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Age Determination of Modern and Archaeological Chinook Salmon (Oncorhynchus tshawytcha) Using Vertebrae

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Age Determination of Modern and Archaeological Chinook Salmon (*Oncorhynchus tshawytcha*) Using Vertebrae

by

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A thesis submitted in partial fulfillment of the
requirements for the degree of

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in
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Abstract

Incremental growth rings in X-rays of salmon vertebrae have been used since the 1980s to age Pacific salmon (*Oncorhynchus spp*.) remains from archaeological sites in the Pacific Northwest. These age estimates, paired with generalized life history patterns, have been used to determine salmon species, season of capture and in turn season of site occupation. This approach relies on a variety of assumptions, the most fundamental of which is that rings represent true years. Archaeologists using vertebral age determination techniques have failed to adequately test this assumption and present their methodologies. This thesis assesses the validity of using incremental growth structures in Chinook salmon *(Oncorhynchus tshawytscha)* vertebrae to determine the age at death of fish represented in archaeological sites. This project develops criteria and a protocol for the identification of true annuli and tests these identifications on a collection of modern Chinook salmon of known age. Finally, this protocol is applied to archaeological remains of Chinook from Cathlapotle (45CL1).

Three collections of modern known age fish (N=121) were used to evaluate and test approaches to aging Chinook salmon with vertebrae. These collections contained juvenile and adult Chinook from Washington and Oregon. I evaluated a variety of methods for viewing rings including magnified surface images, X-ray images and thin sections to determine which is the most accurate, reliable and efficient, also considering the extent of specimen destruction. Rings visible in X-rays were found to reflect the internal structure of vertebrae rather than annular growth. The number of these internal walls did not correspond to the known ages of fish and are therefore not true annuli.

Criteria previously described by salmon fisheries biologist were used to isolate annuli (on the centrum surface) on the Hanford reach collection (N=46). In a test for accuracy 39 (85%) were aged correctly. In a test of reliability utilizing five additional readers all but 14 cases showed discrepancies among readers. Results of the test of reliability were not as successful as other researchers in fisheries biology but given the high accuracy rate the method remains valid.

The final goal of this project was to determine the feasibility of applying the surface ring method of age determination to archaeological collections. Archaeological salmon vertebrae from Cathlapotle (45CL1) on the lower Columbia River, Washington state were utilized. One hundred salmon vertebrae were selected and classified to species according to Huber et al. (2011); 89 were identified as Chinook. Of these, 39 had sufficient preservation of the surface to view and interpret incremental rings. Three ages were identified 3, 4 and 5 year olds. This ageing protocol can be applied to archaeological Chinook salmon vertebrae to estimate age of ancient Chinook salmon. Additional work is needed on other salmon species to demonstrate the methods validity across all salmonid species.

This analysis has great potential for modeling salmon paleo-life history by contributing data from salmon populations prior to the major impacts of the 19th and 20th century. This is particularly valuable for salmon conservation because information on size, the timing and duration of freshwater emigration as well as the age of spawning and death is critical to the management of hatchery and wild salmon populations.

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Chapter 1.

Introduction

This thesis assesses the validity of using incremental growth structures in Chinook salmon *(Oncorhynchus tshawytscha)* vertebrae to determine the age at death of fish represented in archaeological sites. This ageing method has valuable applications for both archaeology and fisheries biology. Incremental growth structures, or growth rings, are found within the skeletal framework of many organisms such as the vertebrae and otoliths of fish, teeth of mammals and the exoskeletons of bivalves. These growth structures are marked by changes in topography, color or opacity within elements and are the result of daily, monthly or seasonal fluctuations in the conditions conducive to growth.

In archaeology, seasonality studies use incremental growth structures such as vertebrae, scales, otoliths and spines to determine the season of death and ages of organisms at death. This information is used to infer the time of year (month or season) of resource procurement and site occupation, contributing to our understanding of prehistoric mobility and land use (Andrus 2011; Brewer 1987; Cannon and Burchell 2009; Casteel 1972; Colley 1990; Higham and Horn 2000; Monks 1981; Morey 1983; Stutz 2002; Van Neer et al. 1999; Wheeler and Jones 1989). This is typically achieved by determining or estimating the amount of growth within one year of the increment and measuring the proportion of a year's growth past the last increment. However, archaeologists have lacked rigor in testing and presenting the validity of these methods.

In the Pacific Northwest, age determination from the incremental growth structures in fish vertebrae has been used for seasonality studies as well as to assign species classification to Pacific salmon (*Oncorhynchus* spp.) remains from archaeological sites. Cannon (1988) was the first to use incremental growth rings on vertebrae to determine age at death and in turn infer species. Cannon suggested, as did others (Carlson 1988; Wheeler and Jones 1989), that rings were often not visible on the surface of archaeological specimens. As an alternative Cannon employed X-ray technology, suggesting that X-rays were a superior way to observe the incremental rings of vertebrae. Cannon connected the age of archaeological vertebrae with the species that tended to spawn at that age, assuming that each species spawned at a specific age and in a distinct season. These species identifications were used to suggest the season of site occupation at Namu (ElSx-1) a coastal British Columbia archaeological. Others have followed Cannon in using fish age estimated from vertebrae for species identification (Berry 2000; Orchard and Szpak 2011; Trost 2005). Such applications rely on various assumptions including that incremental bands on vertebrae in fact represent annual growth. Archaeologists using vertebral age determination techniques have failed to adequately test this assumption and present their methodologies. Fisheries research (e.g. Campana and Thorrold. 2001) even suggests that fish vertebrae may not share the unique ageing properties of other elements, thus reinforcing the need for validation studies.

The use of incremental growth structures, usually on scales and otoliths, for age determination is an integral area of fisheries biology because it contributes to our understanding of fish population dynamics. Campana and Thorrold (2001) estimated from an informal survey of 30 fisheries laboratories from around the world that at least 800,000 otoliths and close to 2 million fish were used in ageing studies in 1999 alone. The information from such studies is used to calculate growth rate, mortality rate and productivity which informs fisheries management options (Campana 2001). Overly optimistic estimates of growth and mortality rates have led to catastrophic effects on populations of fish species (Campana 2001). Generally, these problems arise from underestimates of fish maturity rates which result in overharvesting of populations before they reach reproductive maturity (e.g. Beamish and McFarlane 1995; Smith et al. 1995).

A critical component of fisheries ageing studies concerns validation and controlling for sources of error. The formation of yearly growth rings, or annuli, for northern temperate fish populations has been primarily attributed to differences between slow winter growth (seen as dark narrow bands under reflected light) associated with low water temperature and reduced food supply and more rapid summer growth (seen as broad white bands) associated with an increase in water temperature and a more abundant food supply (Beckman and Wilson 1995; Kusakari 1969; Rojo 1987). However, other factors including reproductive cycles, population densities, local water conditions, the availability of food and temperature variation have also been suggested as determinates of ring growth that may not reflect annual patterns (Beckman and Wilson 1995; Irie 1960; Morey 1983). Incremental growth rings can represent one year of growth but other rings called false annuli or growth checks, caused by these other factors, can be present. True age assessment thus requires distinguishing such checks from true annuli. Even within professional ageing laboratories, mistakes are made and fish ages are inaccurate. Campana (2001) noted interpretation error resulting in the mis-ageing of haddock (*Melanogrammus aeglefinus*) by as much as 50%. Because of these potential sources of variation in the formation and interpretation of incremental rings in fish vertebrae, validation including blind studies are needed to better understand annulus formation and to substantiate age determination.

The validation of vertebral age determination of Pacific salmon and its application to archaeological materials has the potential to inform current fisheries research. This research will allow age to be used in conjunction with other analyses to create detailed life history profiles (e.g. morphometric species identification [Huber et al. 2011], stable isotopes [Zazzo et al. 2006], and skeletal element analysis [Miller et al. 2011; Robinson et al. 2009] to model salmon paleo-life history. This will enable fisheries managers to use this understanding of the variability in age and growth of salmon populations for the management of hatchery and wild salmon populations.

The primary goal of this project was to firmly establish if incremental growth rings present on Chinook salmon vertebrae represent years of growth. My thesis develops criteria and a protocol for the identification of true annuli and tests these identifications on a collection of modern Chinook salmon of known age. To accomplish this, I evaluate a variety of different methods for viewing rings including magnified surface images, X-rays images and thin sections to determine which is the most accurate and reliable. The secondary goal of this project is to determine the feasibility of applying this method of age determination to archaeological collections by identifying protocol, criteria of ring identification and defining limitations of this methodology. I apply this new method of age determination to archaeological collections of salmon vertebrae from Cathlapotle (45CL1) on the lower Columbia River, Washington state.

This thesis is organized into six chapters. In Chapter 2, I describe the life history of Pacific salmon including distribution, habitat and the timing of migrations to and from freshwater. I follow this with a review of ageing studies in fisheries biology, covering the process of validating the accuracy and reliability of methods. I conclude with an overview of ageing studies in archaeology. In Chapter 3, I present the methods and materials used in this project, which includes acquisition of a control sample, specimen preparation and the development of my ageing protocol. Additionally I discuss the archaeological application of my technique including a review of the methods of vertebra selection, species identification and age determination. In Chapter 4, I evaluate the validity of using rings visible in X-ray images of Chinook salmon vertebrae to determine age. To do this, I compare X-rays, thin sections and surface images to identify specifically what X-ray images highlight. In Chapter 5, I present the results of a blind test of my protocol for accuracy and precision. I also apply my method of age determination to archaeological vertebrae from Cathlapotle. In Chapter 6, I present the conclusions of this project and directions for future research.

Chapter 2.

Ageing Studies in Archaeology and Fisheries Biology

Archaeological Fish remains and Conservation Biology

Archaeology has the potential to provide a long history of fisheries prior to major impacts of the 19th and 20th century (e.g. Baisre 2010; Betts et al. 2011; Jackson et al 2001; Pauly 1995; McKechnie et al. 2014). Data concerning fisheries prior to the development of land, rivers and fisheries is limited and although historical records may offer a mention of historic fisheries, these records are often brief and potentially unreliable (Hewes 1973). Fish bones from archaeological sites can be used to reconstruct details about prehistoric fish populations in a quantitative fashion, including the age, size, distribution and life history patterns informing the decisions and management strategies of conservation biologists (Butler and Delacorte 2004; Whyte 2004).

This information can be particularly useful for salmon conservation because information on size, the timing and duration of freshwater emigration as well as the age of spawning and death is so critical for the management of hatchery and wild salmon populations. Some researchers have used archaeological salmon remains to understand demography and life history of populations prior to industrial development. Robinson et al. (2009) used strontium/calcium ratios of archaeological salmon vertebrae to differentiate sea-run from landlocked salmon life history patterns. Stevenson (2011) and

Butler et al. (2010) used a geochemical analysis and mDNA to determine the native status of salmon of the Upper Klamath Basin. Miller et al. (2011) compared the structural and chemical composition of archaeological Chinook salmon otoliths to modern ones to provide information on Chinook salmon life history before and after local population extirpation in the upper Columbia River in North Central Washington.

Historically, most salmonid demographic and life history research has relied primarily on scales (Bali 1959; Campbell 2010; Connor et al. 2005; Dahl 1911Gilbert 1913; Koo 1962; Rich 1920) with some use of otoliths (Murray 1994; Nielson and Geen 1982) and fin rays (Chilton and Bilton 1986) for ageing fish, but little emphasis on exploring the relationship between incremental rings and age in vertebrae (but see Hesse 1994; Wesley 1996). In archaeological contexts, scales and otoliths are rare, but vertebrae are well preserved, abundant and commonly recovered. They offer great potential to investigate life history patterns for prehistoric salmon populations. Reaching this potential requires that we develop valid approaches to determining fish age using vertebrae.

Pacific Salmon Life History

The genus *Oncorhynchus* includes seven anadromous species distributed today along the west coast of North America from Los Angeles, California (34º 03' N) to the Arctic Ocean in Alaska (66º 30' N), and in the northwest Pacific from the Sea of Okhotsk (59º N) to the eastern Korean Peninsula (34º 11' N) (Groot and Margolis 1991; Nelson 1994). Pacific salmon spawn in gravel beds of rivers and streams, or along lake shores. After emerging from the gravels, most young salmon migrate to sea after varying periods of freshwater residency (a few weeks to three years). Maturation occurs during marine residence lasting one to seven years after which adults return to their natal breeding grounds to spawn. As shown in Table 2.1, there is considerable variation in life history between and within species. For example, Chinook salmon migrate to sea during their first year of life, normally within three months of emerging from the spawning gravel (Groot and Margolis 1991). They spend the majority of their lives in coastal waters and return to their natal streams a few days to a few weeks before spawning. Most Chinook salmon return to spawn in the fourth or fifth years of life but some may return as early as the third or as late as the eighth (Hart 1973). This variation in the juvenile and adult behavior of Chinook salmon has developed as an adaptive strategy to spread the risk of mortality across habitats and years (Groot and Margolis 1991; Quinn 2005).

Table 2.1 Life history of Pacific salmon (*Oncorhynchus spp*.) (from Groot and Margolis 1991 unless otherwise noted)

Species	Freshwater Residency	Age at Spawning	Spawning Season
Sockeye (*O. nerka*)	1 month-3 years	2-7 years	Late summer-Fall
Pink (*O. gorbuscha*)	1 month	2 years	August-November
Chum (*O. keta*)	1 month	2-7 years	May-January
Chinook (*O. tshawytscha*)	3 months-2 years	3-8 years	Generally May to October
Coho (*O. kisutch*)	1 year or more	1-3 years of age[1]	March to August
Rainbow/steelhead (*O. mykiss*)	1-3 years1	1-5 years[1]	Highly Variable[1]
Cutthroat (*O. clarkii*)	1-6 years[1]	Highly Variable[1]	Feburary-March[1]

[1] Wydoski and Whitney 2003

Age Determination in Fisheries Biology

As previously discussed, age determination from incremental growth structures in fish is widely used in fisheries biology to learn about age, growth and population dynamics. Age data form the basis for calculations of growth rate, mortalilty rate and productivity and many models used in decisions regarding populations are based on age stock assessments (Campana 2001; Campana and Thorrold 2001). Fisheries research focuses on otoliths and scales, but other elements including fin rays, opercula, cleithra and vertebrae are sometimes used. Otoliths are generally favored because of the precision of age estimates they provide based on annuli and the relative ease of otolith preparation and annuli enumeration (Campana and Thorrold 2001). Whichever element is used, ageing studies emphasize the need to validate methods (Beamish and MaFarlane 1983, 1987; Campana 2001).

