Comparative Age-Determination Techniques for White Sturgeon in California

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Abstract. —We compared growth patterns of clavicles, cleithra, opercles, medial nuchals, dorsal scutes, and pectoral fin ray sections from white sturgeon *Acipenser transmontanus* in California. The legibility and interpretability of growth patterns, ease of collection and processing, and relative precision of age estimates were evaluated for each structure with data collected on skeletal structures and morphometric measurements of 147 individuals ranging in size from 31 to 224 cm total length. Various methods were used to elucidate growth zones (thin-sectioning, oil and water clearing, staining, and X-ray radiography) to determine the most useful ageing technique for each structure. All calcified structures contained concentric growth zones that increased in number with the size of the fish and were interpreted as annual events. There was a direct linear relationship between size of the structures and size of the fish. Pectoral fin sections were the most practical ageing structure in terms of ease of collection, processing, legibility, and precision of interpretation. Age estimates from other structures resulted in poor precision between readers but relatively good intrareader precision. This suggests possible corroborative use with the development of better interpretive criteria and elucidation techniques. The von Bertalanffy growth curve calculated from age estimates based on pectoral fin sections for all samples produced a growth model for white sturgeon that compared favorably with those from previous studies.

Recently, much attention has centered on the white sturgeon *Acipenser transmontanus* in California, primarily due to its increasing popularity as a sport fish and its potential for cultivation. There is increasing interest in the sport fishery because white sturgeon are the largest freshwater fish in North America, they have excellent fighting ability, the quality of their flesh and roe is high, the stocks of other popular game fishes are decreasing, and the life history characteristics of white sturgeon may make them susceptible to overexploitation. Despite the popularity and economic importance of this species, information on size, age composition, and growth is not well developed. Yet, these data are essential for establishing adequate management of the fishery.

Pycha (1956) reported on sturgeon age, growth, size composition, migrations, and abundance, and Kohlhorst et al. (1980) reported on white sturgeon age and growth but found considerable disagreement between individual estimates of age based on transverse sections of marginal pectoral fin rays. The ability to age fish accurately is essential to a complete understanding of the dynamics of fish populations. The most reliable means of estimating age and growth of fishes is by examination of calcified structures for growth patterns (Bagenal 1974) and mark–recapture studies for validation of age estimates (Beamish and McFarlane

1983). Historically, various structures were used to age different species of sturgeons. Among these structures were scutes (the ganoid scales of sturgeons), pectoral fin rays, otoliths, opercles, and several other skeletal parts (Cuerrier 1951). Although the determination of ages from thin sections of pectoral fin rays has been the method of choice, there has been no comparative analysis of all possible structures to determine which methods are the best for any species. In addition, most authors assumed that the growth increments found in skeletal structures were deposited annually, but few verified age estimates, and none have attempted validation. Verification is defined as the repeatability of a numerical interpretation (analogous to precision) that may be independent of age, and validation is the determination of the true temporal meaning of a growth increment (analogous to accuracy; Cailliet et al. 1986; Summerfelt and Hall 1987).

The specific objectives of our study were (1) to assess the use of several calcified structures for determining the age of white sturgeon, (2) to develop techniques that would elucidate growth patterns in these structures and make them more useful for age determination, and (3) to compare age estimates from different structures to determine the most precise method for determining age and growth parameters.

Methods

We collected white sturgeon from the San Francisco Bay and Delta region of California between January 1984 and December 1985. Most samples were obtained from sport fishermen, whereas others were acquired from participants in the University of California at Davis aquaculture program who were collecting white sturgeon for brood stock and other studies. We recorded sex, total length (TL) and fork length (FL) to the nearest centimeter, and weight (WT) to the nearest kilogram for most samples. Because some fish had been eviscerated or had their heads removed before sampling, sex, weight, and length data were not always available.

We attempted to collect a variety of calcified structures from fish over a wide size range. We removed the head, with pectoral girdle and fins attached, and dorsal scutes, labeled them with the appropriate sample number, and returned them to the laboratory for processing. Numerous structures were assessed qualitatively for their usefulness in age determination (Figure 1); these structures included marginal pectoral fin rays, opercles, clavicles, cleithra, medial nuchals, and dorsal scutes because they all had visible calcium deposition patterns. Although saccular otoliths (sagittae) are commonly used for age determination in other fishes and have been used to age other species of sturgeon (Harkness 1923; Schneberger and Woodbury 1944), we found those from white sturgeon difficult to process and read. Therefore, we did not evaluate them further but stored them for later analysis.

We use the terms ring, annulus, and band to describe the growth patterns or growth increments found in the various calcified structures. The term ring describes depositional patterns associated with either winter (translucent) or summer (opaque) growth zones (Chilton and Beamish 1982). Visually, these rings are not simply fine lines that encircle the origin of growth but are of variable width, conform to the shape of each structure, and radiate from a point of origin somewhat parallel to the outer edge.

In many ageing studies, the term annulus refers to the translucent or winter growth zones that are counted for age estimation. Here, an annulus refers to one set of translucent and opaque rings because each translucent zone has a corresponding opaque zone (from the assumption that both are consistent seasonal events). Although the time period for translucent or opaque ring deposition has not been verified, we assumed that each annulus represents a yearly event. This is the basic assumption of all ageing studies in which age validation has not yet been attempted or completed.

The term band describes the wide opaque growth zones in only three of the structures: opercles, clavicles, and cleithra. In these structures, the wide bands were composed of opaque bone with numerous ancillary rings, or calcium layers, that initially were confused with translucent rings. With some practice, these ancillary rings could be distinguished from translucent rings. As with the other structures, each opaque band and translucent ring pair was counted as an annulus.

