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Accuracy of Using Scales and Cleithra for Aging Northern Pike from an Oligotrophic Ontario Lake

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Abstract.—We examined the validity (accuracy and precision) of using scales and cleithra to assess the structure of the small population of northern pike *Esox lucius* (N = 220–270 fish age 3 or older) found in Squeers Lake, Ontario (48°31'N, 90°33'W). The high percent frequency of agreement, low index of average error, and low index of precision indicate that both scales and cleithra are equally suitable tissues for assigning age structure to Squeers Lake northern pike. An examination of the accuracy of age estimates through the use of oxytetracycline (OTC) labeling and mark–recapture of fish confirmed that marks identified on both tissues were in fact annuli. Cleithra, however, yielded more accurate results for northern pike older than age 10. Future northern pike age-assessment studies require further mark–recapture and OTC-labeling programs, particularly when precision levels exceed 5%. The collection of this additional data from any ongoing mark–recapture program would not only quickly validate the population's age structure, but would also ensure the accuracy of any future comparison based on age composition.

Scales have traditionally been used to age northern pike *Esox lucius* (Casselman 1974). However, a number of problems are inherent in the use of scales as the principal aging tissue. Evidence presented by Frost and Kipling (1959) and Casselman (1974), who used other bony tissues, suggested that scale ages often underestimated the age composition of northern pike following postmaturational growth and may therefore affect estimates of growth, population structure, age at maturity, and mortality. Because bony tissues preserve calcium, which is often resorbed from scales, they provide a more valid aging tissue (Simkiss 1974). Casselman (1974, 1979) showed that cleithra yield much more accurate results than do scales for aging esocids. The use of cleithra, however, necessitates killing the fish being sampled.

Our specific objective was to use age validation to ensure the accurate assessment of the age structure of northern pike in a small (384.5-hectare), oligotrophic northwestern Ontario lake. The small size of the lake's population (i.e., 220–270 fish age 3 or older; Laine 1989) required that few fish be killed. Most age data, therefore, were obtained from scales collected from live fish. Initially, scale and cleithrum ages from all sampled fish were compared to determine whether the same number of annuli was formed on both aging tissues. Such a comparison does not validate an age assessment, but simply provides a measure of agreement. It does, however, provide some indication of the confidence to be placed in the interpretations (Beamish and McFarlane 1983, 1987; Casselman 1983). Beamish and McFarlane (1983) encouraged validation of assigned ages to prove accuracy. They emphasized the importance of accurate age assessment in understanding life history traits. Validation is considered successful only when the growth zones considered to be annuli are demonstrated to form annually for all age-groups in a population. This validation can be accomplished either through the use of mark–recapture studies or through the capture of known-age fish from the population (Beamish and McFarlane 1983, 1987). To ensure that zones identified as annuli on both aging tissues actually formed once a year, we examined fish whose time of release was known from (1) a multiyear mark–recapture program and (2) the injection of oxytetracycline (OTC) into a subsample of northern pike during initial tagging to establish a temporal reference mark. Oxytetracycline binds with proteins in the blood and is incorporated only in newly forming and mineralizing bone and cartilage (Frost et al. 1961). The use

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of such mark–recapture techniques and the placing of a spatial and temporal orientation mark in calcified tissue (such as through the use of OTC) not only provides the scientific basis required for age and growth assessments, but also reference material for improving subsequent interpretations (Casselman 1983).

Methods

In 1982, 1984, and 1985, aging tissues were collected from all northern pike sampled from Squeers Lake, Ontario (48°31′N, 90°33′W). During spring population estimates, fish were anesthetized with tricaine (MS-222), sampled, and released. Sampling included determination of sex by external means if possible; recording any previous tagging history; the placement of an individually numbered oval tag (Floy tag FTF 69) with polyethylene filament immediately anterior to the dorsal fin; the administration of an accessory clip to the pectoral fin in 1982, the caudal fin in 1984, and the dorsal fin in 1985; and the collection of a scale sample from above the lateral line, just anterior to the dorsal fin, on the left side of the fish. Scale samples were taken from the same location on the right side of the fish upon subsequent recapture. A portion of the fish captured in 1982 (N = 44) and 1984 (N = 75) were also given an intraperitoneal injection of 0.5 mL oxytetracycline (brand name, Liquimycin)/kg of body weight to produce a temporal reference mark on bony tissues (Weber and Ridgeway 1962; Kobayashi et al. 1964). Cleithra were collected from all northern pike killed during subsequent monthly gillnetting. A more detailed description of the netting activities carried out for this study is provided by Laine (1989).