The process of validating fish ageing techniques involves minimizing two main sources of error (Beamish and McFarlane 1983; Campana 2001). The first is *accuracy* or *process error* and is associated with establishing whether alternating incremental growth structures are in fact annular. Evaluation of this type of error is generally achieved by comparing age estimates from growth structures to the known ages of fish. These known ages are independently determined through captive rearing, the recapture of fish tagged at birth with a coded wire tag (CWT), radiochemical dating or mark-recapture utilizing oxytetracycline (OTC) injection. CWTs are small pieces (0.25 x 0.5 or 1.0 mm) of stainless steel wire that are injected into the snouts of juvenile salmon. Each tag is etched with a binary code that identifies its release group which includes date and location of release. For OTC marking, OTC is injected into a captured fish and is incorporated into the tissue and identifiable upon recapture making it possible to observe the formation of increments since it was marked. The second source of error is *interpretation error* and is associated with the replicability/reliability of the method or what Beamish and McFarlane (1983) call precision. In other words, do multiple observers produce the same results? This type of error is evaluated by comparing age estimates made by multiple analysts reviewing the same specimen (e.g. scales or otoliths) utilizing the same method to estimate age.

Ageing Fish Vertebrae

As with other elements that grow incrementally, growth rings in fish vertebrae result from variations in the density and cellular structure of the ossified elements. On the surface of vertebrae viewed microscopically under reflected light, these annular patterns on vertebrae centrum are seen as an alternate pattern of translucent (dark, absorptive zones) and opaque (white, reflective zones) bands (Kusakari 1969). In his observations of the flatfish, *Kareius bicoloratus,* Kusakari (1969) was the first to note that the most central part of the centrum, the focus, is dark and is followed by the first white band (annuli being formed during the summer months) formed at age one. A second dark band starts to appear during the fall and each successive dark band becomes thinner. This pattern continues until the fish dies.

This pattern has been observed in salmonids as well. Two studies used vertebra growth rings to estimate age for Chinook salmon introduced into Lake Michigan (Hesse 1994; Wesley 1996). The accuracy and reliability of age estimates were tested against

Chinook salmon of known ages which were measured through the use of a CWT and OTC marking. Both studies achieved exceptional results, correctly identifying the age of Chinook salmon over 93% of the time. Wesley noted that most of the error was associated with younger fish (two years or younger) which may be because vertebrae do not yet have strong areas of opaque and translucent zones or that the zones representing the first annulus may be confused with smolt checks. Smolt or accessory checks, also known as growth checks, are rings not associated with a year of life but rather result from slow growth periods as a result of shifting residence locations (Wesley 1996). These smolt checks can appear as one-to-three accessory checks (thin white rings) within the first dark area and are common in individuals less than one year and in some age 1 Chinook salmon. Accessory checks are much thinner than the annular bands and are not visible in Chinook salmon, age 2 and older (Hesse 1994). Wesley also noted that there was some error associated with ageing fish older than four years but because of small numbers of older fish, the accuracy of ageing these fish is still in question. Although life cycles and environmental conditions are different between the introduced species of Chinook salmon in Lake Michigan studied by Hesse and Wesley and the anadromous Chinook salmon in the Pacific Northwest, similarities in age and growth are likely. Overall, these studies give credence to the possibility of using vertebrae for age determination of anadromous Chinook salmon.

Age Determination of Fish in Archaeology

Archaeologists have used the incremental growth of a variety of elements from fish, including vertebrae, to estimate their season of death since the 1970s (e.g. Brewer

1987; Carpenter 2002; Colley 1990; Higham and Horn 2000, Morey 1983; Wheeler and Jones 1989). Casteel (1972; 1976) was the first to discuss using the annuli in fish vertebrae not only for age determination but also for estimating the approximate season of death. Although innovative for the time, Casteel (1972) did not identify the species of fish used in his seasonality study, other than to limit them to temperate freshwater species, and he did not consider validation of his method. More recently, Carpenter (2002) refined the use of fish remains for seasonality studies using a modern collection of Sacramento pikeminnow (*Ptychocheilus grandis*). He developed a marginal increment analysis (MIA) to more precisely determine season of death of archaeological Sacramento pikeminnow using a modern collection of known age individuals to relate increments to true years. MIA analysis assumes that growth zones are formed yearly and by estimating the percentage of completion in the outermost zone of growth yearly, an oscillating cycle of annulus growth will be present when plotted against the season (Carpenter 2002). At least in part because these growth structures are not clearly visible on the surface, researchers have used thin sectioning or radiographic (X-ray) images to recognize and document ring formation.

Cannon (1988, 1991) used radiographic images (Figure 2.1) to observe incremental growth rings on vertebrae from the 7,000 year old archaeological site of Namu located in coastal British Columbia to determine species, estimate season of capture and in turn season of site occupation. He did this by counting growth rings visible in the radiographic images that were assumed to be true annuli, and then matching the estimated fish age to the species which best matched the life history profile. Cannon considered the potential that five species of Pacific salmon were present and each had a specific life history, spawning at a unique age and predictable time of year. Cannon presumed that Pacific salmon were mostly available to native fishers only at the time of their return to spawning streams and therefore, when procured, salmon would be of spawning age. After estimating the age of salmon vertebrae from Namu, Cannon attempted to identify specific species by correlating the species of salmon whose common spawning age most readily fit the estimated age of the vertebra. For example, Cannon states that in British Columbia, chum (*O. keta*) spawn in their third or fourth year of life, sometime in the late summer or fall. Therefore, a salmon vertebra recovered from an archaeological assemblage with an age of three or four years would likely be a chum salmon that was procured sometime in late summer or fall.

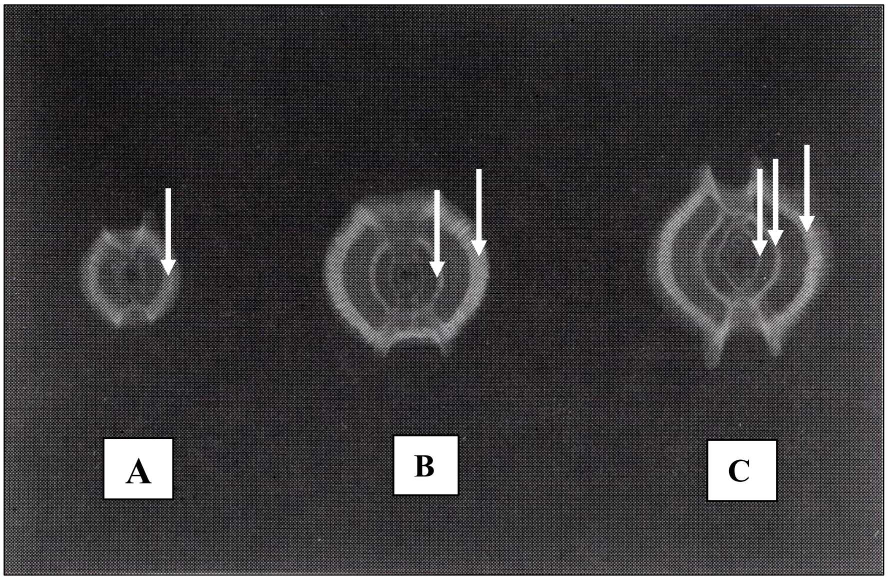

Figure 2.1 Radiographic image used by Cannon (1988, pg. 105) in his original age estimates of salmon vertebrae from the archaeological site at Namu, B.C. (ElSx 1) with rings labeled (from the *Journal of Field Archaeology*). They were identified as 2 (A), 3 (B), and 4 (C) year olds.

Cannon's method relies on a multitude of assumptions. Initially, it relies on the central tendency of the timing of Pacific salmon spawning age. However, as discussed previously there is considerable overlap in many aspects of the life history of these fishes. For instance, a fish said to be three years of age could possibly be represented by four of the five species discussed in Cannon's original study (Table 2.1). This logic also relies on an assumption that the timing of the life history of salmon has remained consistent over thousands of years. This seems doubtful in light of the variation observed in the timing of modern salmon life histories which have likely evolved as adaptations for survival (Groot and Margolis 1991). Perhaps the most fundamental assumption that Cannon makes in his argument is that one ring observed in his X-rays is in fact equivalent to one year of life. Although in his initial study Cannon mentions validating his method with a modern collection of individuals of known age, the particulars of this study were not presented; rates of error remain unknown.

Recent work using ancient DNA has demonstrated problems with Cannon's approach. Yang et al. (2004) tested Cannon’s species identifications from Namu through ancient DNA (aDNA) analysis. Among other discrepancies, sockeye salmon (*O. nerka*) was identified in assemblage from ancient DNA, but not from the X-ray approach. In the archaeological salmonid assemblage from the Keatley Creek site, on the British Columbia Plateau, Speller et al. (2005) found no evidence of pink salmon from DNA, which Berry (2000) had identified as present using the radiographic method. Because of overlapping age profiles, the radiographic method was unable to differentiate three of the four species identified at Namu using aDNA.

Despite these problems, researchers have continued to apply Cannon's approach using rings seen in X-rays to estimate fish age, then identify salmon species and finally infer season of use. Trost (2005) employed Cannon's method to attempt species identification of a small sample of salmon vertebrae from the Cove Cliff site in British Columbia. Recently, Orchard and Szpak (2011) have taken the use of radiographic techniques a step further by employing digital imaging technology to obtain X-rays and assign specimens to a year class. By combining this technology along with classifying vertebrae into size classes associated with each species, they attempted to more accurately determine species. These studies rely on the assumptions outlined previously, the most fundamental being that one year of life is in fact represented by one growth ring visible on an X-ray.

Chapter 3

Methods and Materials

Sample Collection and Preparation

I focus on Chinook salmon in my study for several reasons. First, because of its importance to people of the Pacific Northwest and its threatened/endangered status, Chinook are the focus of considerable research which has resulted in substantial knowledge of its life history, which will aid in the understanding of the formation of incremental rings. In addition, Chinook salmon are the longest-lived species of Pacific salmon allowing me to evaluate age and growth from a broad range of age categories. Chinook salmon also display considerable variation in early life history patterns allowing me to examine age and growth in the context of a variety of residence patterns (Table 2.1). Historically, they are also the most abundant species of salmon in the Columbia River system (Craig and Hacker 1940) and are likely the most abundant species in archaeological sites.

The *Oncorhynchus* vertebral column is comprised of 67-75 vertebrae (Butler 1990) that have been divided into four main types based on distinctive morphology (Butler 1993; Morales 1984) (Figure 3.1). These vertebra types are unique and useful in referencing which area of the column vertebrae are located. Type I is the atlas vertebra and can be easily identified because of the asymmetry between the rostral and caudal surfaces and its two dorso-rostral facets that articulate with the exoccipital of the neurocranium. Type II vertebrae have two holes on the dorsal and ventral surfaces where un-fused neural and haemal spines articulate. Type III vertebrae have fused, lightly built neural and haemal spines whereas type IV vertebrae have more robustly built, fused neural and haemal spines. Initial viewing of vertebrae with radiographic images during my pilot study (Hofkamp and Butler 2011) revealed that the rings were more difficult to interpret on atlas vertebrae than on more caudal vertebrae. These X-rays appeared blurry and ambiguous, likely due to the fact that atlas vertebrae are not symmetrical on the rostral and caudal surfaces (Figure 3.1 A and B). Because of this, I focused on Types II through IV vertebrae.

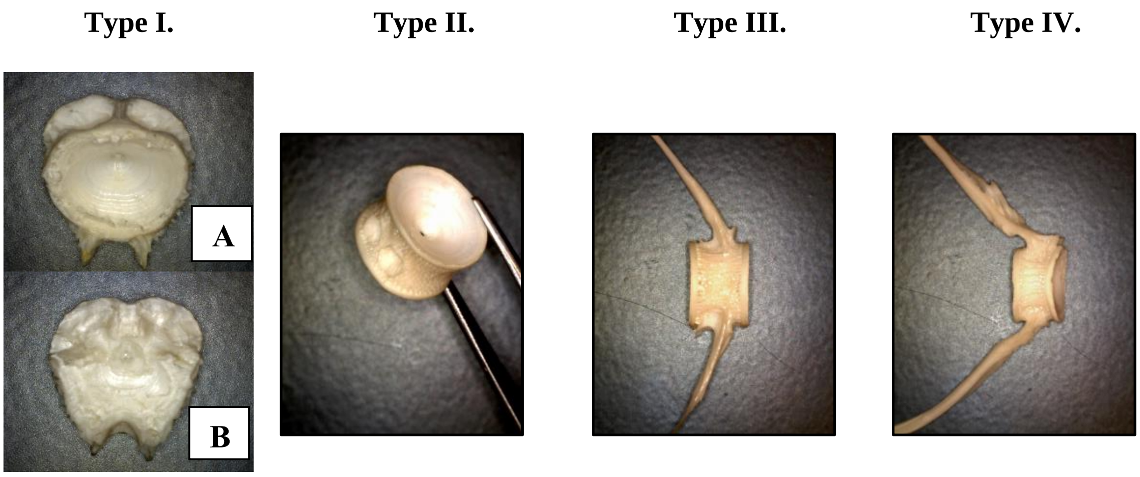
Type I. **Type II.** **Type III.** **Type IV.**

Figure 3.1 Rostral (A) and caudal (B) view of a type I vertebrae. Vertebrae types II, III and IV used in X-ray assessment.

The collections of modern, known-age Chinook salmon used in this study came from three different sources. The first collection was the VLB-85 collection which was created by Dr. Virginia Butler in 1985. The second collection was comprised of Chinook salmon from the Hanford Reach portion of the Columbia River in Washington (hereafter referred to as the Hanford Reach collection). The third, and final, collection was provided by the National Oceanic and Atmospheric Administration (NOAA) as part of their coastal trawls (NOAA collection).

VLB-85 Modern Collection

The VLB-85 collection consisted of 20 Chinook salmon specimens procured from the Washington Department of Fish and Wildlife (WDFW) in February 1985. This collection was mainly used in the present research to evaluate Cannon's X-ray method for determining fish age. This collection represents voluntary sports catch returns for which coded wire tags (CWT) information was available (Table 3.1). Specimens were recovered between July 1984 and January 1985 from locations throughout Washington. All but one (#VLB-85-50) of the recovered Chinook salmon specimens were hatchery-raised.

Table 3.1 VLB-85 Chinook salmon collection used in X-ray assessment.

Case #	Brood Year	Release Site	Recovery Date	CWT Age
85-32	1982	Big Beef Creek	1-27-85	3
85-33	1981	Purdy Creek	1-26-85	4
85-35	1981	Deschutes River	1-29-85	4
85-37	1982	Portage Bay	1-05-85	3
85-40	1981	Green River	1-28-85	4
85-42	1982	Big Beef Creek	1-13-85	3
85-43	1982	Spring Creek	1-14-85	3
85-45	1982	Big Quilcene River	1-22-85	3
85-47	1981	Issaquah Creek	1-28-85	4
85-48	1981	Cowlitz River	9-24-84	3
85-50 (wild)	1981	Nooksack River	1-26-85	4
85-52	1980	Little Qaulicum River	9-07-84	4
85-53	1982	Portage Bay	1-12-84	2
85-54	1981	Finch Creek	10-8-84	3
85-55	1981	Skookum Creek	1-26-85	4
85-56	1982	Big Beef Creek	11-11-84	2
85-70	1982	Big Beef Creek	1-26-85	3
85-73	1982	Cowlitz River	11-7-84	2
85-78	1982	Big Beef Creek	1-13-85	3
85-83	1982	Cowlitz River	8-05-84	2

To facilitate my study, residual soft tissue on the vertebrae needed to be removed. Soaking in water for one week did not help remove the tissue. Next, each vertebrae was soaked in a solution of 0.02 grams of trypsin, a digestive enzyme, and 3.5 ounces of water for 48 hours. This worked only slightly better; many of the vertebrae had been permanently stained over the years. Despite this staining, incremental rings were still distinguishable on the surface of most vertebrae.

