FINAL REPORT

Age Structure and Life History Attributes of

Atlantic Sturgeon (*Acipenser oxyrinchus*) in the Hudson River

HRF GRANT No. 006/93A

SUBMITTED TO

Dr. Dennis J. Suszkowski
Hudson River Foundation
40 West 20th Street, Ninth Floor
New York, N.Y. 10011

REPORT AUTHORS

David H. Secor, Jill T. Stevenson and Edward D. Houde
The University of Maryland System
Center for Environmental and Estuarine Studies
Chesapeake Biological Laboratory
P.O. Box 38
Solomons, MD 20688

11 June 1997

ABSTRACT

Until 1996, Atlantic sturgeon *Acipenser oxyrinchus* supported a commercial fishery in the Hudson River. The effect of fishing mortality on this population was influenced to an unknown, but probably significant, extent by the population's schedule of growth, mortality and reproduction. In this study, demographic parameters of Hudson River Atlantic sturgeon were estimated through microstructural and microchemical examinations of hardparts. Estimated growth, mortality and reproductive schedules were inputs to an age-structured model used to evaluate the vulnerability of Hudson River Atlantic sturgeon to a range of commercial fishing scenarios.

Interpretation of annuli in sectioned pectoral fin rays provided an accurate and precise method for ageing juvenile and adult Atlantic sturgeon (2 - 42 years). Yearly rates for annulus formation were indicated by marginal increment analysis and the examination of annuli in oxytetracycline-marked and known-age laboratory-reared sturgeon. Radiometric ageing of Atlantic sturgeon fin rays, based upon $^{210}Pb:^{226}Ra$ disequilibrium, was applied but without success. A principal assumption of the radiometric ageing method--that the internal structure of the hardpart is a closed system--was violated because fin rays are cellular and heavily vascularized. Annuli in otolith sections were difficult to interpret and under-represented ages estimated from fin ray sections. Based upon fin-ray age estimates, higher growth rates were predicted for males (K = 0.25, L_{∞} = 180 cm) than females (K = 0.08, L_{∞} = 251 cm). Females, however, attained higher maximum observed age (42 years) and size (TL = 277 cm). Modal ages of spawning males and females in the Hudson River fishery were 16 and 19 years, respectively. Spawning frequency was estimated using interpretation of bands of narrow annuli, "spawning belts," and periodogram analysis of annular width measurements. Spawning belt analysis suggested that female Atlantic sturgeon spawn, on average, every four years. However, interpretations of spawning belts were highly subjective. Seasonal patterns of Sr:Ca ratios in fin ray sections were examined to infer spawning cycles. Results indicated that cycles in Sr:Ca ratios were probably related to annulus formation rather than migratory or spawning behaviors.

Life history characteristics and natural and bycatch mortality estimates were included in an age-structured, spawning potential-per-recruit model to estimate the probable response of this population to different fishery management strategies. Size restrictions on harvested sturgeon can effectively preserve reproductive potential of the stock. Elimination of the directed coastal fisheries for sturgeon will allow a higher target fishing mortality in the traditional Hudson River fishery ($F_{50\%}$ = 0.047 and 0.26 for a coastal and Hudson River fishery, or an exclusive Hudson River fishery, respectively). Surprisingly, the model was not sensitive to variability in spawning frequency, but was sensitive to error or variability in estimates of natural mortality and growth parameters, K and L_{∞}. Late age at maturity, low relative fecundity, and infrequent spawning results in extreme sensitivity of Atlantic sturgeon to comparatively low rates of exploitation. Atlantic sturgeon have extremely low replacement rates and are susceptible to multiple fishing gears of other fisheries throughout their life cycle. Despite the current moratorium on Atlantic sturgeon harvests, recovery is expected to require several decades.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Until 1996, Atlantic sturgeon supported a commercial fishery in the Hudson River. The effect of fishing mortality on Hudson River Atlantic sturgeon is influenced to an unknown, but probably significant extent by the population's schedule of growth, mortality and reproduction. In this study, demographic parameters of this population were estimated through microstructural and microchemical examinations of hardparts. Estimated growth, mortality and reproductive schedules were inputs to an age-structured model used to evaluate the vulnerability of Hudson River Atlantic sturgeon to a range of commercial fishing scenarios.