Pectoral fin rays.—We removed the leading (marginal) pectoral fin rays, air-dried them, and made two transverse cuts to remove the proximal (knuckle) and distal ends of the rays (Figure 2). The first cut, to remove the proximal end, was made just distal to the basal recess to improve the uniformity of the structure for mounting and to provide the best sections. This resulted in a 2–5-cm segment, depending on the size of the fish, which was then mounted on a wood block with two-part epoxy. The epoxy was allowed to dry for 6–8 h before the ray segment was sectioned with a low-speed lapidary saw with two closely spaced, high concentration, diamond grit blades. The first section was taken as close to the proximal end as possible. This technique is similar to that described by Kohlhorst et al. (1980). A minimum of three sections, ranging from 0.3 to 0.6 mm in thickness, was taken for each sample to ensure that at least one would be readable. Sections were mounted on a glass slide with clear fingernail polish, and annuli were counted with a binocular dissecting microscope at 16× magnification. Both transmitted and reflected light were used according to the characteristics and legibility of the growth rings. While being viewed, sections were brushed with a thin film of mineral oil to elucidate ring patterns further. We used an optical micrometer to measure the radius of each concentric annulus from the focus to the outside edge of each translucent zone and to the outside edge of the posterior wing of each section.

A subsample of fin sections also was X-rayed for comparison with the technique described above (Cailliet et al. 1983). We viewed the X-radiographs through a dissecting microscope with transmitted light, counted annuli, and assessed the radiographs qualitatively for clarity of ring patterns.

For both techniques, we standardized criteria for defining an annulus. Although the temporal
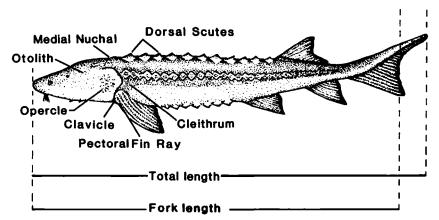
FIGURE 1.—White sturgeon illustration indicating measurements of total length and fork length and location of calcified structures used for age estimation.

periodicity of ring deposition in sturgeon structures never has been truly validated, we assumed that one pair of translucent and opaque rings was formed annually. This has been the basic assumption of all sturgeon ageing studies (Brennan 1988). By definition, an opaque ring is a zone that inhibits the passage of light. Under transmitted light, the opaque zone appeared dark, was usually wider than the translucent ring (especially in earlier years), and was assumed to have formed during the summer months (a period of faster growth). The translucent zone or ring appeared bright under transmitted light, was usually more narrow, and was assumed to have formed during the winter months (a period of slower growth). In addition, each growth zone needed to be distinct and continuous for each discrete pair to be considered an annulus.

We assessed fin sections qualitatively for readability based on clarity of ring patterns and general anomalies that could cause reader error. The outer edge of a section was categorized as translucent or opaque to determine the temporal periodicity of ring formation over the 24-month collection pe-
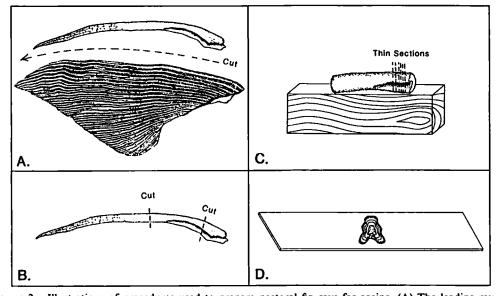

FIGURE 2.—Illustrations of procedures used to prepare pectoral fin rays for ageing. (A) The leading ray from pectoral fin is removed; (B) two transverse cuts are made to provide a segment to be mounted for thin-sectioning; (C) a segment of the leading pectoral fin ray is mounted on a wood block, then thin-sectioned transversely; and (D) thin sections are mounted on glass slides, and annuli are counted.

riod. All fin sections were read at least twice by the senior author, and two readings were compared for precision of intrareader variability. In addition, all fin sections were read by two other readers for analysis of interreader variability. All readings were blind and independent of age estimates by other readers and were based on the same criteria for defining an annulus.

Other structures. —After we removed the pectoral fins, the heads and dorsal scutes of all white sturgeon samples were processed. Opercles were cut away from either side of the head and set aside with the single row of dorsal scutes. After we removed the gills and mouth parts, we made a transverse cut across the head with a bone saw. The posterior portion of the head, which contained the pectoral girdle, the opercles, and dorsal scutes, was then simmered in boiling water for 30–40 min or until muscle and connective tissue could be removed easily from the bones. The medial nuchals, dorsal scutes, clavicles, cleithra, and opercles were then scrubbed clean with a nylon-bristled brush, labeled, and air-dried for at least 48 h, and then stored in resealable plastic bags.

Several techniques were applied to further elucidate the growth rings of these structures, including X-radiography (Cailliet et al. 1983), silver nitrate staining (Stevens 1975), and thin sectioning. The best and simplest technique was to immerse them in a water bath at ambient temperature for 30–60 s just before counting annuli on each structure. The amount of immersion time in the water bath required to clarify ring patterns depended upon the thickness of the structure. Occasionally, a structure dried out and developed a chalky film over its surface while growth increments were being observed. To alleviate this problem, surfaces were brushed with a thin film of mineral oil. This technique was chosen over reimmersion in the water bath, which tended to increase translucency and thereby make the structure "unreadable."

Criteria for determining which sets of growth rings to call annuli varied among structures. Opercles, clavicles, and cleithra have fairly smooth surfaces that reveal concentric growth rings. Procedures for counting these rings were standardized by placing them into two categories, rings and bands (Cailliet et al. 1983). Each band and ring pair was distinguished and counted as an annulus. Making this distinction required some practice.