Sampled fish represented all size-classes within the population. The sample included 77 young of the year and 52 yearling northern pike, which were used to identify and locate the first annulus. A total of 44 (35%) of the 125 northern pike over age 2 from which cleithra were collected had been injected with OTC.

Ages for all northern pike sampled were determined by counting the number of annuli visible on scale impressions made on cellulose acetate slides by a hand-operated roller. Annuli were assigned following the criteria outlined by Frost and Kipling (1959) and Casselman (1967). The scale impressions were read on a microfiche reader at 24–33× magnification. The location of each year increment and the presence of any growth beyond the outermost annulus were marked on a paper slip for future back-calculation.

Cleithra were viewed at 4× magnification through a magnifying lamp against a black background. Annuli were assigned following the criteria outlined by Casselman (1979). All cleithra were also examined under a dissecting microscope at 6× with an ultraviolet light source (Ultraviolet Products, model R51) for the presence of OTC marks. The location of the OTC label on the marked cleithrum with respect to the outside edge was noted, and the number of apparent annuli outside the mark was recorded. To ensure unbiased reading and that the mark identified as an OTC label was not due to natural fluorescence, all cleithra were examined under ultraviolet light in order of collection. Doubtful marks present on any of the cleithra were not considered to be an OTC label.

Precision of interpretation between tissues from the same individual fish and between two readers was calculated initially by determining the percentage of agreement. However, percent agreement will only assess whether an age assigned by one method is equal to that determined by another method. It does not quantify the magnitude of differences (Mills and Beamish 1980), nor does it account for the number of age-classes within the population (Beamish and Fournier 1981). Therefore, alternative indices were also calculated to determine the reproducibility of the ages assigned between tissues and agers. Beamish and Fournier (1981) calculated an average percent error (APE) in aging the jth fish as:

$$\text{APE} = 100\left(\frac{1}{R}\sum_{i=1}^{R}\frac{|x_{ij} - x_j|}{x_j}\right);$$

R is the number of times the sample was aged; x_{ij} is the ith age determination of the jth fish; and x_j is the average age calculated for the jth fish. Alternatively, Chang (1982) proposed the use of the coefficient of variation (V) and an index of precision (D):

$$V = 100 \times \text{SD/mean},$$

and

$$D = V/R^{\frac{1}{2}}.$$

These measurements are not independent of age, but provide a statistical basis to evaluate the reproducibility of age estimates between tissues or agers, or both.

Results

Comparison of Scale and Cleithra Ages

Scales and cleithra from Squeers Lake northern pike exhibited a clear pattern of growth zones.

TABLE 1.—Percent frequency of agreement, average percent error (APE), coefficient of variation (V), and index of precision (D) for northern pike ages from samples collected from Squeers Lake, Ontario, as determined from scales (Sc) and cleithra (Cl) by two readers.

Reader	Structure	N	Percent agreement within				APE (%)	V (%)	D (%)
			0[a]	1 year	2 years	3 years			
			Comparisons between structures						
1	Sc and Cl	155	92	99	100		0.56	0.80	0.57
2	Sc and Cl	57	72	89	97	100	2.51	3.86	2.73
			Comparisons between readers						
1 and 2	Sc	113	78	93	97	100	1.78	2.51	1.73
1 and 2	Cl	61	88	93	98	100	1.15	1.20	0.85

[a] Percent of samples with 100% full agreement.

There was 92% total agreement and 99% agreement within 1 year between the ages assigned to the two tissues by the first reader (Table 1). Although disagreements between the two tissues occurred as early as age 4, there was a high level of precision of ages obtained from the two aging structures. Whenever disagreements occurred, cleithral ages were older than scale ages.

A definite difference was evident with respect to aging fish of different sexes. There was 100% agreement between the scale and cleithral ages in females. However, some aging difficulties were encountered with both tissues collected from males older than age 8. The problems stemmed from the crowding of annuli at the edges of the tissues (as a result of the near cessation in growth of these fish) and the presence of more false annuli, particularly on the cleithra.

Ages assigned to a subsample of 57 fish by a second reader produced an overall lower percentage agreement (72%) between the two tissues. The APE, V, and D values, however, did not exceed 4% (Table 1), which still reflects high precision.

