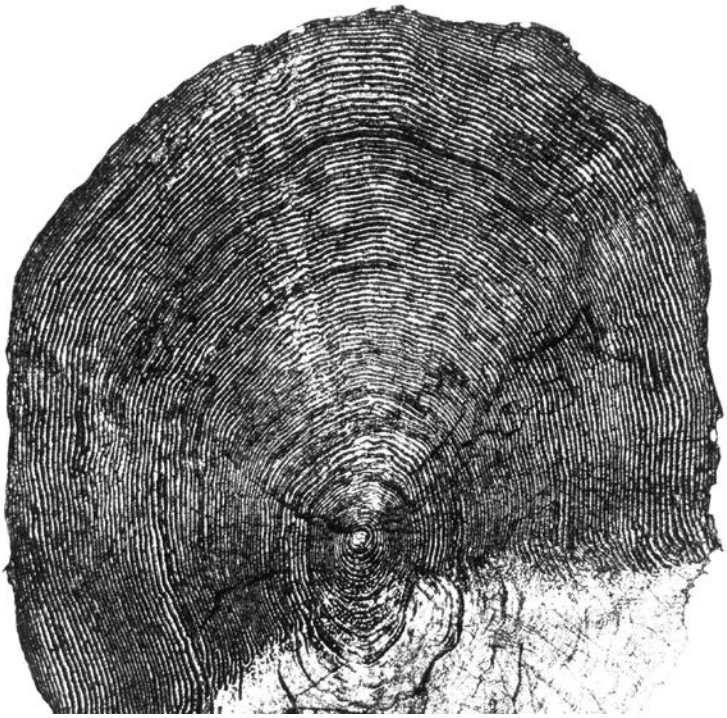
FISHERY RESEARCH

PROCESS AND METHODS FOR ASSIGNING AGES TO ANADROMOUS SALMONIDS FROM SCALE SAMPLES



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ABBREVIATIONS AND ACRONYMS

BPA	Bonneville Power Administration
CWT	Coded Wire Tag
IDFG	Idaho Department of Fish and Game
ISEMP	Integrated Status and Effectiveness Monitoring Program
INPMEP	Idaho Natural Production Monitoring and Evaluation Project
ISMES	Idaho Steelhead Monitoring and Evaluation Studies
ISS	Idaho Supplementation Studies
LGR	Lower Granite Dam
NRAAL	Nampa Research Anadromous Ageing Laboratory
PIT	Passive Integrated Transponder
PTAGIS	PIT Tag Information System

SUMMARY

The Nampa Research Anadromous Ageing Laboratory (NRAAL) supports the Idaho Steelhead Monitoring and Evaluation Studies, the Idaho Natural Production Research Monitoring and Evaluation Project, and other projects by: 1) providing ageing assistance to internal and external cooperators, 2) providing accurate age data in a timely manner, and 3) investigating techniques and issues pertinent to age, growth, and life history studies in the Snake River basin. The NRAAL receives scale samples from wild adult and juvenile Chinook salmon and steelhead collected at weirs and screw traps managed by multiple agencies (Idaho Department of Fish and Game, Nez Perce Tribe, Shoshone Bannock Tribe) across the Snake River basin in Idaho, and at Lower Granite Dam in Washington.

After acquiring field samples, NRAAL personnel perform data entry, sample processing (mounting and imaging), and age determination. Fish data are entered into a Microsoft® Access™ database and quality control checked for accuracy. Scales are mounted between two microscope slides. Digital images are archived on a shared network drive for easy access, allowing multiple readers to view images simultaneously. When assigning ages, readers independently view scale images on a computer monitor and record their observations using electronic worksheets. Age data are entered into the database and quality control checked for age-length outliers.

The NRAAL uses known-age fish sampled through PIT tag mark-recapture to validate saltwater age determination. Freshwater ages are currently not validated; however, we have several projects underway to aid in freshwater validation. For both species, the NRAAL's saltwater ageing accuracy has been greater than 96.00%, which surpasses the 90.00% bias threshold. Accuracy is improved by using multiple readers and training methods, including tests for new readers. The NRAAL has recently started assessing precision for all locations *post hoc* using coefficient of variation and percent agreement. Estimates of saltwater age coefficient of variation (COV) and percent agreement (PA) meet the NRAAL's goal of ≤0.10 COV and ≥80% PA the majority of the time. Freshwater age verification estimates meet goals for Chinook; however, in many cases steelhead do not meet goals. The NRAAL's current research is working to establish freshwater age validation techniques to increase accuracy and precision of freshwater ages.

This report has been prepared by the NRAAL to maintain and distribute its protocols for the benefit of new, supporting, and collaborating staff as well as the fisheries research community as a whole. This report also acts as a source of laboratory history and as an archive of methods and knowledge used by the NRAAL.

INTRODUCTION

Populations of spring-summer Chinook Salmon *Oncorhynchus tshawytscha* and steelhead trout *Oncorhynchus mykiss* in the Snake River basin (hereafter Chinook and steelhead) declined substantially in the late 1960s and early 1970s following the construction of hydroelectric dams in the Columbia and Snake river basins (Raymond 1988). Chinook and steelhead were classified as threatened under the Endangered Species Act in 1992 and 1997, respectively. The long-range goals of the Idaho Department of Fish and Game (IDFG) anadromous fish program, consistent with basinwide mitigation and recovery programs, are to preserve Idaho's salmon and steelhead runs and recover them to provide benefit to all users (IDFG 2007).

Evaluation of population status relative to these goals requires monitoring. Population age structure is an important parameter for assessing status. Primary IDFG monitoring projects for wild fish include the Idaho Steelhead Monitoring and Evaluation Studies (ISMES; project number 1990-055-00) and the Idaho Natural Production Research Monitoring and Evaluation Project (INPMEP; project number 1991-073-00). The Nampa Research Anadromous Ageing Laboratory (NRAAL) supports these and other projects by: 1) providing ageing assistance to internal and external cooperators, 2) providing accurate age data in a timely manner, and 3) investigating techniques and issues pertinent to age, growth, and life history studies in the Snake River basin. Age data are used to assign juveniles and returning adults to specific brood years, for cohort analysis, and to estimate productivity and survival rates (Copeland *et al*. 2007; Copeland *et al*. 2011, 2012; Kennedy *et al*. 2011, 2012; Schrader *et al*. 2011, 2012). ISMES and INPMEP collect scales, fin rays, and otoliths to determine individual ages.

Scales have been used as a tool to age and interpret life history strategies in salmon populations for at least a century (Gilbert 1912). Anadromous salmonids have complex life histories that include variable amounts of time in both freshwater and saltwater environments (Elliott 1994; Quinn 2005). Two distinct portions of an adult scale represent the freshwater and saltwater life histories. Combining the ages for both portions allows the determination of the total age of the adult fish sampled. Determining years within the freshwater region is more difficult than saltwater age determination and in general it is not attempted when using fin rays or otoliths.

There are several other benefits of using scales for age determination: scales can easily be removed, fish can be sampled multiple times, and collection does not require killing or severely harming the fish. However, the use of scales has limitations including regeneration, scale resorption, and false annuli (Quist *et al*. 2012). Regeneration, which partially or wholly destroys the freshwater portion of a scale, and false checks are common contributors to errors in age assignments.

IDFG's anadromous monitoring projects collect many scale samples. The need for NRAAL was recognized in 2006, when the initiation of these projects expanded beyond the scope of current project infrastructure. In 2007 funding was acquired to staff an 8-month technician to improve program stability, maintain trained personnel, and assist in ageing an increasing number of samples. Consultation with other laboratories occurred during this time to organize and set up laboratory protocols. In 2008, the NRAAL added a second 8-month technician; shortly thereafter both positions became year-round. In 2009 the addition of new projects and the need to maintain trained staff resulted in funding to hire a laboratory coordinator and two additional technicians (one year round, one 8-month). Since 2009 laboratory personnel have consisted of a coordinator, three year-round technicians, and one 8-month technician. Methods have evolved in response to new resources, knowledge, and

experience since the NRAAL's formation in 2006. Trained and experienced personnel are required for the teaching of new laboratory personnel to ensure consistency and accuracy. Protocols and consistency in scale data collection and processing are necessary tools for quality control and preserving scientific integrity.

This report has been prepared by the NRAAL to maintain and distribute its protocols to make referencing our laboratory protocols simple and efficient. Its distribution and use will benefit new, supporting, and collaborating staff. The focus is on processing and analysis of scale samples because those are the majority of samples collected annually, and methodology for processing other structures (fin rays and otoliths) is very different. This report also acts as a source of laboratory history and as an archive of methods and knowledge used by the NRAAL. This document should be reviewed and updated as needed on a periodic basis (every five years). We have organized this document into the four major processing phases: scale collection, data entry and quality control, scale processing, and ageing procedures.

METHODS

Scale Collection

Cooperators collect samples from fish captured at remote locations and trap sites, wild adults captured at IDFG hatchery weirs, and wild fish collected at Lower Granite Dam. Prior to scale collection in the field, NRAAL personnel prepare and distribute standardized scale collection envelopes to minimize time handling of fish and minimize human error in data recording. Additionally, because the NRAAL relies on field personnel across many different locations and agencies for scale collection, these envelopes provide quality control and standardization. Collection packets consist of a 2½ inch by 4¼ inch 20 lb. coin envelope, a scale card, and a 2" x 4" shipping label. A scale card is placed in the envelope to prevent sample adhesion to the coin envelope; therefore, it is important that the card is cut from treated paper (Rite-in-the-Rain is recommended) and folded in half before insertion. Labels are placed on the envelopes for data collection. The NRAAL has generated templates for juvenile and adult collection envelopes (Figure 1). Unique sample numbers are assigned each year to prevent duplication (see Table 1 for more information).

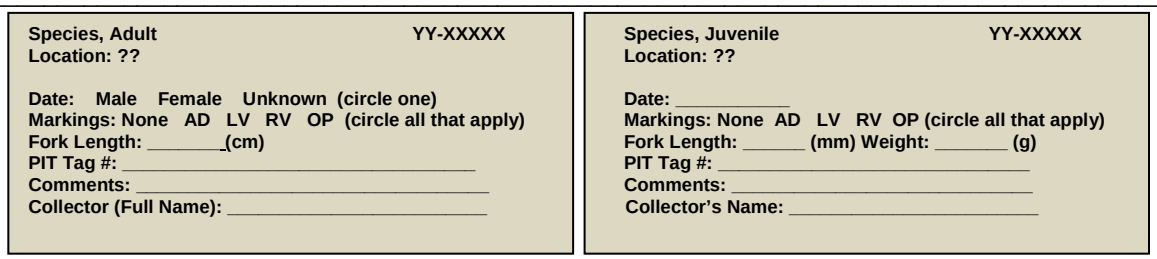

Figure 1. Label templates used for field collection envelopes. The left label is for adult samples, the right label is for juvenile samples. Envelope headers (species, location and sample numbers) change according to collections.

When collecting samples in the field, scales should be taken from a consistent area on each fish to yield scales of similar size and symmetrical shape. Literature suggests the best location for salmonid scale sampling is within six scales on either side of an imaginary line running from the posterior base of the dorsal fin to the anterior base of the anal fin, and within two to three scale rows above the lateral line (Figure 2). Scales are developed in this area first, yielding the highest probability of obtaining complete scale information (Quinn 2005; Shearer 1992; Mosher 1968).

Field sampling procedures are as follows:

1. Follow established protocols for fishing and handling procedures at the specific trap or weir.
2. Take physical measurements including weight, length, condition, etc. from the fish and record on envelope label.
3. Clean tools and work station to remove contamination and previous sample remnants.
4. Prepare the optimal area on the fish for scale removal by gently removing slime with knife and wiping on a towel.
5. Adult: grasp a scale in the preferred area with forceps and pull the scale from the fish. To expedite processing, a knife may be used instead of forceps. With a knife, scrape in a posterior direction in the preferred area.
   Juvenile: gently scrape scales from preferred area with the tip of a small knife.
6. Adult: collect 8-10 scales.
   Juvenile: collect 15-20 scales.
7. Spread scales out on the scale card and then fold the scale card to cover the scales. Place the folded scale card inside the envelope.
8. Double-check data ensuring all required data are recorded.
9. After sampling has been completed, place filled scale envelopes in a safe, ventilated area to dry for 24-48 hrs.

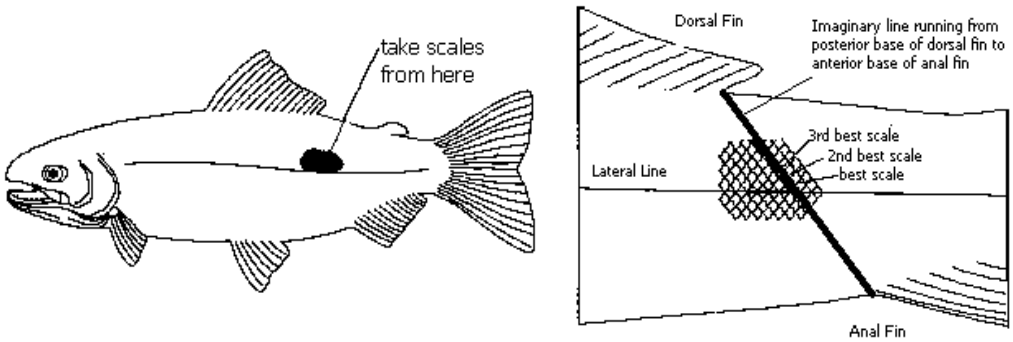

Figure 2. Preferred scale collection location from salmonids. Scales should be taken within six scales on either side of an imaginary line running from the posterior base of the dorsal fin, to the anterior base of the anal fin, and within two to three scale rows above the lateral line. Picture source: https://www.for.gov.bc.ca/hts/risc/pubs/aquatic/fishcol/fish-3.htm.