Sagittal otoliths and pectoral fin rays were examined for age and growth analysis. Interpretation of annuli in sectioned pectoral fin rays provided an accurate and precise method for ageing juvenile and adult Atlantic sturgeon (2 - 42 years). Yearly rate for annulus formation were indicated by marginal increment analysis and the examination of annuli in oxytetracycline-marked and known-age laboratory-reared sturgeon. Some fin ray sections exhibited seasonal deposition of calcium which may be related to annulus formation. Radiometric ageing of Atlantic sturgeon fin rays, based upon ^{210}Pb:^{226}Ra disequilibrium, was applied but without success. Because fin rays are cellular and heavily vascularized, exogenous ^{210}Pb probably entered into the hardpart, invalidating the principle assumption of the radiometric ageing technique - that ^{210}Pb occurs in equilibrium with ^{210}Po, the product of ^{226}Ra decay. It is probable that otolith sections exhibit annuli which under-represent age. On average, we observed five fewer annuli in sagittal otoliths than in fin rays.

Based upon fin-ray age estimates, higher growth rates were predicted for males (K = 0.25, L_∞ = 180 cm) than females (K = 0.08, L_∞ = 251 cm). Females, however, attained a higher maximum observed age (42 years) and size (TL = 277 cm) than males (maximum age = 35; maximum TL = 226 cm). Modal ages of spawning males and females in the Hudson River fishery were 16 and 19 years, respectively.

Growth rates of Acipenseridae may be affected by differences in migratory behaviors. Atlantic sturgeon showed higher growth coefficients and higher relative fecundity than semi-anadromous or freshwater resident species of sturgeon. We speculate that these traits are linked to Atlantic sturgeon's anadromous life history, which is shared only by two other species of

sturgeon (*A. sturio* and *A. medirostris*).

Spawning frequency was estimated using interpretation of bands of narrow annuli, "spawning belts," and periodogram analysis of annular width measurements. Spawning belt analysis, on average, suggested that female Atlantic sturgeon spawn every four years. However, interpretations of spawning belts were highly subjective and imprecise. Annual patterns of strontium and calcium examined using microprobe analysis of hardparts has been shown to be coincident with upestuary spawning runs in striped bass. However, in Atlantic sturgeon fin ray sections, Sr:Ca ratios were probably related to annulus formation rather than migratory or spawning behaviors. Sr:Ca ratios in otolith sections taken from juvenile sturgeon, showed increased ratio with age, a pattern expected for juvenile emigration into coastal habitats.

Life history characteristics and natural and bycatch mortality estimates were included in an age-structured, spawning potential-per-recruit model to estimate the response of this population to different fishery management strategies. By choosing a higher acceptable spawning potential threshold (50% of the unfished egg production per recruit), as recommended by Atlantic States Marine Fisheries Commission, sturgeon fishery managers can avert risk of overfishing associated with managing a species that is long-lived and matures late in life. Size restrictions on harvested sturgeon are an effective control to preserve reproductive potential in the stock. Elimination of the directed coastal fisheries for sturgeon will allow a higher target fishing mortality in the more traditional Hudson River caviar fishery ($F_{50\%}$ = 0.047 and 0.26 for a coastal and Hudson River fishery, or an exclusive Hudson River fishery, respectively). The model was not sensitive to spawning frequency (3 - 5 yrs) but was sensitive to errors or variability in estimates of natural mortality and growth parameters, K and L_∞. Atlantic sturgeon show extremely low replacement rates and occur as bycatch in several coastal fisheries. Consequently, a moratorium on directed fishing will be necessary to rebuild the population.

Chapter 1.   Age and Growth of Hudson River Atlantic Sturgeon

Jill T. Stevenson and David H. Secor

INTRODUCTION

Atlantic sturgeon *(Acipenser oxyrinchus)* are large anadromous fish that use the Hudson River and other Atlantic coast estuaries as spawning and nursery grounds. Population levels throughout the species' range declined appreciably by 1900 due to an increase in the harvest of sturgeon for caviar following the Civil War (Murawski and Pacheco 1977). Overfishing and deterioration of habitat quality, predominantly the blockage of spawning runs, have contributed to the extirpation of several Atlantic sturgeon populations (ASMFC 1990; Waldman and Wirgin 1996). However, unlike most populations of Atlantic sturgeon, the Hudson River stock has recently supported both estuarine and coastal commercial fisheries. Increased harvests of Hudson River Atlantic sturgeon since 1990 generated concern among state and federal management agencies that the population may be overexploited (K. McKown, NYDEC, unpubl. data).