Dorsal scutes and medial nuchals were similar in morphology, although medial nuchals were significantly larger than scutes in bigger fish. Although they were very closely spaced, translucent and opaque, growth rings were fairly distinct. Therefore, these structures were assessed for readability, and annuli were counted according to the same criteria accepted for pectoral fin ray sections.

Calcified structures from a subsample of 44 fish were selected for age analysis from the range of size-classes available. Initially, all structures from each fish were assessed for quality (lack of chips, cracks, or deformities) and readability (clarity of ring patterns). Subsequently, one of each type of structure (i.e., one scute, opercle, clavicle, and cleithrum) was selected from each fish for ageing. All structures were viewed with a large magnifying glass and reflected light against a dark background. Occasionally, a low-power, binocular dissecting microscope and reflected light were required to assess illegible growth rings. All structures selected for age analysis (with the exception of dorsal scutes) were read twice by the first author and once by at least one other reader to determine interreader variability. In addition, annulus counts from other structures were compared to counts for pectoral fin sections from the same fish as a standard to determine intrareader variability.

Age and growth. —After they had dried, all structures were measured to the nearest millimeter across a preselected axis with vernier calipers (Figure 3). We determined the relationships between the size of a structure (fin section radius, medial nuchal width, dorsal scute length, clavicle radius, cleithrum radius, diagonal opercle diameter) and total length by regression analysis (Zar 1974).

We used several criteria to assess the usefulness of each structure for estimating age in white sturgeon. These criteria included the agreement of age estimates, legibility of each structure, and ease of collection and processing.

Age estimates of structures from the subsample of 44 fish were compared to determine the consistency of age estimates within and between readers and variability of ages between corresponding structures from a single fish. We tested the variability between age estimates made by a single reader from different structures from the same fish by regression analysis. The independent variable was assumed to be representative of the true age of the fish.

The relationship between length and age was modeled from pectoral fin ray data. We calculated von Bertalanffy growth parameters L_∞, k, and t_0 (the ultimate length, growth coefficient, and time at length zero for a population, respectively) from one of four different methods (Walford 1946; Al-
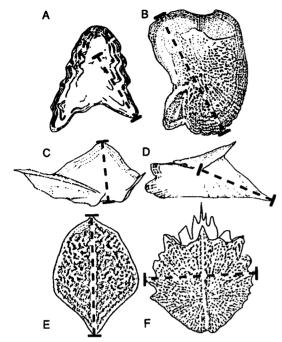
FIGURE 3.—Illustrations of calcified structures of white sturgeon used for age estimation. Lines mark axis of growth measured. (A) Pectoral fin ray section, (B) opercle, (C) cleithrum, (D) clavicle, (E) dorsal scute, and (F) medial nuchal.

len 1966; Gulland 1969; Everhart et al. 1975). We used the technique that produced the least mean square error to fit the von Bertalanffy growth curve and characterized growth by plotting individual total lengths against age estimates and by plotting the predicted von Bertalanffy growth curve based on the parameters L_∞, k, and t_0 for combined sexes.

Results

Summary of Fish Sampled

Of 147 white sturgeon collected between 1984 and 1985, total length and weight data were collected from 143 and 129 fish, respectively. Overall, sizes ranged from 31 to 224 cm TL; most fish were between 100 and 180 cm (Figure 4). The sexes of 66 males and 54 females were identified. Males ranged in size from 52 cm (weight undetermined) to 190.5 cm TL (46.72 kg), whereas females ranged in size from 103 cm (5.44 kg) to 224 cm TL (80.74 kg).

There were statistically significant linear correlations ($P < 0.001$) between TL and FL for males ($N = 50$; $r^2 = 0.98$), females ($N = 39$; $r^2 = 0.98$), and all samples combined (sexed and unsexed, FL $= -3.55 + 0.93$TL; $N = 106$; $r^2 = 1.00$). The functional relationship between TL and WT (kg) was best expressed by the equation: $\log_{10}$ WT $= -5.21 + 2.98 \log_{10}$ TL for males ($N = 63$; $r^2 = 0.94$), $\log_{10}$ WT $= -6.04 + 3.36 \log_{10}$ TL for females ($N = 52$; $r^2 = 0.94$), and $\log_{10}$ WT $= -5.70 + 3.19 \log_{10}$ TL for all samples combined ($N = 124$; $r^2 = 0.94$).

Pectoral Fin Ray Sections as Ageing Material

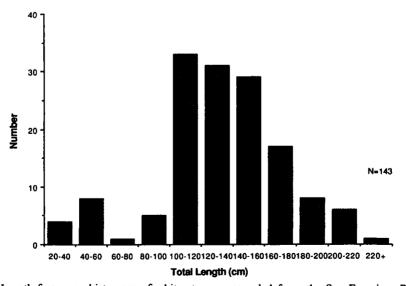Pectoral fin rays were collected from all 147 fish and thin-sectioned. Of those, 122 were measured