Data Entry and Quality Control

Samples and associated data are delivered to the NRAAL after collection for cataloging, data entry, and processing. We request the data in electronic spreadsheet or database format to allow quick and accurate addition of records to the laboratory database. Data are accepted by the NRAAL in any of the four following formats:

(1) Sent by email to the laboratory coordinator,
(2) Uploaded by cooperators to the BioSamples Extranet website: https://collaboration.idfg.idaho.gov/biosamples/default.aspx,
(3) Retrieved from the FINS database,
(4) Retrieved from the IDFG Lower Granite Dam Trapping database: https://collaboration.idfg.idaho.gov/LGTrapping/default.aspx.

Scale envelope data are quality checked and corrected by comparing available information to electronic data. Quality control is essential in data management to ensure accuracy, thereby reducing valuable time spent resolving errors later. Quality control also reduces or eliminates uncertainty for data users. Data such as collection date, length, marks, and tags are checked overall for major discrepancies and checked randomly for minor discrepancies. Major discrepancies include incorrect year, incorrect length units, missing marks or tags, and missing data for samples in hand. Minor discrepancies are generally transcription errors.

Tracking sheets are created for each scale collection location, species, and life stage on an annual basis. Tracking sheets allow NRAAL personnel to record their involvement in each stage of sample processing, creating both a log of progress and a trail of accountability if errors must be retraced. Concurrent with the creation of a tracking sheet, a unique BioSamples identification (ID) number is assigned to each sample. The BioSample ID can either be from the NRAAL prepared collection envelope or can be newly assigned if the NRAAL envelopes are not used. BioSample ID numbers are formatted as YY-XXXXX with YY signifying the last two digits of the year and XXXXX signifying the five digit sample ID (e.g. 14-10001). Sample ID groups are established for species, life stage, and collection locations (Table 1).

Table 1. BioSample IDs assigned to species, life stage and location of sample collections. LGR – Lower Granite Dam. YY is used to denote the last two digits of the year of collection.

Species	Life Stage	Sample Type	Location	BioSample IDs Available
Chinook	Adult	Fin Rays	All Locations	YY-00001 through YY-10000
Chinook	Adult	Scales	LGR	YY-10001 through YY-20000
Steelhead	Adult	Scales	LGR	YY-20001 through YY-30000
Chinook	Adult	Scales	All Locations (except LGR)	YY-30001 through YY-40000
Steelhead	Adult	Scales	All Locations (except LGR)	YY-40001 through YY-50000
Chinook	Juvenile	Scales	All Locations	YY-50001 through YY-60000
Steelhead	Juvenile	Scales	All Locations	YY-60001 through YY-80000
Any	Any	Any	Overflow numbers	YY-80001 through YY-99999

The BioSamples Database (BSDB; see Appendix A for metadata) was established to house the anadromous ageing information generated by the NRAAL including scale, fin ray, and otolith age data. Data are uploaded using a standardized Excel spreadsheet template, reducing transcription errors that might occur from entering data manually in the BSDB interface (Figure 3). Sample information is ready for upload after formatting and quality control are complete. Uploads are posted on the BioSamples Extranet website https://collaboration.idfg.idaho.gov/biosamples/default.aspx, where the database coordinator retrieves the spreadsheet and appends data to the BSDB. As of January 2015, approximately 218,000 records of archived data can be retrieved by laboratory personnel, cooperators, biologists, and managers that are granted access. For access to the BSDB, contact Paul Bunn (paul.bunn@idfg.idaho.gov), the current Nampa Research Database Coordinator.

Scale Processing

Scales must be processed prior to age determination. Processing consists of sorting and selecting, mounting, and imaging. Sorting and selecting is performed to identify adult scales that are best suited for ageing. Mounting of adult and juvenile scales is performed by securing them between two glass microscope slides. Imaging is done digitally using a computer and digital camera attached to a microscope. The following sections detail the NRAAL's mounting and imaging processes.

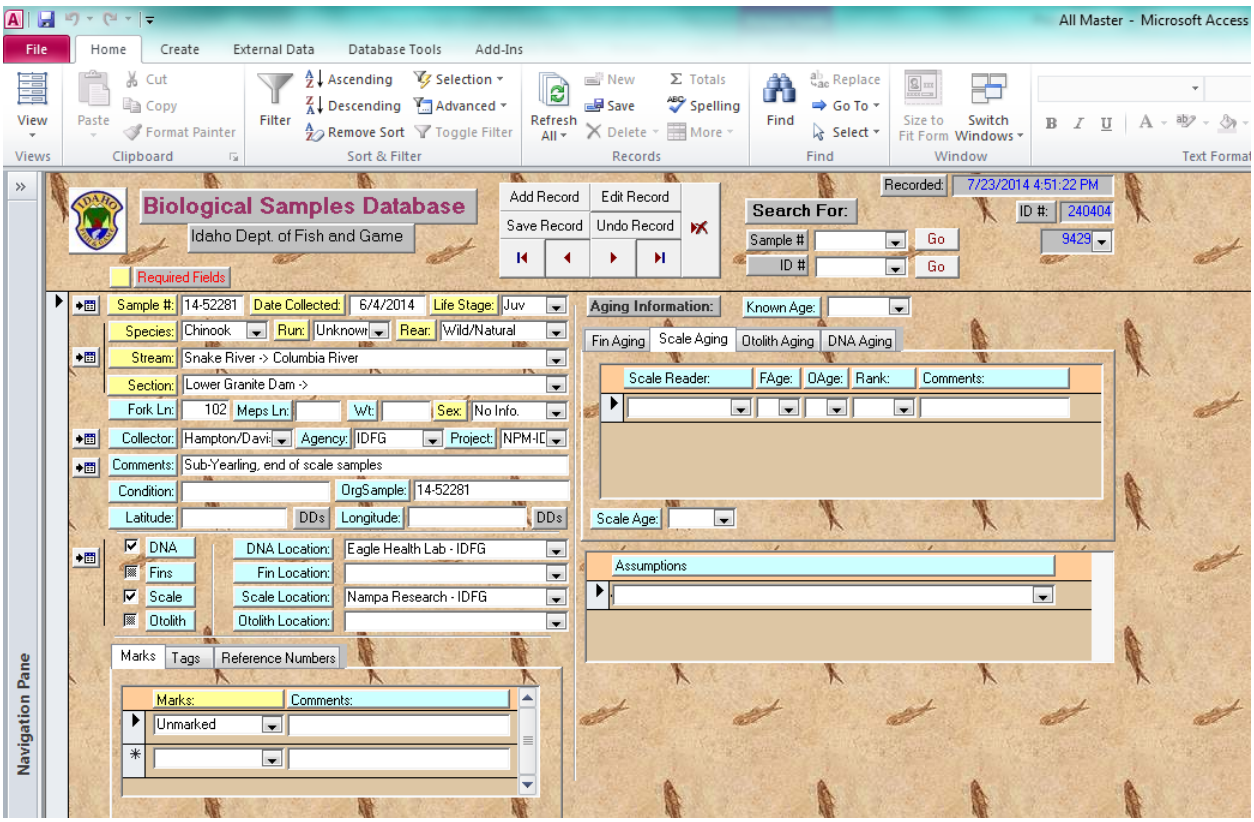

Figure 3. Screen shot of the BioSamples Database All Master data entry form.

Mounting

Scales are mounted between two glass microscope slides for ease of handling, imaging, and archiving. Care must be taken to prevent sample contamination and to keep slides free from fingerprints and smudging. Mounting is a multi-step process and varies between adults and juveniles. Adult scales are larger and require more processing time through sorting, selecting, and cleaning. Juvenile scales are smaller and therefore cannot be cleaned; however, more scales are mounted due to the increased possibility of regeneration and damage from the collection process.

To both expedite and safeguard the mounting process, it is essential for personnel to establish clean and organized work stations. NRAAL personnel perform the majority of mounting in house; however, in some cases cooperating field personnel are encouraged to mount samples. When establishing a work station, the area should be well-lit and protected against air drafts to prevent sample loss. Equipment for mounting scales includes the following:

- Glass microscope slides with frosted ends (to secure scales for handling and imaging)
- Marker (for labeling slides; the NRAAL suggests a fine-tip Sharpie®)
- Scotch® brand transparent tape (to secure slides together while keeping sample number visible)
- Scalpel (for opening envelopes and removal of scale samples)
- Forceps (for removal and manipulation of scale samples)
- Probes (for removal and manipulation of scale samples)
- Dark surface (optional; best for contrasting scales while on slides)

Additional resources are required for the mounting of adult scales:

- Dissecting microscope (NRAAL personnel use ~2.0x objective to sort and select scales, microscope is capable of 1.0x – 4.0x)
- Small wash basin (small bowl or furniture coaster; for cleaning mucus and debris from a scale's surface)
- Damp towelette (to keep clean scales from drying and curling before mounting)
- Dry towelette (to dry scales immediately before mounting; the NRAAL suggests using Kimwipes® or other optics cleaning towelettes to avoid fiber deposits on scales)

Procedures for mounting <u>adult</u> scales:

1. Select a sample and label the frosted-ends of two microscope slides with the laboratory sample number.
2. Carefully remove the scale card from the sample envelope, as all scales may not have adhered to the card.
3. Remove scales from the card using forceps or a scalpel, and place them on a stereo microscope stage.
4. Sort scales based on quality of ageing features. Select the six best scales with the least regeneration (Appendix B-1) and resorption for mounting (Appendix B-2).
5. Place un-used scales back in scale card and secure the card inside sample envelope.
6. Soak selected scales in water to loosen mucus and debris. Generally allow at least 30 seconds.
7. Clean a scale by rubbing it between your fingertips. If scales cannot effectively be cleaned with fingers alone, grasping them gently with forceps and pulling away debris

may yield better results, but this process also runs the risk of scraping or tearing the scale.

8. Once a scale has been cleaned, place it on a damp towelette. Moisture will keep the scale from drying and curling up on its edges while subsequent scales are being cleaned.
9. Repeat steps 7-8 for each scale in the wash basin. Once all scales have been cleaned, examine the wash basin a second time ensuring no scales remain.
10. Remove any excess water from scales using a dry towelette.
11. With forceps, place scales on microscope slide aligning posterior fields downward (Figure 4).
12. Once all scales have been mounted, carefully cover the scales with the second labeled slide. Secure slides together with a piece of clear tape wrapped around the ends of the long edges (Figure 5).
13. Place the mounted sample back in its sample envelope taking care not to split the scale card open.
14. Properly inventory the mounted sample packet.

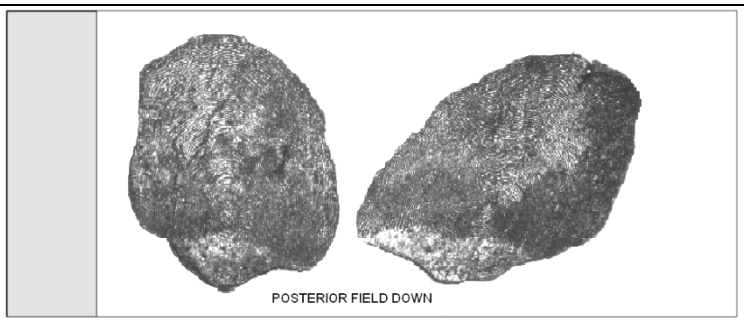

Figure 4. Mounting of cleaned adult scales with posterior fields aligned in the same direction.

Procedures for mounting <u>juvenile</u> scales:

1. Select a sample and label the frosted-ends of two microscope slides with the laboratory sample number.
2. Carefully remove the scale card from the sample envelope, as all scales may not have adhered to the card.
3. Situate slides label-down with frosted ends on opposing sides.
4. Remove scales from card using forceps or a scalpel and place 15-20 scales onto a single microscope slide. Keep the other slide free from scales.
5. Securely place the scale card with remaining unused scales, if any, back inside the sample envelope.
6. On the single slide with scales, separate scales so they are in a single layer and do not overlap.
7. Carefully place the clean slide on top of the slide with scales. Secure the slides together with a piece of clear tape wrapped around the ends of the long edges (Figure 5).
   Note: juvenile scales are easily disturbed by air drafts and static between slides.

8. Place the mounted sample back in its sample envelope taking care not to split the scale card open.
9. Properly inventory the mounted sample packet.

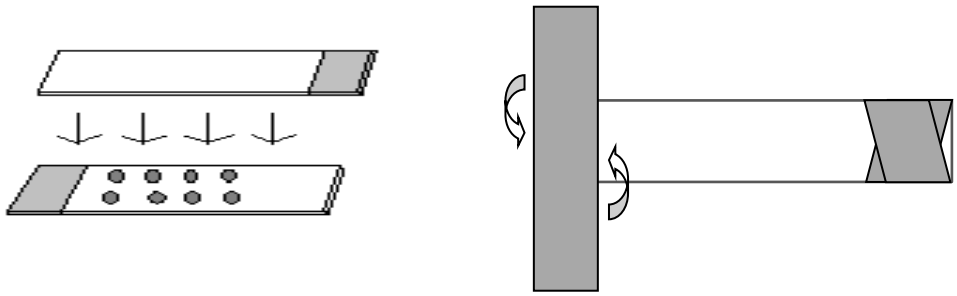

Figure 5. Place empty slide on top of the slide with scales. The frosted ends should be on opposite sides so the sample number is visible on either side. Secure slides together with tape over the frosted ends.

Imaging and Electronic Archival

Digital images are archived on a shared network drive for easy access, allowing multiple readers to view images simultaneously. Imaging and ageing are performed separately to maximize efficiency in each activity. Scales are imaged on a computer monitor using a Leica® DM4000B compound microscope and a Leica® DC500 digital camera. The NRAAL currently uses the software program Image Pro® Express to capture and archive scale images.

NRAAL personnel examine scales for cleanliness, regeneration, resorption/erosion, symmetry (proper scale collection location), and other damage (tearing, scuffs, etc.) when imaging and electronically archiving samples. Based on these qualities, the three best scales from each sample are chosen for imaging. Whole adult scales are imaged using a 12.5x objective, while adult freshwater regions and juveniles are imaged using a 40x objective. Overall, six images are taken for adults (three whole scale images and three close up freshwater images) and three images are taken for juveniles. Images are saved in JPEG format. The NRAAL's naming convention includes the BioSample ID followed by an "a", "b", "c" (scale 1, 2, 3; e.g. 14-10001a). For adults only, freshwater images are distinguished with a "_fw" suffix (e.g. 14-10001a_fw). These procedures are for standard monitoring needs and may be modified for specific investigations.