The VLB-85 collection has its limitations. It is comprised primarily of hatchery-raised fish. Because incremental rings are formed by cessations in growth related to annual availability of food sources, consistent hatchery conditions may not be conducive to incremental growth. If this occurred it would mean a salmon, reared in the hatchery, which is the same age as a wild salmon may have a different number of incremental rings than a fish of the same age born in the wild. A deeper understanding of the process of growth and ring formation during this early life history of hatchery reared fish is needed. Understanding the differences in incremental growth between wild and hatchery populations especially important when examining prehistoric populations represent wild fish.

Hanford Reach Collection

The Hanford Reach collection was utilized mainly for the development and testing of my ageing protocol but was also used in my evaluation of the X-ray method of ageing. This collection was obtained in fall of 2011 in collaboration with the WDFW who have a long term program to track fall run Chinook salmon which spawn in the Hanford Reach portion of the Columbia River (Figure 3.2). This roughly 50 mile (80.5 km) portion of the river in central Washington, from the Priest Rapids dam to the city of Richland, is free flowing and contains the largest remaining population of naturally spawning fall Chinook salmon in the Columbia River. This collection was ideal since it allowed me to study wild fish and compare patterns with hatchery-reared individuals. Also, the fish are part of the CWT program, providing information on “true” age; scales were also collected providing an opportunity to compare-contrast incremental growth on different structures. Finally, because I was able to collect and prepare complete carcasses I was able to examine growth along the entire vertebral column.

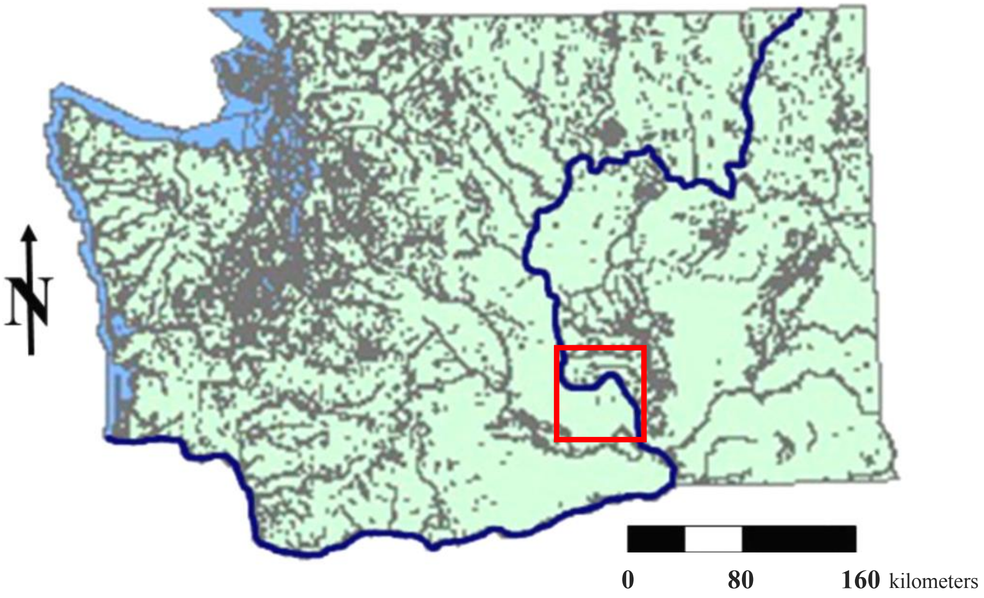

Figure 3.2 Location of the Hanford Reach of the Columbia River in Washington State.

The Hanford Reach collection consisted entirely of fall run adults (Table 3.2), which had been marked with CWTs. The WDFW provided me with all details regarding each individual's life history including origin, age, recovery and release date, and sex. These fish were collected by WDFW crews (Paul Hoffarth, supervisor) and myself in November 2011 during the annual fall spawning ground surveys. As each fish was retrieved it was assigned an identification number, fork length, species, and sex was recorded and a scale sample was removed. All collected scales were affixed to a scale card and given a unique scale card number. For those fish with a CWT, the cranium was removed, then taken back to a lab so the CWT could later be located and retrieved. For each cranium, a unique identification tag called a snout card was filled out with information about that individual such as fork length, sex, the location of recovery, species and generally the unique scale card number. Once the fish were harvested and the crania collected, most of the trunk soft tissue was removed from each spinal column and discarded back into the Columbia River. Each spinal column was individually bagged, sealed and later frozen. A label with the corresponding head tag number and scale card number was affixed to each bag so that all specimens could later be matched to their CWT and scale age. All of these specimens were transported back to Portland and placed in a freezer in the Portland State University (PSU) Biology department. To facilitate blind-testing I assigned a “case number’ to each fish, which corresponded to each fish’s formal identification number, so no reader had knowledge of the collection.

Table 3.2 CWT and scale age details for Hanford Reach Chinook.

ID#	Stock	Brood Year	Recovery Date	Fork Length	CWT Age	Scale Age
1058-1	Ringold Springs Hatchery	2007	11/9/2011	78	4	4_1
1061-1	Ringold Springs Hatchery	2007	11/13/2011	81	4	4_1
1061-12	Oxbow Hatchery	2009	11/13/2011	49	2	2_1
1061-2	Lyons Ferry Hatchery	2007	11/13/2011	79	4	4_1
1061-9	Lyons Ferry Hatchery	2008	11/13/2011	58	3	3_2
1063-19	Hanford URB Wild	2006	11/13/2011	90	5	5_1
1063-20	Umatilla Hatchery	2008	11/15/2011	58	3	3_2
1064-11	Lyons Ferry Hatchery	2006	11/13/2011	75	5	5_2
1064-12	Ringold Springs Hatchery	2007	11/13/2011	78	4	4_1
1064-13	Priest Rapids Hatchery	2009	11/13/2011	53	2	2_1
1067-16	Bonneville Pool	2007	11/18/2011	90	4	4_1
1068-7	Priest Rapids Hatchery	2007	11/16/2011	79	4	4_1
1071-18	L White Salmon	2007	11/20/2011	69	4	4_1
1091-14	Hanford URB Wild	2006	11/17/2011	87	5	5_1
1092-2	Hanford URB Wild	2007	11/18/2011	75	4	4_1
1092-4	Priest Rapids	2008	11/18/2011	72	3	3_1
1102-2	Priest Rapids Hatchery	2009	11/21/2011	45	2	2_1
1102-3	Umatilla Hatchery	2008	11/21/2011	70	3	3_1
1123-10	Priest Rapids Hatchery	2009	11/10/2011	52	2	2_1
1123-11	Priest Rapids Hatchery	2009	11/10/2011	43	2	2_1
1123-12	Hanford URB Wild	2008	11/10/2011	69	3	3_1
1123-13	Priest Rapids Hatchery	2008	11/10/2011	71	3	3_1
1135-7	Hanford URB Wild	2007	11/10/2011	N/A	4	4_1
1139-5	Hanford URB Wild	2007	11/13/2011	84	4	4_1
1139-6	Priest Rapids Hatchery	2009	11/13/2011	54	2	2_1
1141-20	Umatilla Hatchery	2007	11/15/2011	80	4	4_1
1142-11	Hanford URB Wild	2007	11/15/2011	99	4	4_1
1144-1	Priest Rapids Hatchery	2009	11/16/2011	48	2	2_1
1144-13	Hanford URB Wild	2007	11/16/2011	91	4	4_1
1151-14	Hanford URB Wild	2007	11/18/2011	89	4	4_1
1151-15	Umatilla Hatchery	2008	11/18/2011	67	3	3_1
1152-15	Ringold Springs Hatchery	2007	11/18/2011	85	4	4_1
1157-17	Umatilla Hatchery	2008	11/21/2011	79	3	3_1
1239-3	Irrigon Hatchery	2008	11/20/2011	74	3	3_1
1241-3	Ringold Springs Hatchery	2007	11/20/2011	73	4	4_1
1241-8	Hanford URB Wild	2007	11/20/2011	81	4	4_1
1241-9	Irrigon Hatchery	2008	11/20/2011	80	3	3_1
1449-14	Hanford URB Wild	2007	11/15/2011	N/A	4	4_1
1449-15	Umatilla Hatchery	2008	11/15/2011	53	3	3_2
1450-4	Hanford URB Wild	2007	11/13/2011	85	4	4_1
1450-5	Hanford URB Wild	2007	11/13/2011	85	4	4_1
8232-1	Hanford URB Wild	2007	11/20/2011	88	4	4_1
8238-2	Hanford URB Wild	2008	11/17/2011	67	3	3_1
8233-13	Hanford URB Wild	2007	11/14/2011	86	4	4_1
8237-5	Irrigon Hatchery	2008	11/17/2011	77	3	3_1
8241-4	Priest Rapids Hatchery	2007	11/14/2011	80	4	4_1

A total of 55 fish with CWTs were recovered during the Hanford Reach surveys. Of these, five had blank CWT's, which meant they lacked independent age records and thus could not be included in my control study. Four of the fish originally identified in the field as Chinook were actually coho (*O. kisutch*). Of the remaining 46 fish, 16 (35%) were wild fish known as Hanford Upriver Brights (Hanford URB) and 30 (65%) were hatchery fish originating from eight different hatcheries across Washington and Oregon (Table 3.3). The collection of 46 fish consisted of 23 (50%) females and 23 (50%) males, including 8 jacks (male Chinook which return to freshwater after only 1 year in the ocean).

Table 3.3 Summary of stock/age class for Hanford Reach Chinook salmon specimens.

	Age Class				
Stock	**2 year**	**3 year**	**4 year**	**5 year**	**Total**
Hanford URB Wild		2	12	2	16
Priest Rapids Hatchery	6	2	2		10
Umatilla Hatchery		5	1		6
Ringold Hatchery			5		5
Irrigon Hatchery		3			3
Lyons Ferry Hatchery		1	1	1	3
Oxbow Hatchery	1				1
Bonneville Pool			1		1
L. White Salmon			1		1
Total	7	13	23	3	46

One goal was to obtain the widest range of age classes possible from extant wild populations so it would be possible to validate across all age classes as stressed by Beamish and McFarlane (1983; 1987). The CWT records show the age structure of this sample is represented by four age classes with 4 year olds dominant. It is possible for

Chinook salmon to exceed the age of 5, but I assume the age range of the fish in this collection was adequate for identifying criteria for annular growth.

Scale ages were determined (under the supervision of Lance Campbell, WDFW) for all 46 fish at the WDFW ageing laboratory in Olympia, WA (Table 3.2). Ages were reported using the Gilbert-Rich system, which uses an Arabic numeral followed by a subscript numeral (e.g. 4_2 and $3_{1,}$ described as "4 sub 2" and "3 sub 1"). The first numeral indicates the total age of the fish and the subscript indicates the time spent in freshwater prior to saltwater immigration. The assigned scale ages of the Hanford Reach assemblage matched the CWT ages 100% of the time. Scale patterns make it possible to determine the total age of the fish as well as the period of time a fish spent in freshwater prior to immigration to saltwater giving further insight into life history patterns. A total of 42 fish in my assemblage had a freshwater residency of one year, denoted "sub 1", and four of the fish had two year freshwater residencies, denoted "sub 2".

NOAA Collection

To learn details about the structure of vertebrae during emergence and in the first year, additional younger fish also of known age were needed. Such fish were obtained from the NOAA coastal test trawls (Hatfield Marine Science Center, Newport, OR) with the assistance of Dr. Jessica Miller (Oregon State University), harvested in June 2011. The collection consisted of 55 individuals of mixed age: juveniles, yearlings and subyearlings (Table 3.4); whether they are hatchery or wild is unknown. Age categories were assigned by NOAA personnel according to length, which is common practice (Dr. Jessica Miller; personal communication). I acquired these fish during a "cutting party" at the NOAA research station/ Hatfield Marine Science Center in Newport, Oregon in October of 2011. All specimens which had been frozen were thawed. Next, the volunteers removed the otoliths and internal organs, then sampled scales and muscle for DNA. I then collected and bagged the remaining portions of each fish and returned them to Portland State University for maceration.

Table 3.4 Fork length (mm) and age class for NOAA Chinook salmon.

ID#	Age Class (length)	Fork Length (mm)
29763	subyearling	87
29693	subyearling	88
29759	subyearling	89
29707	subyearling	93
29695	subyearling	96
29675	subyearling	100
29690	subyearling	103
29710	subyearling	103
29782	subyearling	104
29678	subyearling	106
29678	subyearling	106
29713	subyearling	107
29706	subyearling	108
29669	subyearling	109
29712	subyearling	110
29676	subyearling	111
29760	subyearling	112
29623	subyearling	118
29714	subyearling	122
29605	subyearling	126
29716	subyearling	132
29423	subyearling	134
29657	yearling	141
29614	yearling	144
29681	yearling	146
29729	yearling	148
29632	yearling	151
29633	yearling	153
29680	yearling	153
29635	yearling	158
29645	yearling	158
29700	yearling	160
29539	yearling	162
29640	yearling	162
29436	yearling	163
29731	yearling	167
29658	yearling	168
29734	yearling	173
29737	yearling	174
29739	yearling	187
29751	yearling	193
29789	yearling	195
29480	yearling	207

29701	yearling	217
29703	yearling	218
29626	yearling	226
29794	yearling	250
29615	yearling	263
29685	mixed age juvenile	278
29686	mixed age juvenile	284
29770	mixed age juvenile	305
29468	mixed age juvenile	339
29518	mixed age juvenile	354
29688	mixed age juvenile	390
29705	mixed age juvenile	391
29472	mixed age juvenile	435

Hanford Reach and NOAA Specimen Preparation

To remove flesh and isolate vertebrae in both the Hanford Reach and NOAA collections, salmon carcasses were prepared using a simple warm water maceration procedure. All maceration took place in the PSU Zoology Lab facilitated by Dr. Deborah Duffield and Dalin D'Alessandro. Each carcass was placed in a large glass jar or plastic bucket. The original identification card created in the field was affixed to the side of the jar or bucket and kept with the specimen at all times. Warm tap water was added to each container covering the carcass. These were allowed to sit for a period of two-to-four weeks at ambient temperature (~65°F). After this time period, the water was decanted and clean water was added. This process was repeated two-to-three times until all residual soft tissue was removed from the skeleton. Next, the contents of each jar was poured through fine mesh window screen. Next, each specimen was rinsed with fresh tap water, then soaked in a 15% ammonia and water solution for 10 minutes to reduce odor. Each specimen was rinsed one more time with tap water to remove trace ammonia, placed on a paper towel lined tray and allowed to dry for several days. Next, each specimen was placed in a plastic bag along with its original identification card and cataloged by its unique scale card number or NOAA salmon identification number.