FIGURE 4.—Length-frequency histogram of white sturgeon sampled from the San Francisco Bay and Delta, California.
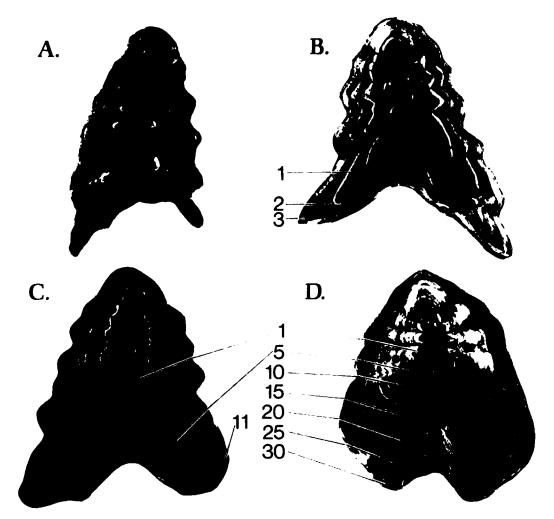
FIGURE 5.—Transverse sections of first pectoral fin rays of white sturgeon of four estimated ages. White rings are translucent and are assumed to be deposited during the winter growth period. Annuli are numbered for reference. (A) Age 0 (total length [TL], 31 cm), (B) age 3 (TL, 60 cm), (C) age 11 (TL, 119 cm), (D) age 30 (TL, 191 cm).

from the origin to the outer edge of one posterior lobe. There was a significant positive linear relationship between TL and fin section width (r^2 = 0.81), although there were differences in the morphology of each section due to the generally inconsistent formation and width of annuli between samples. The width of fin sections ranged from 1.67 mm (29 cm TL) to 8.62 mm (173 cm TL).

Growth rings were present in all fin sections examined and increased in number with increasing section width and fish size. Annuli were more widely spaced near the origin and usually more tightly grouped toward the outer edge, especially in larger (older) fish (Figure 5). Tight groupings of

annuli occurred frequently, even in earlier years, and was a common problem in annulus delineation. Therefore, it was occasionally necessary to get a consensus of more than one reader to define annuli in some samples.

Incorporation of secondary fin rays into the posterior lobe of the first ray, a phenomenon which has not been described previously for white sturgeon, also reduced the readability of fin sections because of crowding of annuli (Figure 6). This potentially could lead to errors in age determination. The inspection of samples with secondary rays incorporated and unincorporated into the first ray revealed annulus counts that corresponded to
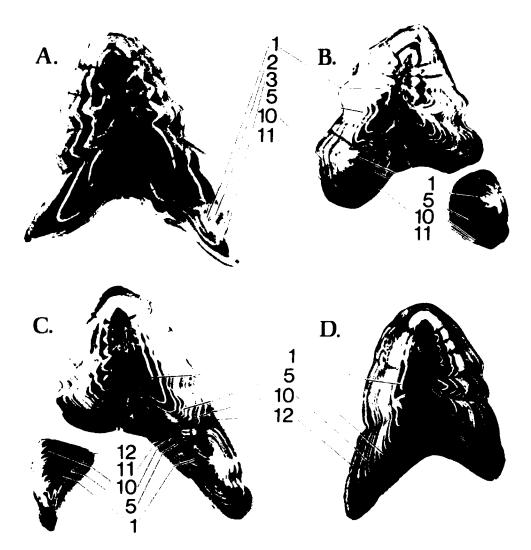
FIGURE 6.—Transverse sections showing secondary rays incorporated with first rays in pectoral fins of white sturgeon. Secondary ray (A) incorporated during year 2 of growth in an age-3 fish (total length [TL], 60 cm), (B) incorporated during year 4 in an age-11 fish (TL, 122 cm), (C) incorporated during year 11 in an age-12 fish (right fin ray, sample 3122; TL, 127 cm), and (D) not incorporated (left fin ray, sample 3122). Note that in all samples annulus counts of incorporated and unincorporated secondary rays agree with annulus counts of primary rays.

counts of the first ray alone. In addition, the incorporation of secondary rays did not always occur bilaterally (in both right and left fin rays) nor did it always occur in both lobes of the same first ray. Secondary fin ray incorporation occurred in 52% of all fin sections examined.

The seasonality of depositional patterns often is determined by characterizing the clarity of the edge during the course of the year. It was difficult, however, to determine whether the edge was translucent or opaque in fin ray sections from white sturgeon. This was particularly true in larger (older) fish that tended to have more narrowly spaced annuli in the posterior portion of the fin section. In the 125 fin sections examined, distinct trends in seasonal formation were not apparent.

The precision of age estimates based on two independent blind counts of fin-section annuli by a single reader resulted in 38% exact agreement, 74% agreement within one annulus count, and 84% agreement within two annulus counts. Discrepancies in counts ranged from −4 to +11 (Figure

Within Reader

a.

y-axis: Number (50, 40, 30, 20, 10, 0)
x-axis: -4 -3 -2 -1 0 1 2 3 4 5 7 8 11

N=118

Between Readers 1-2

b.

y-axis: Number (40, 30, 20, 10, 0)
x-axis: -7 -6 -4 -3 -2 -1 0 1 2 3 4 5 6 7 9

N=131

Between Readers 1-3

c.

y-axis: Number (50, 40, 30, 20, 10, 0)
x-axis: -10 -9 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 6 10

N=131

Discrepancies (annulus counts)

Between Readers 2-3

d.

y-axis: Number (30, 20, 10, 0)
x-axis: -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 6 8
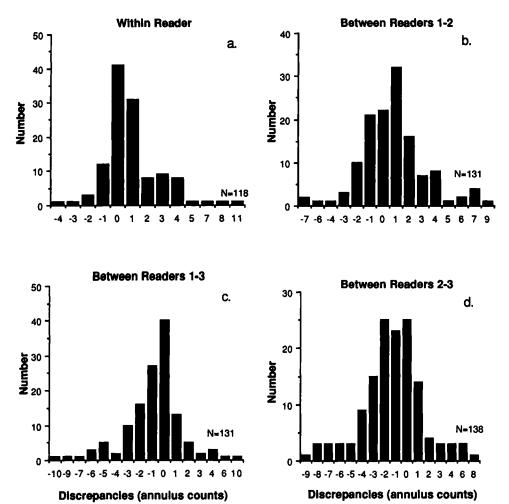
N=138

Discrepancies (annulus counts)

FIGURE 7.—Discrepancies between annulus counts of fin sections from white sturgeon. Each comparison represents independent counts by (a) reader 1 versus reader 1, (b) reader 1 versus reader 2, (c) reader 1 versus reader 3, and (d) reader 2 versus reader 3. 0 = no discrepancies, i.e., exact agreement.

