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Manual for Aging Steelhead Trout Scales Based on Scale Sampling from Sitkoh Creek, Big Ratz Creek, and Other Southeast Alaska Streams, 2003–2011

by

David C. Love

December 2016

Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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Weights and measures (metric)		General	Mathematics, statistics
centimeter	cm	Alaska Administrative Code	<i>all standard mathematical signs, symbols and abbreviations</i>
deciliter	dL	all commonly accepted abbreviations	alternate hypothesis
gram	g	e.g., Mr., Mrs., AM, PM, etc.	base of natural logarithm
hectare	ha		catch per unit effort
kilogram	kg		coefficient of variation
kilometer	km	all commonly accepted professional titles	common test statistics
liter	L	e.g., Dr., Ph.D., R.N., etc.	(F, t, χ^2 , etc.)
meter	m		confidence interval
milliliter	mL	at	correlation coefficient
millimeter	mm	compass directions:	(multiple)
		east	R
		north	correlation coefficient
		south	(simple)
		west	covariance
		copyright	degree (angular)
		corporate suffixes:	degrees of freedom
		Company	expected value
		Corporation	greater than
		Incorporated	greater than or equal to
		Limited	harvest per unit effort
		District of Columbia	less than
		et alii (and others)	less than or equal to
		et cetera (and so forth)	logarithm (natural)
		exempli gratia	logarithm (base 10)
		(for example)	logarithm (specify base)
day	d	e.g.	\log_2 , etc.
degrees Celsius	°C	Federal Information Code	'
degrees Fahrenheit	°F	id est (that is)	not significant
degrees kelvin	K	latitude or longitude	null hypothesis
hour	h	monetary symbols	percent
minute	min	(U.S.)	probability
second	s	months (tables and figures): first three letters	probability of a type I error
		United States	(rejection of the null hypothesis when true)
		United States of America (noun)	probability of a type II error
		U.S.C.	(acceptance of the null hypothesis when false)
		U.S. state	second (angular)
		use two-letter abbreviations (e.g., AK, WA)	standard deviation
			standard error
			variance
			population
			sample
Physics and chemistry			Var
all atomic symbols			var
alternating current	AC	registered trademark	
ampere	A	trademark	
calorie	cal	United States	
direct current	DC	(adjective)	
hertz	Hz	United States of	
horsepower	hp	United States	
hydrogen ion activity (negative log of)	pH	America (noun)	
parts per million	ppm	U.S.C.	
parts per thousand	ppt, ‰	U.S. state	
volts	V	use two-letter abbreviations (e.g., AK, WA)	
watts	W		

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**MANUAL FOR AGING STEELHEAD TROUT SCALES BASED ON
SCALE SAMPLING FROM SITKOH CREEK, BIG RATZ CREEK, AND
OTHER SOUTHEAST ALASKA STREAMS, 2003–2011**

by
David C. Love,
Alaska Department of Fish and Game, Division of Sport Fish, Douglas

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

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*David C. Love,
Alaska Department of Fish and Game, Division of Sport Fish,
802 3rd Street, Douglas, USA*

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ABSTRACT

This manual documents methods useful for standardized sampling and aging of steelhead trout (*Oncorhynchus mykiss*) scales in Southeast Alaska. Steelhead trout scale aging can be a subjective process without standardized methods and criteria for aging and known-age samples for comparison; these methods improve precision and accuracy of estimated ages and overall consistency. Methods and criteria based on observed scale morphology were developed from samples of 2,557 scales collected from resident parr, emigrant smolt, and migratory rainbow trout, and also from 2,315 immigrant adult steelhead trout collected from several different locations in Southeast Alaska from 2003 to 2011. Typical scale characteristics used to age steelhead trout include identification of first annulus, interpretation of plus growth, representative circuli counts and fork length criteria for estimating smolt ages, and comparison to morphology from known-aged adult samples. Characteristics of regenerated scales, spawning checks, false checks, and plus-growth useful for interpretation of scale morphology are also discussed. Variable life history of steelhead trout as related to scale morphology is discussed. Steelhead trout juvenile and smolt scale aging methods for freshwater life history include triplicate reads, use of criteria to improve precision, and aging practice from validated smolt scales. Expected scale morphology for the marine portion of adult steelhead trout scales collected from known ocean-age fish is presented. Methods for evaluating scale aging consistency through testing within-reader precision (repeatability), and between-reader relative accuracy are suggested. Annotated images of representative scale samples from parr, smolt, and adult steelhead trout are provided for reference as is a list of known-age, or matching-age scale, images to be used for training. Standardizing protocols and criteria used to age Southeast Alaska steelhead trout scales along with methods to evaluate technician performance are suggested as a means to ensure consistency and continuity in aging methods for future scale aging of steelhead trout in Southeast Alaska.

Key words: steelhead trout, *Oncorhynchus mykiss*, standardized scale sampling methods, age estimation criteria, scale morphology, triplicate reads, known-age samples, representative scales, within-reader precision, between reader relative accuracy, annotated images, consistency and continuity

INTRODUCTION

Scale sampling is a non-lethal method routinely used to age individual fish and to determine age composition in fish populations. Compared to other bony structures, collection and preparation of scales is relatively easy and inexpensive. Age can be estimated from scale morphology by evaluating growth patterns, stress marks, circuli counts, and annular marks, but can be subjective if consistent and informed methods are not used. Due to their complex life history and unique stream and river habitats, steelhead trout (*Oncorhynchus mykiss*) scale formation can be variable and scale aging can be challenging. Standardized procedures for aging steelhead establish a baseline method that can be evaluated, will minimize within- and between-reader variability (improve precision), and will improve consistency (limit bias) in aging by scale aging technicians, especially with changes in staff. The methods and aging criteria documented here are proposed as guidelines to define a more standard, repeatable process and provide defined scale aging interpretation, especially when estimates based only on observed scale morphology are inconsistent. The purpose of this manual is to document standardized methods utilized to sample, image, and consistently age scales collected from steelhead trout populations in Southeast Alaska. Suggestions for evaluating within- and between-reader precision and accuracy are provided.

Tools useful in consistently aging scale samples in a more precise, standardized manner are provided here in the following forms: 1) defined methods of collection, scale preparation, and imaging, 2) modal age estimation using triplicate aging reads combined with corroborated criteria to assist the technician in assigning age, 3) annotated example scale images useful for initial orientation and for annual retraining of technicians, 4) use of known-age and consistently-aged scales for practice using the standardized methods, and 5) evaluation of precision and bias

within and between technicians to assess consistency in aging using methods described by Compana et al. (1995). Electronic images of verified-age smolt scales and known-age adult scales from Sitkoh and Big Ratz creeks are available for practice and training from Alaska Department of Fish and Game (ADF&G), Sport Fish Division (DSF) Region 1, Douglas office.

RELEVANT STEELHEAD LIFE HISTORY

Unlike salmon, steelhead trout can exhibit a variety of life histories ranging from non-migratory to strongly anadromous iteroparity (Withler 1966). Variable freshwater and marine residency, multiparous adults with variable spawning histories, and diverse life-history forms (life-history polymorphism) all contribute to variable scale morphology and possibly subjectivity in interpretation of scale patterns. Southeast Alaska steelhead trout life-history variability may seem to make scale aging a challenge, although atypical scale patterns are probably not as common as technicians might envision. Although an understanding of various life history forms is necessary, scale technicians should be careful not to over-interpret scale pattern as being more complex than may actually be evident for the scale being aged. Scale ages estimated for systematically sampled Sitkoh Creek smolts from 2003 to 2009 having unique life-histories represented 1% or less of the total smolt emigration during any given year (Love et al. 2013). Scale aging technicians should keep in mind that the majority of the scale samples collected from Southeast Alaska were not from the highly different individuals described above. But knowing about the variations in life-history is important for a technician aging scales, since occasionally the technician may encounter these very different, and difficult-to-age, scales.

Although variable life-history in steelhead trout results in differences in scale morphology, samples collected from populations displaying more uniform life-histories result in greater conformity in scale morphology and less variable aging results. The scales used to develop steelhead trout aging methods described herein come from systematic collections taken from spawning adult immigrants and emigrating smolt at the Sitkoh Creek and Big Ratz Creek weirs operated each spring (April–June) from 2003 to 2011 (Love and Harding 2008–2009; Love et al. 2012a, 2012b; Love et al. 2013). These systems support predominantly spring spawning (ocean maturing) populations of steelhead trout of between 350–500 immigrant adults and 1,500–2,500 emigrant smolts. Scale samples collected from Sitkoh and Big Ratz creeks were from freshwater-age-3 and -4 outmigrant smolts, with only a few freshwater-age-2 and essentially no freshwater-age-1 or freshwater age-5 fish. Since few overwintering fall run adults appear to occur in the Sitkoh Creek and Big Ratz Creek populations, scale morphology and aging estimates of samples from adult steelhead trout were somewhat less variable than would be found in populations that include fall run immigrants. Based on PIT-tag recaptures at Sitkoh and Big Ratz creeks, the majority of adults from these creeks spent 2–3 years maturing in the ocean before returning to spawn for the first time. Ocean-age-2 steelhead are reported to be the dominant adult age class in North America (Busby et al. 1996), while ocean-age-2 and ocean-age-3 were the 2 most abundant ocean ages for several Southeast Alaska steelhead streams (Lohr and Bryant 1999). Most of the first-time spawning adults were, in fact, freshwater-age-3 and freshwater-age-4 and ocean-age-2 and ocean-age-3, whereas single-repeat spawning adults were the most common repeat spawners in both Sitkoh and Big Ratz creeks (Love and Harding 2008–2009; Love et al. 2012a, 2012b; Love et al. 2013). In 2007, a small 465 mm fork length (FL) male steelhead trout adult tagged as a smolt in 2006 was recaptured moving upstream through the weir at Sitkoh Creek. This was the first known ocean-age-1 precocious male steelhead trout to be recaptured at Sitkoh Creek. Unfortunately, this tagged fish was noted, but not scale sampled. Annotated

images of scales from an ocean-age-1 adult sampled as a smolt in 2010 and then resampled the next spring as an adult in Big Ratz Creek are provided in Appendix A4. This was the second of only 2 ocean-age-1 PIT-tagged individuals that were recaptured during 9 years of spring weir operations that passed over 4,500 adults upstream into Sitkoh and Big Ratz creeks. Populations in Oregon and California have higher frequencies of ocean-age-1 adults than populations to the north. In Southeast Alaska a few ocean-age-1 adults have also been reported in the Karta, Thorne, Klawock, and Situk rivers, and also in Petersburg and Peterson creeks (Lohr and Bryant 1999).

Although most of the scale images and aging estimates presented here come from Sitkoh Creek steelhead trout samples, a few samples collected elsewhere are also included for clarification and documentation purposes and to illustrate scale morphology differences in samples from different steelhead trout and/or rainbow trout life-history forms. Two images from freshwater-age-0 juveniles collected from Tye Creek, in the Staney Creek drainage on Prince of Wales Island, Southeast Alaska, in August 2011 are included to illustrate freshwater-age-0 scale circuli patterns for young-of-the-year steelhead trout. Examples of freshwater-age-0 scale morphology are important, especially if circuli count criteria are used to standardize aging efforts. Misidentification of the first annulus, which can be difficult to distinguish in emigrant smolt, can result in inaccurate overall scale age. Any steelhead project collecting scales should also attempt to collect young-of-the-year samples to verify first annuli appearance.

Emigration of immature smolts/rainbow trout and mature rainbow trout can also confuse smolt sampling and aging results for scales collected during the spring emigration. In Sitkoh Creek, PIT-tagged nomadic smolt-like rainbow trout were recaptured as emigrants through the Sitkoh Creek weir in 2003/2004 and 2006/2007. During these emigration events, the sexually immature smolts/rainbow trout had the typical silvery smolt coloration of the emigrant smolt population, but were recaptured subsequently as smolt in following years. Nomadic smolts such as these have also been reported in Scott Creek, central California, utilizing the estuary during the summer when rearing conditions were good then migrating upstream in the fall when estuarine water quality declined before emigrating a second time the following spring (Hayes et al. 2011). It is not known if these Sitkoh Creek emigrants were immature anadromous rainbow trout or possibly precocious steelhead trout smolt. Small numbers of resident rainbow trout have also been counted as they emigrating through the Sitkoh Creek weir each year of its operation. These were typically not silvery in coloration, were sexually mature, and had scale ages estimated as freshwater-age-5 and older.

A final life history type that might confuse scale technicians, but is collected as an immigrant following the spring smolt emigration, is the “half-pounder” (Behnke 2002) originally described in the Rogue, Klamath, Mad, and Eel rivers of southern Oregon and northern California (Snyder 1925; Kesner and Barnhart 1972; Everest 1973; Barnhart 1986). Half-pounders are generally less than 400 mm FL, are immature, and migrate upstream in the late summer or early fall. Two immature immigrants from Chuck Creek, Heceta Island and one from Log Jam Creek, Prince of Wales Island (Steve McCurdy, retired Fishery Biologist, ADF&G, Craig, personal communication) have been sampled as they moved into these streams from marine waters. These fish were not sexually mature and had less than ocean-age-1 marine scale signatures. Increased circuli spacing and good growth near the outer margin of the scale and greater size at recapture indicated they emigrated in the spring and used the marine environment, including possibly using nearshore estuarine areas, before returning to fresh water the same season. Annotated images

documenting Southeast Alaska immigrant immature half-pounder steelhead trout from Prince of Wales Island and Heceta Island, adult anadromous rainbow trout captured from Sitkoh Creek, and an ocean-age-1 adult from Big Ratz Creek similar to the one observed, but not sampled at Sitkoh Creek, are all presented in Appendix C.

SAMPLE SITES

An understanding of the sample sites for which objective criteria were developed is important for evaluating the use of the aging methods described herein in other stream systems and steelhead trout populations. The Sitkoh Creek system is located on southeastern Chichagof Island in Southeast Alaska (Figure 1) and empties into Chatham Strait via Sitkoh Bay. Sitkoh Creek (ADF&G Anadromous Stream Catalog No. 113-59-10040) is about 6.4 km long and drains Sitkoh Lake, which has a surface area of about 2.00 km². Sitkoh Creek is a snowmelt- and rainfall-driven stream system with an east-west aspect that often freezes bank-to-bank during severe winters, dewatering large sections of the creek.

Methods initially developed at Sitkoh Creek were also applied to scales collected from Big Ratz Creek, Prince of Wales Island, from immigrant adult and emigrant smolt steelhead trout during weir operations conducted in 2010–2011. The Big Ratz Creek system (Anadromous Stream Catalog No. 106-10-10100) is located on northeastern Prince of Wales Island and empties into Chatham Strait via Ratz Harbor (Figure 1). Big Ratz Creek is about 6.4 km long and drains Big Lake, Trumpeter Lake, and Little Lake, which have a combined surface area of 1.31 km². On the continuum, the Big Ratz drainage seems to be more rainfall-driven than snowmelt-driven. Big Ratz Creek may support more ocean-age-1 adult steelhead trout than were observed at Sitkoh Creek due to these somewhat warmer conditions. One such known ocean-age-1 adult tagged as a smolt in 2010 and recovered in 2011 as an adult at Big Ratz Creek is presented in this report (Appendix A4). During most years, the creek does not freeze bank-to-bank for the entire winter.

Scales samples of summer and fall immigrant immature anadromous steelhead/rainbow trout were also collected serendipitously by USFS personnel (Sheila Jacobsen, Fishery Biologist, USFS, Craig Ranger District, personal communication) at Tye Creek in the Staney Creek drainage on Prince of Wales Island, and by the ADF&G area management biologist from Log Jam creek on Prince of Wales Island and Chuck Creek (Figure 1) on Heceta Island (Steve McCurdy, former Craig Area Management Biologist, ADF&G, personal communication). Images of these samples are also presented in this report. Reports of these smaller, half-pounder steelhead trout measuring from 300 to 400 mm FL have also been reported from other Southeast Alaska streams: 12-Mile Creek, Prince of Wales (Steve Hoffman, retired Ketchikan Area Management Biologist, ADF&G, personal communication), in the gillnet fisheries at the mouth of the Stikine River (Doug Fleming, retired Petersburg Area Management Biologist, ADF&G, personal communication), and Nakwasina River, Baranof Island (Patrick Fowler, Sitka Assistant Area Management Biologist, ADF&G, personal communication). These various stream systems vary between rainfall and snowmelt in their water sources.