Ageing Procedures

From 2007 to 2014, the NRAAL received 127,687 scale samples, of which 90,505 have been aged (Table 2). Samples were collected from numerous tributaries throughout Idaho and at LGR in Washington. The NRAAL currently provides age estimates for management and research purposes and for a number of projects including ISMES, INPMEP, Potlatch River Steelhead Monitoring and Evaluation Project, Integrated Status and Effectiveness Monitoring Program (ISEMP), and several other studies throughout the Snake River basin. Age data are stored in the IDFG BSDB and have been reported in numerous documents. Examples of current

reported data include age frequencies (Table 3), age compositions (Figure 6), and mean length at age (Table 4). See Schrader *et al.* 2011 and 2012, Copeland *et al.* 2011 and 2012, Kennedy *et al.* 2012, and Bowersox *et al.* 2011 for more results.

Scale Patterns

Age determination is largely a practice of pattern recognition (Appendix B-3) and has an inherent element of subjectivity (Campana 2001). As scales increase in size, correlating to fish growth, concentric rings known as circuli form around the margin of the scale. Patterns observed in salmonid scales are most prominent in the anterior portion (Appendix B-3), as circuli in the posterior region are worn from exposure. Salmonids are subject to a multitude of factors affecting their growth and consequently affecting patterns on scales. Circuli are widely spaced during periods of fast growth, and narrowly spaced when growth slows (Fisher and Pearcy 1990, 2005). An annulus, the mark signifying one year of growth, is defined as a dark band of narrowly spaced circuli typically formed during slow winter growth. Also, annuli often exhibit cutting over (the abrupt ending of one circuli when it crosses over or is compressed into another circuli) as a result of slow growth. Spacing between annuli will depend on location. Annuli must appear continuously from both lateral edges through the anterior of the scale. Age is assigned by counting annuli that appear on scales.

Table 2. Species and life stage of scale samples received and aged by the NRAAL between 2007 and 2013.

Year	Species	Life Stage	Samples Received	Samples Assigned Age
2013	Chinook	Adult	3,156	2,593
		Juvenile	207	205
	Steelhead	Adult	4,877	3,113
		Juvenile	7,100	6,692
2012	Chinook	Adult	3,634	2,566
		Juvenile	200	200
	Steelhead	Adult	5,401	4,042
		Juvenile	4,362	4,362
2011	Chinook	Adult	6,864	2,963
		Juvenile	3,935	2,141
	Steelhead	Adult	7,060	3,857
		Juvenile	14,297	10,021
2010	Chinook	Adult	2,050	1,889
		Juvenile	1,660	1,660
	Steelhead	Adult	6,770	4,082
		Juvenile	11,872	10,876
2009	Chinook	Adult	6,839	2,175
		Juvenile	2,085	2,085
	Steelhead	Adult	5,447	2,696
		Juvenile	7,885	7,357
2008	Chinook	Adult	3,913	1,892
		Juvenile	1	-
	Steelhead	Adult	3,075	1,470
		Juvenile	3,993	3,831
2007	Chinook	Adult	5,283	3,100
		Juvenile	2	-
	Steelhead	Adult	603	136
		Juvenile	5,116	4,501
Grand Total			**127,687**	**90,505**

Table 3. Number of fish by age of adult steelhead sampled at weirs during spring 2011 (Copeland *et al*. 2012). Age values before the period denote freshwater ages and values after the period denote saltwater ages. A '?' signifies that a freshwater age was not assigned and 'S' signifies a spawn check.

Location	Population	N	1.1	1.2	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	3.2S1	?.1	?.2	?.3
Fish Creek	Lochsa	216	1	1	1	68	1	-	94	3	-	4	-	1	41	1
Rapid River	Little Salmon	132	1	2	15	45	1	12	31	-	1	1	-	5	18	-
Big Creek	Lower MF Salmon	94	-	-	2	22	-	10	44	-	1	6	1	1	7	-
Pahsimeroi River	Pahsimeroi	189	12	53	55	39	-	3	2	-	-	1	-	9	15	-
EF Salmon River	East Fork Salmon	35	-	-	5	24	-	3	-	-	-	-	-	1	1	1
Salmon River (Sawtooth)	Upper Salmon	96	2	3	31	44	-	3	5	-	-	-	-	1	7	-

Figure 6. Estimated escapement (42,773) by brood year of wild adult steelhead at Lower Granite Dam, spawn year 2010 (Schrader *et al*. 2012). Confidence intervals are at 95%.

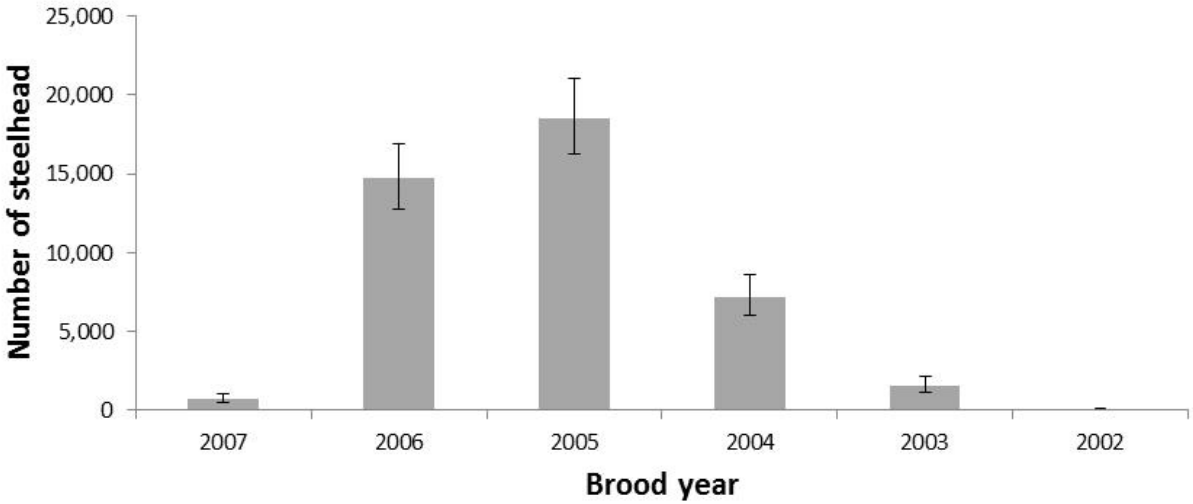

Table 4. Mean fork length at age of juvenile steelhead captured at screw traps in locations within the Clearwater and Salmon drainages during fall 2011 (August 15–November 31; Copeland *et al*. 2012). Number of fish aged is in parentheses.

		Mean fork length at age (mm)					
Location	**Population**	**Age-0**	**Age-1**	**Age-2**	**Age-3**	**Age-4**	**Age-5**
Fish Creek	Lochsa	-	120 (28)	155 (67)	184 (1)	-	-
Rapid River	Little Salmon	-	156 (18)	178 (30)	189 (12)	202 (1)	-
SF Salmon at Knox Bridge	SF Salmon	87 (1)	118 (58)	173 (48)	187 (1)	-	-
Johnson Creek	SF Salmon	65 (11)	112 (71)	170 (93)	189 (6)	245 (1)	-
Lake Creek	Secesh	-	-	157 (10)	187 (2)	238 (3)	-
Upper Secesh	Secesh	-	126 (5)	160 (23)	190 (7)	202 (6)	273 (2)
Lower Secesh	Secesh	70 (3)	104 (16)	159 (97)	184 (85)	192 (6)	-
Big Creek	Lower MF Salmon	-	106 (18)	172 (49)	197 (18)	228 (3)	-
Upper Lemhi	Lemhi	98 (2)	182 (191)	193 (4)	-	-	-
Lower Lemhi	Lemhi	109 (5)	187 (144)	204 (39)	210 (1)	198 (1)	-
Hayden Creek	Lemhi	70 (100)	135 (50)	190 (49)	225 (1)	-	-
Pahsimeroi River	Pahsimeroi	80 (1)	143 (6)	-	144 (2)	-	-
Salmon River at Sawtooth	Upper Salmon	88 (10)	151 (62)	172 (2)	-	-	-

Juvenile scales are collected continuously during the field season, which can create difficulties for assigning ages as patterns change with fish growth. Familiarization with common freshwater patterns is advantageous when ageing and is encouraged if sampling occurs in numerous tributaries that exhibit varying environmental conditions. Seasonal variability and diverse stream conditions cause wide variations in freshwater growth increments throughout Idaho. Fish in the northern hemisphere are given a birthdate of January 1st (Quist *et al*. 2012, Bagenal and Tesch 1978). If an annulus is not obvious on the scale margin of samples collected after January 1st and there is substantial growth past the last visible annulus (known as plus growth), this growth is considered evidence that an annulus will manifest in spring and is therefore counted as an annulus (Appendix B-4a). However, plus growth seen late in the summer and fall is not counted because an annulus will not form until late fall or spring (Appendix B-4b). If an annulus is seen on the edge of a fall collected scale it is not counted (Appendix B-4c). In general, Idaho screw trap collected juvenile Chinook ages (freshwater) are largely age-1, but they can range from young-of-year (zero) to two years and juvenile steelhead ages range from young-of-year to six years.

Adult salmon scales are more complex than juvenile scales because they exhibit two distinct regions of growth, a freshwater/river region and a saltwater/marine region (Quinn 2005, Shearer 1992). A transition from freshwater to saltwater growth is seen at ocean entry. Often smolts exhibit faster growth as they travel through main-stem rivers and the estuary. The physiological change required of the fish once it enters the saltwater environment produces a noticeable mark called the ocean entrance followed by increased growth as fish acclimate to the new marine environment. Saltwater growth results in wider circuli spacing and thicker circuli, but in principle their annuli are similar to freshwater annuli in that they exhibit narrower spaced circuli and cutting over (forking) of circuli.

The NRAAL uses the European system to designate final ages: two numerals separated by a decimal. The first numeral (left of decimal) is the freshwater age; the second numeral (right of decimal) is the saltwater age. For example, a Chinook aged as a 1 freshwater and 3 saltwater, will have a final age of 1.3. Variations to the numeral system are minijacks and repeat spawners. To designate Chinook minijacks from juvenile samples, the abbreviation MJ is used for saltwater age instead of zero (0) (e.g. 1.MJ). For repeat spawning steelhead, an 'S' designates a winter spent in freshwater while on a spawning run. The 'S' is positioned on the right side of the decimal (saltwater) and is in chronological order in which it occurred with saltwater annuli. For example, a fish assigned a final age of 2.1S1 spent two (2) years in freshwater, one (1) year in saltwater, returned the next winter for a spawning run (S), then spent one (1) more year in saltwater after spawning. If a fish has spawned two times, there will be two 'S' symbols signifying those years. Each 'S' signifies one year of life. If we are unable to assign an age for freshwater, a question mark (?) is used as a place holder (e.g. ?.2). If a sample is deemed entirely unreadable or contaminated (sample contains scales from more than one fish), it is assigned an age of N.A. (Not Ageable). Total age at spawning is the sum of freshwater and saltwater ages, plus 1. For Chinook the addition of an extra year is for the winter spent in the gravel before hatching; for steelhead it is the time spent overwintering in freshwater before spawning. The NRAAL determination of Chinook and steelhead total ages as related to their life cycle can be expressed graphically (Figures 7 and 8). Total age can be viewed as how many winters an individual has lived in freshwater and saltwater combined.

In Columbia basin Chinook populations, age at smoltification can range from zero to two years and saltwater ages can range from one to six years (Myers *et al.* 1998). The majority of Snake River spring-summer Chinook smolt as yearlings (age-1) and those exhibiting zero (age-0) and two (age-2) freshwater annuli are rare. Freshwater ages discussed here represent distinct annuli on scales and do not include the winter spent in gravel before emerging. The majority of spring-summer Chinook return as one-, two-, or three-saltwater fish. Four-saltwater and zero-saltwater (minijack) Chinook have been noted but are rare, and only one Chinook has ever been assigned as a five saltwater in the NRAAL (aged from a fin-ray sample, not reported). Snake River spring-summer Chinook total age at spawning typically range from three to five years but they can range from one to seven years. Chinook that exhibit rapid saltwater growth after entering the ocean but lack a saltwater annulus are known as minijacks (Johnson *et al.* 2012; Appendix B-5). Minijacks make long-distance migrations to the ocean or estuary, then return to spawn the same year as emigration.

Steelhead can spend up to seven years prior to smoltification, and spend up to 3 years in saltwater prior to spawning (Good et al. 2005). In Snake River steelhead populations the NRAAL have assigned age at smoltification from one to six years, saltwater ages from one to three years, and a small percentage have been assigned as repeat spawners. Total age generally ranges from three to eight years. There is potential for older individuals but they are rare. Freshwater variability results from diverse stream conditions and seasonal variability. Steelhead can exhibit iteroparity (repeat spawning), which results in a scar mark on their scales (Appendix B-6). These scars, known as a spawn checks, exhibit surface erosion and generally have an uneven shape resulting from erosion on the scale margin. Spawn checks are caused by resorption of circuli that occurs during their return to freshwater for spawning (Davis and Light 1985). After resorption occurs and the fish returns to saltwater, scale growth resumes, forming a spawn check (White and Medcof 1968). Currently the NRAAL age determinations suggest that most repeat spawners in the Snake River basin show one of two patterns; 1) consecutive spawners, who spawn two years in a row (Appendix B-6a), and 2) skip (alternate) spawners, who spend one year in saltwater between spawning years (Appendix B-6b).