7a). Comparisons between the two independent blind counts of fin-section annuli by more than one reader also were high (17–31% exact agreement, 57–63% agreement within one annulus count, and 77–84% within two annulus counts; Figure 7b–d).

Other Structures

Opercles from 126 fish were measured diagonally across the widest axis. There was a positive linear relationship between TL and opercle width ($r^2 = 0.94$), and opercle width ranged from 14 mm (28.6 cm TL) to 144 mm (224 cm TL).

Concentric rings and bands, observed on the medial side of all opercles, increased in number with the size of structure, but unlike the more

well-defined translucent and opaque rings of pectoral fin sections, opercular patterns consisted of wide opaque bands bordered by very narrow translucent rings. Opaque bands were often composed of numerous rings that could be confused with a translucent ring. Therefore, annuli were defined as wide opaque bands with a distinct break at the formation of a translucent ring. Translucent rings were discrete and continuous. Extraneous ring patterns were not counted.

Comparisons of the two independent blind counts of annuli from a subsample of opercles resulted in a low level of agreement: only 15% exact agreement, 30% agreement within one annulus count, and 53% agreement within two annulus counts. Discrepancies in counts ranged from −9 to +7 (Figure 8a).
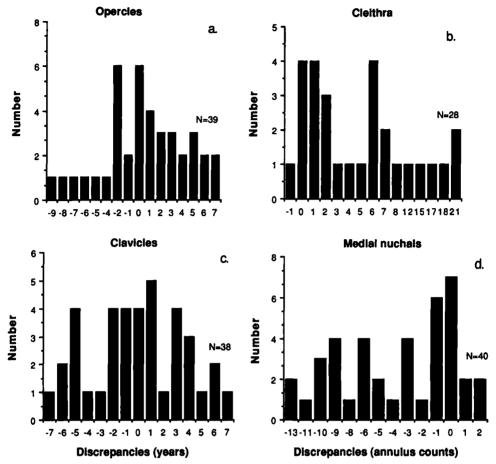
FIGURE 8.—Discrepancies between annulus counts of four bony structures from white sturgeon. Each comparison represents two independent counts by different readers. 0 = no discrepancies, i.e., exact agreement.

Cleithra and clavicles from 126 fish were measured across the widest axis from the origin to the outer edge of the portion of the structure used for age analysis. There was a positive linear relationship between TL and cleithrum width ($r^2 = 0.94$) or clavicle width ($r^2 = 0.90$). Width ranged from 9 mm (28.6 cm TL) to 87 mm (224 cm TL) for cleithra and from 10 mm (28.6 cm TL) to 83 mm (202 cm TL) for clavicles.

Comparisons of the two independent blind counts of a subsample of cleithra or clavicles resulted in only 14% exact agreement, 32% agreement within one annulus count, and 43% agreement within two annulus counts ($N = 29$) for cleithra; and 11% exact agreement, 35% agreement within one annulus count, and 48% agreement within two annulus counts ($N = 38$) for clavicles. Discrepancies in counts ranged from −1 to +21 for cleithra and from −7 to +7 for clavicles (Figure 8b, c).

Medial nuchals from 110 fish were measured transversely, and dorsal scutes from 111 fish were measured longitudinally across the widest axis of the structure. There was a positive linear relationship between TL and both medial nuchal width ($r^2 = 0.76$) and dorsal scute length ($r^2 = 0.61$). Medial nuchal width ranged from 11 mm (31 cm TL) to 77 mm (199 cm TL), whereas dorsal scute length ranged from 14 mm (31 cm TL) to 60 mm (158 cm TL). Comparisons between the two independent blind counts of a subsample of medial nuchals resulted in only 18% exact agreement, 28% agreement within one annulus count, and 35% within two annulus counts ($N = 40$). Discrepancies in counts ranged from −13 to +2 (Figure 8d).

Single-Reader Comparison of All Structures

There were positive linear relationships in all 15 comparisons of annulus counts based on different structures from the same fish. All regres-

TABLE 1.—Regression analysis of annulus counts from six calcified structures of white sturgeon used for age estimation. b = regression coefficient or slope of the best-fit regression line.

Structures compared	r^2	b
Pectoral fin section versus medial nuchal	0.88	1.10
Clavicle versus operculum	0.96	1.01
Cleithrum versus operculum	0.94	1.01
Clavicle versus cleithrum	0.96	0.98
Pectoral fin section versus dorsal scute	0.83	0.93
Pectoral fin section versus operculum	0.94	0.90
Pectoral fin section versus clavicle	0.92	0.89
Pectoral fin section versus cleithrum	0.88	0.88
Dorsal scute versus operculum	0.81	0.87
Dorsal scute versus clavicle	0.88	0.85
Medial nuchal versus dorsal scute	0.90	0.84
Dorsal scute versus cleithrum	0.85	0.80
Medial nuchal versus clavicle	0.86	0.74
Medial nuchal versus cleithrum	0.83	0.73
Medial nuchal versus operculum	0.85	0.72

sions were highly significant. Coefficients of determination ranged from $r^2 = 0.96$ for opercle versus clavicle annulus counts to $r^2 = 0.81$ for opercle versus dorsal scute counts, and the slopes of all regressions did not differ significantly from 1 ($P < 0.001$; Table 1).