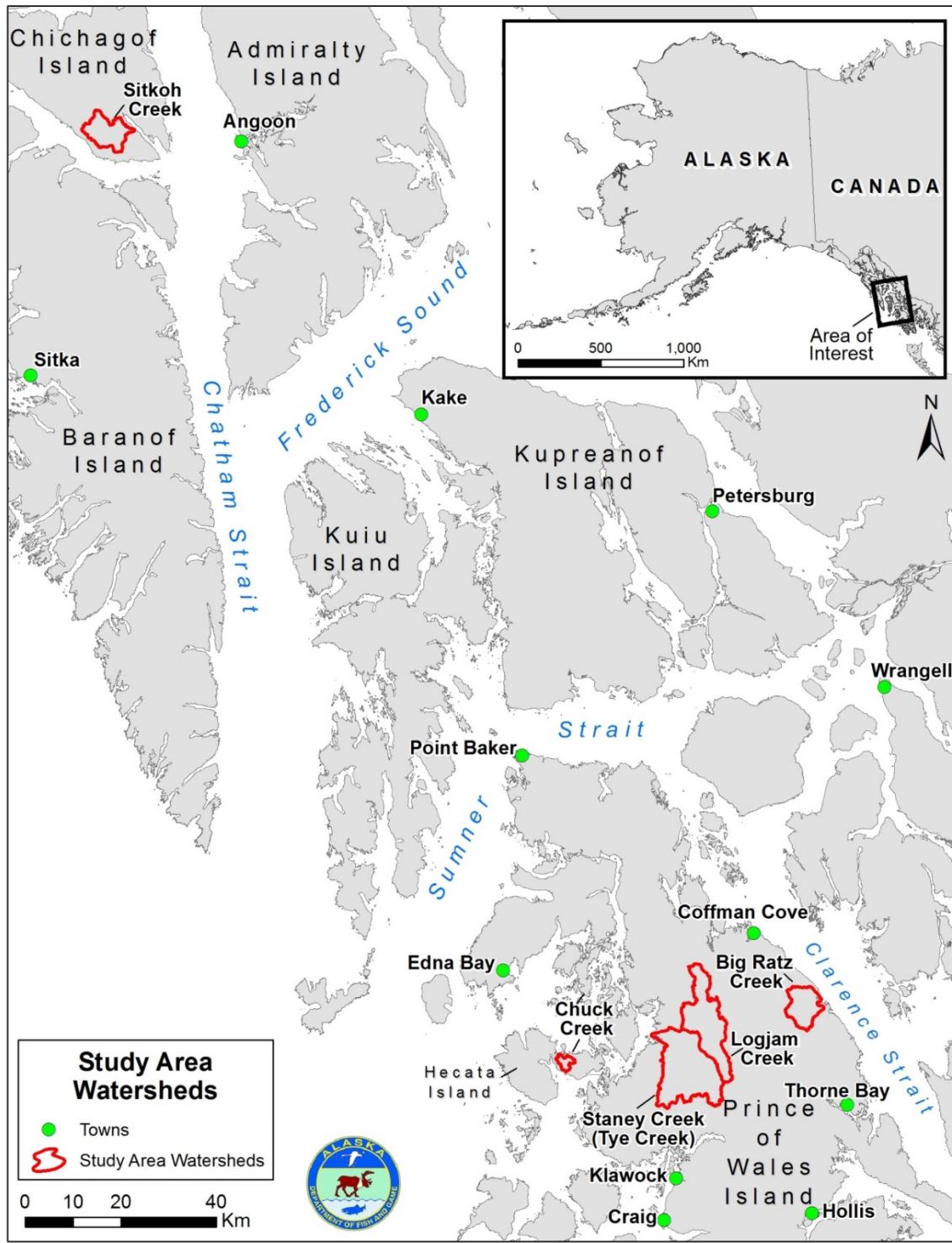


Figure 1.—Location of study areas in Southeast Alaska that are relevant to steelhead trout scale aging methods described.

OBJECTIVES

Objective 1: Document standardized protocols for scale sampling, collection, preparation, and imaging for smolt and adult steelhead trout.

Objective 2: Document established aging protocols and aging criteria for smolt and adult steelhead trout.

Objective 3: Provide annotated images of steelhead trout smolt and adult steelhead trout scales to assist aging technicians in learning pertinent scale characteristics and scale aging method.

Objective 4: Provide a variety of verified-age steelhead smolt and adult steelhead trout scale images for technicians to practice use of the scale aging method.

Objective 5: Present methods to evaluate scale aging technicians' obtained age estimates for precision, accuracy, and consistency.

METHODS

SAMPLING AND AGING PROTOCOLS

Standardized scale collection, preparation, imaging, and modal aging methods, scale aging criteria based on relevant scale features, and methods to estimate within-reader precision and between-reader accuracy are described below. The goal here is to obtain unbiased estimates of age obtained in a standardized, consistent manner. Technicians aging scales should use the guidelines presented here when they are uncertain about the age of a sample, or to double-check themselves when aging scales. Although the criteria range(s) described here are useful to technicians for assigning estimated scale ages, if scale morphology clearly indicates scale age other than predicted by criteria range(s), then criteria should not be considered as absolute for assigning age.

Scale Collection and Preparation

Steelhead Trout Smolt

Scale samples from smaller fish, such as parr and smolt, are more easily collected using a scalpel blade gently scraped in a posterior to anterior direction over the “preferred” area (Scarneccia 1979) of the fish’s left side. Without turning the scalpel over, the scales can then be deposited onto microscope slides by dragging the blade edge back over the slide surface. Scales should then be teased apart with probes or scalpel tips before sandwiching between 2 microscope slides. Excess mucous should be cleared away from the scales using the back of the scalpel. Due to high scale regeneration rates, about 15–20 scales should be sampled from each fish. If collected and mounted carefully in the field, scale samples from steelhead trout smolt typically do not need further cleaning or remounting onto glass slides. Slides should be allowed to dry, then should be stored inside a coin envelope inscribed with sample date, sample number, species sampled, location, length (mm FL), and tag number (if any). This information should match accompanying data sheets, and coin envelopes should be stacked and bundled in sequential order for subsequent imaging and/or aging.

Steelhead Trout Adults

Systematic subsamples of scales should be collected from populations of adult steelhead trout, rainbow trout, and/or steelhead trout smolt, in order to more accurately describe ages, growth,

survival, and extent of freshwater/marine residence in the populations sampled. Scale images provided in this manual were from returning steelhead trout adults, either untagged or previously PIT-tagged. Recaptures allowed for age estimates and annotated images (Appendix D) to be made on known-age adults. Scales from adult steelhead should be removed from the preferred area on the left side of the fish (Scarneccia 1979). The preferred area in adults is between approximately 4 to 6 scale rows below and behind the dorsal fin, but above the lateral line. If the fish had been recaptured, scale samples would have been taken from the right side in the same preferred area. Remove scales using forceps, being sure to clean off any dirt or skin, then place the scale exterior out and anterior up onto labeled gum cards covered by blotter paper. Cards should be carefully and clearly labeled using pencil or waterproof pen, including species, location sampled, date, gear type, sampler's name, and sample identification number. Completed cards should be carefully dried and pressed flat. Alternatively, if electronic imaging is to be used, scales can be collected and pressed between microscope slides. If this method is employed it is important to carefully clean the scales and dry as best as possible in the field before pressing between the slides to avoid molding. Be sure that datasheets and scale identification numbers match.

Scale Imaging and Modal Aging

Steelhead Trout Smolt Scale Imaging

If collected and mounted carefully in the field, scale samples from steelhead trout smolt typically do not need any further cleaning or remounting onto glass slides. Of the 15–20 scales sampled, the most legible one or two should be chosen for imaging. Scales that appeared to have developed normally and were similar in size to most of the other scales in the sample are considered representative. Ideal scales are clean, mucus-free, and do not have a regenerated focus. Regenerated scales are not to be used for aging. These are new scales that grew rapidly to replace a scale that was lost, and have a clear, grainy, or irregularly formed center (focus). Legible scales are those that are not regenerated, are not reabsorbed on the edges, and have clearly visible circuli. Smolt scale images should be made using the 4X objective of the Leica DMD108¹ imaging microscope located at the ADF&G DSF office in Douglas, Alaska. Electronic scale images of past and future samples should be archived on the “S” drive at the ADF&G DSF office in Douglas, Alaska.

Steelhead Trout Adult Scale Imaging

If not already collected onto microscope slides, the most legible adult scales should be selected from the gum cards, cleaned, and mounted onto microscope slides for imaging. Clean, legible scales are mounted rough side (exterior) up with the anterior end toward the top (short side) of the microscope slide. A second glass slide should be secured over the first to protect the newly mounted scales. Each glass slide and scale sandwich should then be labeled with sample number, sampling location, sampling date, species, PIT tag number, and length (mm FL). Images should electronically imaged for archive. The Indus International Microfiche reader and electronic imaging software located at the ADF&G Mark, Tag, and Age Laboratory in Juneau, Alaska is available for imaging. If no legible scales are found on the gum card for the sample, a place marker with the label NGS (“No Good Scale”) should be imaged for that sample. Electronic

¹ Product names used in this report are included for scientific completeness but do not constitute a product endorsement.

images of scales of past samples are archived on the “S” drive at the ADF&G DSF office in Douglas, Alaska.

Modal aging and aging format

Once scale samples have been prepared and electronically imaged, scale aging technicians should make triplicate reads of each image to obtain modal ages. Triplicate, independent aging reads to obtain a modal age should be employed for aging both adult and smolt scales. Scale ages should be estimated primarily from the area of the scale lying 45° off either side of an imaginary reference line drawn along the longest axis of the scale, from the focus to the anterior edge. Since scale growth is typically greatest along the long axis of the scale, patterns are often more evident in this area of the scale. Between readings, the scale images should be randomized by the project biologist and scale aging results from the previous reading(s) should not be known during subsequent readings. The modal age of each scale is assumed to be the most consistent age and is thus reported. If none of the 3 independent reads agree, the sample should be considered unreadable and be omitted from the age composition. If multiple scale aging technicians will be aging scales, efforts should be made to train and use standardized techniques, and to evaluate reproducibility of age estimates by evaluating within-reader precision and repeatability as well as between-reader bias.

Smolt scale ages should be recorded in the format of X.X, where the number preceding the decimal would be the age followed by the decimal and a zero. The aging format used for adults should be recorded as X.XSX SX after Koo (1962). For example, an adult steelhead trout scale aged as 3.2S1S1 would have been from a fish that spent 3 years in freshwater, 2 initially in the ocean, before returning for the first time to spawn, had an evident spawning check, spent 1 additional year in the ocean, spawned again, then was recaptured as an immigrant adult on its third spawning migration after 1 additional year in the ocean. “S” denotes the reabsorption of annuli in a spawning check. All scale images that showed regenerated focus, poor images, or those scales obscured by excess slime or were damaged such that they cannot be read should be omitted from aging. If possible and available, multiple scales of similar size from the same fish should be reviewed to verify aging, with the intent that representative scales having similar growth structure be aged. Circuli counts are made through an annulus in determining annuli counts. Definitions are as follows: 1) “circuli” are complete, or mostly complete, rings of growth, with relatively even spacing between each ring; 2) “annuli” are groups of circuli spaced closer together during winter, or colder water, slower-growth periods. Annular marks can be eroded together, or be resorbed and obscured by spawning checks especially in spring spawning steelhead trout.

Relevant Features Important to Aging

In addition to defined criteria useful for scale aging, other morphological features that should be considered when aging steelhead trout smolt scales include the following: rejection of regenerated scales, identification of first annuli, overall scale size, interpretation of “plus growth,” identification of spawning checks, and false checks. Standardized interpretation of these features should improve consistency (i.e., relative accuracy) and precision.

Regenerated Scales

When a scale is lost, a replacement scale grows rapidly to replace it. These regenerated scales can be identified by a center that is clear, irregularly formed, or grainy in appearance. No circuli

will be present during this period of rapid growth. Regenerated scales should not be used because they are missing circuli.

First Annulus Formation

Scale aging criteria are based on a combination of the observed scale morphology and the relationship of total circuli counts to estimated scale ages. To be consistent in estimating scale age of steelhead trout smolts, identification of the first annulus is critical. The first scales to form in juvenile rainbow trout were found just above the lateral line between the dorsal and anal fins, the preferred area to sample scales (Paget 1920; Scarneccia 1979). Sampling from this area is critical for estimating ages of young-of-the-year trout. Not having known-aged juvenile scales for the stream being studied (i.e., Sitkoh and Big Ratz creeks), an observed number of circuli to the first annulus from scales collected from the preferred area must be assumed to represent growth to the first annulus. In Southeast Alaska, steelhead trout juveniles produced by spring-run adults (March–May) are reported to emerge from the gravels in about mid-July at a size of 30–40 mm. Based on samples from Petersburg Creek collected in 1972, Peterson Creek in 1980, Situk River in 1968, and Sitkoh Creek 1982, the formation of the first 6–8 circuli occurs by the end of the first growing season and at a size greater than 40 mm FL (Jones, *Unpublished document*). Steelhead trout button-up fry collected from Sitkoh Creek in 2008 that were 28–40 mm FL had no evident scales. Samples of 50 mm and 55 mm FL steelhead trout parr assumed to be young-of-the-year (YOY) that were sampled late in August from Tye Creek had total circuli counts of 4–6 circuli and are presented in Appendices A1 and A2. Possibly, because this is a productive southern Southeast rainfall-dependent system with warmer water and less winter-time icing, it may be that these images may represent maximum growth through August 26, 2011, in what appear to be freshwater-age-0 parr. By contrast, an example of a 1.0 freshwater scale with 10 circuli from a steelhead trout parr that was 58 mm FL on 4/17/07 from the Sitkoh Creek system, which has an east-west aspect, late melting snowmelt, and strong winter icing and colder water temperatures, can be seen in Appendix A3.

Interpretation of Plus Growth

Steelhead trout smolt scale samples having complete circuli laid down outside the last annulus exhibit what has been called “plus growth.” All Sitkoh Creek steelhead trout smolt were sampled in the spring during 2003–2009, no earlier than about May 1 and no later than June 25. Observed plus growth of 3 or more circuli was more typically observed in emigrant Sitkoh Creek smolts that were sampled later in June. Occasionally, a smolt scale sampled in early May exhibited plus growth, but these samples were not common. If an early- to mid-May sample had plus growth of more than 3 circuli, this was considered evidence of another year, even if there was no evident annulus. June samples with plus growth of 3 circuli or less should not be counted as an extra year.

Spawning Checks

A spawning check is defined as a “break” in contiguous circuli, or resorption (clear area) of circuli within or just preceding an annulus, especially in older, mature fish. At Sitkoh Creek, once adult steelhead trout made the initial spawning migration, they normally returned on an annual basis, as indicated by annual spawning checks. However, a small number (less than 1%) “skip-spawned,” or skipped a year before returning to spawn. See annotated images in Appendix D for examples.

False Checks

False checks are observed in scales exhibiting a period of reduced growth, stress, or shock (Lux 1971). One type of false check, or “accessory check” (Narver and Withler 1971), was sometimes observed in Sitkoh Creek adult scales prior to the first ocean winter annulus. Unlike spawning checks, accessory checks occurred before the annulus. False checks were thinner than annuli and were often more pronounced near the dorsal and ventral sides of the scale than were annuli (Appendix D).

Freshwater Age Estimation for Steelhead Trout Smolt

Precision of freshwater aging methods benefit from systematic methods and estimating modal ages via triplicate reads. Relative accuracy can be improved through comparison to known age and validated age examples and annotated images of known age samples. By systematically sampling, modal aging, identifying first annuli, and using defined criteria such as total circuli count and fork length ranges by estimated age, technicians should expect to obtain consistent estimates for age 2–4 freshwater scale samples. About 80% of the variation in age estimates may be explained by total circuli counts; multiple regression analysis of the combination of circuli count and length measurement criteria as indicators of scale age (Table 1) correlated similarly ($r^2 = 0.8071$, $P < 0.005$), as did circuli counts alone ($r^2 = 0.8047$, $P < 0.005$).