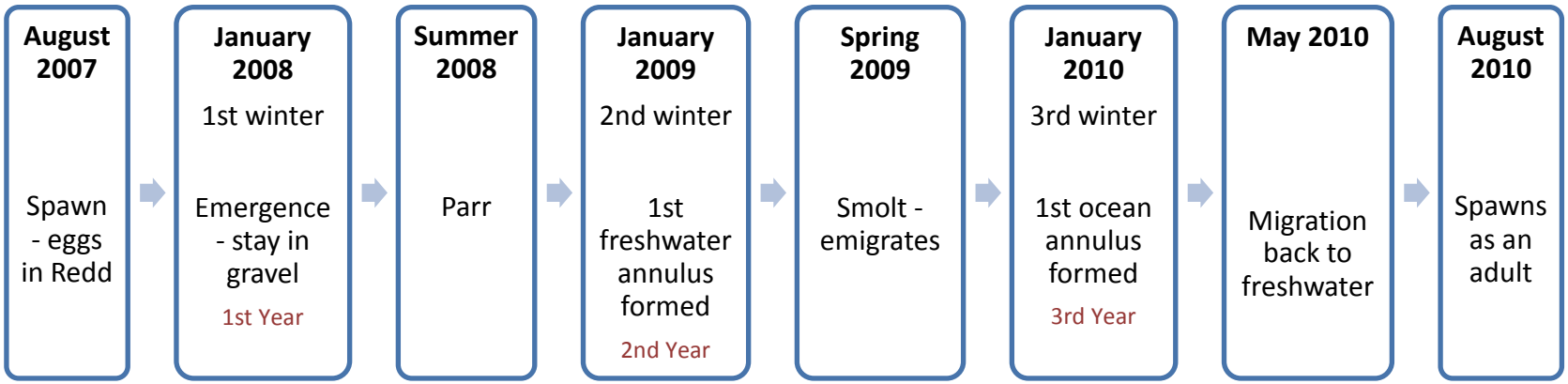

Figure 7. Example of how to determine total age, egg to spawn for an adult Chinook salmon. The individual in this example is aged as a 1.1, and total age is 3.

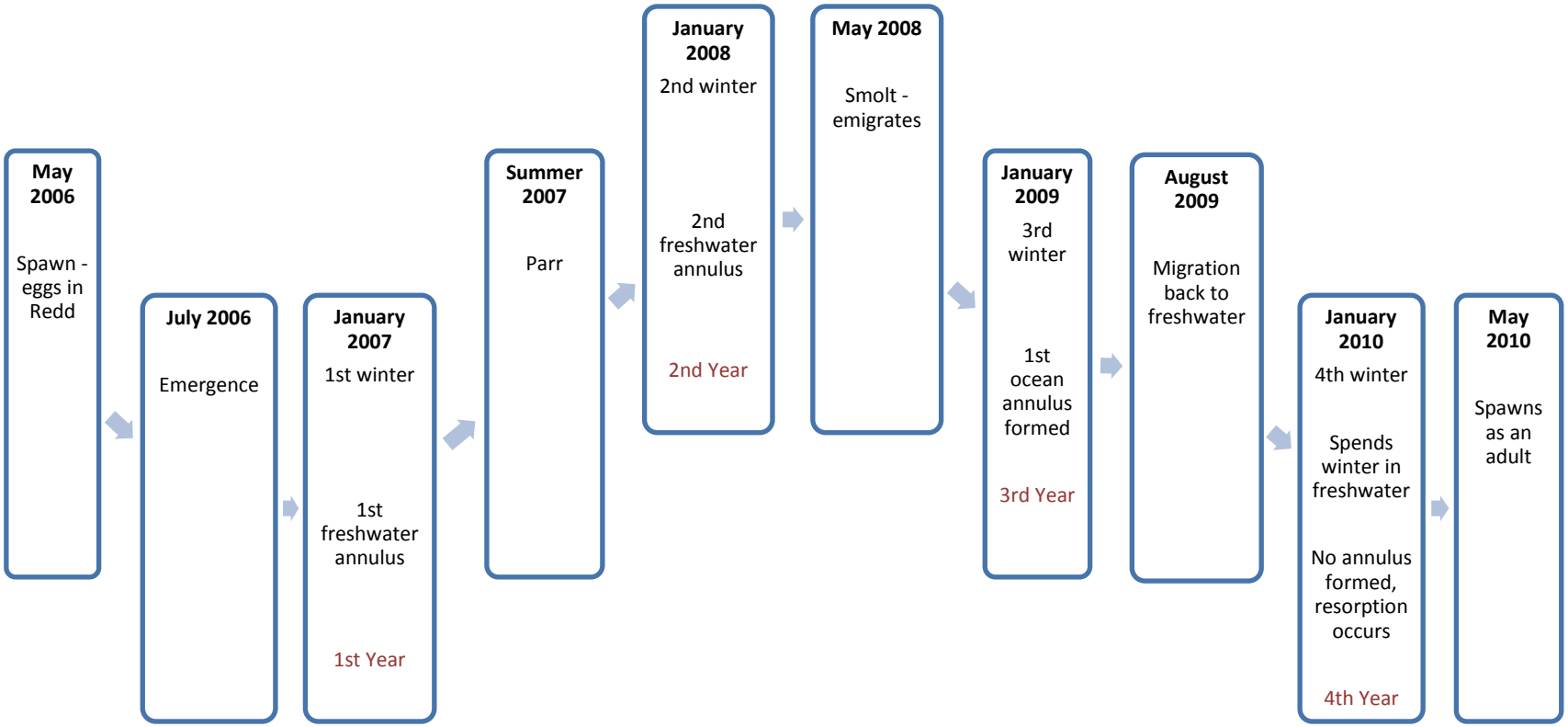

Figure 8. Example of how to determine total age, egg to spawn, for an adult steelhead. The individual in the example is aged as a 2.1, and total age is 4.

There are several factors that present potential issues when reading scales. Regeneration occurs when a lost scale is replaced by a new scale which lacks growth and age information prior to scale loss (Appendix B-1); thus, regeneration can lead readers to underestimate age. Formation of the first annulus, or lack thereof, can also cause ages to be underestimated (Lentsch and Griffith 1987). Furthermore, resorption on the scale margin can cause annuli to be obscured or lost (Hernandez *et al*. 2014; Appendix B-2), causing an underestimated age. Identification of freshwater annuli, interpretation of freshwater plus growth, and ocean entrance in adults can be difficult to interpret due to differing transition zones (Appendix B-7). This can lead to either overestimation or underestimation of age. False annuli (check) identification can be an issue in all life stages and species (Appendix B-8). Checks are usually thinner than annuli and many times do not maintain continuity between lateral axes. Because checks are mistaken for true annuli, they can result in an overestimation of age. For example, hatchery fish can exhibit numerous checks in their freshwater region (Appendix B-9) due to unnatural growth patterns caused by disease, high rearing densities, changes in environment (e.g. stocking events), and other hatchery conditions. Therefore, the occurrence of hatchery fish can cause confusion in freshwater age determination and an overestimation of age.

Assigning Ages

When assigning ages, readers independently view scale images on a computer monitor and record their observations using electronic worksheets. Worksheets contain information pertinent to age determination, including location, sample ID, and collection date. Length is intentionally omitted to control bias, as length and age tend to correlate. For quality assurance, each sample is assigned to two independent readers. Readers assign freshwater and saltwater ages in separate columns. When ageing juveniles, only freshwater age is determined and a zero is entered for saltwater. Samples can be deemed unreadable and removed from further analysis if an age cannot be determined due to regeneration, poor scale quality, or if readers cannot agree. If a freshwater age has been discarded, readers can still assign a saltwater age. Readers also assign a confidence rank to their age and make comments pertaining to their age interpretation. Confidence ranks allow us to determine the conviction in readers' assigned ages and comments allow *post hoc* insight why readers assigned the age they did.

Confidence rankings are as follows:

Rank 1 – High: Highly confident in assigned age, and highly confident in ability to reproduce age.

Rank 2 – Moderate: Moderately confident in assigned age, and good chance of repeating age. Sample may have a quality issue that decreases confidence, such as faint annuli, split annuli, or slight regeneration.

Rank 3 – Low: Not confident in assigned age, fairly high level of uncertainty in ability to reproduce age. Sample may have a quality issue that greatly decreases confidence such as heavy checks or unclear/diffuse annuli. If a sample is assigned a rank of 3 that sample will be re-examined by readers in the referee session (see Accuracy and Precision) even if reader ages agree.

Rank 4 – Unreadable – Unable to provide an age due to condition issue. Both freshwater and saltwater sections must be affected. Clarify with a comment.

Once initial ages are assigned, ages from each reader are compared and discrepancies between readers are resolved (see Accuracy and Precision section below). Reader ages, ranks, comments, and the consensus final age are entered into BSDB.

A quality control check of final ages is completed by examining length and age data in a length age comparison. Outliers are re-examined using the methods described above; length information is still omitted from the process. Ages are corrected if readers agree on an age that differs from the original assigned age. Major outliers may be discarded *post hoc*.

Age Accuracy and Precision

The use of validation and verification in ageing studies is a crucial component of data integrity (Quist *et al*. 2012). Validation refers to the accuracy of ages assigned, whereas verification refers to precision (Beamish and McFarlane 1983). The NRAAL uses known-age fish sampled through mark-recapture to validate saltwater age determination; freshwater ages are currently not validated. Verification is achieved using multiple readers and training methods, including tests for new readers. The NRAAL has recently started assessing precision for all locations *post hoc* using coefficient of variation and percent agreement.

To improve accuracy, laboratory personnel complete age training and testing before they officially start contributing to the ageing process. Training and testing enhances the accuracy and precision of age readers. New and returning personnel train using PowerPoint® presentations as well as examining collections from past years. Tests are used for both juvenile and adult Chinook and steelhead ageing. Bias is negligible for readers scoring 90% or better on tests for accuracy (Buckmeier 2002); therefore, readers must pass tests with at least a 90% before they start contributing to the ageing process. Adult tests were produced using known saltwater age fish. Juvenile tests were produced by experienced readers to enhance consistency; however, a set of known freshwater age fish is currently unavailable.

An unbiased age is the preferred goal for any age validation study (Campana 2001). Saltwater age validation is achieved using recaptured fish that were marked as juveniles with passive integrated transponder (PIT) tags or coded wire tags (CWT), or have been genotyped using parentage based tagging (PBT). PIT tag information is available on the PTAGIS Information System website http://www.ptagis.org/. CWT information is available on the RMIS website http://www.rmpc.org/. PBT information is available through the IDFG Eagle Fish Genetics Laboratory in Eagle, Idaho. To obtain a known saltwater age for PIT-tagged fish, there must be evidence of out-migration (juvenile detections at dams) and return migration (adult ladder detections at Columbia basin dams). Repeat spawners must have adult detections in multiple years (skip spawner) or several months later (consecutive spawner). CWT and PBT are used with hatchery fish and therefore are currently less useful than PIT tags for wild age validation. CWT information is available from the CWT technician at Nampa Research and PBT results are available from the IDFG Eagle Fish Genetics Laboratory. The NRAAL's current sample size goal for validation is 100 known-age fish per collection year with a minimum of 30.

Familiarizing readers with growth patterns can be an important step in successful ageing. Readers are required to familiarize themselves with current patterns for LGR adult collections by examining known-age samples collected that year. This process is part of our training each year to give laboratory personnel the opportunity to discuss new prevalent markings and changing saltwater patterns. Readers complete known-age sample examinations and discussions before age determination begins. Known-saltwater age samples from LGR are also a useful tool for ageing adult weir samples, as all patterns should be represented in the

LGR sample. To determine our yearly saltwater accuracy rate, known-age fish are returned to the sample at large and aged blindly, like all other samples. Examination of a location's previous year's samples can also be useful both for adults and juveniles. If possible, it is also beneficial to age fall collected juveniles prior to spring collections. This allows readers to examine the amount of growth and possible checks seen throughout the year.

For both species, the NRAAL's saltwater ageing accuracy has been greater than 96.00%, which surpasses the 90.00% bias threshold (Table 5) based on known-age fish, and we are working to expand our validation process to include freshwater ages. The NRAAL currently has several validation and verification projects underway to improve our assessment of juvenile and freshwater ageing accuracy and precision (Table 6). These projects are mainly focused on steelhead, as they exhibit a more complex life history than Chinook in Idaho. Two promising validation techniques incorporate genetic PBT information. PBT is a genetic method that can be used to assign hatchery fish back to their parents (Steele *et al*. 2013), and in turn back to their brood year. In its early stages of use, this method of total age validation may prove useful in aiding identification of hatchery fish via scale patterns. Alternate methods are still required to conduct freshwater age validation on fish of wild origin because hatchery fish exhibit considerably different freshwater growth patterns than wild fish. Currently the Shoshone Bannock Tribe has a pedigree study underway using PBT and their hatchery egg box program in the Yankee Fork River (Lytle Denny, personal communication), so we are collaborating with them to obtain scale age validation. The use of PBT with egg box hatchery fish can provide both freshwater and total age validation (Seamons and Dauer 2009). Egg box hatchery fish should exhibit similar growth patterns with wild reared fish and thus could be used as a metric for validating wild fish total age. Sampling egg box planted fish at screw traps can provide samples for freshwater (juvenile) age validation, while fish sampled at weirs or LGR can provide total age validation. Another encouraging research project the NRAAL is working on with the IDFG Eagle Fish Genetics Laboratory uses PBT in wild populations. By genotyping wild adult fish collected at weirs we can assign their offspring as juvenile out-migrants, or as adults returning, to their parents (Rawding *et al.* 2014). This also can provide freshwater and total age validation.

To assess precision, all samples are read double-blind, i.e. independently by two readers. If more than two readers are ageing scales from a given location, samples are divided evenly among and between readers. Therefore every reader will age the same number of samples and will be equally paired with every other reader. Age discrepancies between readers are resolved with a referee session. All readers accountable for the subset of samples aged in a session collectively resolve their differences in assigned ages and a final age is determined for each sample in contention. If a consensus is not attained in the referee session, the sample is deemed unreadable and removed from further analysis. Independently assigned ages are not known in the referee sessions; however, reader comments are used. Final ages are recorded and noted as the "referee" age.