Annulus counts in the calcified structures of each fish also were compared to determine the amount of single-reader variability, and then each structure was ranked in ascending order based on the level of agreement within three annulus counts. Annulus counts for pectoral fin sections were used as a standard due to the low degree of interreader and intrareader variability for this structure (Table 2). Exact agreement of paired annulus counts was relatively consistent and ranged from 24 to 28% for all structures compared to fin section counts (Table 2). The percent agreement within one annulus count increased markedly for cleithra (45%), clavicles (36%), and opercles (36%) with a

cumulative percent agreement of 70, 63, and 62%, respectively. Agreement within two annulus counts was again highest for clavicles (18%), cleithra (11%), and opercles (12%) with cumulative percent agreement figures (81, 81, and 74%, respectively) almost equal to that of annulus counts for pectoral fin sections alone (84%).

Each structure was ranked according to the cumulative percent agreement within three annulus counts to provide a relative index of interpretability. Annulus counts for pectoral fin sections ranked highest with 92% cumulative agreement and were followed by cleithra (90% cumulative agreement with the standard), clavicles (88%), opercles (86%), dorsal scutes (67%), and medial nuchals (51%).

Assessment of Elucidation Techniques

Of the two techniques that were used to elucidate the ring patterns, X-radiography of fin sections was less successful than clearing with oil. Although several radiographs showed discernible annuli, most samples produced poor-quality radiographs, probably due to the lack of uniformity in the thickness of each section and the fluctuations in X-ray emissions at such low energy levels.

Clearing with oil was the best and simplest technique for elucidating ring patterns in fin sections. The oil not only helped to clear the surface of the dried sections, which often developed an opaque haze, but also penetrated the section to help delineate the growth zones. Occasionally, the thinnest sections became too transparent when the oil was applied. Under these circumstances, clearing with oil was not used, or a thicker section was selected.

For other structures, the X-radiography of all structures produced poor and inconsistent images. Silver nitrate was poorly absorbed into the bone

TABLE 2.—Percent agreement of annulus counts for each structure with annulus counts for sections of pectoral fin rays of white sturgeon used as a standard. Each comparison of annulus counts was between different structures from the same sturgeon and was of counts by a single reader. For fin sections, the data represent two independent counts of annuli from the same structure.

Agreement or rank	Structure					
	Pectoral fin section	Medial nuchal	Dorsal scute	Clavicle	Cleithrum	Opercle
Agreement						
Complete	46	24	28	27	25	26
Within 1 annulus count	36	10	14	36	45	36
Within 2 annulus counts	2	7	11	18	11	12
Within 3 annulus counts	8	10	14	7	9	12
Cumulative percent	92	51	67	88	90	86
Rank	1	6	5	3	2	4

resulting in superficial, patchy stains that did not accentuate growth patterns.

Immersion in water and clearing with oil was the best and simplest technique used to elucidate growth patterns. A 15–30-s water immersion of the structures before annuli were counted was usually sufficient. After a short drying time, application of a thin film of mineral oil improved translucency. The application of oil was most often required for larger (thicker) samples that had less discernible early rings and bands, and more closely spaced marginal rings and bands.

Age–Length Relationship

The most consistent estimates of age were derived from pectoral fin ray sections. The von Bertalanffy growth curve for 134 white sturgeon (31–224 cm TL) rose gradually and leveled at an estimated TL of 230.59 cm for both sexes combined. Females were estimated to reach a longer length (224 cm TL) than males (190.5 cm TL). These lengths corresponded to the oldest female (34 years) but not the oldest male. The highest age estimate for males was 28 years at sizes between 156 and 167 cm TL. Based on the results of this study, growth of white sturgeon in California was best described by the equation $l_t = 230.59[1 - e^{-0.0533(t+2.9176)}]$; l_t is length at age t (years).

Discussion

Size frequencies (TL in cm) of white sturgeon sampled for this study were similar to the size frequencies presented by Kohlhorst et al. (1980) who sampled 2,203 white sturgeon from the sport-fishery and net surveys throughout the San Francisco Bay and Delta of California. Although white sturgeon are reported to reach a total length of 610 cm and about 820 kg (Miller and Lea 1972; Hart 1973; Eschmeyer et al. 1983), specimens larger than about 225 cm and 90 kg are uncommon in the San Francisco Bay and Delta system and were not available for this study.

The initial step to be taken when any age and growth study is conducted is selection of a bony structure that will provide the most accurate information about the age of a fish. The selection of an optimal ageing structure should be based on the comparison of a variety of structures according to clarity of ring patterns, percent agreement or precision of pattern interpretability, ease of collection and processing, and ultimately, accuracy of ageing a specific structure.

Historically, sturgeons have been aged by examination of a variety of structures (Cuerrier

1951), but no single author has reported on a cross comparison of several structures for determining age and growth parameters. For white sturgeon, assessment of structures other than pectoral fin sections has never been attempted, and verification of age estimates has resulted in considerable disagreement between readers interpreting ages.

An important assumption inherent in growth studies based on skeletal structures is that fish size and structure size are closely related throughout the entire life cycle (Lee et al. 1983; Smith 1983). Although many attempts have been made to estimate the age of sturgeons from skeletal structures, few studies have examined this relationship. Sunde (1961) found a poor relationship between fin section measurements and fork length of lake sturgeon *Acipenser fulvescens,* but Semakula and Larkin (1968) found a significant relationship between fin section width and fork length of white sturgeon ($r^2 = 0.81$). Other studies have not used this procedure for fin sections or any other structure.