Age and growth rate estimates are essential inputs to stock assessment models used to evaluate the effect of fishing on population dynamics (Pitcher and Hart 1982). Inaccurate age determination can result in biased estimates of growth, recruitment, mortality, and production. Management decisions based upon inaccurate age estimates have led to overexploitation in several fisheries (Beamish and MacFarlane 1983). Age validation in hardparts is the determination of the true temporal meaning of a growth increment (Cailliet et al. 1986; Kalish 1995). Precision is defined as the repeatability of an age estimate, rather than determination of the actual age. Unbiased age estimates verify the age determination technique (Kalish 1995; Brennan and Cailliet 1991).

The life history strategy of Atlantic sturgeon indicates that age structure and vital rates are especially critical in guiding conservation efforts. Atlantic sturgeon exhibit many 'K'-selected traits (Ricklefs 1976; Adams 1980) such as large body size, high maximum age, late maturation, and probable low mortality rates. However, they exhibit high growth rates. These traits as well as their low relative fecundity and less-than-annual spawning frequency make sturgeon especially

1

susceptible to overexploitation (Boreman 1994).

Life History

Atlantic sturgeon are spawned near or above the salt wedge in the Hudson River (approximately Rkm 90, Fig. 1.1) from late May through early July (Bain 1994). Juveniles are thought to remain in the estuary for 2 - 4 years before they migrate down- estuary and subsequently range widely in and out of East Coast estuaries (Dovel and Berggren 1983). The older juvenile ("sub-adult") and adult fish reside in shelf waters along the Atlantic Coast and return up-estuary to spawn. Males mature within 10 - 12 years of age and females mature within 14 - 17 years of age (Doroshov et al. 1994). Little is known about sex-specific spawning schedules but it is thought that males return to the estuary annually to spawn and females probably spawn less frequently (Doroshov et al. 1994: See Ch. 3). Until recently, age-specific growth rates, survival rates, and reproductive schedules of Hudson River Atlantic sturgeon had been estimated using poorly validated techniques. Estimates of age-specific rates of growth, reproduction, and mortality were insufficiently developed to support management models for this declining population (ASMFC 1990).

Sturgeon Hardparts

Annuli in calcified structures have been used to age acipenserids; including scutes (Brennan and Cailliet 1989), fin rays (Harkness 1923; Bajkov 1930; Cuerrier 1951; Pycha 1956; Roussow 1957; Semakula and Larkin 1968; Huff 1975; Kohlhorst et al. 1980; Taubert 1980; Dovel and Berggren 1983; Wilson 1987; Brennan and Cailliet 1991; Guenette et al. 1992; Keenlyne and Jenkins 1993; Doroshov et al. 1994; Rien and Beamesderfer 1994), otoliths (Greeley 1937; Harkness 1923), operculi (Huff 1975; Brennan and Cailliet 1989, 1991), and other skeletal parts (Kleer 1916; Brennan and Cailliet 1989, 1991). Annuli in these structures are defined as a pair of optical zones that are formed as fish grow. In fin ray sections, a translucent zone (viewed with transmitted light) is thought to form during the winter months when somatic growth is slow. An opaque zone is generally wider and is thought to form during rapid growth in

2

the warmer months (Lai et al. 1996).

Pectoral fin ray sections have been preferred for use in ageing sturgeon because annuli in sections can be consistently interpreted. Fin rays are easily collected and processed without the need to sacrifice the fish (Brennan and Cailliet 1989). Pectoral fin rays have been used in the past to age Atlantic sturgeon but due to the perceived difficulty in ageing older fish (>20 years), age estimates were deemed unreliable (Magnin 1964; Huff 1975; Dovel and Berggren 1983). Difficulty in interpreting annuli in older fish was a result of the narrowing of the distal translucent zones and the presence of "false" annuli. We expected that improved methodology in thin-sectioning and polishing fin rays, and increased resolution through computer imaging would contribute to increased readability and precision in age estimates.

Otoliths are often the preferred structures for ageing but the rate of annulus formation has not been evaluated. Greeley (1937) enumerated ridges on the external surface of the otolith without examination of an internal plane. Subsequent studies indicated that interpretations of annuli on an internal plane of otoliths did not provide age estimates as precise as those from fin ray sections (Schneberger and Woodbury 1944; Brennan and Cailliet 1991).

Validation of Annulus Formation

Fin ray based ageing has been validated for white sturgeon *Acipenser transmontanus,* (Brennan 1988; Brennan and Cailliet 1991) and lake sturgeon *Acipenser fulvescens* (Rossiter et al. 1995). However, no studies have validated the periodicity of annulus deposition in Atlantic sturgeon. Marginal increment analysis is a common method used to determine the frequency of annulus formation (Wenner et al. 1986; Maceina et al. 1987; Hyndes et al. 1992; Barbieri et al. 1994; Lowerre-Barbieri et al. 1994; Tserpes and Tsimenides 1995; Crabtree et al. 1996). The marginal increment is the opaque zone deposited subsequent to the last identifiable translucent zone at the margin of a structure used for age estimation (Kalish 1995). Seasonal growth of the opaque zone is used to determine the timing of annulus formation (Cailliet et al. 1986; Beamish and McFarlane 1987; Brennan and Cailliet 1989). This analysis assumes the translucent annuli are formed when the marginal increment is zero.