Documenting Rings

To identify the best method of viewing incremental rings, I examined a collection of vertebrae comparing surface images, X-ray images and thin sections. These methods all vary in difficulty of preparation, extent of specimen destruction and aspect of vertebra highlighted. The simplest approach, which is completely nondestructive, involves examining the centrum surface and then taking an image for permanent documentation. This method only allows viewing of the surface rings. The more difficult, but also nondestructive approach, uses X-ray technology to view internal rings in vertebrae. The most time consuming method, which also permanently destroys most of the specimen, involves thin sectioning the vertebra by cutting it in half, mounting it on a glass slide and then polishing the surface. In thin sections, a view of both the profile of rings visible on the surface as well as the internal structure of vertebrae is obtained. All three of these techniques were attempted on at least a sample of vertebrae to determine which was the most practical in terms of degree of difficulty, efficiency, extent of specimen destruction, and most importantly, which one revealed annular growth rings.

The surface images of the VLB-85 vertebrae were taken with a microscope (Leica MZ 125) at 10X magnification and digital camera (Leica DFC 320) housed in the Portland State University Biology Department in Dr. Luis Ruedas's lab. Vertebrae were placed on the microscope stage as level as possible and both the rostral and caudal surfaces were photographed.

The Hanford Reach vertebrae were viewed with either the naked eye or with a 1.5X magnification. A 95% ethanol solution was applied to each vertebra during the development of this ageing protocol and during testing to enhance incremental rings.

Radiographic images of the VLB-85 collection of vertebrae were taken at Oregon Health and Science University (OHSU) with the help of Dr. Raymond Friedman using a Holologic Mammographic Selina with HTC technology at double magnification with a radiographic exposure of 25kV and 100 mAs. Images were saved as a *Dicom* file on a compact disk and were later viewed in Photoshop 8.0.

Thin sections were cut using an Isomet slow speed saw housed in Dr. Martin Streck's petrology lab at Portland State University or an X-ACTO fine tooth pull saw. Both methods worked equally well; the hand saw was used when the Isomet saw was not available. Thin sections were cut one of two ways, either on the sagittal plane or on the frontal (Chiasson 1966) plane also known as the longitudinal plane (Cailliet et al. 1986) (Figure 3.3). All of the VLB thin sections were cut sagitally. However, after completing these, for type II vertebrae, I discovered that the foramen where the unfused neural and haemal spines articulate can distort certain growth features. Therefore, the remainder of the thin sections were cut frontally to avoid these foramen. Thin sections were created by first cutting the vertebrae perpendicular to the centrum face, just to one side of the notochord opening. The side which retained the notochord opening was wet sanded down with 400 and 600 grit waterproof sandpaper until the center of the vertebra was exposed, just barely sanding into the notochord opening. Next, the sanded surface was polished on 12, 3 and 0.05 micron lapping film. This polished surface was then fastened to a glass slide using a two-part epoxy resin. After twenty-four hours for the resin to cure, another cut was made parallel to the first, leaving only a thin strip of vertebra left the glass slide. This new surface was then sanded with 400 and 600 grit sandpaper followed by the 12, 3 and 0.05 micron lapping film to polish. Completed specimen thickness (0.15 to 0.25 mm) was very close to those obtained by Carpenter (2002), in his Sacramento pikeminnow study. Thin section images were photographed using a microscope (Zeiss Accuscope) at 10X magnification and a digital camera (Ziess AxioCam ERc5s) utilizing *ZEN* 2012 imaging software housed in the Department of Anthropology at Portland State University.

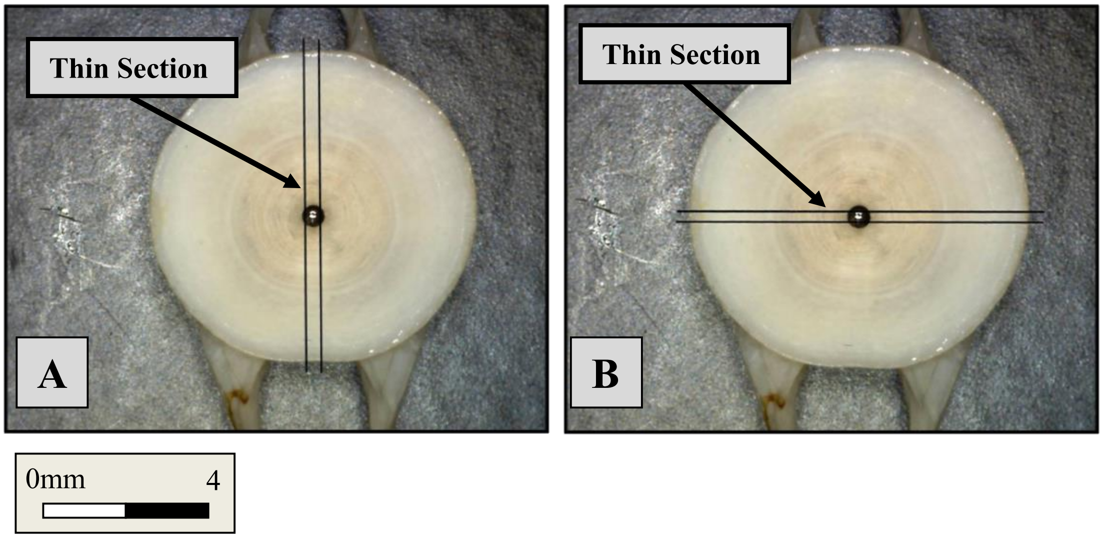

Figure 3.3 Position of sagittal (A) and frontal/longitudinal (B) thin sections on a type III modern vertebra (2 year old, catalog # 1064-13).

Developing a Protocol of Ring Identification

The protocol for ring identification used in this study was developed with the help of Lance Campbell and John Sneva from the WDFW Fish Ageing Unit, Olympia, WA. We drew on the work of Hesse (1994) (based on Kusakari 1969) and Wesley (1996) whose work with landlocked Chinook salmon demonstrated the feasibility of using vertebral growth rings to age salmonids, with some subtle protocol adjustments concerning the location of growth checks to account for the differences in landlocked vs. anadromous life history patterns.

The centrum surface of Chinook vertebrae, seen with reflected light, presents a multitude of rings of various thicknesses and color that encircle the notochord opening (Figure 3.4). My goal was to try to identify which of these rings correlated with annular growth. Using the Hanford Reach and NOAA assemblages this was done through an iterative process of comparing rings thought to be annular, on the vertebrae of known age fish, to the life history data of these same fish obtained from the CWTs. Through this process, I was able to determine which rings correlated with annular growth and what the characteristics of these "annular" rings were.

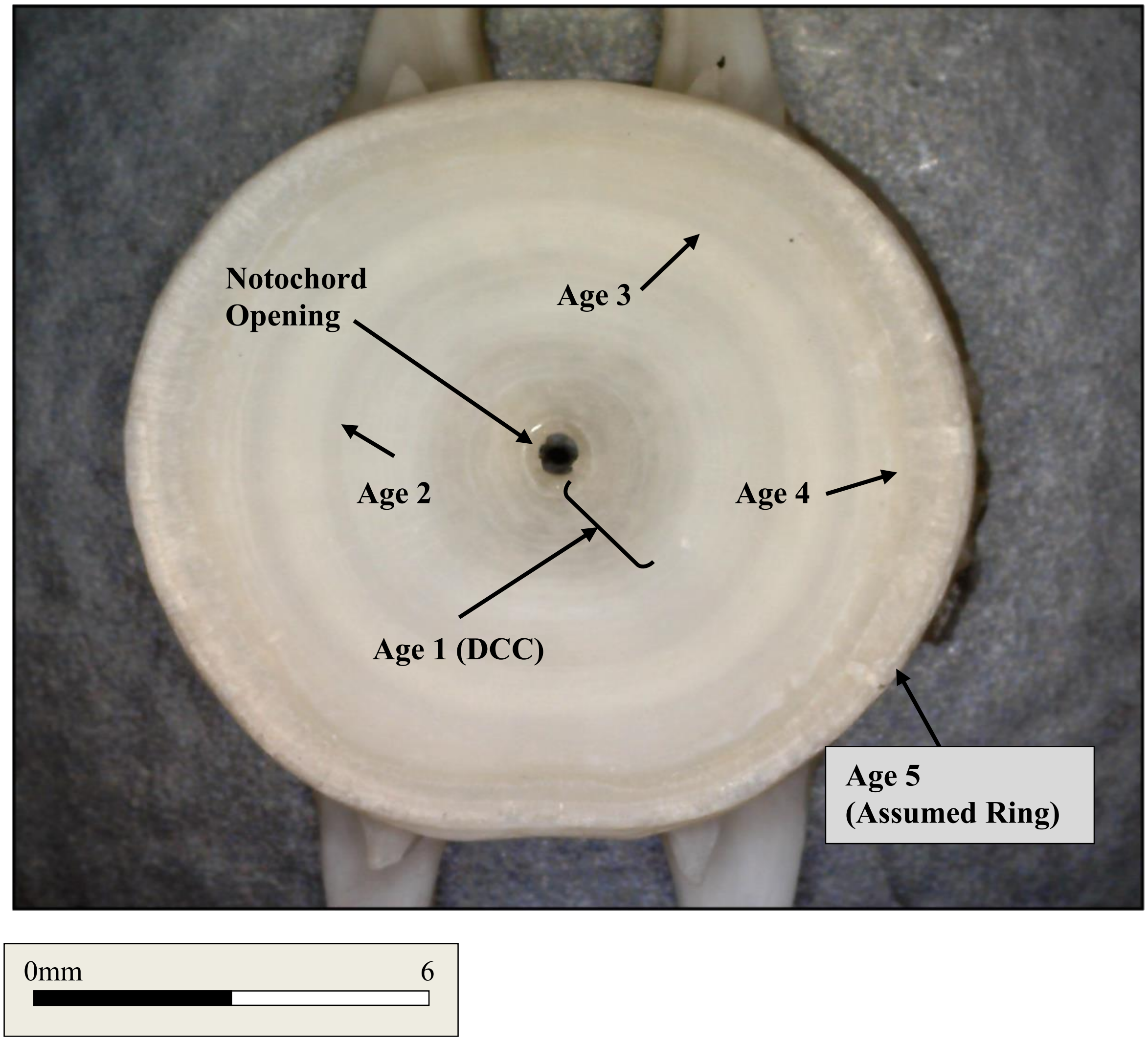

Figure 3.4 Vertebra from a five year old fish with the DCC and annuli labeled. (catalog number 1063-19, type III)

I used a generalized Chinook salmon life history model, based on Groot and Margolis (1991) and Quinn (2004), to relate growth to incremental rings. At the focus of the centrum face is the notochord opening (Figure 3.4). Surrounding this hole is a dark central core (DCC). The DCC is present when the young Chinook emerges from the gravels as free swimming fish in spring and can be observed in vertebrae from subyearling Chinook salmon from the NOAA collection that emerged from the gravels just months before they were collected (Figure 3.5). Fisheries protocol assigns the age of the fish at emergence as “age 1” (Cailliet et al. 1986). After emergence during its first spring and summer the young Chinook accumulates a zone of opaque (white) growth. During its first winter, after emerging from the spawning gravels, the salmon experiences slower growth expressed as a narrow dark zone. This pattern continues throughout the individual’s lifetime until it returns to spawn, accumulating additional dark bands in winter and white bands in spring and summer.

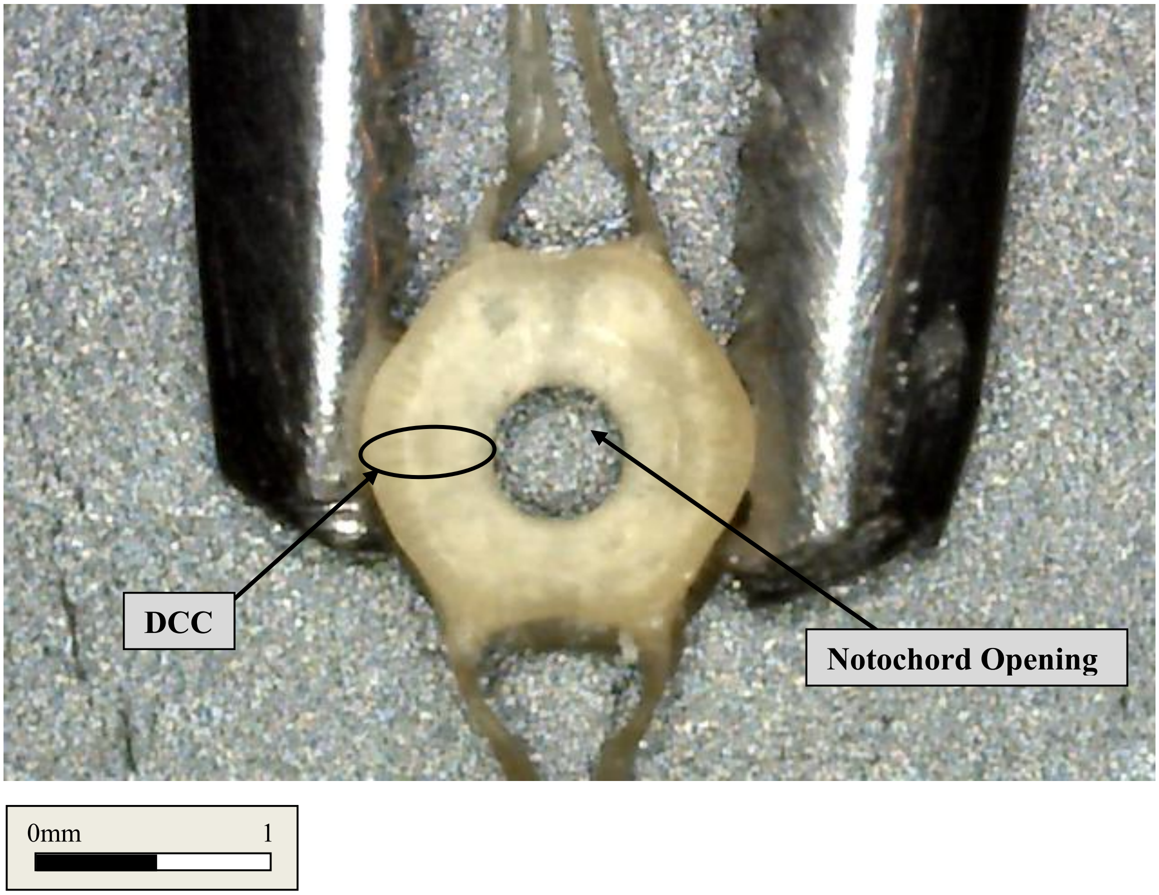

Figure 3.5 Modern subyearling vertebra with no growth beyond the dark central core (DCC) (Catalog number 29716, type III)

In many fish populations there is a strong correlation between vertebrae size and fish size as well as between age and fish size (Casteel 1976; Cailliet et al. 1986; Wheeler and Jones 1989). For further development of the protocol I modeled the relationship between vertebrae diameter (VD) and fork length (FL) using a scatterplot (Figure 3.6). A single type II vertebra was selected from each of the Hanford Reach fish carcasses. The diameter of each vertebra was measured using digital calipers (details in Appendix A). VD was plotted along the X-axis. The corresponding FL for each vertebra was plotted along the Y-axis. The age class for each fish, known from CWT's, is differentiated in the scatterplot.