Precision metrics calculated *post hoc* are coefficient of variation (COV) and percent agreement (PA). Precision metrics are used to assess the ease or difficulty of determining ages for a specific collection of samples and for comparison of uniformity among readers. The NRAAL's precision goals are ≤0.10 for COV and ≥80% for PA. The coefficient of variation is shown to be more rigorous and more flexible than average percent error (Chang 1982, Campana et al. 1995); thus, it is used as the NRAAL's standard metric for verification. Its value, however, is proportional to the difference in age disagreement between readers over the total age of the sample. The value of the coefficient, for example based on a one-year difference between readers, will be much smaller for a fish with 30 years total age compared to a fish with 3 years total age. When internally comparing "agree" to "disagree," the NRAAL uses percent

agreement as its metric, a binary value with no weight adjustment that simply shows whether or not a discrepancy has occurred. Within-reader variability is an additional metric that the NRAAL would like to calculate in the future because it will help determine reader drift and bias.

The NRAAL's verification calculations of COV and PA have recently become a standard part of our laboratory processing methods. Among-reader COV is being reported annually for adults collected at LGR as a precision metric (Table 5) and will be reported for other locations in the future. LGR Chinook freshwater and saltwater estimates in 2012 and 2013, as well as steelhead saltwater estimates in 2012 and 2013, met our goals of COV ≤0.10 and PA ≥80%. However, steelhead freshwater age estimates did not always meet our goals, producing COV = 0.124 in 2013; PA was 74.17% and 68.28% in 2012 and 2013, respectively. Adult steelhead collected at weirs in 2012 and 2013 proved even more difficult to age and therefore many precision estimates did not meet our goals (Table 7). The COV freshwater estimates met our goal of ≤0.10 in only half of all cases during 2012 and 2013. Saltwater COV estimates were more acceptable, meeting our goal in all but two cases. The PA saltwater estimates met our goal two-thirds of the time over all cases. The PA freshwater estimates did not meet our goal of ≥80% except for one case, the EF Salmon, in 2013. Juvenile steelhead collected at screw traps in 2012 and 2013 almost always met our COV goals but many did not meet our PA goals (Table 8). Lolo Creek and Pahsimeroi River are the only locations that did not meet our goal of COV ≤0.10. However, Crooked River, LGR, Marsh Creek, Rapid River, and Red River did not meet our goal of PA ≥80% between readers in one or more years. The freshwater environment produces more variability than the saltwater environment; therefore, we expect that freshwater ages will always be more variable. We hope to meet our verification measurements standards with improved reference collections and the creation of ageing summaries for each location so readers can examine and calibrate themselves on a yearly basis prior to ageing. This is especially important for new readers, as we have seen reductions in our CV and PA estimates when new readers begin ageing (i.e. 2013 results). Other verification projects (Table 6) include a longitudinal study of the formation of a freshwater annulus and evaluating the presence or absence of a first annulus based on circuli counts and temperature data. Additionally, while not being actively pursued by the NRAAL in lieu of other projects, scales from wild resident rainbow trout sampled longitudinally could provide insight into the formation of freshwater annuli.

Table 5. Precision and saltwater accuracy estimates for adult steelhead and Chinook collected at Lower Granite Dam in 2012 and 2013. The NRAAL coefficient of variation goal is ≤0.10 and the percent agreement goal is ≥80%.

Species	Sample Count		Saltwater Accuracy		Coefficient of Variation				Percent Agreement			
					FW Age		SW Age		FW Age		SW Age	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Steelhead	2,264	2,563	97.56%	96.47%	0.087	0.124	0.033	0.080	74.17%	68.28%	90.78%	83.14%
Chinook	2,344	2,227	98.78%	97.89%	0.020	0.031	0.015	0.039	97.27%	95.21%	93.49%	90.59%

Table 6. Summary of research topics the NRAAL is currently working on. The purpose of each project is based on whether the project is important for annual monitoring or whether it is a special topic. Annual monitoring is considered important for our overall age estimates provided for species management and age composition estimates.

Topic	Purpose	Description
Freshwater Validation	Annual Monitoring	Assign parentage of wild juvenile steelhead sampled at screw traps to adults sampled at efficient weirs using Parental Based Tagging (PBT) genetic information
Freshwater Validation	Annual Monitoring	“Wild” surrogate fish via PBT egg box release hatchery fish
Total Age Validation	Annual Monitoring	Hatchery adult steelhead or Chinook collected at Lower Granite Dam that assigned to PBT and were reared (released) in egg boxes
Spawn Check Validation	Annual Monitoring	The use of known repeat spawning steelhead to validate spawn check identification and describe spawn check characteristics
Freshwater Verification	Annual Monitoring	Partial validation of freshwater annuli in longitudinal samples of juvenile steelhead scales
Freshwater Verification	Annual Monitoring	Validation of the first growth increment for Snake River Basin steelhead
Historic Dworshak Hatchery Samples	Special Topic	Examine historic scale samples collected from the original broodstock of Dworshak hatchery.
Resorption Measurements (Completed)	Special Topic	Quantitative assessment of scale resorption in migrating and spawning Steelhead of the Snake River basin (Published Manuscript Hernandez *et al.* 2014)
Chinook life histories (Completed)	Special Topic	Defining Life Histories of Precocious Male Parr, Minijack, and Jack Chinook Salmon Using Scale Patterns (Published Manuscript Johnson *et al.* 2012)

Table 7. Precision estimates for adult steelhead collected at weirs in 2012 and 2013. The NRAAL coefficient of variation goal is ≤0.10 and the percent agreement goal is ≥80%. 2012 Big Creek results were not included due to low sample size.

Species	Sample Count		Coefficient of Variation				% Agreement			
			FW Age		SW Age		FW Age		SW Age	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Big Creek	3	25	-	0.124	-	0.098	-	72.00%	-	80.00%
Crooked River	41	14	0.150	0.077	0.069	0.024	60.98%	75.61%	71.43%	92.86%
EF Salmon	80	30	0.063	0.035	0.037	0.113	78.75%	86.67%	90.00%	73.33%
Fish Creek	136	91	0.090	0.114	0.023	0.051	72.06%	65.93%	92.65%	87.91%
Pahsimeroi River	284	177	0.099	0.111	0.031	0.044	78.17%	75.14%	92.96%	89.83%
Rapid River	82	27	0.104	0.050	0.045	0.185	68.29%	77.78%	89.02%	59.26%
Sawtooth River	61	39	0.116	0.082	0.066	0.100	73.77%	79.49%	83.61%	74.36%
Averages:			10.36%	8.48%	4.72%	8.79%	72.00%	76.09%	86.61%	79.65%

Table 8. Precision estimates for juvenile steelhead collected at screw traps in 2012 and 2013. The NRAAL coefficient of variation goal is ≤0.10 and the percent agreement goal is ≥80%.

Location	Sample Count		Coefficient of Variation		Percent Agreement	
	2012	**2013**	**2012**	**2013**	**2012**	**2013**
American River	162	77	0.022	0.027	93.21%	90.91%
Big Creek	352	469	0.028	0.036	89.20%	87.63%
Crooked River	537	308	0.025	0.096	89.94%	72.40%
Fish Creek	420	384	0.021	0.047	93.10%	85.94%
Lower Granite Dam	1,274	1,807	0.052	0.078	82.42%	75.82%
Lolo Creek	224	250	0.103	0.138	84.82%	67.60%
Marsh Creek	209	245	0.053	0.069	84.69%	80.00%
Pahsimeroi River	185	227	0.159	0.266	77.84%	66.08%
Rapid River	185	313	0.090	0.071	70.81%	75.72%
Red River	79	203	0.083	0.072	70.89%	81.77%
Salmon River (Sawtooth)	188	189	0.010	0.071	95.74%	85.19%
Averages:			0.059	0.088	84.79%	79.01%

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APPENDICES

Appendix A. Glossary and BioSamples Database Metadata

Glossary

Age accuracy – Proximity of an age estimate to the actual age.

Annulus – (annuli, plural) Area of closely-spaced circuli associated with reduced winter growth. Opposite of area of widely-spaced circuli associated with summer growth. Winter and summer growth represent one year, indicating one year of life.

Band – Concentric region of a scale which is formed during a particular time of year.

Bias – The difference between an estimator's expectation and the true value of the parameter being estimated.

Brood Year – The year in which a group of fish were spawned.

Check – A reduction of circuli spacing or a disruption in a growth zone.

Circulus – (circuli, plural) Dark concentric circles or rings that radiate out from the scale focus.

Coded wire tags (CWT) – A small wire engraved with a code and inserted in the snout of fish, which, when retrieved, allows for the identification of the brood year and origin of the fish.

Focus – Scale formation point of origin, should form a tight circle.

Final Age – Consensus age assigned by multiple readers to a fish.

Freshwater/River Zone – Portion of the scale formed while the fish was rearing in freshwater before migrating to ocean.

Jack – Male Chinook salmon returning to spawn as a one-ocean fish. Total age is usually 3 for spring/summer Chinook and 2 for fall Chinook.

January 1st Birthdate – Used to determine total age for fish collected in the northern hemisphere. It is important to use in determination of whether to include the final annulus or the growth on edge of structure in age determination.

Known-age – The true age of a fish used to determine the validity of ageing methodology.

Margin – The edge of the ageing structure. Interpretation of growth and annuli on the margin can influence age determination.

Minijack – Chinook salmon that migrates to the estuary/ocean and as a mature male to spawn in freshwater within the same run year. They return to freshwater before forming an ocean annulus.

Ocean Entrance – Portion of scale following the freshwater zone. Occurs due to transition from freshwater to saltwater, and results in an increase in circuli spacing and thickness.

Percent Agreement – Computing of the percentage of times in which the age readers agree on age estimation.

Precision – Repeatability of an age estimate.

Plus Growth – Growth following the last annulus on a scale. The January 1st birthdate is very important in determining whether to include growth on edge of the scale in age determination.

Quality Control – A system for verifying and maintaining a desired level of data quality.

Regeneration – Area of a scale with missing circuli caused by the reforming of a previously lost scale. This might cause a scale to be useless in age determination; especially in the freshwater region.

Resorption/Erosion – Loss of some or all of the scale edge during migration and spawning. This is a condition issue and might cause a scale to be underaged due to loss of scale material.

Saltwater/Ocean Zone – Portion of scale formed while the fish was in saltwater.

Spawn Check/Spawn Mark/Scar Mark – Indicates return to freshwater and an attempt at spawning. Visible scar left on scale after resorption causes loss of scale material and then scale growth resumes.

Total Age – Age of an adult fish at spawning. Calculated by addition of freshwater age, saltwater age, and one year. The addition of one year is made to Chinook salmon to account for the winter spent as an egg before hatching. The addition of one year is made to steelhead to account for the winter spent in freshwater before spawning.

Validation – Process of determining accuracy of an ageing methodology.

Verification – Process of determining precision of an ageing methodology.

Year Class – Age class in which a group of fish belongs. Often referred to as a brood year in the case of anadromous salmonids.

BioSamples Database Metadata

ID – The unique key number generated for each database record.

Sample # – Unique BioSamples ID number assigned by ageing laboratory.

Formatting:	YY-XXXXX
	YY = Last two digit of collection year.
	XXXXX = Five digit number assigned to sample.

Date – Date the fish was collected and sampled, MM/DD/YYYY.

Year – Year the fish was collected and sampled.

Stream ID – Code number representing a “Stream and Parent” and “Stream Section” combination.

Stream and Parent – Stream and parent stream of the collection location.

Stream Section – Section of the stream in which the fish was collected.

Species – Species of fish collected. Code corresponds to species codes in PTAGIS database.

Species	PTAGIS Code
Unknown Origin	0
Chinook	1
Coho	2
Steelhead	3
Sockeye	4
Chum	5
Bull trout	7
No Info.	NI (not PTAGIS code)

Run – Run timing of fish collected. Code corresponds to run codes in PTAGIS database.

Run	PTAGIS Code	Notes, timing at Lower Granite Dam
Spring (Chinook)	1	Chinook 3/1 - 6/17
Summer Chinook & Steelhead	2	Chinook 6/18 to 8/17, All Snake River basin steelhead
Fall (Chinook)	3	Chinook 8/18 - 12/1
Winter	4	
Unknown	5	
No Info.	NI	
Resident	R	

Rear Type – Origin or rearing type of fish collected. Code corresponds to rear codes in PTAGIS database.

Rear Type	PTAGIS Code
Hatchery	H
Wild/Natural	W
Unknown Origin	U
No Info.	NI

Sex – Gender of fish collected. Code corresponds to rear codes in PTAGIS database.

Sex	PTAGIS Code	Notes
Female	F	
Male	M	
No Info.	NI	Used if no data was recorded.
Unknown	U	Used if collector attempted to determine sex the fish but it could not be determined.

Fork Length – Fork length in cm of fish collected.
Note: If lengths are originally recorded in mm, they are converted to cm before entering into the database.

Life Stage – Stage of life in which fish was collected. Recorded as either Juvenile or Adult.

Spawn Year – Determination of the year in which an adult fish will spawn from sample collection date.

Species	Corresponding Spawn Year
Steelhead (@ Lower Granite)	July 1 to June 30 of the following year.
Chinook (Spring/Summer @ Lower Granite)	March 1 to August 17
Sockeye	Calendar Year

Collecting Agency – Agency of personnel that collected/sampled the fish.

Agency	Code
Idaho Department of Fish & Game	IDFG
National Marine Fisheries Service	NMFS
National Oceanic and Atmospheric Administration	NOAA
Nez Perce Tribe	NPT
No information was recorded	NI
Not found in listed above	Other
Oregon Department of Fish & Wildlife	ODFW
Shoshone-Bannock Tribe	SHOBAN
United State Fish & Wildlife	USFW
United State Forest Service	USFS
Integrated Status & Effectiveness Monitoring Program	ISEMP
Quantitative Consultants Inc.	QCI

Collector Name – Name or initials of personnel who collected/sampled the fish. Entered as NI if “No Information” is provided.

Project – Name of the project that collected the fish. Includes project name, agency and region (if applicable).