Freshwater-age-0 Criteria

Young-of-the-year Sitkoh Creek steelhead trout parr in the 30 to 40 mm FL range have been observed as button-up fry with evident yolk and poor swimming abilities. These newly hatched fry had no evident scales, even following a hematoxylin-eosin histological stain of smear samples from the entire side of several of the fish. During minnow trapping in the spring of 2006 and 2007, no juveniles below 50 mm FL were collected; however, all that were captured had scales indicating that they were 0+ fish that had hatched in the late summer/fall of the previous year. There is a need for known aged juvenile steelhead trout freshwater scale samples from Southeast Alaska streams in order to verify formation of the first annulus, but for freshwater scale aging of samples for this method, age 0 fish were assumed to be less than 50 mm FL and had scales with 6 circuli or less. Tye Creek steelhead trout scale images (Appendices A1 and A2) provide examples of samples from 50 and 55 mm FL fish with 4–6 circuli that were collected in Southeast Alaska in August. The circuli count range for age 0 would be estimated to be 6 circuli or less (Table 1).

Freshwater-age-1

Based on estimated ages of scale samples of Sitkoh Creek steelhead trout parr collected from stream trapping during 2006 and 2007, steelhead trout parr estimated as age 1 ranged from 53 to 86 mm FL. Annuli formation in age 1 fish appears to be variable. Annuli were often evident following age 0 summer growth of up to 6 circuli, but sometimes 12 circuli or more are present before the first evident annulus, making differentiating between the age 1 and age 2 difficult without the use of circuli counts. Scales from 1st winter (age 1 on January 1) fish with evident annuli had a minimum of 7 and maximum of 15 circuli. Circuli ranges for Sitkoh and Big Ratz creeks indicated that 7–12 circuli were likely age 1 criteria range. Scale samples from the smallest steelhead trout parr were estimated as age 1 if there were up to 12 circuli, but no evident annuli (Table 1). If the fish was collected earlier in the spring (prior to end of May, especially if water temperatures were low), had an evident 1st winter (age 1) annulus, and there were more

than 12–15 total circuli, or plus growth was more than 3 circuli, then this fish was marked as age 2.

Freshwater-age-2

Freshwater-age-2 parr/smolt were typically greater than 70 mm FL, with a range of 57–128 mm FL. Scales from 2nd winter (freshwater-age-2) fish with 2 evident annuli had a minimum of 10 and maximum of 20 circuli. If only 1 annulus was evident, there were 20 or more total circuli, and the fish was greater than 86 mm FL, the 2nd annulus could possibly be present within the first 20 circuli. Regression of circuli counts on age (criteria range) indicated that the estimated age 2 samples would be between 13–21 circuli (Table 1).

Freshwater-age-3

Freshwater-age-3 smolt with 3 evident annuli averaged 179 mm FL and ranged in size from 110 to 250 mm FL. Scales from 3rd winter (age 3) fish with 3 evident annuli had between a minimum of 18 and maximum of 39 total circuli. Estimated circuli criteria range for age 3 were 22–34 (Table 1). If only 2 annuli were evident, there were 34 total circuli, and the fish was greater than 128 mm FL, the 3rd annulus was likely present within the 20–34 circuli. Scale ages from freshwater-age-3, freshwater-age-4, and freshwater-age-5 smolt were likely best estimated by circuli counts since they had better sample sizes.

Freshwater-age-4

Freshwater-age-4 smolt with 4 evident annuli averaged 199 mm FL and range in size from 152 to 304 mm FL. Scales from 4th winter (freshwater-age-4) fish with 4 evident annuli could possibly have 27–60 circuli. Regression of circuli counts by age estimates indicated a range of 35–45 circuli for age 4 (Table 1). If only 3 annuli were evident, there were more than 44 circuli, and the fish was greater than 250 mm FL, the 3rd annulus was very likely present within the first 35–45 circuli.

Freshwater-age-5

Freshwater-age-5 fish with 5 evident annuli ranged in size from 175 to 350 mm FL. Scales from 5th winter (freshwater-age-5) fish with 5 evident annuli were estimated by circuli criteria to have 46–56 circuli (Table 1). If only 4 annuli were evident, there were more than 46 circuli, and the fish was greater than 304 mm FL, the fish might possibly be a freshwater-age-5 fish. Most fish aged as freshwater-age-5 were quite probably migratory rainbow trout. In Sitkoh Creek from 2003 to 2009, 13% of all emigrant smolt were estimated to be freshwater-age-5 (11% were estimated as age 5 in Ratz Creek). However, very few (less than 1%) of the adult steelhead trout that were scale-sampled as returning immigrants were estimated as freshwater-age-5, indicating that most of these fish did not survive in the ocean well or were likely not steelhead trout.

Freshwater-age-6

Freshwater-age-6 fish were likely anadromous rainbow trout, observed with 6 evident annuli, ranging in size from 163 to 375 mm FL. If the fish was greater than 350 mm FL and its scale had more than 56 circuli and 5 annuli were present, then it may be considered to be a freshwater-age-6 fish. These larger, older rainbow trout often have characteristics of weak 1st ocean, or “estuarine,” growth. Circuli spacing for the estuarine part of the scale for these anadromous rainbow trout was not as wide and circuli were not as numerous as in the typical first year of stronger marine growth in steelhead trout smolt.

Freshwater ages greater than age-6

If evident annuli indicated ages greater than 6, or if the fish was greater than 375 mm, and the scale had greater than 56 circuli, then the fish could be between 7 and 10 years old. The few fish sampled of this age ranged in size were from 276 to 478 mm FL and circuli counts could be between 48 and 95. These fish were likely not steelhead trout smolt but were migratory rainbow trout.

Table 1.—Steelhead trout smolt aging criteria: scale circuli counts and fork length (FL) measurements for age 0 to age 6 or older steelhead trout smolts provided to aid scale technicians in maintaining consistency.

Scale age	Circuli count sample size	Min.-max. circuli	Regression eqn. ^a circuli range	FL sample size	Min.-max. FL	FL range based on SD
Age 0	2	1-7	0-6	2	30-55	<55
Age 1	34	7-15	7-12	34	53-86	59-78
Age 2	64	10-20	13-21	64	57-128	75-104
Age 3	304	18-39	22-34	306	110-250	158-199
Age 4	682	27-60	35-45	676	152-304	176-220
Age 5	203	32-64	46-56	200	175-350	196-257
Age 6 or older	74	42-95	>56	69	173-478	218-453

Note: Minimum and maximum circuli counts and fork lengths by estimated scale age, regression-estimated circuli count ranges by age, and fork length ranges based on one standard deviation (SD) about the mean by age.

^a Regression analysis of independent variable (total circuli count) as predictive of dependent variable (estimated age) resulted in $r^2 = 0.8047$ for a sample of 1,363 ($P < 0.005$).

Marine Age Estimation of Steelhead Adults

Marine aging methods incorporate freshwater criteria as well as initial marine growth, identification of first ocean winter annuli, and identification of spawning and false checks in the marine portion of the scale. Marine scale aging methods were established based on aging of known ocean-age recaptured adults that had previously been PIT-tagged as smolts or adults at the Sitkoh and Big Ratz creeks weirs. Because initial marine growth and spawning checks are very distinctive and consistent morphologically, the marine portion of scales from first-time spawners and first-repeat spawners are easier to age. In fact, most first-time spawners and first-repeat spawners could be accurately aged a high proportion of the time; accuracy of triplicate scale aging reads of scales from known age Sitkoh Creek adults ($n=684$) appear to agree with known PIT-tag ages on average 88% for 1st repeat spawners or 90–91% for first-time spawners (Table 2). Based on Chi-square analysis, observed ages (PIT tag) compared to scale-ages (including poor and good scales) for the marine portion of the scale were not significantly different for first-time spawners and first-repeat spawners, but were for spawning adults that had spawned more than twice ($P < 0.05$). Since first-time and first-repeat spawners are also the most abundant steelhead trout in most adult populations (about 80% of the total populations from Sitkoh and Big Ratz creeks), most of the marine ages estimated from Southeast Alaska samples are likely to be accurately and precisely aged. Total circuli counts for the marine portion of the

scales were not found to be as useful, but are also provided to aid scale technicians in evaluating their aging results.

Ocean-age-1 Adult Criteria

Large circuli spacing, first-ocean summer growth followed by an annuli were typical of most steelhead trout aged (Southeast and Southcentral samples) and seems to be the typical first year pattern of faster marine growth. Sometimes a false “accessory check” or stress mark may be present following the first-ocean summer growth but prior to the first saltwater annuli. If annuli are faint immediately after the strong first year of marine growth, it is still likely that there is a first annulus present, especially if there are a large number of circuli following the first-ocean summer growth. Few fish tagged as smolt returned the following year as adult upstream migrants during all years that the Sitkoh Creek and Big Ratz Creek weirs was operated (Appendices D1 and D2). For reference, circuli counts to the first ocean annulus (ocean-age-1) on 203 scales averaged 80 circuli with a range of 45–113.

Ocean-age-2 and ocean-age-3

After the fast first-ocean summer growth (widely spaced circuli), second-ocean summer growth was typically slower, with the second-widest spacing and number of circuli observed for all adult scales compared and for all years sampled (Appendices D3 and D4). In general, spacing between annuli (fewer circuli) appeared to diminish with each passing saltwater year, but this was not always true when comparing 1st to 2nd year of saltwater growth. Ocean-age-2 and ocean-age-3 adults were the most common first-time spawning adults at Sitkoh and Big Ratz creeks, comprising 42–50% of spawning migrations (Love et al. 2013). These age classes could also be accurately aged better than 90% of the time (Table 2). Early ocean residence is often consistently evident in the morphology of adult scales, but if the first few annuli are ambiguous, then circuli count ranges could be used to corroborate age estimates.

Ocean-age-3 or greater

Spacing between annuli diminished after the second saltwater annulus and especially once spawning checks become evident, and scale growth reflected the stress of repeat spawning. The outer perimeter of the scale on repeat-spawning adults was counted as the last saltwater annulus and last year’s spawning check was often observed within this annulus for a spring sampled fish. First-time and first-repeat spawners were likely to be accurately aged better than 88 out of 100 samples aged. First-time spawners that were 3-ocean were also accurately identified better than 90% of the time (Table 2). Ocean-age-3 (X.2S1) were the most common repeat spawning adults. These 3-ocean adults that had spawned once and were on their second spawning migration represented 20–25% of the total adult spring spawners. These were accurately identified and the estimated scale age matched the PIT tag age an average of 90 out of 100 reads (Table 2). Normally, once adult steelhead trout made the initial spawning run, they returned on an annual basis if they survived the second year in the ocean. There were a few tag returns (less than 1%) from adults that had skipped a year (“skip-spawned”) following an initial spawning run.

Table 2.—Adult steelhead scale aging criteria: accuracy and circuli counts for first-time and repeat-spawners.

Ocean age (PIT tag recapture) ^a	No. recaptured	Avg % agreement with known ocean age ^b	Ocean age	Average circuli count ^c (range)
1st-time spawner 1-ocean	1	only 1 sample recaptured	1-ocean (n = 203)	80 (45-113)
1st-time spawner 2-ocean	210	90	2-ocean (n = 202)	107 (74-142)
1st-time spawner 3-ocean	238	91	3-ocean (n = 85)	124 (90-153)
1-repeat spawn (all ocean ages)	95	88	4-ocean (n = 6)	133 (110-146)
2 or more repeat spawner	140	65	> 4-ocean	>150

Note: Expected scale aging accuracy for the marine portion of adult scales for 1-, 2-, and 3-ocean first-time spawners and repeat spawners are reported. Bad scales or scales for which no two of the three aging reads matched are not included.

Note: Circuli counts by predominant ocean ages are provided to corroborate any aging estimates obtained.

^a Recaptured fish were initially tagged at Sitkoh Creek weir during 2003–2008 either as smolts or as adults

^b Estimated scale ages not significantly different from known ages based on PIT-tags ($\alpha = 0.05$) for all ocean ages based on Chi-square analysis.

^c Average circuli counts based on samples of Big Ratz Creek adult immigration during 2010–2011 for which there were readable scales.

Evaluating Aging Consistency

Evaluating scale aging consistency verifies that age interpretations do not change through time, that the age estimates obtained are comparable between agers, and can be useful in training new scale agers. Precision can be evaluated using the Coefficient of Variation (CV) to compare within- and between-reader precision, while accuracy can be monitored by practice aging of scale images and evaluation of aging estimates relative to reference samples.

Within-, and Between-Reader Precision

CV can be used to evaluate the reproducibility of the method for estimating steelhead trout scale ages and for evaluating within- and between-reader precision. Reproducibility of scale reading techniques (i.e., precision) for scale aging estimates calculated using CV, expressed as the ratio of the standard deviation (SD) over the mean (Campana 2001) using the following equation:

$$CV_j = 100\% \times \sqrt{\frac{\sum_{i=1}^R (X_{ij} - X_j)^2}{\frac{R-1}{X_j}}} \quad (1)$$

where CV_j is the age precision estimate for the j th fish, X_{ij} is the i th age determination of the j th fish, X_j is the mean age estimate of the j th fish, and R is the number of times each fish is aged. Averaging CV_j across all fish will give an overall mean CV for the method used. Mean CV

should be calculated for total ages estimated for smolt scales. For adult scales, mean CV should be calculated for total age, freshwater age, ocean age, and total number of spawning checks. CVs of about 5% are considered reference precision for many fish species (Campana 2001). Within-reader and between-reader precision can also be estimated using CV for monitoring each reader's age estimates and for comparing between readers. An example of precision analysis for 2 scale technicians that aged the same set of scales each year is provided in Table 3.

Table 3.—Example of precision analysis detailing the number of paired steelhead trout smolt scales successfully aged by 2 scale aging technicians for the 2004, 2007, and 2008 Sitkoh Creek samples ($n = 673$, of which 92 were not ageable) and the coefficient of variation (CV_j) for each scale technician and all estimated ages by sample year.

Year	Number pairwise aged	Ager 1 CV _j	Ager 2 CV _j
2004	239	2.3250	0.9752
2007	158	2.5068	1.6504
2008	184	1.9363	0.9930
Total No. aged all yrs	581		
Average CV _j all yrs		2.2560	1.2062

Within-Reader and Between-Reader Accuracy

Absolute accuracy (i.e., age matches true value) of scale ages is often not possible since known-age smolt or adult scale samples from the population being studied are often not available. Lacking known-age samples, reference collections can provide comparisons useful for interpreting scale aging methods and relative aging accuracy. Practice with reference freshwater scale images which represented the expected “ideal” image for the estimated scale age (i.e., assigned the same modal age unanimously for 3 or more replicate reads) should provide comparable results for new aging technicians (Appendices E1 and E2). Comparison to known ocean ages for scale images from Sitkoh Creek and Big Ratz Creek made from recaptured PIT-tagged adults previously scale sampled as emigrant smolts is also possible (Appendix D, E3). This allows for verification of ocean-age estimation methods through assessment of accuracy for the marine component of some of the scales. To quantify interpretation error, accuracy relative to reference scales can be evaluated by probability of assigning modal age for triplicate reads. This provides a test of within-reader accuracy. Chi-square testing of the null hypothesis that the reference age frequency of practice scales is equal to the estimated modal age frequency distribution created by a scale technician could also be used to test whether these aging estimates are acceptably accurate (Minard and Dye 1998). This provides feedback for new technicians to monitor their interpretation of scale morphology. Steelhead trout aging estimates for smolt and adult scales relative to accuracy can also be compared using age bias plots (Campana et al. 1995) to evaluate between-reader relative bias. This evaluates consistency in aging between readers or between reference ages. An example of an age bias plot comparing aging results between 2 technicians aging the same set of reference scales is provided in Figure 2. Note estimation bias.