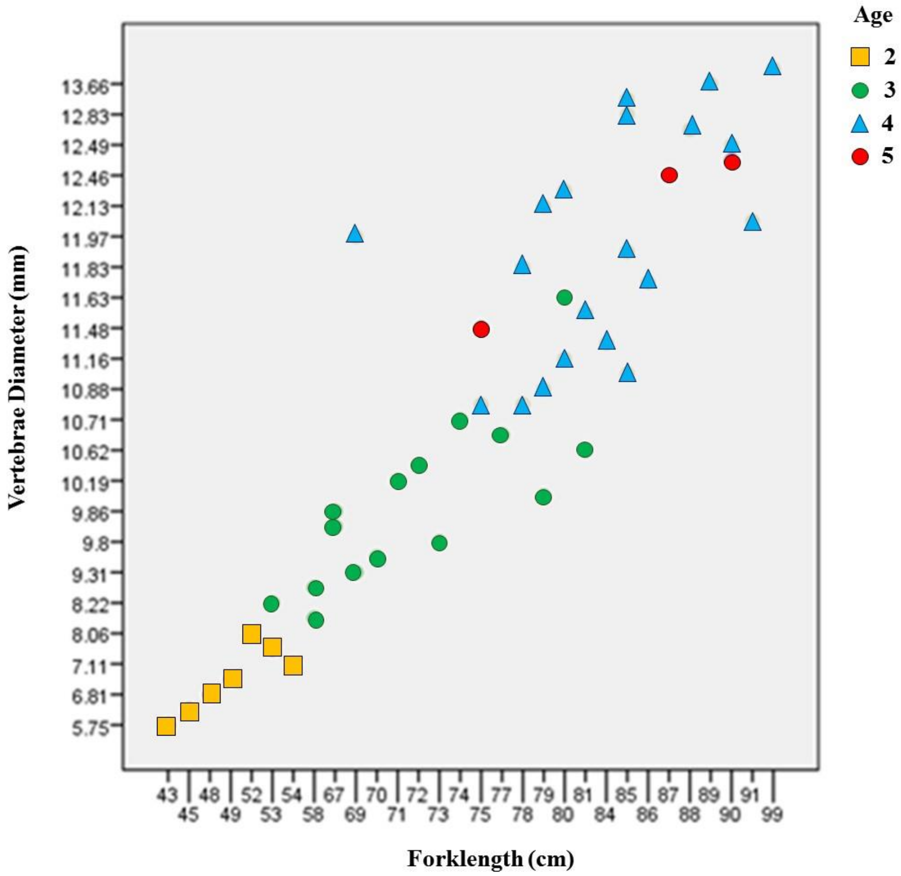

Figure 3.6 Scatter plot between fork length and type II vertebrae diameter for Hanford Reach collection (r=.951, p=.000).

Salmon from the Hanford Reach appear to have grown in a rapid and linear manner up through their third year of life, at which point growth significantly slows. Most of the overlap between age groups is between the 4 and 5 year old fish. This is demonstrated in the scatterplot in Figure 3.6 where all three year olds have a greater FL than all two year olds and all four year olds have a greater fork length than all three year olds. This suggests that annular rings formed in the first three years of life, a period of rapid growth, will tend to be further apart than rings formed during the 4th or 5th year of life when growth is much slower.

This relationship between age and growth was used as a check to help recognize obscure or difficult-to-identify rings by estimating the likely amount of growth that should take place between rings, either farther apart in early life or closer together in later life. This size-age study was helpful in distinguishing true annuli from false checks. For example, additional rings or accessory checks were occasionally observed (Figure 3.7). These are smaller and thinner than what I have defined as annuli. Hesse (1994) noted these as common in Chinook salmon age 1 and younger from Lake Michigan. I observed these accessory rings occasionally in the Hanford Reach and VLB-85 collection as thin white bands in the DCC and as darker bands visible in the first spring/summer's growth.

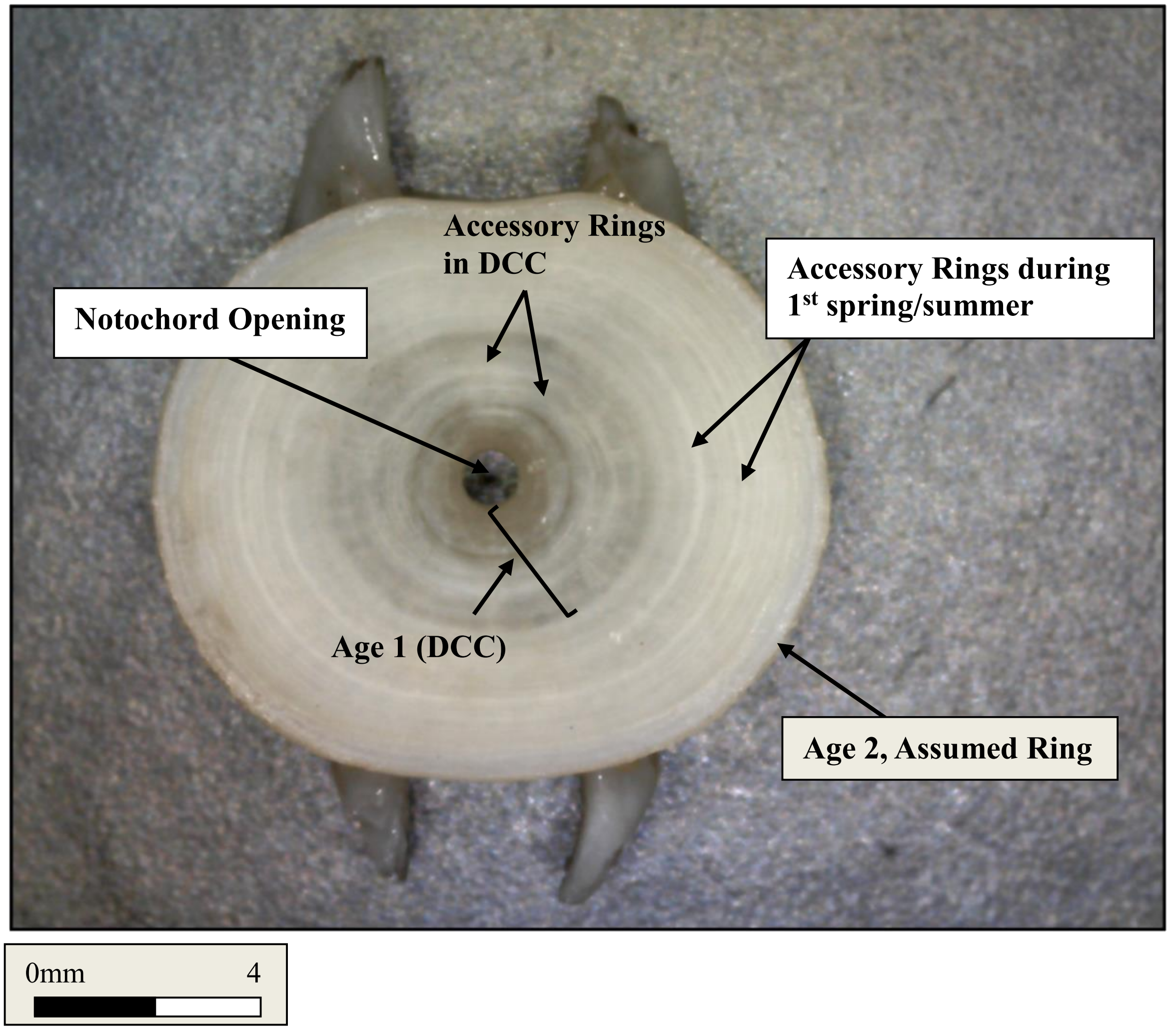

Figure 3.7 Vertebra from a two year old fish with accessory rings, DCC and assumed ring labeled. (Catalog number 1123-11, type II)

Age was assigned to vertebrae according to the following rules (labeled in Figure 3.4). The DCC is counted as age one. If there was significant growth after the DCC but no obvious dark bands then an annular ring is assumed and the individual is said to be a two year old. After the first period of spring/summer growth, comes a period of dark winter growth. These were counted as subsequent annular rings, and in my modern control samples, range in number from 0 to 3. For example, a 6 year old salmon would have 4 winter growth bands. A final ring is always assumed because all of these individuals were harvested in the fall of their final year of life before they accumulated a winter's growth band for that year (Cailliet et al 1986). The total age of the fish is combined as:

DCC(1) + Subsequent Dark Rings(0-3) + Assumed Ring(1) = Age of Fish (2-5)

Test of Accuracy and Precision

After developing my protocol I conducted an initial test of accuracy. I used the ageing method described above to age type II Chinook salmon vertebrae and compared these ages to the known CWT age in the Hanford Reach collection. Although I had used the Hanford Reach assemblage to develop the protocol, the CWT age of any particular fish was unknown to me during testing. Fisher's exact tests were used to test whether discrepancies between CWT age and vertebrae annuli age varied by age class, sex, and early freshwater residency patterns or between hatchery and wild stocks. A Fisher's exact test is used in lieu of a chi square test when sample sizes are small and some cells have values less than five (Zar 1974).

To test the reliability or precision (*sensu* Beamish and McFarlane 1983) of this method, I designed an experiment wherein five readers estimated the ages of the same set of control samples. These readers, all PSU anthropology graduate students, had varying levels of familiarity with archaeological fish remains. I explained the protocol in a one hour workshop during which the readers were allowed to examine several vertebrae from one each of a 2, 3, 4 and 5-year old fish.

To estimate precision in age estimations across the readers, I applied a method commonly used in fisheries biology ageing laboratories, average percent error (APE) (Beamish and Fournier 1981). It is calculated for a repetitive series of determinations, either by the same reader or by different readers. The calculation is defined as:

$$\boldsymbol{APE = \frac{1}{R}\sum_{i=0}^{n}\frac{|X_{ij} - X_j|}{X_j}}$$

The average age of each fish ($\boldsymbol{X_j}$) is calculated from all age estimates from all readers ($\boldsymbol{R}$). $\boldsymbol{R}$ in this case is 5. $\boldsymbol{X_{ij}}$ is the $_i$*th* determination of the $_j$*th* fish and is calculated for every age determination.

Archaeological Application

The goal of this aspect of the project was to assess the feasibility of applying the ageing protocol to archaeological specimens. Fish remains from Cathlapotle (Appendix B), located on the lower Columbia River near Ridgefield, WA, were available for my study (Figure 3.8). Cathlapotle is a ~600 year old Chinookan plank-house village excavated by Dr. Kenneth M. Ames in the 1990's (Ames et al. 1999). Because my ageing protocol focused on Chinook salmon, I needed to insure that archaeological samples were from Chinook. Huber et al. (2011) have suggested that Chinook salmon can consistently be identified to species using a simple morphometric technique utilizing the ratio between vertebra length and height (Figure 3.9). Moss et al. (2014) challenged this method, testing it against aDNA analysis of archaeological specimens from the Coffman Cove site

(49-PET-067) in southeast Alaska, and found it correctly identified species only 57% of the time. Their study mainly showed the morphometric method misidentified pink salmon as sockeye. Moss et al.'s archaeological samples did not include Chinook salmon for which Huber et al. had the best results. Therefore, I will use the Huber et al. method to isolate Chinook salmon vertebrae, recognizing there may be some non-Chinook included.

Figure 3.8 Location of Cathlapotle (45CL1) in Washington State. (from Shepard 2014)

Figure 3.9 Illustration of length (left) and height (right) measurements obtained to determine salmon species in Cathlapotle samples. (From Huber et al. 2011, used with permission from Elsevier publishing)

I selected 100 type II (31) and type III (69) vertebrae, chosen because of their high level of preservation, with a complete central margin of the centrum face. Vertebrae were from one of two proveniences; either level 9, Feature 728 in the SW quadrant of unit N160 W91or level 8 in the SE quadrant of unit N52 W101. Vertebrae should be considered "grab" samples, not representative of the fish assemblage overall. Vertebrae were next cleaned with a dry brush. The length and height was measured for each vertebra using digital calipers to the nearest 0.01mm. Using vertebral length, height, and the ratio of length/height all vertebrae were classified using a classification and regression tree (CART) in the statistical package R according to Huber et al. (2011) utilizing a preloaded workspace: http://conserver.iugocafe.org/user/jorg/Salmonid%20verebrae%20identification.

The salmon vertebrae classified as Chinook were then aged using the protocol developed using modern fish.

CHAPTER 4

Results: Ageing Chinook Salmon Vertebrae Using Radiographic Images.

As noted previously, Cannon (1988) and others have assumed vertebral rings from X-rays represent true years or annuli. Here, I evaluate the validity of this assumption using the Hanford Reach and VLB-85 modern reference collections by comparing and contrasting vertebral architecture visible in X-rays with vertebral thin sections of known age fish. These comparisons have revealed that white bands, visible in X-rays, do not actually represent annular growth.

To reveal the internal architecture X-rays highlight, I compared thin sections to X-rays of the same vertebrae from 13 fish from the VLB-85 collection. One of these comparisons is shown in Figure 4.1, the white bands visible in X-rays correspond to the internal walls visible in thin sections. These walls, made of bone, separate multiple internal compartments which are likely filled with fluid or soft tissue during the fish's life. These walls create a series of nested cylinders encircling the notochord opening (Figure 4.1). The question still remains though, do these rings reflect the age of a particular fish?

