Fisheries Management
and Ecology

Fisheries Management and Ecology, 2011, 18, 424–430

Precision of age estimates derived from scales and pectoral fin rays of blue sucker

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Abstract Evaluating the response of fish populations to environmental influences requires precise age estimation. Pectoral fin rays and scales were compared to determine which is more precise for estimating age of blue sucker, *Cycleptus elongatus* (Lesueur), from the Missouri River, USA. Mean coefficient of variation was significantly lower for fin rays both among three novice readers (rays 19.9%; scales 23.1%) and among three reads by a single experienced reader (rays 11.8%; scales 18.6%). Additionally, exact per cent agreement (rays 20.0%; scales 14.3%) and within-1-year per cent agreement (rays 89.1%; scales 73.9%) were higher for fin rays compared with scales for multiple reads by the same experienced reader. Age–bias plots revealed that scale ages were lower than fin-ray ages > 7. The use of fin rays is recommended for estimating ages of blue sucker until validation procedures are conducted.

KEYWORDS: ageing procedures, *Cycleptus elongatus*, Missouri River.

Introduction

The blue sucker, *Cycleptus elongatus* (Lesueur), is native to large rivers of North America from Montana to Pennsylvania, USA, and south to Mexico. Although sufficiently abundant to support commercial fisheries in the past, blue sucker currently are a species of special concern throughout most of their range (Jelks *et al*. 2008). Assessment of population viability and population responses to habitat alteration and conservation efforts requires effective methods for ageing blue sucker.

Accuracy is critical effective age determination procedures. Validation of accuracy can be assessed through recovery of marked fish, monitoring modal progression, marginal increment analysis, or captively rearing fish from hatch (Campana 2001). This is difficult to achieve for blue sucker because of the wide geographic range, high mobility (Neely *et al*. 2009), long life (Peterson *et al*. 1999; Bednarski & Scarnecchia 2006) and lack of captive-rearing protocols (Jeff Powell, personal communication). Nevertheless, blue sucker age has been estimated using scales (Beal 1967; Rupprecht & Jahn 1980; Moss *et al*. 1983; Hand & Jackson 2003; Morey & Berry 2003; Vokoun *et al*. 2003), pectoral fin rays (Rupprecht & Jahn 1980; Bednarski & Scarnecchia 2006; Eitzmann *et al*. 2007; Bacula *et al*. 2009), opercles (Bacula *et al*. 2009) and otoliths on the daily level (Adams *et al*. 2006). Unfortunately, validation has not been attempted for any of these structures. However, fin rays have been validated for white sucker, *Catostomus comersonii* (Lacepède) (Beamish & Harvey 1969; Quinn & Ross 1982).

Ageing structures that require sacrifice of the specimen are increasingly used to age many species (Beamish & McFarlane 1987). Otoliths have been found to be accurate for arctic grayling, *Thymallus arcticus* (Pallas) (DeCicco & Brown 2006), common carp, *Cyprinus carpio* (Linnaeus) (Brown *et al*. 2004) and white sucker (Thompson & Beckman 1995). Opercles have been validated for the south-eastern blue sucker, *Cycleptus meridionalis* (Burr and Mayden) (Peterson *et al*. 1999), and the Cui-ui, *Chasmistes cujus* (Cope) (Scoppettone 1988). However, sampling protocols often do not always allow sacrificing of the fish. Considering the 'vulnerable' conservation status (Jelks

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 doi: 10.1111/j.1365-2400.2011.00804.x

et al. 2008) and federal designation of blue sucker as a ‘species of concern’, managers should be concerned that the removal of blue sucker for ageing could adversely affect already-low blue sucker population densities. As an alternative, scales and fin rays allow for estimating age without lethal take. Therefore, precision estimates of scales and pectoral fin rays are essential components for selecting the proper age estimation method for blue sucker.

The objective of this study was to compare age estimates and precision between scale and pectoral fin rays. Further, this assessment also provides estimates of removal, processing, viewing and measuring times for scales and fin rays to allow assessment of the efficiency of the structures for ageing blue sucker.