Name	Code
Chinook Captive Rearing-Nampa	CCR-IDFG-Nampa
Integrated Status Effectiveness Monitoring Program-Lewiston	ISEMP-IDFG-Lewiston
Integrated Status Effectiveness Monitoring Program-McCall	ISEMP-IDFG-McCall
Integrated Status Effectiveness Monitoring Program-Nampa	ISEMP-IDFG-Nampa
Integrated Status Effectiveness Monitoring Program-Salmon	ISEMP-IDFG-Salmon
Integrated Status Effectiveness Monitoring Program-NOAA	ISEMP-NOAA
Integrated Status Effectiveness Monitoring Program-NPT	ISEMP-NPT
Integrated Status Effectiveness Monitoring Program-QCI	ISEMP-QCI
Integrated Status Effectiveness Monitoring Program-SBT	ISEMP-SBT
Integrated Status and Effectiveness Monitoring-FWS	ISEMP-USFWS
Idaho Steelhead Monitoring & Evaluation Studies-Nampa	ISMES-IDFG-Nampa
Idaho Salmon Supplementation-Lewiston	ISS-IDFG-Lewiston
Idaho Salmon Supplementation-McCall	ISS-IDFG-McCall
Idaho Salmon Supplementation-Nampa	ISS-IDFG-Nampa
Idaho Salmon Supplementation-Salmon	ISS-IDFG-Salmon
Idaho Salmon Supplementation-Nez Perce Tribe	ISS-NPT
Idaho Salmon Supplementation-Shoshone Bannock Tribe	ISS-SBT
Idaho Salmon Supplementation-USFWS	ISS-USFWS
Johnson Creek Artificial Propagation Enhancement	JCAPE-NPT
License dollars-IDFG-Lewiston	LIC-IDFG-Lewiston
License dollars-IDFG-McCall	LIC-IDFG-McCall
License dollars-IDFG-Nampa	LIC-IDFG-Nampa
License dollars-IDFG-Salmon	LIC-IDFG-Salmon
National Marine Fisheries Service	NMFS-NOAA
Natural Production Monitoring-IDFG-Lewiston	NPM-IDFG-Lewiston
Natural Production Monitoring-IDFG-McCall	NPM-IDFG-McCall
Natural Production Monitoring-IDFG-Nampa	NPM-IDFG-Nampa
Natural Production Monitoring-IDFG-Salmon	NPM-IDFG-Salmon
Rocky Mountain Research Station-Forest Service	RMRS-USFS

Known-Age – Known saltwater age based on PIT tag or coded wire tag information extracted from the PTAGIS database.

Known-Age	Code
1 Ocean	Jack
2 Ocean	2
3 Ocean	3
4 ocean	4
Tagged as Adult (recorded instead of leaving blank to distinguish we have looked it up)	Adult
Repeat Spawner	Repeat
No tag information or no juvenile information in PTAGIS to confirm known-age.	Orphan

Scale Final Age – Final age assigned to scales. In freshwater:saltwater format.
Note: A colon is used to separate freshwater and saltwater in the database, however; the European system of designating final ages uses a decimal. Ages retrieved from the database should be reported using the European system.

Total Age for adults (calculated brood year) = Freshwater Age + Saltwater Age + 1. See BioSamples Database for total list of final ages.

Capture Method – Method of capture used to collect the fish.

Capture	Code
Spawning Ground Survey	SURVEY
Hatchery Rack	HATCH
Sport Fishery Survey	CREEL
Adult Passage Ladder	LADDER
Screw Trap	SCREWT
Weir	WTRAP
Hook & Line	HOOK
Beach Seine	BSEINE
Dip Net	DIPNET
Electroshock	SHOCK
Box Trap	BTRAP
Scoop Trap	SCOTRP
Minnow Trap	MTRAP
Snorkel	SNORK
LGR Juvenile Facility	JFACILITY

Comments – Comments or concerns about the fish/sample, fish condition, and trap condition.

DNA Collected – Records whether a genetic tissue sample was collected.

Option	Code
Yes	-1
No	0

DNA Location – Location where genetic tissue sample is stored (if collected).

Location	Code
Unknown	Unknown
Eagle Health Lab - IDFG	EAGLE
Jay Hesse - NPT	HESSE
Nampa Research - IDFG	NAMPA
Nanaimo Lab	NANAIMO
National Marine Fisheries Service	NMFS
Nez Perce Tribe	NPT
Oregon Department of Fish & Wildlife	ODFW
Paul Moran	MORAN
Phaedra Budy	PHAEDRA
Russ Thurow - USFS	USFS
Shoshone - Bannock Tribe	SHOBAN
Steve Achord - NMPS	ACHORD
U.S. Fish & Wildlife Service	USFW
Washington Department of Fish & Wildlife	WDFW

Fin Collected – Records whether a fin ray sample was collected. See DNA Collected for options.

Fin Location – Location where fin ray sample is stored (if collected). See DNA Location for options.

Scale Collected - Records whether a scale sample was collected. See DNA Collected for options.

Scale Location – Location where scale sample is stored (if collected). See DNA Location for options.

Otolith Collection – Records whether a scale sample was collected. See DNA Collected for options.

Otolith Location – Location where otolith sample is stored (if collected). See DNA Location for options.

Original Sample – Trap record or reference number originally assigned to fish. Can be a BioSample ID if assigned prior to collection.

Carcass Condition – Condition assigned to Chinook carcasses and carcass tissues collected on spawning grounds.

Description	Code
Freshly dead, gills are still dark red or >50% dark red. Flesh is firm and colors externally are bright/dark (not faded).	Excellent
Dead for 2-5 days, gills still have some blood in them and are typically not yet white. External color is still good, but not as bright as one that just died. Flesh is still moderately firm.	Good
Dead for 6-7 days, gills are white, flesh is slightly firm, and there may be spots of fungus.	Fair
Dead for greater than 7 days. Flesh is very soft; it may be a bag of liquid, Cannot determine sex from internal organs because they are mush, fish elongates when you pick it up, highly odiferous, and might be mostly covered by fungus.	Poor
Fin is dried out because it has been exposed to the air.	Dry

Marks – Fin clips that are exhibited by fish collected.

Description	Code
No information regarding fin clips	NI
Adipose fin clip	AD
Adipose fin present on trapped fish (not ad-clipped)	UM
Opercule punch (right or left, record in comments)	OP
Right ventral fin clip	RV
Left ventral fin clip	LV
Right Pectoral fin clip	RP
Left Pectoral fin clip	LP

Marks Comments – Any comments associated with the marks. (Example: partial AD clip).

Tags – Tags that are seen or detected in the fish collected.

Tag	Code	Number
Passive Integrated Transponder Tag	PIT	Hexadecimal Format (Ex: 3D9.480E215631)
Radio Tag	Radio	Channel #, Code #
Coded Wire Tag	Wire	Snout Bag ID or Blank
Jaw Tag	Jaw	Number if applicable
Visible Implant Tag	VI	Number or color of tag
Floy Tag	Floy	Number on tag
Opercule Tag	OP	Number or color of tag

Tag Comments – Any comments associated with the tag. (Example: Tag hard to read).

Reference Numbers – Numbers to reference multiple samples taken from the same fish if their original sample numbers are not all identical.

Reference Type	Code	Number
Generalized Random Tesselation Stratified Number	GRTS	Collection location in GRTS format
Genetic Sample	GEN	Original genetic sample ID
Scale Packet	ScalePac	Original scale packet ID
Slide Box	Slide Box	Number of Slide Box where sample is stored

Valid Sample – Only used for Lower Granite Dam adult Chinook and steelhead.

Valid	Code	Description
Yes	-1	Sample is valid if it was chosen as part of our systematic subsample.
No	0	Sample is invalid only if the LGR trap sample is invalid due to Sort-by-code, length <300 mm fork length, or missing 5 critical data fields.
Null	0	All samples that were not chosen as part of the systematic subsample and are not invalid.

Latitude – Latitude of collection location of fish. Mainly recorded for fish collected during spawning ground surveys.

Longitude – Longitude of collection location of fish. Mainly recorded for fish collected during spawning ground surveys.

SGS Fish ID – Fish ID of corresponding record in the Spawning Ground Survey Database. Allows the transfer of age determined and recorded in BioSamples to the Spawning Ground Survey Database. Only recorded for fish collected on spawning ground surveys.

Reader Age – Age assigned by independent readers.

Reader Rank – Rank assigned by independent readers.

Reader Comments – Comments about age determination made by independent readers.

LLID – Latitude Longitude Identification Number. Pulls coordinates together and routes them to GIS stream layer (hydrography layer).

STHD MPG – Steelhead (ESU) Major Population Group name based on collection location.
https://collaboration.idfg.idaho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

CHN MPG – Chinook (ESU) Major Population Group name based on collection location.
https://collaboration.idfg.daho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

STHD Basin – Minor population within a steelhead major population group.
https://collaboration.idfg.idaho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

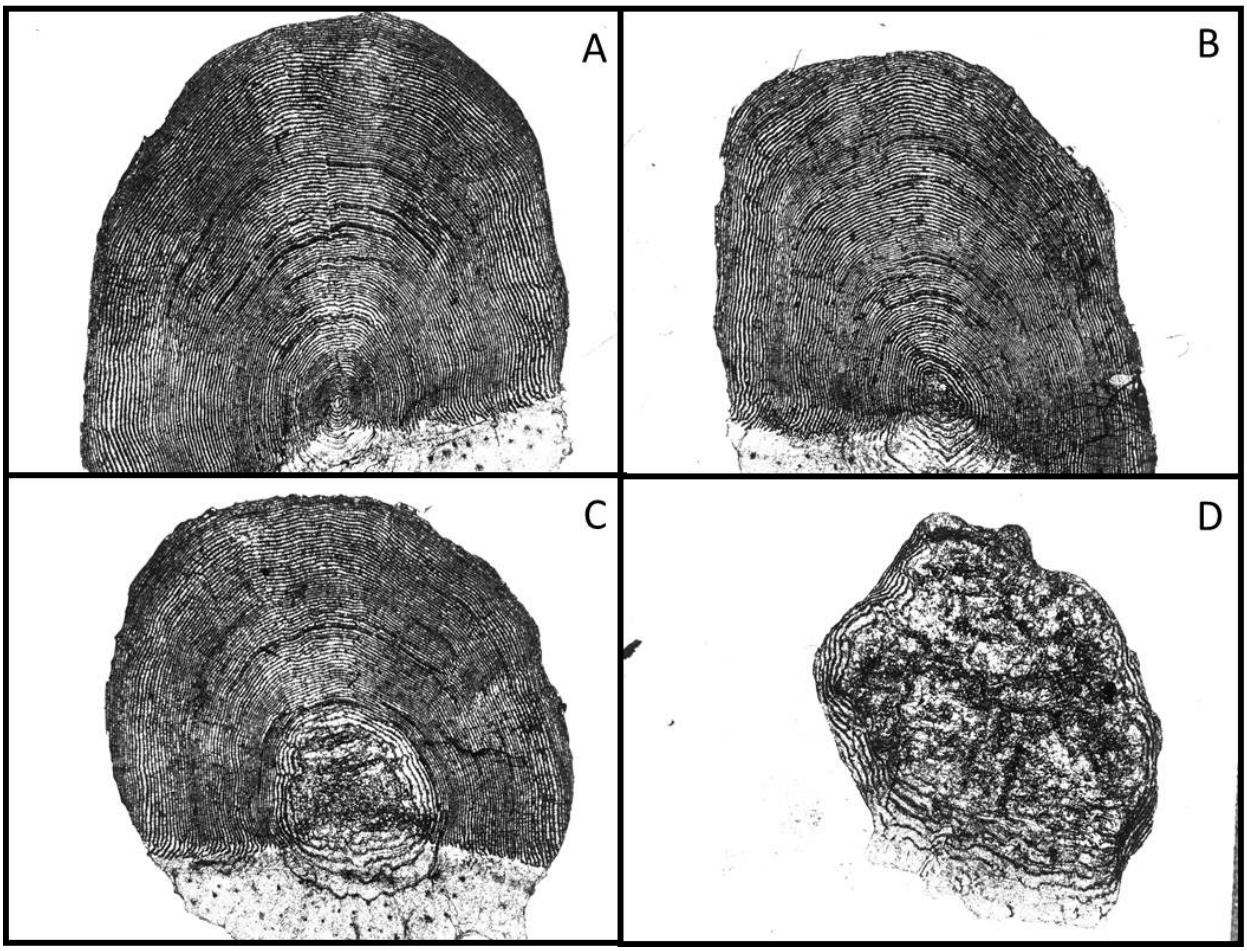
CHN Basin – Minor population within a Chinook major population group.
https://collaboration.idfg.idaho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

STHD Label – Code for the STHDBasin (minor population in MPG).
https://collaboration.idfg.idaho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