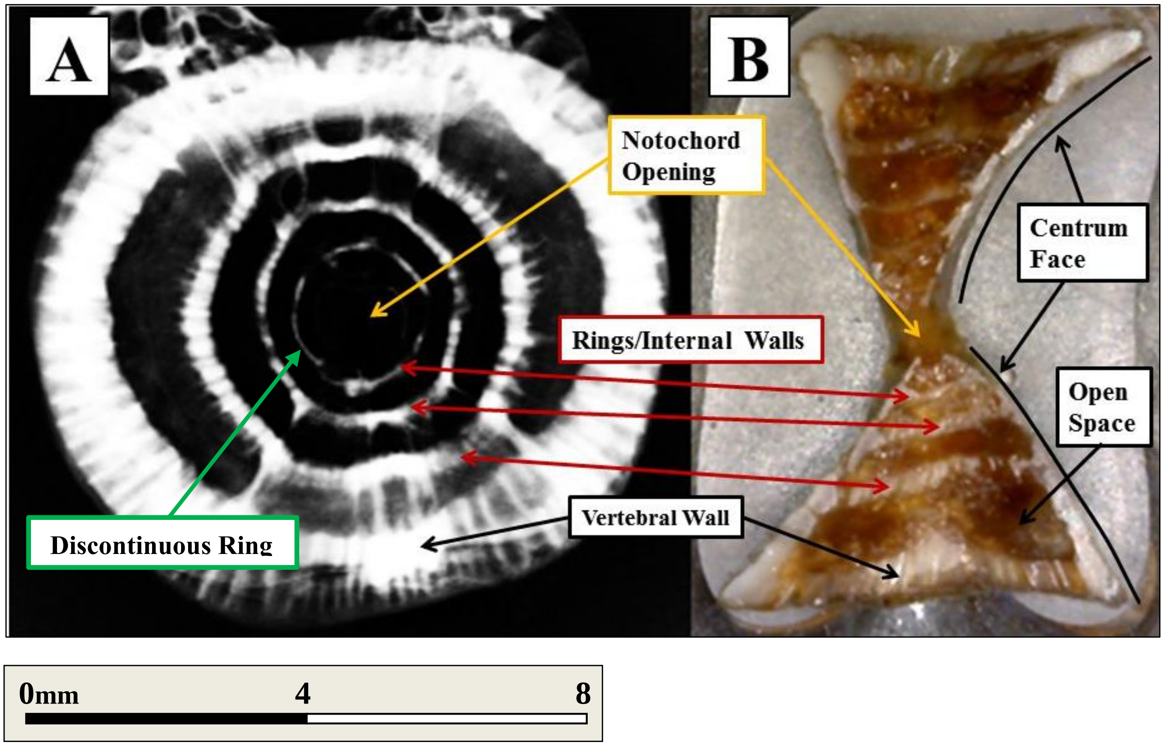

Figure 4.1 Two views of the same vertebra (VLB-85-47). X-ray image (A) and sagittal thin section profile (B) of a 4 year old, type II vertebra showing the corresponding notochord opening, rings/internal walls and vertebral walls.

If the internal walls are annular, I would expect the number of these walls to match the known age based on CWTs. Comparing the number of internal walls from vertebrae (either thin sections or X-rays) with known CWT reveals that they are unrelated. As shown in Figure 4.2, two fish of different ages have the same number of internal walls and this expectation is not met. A 5 year old and a 2 year old individual both have three internal walls, at least on one portion of the centrum. According to Cannon’s (1988) method, the 5 year old fish (A) should have four internal walls and the 2 year old fish (B) should have one internal wall.

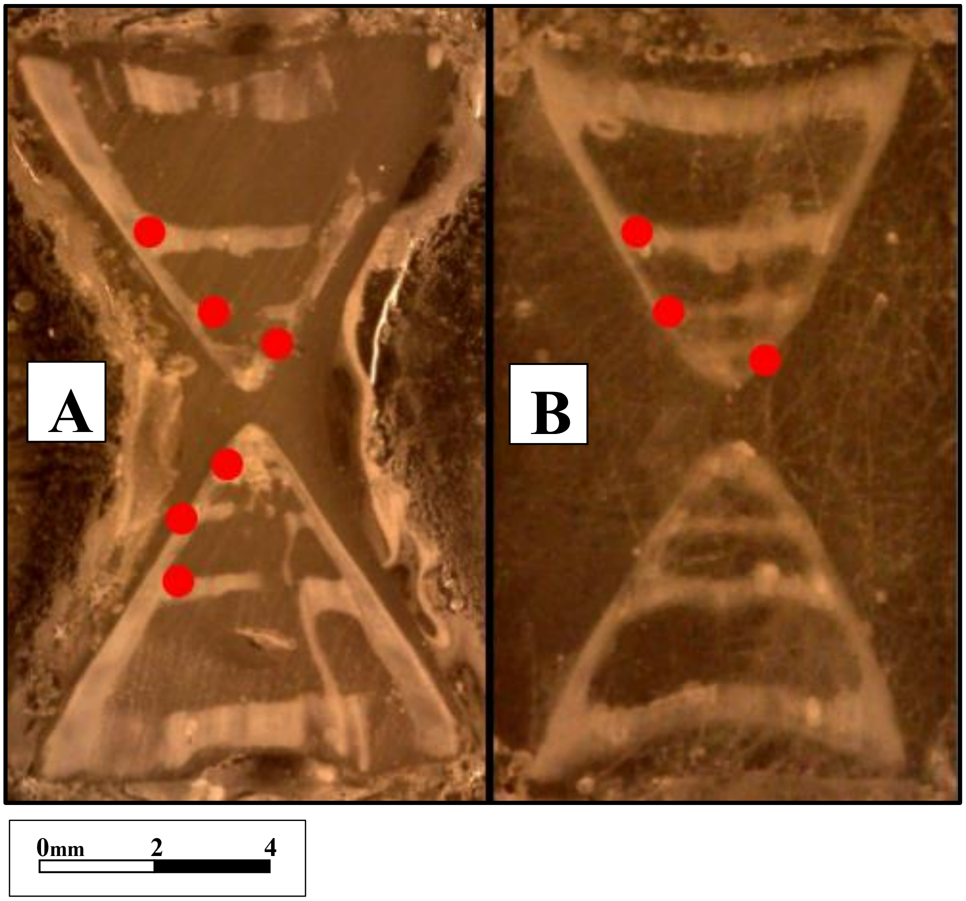

Figure 4.2 (A) A 5 year old fish (#1063-19, type III) with 3 internal walls and (B) a 2 year old fish (#1123-11, type III) with 3 internal walls.

My working hypothesis is that the internal walls are formed and set early in development, possibly at some point in the juvenile stage of their life cycle or even at emergence. This would account for why a 2 year old and a 5 year old both have the same number of internal walls.

Using the number of internal walls to estimate fish age is also a problem because there are varing numbers of internal walls between vertebrae on a single fish and in fact within a single vertebra (Figure 4.3, Table 4.1). As seen in Table 4.1 the number of internal walls varies by type, age and the face being examined. None have a consistent number of internal walls for all three vertebra types. This problem is illustrated in Figure 4.3, which shows a type II, III and IV vertebrae from the same fish. The type II and III have three internal walls and the type IV vertebra has four internal walls. In some cases there are even a differeing number of walls on different faces of the same vertebra. This can be seen in Figure 4.2B, in which a type IV vertebra from a 2 year old fish has three walls on one half of the centrum and two on the other.

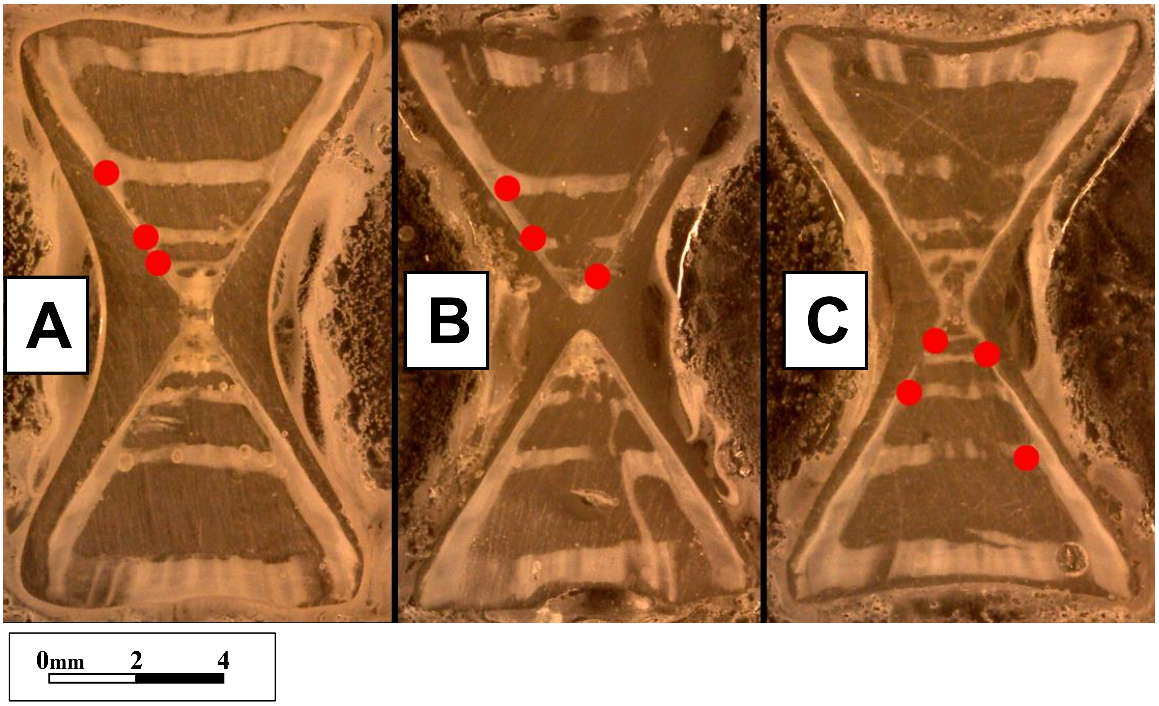

Figure 4.3 Thin sections of three different vertebrae from the same fish (types II[A], III[B] and IV[C]), specimen #1063-19 showing a differing number of internal walls.

Table 4.1 Number of internal walls by vertebra type from six Chinook salmon from the Hanford Reach.

	Vertebra Type		
Fish Catalog Number	**II**	**III**	**IV**
1123-11 (2 yr old)	2	2,3	2,3
1063-20 (3 yr old)	2,3	2,3	3
8241-4 (4 yr old)	3	3,4	4
1058-1 (4 yr old)	3,4	3	3
1063-19 (5 yr old)	3	3	4
1091-14 (5 yr old)	4	3	3,4

Several factors may be contributing to the variation in the number of walls. First, it may be that there are true differences in the number of walls between vertebrae of the same fish. It may also be that there are actual differences in the number of rings in different portions of the same vertebra. However, this would requre that some walls are discontinuous around the circumference of the vertebra and seems unlikely. Some of the walls, especially those most internal and closest to the notochord opening, are ambigous and can appear discountinous in X-rays (e.g. Figure 4.1 A). These innermost rings are likely not discontinous but rather in some areas are so gracile and fenestrated that their density is not detected in X-rays. Additionally, thin sections may be too crude a method for preserving these fragile innermost rings. Improper preparation as well as specimen fragility could affect the number of walls visible.

As described earlier, my work building on Hesse (1994) and Wesley (1996), suggests rings visible on the centrum surface reflect yearly growth. With this in mind, demonstrating that the surface rings are completely independent of the internal walls would further challenge that the internal walls are linked in any way to yearly growth. A

30X magnification of one thin section reveals that the internal walls of vertebrae do not correspond with surface rings (Figure 4.4). Indeed, the internal wall is separated from the centrum surface rings by a barrier, further indicating that growth rings on the centrum face and the internal walls are part of different developmental processes.

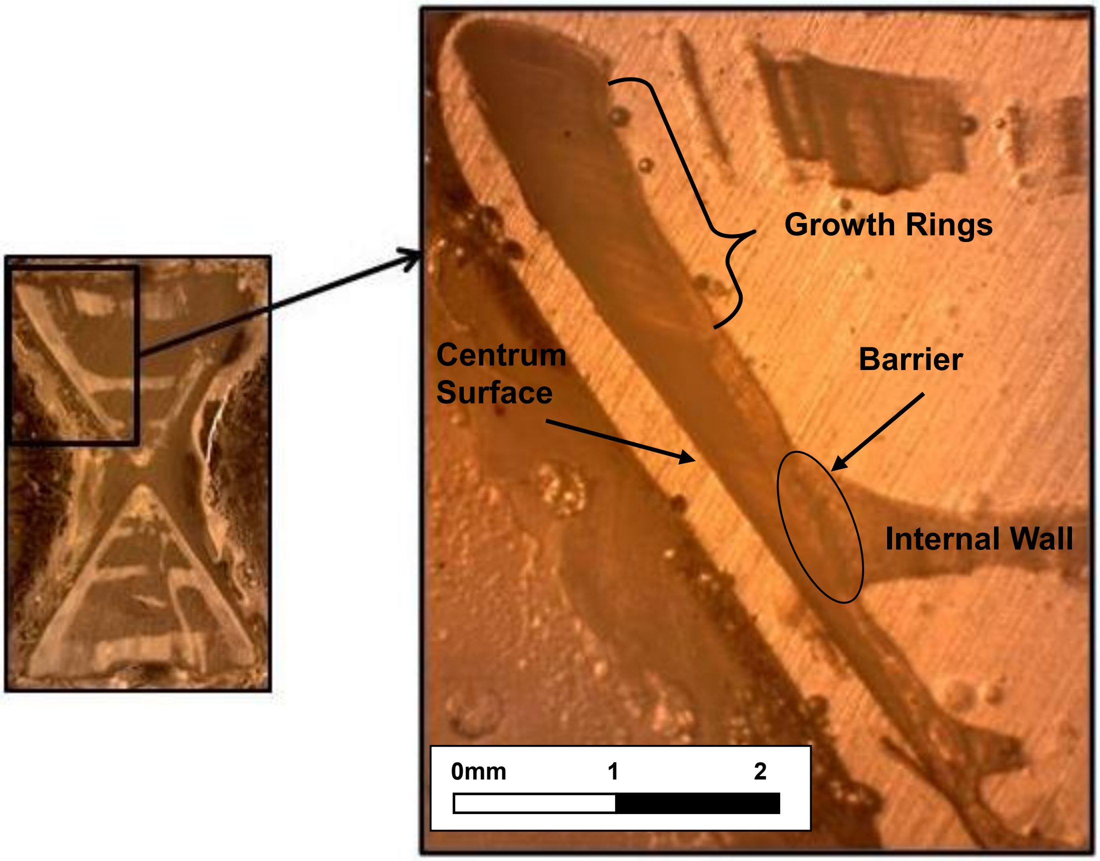

Figure 4.4 Magnified (30X) image of a thin section (catalog # 1063-19, 5 year old), highlighting the boundary between internal walls and the centrum surface.

In sum, comparing X-rays and thin sections of Chinook salmon shows the white rings visible in X-rays that correspond to internal walls, which separate multiple compartments inside the vertebrae. These internal walls are unrelated to fish age and are therefore not annuli. These walls do not appear to exhibit developmental connection to surface rings visible in the profile of thin sections and although they are most likely related to growth and bone development it is in a matter independent of any kind of annular cycle. Furthermore, the CWT ages of the fish used in this study do not correlate with the number of wall visible in X-rays or thin sections.

Chapter 5

Results: Ageing Modern and Ancient Chinook Salmon Using Annular Rings on the Centrum Surface

This chapter reviews the results from testing the accuracy and reliability of my protocol for aging fish using growth rings on the centrum surface on modern fish of known age. I then apply this protocol to archaeological salmon vertebrae from Cathlapotle (45CL1).

Test of Accuracy

For the first part of my ageing study, I aged one type II vertebra from each of the 46 Chinook salmon from the Hanford Reach collection (Table 5.1). Of the 46 fish aged, 39 (85%) were aged correctly using the protocol developed with the Hanford Reach Chinook salmon. Five of the seven discrepancies were under-aged and two were over-aged. All errors were within one year of the actual age. A deviation by more than one year would have indicated poor precision because Chinook salmon in this collection only achieved a maximum age of 5 years

Table 5.1 Assigned vertebral ages for 46 Chinook salmon using my aging protocol.