Materials and methods

Field collection procedures

Blue sucker were collected from the Missouri River below Gavin’s Point Dam [river kilometre (rkm) 1305], South Dakota, to the mouth at St. Louis, Missouri (rkm 0), USA using trammel nets, otter trawls and gill nets according to the methods and specifications outlined in Drobish (2005) during July through November 2005. Total length (TL) was measured to the nearest mm. A 1- to 2-cm portion of the left pectoral fin ray was removed by cutting approximately 1 cm distal to the articulation with the pectoral girdle using a gardener’s shears. This location was selected because of concerns that removing the fin ray at the articulation could be lethal to the fish, which would be unacceptable because of the imperilled status of the species. A minimum of 10 scales were removed from the left side of the fish between the lateral line and dorsal fin (DeVries & Frie 1996) with a knife. Scales and fin-ray sections were stored in paper coin envelopes.

Among-reader analysis

Scales from each fish were pressed between acetate slides (0.5-mm thick) using a scale press. Fin rays were boiled in de-ionised water to separate the first fin ray from the second fin ray and other attached tissues (Fig. 1) and brushed clean. Once dried, rays were embedded in epoxy (Allied High Tech, Rancho Dominguez, CA, USA), and three transverse sections approximately 0.15–0.55-mm thick were cut from the proximal end using a low-speed saw.

Fin rays and scales (acetate impressions) were viewed under a dissecting microscope at 6.3–40× magnification. Criteria for identifying pectoral fin-ray annuli followed the description of zonation under reflected light in Casselman (1983). The reader assigned age from the fin-ray section with the most easily discernable presumptive annuli (hereafter, annuli, i.e. sections having complete concentric annuli and distinct transitions between opaque and translucent growth zones). Criteria for identifying scale annuli followed DeVries and Frie (1996); an annulus was identified where broken circuli and ‘cutting over’ occurred on the posterior half of the scale. Scales were viewed using transmitted light. An age was assigned for each fish when a reader assessed three scales that agreed in age.

Possible bias between scales and fin rays was examined with age–bias plots (Campana *et al.* 1995). Mean scale age was calculated for each age assigned from fin rays. Linear regression was used to model the relationship between mean scale age and fin-ray age. Bias between scales and fin rays was concluded whether the slope differed from one or the intercept differed from 0 (Isermann *et al.* 2003). Statistical significance for this and all tests was declared at $\alpha = 0.05$. Separate age–bias plots were evaluated for each reader.

Three readers independently estimated age for each fish from both scales and fin rays. Readers had

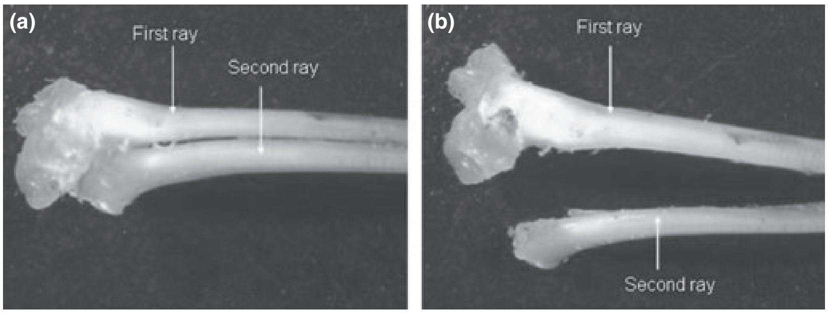

Figure 1. Anterior view of the first pectoral fin ray from the left side of a blue sucker (a). The first ray was disarticulated from the second ray before being placed into epoxy moulds (b).

experience ageing other fish species using scales and otoliths but lacked experience ageing fish from spines and ageing blue sucker. Each reader assigned ages from scales for all fish before ageing fin rays. Age assessments of all fish were done in random order and without information about fish length.