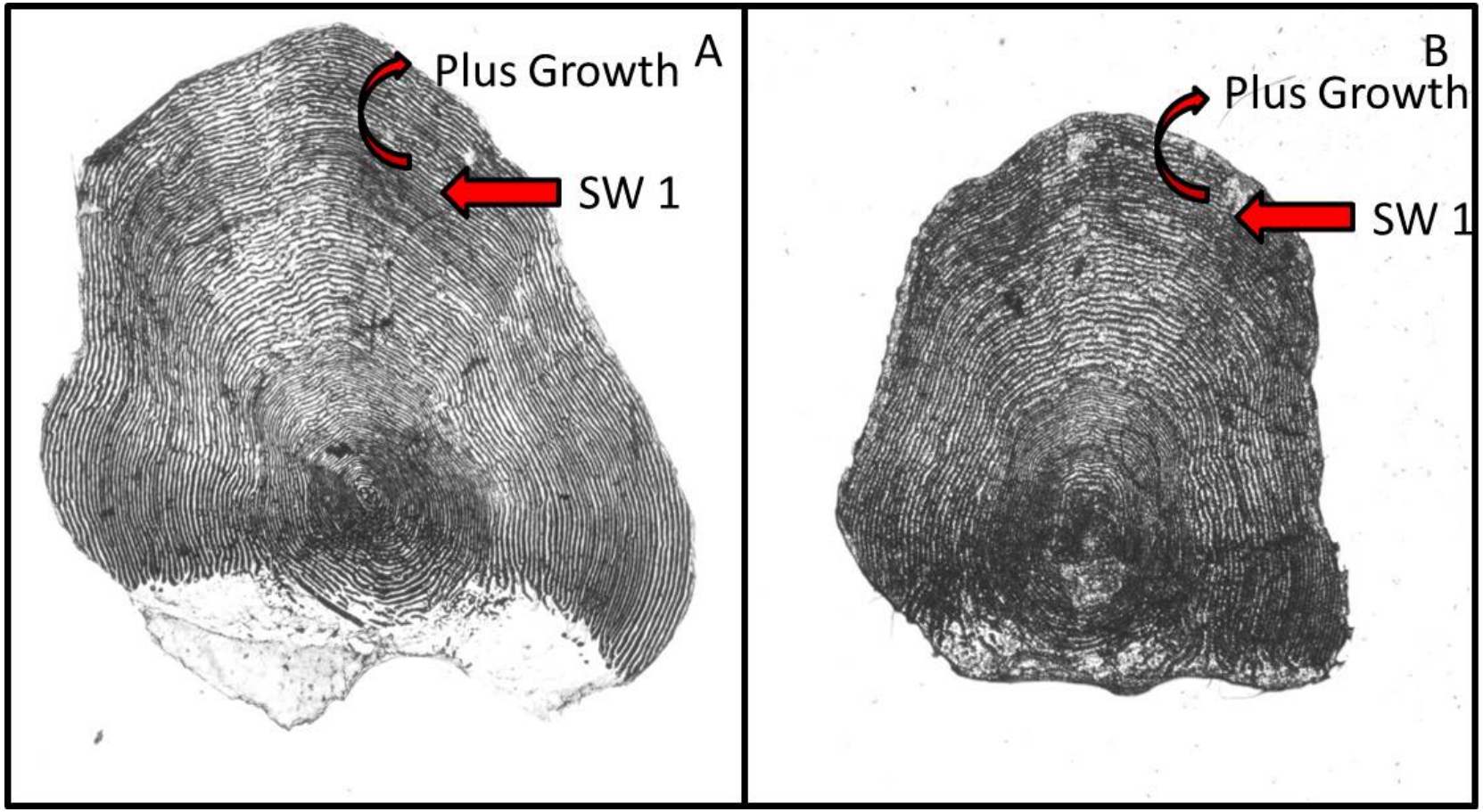
CHN Label – Code for the CHNBasin (minor population in MPG).
https://collaboration.idfg.idaho.gov/biosamples/Shared%20Documents/ESUMPGList.xlsx

HUC – Hydrologic Unit Code. HUC - 4 Code (Subregion).

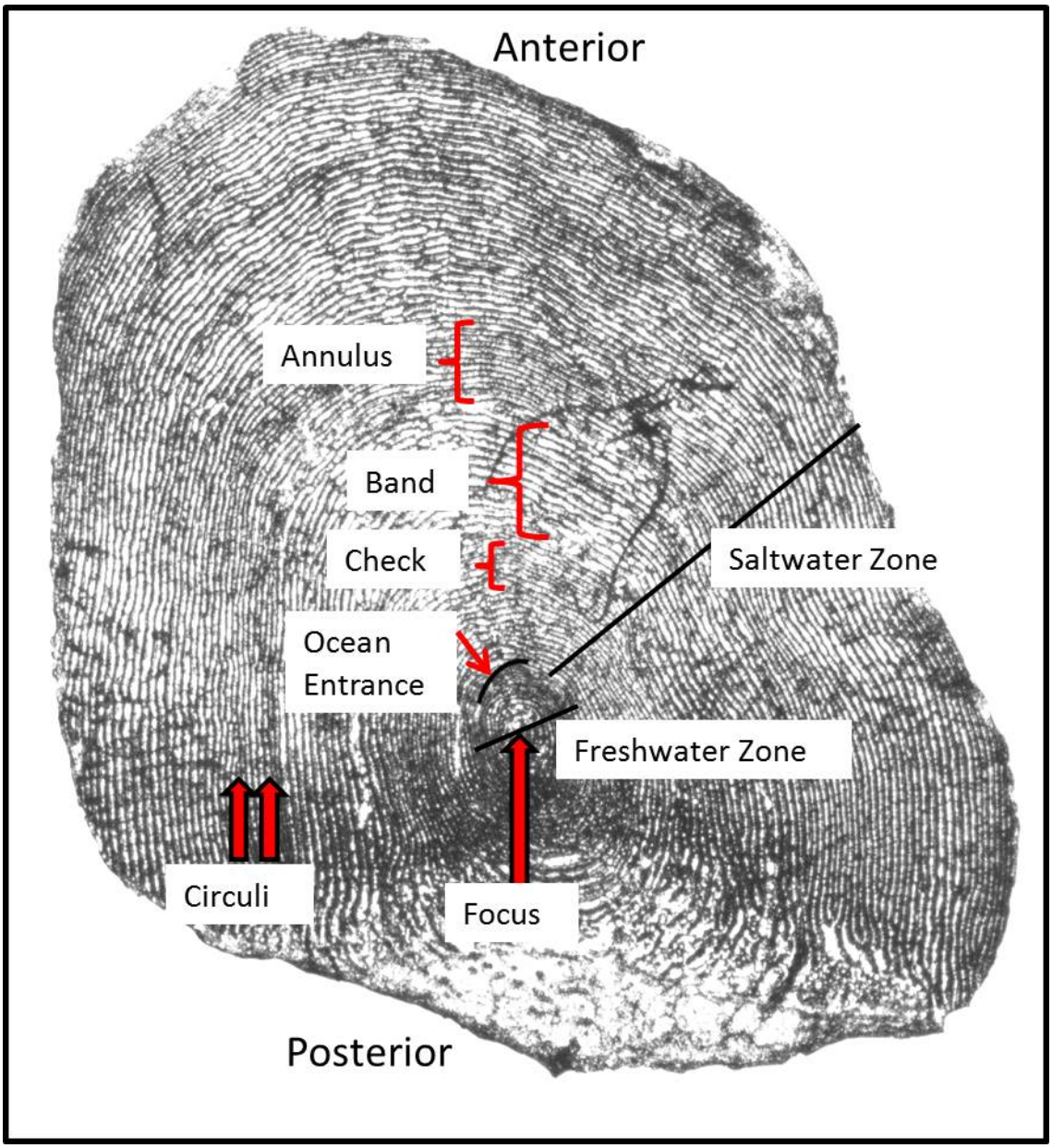
Created Date – Date the record was added to the database.



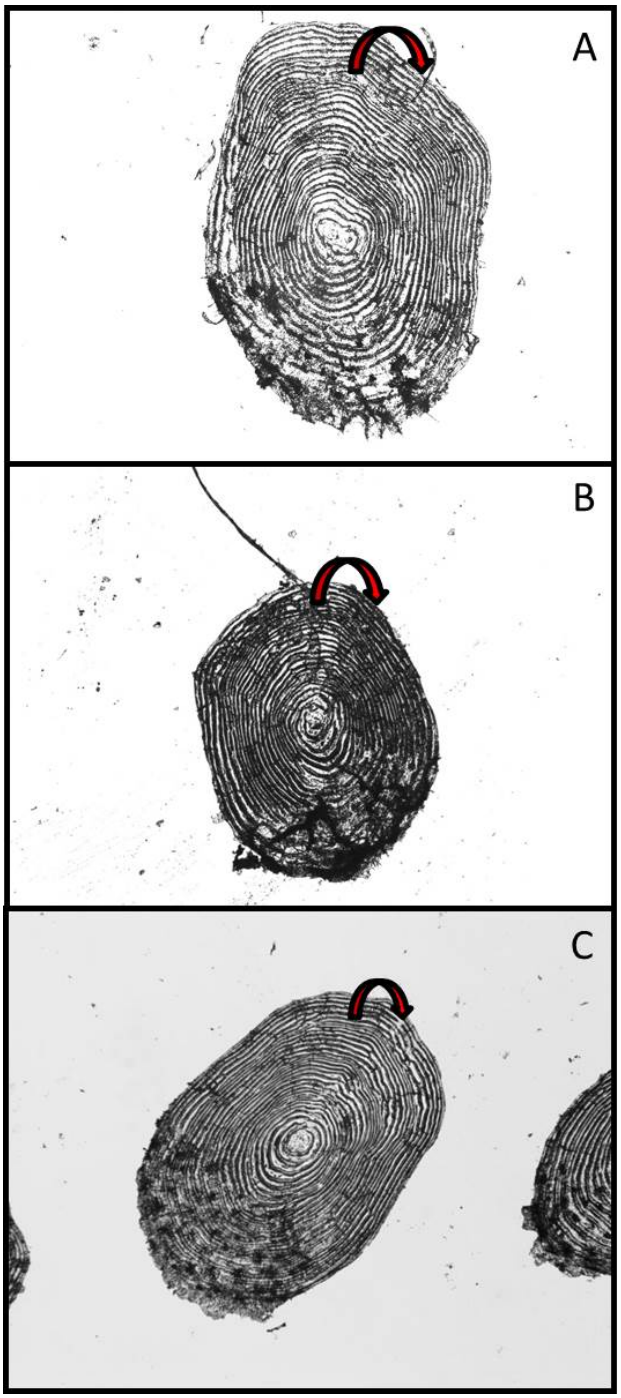
Appendix B-1. Differing degrees of regeneration seen in adult scales. Panel A: no regeneration, total age can be determined. Panel B: the freshwater region is regenerated; only saltwater age can be assigned. Panel C: the freshwater region is completely regenerated and regeneration continues into the saltwater region. Freshwater age cannot be determined and saltwater age would be questionable. Panel D: the scale is completely regenerated, age cannot be determined.



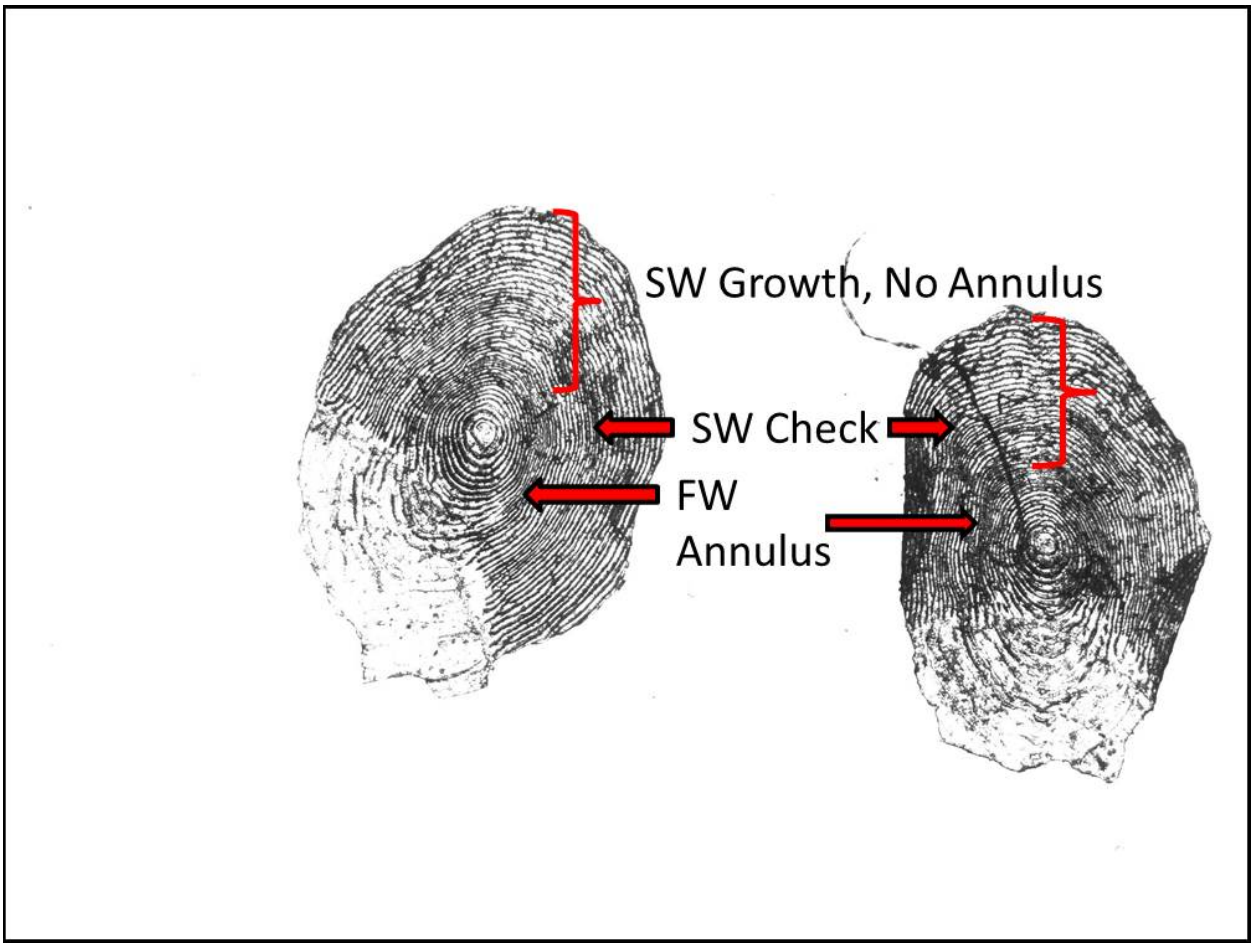
Appendix B-2. Illustration of scale resorption. Adult scales collected at Lower Granite Dam from the same steelhead prespawn (Panel A) and as a post-spawn kelt (Panel B). Scale in Panel A was collected on 9/15/09 and scale in Panel B was collected on 6/2/10. Panel B illustrates the amount of resorption that occurs during overwintering and spawning in freshwater.