Case #	ID #	Sex	Origin	Scale Age	CWT Age	Hofkamp
1	8241-4	F	Wild	4_1	4	4
2	8238-2	M	Wild	3_1	3	3
3	8237-5	M	Hatchery	3_1	3	3
4	8233-13	F	Wild	4_1	4	4
5	8232-1	F	Wild	4_1	4	4
6	1450-5	M	Wild	4_1	4	4
7	1450-4	F	Wild	4_1	4	4
8	1449-15	M(jack)	Hatchery	3_2	3	**2***
9	1449-14	M	Wild	4_1	4	4
10	1241-9	F	Hatchery	3_1	3	3
11	1241-8	F	Wild	4_1	4	4
12	1241-3	F	Hatchery	4_1	4	**3***
13	1239-3	M	Hatchery	3_1	3	3
14	1157-17	M	Hatchery	3_1	3	3
15	1152-15	F	Hatchery	4_1	4	4
16	1151-15	M	Hatchery	3_1	3	3
17	1151-14	F	Wild	4_1	4	4
18	1144-13	M	Wild	4_1	4	4
19	1144-1	M(jack)	Hatchery	2_1	2	2
20	1142-11	M	Wild	4_1	4	**5***
21	1141-20	F	Hatchery	4_1	4	4
22	1139-6	M(jack)	Hatchery	2_1	2	2
23	1139-5	M	Wild	4_1	4	4
24	1135-7	M	Wild	4_1	4	4
25	1123-13	F	Hatchery	3_1	3	3
26	1123-12	M	Wild	3_1	3	3
27	1123-11	M(jack)	Hatchery	2_1	2	2
28	1123-10	M(jack)	Hatchery	2_1	2	**3***
29	1102-3	F	Hatchery	3_1	3	3
30	1102-2	M(jack)	Hatchery	2_1	2	2
31	1092-4	F	Hatchery	3_1	3	3
32	1092-2	M	Wild	4_1	4	4
33	1091-14	F	Wild	5_1	5	5
34	1071-18	F	Hatchery	4_1	4	4
35	1068-7	F	Hatchery	4_1	4	4
36	1067-16	F	Hatchery	4_1	4	4
37	1064-13	M(jack)	Hatchery	2_1	2	2
38	1064-12	F	Hatchery	4_1	4	4
39	1064-11	F	Hatchery	5_2	5	**4***
40	1063-20	M	Hatchery	3_2	3	**2***
41	1063-19	F	Wild	5_1	5	5
42	1061-9	F	Hatchery	3_2	3	3
43	1061-2	F	Hatchery	4_1	4	**3***
44	1061-12	M(jack)	Hatchery	2_1	2	2
45	1061-1	F	Hatchery	4_1	4	4
46	1058-1	M	Hatchery	4_1	4	4

* denotes errors in vertebrae age estimate

These results were comparable to other projects ageing Chinook salmon elements (Table 5.2). My results are slightly better than Godfrey (1968) who aged the scales of Chinook salmon from the Pacific Ocean correctly at a rate of 75% and were consistent with Murray (1994) who aged adult Chinook otoliths successfully 83% of the time. Other researchers have achieved slightly better results using the surface rings of Chinook salmon vertebrae such as Hesse (1994) who aged 365 Chinook and mis-aged only 11, achieving an accuracy rate of 97%, and Wesley (1996) who aged 181 Chinook and mis-aged 11 achieving an accuracy rate of 93.9%. These higher success rates may be due to their projects using collections that had a less variable, less complex non-anadromous life history pattern, as was noted by Hesse (1994). Rates of the WDFW ageing laboratory which have achieved of 90-95% using Chinook scales (Lance Campbell personal communication) are also slightly higher than those demonstrated in this study.

Table 5.2 Comparison of success rates in Chinook salmon ageing projects.

Reference	Element	Success Rate (%)
Godfrey (1968)	Scales	75
Murray (1994)	Otoliths	83
Hesse (1994)	Vertebrae	97
Wesley (1996)	Vertebrae	94
Hofkamp (this thesis)	Vertebrae	85

A Fisher’s exact test [two-tailed, conducted at a 95% level of confidence (alpha of 0.05)] was performed to examine whether the error was either random, or associated with any particular age class, origin, sex or freshwater residency type (Table 5.3). Results for age class (p = 0.8571), origin (p = 0.4075) and sex (p = 0.7534) were not significant. Given my previous concerns with the effect of hatchery rearing on incremental growth rings, that error is not linked to origin is noteworthy.

Table 5.3 Number and percentage of errors per age class, origin, sex, residency type and P value.

Age Class	**# of Individuals**	**# of Errors**	**% of Error**	**p value=**
2	7	1	14%	
3	13	2	29%	
4	23	3	43%	
5	3	1	14%	
Total	46	7	100%	.8571
Origin				
wild	16	1	14%	
hatchery	30	6	86%	
Total	46	7	100%	.4075
Sex				
male	15	2	29%	
female	23	3	42%	
jack	8	2	29%	
Total	46	7	100%	.7534
Freshwater Residency (yrs)				
1	42	4	57%	
2	4	3	43%	
Total	46	7	100%	.0406

The one variable that showed significant error was related to duration of freshwater residency (p = 0.0406). Of the seven errors, three were classified as “sub-twos”, based on scale age determinations. Two of these “sub-two” errors were 3_2 (having only spent one year in the ocean) and one was a 5_2. The remaining four errors were "sub-ones". A total of 75% of all sub 2s in the collection were miss aged. The disproportionate error associated with extended freshwater residency highlights the need for additional criteria development for this life history pattern so that it can be identified. My method preformed well in terms of accurately aging specimens with the exception of those individuals that spent a longer period rearing in freshwater.

Test of Reliability

To examine how well the annulus criteria on the centrum surface can be applied by other researchers, I carried out a test of reliability. One type II vertebra from each of the 46 individual Chinook salmon in the Hanford Reach assemblage was also independently aged by 5 readers. Of the 46 fish, all but 14 cases showed discrepancies among readers (Table 5.4). The resulting index of APE was 8.38%.

Table 5.4 List of estimated vertebral ages and associated APE from five readers for 46 Chinook salmon.

	Reader					
Fish #	**#2**	**#3**	**#4**	**#5**	**#6**	**APE**
33, 41	5	5	5	5	5	0
1,7,11,23,35,45	4	4	4	4	4	0
2, 14, 25	3	3	3	3	3	0
27, 30, 37	2	2	2	2	2	0
3	3	4	3	3	3	0.1
4	4	5	4	4	4	0.076
5	5	5	4	5	4	0.104
6	3	4	4	4	4	0.084
8	2	3	2	2	2	0.145
9	4	4	4	4	3	0.084
10	3	4	3	3	3	0.1
12	4	3	4	4	4	0.084
13	3	3	4	3	3	0.1
15	4	4	4	4	5	0.076
16	3	3	4	3	3	0.1
17	5	5	5	4	5	0.067
18	4	5	4	4	4	0.076
19	2	2	4	3	2	0.277
20	5	5	5	4	5	0.067
21	4	4	5	5	4	0.109
22	2	3	3	3	2	0.185
24	4	4	4	3	3	0.133
26	3	4	3	3	3	0.1
28	3	3	3	2	3	0.114
29	3	5	3	3	3	0.188
31	3	3	4	4	3	0.141
32	3	4	4	4	4	0.084
34	4	5	4	4	4	0.076
36	4	4	5	5	5	0.104
38	4	4	4	4	5	0.076
39	3	4	4	3	4	0.133
40	2	3	2	3	2	0.2
42	2	3	3	3	2	0.184
43	4	3	3	5	3	0.2
44	3	2	3	3	3	0.114
46	3	3	4	5	4	0.168
Total						8.38

This APE was much higher than that noted for other ageing projects. Hesse (1994), in a reliability test using three readers on 100 vertebrae achieved an APE of 2.17, with only 8 discrepancies. Wesley (1996) did not test his vertebral ageing method for reliability but did test three readers in the application of their scale age method. On an assemblage of 76 modern known age fish, only 10 discrepancies occurred, achieving an APE of 3.63.

One likely explanation for the high error rate for my study is the inexperience and limited training of the readers, who were graduate students in archaeology, not fisheries. Hesse (1994) noted that it takes less time to become a proficient reader with vertebrae than with scales. The relatively low error rate I achieved in aging the modern specimens after considerable study suggests that with more training and review, mis-aging would diminish for other readers.

Archaeological application

Of the 100 vertebrae from the Cathlapotle site measured and assigned to species using the Huber et al. (2011) morphometric method, 89 were identified as Chinook: 25 were type II vertebrae, 64 were type III vertebrae (Appendix C and D). Once again, this should not be considered representative of the Cathlapotle assemblage. Although all 89 vertebrae classified as Chinook salmon were complete, not all were sufficiently preserved to interpret incremental rings. For 39 of the Chinook vertebrae, rings were prominent and, in some cases, more so than in modern specimens (Figure 5.1). However, centrum surfaces on 50 vertebrae were too eroded to identify rings (Figure 5.2), a problem previously noted by Cannon (1988), Carlson (1988) and Wheeler and Jones (1989). In fact, it was this problem which initially led Cannon (1988) to begin experimenting with X-rays.

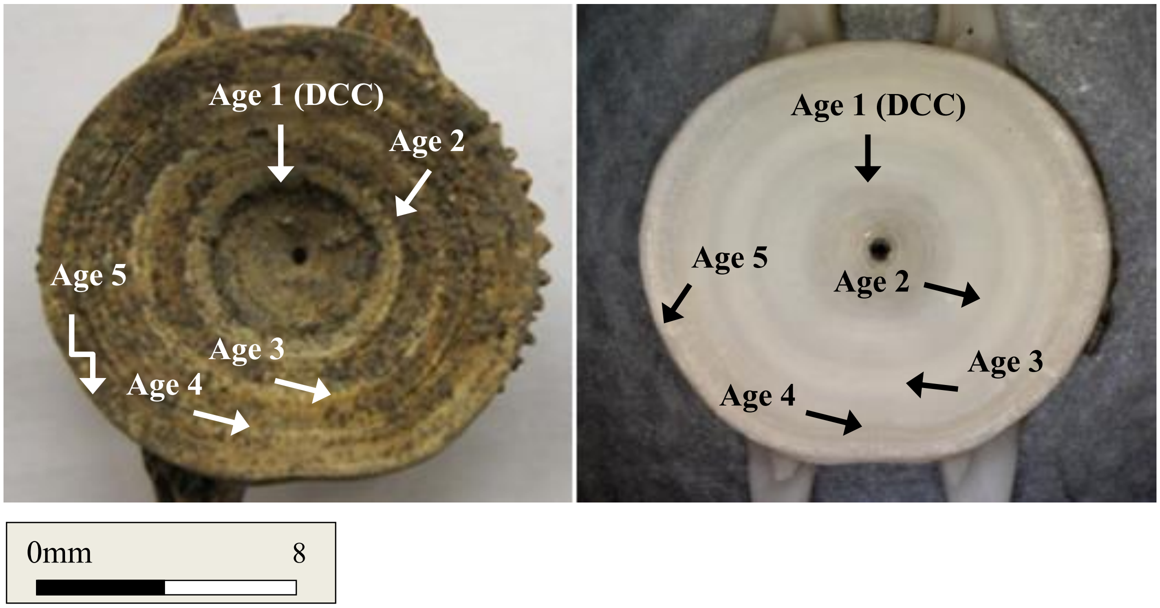

Figure 5.1 Image showing rings visible in archaeological vertebra (left) (photo by Shoshana Rosenberg) and a modern vertebra (right).

Figure 5.2 Archaeological salmon vertebra showing eroded centrum which precludes ageing.

I assigned ages to the 39 Chinook vertebrae with sufficient preservation to view rings (Figure 5.3). Most of the vertebrae were age 4 (46%) and age 5 (44%) with many fewer age 3 (10%).

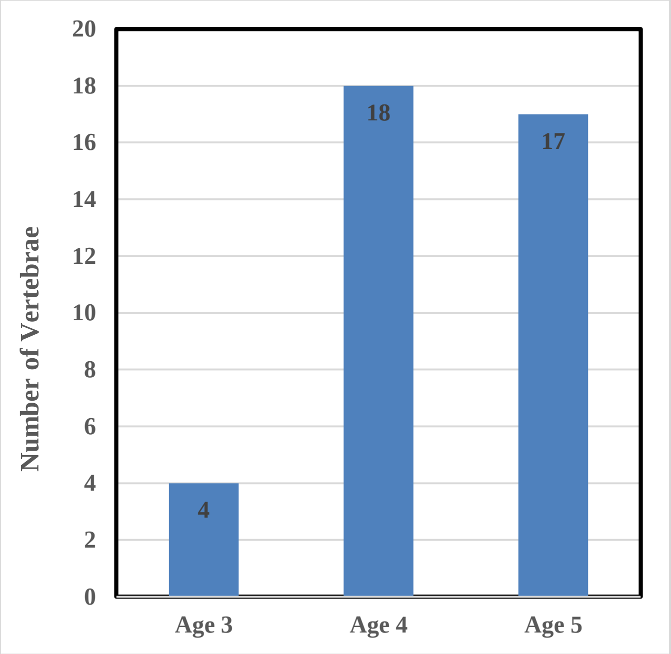

Figure 5.3 Frequency of type II and III Chinook salmon vertebrae by age class, Cathlapotle site.

Chapter 6

Discussion and Conclusions

I have demonstrated that using vertebral rings visible in X-rays as a proxy for age is not a valid method for determining salmonid species. In the course of my research I have evaluated the method of using X-rays of salmon vertebrae to determine fish age, determining that rings visible in X-rays are not associated with annular growth. I developed and tested a protocol of ageing fish using rings visible on the centrum surface of Chinook salmon vertebrae. I evaluated the feasibility of applying this method to archaeological collections by ageing a portion of Chinook salmon vertebrae from Cathlapotle (45CL1). This application of my vertebra ageing protocol to archaeological collections will allow archaeological salmon remains to contribute to our knowledge of long term trends on the life history and demography of salmon populations.

Researchers in Pacific Northwest archaeology have drawn many conclusions related to Native American salmon fisheries based on untested assumptions linking salmon age to vertebral rings observed in X-rays (Berry 2000; Cannon 1988, 1991; Orchard and Szpak 2012; Trost 2005). These assumptions have probably led to inaccurate interpretations concerning prehistoric economies and patterns of salmon use. Specifically the season of site occupation (based on estimating salmon age, then linking that with likely species, then spawning season) and duration has been challenged by my study. Rings visible in X-rays of salmon vertebrae are not connected to incremental growth rings on the centrum surface and thus are not useful for estimating fish age. I observed that rings visible in X-rays correspond to internal bone walls and these walls are likely formed early in life.