Exact and within-1-year per cent agreements were used to quantify precision (Beamish & Fournier 1981; Hoenig *et al.* 1995). Exact per cent agreement was defined as the proportion of fish that were assigned the same age by all three readers. Within-1-year agreement was defined as the proportion of all fish for which age estimates among the three readers were ±1 year of the median assigned age. Partial per cent agreement was also used to assess precision and was defined as the proportion of fish for which two of three readers assigned the same age. Fish for which all three readers estimated the same age were not included in the partial agreement calculation. Among-reader coefficient of variation (CV) ([standard deviation/mean] × 100) was calculated following Chang (1982).

Within-reader analysis

Scales were brushed free of debris in preparation for wet-mount viewing between two microscope slides. Scales were viewed under transmitted light with varying magnifications from 6.3 to 40×. Fin rays were prepared the same as in the among-reader analysis, but three additional sections were cut from each fin ray. Hence, the sections examined for the within-reader analysis were taken more distally from the body than the samples from the among-reader analysis.

One reader assigned ages to both scales and rays from each fish three times (i.e. replicates). The reader was experienced in estimating ages from more than 1500 blue sucker using fin rays and scales. Age assessments of all fish were performed in random order and without knowledge of fish length or time of collection, and fish were rerandomised before each reading. Similar to the among-reader analysis, exact, within-1-year and partial per cent agreements were determined, and within-reader mean CV was used to quantify precision between the two methods. A paired *t*-test was used to test for differences in CV between scales and fin rays.

Field removal, processing and viewing times

Field removal time was defined as the time to remove at least 10 scales or one fin-ray portion from the fish. The processing of fin rays required four stages of processing: cleaning, plugging moulds, loading epoxy and sectioning. Plugging the moulds with petroleum jelly was required to prevent epoxy from running out of the mould. Loading epoxy was time that was required to inject each mould with epoxy. Each phase was timed by recording the time required to complete fin-ray processing for a group of five fish. Processing time for scales included the time required to clean and press at least 10 scales from each fish for a group of five fish. Viewing time was defined as the time required to make age determinations for a group of five fish. Removal times and viewing times were compared with a paired *t*-test.

Results

Scales and fin rays were collected from 230 blue sucker. Captured fish ranged in length from 289 to 797 mm. Fish were aged 0–16 years with scales and 0–22 years with fin rays. Age assignments for an individual fish differed by as much as 9 years for scales and 11 years for fin rays among the three readers. Thirty per cent of all scales collected were regenerated, and age could not be assigned.

Among-reader analysis

All three readers assigned greater scale ages than fin-ray ages for fish aged 1–3 by fin rays (Fig. 2). All three readers assigned lower ages with fin rays for fish with fin-ray ages <7 years and with more than three data points. The slopes and intercepts of the scale age–fin-ray age relationships were significantly different from 1, and intercepts were significantly different from 0 for all three readers.

Exact per cent agreement and partial agreement were greater for fin rays than for scales (Table 1). Within-1-year agreement was greater for scales. Among-reader CV was significantly lower for fin rays than for scales ($t = -2.62$, $P = 0.009$).

Within-reader analysis

Exact, within-1-year and partial per cent agreement were greater for fin rays than for scales (Table 2). CV was significantly lower for fin rays than for scales ($t = -6.01$, $P < 0.001$; Table 2).

Field removal, processing and viewing time results

Mean (±SE) field removal time for scales (13.0 s ± 0.28) was not significantly different ($t = 1.83$; $P = 0.068$) from fin rays (13.7 ± 0.27 s; Table 3).