An alternative method of validation is based upon known-age individuals or a known

3

interval since a calcified structure was marked. Because the time or age since birth or marking is known, direct observations of growth zones subsequent to the first annulus or mark can validate annual deposition of growth rings. Oxytetracycline (OTC) has been used to mark sturgeon fin rays to validate that annuli are deposited annually (Beamish and MacFarlane 1987; Francis 1995).

Microchemical analysis of opaque and translucent zones is an emerging method of verifying the periodicity of annuli (Jones and Geen 1977; Casselman 1983; Radtke and Targett 1984; Cailliet and Radtke 1987). In this method, variations in calcium concentrations are correlated with optically defined growth zones in the hardpart. The opaque zone, formed when growth is rapid, should contain higher calcium carbonate concentrations (Cailliet and Radtke 1987; Lai et al. 1996). Conversely, the translucent zone is expected to contain lower concentrations of calcium. Therefore, the most recently formed material in fin rays should comprise high and low concentrations of calcium in fish collected during summer months and winter months, respectively. Other elements (e.g., K, Mg, Na) might also indicate seasonal trends in deposition correlated to the optical zones of annuli.

In our study, four approaches were used to verify the accuracy in age estimation using fin ray and otolith sections: 1) marginal increment analysis of fin rays of sturgeon collected throughout a 12 month period; 2) laboratory rearing of OTC-marked and known-age fish; 3) fin ray microchemical analysis of peripheral regions to verify the visual interpretations of seasonal growth zones (i.e., opaque and translucent zones); and 4) microchemical life history analysis of fin ray and otolith sections to identify seasonal trends in calcium which would indicate annulus formation.

We used interpretations of annuli in fin rays to estimate growth rate of Hudson River Atlantic sturgeon using the von Bertalanffy growth model. Dovel and Berggren (1983) measured growth of Hudson River Atlantic sturgeon. However, their sample size was small and sexes were not differentiated. Sex-specific growth models were needed to support yield per recruit and egg production potential ratio management models (Ch. 4).

Objectives

1)   Develop a technique using hardparts to age Atlantic sturgeon from the Hudson River.

4

2)   Assess the accuracy and precision of this technique.

3)   Model the sex-specific growth patterns (length at age) for input into a yield-per-recruit
        analysis (Ch. 4).

METHODS

Pectoral fin rays from juvenile (70- 149 cm total length [TL]) and adult Atlantic sturgeon
were collected during fishery harvests (1992-1995) in New York and New Jersey waters in
cooperation with New York Department of Environmental Conservation (NYDEC) and New
Jersey Department of Environmental Protection (NJDEP). Commercial fishermen were required
to submit one pectoral fin ray from every harvested sturgeon to NYDEC with dressed (head, tail,
fins, and entrails removed) carcass length and weight information. Research scientists (J. Van
Eenennaam, Univ. of CA, Davis; M.Bain, Cornell Univ.; and J. Stevenson,Univ. of MD) assisted
in these collections in 1993-1995.

Prior to 1993, 122 cm TL (4 feet) and 107 cm TL minimum size limits were enforced for
New York and New Jersey fisheries, respectively. Fishing seasons were limited to a spring and
fall season. In 1993, the minimum size increased to 152 cm (5 feet) and entry to the fishery was
limited. Furthermore, the combined New York and New Jersey Atlantic sturgeon harvests were
capped at the 1990 level. Principle gears were anchored and drift gillnet (25-36 cm stretched
mesh) in the Hudson River and gillnet and trawls in coastal waters (Young 1994). Fin rays were
obtained from juvenile sturgeon in the Hudson River (1993-1995) which were collected using 3 -
13 cm (1-5") stretched mesh monofilament anchored gillnets set during slack tide for a maximum
of one hour. In addition, fin rays were obtained from: 1) commercial fishermen in Chesapeake
Bay (1996); 2) by National Marine Fisheries Service (NMFS) personnel on spring trawl surveys
in the Mid-Atlantic Bight (1994, 1996, J. Fields, NMFS Northeast Science Center, Woods Hole,
MA); and 3) by USFWS personnel in Delaware Bay (J. Mohler, USFWS, Northeast Fishery
Center, Lamar, PA).