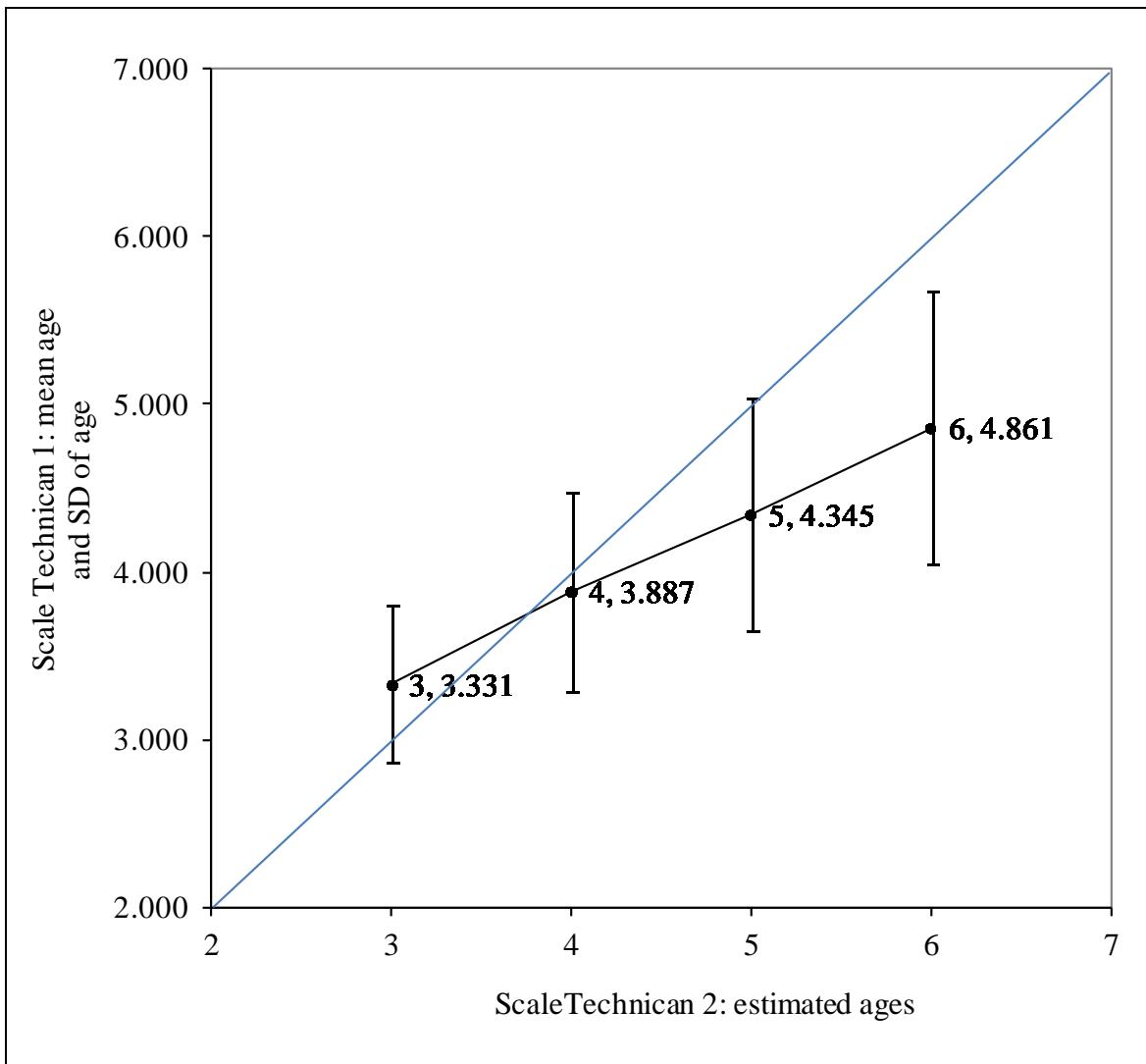


Figure 2.—Example of age bias graph of pairwise age comparisons between scale aging Technician 1 mean ages and associated SD and scale aging Technician 2 estimated ages for smolt steelhead trout from Sitkoh Creek 2003–2008.

Notes:

- The 1:1 equivalence line (blue color) is also indicated.
- Age estimation bias appears to be present relative to scale Technician 2 estimates.
- Paired aging estimates were collected for 2 scale aging technicians during 2004, 2007, and 2008 for a total sample size of 673.

Annotated Scale Images

Annotated scale images are provided in Appendices A–D. Scale morphology is relative to the different conditions likely experienced by the fish. Seasonal variability in conditions such as water temperature, food supply, and growth translate to variations in circuli spacing on the scale. Warmer temperatures and more food during the spring and summer generally produce wider, complete circuli, sometimes wavy in appearance. Colder, winter growth conditions result in the formation of tightly spaced circuli in the formation of an annulus. Circuli at the annulus also sometimes “cross over” previously deposited circuli, resulting in incomplete, broken circuli. Circuli counts for an annulus are always made to the outer circuli, or outer edge, of the annulus. Steelhead trout smolts appeared to grow similarly during their 3rd and 4th year, with a similar number of circuli deposited, and generally fewer circuli deposited than during the very rapid growth they typically experienced once they first reached the marine environment. Annotated images of representative scales from smolt estimated as freshwater ages 1–5 are provided in Appendix A.

To illustrate differences in appearance of scales from resident rainbow trout and steelhead trout smolt, annotated images of PIT-tagged and recaptured anadromous rainbow trout are included in Appendix B. Recaptures of anadromous rainbow trout indicated migration to and/or use of the estuarine-influenced portion of Sitkoh Creek below the weir. Unlike steelhead trout half-pounders, anadromous rainbow trout described here are differentiated because they appeared to be freshwater residents that were recaptured as repeat spring emigrants, often multiple times. All but one of these was sexually mature. Immature summer and fall immigrant juvenile half-pounders were also occasionally observed in Southeast Alaska stream systems. Annotated images of immigrant half-pounders that returned in July to Log Jam Creek on Prince of Wales Island, and in October to Chuck Creek on Heceta Island, are provided in Appendix C. Both of these were also not sexually mature. Annotated images of scales from freshwater-age-3 and freshwater-age-4 smolts, which were initially PIT tagged as smolts then recaptured as first-time, and repeat spawning adults are presented in Appendix D. Some of these images are from PIT-tagged fish (initially tagged as smolt) that were recaptured and scale sampled multiple times.

Freshwater and Marine Aged Examples for Practice

At the beginning of the season, scale technicians should practice scale aging of these smolt scales using relevant features and standardized methods prior to aging new samples and should occasionally review the reference set and methods. When scale aging criteria are not used for a period of time, readers may forget or alter them and consistency in aging may suffer (Erickson 1999). Annotated scale images provide visual examples of the variation in scale images from various life-history stages of steelhead trout and are useful in training new scale aging technicians to be more standardized in their approach to aging, and more precise in their aging methods of steelhead trout smolt, anadromous rainbow trout, half-pounders, and adult steelhead (Appendices A–D).

Potential readers should review the smolt scale images to learn the relevant features discussed and compare to the aging criteria to understand the estimated total age. The reader then should apply the methods learned to the verified-age smolt practice scale images available at ADF&G DSF off in Douglas. Equivalent-aged (same reads 5 or more times by multiple technicians) samples of smolt scales are provided in Appendix E1. Parr and smolt scale images that were verified by comparison to otolith samples (i.e., same age as paired otolith) are provided in

Appendix E2. Examples of known ocean-age scale samples from adults that were recaptured at Sitkoh Creek weir during 2005–2009 that had matching ages each time they were read are listed in Appendix E3. Images are available from for review from the ADF&G DSF office in Douglas.

DISCUSSION

Consistency in scale aging involves process and interpretation (Campana 2001). Standardized processes aid the scale technician with precision, or in making reproducible age estimates. Reference or known-age samples provide relative comparisons that are useful for interpreting scale morphology accurately. Standardized processes for aging scales from Southeast Alaska steelhead, including modal aging, identification of first annuli, interpretation of plus growth, and use of circuli count and fork length criteria, should help to maintain and promote consistency in estimating ages. The protocols documented here should allow new technicians to make repeatable and comparable estimates to previous samples, to evaluate aging estimates, and to monitor consistency in age estimation.

Steelhead trout scale aging is complicated by variable life history patterns exhibited during residence in freshwater and marine habitats. Although freshwater residence of juvenile steelhead trout in Southeast Alaska may range from 2–6 years, freshwater-age-3 and freshwater-age-4 juveniles dominate the age structure, which in turn leads to scale samples from these ages dominating the scale collection (approximately 70–80%) to be used for reference material (Love et al. 2012b). Using documented methods and criteria, precision in aging these ages of steelhead trout should be consistent for about 80 samples for each 100 evaluated. Relatively large sample sizes are available for these more commonly encountered ages, which in turn contributes to greater precision. However, as with any reference collection there is significant benefit in having sufficient samples representative of the entire population—or, in this case, all ages. In Southeast Alaska, additional samples from freshwater-age-1, freshwater-age-2, and freshwater-age-5 steelhead trout smolt are needed. Any new steelhead trout project should also attempt to identify the first freshwater winter, because not knowing that first annulus will mean inaccuracies in aging by at least 1 year. Precision and relative accuracy of age estimates (i.e., relative to reference collections) for freshwater ages should also be reported for all new studies that are initiated. This would provide a measure of continuity in age estimates for future projects and years. Additional scale samples need to continue to be collected, imaged, archived, and aged from a variety of unique life history variants from different populations and steelhead trout habitats.

The most common marine ages encountered in Southeast Alaska steelhead were ocean-age-2 and ocean-age-3 fish, which represented adults that returned as either first-time spawners or first-repeat spawners (Love et al. 2012b). Known ocean-age samples from these predominant age classes were used to develop accurate and standardized processes. Scale technicians should be expected to age most first-time spawners and first-repeat spawners accurately for a high proportion of the time—on average 90% for first-time spawners and 88% for first-repeat spawners. Estimated ages should not be significantly different from expected, and this assumption should be tested and reported. Practice with known-aged scale images prior to the onset of aging efforts should help in training new technicians and in maintaining accuracy. Practice images should periodically be re-visited and consistency re-evaluated for all technicians. Employing unique tagging methods (e.g., PIT tags) during steelhead trout sampling projects provides the ability to obtain known-age samples for verifying accuracy, and as such this is very

much encouraged. The need for experienced scale aging technicians, training, the use of standardized methods, and evaluation of consistency in scale aging cannot be overemphasized. Evaluating aging consistency throughout a project is important if age estimates are to be meaningful relative to other years of the same project and to other projects aging steelhead trout scales. Although this document is not expected to be the last word on describing consistent aging methodology for steelhead trout scales, it is intended to serve as a standardized origin and reference manual for future studies of steelhead trout when age composition data are required.

ACKNOWLEDGMENTS

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**APPENDIX A: ANNOTATED IMAGES OF SITKOH CREEK
AND TYE CREEK STEELHEAD PARR AND SMOLT
SCALES, SOUTHEAST ALASKA, 2004–2011**

Minnow Trapping sample

TYESH 2011_18_B



1.0 mm

0.0

Appendix A1.—Freshwater aging example: scale image estimated as age 0.0 (freshwater duration < 1 year) from steelhead trout parr captured in Tye Creek, Prince of Wales Island, in August of 2011.

Note: This fish was likely hatched in the spring of 2011 and grew to 55 mm FL when it was sampled on 8/26/11.

Minnow Trapping sample

TYESH_2011_20A



1.0 mm

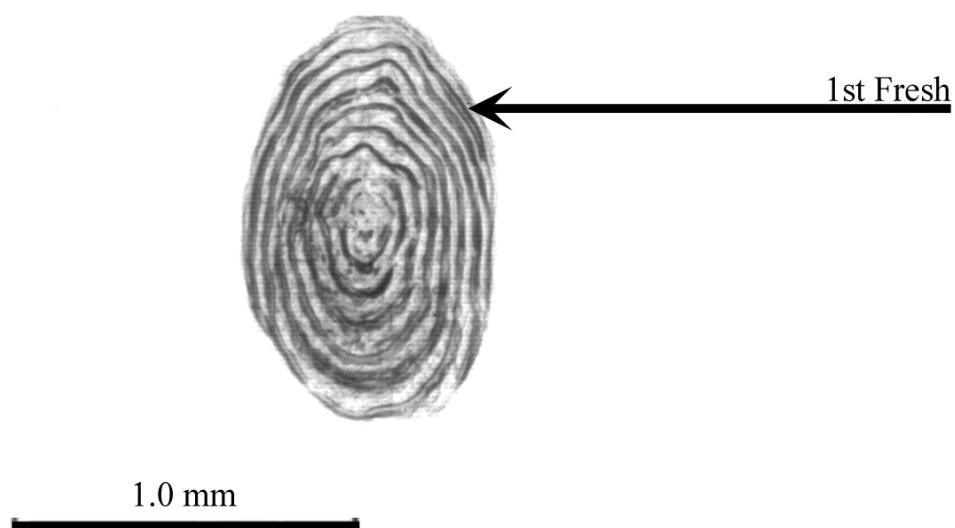
0.0

Appendix A2.—Freshwater aging example: scale image estimated as age 0.0 from steelhead trout parr captured in Tye Creek, Prince of Wales Island, in August of 2011.

Note: This fish was likely hatched in the spring of 2011 and grew to 50 mm FL when it was sampled on 8/26/11.

Minnow Trapping sample

07sitSHM_018-c



1.0

Appendix A3.—Freshwater aging example: scale image estimated as age 1.0 (58 mm FL) from a steelhead trout parr captured on 4/17/07 in Sitkoh Creek minnow trapping samples.

Minnow Trapping sample

07sitSHM_002-c



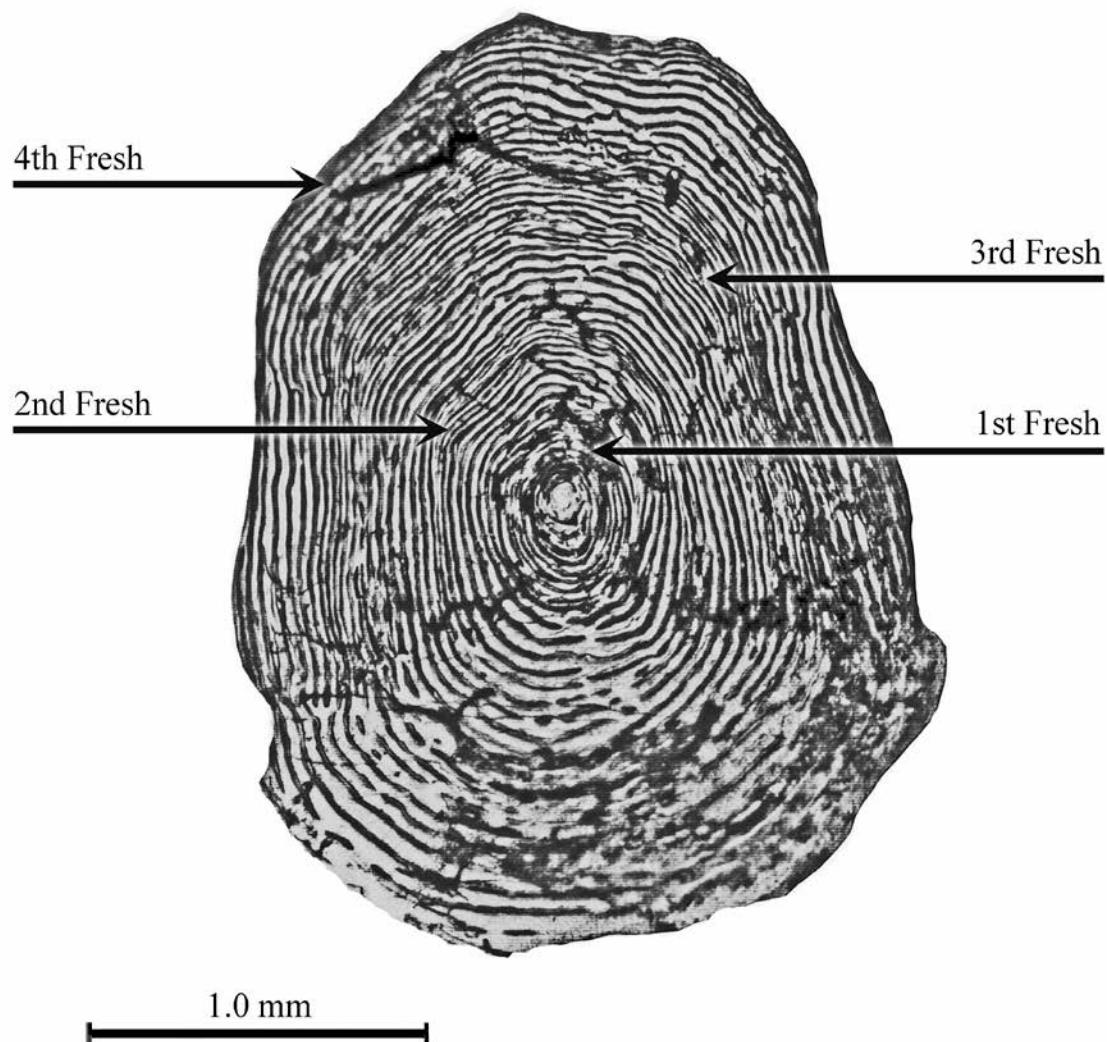
Appendix A4.—Freshwater aging example: scale image estimated as age 2.0 (66 mm FL) from a steelhead trout parr captured on 4/15/07 in Sitkoh Creek minnow trapping samples.