In comparisons of the ages assigned by two readers to the same structure, cleithra gave the best results, with 88% total agreement. Both scale and cleithral ages, however, had 93% and 97–98% agreement within 1 and 2 years, respectively. Furthermore, the APE, V, and D values were all less than 3% (Table 1). Overall, reader 1 assigned older ages to the same tissue relative to reader 2 (Laine 1989).

Time of Annulus Formation

The major source of discrepancy in ages assigned by the two readers lay in interpreting whether an annulus had been formed on samples collected during early summer. Therefore, the time of annulus formation for Squeers Lake northern pike was determined by examining scales and cleithra collected during April–September. This information was supplemented by the multiple recapture of tagged fish over the course of the same year.

The time of annulus formation varied with both age and type of aging tissue. No new growth (as defined by the presence of a minimum of one to three circuli on scales or any opaque material on cleithra after the outermost annulus) was evident at the outer edge of either tissue collected from immature (age-1 and -2) northern pike until mid-May to early June (Figure 1). Mature fish (≥age 3) did not display annulus formation until mid to late June, and annulus formation was not fully complete on either aging tissue until early August. There was still some difficulty in interpreting the edge of the aging structure at this time, particularly with samples collected from older, slower-growing males. In several instances it could not be determined whether the narrow band of translucent material on the outside edge on these tissues had been formed previously or within a given calendar year. This strongly influenced interpretations of whether annulus formation had actually occurred for a number of the fish sampled in July and August (Figure 1). In all cases, annulus formation on scales preceded such development on cleithra.

Validation of Scale and Cleithral Age Increments

Comparing age at release with age at recapture of tagged fish indicated that either scales or cleithra are valid for aging Squeers Lake northern pike to at least age 10 (Table 2). The increase in the number of scale-annulus increments agreed with the total time (in years) between tagging and recapture 90.5% of the time. Of those fish aged incorrectly, 84% (16) were underaged by 1 year. There was 100% agreement between assigned cleithra age and expected age.

Of the 139 fish injected with OTC in 1982 and

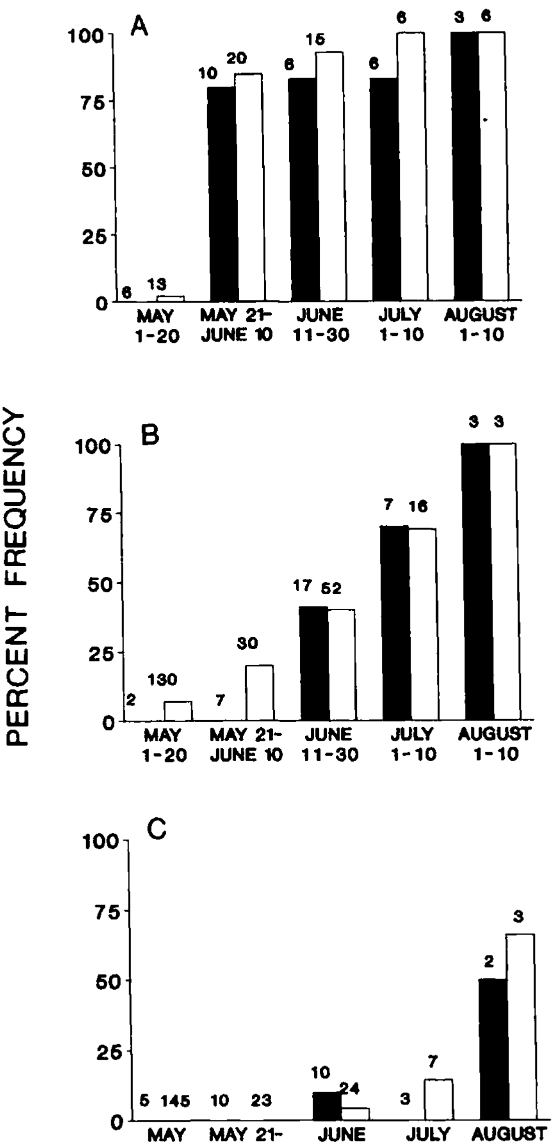

FIGURE 1.—Time of annulus formation for (A) subadult (age-1 and -2), (B) mid adult (age-3–5) and (C) old adult (older than age-5) northern pike as determined by the presence of circuli following the outermost annulus on cleithra (solid bars) and scales (open bars) collected from fish sampled from Squeers Lake, Ontario. The number above each bar indicates sample size.

1984, a total of 44 (37%, including two 1982 and one 1984 tag losses) were recaptured between 1982 and 1985. A clear OTC mark was apparent on 33 (75%) of these cleithra. The mark was visible on the majority of the samples as a bright yellow band under ultraviolet light, particularly on the anterior third of the cleithrum.