I evaluated the several methods of viewing vertebrae. I found that examining the centrum surface was the quickest, least destructive method of viewing incremental growth. More importantly, this method proved to also be the most accurate for examining annular growth rings.

Drawing on previous studies by Hesse (1994) and Wesley (1996) that had suggested the feasibility of ageing salmonids using centrum surface rings, I obtained modern reference materials for anadromous salmonids, particularly Chinook salmon. I developed a protocol for separating true annuli from other incremental rings. I tested this protocol for accuracy and reliability substantiating that using surface rings is a valid approach for ageing Chinook salmon.

This contributes to a larger goal of accountability in zooarchaeology. Researchers have suggested that zooarchaology as a discipline has generally lacked efforts from analysts to validate and substantiate their results (Driver 1992; Gobalet 2001; Wolverton 2014) Procedures for faunal analysis are rarely explicit nor are they susceptible to testing or critical evaluation (Driver 1992). By presenting my protocol for ring identification and presenting my results for accuracy and reliability other researchers are able to evaluate, compare or attempt to repeat my results.

The ultimate goal of this project was to access the feasibility of applying the ageing protocol to archaeological specimens. Contrary to what other researchers have suggested (Cannon 1988; Carlson 1988; Wheeler and Jones 1989), I demonstrated that rings are visible on the surface of archaeological vertebrae. After identifying Chinook salmon vertebrae from the Cathlapotle assemblage, I aged those which were in a reasonably good state of preservation.

If we can further refine this method, it will be possible to study life history variation using salmonid vertebrae from archaeological sites all along the Pacific Rim, dating to the last 10,000 years or more. Specifically, it will be possible to model changes in salmon life history, focusing on timing and duration of juvenile estuary residence, timing of ocean migration and the age of spawning and death Some efforts have been made to do this using historic records (Campbell 2010) but utilizing older archaeological collections [e.g. Dallas Roadcut site, 7500-9500 BP (Cressman 1960; Butler 1993)] would allow fisheries managers to evaluate salmon life history associated with environments that pre-date hatcheries, intensive commercial fishing and hydroelectric dams.

Simply further establishing that age can be determined from vertebrae has value to fisheries ageing projects, especially as an alternative to scales, where the edges can be eroded on older individuals (Hesse 1994). Vertebral age determination can also be useful to fisheries biologists by allowing them to use a combination of multiple elements for ageing which may reduce errors resulting in the improper management of fish stocks (Chilton and Bilton 1986).

Additional work needs to be carried out to substantiate these findings. Specifically, a larger sample of known-age modern specimens are needed to address errors associated with longer initial freshwater residency. More sub-two individuals are needed to further characterize this error and could make it possible to isolate patterns for identification and interpretation. Excluding the sub- two residency pattern errors, the accuracy rate for my readings in this study would have been 93%, comparable to rates achieved by Hesse and Wesley. A reliable protocol for identifying these sub-twos will be especially important to use archaeological vertebrae to reconstruct the early life history patterns of salmon.

The additional samples should also include fish > 5 years old, as Chinook are known to attain higher ages (Groot and Margolis 1991; Hart 1973; Quinn 2005). Beamish and McFarlane (1983; 1987) stress the importance of validating across all possible age classes.

In order for this method to be used by others, additional time needs to be spent testing the reliability of the method in terms of inter-observer error. The majority of ageing done with fish elements is undertaken by professionals, and potential analysts need more time than just my hour long workshop to practice and become more proficient interpreting the rings on vertebrae. A larger sample of known age fish would allow for the development of a larger, more complete teaching collection.

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APPENDIX

Appendix A. Type II and type III vertebrae widths and fork lengths of Hanford Reach collection.

Scale Card #	Fork Length	Type II width (mm)	Type III width (mm)
1058-1	78	10.74	11.61
1061-1	81	11.49	11.8
1061-12	49	7.07	7.1
1061-2	79	12.13	12.59
1061-9	58	8.49	8.65
1063-19	90	12.47	13.65
1063-20	58	8.09	8.54
1064-11	75	11.48	12.29
1064-12	78	11.83	12.54
1064-13	53	7.59	8
1067-16	90	12.49	13.68
1068-7	79	10.88	11.74
1071-18	69	11.97	13.49
1091-14	87	12.46	12.7
1092-2	75	10.74	11.21
1092-4	72	10.24	10.02
1102-2	45	5.86	6.27
1102-3	70	9.51	10.64
1123-10	52	8.06	8.13
1123-11	43	5.75	6.28
1123-12	69	9.31	10.31
1123-13	71	10.19	10.87
1135-7	85	11.96	11.59
1139-5	84	11.22	12.71
1139-6	54	7.11	7.86
1141-20	80	12.22	12.52
1142-11	99	14.28	15.15
1144-1	48	6.81	7.55
1144-13	91	12.1	13.5
1151-14	89	13.66	13.89
1151-15	67	9.86	9.77
1152-15	85	13.04	13.75
1157-17	79	10	10.45
1239-3	74	10.71	10.7
1241-3	73	9.8	10.33
1241-8	81	10.62	12.28
1241-9	80	11.63	11.76
1449-14	74/78	Did not include	Did not include
1449-15	53	8.22	8.13
1450-4	85	12.83	13.28
1450-5	85	11.12	12.27
8232-1	88	12.6	13.24
8233-13	86	11.7	12.74
8237-5	77	10.63	11.54
8238-2	67	9.81	9.84
8241-4	80	11.16	11.74

Appendix B Summary of Cathlapotle vertebrae provenience.

Catalog #	Provenience	Species	Vert Type	Age
1	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
2	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
3	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	UC
4	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
5	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	UC
6	N160 W91, SW quad, lvl 9, Fea. 728	Steelhead	II	N/A
7	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
8	N160 W91, SW quad, lvl 9, Fea. 728	Steelhead	II	N/A
9	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	4
10	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
11	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	UC
12	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	4
13	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
14	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	4
15	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	4
16	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
17	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	3
18	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	II	5
19	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
20	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
21	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
22	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
23	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
24	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
25	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
26	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
27	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
28	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
29	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
30	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
31	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
32	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
33	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
34	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
35	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
36	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
37	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
38	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	3
39	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
40	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4

41	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
42	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
43	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
44	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
45	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
46	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
47	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
48	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
49	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
50	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
51	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
52	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
53	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
54	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
55	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
56	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
57	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
58	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
59	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
60	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
61	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
62	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
63	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
64	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
65	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
66	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
67	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
68	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
69	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
70	N160 W91, SW quad, lvl 9, Fea. 728	Chum	III	N/A
71	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
72	N160 W91, SW quad, lvl 9, Fea. 728	Steelhead	III	N/A
73	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	4
74	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	5
75	N160 W91, SW quad, lvl 9, Fea. 728	Chinook	III	UC
76	N52 W101, SE quad, lvl 8	Steelhead	II	N/A
77	N52 W101, SE quad, lvl 8	Chinook	II	4
78	N52 W101, SE quad, lvl 8	Chinook	II	UC
79	N52 W101, SE quad, lvl 8	Chinook	II	UC
80	N52 W101, SE quad, lvl 8	Coho	II	N/A
81	N52 W101, SE quad, lvl 8	Chum	II	N/A
82	N52 W101, SE quad, lvl 8	Steelhead	II	N/A
83	N52 W101, SE quad, lvl 8	Chinook	II	UC
84	N52 W101, SE quad, lvl 8	Chinook	II	UC

85	N52 W101, SE quad, lvl 8	Chinook	II	UC
86	N52 W101, SE quad, lvl 8	Chinook	II	4
87	N52 W101, SE quad, lvl 8	Chinook	II	UC
88	N52 W101, SE quad, lvl 8	Chinook	II	4
89	N52 W101, SE quad, lvl 8	NA	III	NA
90	N52 W101, SE quad, lvl 8	UC	III	UC
91	N52 W101, SE quad, lvl 8	NA	III	NA
92	N52 W101, SE quad, lvl 8	UC	III	UC
93	N52 W101, SE quad, lvl 8	NA	III	NA
94	N52 W101, SE quad, lvl 8	UC	III	UC
95	N52 W101, SE quad, lvl 8	4	III	4
96	N52 W101, SE quad, lvl 8	5	III	5
97	N52 W101, SE quad, lvl 8	UC	III	UC
98	N52 W101, SE quad, lvl 8	5	III	5
99	N52 W101, SE quad, lvl 8	UC	III	UC
100	N52 W101, SE quad, lvl 8	UC	III	UC

Appendix C. Results of Morphometric Classification, Type II vertebrae from Cathlapotle

Index	Mean L	Mean H	L_H_ratio	R species
1	10.02	14.81	0.67657	[1,] "Chinook"
2	8.82	13.09	0.673797	[2,] "Chinook"
3	10.01	14.2	0.70493	[3,] "Chinook"
4	10.09	14.38	0.701669	[4,] "Chinook"
5	9.8	14.73	0.665309	[5,] "Chinook"
6	9.87	12.48	0.790865	[6,] "steelhead"
7	8.05	11.6	0.693966	[7,] "Chinook"
8	9.04	11.54	0.783362	[8,] "steelhead"
9	6.94	12.12	0.572607	[9,] "Chinook"
10	8.72	12.41	0.702659	[10,] "Chinook"
11	8.19	12.04	0.680233	[11,] "Chinook"
12	9.35	13.32	0.701952	[12,] "Chinook"
13	9.21	12.73	0.723488	[13,] "Chinook"
14	8.76	12.83	0.682775	[14,] "Chinook"
15	6.93	11.77	0.588785	[15,] "Chinook"
16	8.57	12.4	0.691129	[16,] "Chinook"
17	6.9	9.88	0.698381	[17,] "Chinook"
18	10.24	14.42	0.710125	[18,] "Chinook"
76	8.69	10.55	0.823697	[1,] "steelhead"
77	7.87	10.12	0.777668	[2,] "Chinook"
78	7.39	11.06	0.668174	[3,] "Chinook"
79	7.77	10.89	0.713499	[4,] "Chinook"
80	6.06	8.38	0.72315	[5,] "coho"
81	7.72	9.74	0.792608	[6,] "chum"
82	9.67	10.74	0.900372	[7,] "steelhead"
83	7.65	10.66	0.717636	[8,] "Chinook"
84	7.63	11.77	0.648258	[9,] "Chinook"
85	8.21	11.53	0.712056	[10,] "Chinook"
86	7.56	10.05	0.752239	[11,] "Chinook"
87	7.7	11.45	0.672489	[12,] "Chinook"
88	7.75	11.21	0.691347	[13,] "Chinook"

Appendix D. Results of Morphometric Classification, Type III vertebrae from Cathlapotle

Index	Mean L	Mean H	L_H_ratio	R species
19	9.65	12.39	0.778854	[1,] "Chinook"
20	10.33	14.12	0.731586	[2,] "Chinook"
21	9.62	12.4	0.775806	[3,] "Chinook"
22	12	15.66	0.766284	[4,] "Chinook"
23	12.03	15.79	0.761875	[5,] "Chinook"
24	9.64	13.23	0.728647	[6,] "Chinook"
25	10.03	12.7	0.789764	[7,] "Chinook"
26	10.3	14.94	0.689424	[8,] "Chinook"
27	12.63	16	0.789375	[9,] "Chinook"
28	9.88	15.21	0.649573	[10,] "Chinook"
29	9.81	13.47	0.728285	[11,] "Chinook"
30	12.18	15.73	0.774317	[12,] "Chinook"
31	8.59	12.63	0.680127	[13,] "Chinook"
32	9.52	12.43	0.765889	[14,] "Chinook"
33	9.98	13.44	0.74256	[15,] "Chinook"
34	10.68	14.2	0.752113	[16,] "Chinook"
35	9.39	14.17	0.662668	[17,] "Chinook"
36	9.52	12.35	0.77085	[18,] "Chinook"
37	12.17	15.97	0.762054	[19,] "Chinook"
38	9.67	12.66	0.763823	[20,] "Chinook"
39	9.65	13.9	0.694245	[21,] "Chinook"
40	12.62	15.62	0.807939	[22,] "Chinook"
41	9.17	12.91	0.710302	[23,] "Chinook"
42	9.69	12.38	0.782714	[24,] "Chinook"
43	10.31	13.46	0.765973	[25,] "Chinook"
44	9.77	12.55	0.778486	[26,] "Chinook"
45	11.14	15.17	0.734344	[27,] "Chinook"
46	10.22	13.89	0.735781	[28,] "Chinook"
47	9.33	12.26	0.761011	[29,] "Chinook"
48	8.75	13.84	0.632225	[30,] "Chinook"
49	8.37	11.97	0.699248	[31,] "Chinook"
50	8.66	12.71	0.681353	[32,] "Chinook"
51	9.28	12.82	0.723869	[33,] "Chinook"
52	9.55	12.46	0.766453	[34,] "Chinook"
53	9.72	13.66	0.711567	[35,] "Chinook"
54	8.73	13.15	0.663878	[36,] "Chinook"
55	9.32	13.34	0.698651	[37,] "Chinook"
56	10.47	14.98	0.698932	[38,] "Chinook"
57	9.72	13.04	0.745399	[39,] "Chinook"
58	8.62	11.06	0.779385	[40,] "Chinook"

59	9.41	12.42	0.757649	[41,] “Chinook”
60	9.78	12.77	0.765857	[42,] “Chinook”
61	12.15	15.6	0.778846	[43,] “Chinook”
62	9.43	13.09	0.720397	[44,] “Chinook”
63	8.55	11.1	0.77027	[45,] “Chinook”
64	9.92	13.35	0.743071	[46,] “Chinook”
65	8.82	12.48	0.706731	[47,] “Chinook”
66	11.09	15.14	0.732497	[48,] “Chinook”
67	8.74	12.38	0.705977	[49,] “Chinook”
68	9.67	12.13	0.797197	[50,] “Chinook”
69	9.09	12.31	0.738424	[51,] “Chinook”
70	7.29	9.19	0.793254	[52,] “chum”
71	6.83	9.72	0.702675	[53,] “Chinook”
72	6.48	7.48	0.86631	[54,] “steelhead”
73	9.63	13.32	0.722973	[55,] “Chinook”
74	8.82	13.11	0.672769	[56,] “Chinook”
75	11.12	14.5	0.766897	[57,] “Chinook”
89	6.93	8.97	0.772575	[1,] “coho”
90	8.18	10.98	0.744991	[2,] “Chinook”
91	9.35	10.71	0.873016	[3,] “steelhead”
92	9.06	12.78	0.70892	[4,] “Chinook”
93	7.34	8.6	0.853488	[5,] “steelhead”
94	8	12.44	0.643087	[6,] “Chinook”
95	9.56	13.94	0.685796	[7,] “Chinook”
96	10.74	14.66	0.732606	[8,] “Chinook”
97	7.89	11.4	0.692105	[9,] “Chinook”
98	10.81	15.35	0.704235	[10,] “Chinook”
99	8.12	12.42	0.653784	[11,] “Chinook”
100	8.82	12.06	0.731343	[12,] “Chinook”