Reported measurements of dressed carcasses were converted to total length for growth
analysis based on our measurements of Atlantic sturgeon in the field (Table 1.1). The relationship
between dressed weight and dressed length was calculated for sturgeon from the New York and

New Jersey coastal fisheries for both spring and fall-captured fish (1993-1995). Sturgeon included in the allometric regression sample were collected during spawning season in the Hudson River and all fish but five possessed mature gonads. Condition factor was estimated as:

$$C = TW/TL^3 *100$$

where TW = total weight (g) and TL = total length (cm) (Weatherley and Gill 1987).

Removal and Preparation of Ageing Structures

The first pectoral fin ray was removed at the point of its articulation and air-dried (n = 634). Fin rays were sectioned no more than 1 cm distal to the articulation point to reduce the risk of 'losing' annuli in narrow peripheral regions of the ray (Figure 1.2). Soft tissue which adhered to the fin rays was allowed to decompose through microbial decay. A section of each fin ray (1-cm wide) was then embedded in a block of Spurr epoxy, sectioned using an Isomet saw (Buehler, Lake Bluff, IL) and mounted on glass slides (see Secor et al. 1991). Some fin rays were not embedded but were sectioned using a jeweler's saw. All sections were then polished using a Minimet 1,000 automatic grinder/polisher (Buehler, Lake Bluff, IL) with fine grit carborundum paper and a 0.3 μm alumina slurry on a polishing cloth. Final sections were ca.1-2 mm in thickness.

Sagittal otoliths were removed from the severed heads of a subset of fish (1994-1995, n = 114) by making a cranio-caudal mid-sagittal cut from the snout to a position posterior to the operculum (Secor et al. 1991). The intercranial cavity was then exposed and the semicircular canals and otoliths were located and removed (Figure 1.3). It was important to bisect the cranium precisely because of the medial position of the sagittal otoliths. We were consistently successful at finding the sagittae and occasionally located the lapilli. Due to their small size, the asteriscii were retrieved in only a few instances. Lapilli were not used for ageing purposes due to their small size and brittleness which made handling difficult. Sagittae were cleaned in 10% bleach, rinsed several times with deionized water, and air-dried. One sagittal otolith from each pair was weighed to the nearest 0.01 mg, embedded in Spurr epoxy, and sectioned as described in

6

Secor et al. (1991). Due to their fragility, otolith sections were polished by hand using a variety of fine sandpapers and 0.03 μm alumina slurry. In contrasts among several sectioning planes, we observed that transverse sections displayed the most consistently interpretable annuli.

Annuli were interpreted in thin sections viewed under reflected light at 15X magnification. Annuli in fin rays and otoliths were defined as bipartite zones, each comprised of an opaque and a translucent zone (Figure 1.4). The first translucent zone was counted as the beginning of the first year of life. In some instances, a secondary fin ray was incorporated within the primary fin ray (see Figure 1.5a). In these cases, care was taken to enumerate annuli only in the primary ray. "False" annuli appear in fin ray sections of older individuals. These structures were not continuous around the entire circumference of the section and were thus distinguishable from counted annuli (Prince et al. 1985; Doroshov et al. 1994).

Precision and Bias in Annulus Interpretation

Two experienced readers interpreted annuli in samples of both fin rays and otoliths without knowledge of collection date, fish size, or previous age determination. Personnel were trained with the aid of an imaging system which permitted simultaneous observation and discussion of annular growth zones. Additionally, an experienced reader from a University of California laboratory (J. Van Eenennaam, UC, Davis), provided age estimates from independently prepared fin ray sections taken from the same sample. Paired difference tests were used to statistically evaluate bias and precision among readers.

Bias was assessed visually using an age-bias plot. The coefficient of variation was used to measure precision and age frequency histograms were used to document matched age estimates. In the absence of bias, coefficient of variation is the most sensitive statistic in documenting precision among multiple readers and is preferred over correlation coefficients and percent agreement values (Campana et al. 1995a). The age-bias plot visually illustrates one age reading versus another and is interpreted through reference to the equivalence line Reader 1 = Reader 2. The age estimates of Reader 2 are represented as the mean age and 95% confidence intervals corresponding to each of the age classes estimated by Reader 1. For example, if Reader 1 estimates 5 fish to be 15 years old, the age-bias plot indicates the mean age of those 5 fish as

7

estimated by Reader 2. Divergence from the equivalence line indicates systematic differences between the two readers. Additionally, age estimates derived from otolith sections and fin ray sections from the same individual were compared to identify the most useful hardpart for ageing purposes.