PIT 445A047520
04sitSH0066



Appendix A5.—Freshwater aging example: scale image estimated as age 3.0 (192 mm FL) from an emigrant steelhead trout smolt captured 5/23/04 at Sitkoh Creek weir.

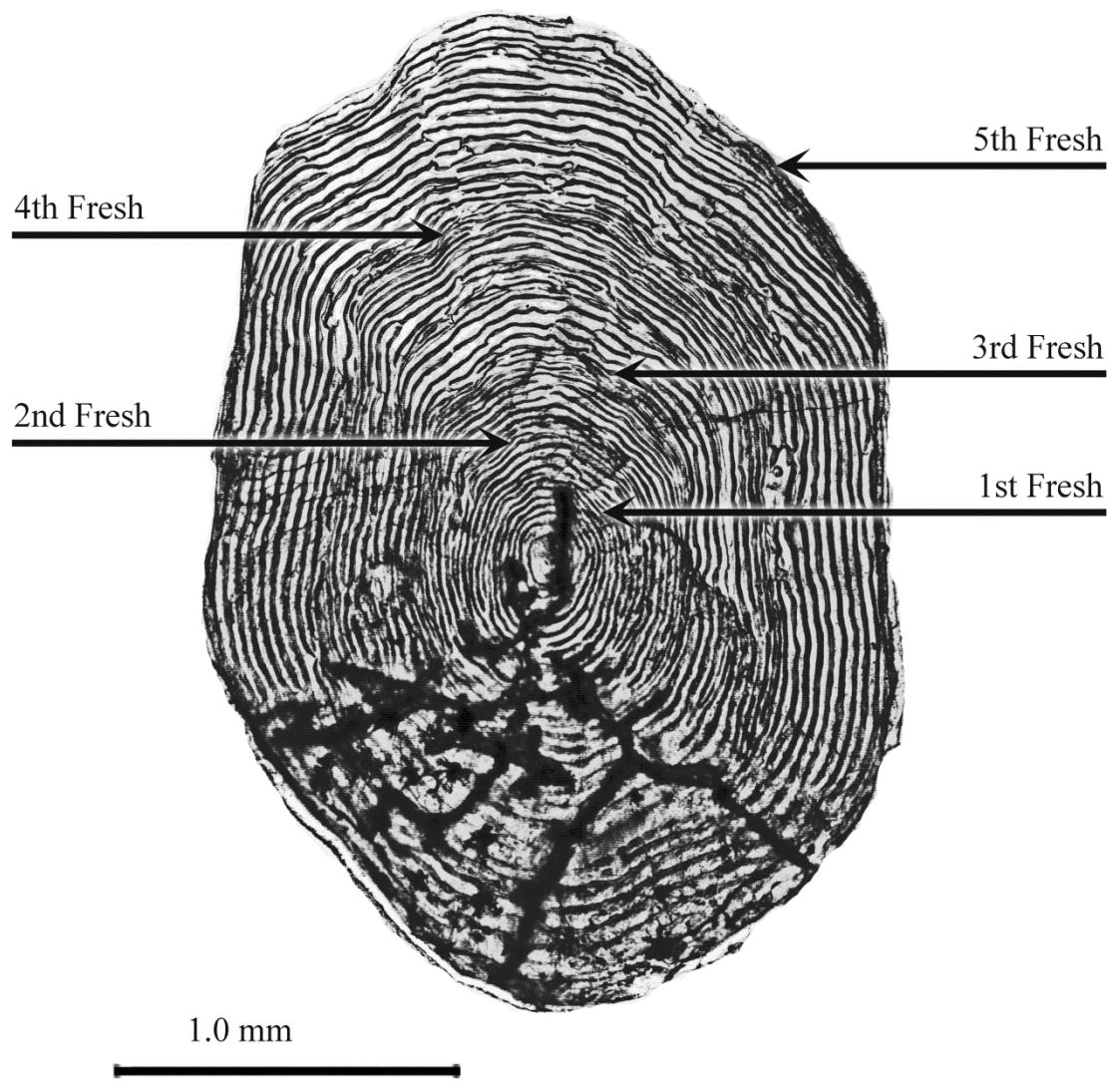
PIT 4462461045
04sitSH0029



4.0

Appendix A6.—Freshwater aging example: scale image estimated as age 4.0 (206 mm FL) from an emigrant steelhead trout smolt captured 5/20/04 at Sitkoh Creek weir.

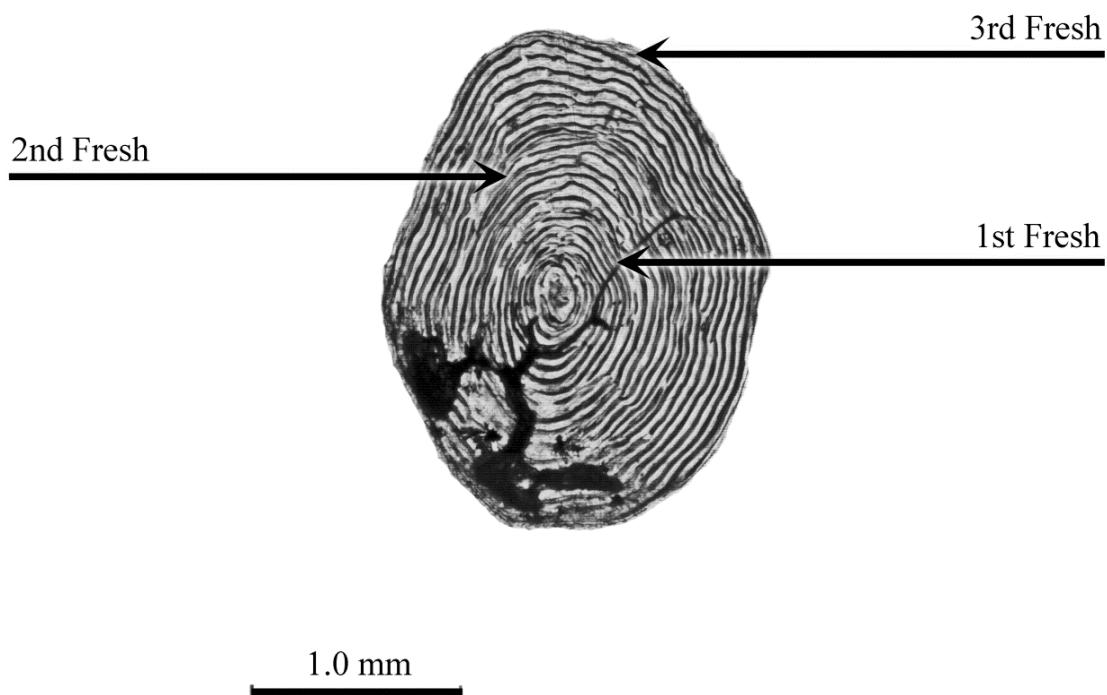
PIT 3D91BF239BC7
06sitSH0319



Appendix A7.—Freshwater aging example: scale image estimated as age 5.0 (261 mm FL) from an emigrant steelhead trout smolt captured 6/3/06 at Sitkoh Creek weir.

**APPENDIX B: ANNOTATED IMAGES OF ANADROMOUS
RAINBOW TROUT SCALES FROM SITKOH CREEK,
2006–2009**

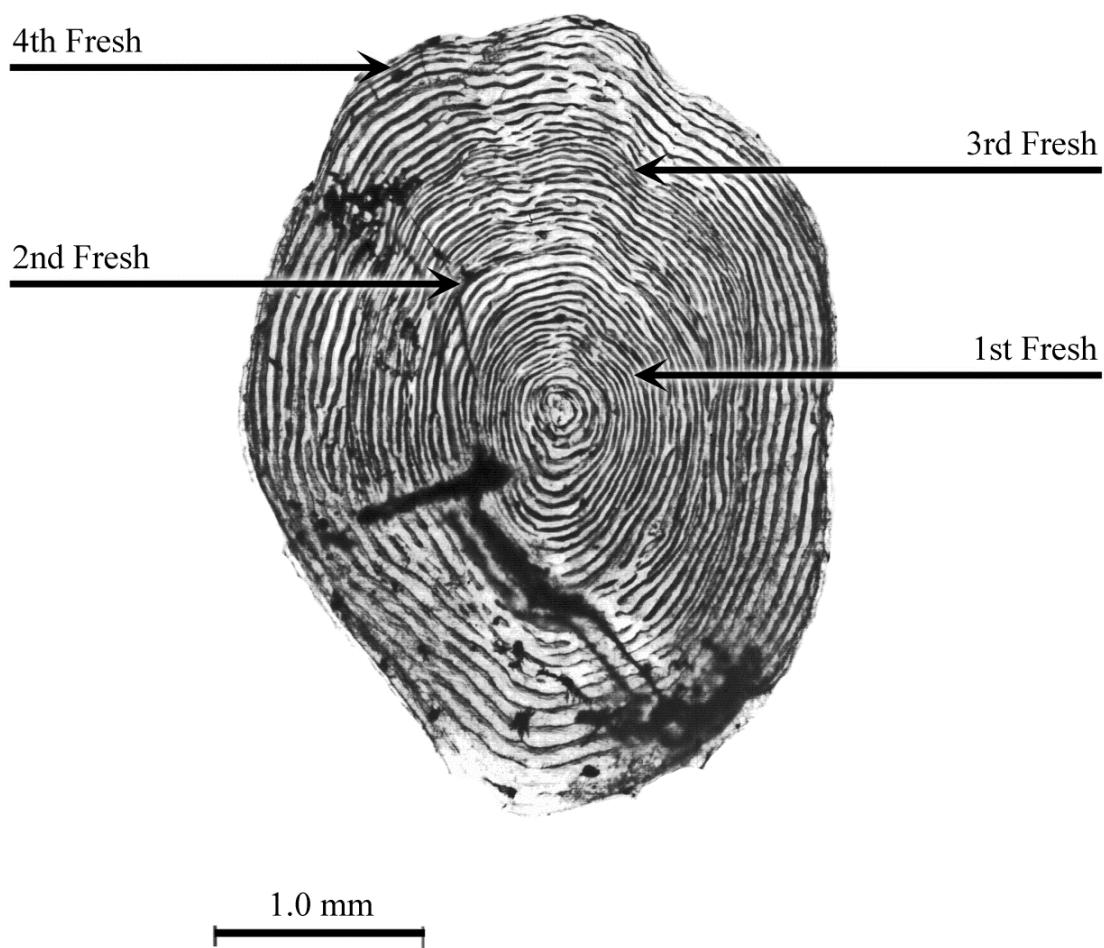
PIT 3D91BF23AF093
06sitSHJ0017



3.0

Appendix B1.—Rainbow aging example: scale image estimated as age 3.0 from immature (140 mm FL) emigrant rainbow trout smolt implanted with PIT tag 3D91BF23AF093 captured at Sitkoh Creek weir, 2006.

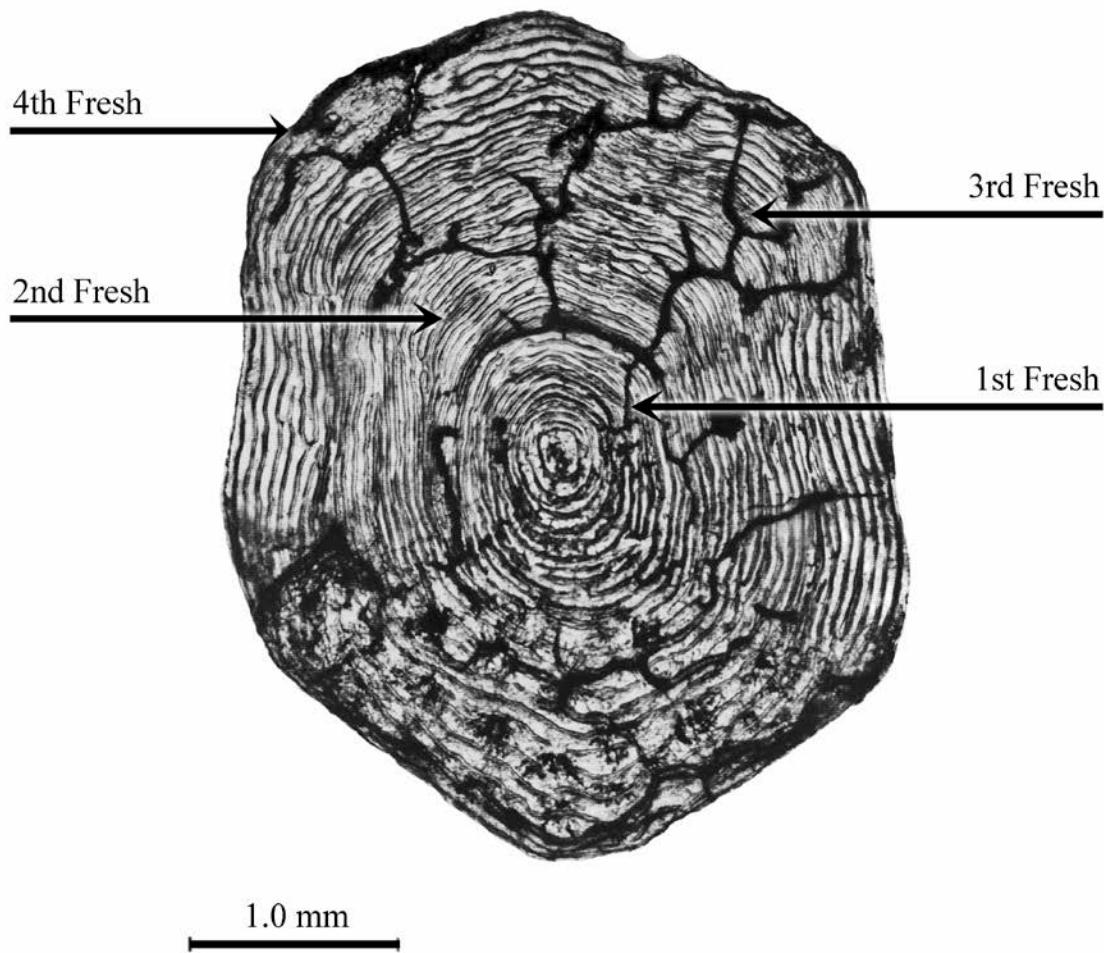
PIT 3D91BF23AF093
07sitRBT0004



4.0

Appendix B2.—Rainbow aging example: scale image estimated as age 4.0 from a mature male (190 mm FL) immigrant rainbow trout recaptured in 2007 that was previously captured, scale sampled, and PIT-tagged at Sitkoh Creek weir as an emigrant in 2006 (Appendix B1).