TABLE 2.—Comparison of the expected and actual numbers of annuli formed on (1) scales collected from recaptured northern pike initially tagged in Squeers Lake, Ontario, during 1982, 1984, and 1985, and (2) cleithra following oxytetracycline (OTC) labeling for northern pike recovered 1 month to 3 years following initial capture and intraperitoneal injection of 0.5 mL OTC/kg body weight in Squeers Lake, Ontario. Underlined values are numbers of northern pike that formed the expected number of annuli during the time at large before recapture.

Number of annuli formed	Number of years after tagging 0	1	2	3	Age-groups represented
Validation of scales by mark-recapture					
−1	8				
0	87	4			1–12
1		67	2	2[a]	3–9
2	1	1	10	2	6–10
3				7	7–11
Validation of cleithra with OTC marks					
0	7				3–7
1		20			3–8
2			1		6
3				5	7–11

[a] Sample number represents the same fish caught twice over the 1985 field season.

The cleithral age of fish recaptured 1–3 years after initial release had correspondingly increased by 1–3 years at recapture (Table 2). Scale ages for 30 of the 33 fish sampled also agreed with cleithral ages assigned.

Discussion

The high percent frequency of agreement, low index of precision, and low index of average error among scales, cleithra, and readers (Table 1) indicate that both scales and cleithra are equally suitable tissues for assigning ages to Squeers Lake northern pike. Some disagreement occurred in assigned ages given by the two readers for both aging tissues. However 35 and 66% of the ±1-year disagreement between scales and cleithra, respectively, was accounted for by the presence or absence of growth at the margin. This type of disagreement underlines the importance of using a coding system, such as the one developed by Casselman (1983), that describes the number of annuli present and the condition of the outside edge of the scale following the last annulus, and documents the time of annulus formation of all age-groups within a given population. The results follow a general trend for most fish species such that, when differences between aging tissues occur, scale ages are generally lower than corresponding ages determined from an alternative bony tissue.

Results from the present study strongly support previous findings that the time of annulus formation depends on age and occurs earlier in young, sexually immature fish (Casselman 1967, 1974). Casselman (1967) believed that temperature was the most important factor controlling linear growth and annulus formation for immature northern pike. However, in mature northern pike, Casselman (1967) believed that annulus formation represented a combination of a cessation in growth related to decreasing water temperature; the energetic demands of gonadal recrudescence; and a delay in the resumption of growth in the spring due to spawning.

The problems associated with not validating age determinations have been discussed by Beamish and McFarlane (1983, 1987) and Casselman (1983). For example, the wide range in growth rates exhibited by northern pike in a localized region such as northwestern Ontario (Laine 1989) emphasizes the importance of age validation to ensure the accuracy of the age determinations. However, despite the number of studies examining the age and growth of northern pike, only three involved actual validation of the aging techniques either through the use of mark–recapture (Frost and Kipling 1959; Casselman 1967) or OTC-marking methods (Casselman 1974).

The results from the present mark–recapture and OTC-marking experiments demonstrated the validity of using either scales or cleithra to assign ages to Squeers Lake northern pike. Upon recovery of these fish and examination of the scales or cleithra, (1) the number of expected zones had formed beyond the previously identified age and (2) the number of zones following the OTC mark always equaled the number of years at liberty. Our results did fail to validate assigned scale ages greater than age 10. Therefore, these fish could not be included in the assessment of any age-related characteristics for the population.

On the basis of our results, any future study to assess ages of northern pike populations should at least initially include the use of both scales and cleithra for age assignment. Should the results demonstrate that precision levels between readers and tissues are high, scales should be the favored tissue because they still provide a much quicker method of age assessment. This also allows for the opportunity of live release and thereby substantially lowers sampling mortality. Ricker (1975) and Powers (1983) both felt that 10% precision is acceptable. However, they were dealing with marine fisheries having species with 20 or more age-classes. When examining populations of shorter-lived species such as northern pike, which rarely have more than 10 major age-classes, a maximum APE, V, or D of 5% would ensure that the method is both consistently repeatable and practical (Prince et al. 1985). A mark–recapture or OTC-labeling program should also be considered in all cases, particularly when precision exceeds 5%. The ease of analysis of this additional data collected from any ongoing mark–recapture program would not only quickly validate the population's age structure, but also would ensure the accuracy of any comparisons based on age composition.

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