Validation of Annulus Formation

The timing of annulus formation in Atlantic sturgeon fin rays and otoliths was validated based upon chemical marking of juvenile hatchery-reared fish, marginal increment analysis, chemical microanalysis of marginal increments, and seasonal trends in microchemistry throughout the life of the fish. Our efforts were concentrated on fin ray age validation because annuli in otolith sections were difficult to consistently interpret in otolith sections (Table 1.2, See Discussion).

1. OTC Marking of Fin Rays

A solution of oxytetracycline (25 mg/kg body weight) was injected into the dorsal musculature of five juvenile, known-age, laboratory-reared fish (55 - 65 cm TL) from Hudson River broodstock which were spawned at the USFWS Northeast Fishery Center, Lamar, PA. Juveniles were reared in circular fiberglass tanks in a recirculating system at Leetown National Science Center (A. Henderson-Arzapalo, U.S. Geological Survey, Leetown, WV). All fish were fed a mixture of commercial pellet feeds. Fish were subjected to a 12-hour day: 12-hour night photoperiod and water temperature ranged from 15 to 20°C. At 3 months post-injection, the first fin ray section was removed with a jeweler's saw and silver nitrate was applied to the fin to encourage clotting of the wound. At fifteen months post-injection, a second section was removed from the opposite pectoral fin ray. Fin ray segments were dried, sectioned, mounted on glass slides, and polished. Thin sections were viewed with epifluorescent microscopy to identify the OTC marks. The position of the OTC mark with respect to opaque and translucent zones was recorded and a micrograph was taken. In all cases, the reader was aware that the fish had received an injection of OTC but had no knowledge of the date of collection of the section.

2.   Marginal Increment Analysis

Increment widths were measured with an image analysis software package (Optimas, Inc.) to the nearest 0.001 mm. The marginal increment ratio (R_{mi}) was estimated for each fin ray according to the formula:

$$R_{mi} = M * X^{-1}$$

where M = the width of the outermost opaque zone (marginal increment) and X = the mean of the 3 opaque zones deposited previous to M. This method reduced biases associated with ontogenetic variation in widths of annuli and inconsistencies in the location where the fin ray was severed from the torso. Age and sexes were combined and mean R_{mi} and standard error computed for each month separately. A Kruskal-Wallis non-parametric test was used to determine differences among months ($\alpha = 0.05$).

3.   Microchemistry of Hardparts

Wavelength dispersive spectrometry (WDS) was used to measure the microchemistry of fin rays and otoliths. Calcium, phosphorus (fin rays only), and magnesium (otoliths only) concentrations were measured in hardparts using a JEOL JXA-840A wavelength - dispersive electron microprobe (Center for Microanalysis, College Park, MD). Phosphorus was measured in fin rays only because it comprised a large fraction of the fin ray's hydroxyapatite structure (See Ch. 2). Magnesium analyses in otoliths were time consuming and expensive. Therefore, only 3 otoliths were examined. Accelerating voltage was 25 kV, cup current was 20 nA, and each measured concentration represented a 5 x 5 μm rastered square area in the hardpart section.

The microchemistry of peripheral regions of hardparts, representing the most recently deposited calcium and phosphorus, was measured in a sample of 20 fin rays taken from fish ranging 3-18 years of age collected in March, June, September, and November. Three points were measured on each section and a mean computed. Mean elemental concentrations for each month of collection were compared to estimate the timing of annulus formation.

Elemental chronologies of 15 fin rays and 11 otolith sections from mature fish (Table 1.3) were also measured using WDS microanalysis. These chronologies were series of point

measurements along axes that traversed several annuli. Chronologies were set up so that at least 5 points were measured per annulus. Points were assumed to sample season in linear proportion. The point immediately succeeding the translucent zone was labeled time 0 and subsequent points throughout the opaque zone were additively combined. For example, if 5 points were probed in one annulus, points would be labeled as $t + 0$, $t + 0.2$, $t + 0.4$, etc. where t is the number assigned to each annulus. Due to expected autocorrelation of elemental concentrations between adjacent points, data were analyzed with a repeated measures, multivariate analysis of variance (MANOVA, $\alpha = 0.10$) to identify seasonal trends within years (Chambers and Miller 1995).