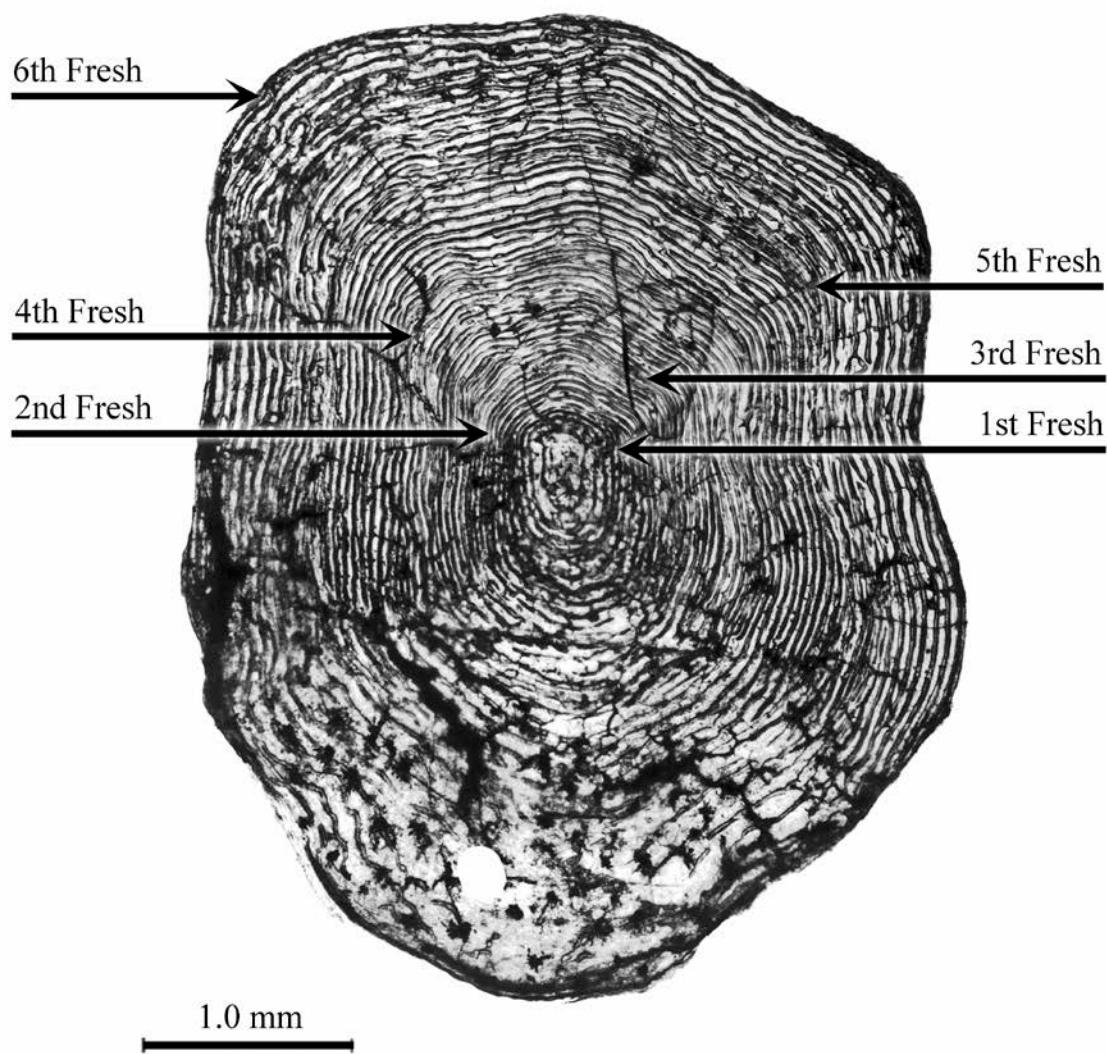
3D91BF237AA4C
06sitRBT0008



4.0

Appendix B3.—Rainbow aging example: scale image estimated as age 4.0 from a mature male (232 mm FL) emigrant rainbow trout captured and PIT-tagged with tag 3D91BF237AA4C at Sitkoh Creek weir, 2006.

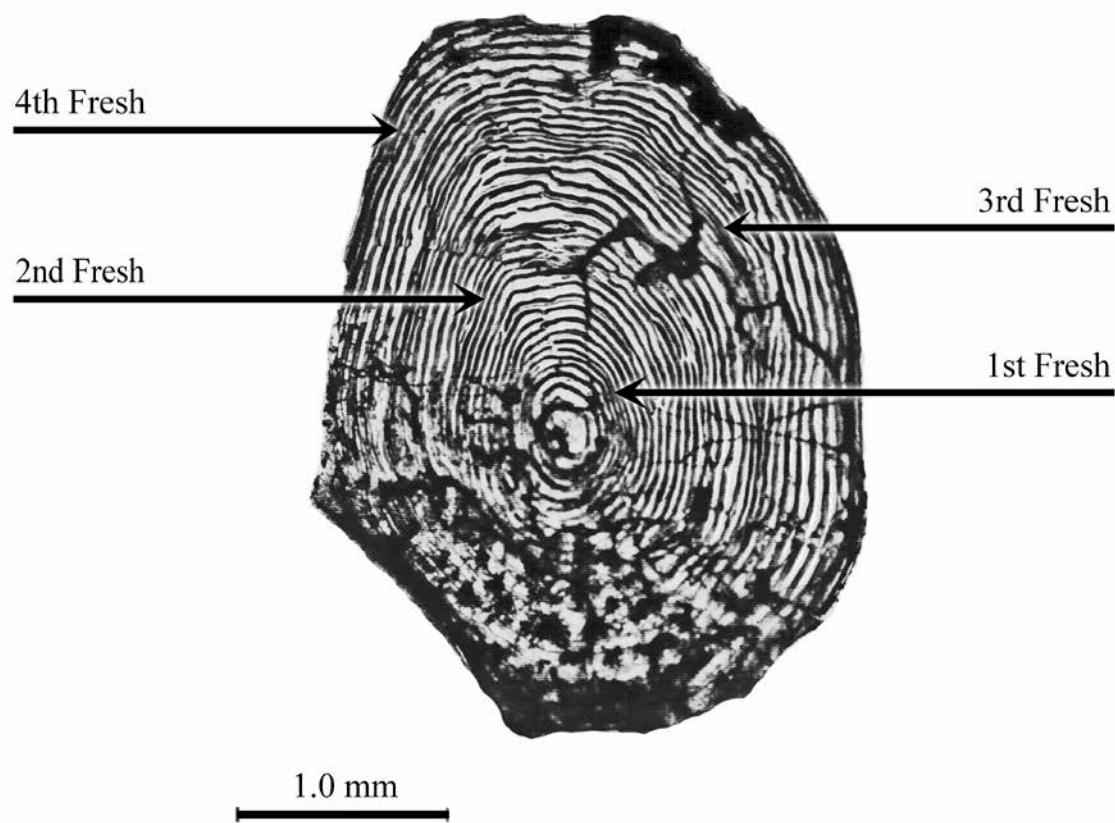
3D91BF237AA4C
08sitRBT0003



6.0

Appendix B4.—Rainbow aging example: scale image estimated as age 6.0 from a mature male (350 mm FL) emigrant rainbow trout recaptured in 2008 that was PIT-tagged and scale sampled two years previously at Sitkoh Creek weir (Appendix B3).

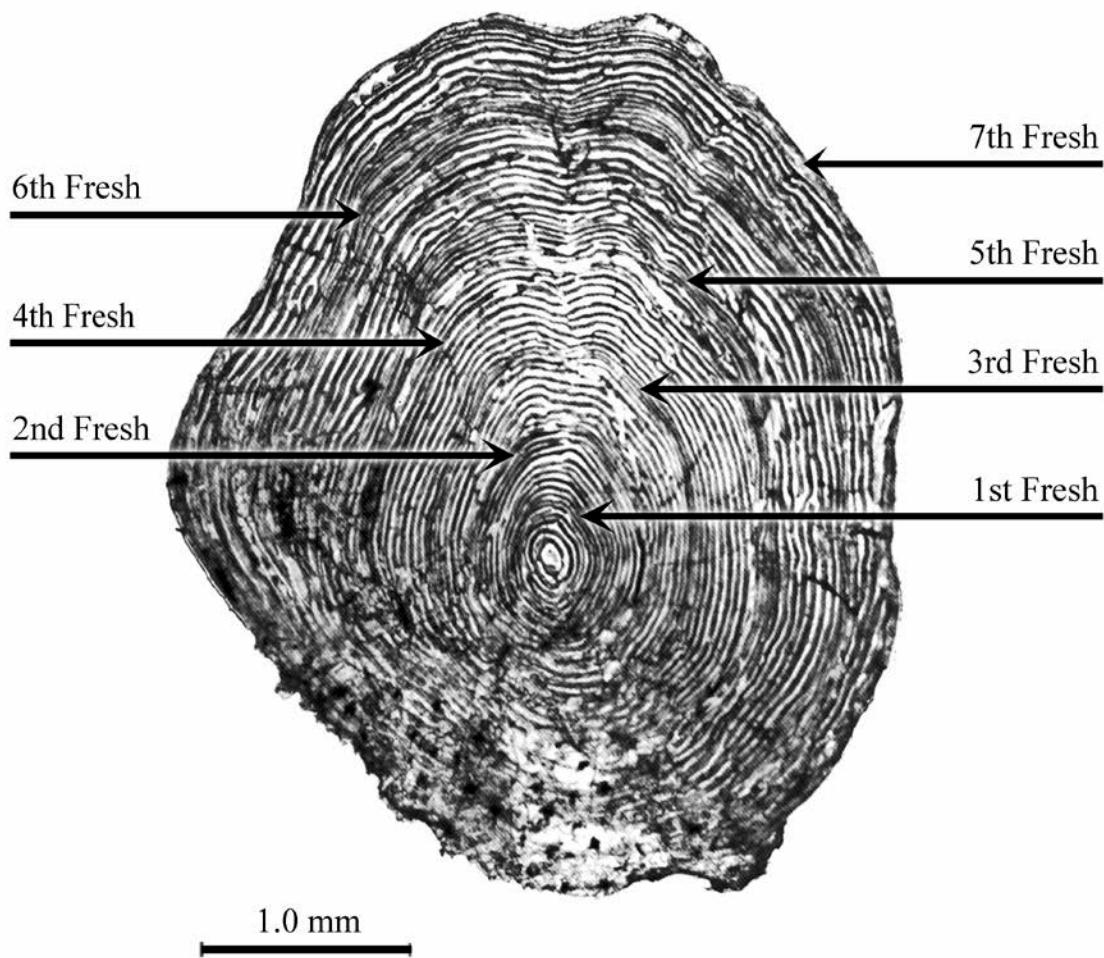
3D91BF237A146
06sitRBT0012



4.0

Appendix B5.—Rainbow aging example: scale image estimated as age 4.0 from mature male (210 mm FL) emigrant rainbow trout PIT-tagged with tag 3D91BF237A146 at Sitkoh Creek weir, 2006.

3D91BF237A146
09sitRBT_014R_02

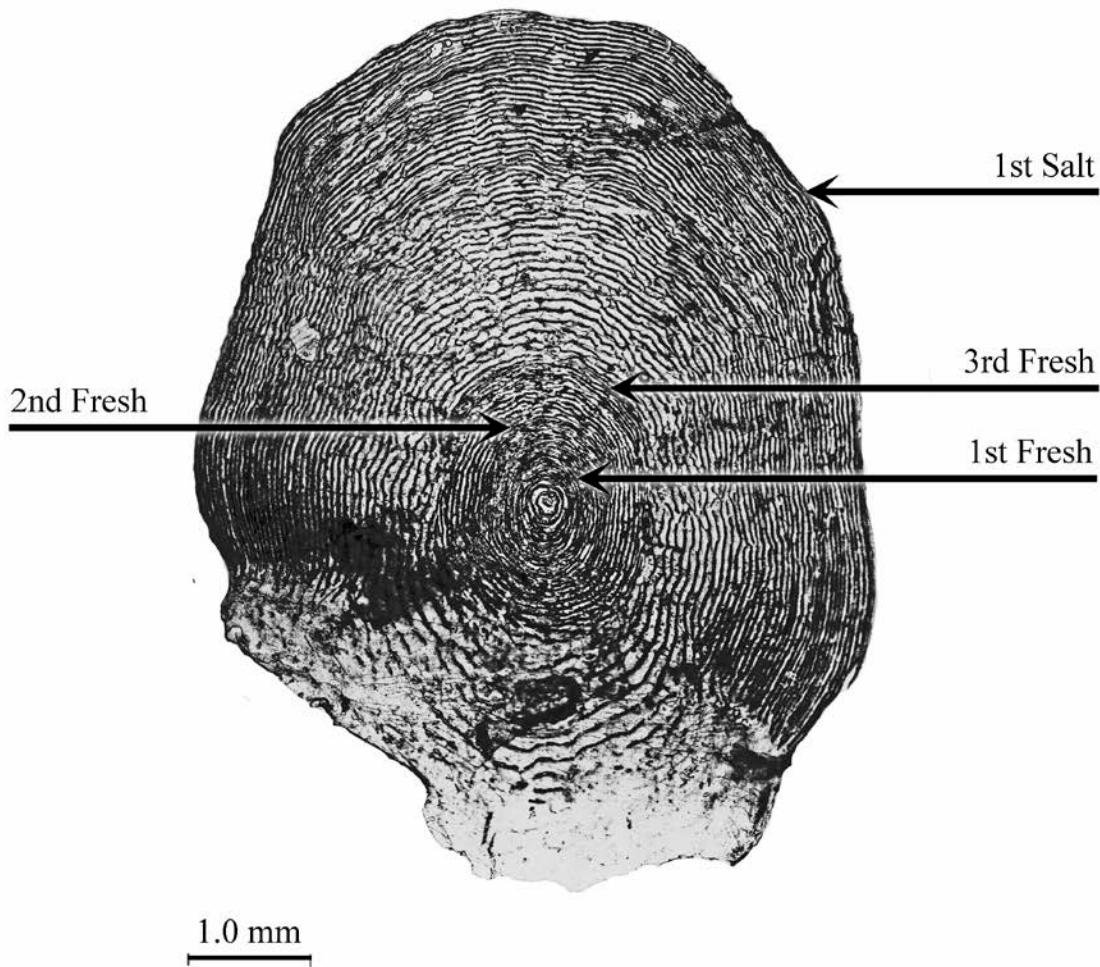


7.0

Appendix B6.—Rainbow aging example: scale image estimated as age 7.0 from a mature male immigrant rainbow trout (378 mm FL) recaptured in 2009 that was initially sampled at Sitkoh Creek weir 3 years previously as an emigrant adult (Appendix B5).

**APPENDIX C. ANNOTATED IMAGES OF 1-OCEAN
SUMMER/FALL IMMATURE IMMIGRANT STEELHEAD
TROUT SCALES COLLECTED FROM LOG JAM CREEK
AND CHUCK CREEK, SOUTHEAST ALASKA, 2004–2011**

Log Jam creek-Summer Steelhead (sampled 7/16/11; 485mm TL)



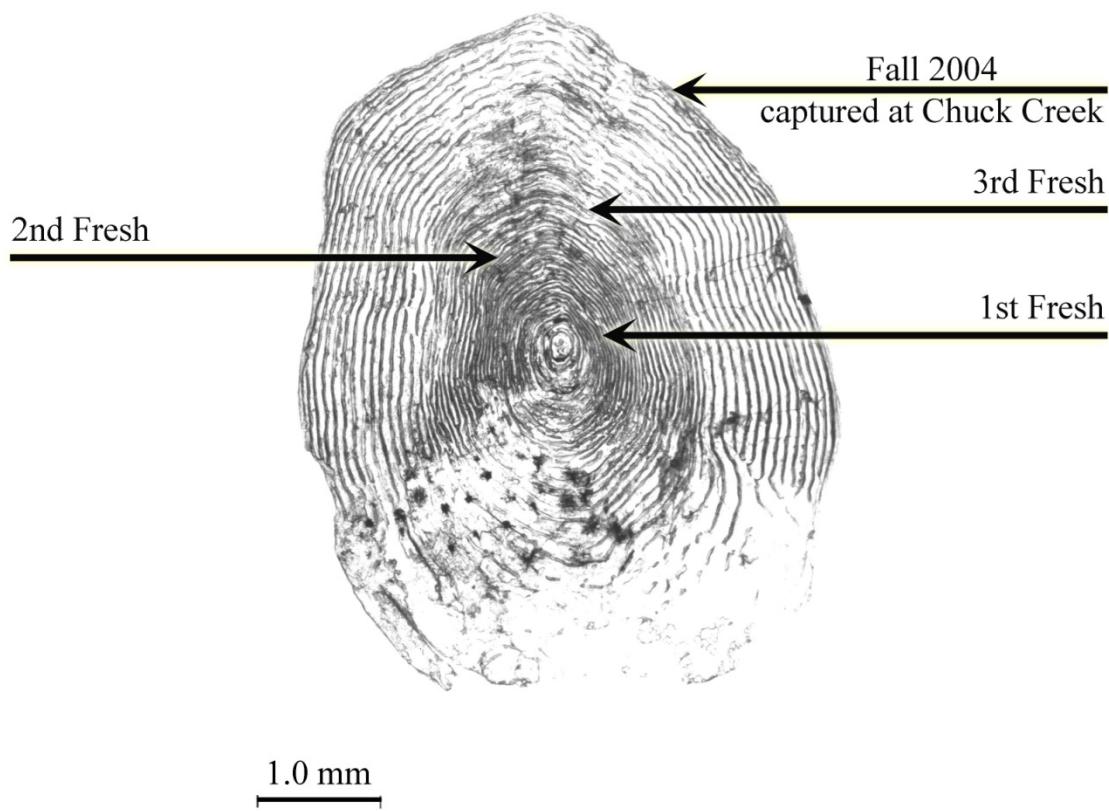
3.1

Appendix C1.—Summer steelhead trout example: scale image estimated as age-3.1, or 3-freshwater, 1-ocean.