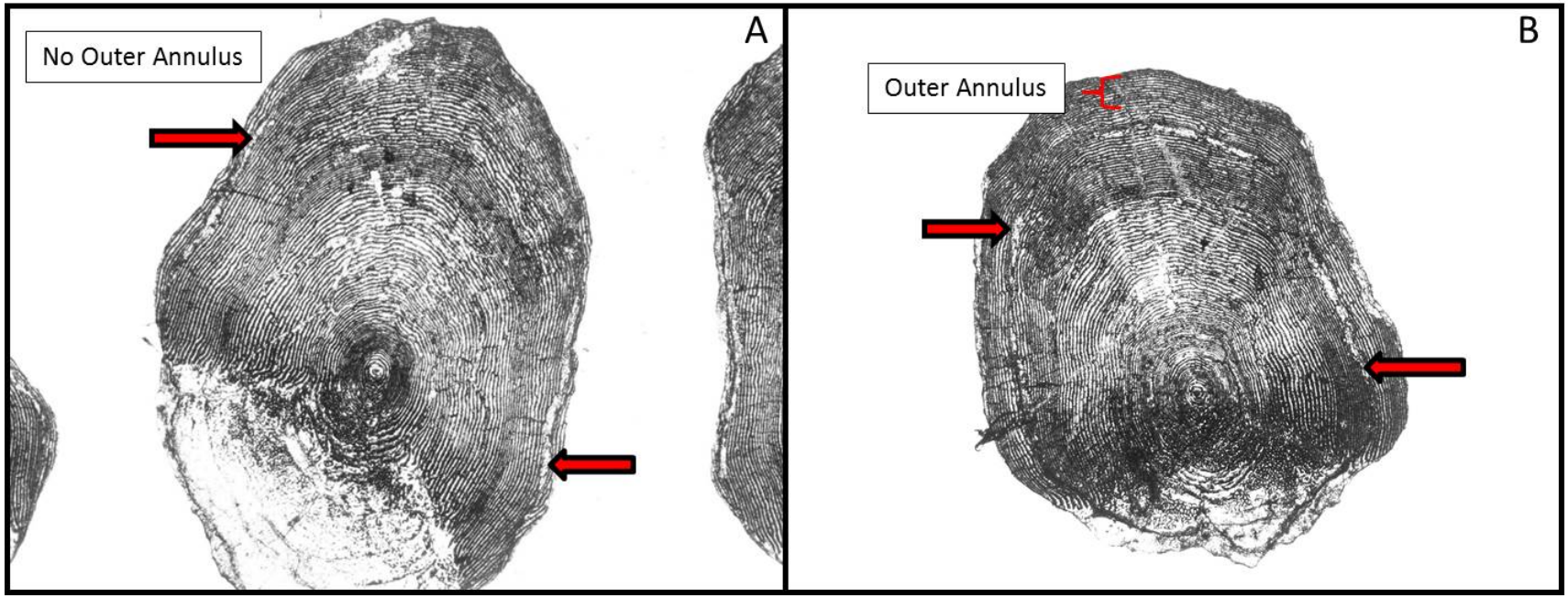
Appendix B-3. Illustration of an adult salmon scale pattern and morphological characteristics.



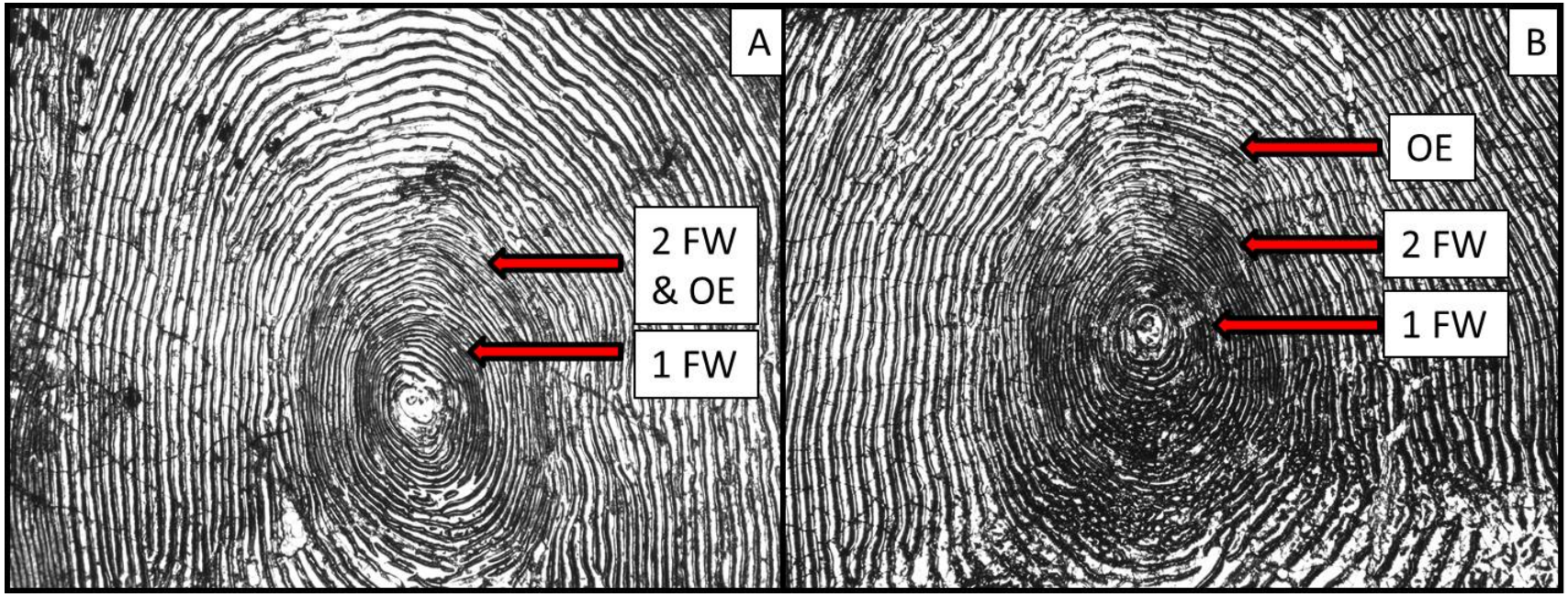
Appendix B-4. Age assignments with differing amounts of plus growth. Panel A: Spring collected juvenile steelhead showing plus growth after 2nd annulus. Spring growth counted, thus age is 3. Panel B: Summer collected juvenile steelhead showing plus growth after 2nd annulus. In this case plus growth is not counted because growth was put on during spring and summer, thus age is 2. Panel C: Fall collected juvenile steelhead showing plus growth after 3rd annulus. Plus growth is not counted because growth was put on during spring, summer and fall. Thus age is 3.



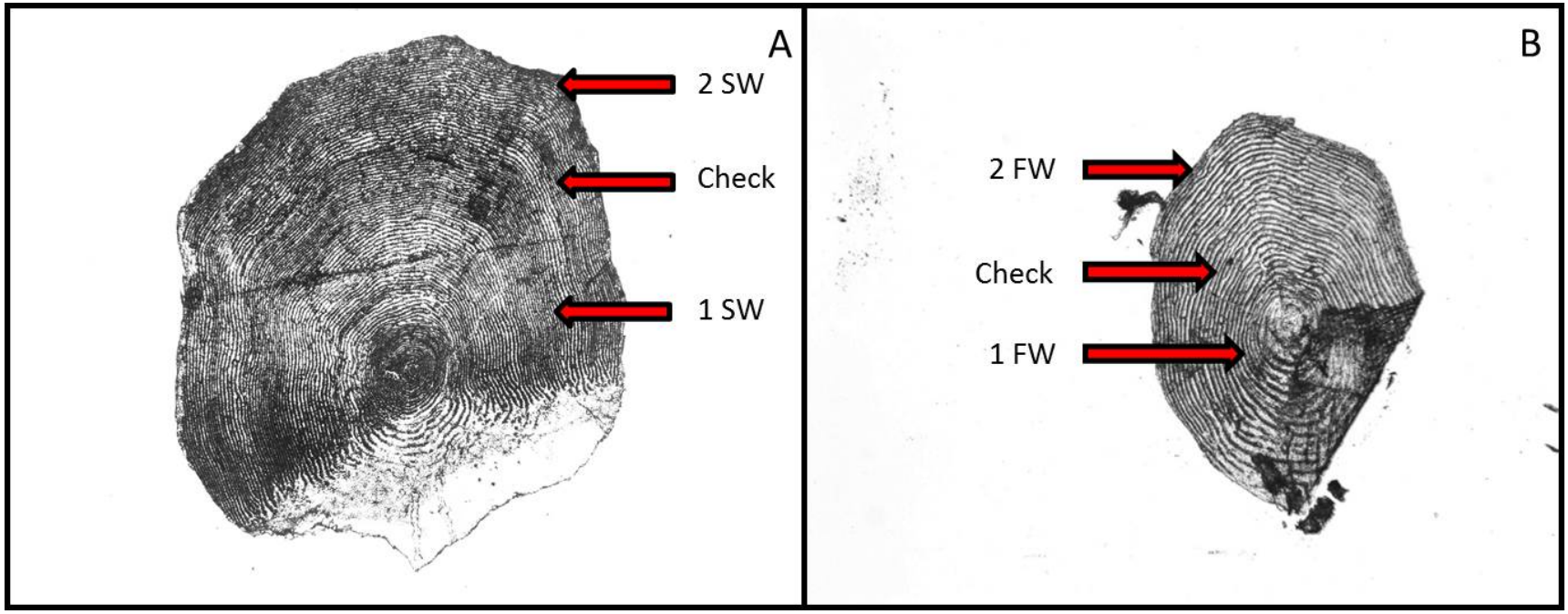
Appendix B-5. Chinook minijack scale exhibiting 1-freshwater annulus, a saltwater summer check, and no saltwater annulus.



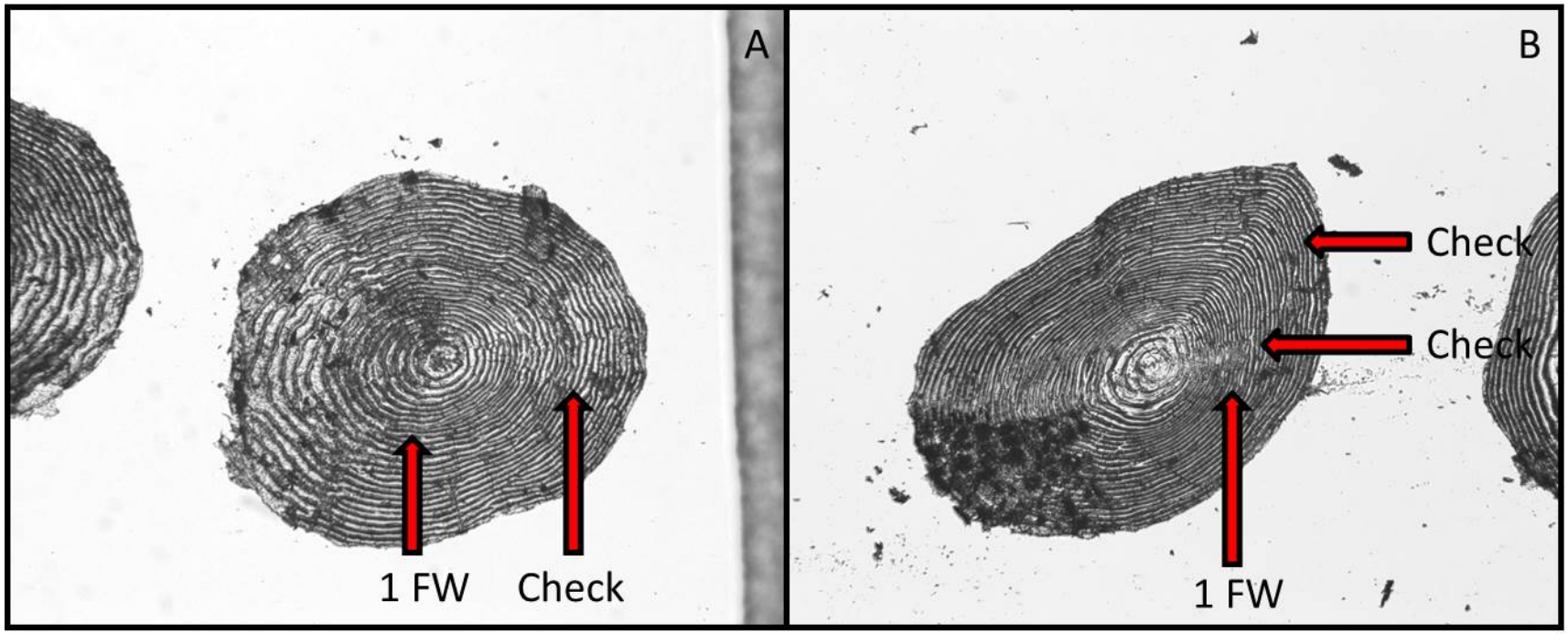
Appendix B-6. Adult steelhead scales showing a spawn check (red arrows) resulting from spawning. Panel A illustrates a steelhead who has returned to spawn in two consecutive years. Notice how close the spawn check is to the edge of the scale because there is not a winter annulus between the visible spawn check and the edge of the scale. Panel B illustrates a steelhead who has spent a winter in saltwater after its first spawning run and before returning to spawn for a second time (skip spawner). Notice there is scale growth and an annulus near or on the edge of the scale that occurs after the visible spawn check.



Appendix B-7. Freshwater sections of steelhead showing differences in ocean entrance (OE) identification. Panel A shows a steelhead in which the 2nd freshwater annulus and OE are synonymous. Panel B shows a steelhead in which there was plus growth between the 2nd freshwater annulus and its OE. Therefore OE is not counted as an annulus.



Appendix B-8. Adult and juvenile steelhead scales displaying checks. Panel A shows check in the saltwater region of an adult scale. It was determined to be a check because of its close proximity to the first annulus, and there are many fewer circuli narrowed compared to that of the first annulus. Panel B shows a freshwater check on a juvenile scale. It was determined to be a check due to the irregular narrowing around the anterior and lateral margin of the scale.



Appendix B-9. Juvenile hatchery steelhead known-age-1 scales exhibiting (Panel A) one check and (Panel B) multiple checks. Unmarked ad-intact hatchery fish complicate age determination due to strong checks. Panel A was assigned the correct age, while Panel B was aged incorrectly.

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