The model used for the MANOVA analyses designated dependent variables with respect to independent factors of interest (SAS 1987) and evaluates the matrix equation:

$$Y = X\beta + E$$

where Y is a matrix of dependent variables (columns) and n observations (rows), X contains k treatment levels for each factor (columns), and n observations (rows), β contains k treatment levels (columns) for each dependent variable (rows), and E is the matrix of model residuals. A disadvantage of this analysis is that all cells of the matrix must contain data. An entire row was omitted from the analysis if data were missing from that row.

The choice of hardpart for an analysis may affect seasonal patterns of elemental concentrations. These effects were evaluated by the model:

$$M_{0.0}, \; M_{0.2}, \; M_{0.4}, \; M_{0.6}, \; M_{0.8} = \text{Hardpart (fin ray or otolith)}$$

where M_t = microconstituent concentration at seasonal point t. Separate MANOVA's were performed for each age.

Growth

Ages derived from fin ray sections were used to construct sex-specific von Bertalanffy growth models. Growth parameters were estimated iteratively using a least squares method (Statgraphics 1989). The model is described by the following equation:

$$L_t = L_\infty [1 - e^{(-K(t-t0))}]$$

where L_t is the mean length at age t, t is the age in years, K is the Brody growth coefficient (Ricker 1975), L_∞ is the asymptotic length, and t_0 is the age at which the length of the fish is equal

to 0. Length-at-age relationships were first examined to determine their variance structure and progression of modal lengths with age. Because of the tremendous variability of points at the inflection point, it was unlikely that a single growth model would fit all stanzas of the growth curve. Therefore, juvenile growth was modeled separately with a multiplicative function applied to sex-specific length data.

All statistical calculations were completed by PC-SAS (SAS Institute, Inc.) and Statgraphics (STSC, Inc.). Data that did not satisfy assumptions of homogeneity of variances (Bartlett's test, $\alpha = 0.05$) were transformed to satisfy this assumption. Transformed data which still did not satisfy this assumption were analyzed using a Kruskal-Wallis non-parametric test to examine differences between groups.

RESULTS

Allometric Relationships (Table 1.1)

Sex-specific length-weight relationships had exponents <3.0 (males, $\beta = 2.84$ and females, $\beta = 2.42$). Other relationships had significant fits and supported conversions of data supplied by fishermen for our growth analysis. Dressed weight was the most useful predictor of total length ($r^2 = 0.73$) and total weight ($r^2 = 0.99$) needed for growth models.

Females had a significantly higher condition factor in spring than in fall (ANCOVA, covariate = age, n = 260, p = 0.01; Figure 1.6). Males did not exhibit significant seasonal differences in condition factor (ANCOVA, covariate = age, n = 345, p = 0.48). Condition factor increased slightly with increasing age. Variability in condition factor at age increased with increasing ages for both males and females (Figure 1.7). High variability was observed for mature age-classes (≥ 10 years, males; ≥ 13 years, females).

Optical Interpretation of Annuli

Otoliths were irregularly shaped and their annuli were difficult to interpret. The first 3-9 growth zones showed clear alternation of opaque and translucent zones. Thereafter, translucent

11

zones were irregularly spaced and often appeared to 'overlap.' These features decreased confidence in assigning annuli (Figure 1.4).

Fin ray sections exhibited concentric optical bands: narrow translucent zones and wide opaque zones when viewed with transmitted light (Figure 1.4). Fin rays contained a vascularized core and deposits of organic material in lobe regions. Interspersed were fibrils which we interpreted to be collagen or some other structural protein. Cells and small canaliculi were observed throughout the fin ray section. Bipartite annuli were apparent in all fin ray sections. Annuli became much more narrow towards the outer edge in larger (and presumably older) fish.

False annuli were apparent in fin ray sections, especially those taken from older fish. These ill-defined gray zones (slightly opaque) had an appearance similar to translucent zones. Because these gray zones were not continuous around the circumference of the fin ray section, they could be consistently identified and excluded from annuli counts. "Belts" of two to five narrow annuli (Figure 1.5b) were apparent in most female fin ray sections (96% of a subsample of 48 female fin rays). These 'belts' may be related to spawning history (Ch. 3).

Secondary fin rays were embedded in most primary fin ray sections (84%). Secondary rays did not consistently occur in either the left or right lobe, or in both lobes. Annuli of the primary ray were easily distinguished from those of secondary rays (Figure 1.5a). Ages estimated from embedded secondary fin rays were similar to ages estimated from the primary ray in individuals up to ca. 12 years old. In older fish, the number of annuli in secondary fin rays was generally less than that enumerated in the primary fin ray.