Note: This immature summer immigrant fish (485 mm TL) was captured 7/16/11 in Log Jam Creek, Prince of Wales Island.

Chuck Creek fall steelhead

04chuckSH_001



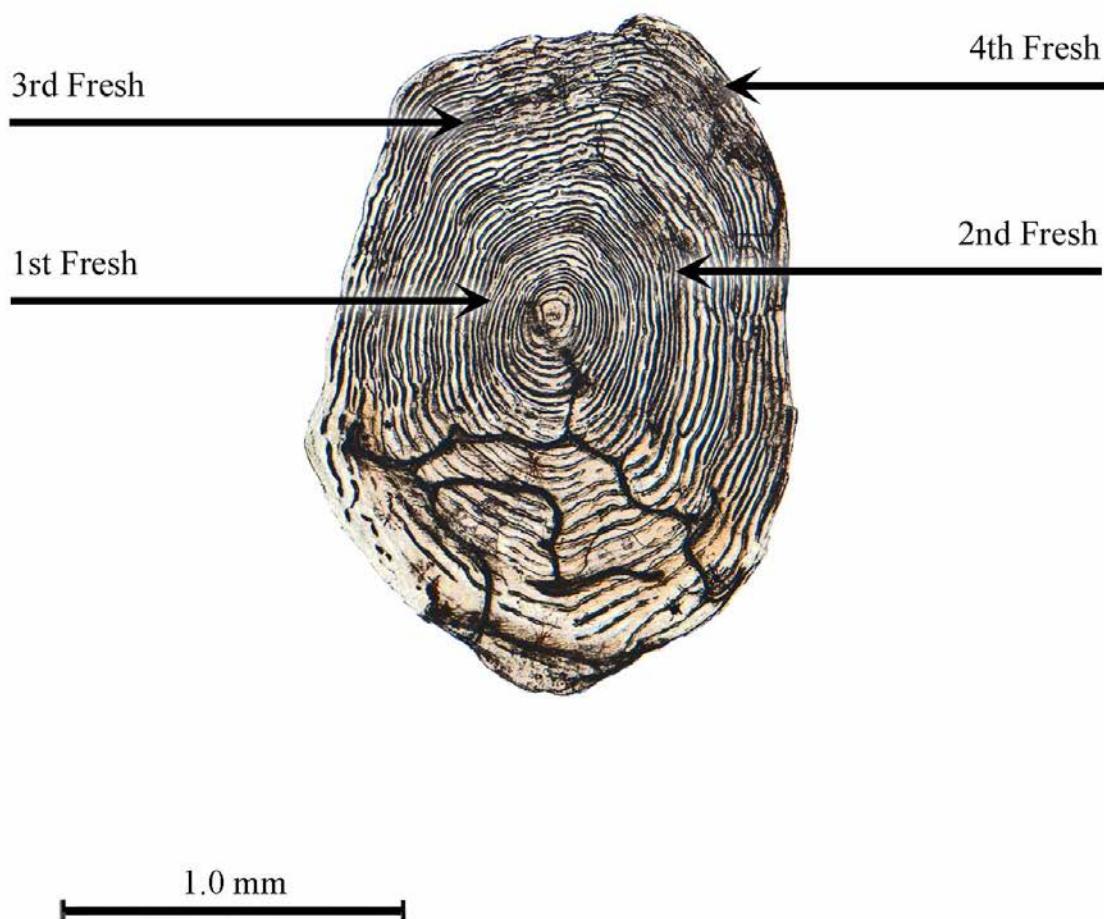
3.1

Appendix C2.—Fall steelhead trout example: scale image estimated as age-3.1, or 3-freshwater, 1-ocean (less than a full annulus).

Note: The annulus appears just to be forming. This immature immigrant “half-pounder” (315 MEF) was captured 10/5/04 in Chuck Creek, Heceta Island.

APPENDIX D. ANNOTATED SCALE IMAGES OF PIT-TAGGED STEELHEAD TROUT SMOLT THAT WERE RECAPTURED FROM THE SITKOH CREEK AND BIG RATZ CREEK WEIRS, SOUTHEAST ALASKA, 2003–2011

PIT 3D9257C665CD8
2010 RatzSHJ_0315

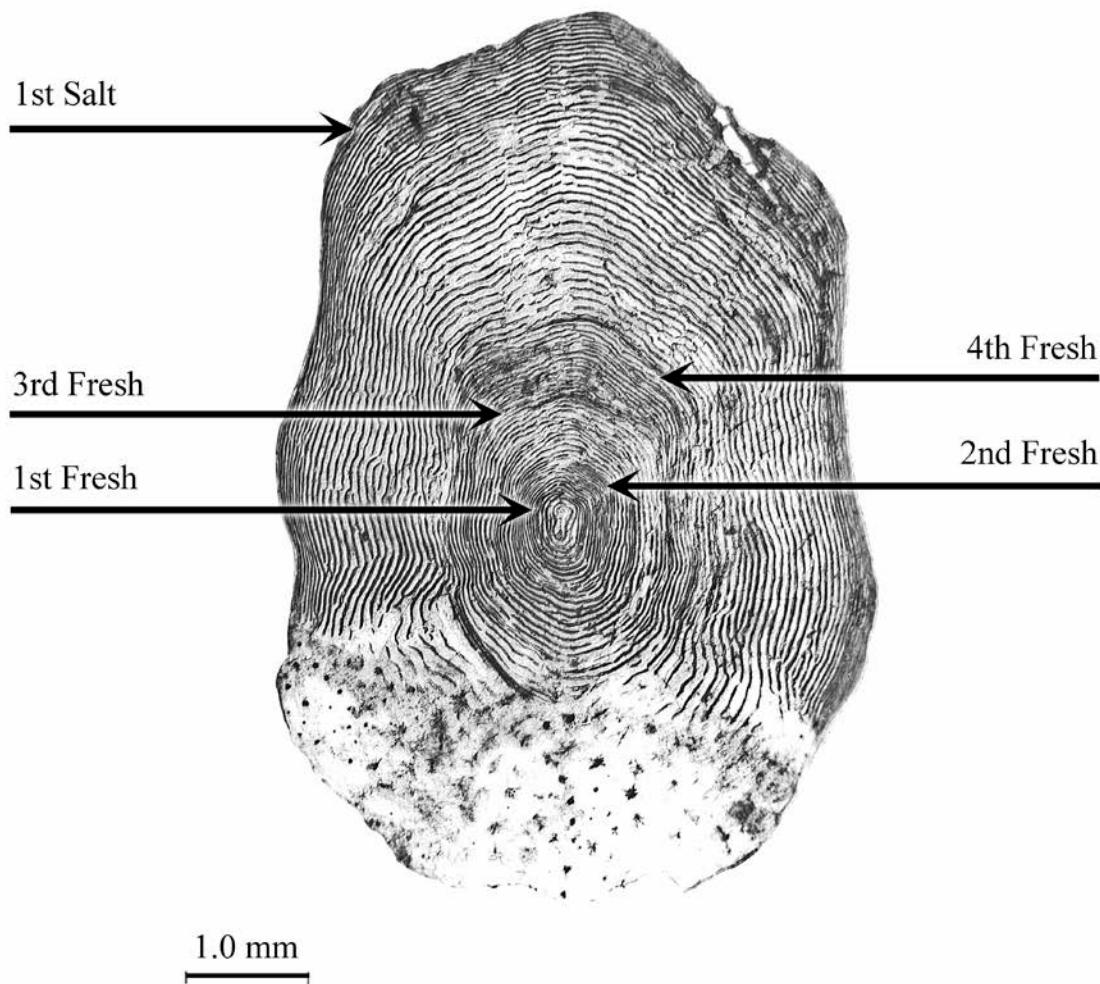


4.0

Appendix D1.—Steelhead trout aging example: scale image estimated as age 4.0 (238 mm FL) from emigrant steelhead trout smolt initially tagged with tag 3D9257C665CD8 on 5/13/10 at Big Ratz Creek weir.

PIT 3D9257C665CD8

2011RatzUpRecap_0303

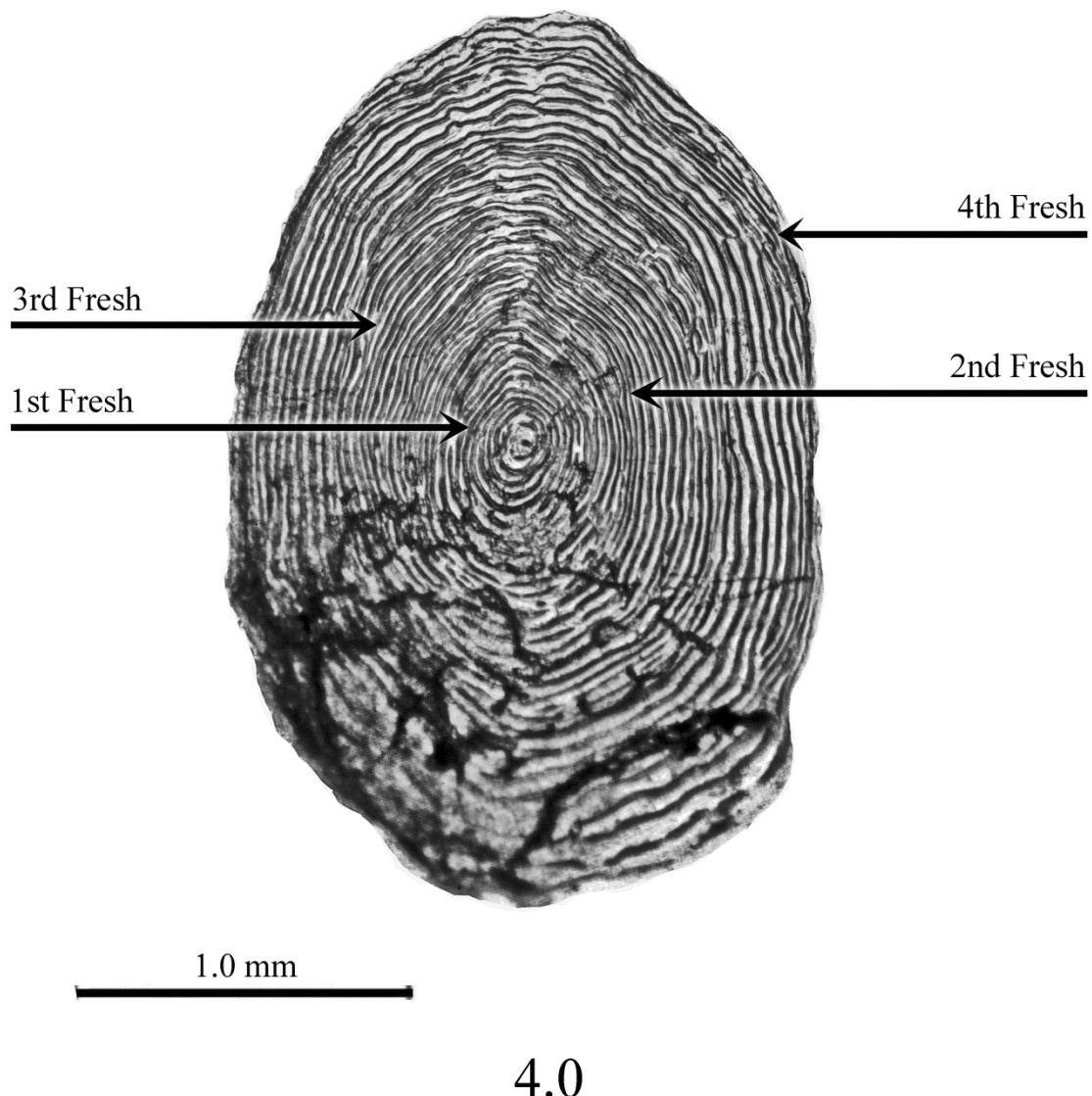


4.1

Appendix D2.—Steelhead trout aging example: scale image estimated as age-4.1, or 4-freshwater, known 1-ocean spring immigrant adult male (500 mm FL) steelhead trout on first spawning migration that was sampled the previous year at Big Ratz Creek weir (Appendix D1) and was recaptured 4/28/11.

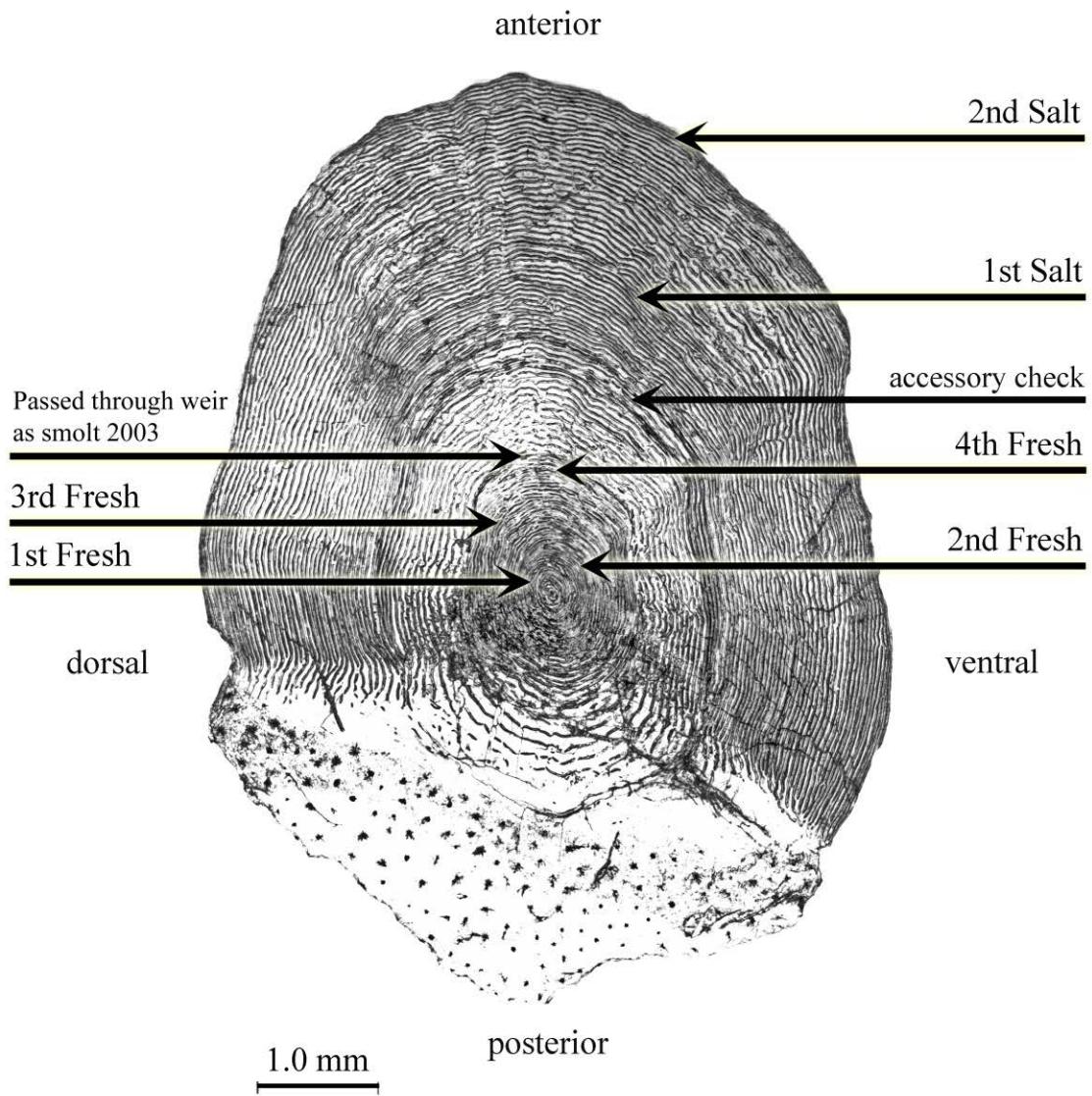
Note: Strong first-ocean growth is evident.

PIT 134554111A
03 sitSH2379



Appendix D3.—Steelhead trout aging example: scale image estimated as age-4.0 (200 mm FL) from emigrant steelhead trout smolt initially PIT-tagged (PIT 134554111A) at Sitkoh Creek weir on 6/6/03.