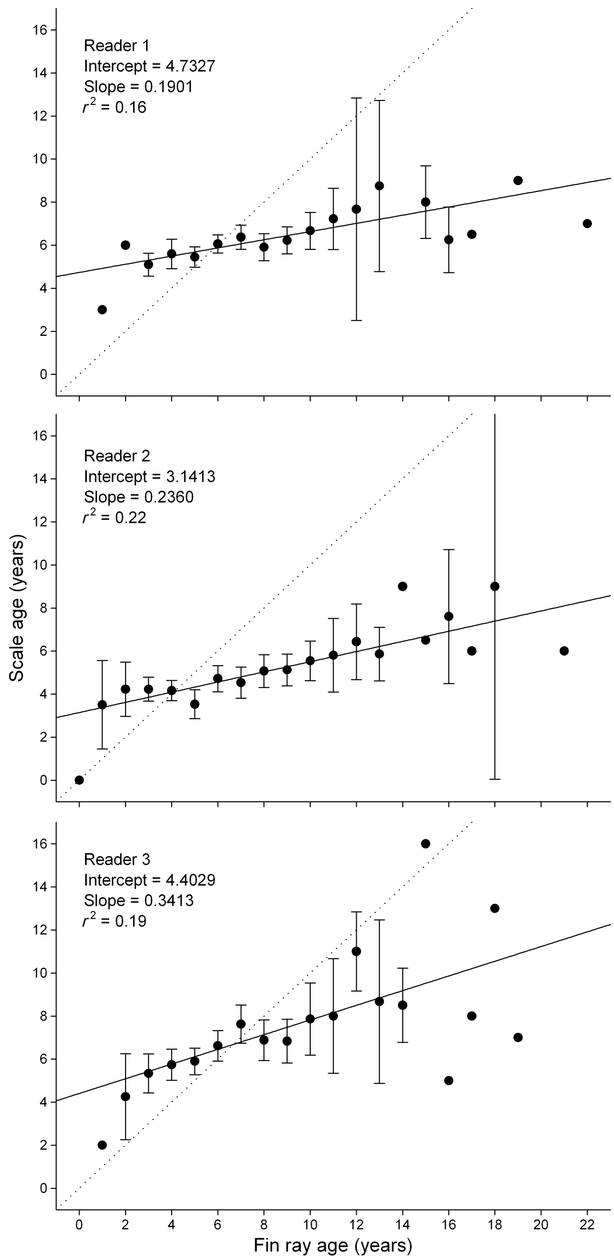

Figure 2. Age–bias plots for 230 blue sucker aged by scales and pectoral fin rays by three readers. The dashed line represents perfect agreement between ages assigned from scales and fin rays. The solid line and associated statistics represent the relationship between scale age and fin ray age. Intercepts were significantly different from 0 ($P < 0.001$), and slopes were significantly different from 1($P < 0.001$) for all three readers.

Mean viewing time was significantly less for fin rays than it was for scales ($t = 3.24$; $P = 0.002$). Total processing time was greater for scales than it was for rays because of the time required to clean scales.

Table 1. Exact per cent agreement, within-1-year per cent agreement, per cent partial agreement and mean among-reader coefficient of variation (CV) calculated from age estimations made among three novice readers on 230 pairs of blue sucker scales and pectoral fin rays

Ageing structure	Percent agreement: Exact	Percent agreement: Within-1-year	Percent partial agreement	CV
Fin rays	8.3	57.8	50.2	19.9 (0.92)
Scales	6.1	58.3	46.6	23.1 (0.93)

Percent partial agreement is the proportion of fish for which only two of three reader replicates agreed in age. Standard errors are in parenthesis. CV values are significantly different ($t = -2.62$, $P = 0.009$).

Table 2. Exact percent agreement, within-1-year per cent agreement, per cent partial agreement and mean within-reader coefficient of variation (CV) calculated from age estimations made among three replicated readings from one experienced reader on 230 pairs of blue sucker scales and pectoral fin rays

Ageing structure	Percent agreement: Exact	Percent agreement: Within-1-year	Percent partial agreement	CV
Fin rays	20.0	89.1	80.9	11.8 (0.56)
Scales	14.3	73.9	63.9	18.6 (1.00)

Percent partial agreement is the proportion of fish for which only two of three reader replicates agreed in age. Standard errors are in parenthesis. CV values are significantly different ($t = -6.01$, $P < 0.001$).