The level of precision was quite good between the two Chesapeake Biological Laboratory (CBL) readers ageing the same fin ray sample (C.V. = 4.8%; Figure 1.8). Ages were not significantly different between two CBL readers and mean imprecision was 1.2 years (t= -1.97, p>0.05; Table 1.4). Replicate age estimates were less precise between readers from different labs viewing different specimen sections (mean imprecision = 4.6 yrs., C.V.= 7.2%). The discrepancies were generally in older fish, and were randomly distributed between readers indicating no bias (t = 0.21, p>0.05). Pairwise comparisons of age estimates from otolith sections were less precise than fin ray interpretations (Figure 1.9). Although the absolute imprecision, 3.3 yrs., was similar to fin ray section interpretations, C.V. (14.8 %) was much higher.

In a comparison of otolith and fin ray sections, ages estimated from otolith sections were

significantly lower than those estimated from corresponding fin ray sections (Figure 1.10). The precision was poor (C.V. = 19.8%) and the bias was most apparent in presumed older fish (mean difference = 5.5 yrs., t = 9.01, p<0.05). Annuli were difficult to identify in otoliths from fish with >20 annuli due to a lack of optical contrast between opaque and translucent zones.

Accuracy of Ageing Techniques

1.   Known-Age and OTC-Marked Juvenile Atlantic Sturgeon

Annuli interpretations of known-age hatchery-reared sturgeon resulted in exact age estimates. The sturgeon were 4+ years old and annuli were clearly defined. There was a distinct difference in the morphology of hatchery-reared and wild juvenile sturgeon fin rays. Hatchery-reared fish exhibited irregularly shaped, compressed annuli which may indicate erosion or injury (Figure 1.11) but annuli were nonetheless easily recognized. A distinct OTC mark followed by an opaque zone was apparent in all of the fin rays examined 3 months post OTC-injection (N = 4, Figure 1.12). The fish did not show any symptoms of stress following fin ray removal. At 15 months post-OTC injection, a single fin ray section exhibited a clear OTC mark, followed by an opaque and translucent zone.

2.   Marginal Increment Analysis

Marginal increment ratio for spring-collected fish (February, March, April) were apparently lower than for other months (Figure 1.13). Because very few fish were collected in "other" months, monthly samples were pooled for each season and compared among seasons. Kruskal-Wallis test (p = 0.04) results supported the apparent decline in marginal increment ratio in spring months. Mean MIR was 18% for spring months (Feb.- May), 33% for summer months (June-Sept.), and 36% for winter months (Oct.-Jan.). Highest rates of marginal increment 'completion' were observed in winter months (November-December).

3.   Microchemical Analysis

    Microchemical analysis of the peripheral regions of fin rays demonstrated a significant seasonal effect on concentrations of calcium and phosphorus in fin ray sections collected throughout the year (Kruskal-Wallis test, $p \leq 0.04$, Figure 1.14). November-collected fin rays were significantly lower in calcium and phosphorus than March, June, and September-collected samples. Ca/P ratios increased significantly throughout the year (Kruskal-Wallis test, $p = 0.04$).

    Seasonal trends of calcium chronologies of fin ray sections could not be analyzed using repeated measures MANOVA's because they did not satisfy the assumption of multivariate homogeneity (Mauchley's criteria applied to orthogonal components, $\alpha = 0.05$). In all cases, non-optimal transformations did not correct for this problem. Fin ray plots did, however, reveal cyclical trends in Ca and P associated with translucent zones (Figure 1.15). Cyclical trends were especially apparent in Ca/P chronologies of fin rays. In 5 of 15 fin ray sections analyzed, Ca/P increased with increasing age.

    For otolith sections, the MANOVA test for seasonal trends in calcium chronologies in otoliths sections revealed no significant seasonal effect ($p = 0.18$). These trends, however, may have been obscured due to the omission of data required for a balanced MANOVA analysis. "Unedited" transect data showed cyclical patterns in Ca with periodic nadirs which were consistently associated with translucent zones (Figure 1.16). Magnesium in otolith sections also exhibited seasonal trends in two of the three specimens examined, with low Mg in summer months (opaque zone of fin ray) and higher concentrations of Mg associated with the time of translucent zone formation, although sample size was small (Figure 1.17).

Growth

    Based upon interpretations of annuli in fin ray sections, there was considerable variation in length-at-age among Atlantic sturgeon. Normal probability distributions of total length for age classes were constructed for each sex (Figures 1.18 and 1.19). With the exception of age classes 18 - 19 years for males ($p = 0.004$) and 10-11 years for females ($p = 0.003$), age class distributions were not significantly different than a normal distribution ($x^2 > 0.05$). x^2 tests could

14