The positive relationships between total length and the size characteristics of each structure supported the use of the structures for age estimation. However, it should be noted that this important procedure is not definitive for determining the overall acceptability of one particular structure over another. For example, the coefficient of determination was highest for opercles ($r^2 = 0.94$); pectoral fin sections ($r^2 = 0.81$) ranked fourth, although the pectoral fin rays ultimately were the structure of choice. Thus, rather than depend on structure morphology, it is more important to concentrate on the accuracy and reproducibility of annulus counts within a particular structure.

Information on growth was available from each of the structures studied. All had apparent rhythmic depositional patterns that were, to varying degrees, recognizable and quantifiable. These growth patterns increased in number with the size of the fish and thus met another important criterion for determining the usefulness of each structure for ageing. Although each structure had recognizable growth patterns, the delineation of annuli was often difficult, and the nature of ring depositions is not well understood.

To fully understand the timing and frequency of ring deposition throughout a year for a particular species, investigators must collect samples throughout the year and correlate edge characteristics with a particular time of the year, first characterize the edge without knowledge of the date of capture, or use the internationally accepted

birthdate of January 1. Inspection of edge characteristics on pectoral fin sections of white sturgeon revealed no distinct trends in the time of translucent or opaque ring formation. The inability to fit a definitive edge characteristic (translucent or opaque) to the assumed proper season was probably due to the differential width of growth rings. Translucent rings were narrow compared to opaque rings and were very difficult to distinguish at the outer edge, especially if it was early in the time of formation or if the outer ring pairs were narrowly spaced, as in larger (older) fish. Also, opaque ring widths varied within samples and were generally wider in early years and more narrow and closely spaced in later years. This may indicate variable rates of growth throughout the life of a particular individual but may not be a good indicator of time of annulus formation. Berkeley and Houde (1983) suggested that the distance between the last annulus and the edge of the spine is an indicator of the time of annulus formation. The smaller this distance, the closer the capture date is to the date of annulus formation. This technique probably would not work for white sturgeon due to variable growth throughout the life of an individual and individual growth variability between fish. Therefore, for white sturgeon, the timing of ring formation must be assumed until validation studies have been completed.

Although opercles have been used to determine the ages of sturgeons in Russia (Chugunova 1959), their usefulness for age estimation of white sturgeon appears to be limited, based on the precision of our age estimates. Even though opercle growth was significantly related to the size of the fish and this structure was easy to collect and process, the agreement among readers in their interpretation of patterns was low. Numerous checks or ancillary rings may have been interpreted as annuli; lateral surface denticulations created a background matrix that tended to obscure growth zones; the incorporation of natural oils in the bony matrix also decreased readability, especially in larger fish; the thickness of larger opercles created problems with the interpretation of early annuli; and delineation of marginal annuli was difficult for larger fish samples due to the decreased width of growth increments. Thus, more complex preparation techniques and the development of more accurate and reproducible criteria for describing annuli in opercles are needed to improve the usefulness of this structure for age determination.

Concentric ring and band patterns increased in number with the width of clavicles and cleithra.

Annulus patterns similar to those described for opercles were evident in all clavicles and cleithra observed, although growth patterns were easier to interpret in the latter two structures due to the lower density of calcium deposition (thickness) of structures with increasing size, the absence of natural oils in the bony matrix of larger samples, and the absence of denticulations in cleithra. Both structures met the initial criteria for selection as a structure for age estimation. Each structure increased in size relative to the size of the fish, was relatively easy to collect and process, and had enumerable growth zones that appeared to increase with the size of the fish. However, analysis of both structures resulted in a low level of agreement and a wide range of discrepancies in annulus counts between readers who interpreted growth increments.

Growth increments observed in clavicles were not well defined, which was probably the major source of reader error. The distinction between true and false annuli was often difficult. The formation of false annuli was probably a result of the normal depositional process, which appeared to be a layering of mineralized bony tissue. The problem was compounded by the differential width of growth increments, especially in larger fish, and ventral denticulations that tended to obscure annuli. Similarly, annuli in cleithra were difficult to distinguish, although surface denticulations were not present in this structure. Delineation of annuli was most difficult near the margin of both structures. Crowding of annuli near the margins of larger samples was probably the greatest source of error between readers. Until techniques (e.g., Casselman 1983) are used to define and confirm depositional patterns in clavicles and cleithra of white sturgeon, the usefulness of these structures for age determination appears limited.

Dorsal scutes and medial nuchals exhibited similar morphological characteristics and had growth patterns similar to those described for pectoral fin sections, although the relationship between total length of the fish and width of the structure was stronger for medial nuchals. The variability in size of dorsal scutes in relation to total length may be due to degeneration in larger specimens. Delineation of growth rings in dorsal scutes and medial nuchals was more difficult than in other structures due to denticulations on the dorsal surface of each structure, increased density of calcium deposition in larger structures that often obscured early annuli, and severe crowding of annuli near the edges of larger structures.

Based on the other criteria for selection, both scutes and medial nuchals showed promise as structures for ageing white sturgeon. Both were easy to collect and process, and each had enumerable growth rings that appeared to increase with the size of the fish. However, as with the other calcified structures, between-reader comparisons indicated a low level of agreement and a wide range of discrepancies in annulus counts, and the true nature of the growth patterns observed in medial nuchals and dorsal scutes remains to be solved.

