickson 1983; Logsdon 2007; Koenigs et al. 2013) and has been attributed to difficulty in identifying outer annuli that may be more crowded in the spines of older

fish (Frie et al. 1989; Kocovsky and Carline 2000). As suggested previously, use of whole dorsal spines and a fiber optic light may allow some readers to better discern outer annuli on spines (e.g., Logsdon 2007; Williamson and Dirnberger 2010), which could alleviate this problem to some extent. However, Logsdon (2007) showed that ages estimated from whole dorsal spines using a fiber optic light underestimated otolith ages for Walleye from

Mille Lacs Lake, Minnesota, for fish that were assigned consensus otolith ages  $\geq 10$ . Our results verify those reported by Logsdon (2007), in that although whole dorsal spines yield slightly older age estimates than thin cross sections, they still underestimate fish age relative to otolith sections.

Comparisons of age assignments between structures are informative, but age-based sampling guidance is not always applicable to structure selection in the field as fish age is typically not known at the time of capture. Rather, guidance on structure selection related to Walleye TL and sex is more relevant to biologists as these metrics can be measured at time of capture. Although sex cannot always be identified, many state agencies (e.g., Iowa, Michigan, Minnesota, Wisconsin) collect structures for Walleye age estimation during springtime assessments when sex can be determined by extrusion of gametes from live spawning fish. Our results suggest that mean ages from sectioned dorsal spines and sectioned otoliths were generally similar for males  $< 450$  mm TL and females  $< 600$  mm TL, although spine ages tended to be slightly higher than otolith ages. Above these TL thresholds, dorsal spine ages tend to underestimate sectioned otolith ages. Similarly, Koenigs et al. (2013) reported that ages assigned to Walleye from Lake Winnebago, Wisconsin, by using dorsal spines and otoliths generally agreed for males  $\leq 457$  mm TL and females  $\leq 508$  mm TL, and Logsdon (2007) recommended that otoliths be used to estimate ages of Walleye  $\geq 530$  mm TL in Red and Mille Lacs lakes, Minnesota.

Similar to Koenigs et al. (2013), we suggest that length- and sex-based guidelines can be used to reduce the number of fish that are sacrificed for otolith removal in situations where this is a concern. Although some overestimation of otolith ages may occur, we suggest that dorsal spines can be used to estimate ages for male Walleye  $< 450$  mm TL and females  $< 600$  mm TL. If sex is unknown, we suggest dorsal spines be used to estimate ages for Walleye  $< 450$  mm TL, but that otoliths be used for fish  $\geq 450$  mm TL. Our length- and sex-based thresholds may be more broadly applicable to North American Walleye populations than those reported by Koenigs et al. (2013) for Walleye in Lake Winnebago, Wisconsin, because we incorporated samples of fish from many populations exhibiting different growth rates and longevity.

### Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

**Data S1.** All data are organized in a single tab, “Data S1 – Supplemental data.” The supplemental data tab contains the locations, fish identification number, total length, sex, growth category, age estimates assigned by two readers for sectioned otoliths and dorsal spines;

differences in age assignments between structures for each reader; and coefficients of variation for otolith and dorsal spine age assignments for Walleye *Sander vitreus* collected from 35 Wisconsin water bodies during 2011–2014. All headings and terms used in this data file are defined in the first tab, “Data dictionary.”

Found at DOI: <http://dx.doi.org/10.3996/052017-JFWM-038.S1> (58 KB XLSX).

### Acknowledgments

This research was funded by the Wisconsin Department of Natural Resources and was completed with substantial input from members of the Wisconsin Department of Natural Resources Fish Age Task Group. We thank all of the personnel with the Wisconsin Department of Natural Resources and the Great Lakes Indian Fish and Wildlife Commission that collected fish or structures that were used in our evaluation and C. Isermann for preparing all of the structures. B. Blackwell provided helpful comments on an earlier draft of the manuscript. This manuscript is dedicated to the memory of Eric Nothem, who along with B. Kissinger, completed pilot work for this project.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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