Table 3. Mean (SE) processing times (minutes) for preparing and assigning ages from scales and pectoral fin rays from blue sucker

Process	Scales: n	Scales: Processing time	Fin rays: n	Fin rays: Processing time
Pressing	45	11.3 (0.25)		
Cleaning	48	41.5 (1.81)	38	9.5 (0.34)
Mould plugging			22	1.6 (0.14)
Epoxy loading			45	1.7 (0.13)
Sectioning			45	21.1 (0.48)
Viewing	48	6.1 (0.23)	48	5.5 (0.17)
Total		58.9		39.3

All times are for processing groups of five fish; n is the number of five fish groups.

Discussion

The results of this study demonstrated that fin rays are a more precise structure than scales for age estimation of blue sucker from the Missouri River. All readers reported that annuli were easier to recognise on fin-ray sections, and this is substantiated by the shorter viewing and measuring times. Scales were often unreadable because of the damage caused by regeneration. Fin rays have been found to be more precise than scales to estimate age of other catostomids such as carpsuckers, *Carpoides* spp. (Braaten *et al*. 1999; Spiegel *et al*. 2010), white sucker (Sylvester & Berry 2006; Quist *et al*. 2007), bluehead sucker, *Catostomus discobolus* (Cope), and flannelmouth sucker, *Catostomus latipinnis* (Baird & Girard) (Quist *et al*. 2007).

Fin rays likely provided more accurate estimates of true ages based on structure bias. Scale ages have been found to overestimate fin ray ages in younger common carp (Phelps *et al*. 2007). In this study, it was likely that some annuli identified on scales were not counted on the fish estimated less than age 4 by fin rays. This could have been caused by the prevalence of broken circuli that were difficult to distinguish from an annulus on the smaller scale. Readers may have been able to discern annual marks from presumed false annuli more effectively on fin rays than scales. Comparisons of age estimation structures of blue sucker (Rupprecht & Jahn 1980) and other species (Beamish 1981; Erickson 1983; Welch *et al*. 1993; Braaten *et al*. 1999; Isermann *et al*. 2003) found that scales provided lower estimates of age than fin rays or spines. Bias assessment results were similar to those of Rupprecht and Jahn (1980) who identified that scale ages were lower than fin-ray ages for blue sucker aged 7 and older. Other studies that estimated blue sucker ages using fin rays (Bednarski & Scarnecchia 2006) and opercles (Peterson *et al*. 1999) found greater longevity for this species than previously reported based on scale ageing. Scale age underestimation of fin-ray ages at older ages has been validated in white sucker (Beamish & Harvey 1969). Therefore, it is plausible that scales underestimate blue sucker ages, making fin rays a better choice than scales to obtain more accurate age estimates.

In addition to greater precision, time spent for fin rays on total processing and assigning ages (viewing) was less for fin rays. The results of this study corroborate others that found fin rays or spines (morphologically similar) were quicker to process and view (Welch *et al*. 1993; Isermann *et al*. 2003; Stolarski & Hartman 2008; Vandergoot *et al*. 2008) than scales. Therefore, fin rays are both a more precise and efficient way to estimate ages of blue sucker.

Although fin rays are the more precise structure, validation of age estimates is still needed. With the recent increase in interagency collaboration for benthic fish sampling on the lower Missouri River (Welker & Drobish 2009), mangers have an opportunity to conduct age validation on a large geographic scale. Additionally, further evaluation of effects of fin-ray removal closer to the pectoral girdle articulation on estimated age, precision and specimen survival is needed.

Acknowledgments

Thanks are extended to the U. S. Army Corps of Engineers for funding this project. The Missouri Department of Conservation, Chillicothe and the U. S. Fish and Wildlife Service, Columbia National Fish and Wildlife Conservation Office greatly helped to collect additional fish. Ron Gunderson and Nathan Locker provided assistance with structure processing. Appreciation is given to Kevin Buhl and the Columbia Environmental Research Center (USGS), Yankton Field Office, for providing laboratory space. Additionally, Pallid Sturgeon Population Assessment Team colleagues and several anonymous reviewers are thanked for their insightful comments while preparing this manuscript.

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