Pectoral fin sections appeared to be the simplest and most consistently reliable material for age estimation of white sturgeon in California. Compared to the other calcified structures, fin rays were relatively simple to collect, process, interpret, and store. The number of growth increments appeared to increase with the size of the fish, and the measurement of each section was significantly related to the corresponding total length. Although processing time was slightly greater than for other structures, fin rays were fairly simple to process and required little expertise once a specific technique had been determined. There was a relatively high level of agreement among readers, and pectoral fin sections ranked above all other structures for this criterion. Growth rings found in fin sections were a consistent feature from specimen to specimen, but readers required some training in the interpretation of these rings before becoming proficient at the estimation of ages.

Difficulties in the interpretation of growth rings in fin sections of white sturgeon included the variability of spacing between successive annuli, the incorporation of secondary rays, and the lack of definition in marginal annuli. Crowding of annuli occurred frequently, even in earlier years. This seems to indicate a reduced rate of growth or calcium deposition, which may have been a result of intrinsic or extrinsic factors. Crowding of annuli in earlier years may have been a result of environmental stresses (lack of food, temperature fluctuations, etc.), whereas with older fish, the narrow spacing of annuli may indicate a spawning period (Roussow 1957). Perhaps these groupings were simply natural breaks in the growth process that resulted in slower growth of the calcified structure.

The readability of fin sections also was hampered by the incorporation of secondary rays into the posterior lobes of the first ray in 52% of all fin sections examined. Roussow (1957) noted that these occurred in fin ray sections of the lake sturgeon and Chugunova (1959) stated that these "su-pernumerary centers" appeared very often in both the beluga *Huso huso* and the sevryuga *Acipenser stellatus*.

Initially, it was difficult to determine the cause of what appeared to be additional rings radiating from another origin. Three lines of evidence suggested that they were the incorporation of secondary rays. First, they occurred in other species (Roussow 1957; Chugunova 1959). Second, annulus counts from the secondary origin to the outermost secondary annulus corresponded to annulus counts from the origin of the first ray to the outermost secondary annulus. Subsequent rings of the first ray were formed around the secondary rings and radiated out into the posterior lobes somewhat parallel to the curvature of the outer edge. Third, counts of annuli in recently incorporated secondary rays agreed with annulus counts of the first ray from the origin to the outer edge, both of which agreed with annulus counts taken from the opposing, unincorporated secondary ray. This is not to say that the secondary rays were as good for ageing as the first ray but that they occasionally may be used for verification, especially in smaller fish where growth rings are more easily distinguished. The cause of secondary ray incorporation remains to be determined.

Differences among annulus counts from different structures of each fish by single or multiple readers varied greatly. The higher precision of single reader versus interreader annulus counts probably resulted from more experience and familiarity with white sturgeon structures by an individual reader. The level of precision for intrareader counts, within three annulus counts, was considered an acceptable level for white sturgeon, a long-lived species. Counts from medial nuchals and dorsal scutes had a low percentage of agreement, which demonstrated the inability to reproduce age estimates from these structures. Opercles, cleithra, and clavicles had high levels of intrareader agreement but low interreader agreement; therefore, the criteria for interpreting growth increments by multiple readers may need to be better defined or understood.

Verification and validation of age estimates are now considered to be essential, yet often overlooked, elements in age and growth studies (Beamish and McFarlane 1983). In this study, ages of individual white sturgeons were verified at least partially by comparing age estimates from several structures. The high level of interreader agreement for annulus counts of pectoral fin sections provided good evidence of similar age determinations.

In addition, high intrareader agreement among structures suggests corroboration. Unfortunately, without valid evidence of the temporal periodicity of growth increment formation, these interpretations may be biased if not meaningless.

Finally, according to the stated assumptions and our evidence, the longevity of white sturgeons is at least 28 years for males and 34 years for females. The largest female sampled was also the oldest fish and weighed 80.74 kg. The largest female white sturgeon recorded from California was 272 cm (TL) and weighed 212.28 kg, so the age of females in this population probably greatly exceeds this estimate of 34 years. The largest female white sturgeon ever aged was found dead in November 1987 in Lake Washington, Seattle, Washington. This fish measured 336 cm TL, weighed about 304 kg, and was about 70 years old (J. S. Brennan, unpublished data). Miller (1962) reported that the oldest female white sturgeon ever encountered in California had an estimated age of 45–47 years. The largest male we sampled (46.72 kg) was only 23 years of age. The oldest males we sampled (28 years) were only slightly larger than the average size, so it is probable that the age of males also exceeds our estimate of age. It was difficult to characterize growth for both sexes due to the considerable variation in length for an estimated age. Growth variability is probably a normal characteristic of white sturgeon populations (Semakula and Larkin 1968; Kohlhorst et al. 1980; Hess 1984). The largest white sturgeon ever reported came from the Columbia River, Oregon, and weighed in excess of 818 kg (Miller 1962). Because these populations cannot be distinguished genetically (D. Bartley, personal communication) and the populations intermix, as evidenced from tagging studies (Miller 1962; Miller 1972a, 1972b; Brennan, unpublished data), it is probable that results from this study generally underrepresent age and growth characteristics of white sturgeon. However, the similarity between the von Bertalanffy growth function in this study and that of Kohlhorst et al. (1980; $l_t = 261.2[1 - e^{-0.04027(t + 3.638)}]$) seems to indicate an adequate representation of white sturgeon growth in the San Francisco Bay and Delta system for the size-classes represented. A more accurate model will not be possible until fish from a wider range of sizes are collected and the true nature of ring deposition in calcified structures is determined through validation studies. We have initiated validation studies and are awaiting returns. Results from these studies will provide more accurate data to better understand growth and growth increment formation in calcified structures of white sturgeon.

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