PIT 134554111A
05sitSH010R_07

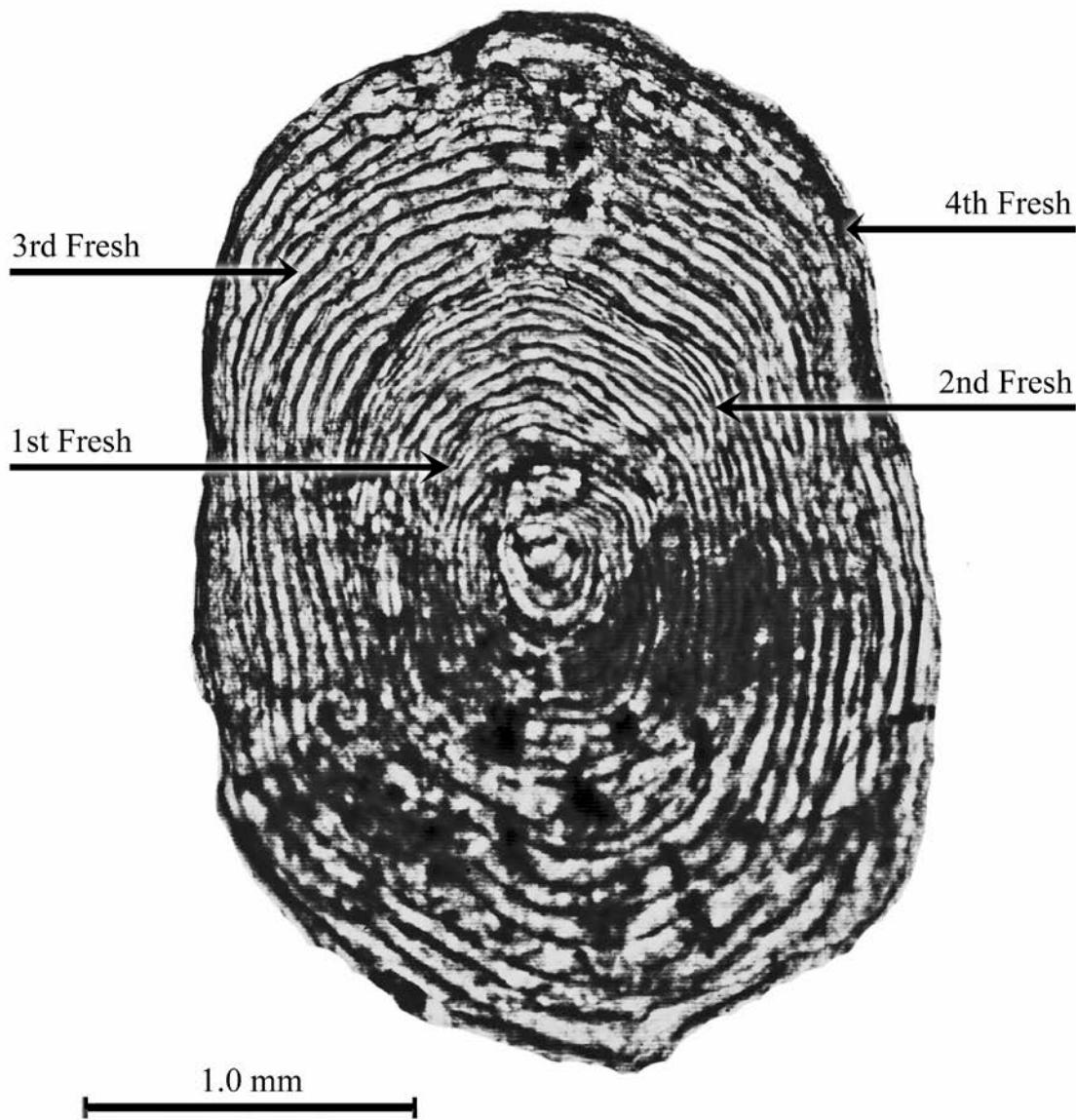


4.2

Appendix D4.—Steelhead trout aging example: scale image estimated as age-4.2 (752 mm FL), or 4-freshwater (Appendix D3) spring immigrant adult steelhead trout on first spawning migration that was recaptured at Sitkoh Creek weir on 5/6/05 as a known 2-ocean adult.

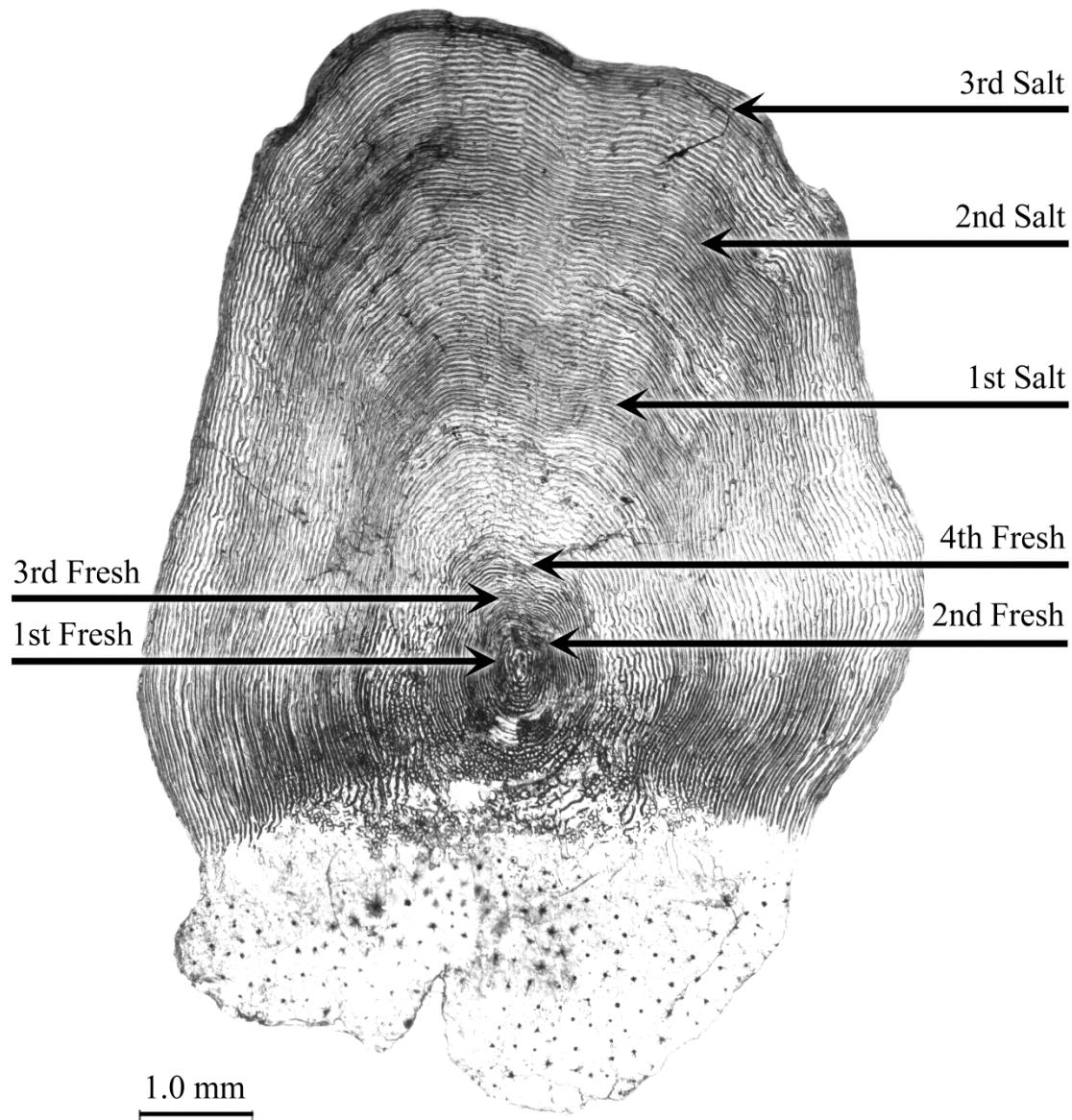
Note: Accessory check prior to first saltwater annulus is evident.

PIT 133655296A
03sitSH2385



Appendix D5.—Steelhead trout aging example: scale image estimated as freshwater age-4.0 (179 mm FL) from emigrant steelhead trout smolt PIT tagged with PIT tag 133655296A at Sitkoh Creek weir on 6/6/03.

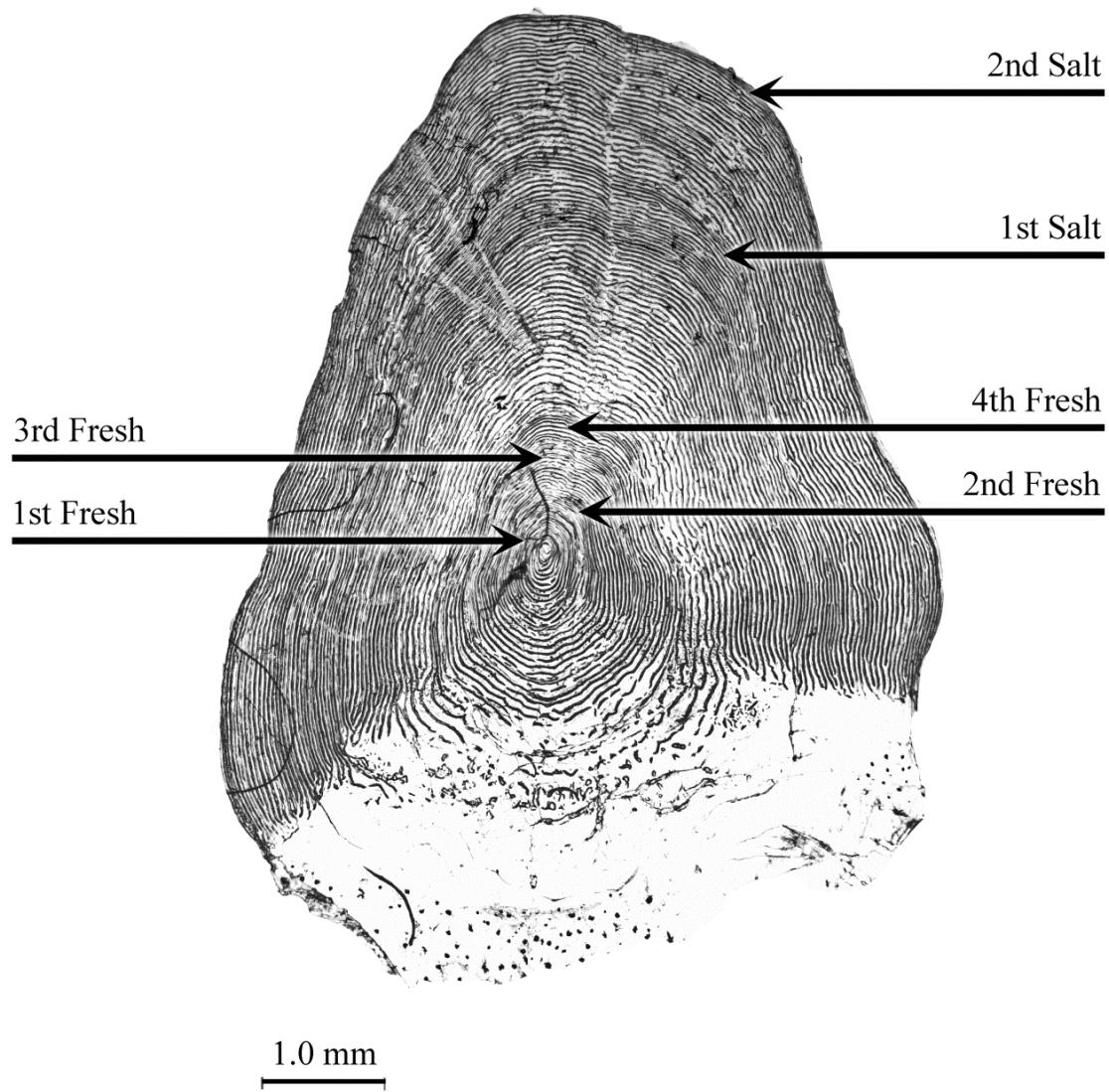
PIT 133655296A
06sitSH006R_01



4.3

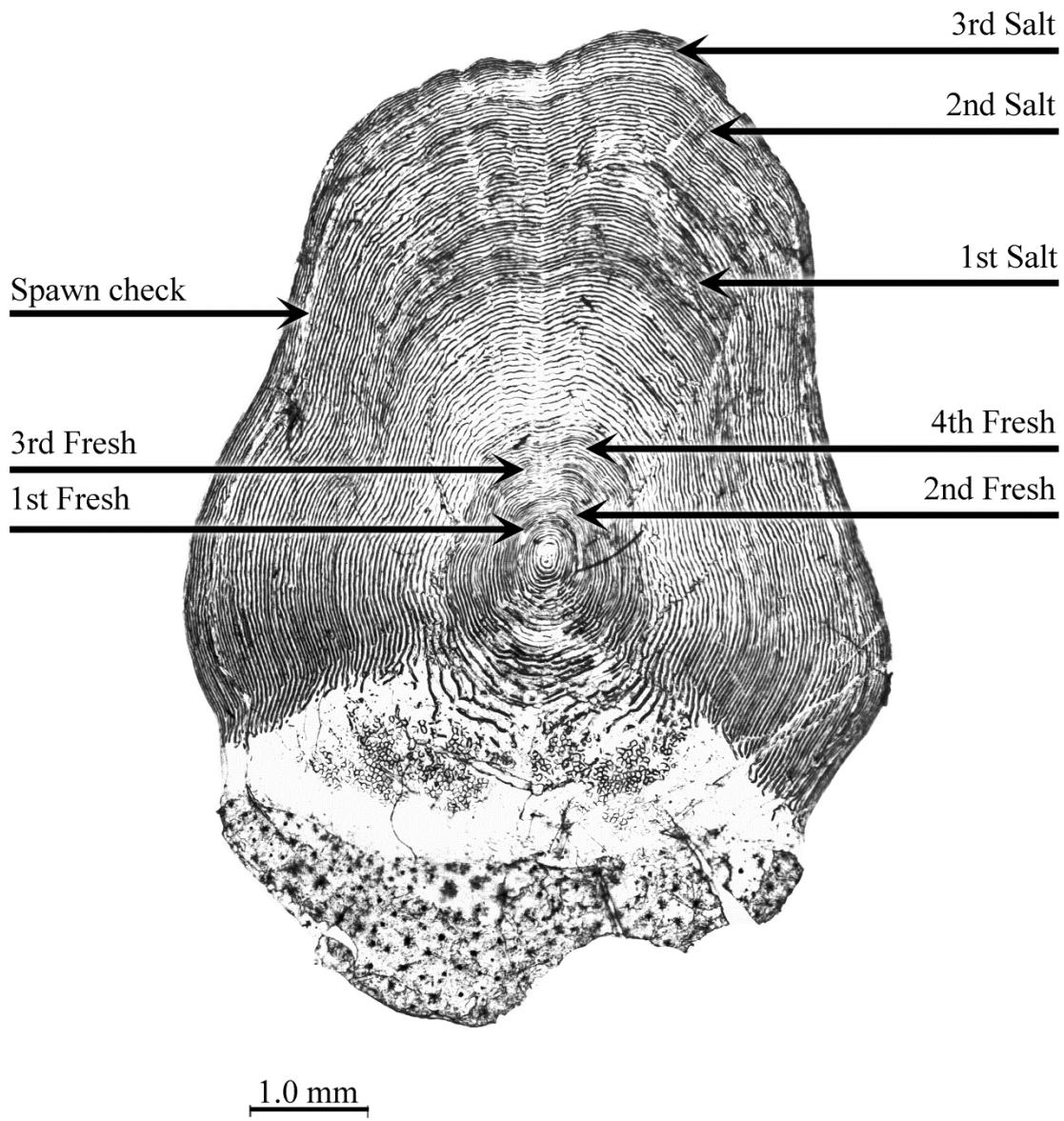
Appendix D6.—Steelhead trout aging example: scale image estimated as age-4.3 (713 mm FL), or 4-freshwater, known 3-ocean spring immigrant adult steelhead trout on first spawning migration that was recaptured at Sitkoh Creek weir on 5/6/06.

PIT 44607D5148
06sitSH014R_02



Appendix D7.—Steelhead trout aging example: scale image estimated as age-4.2 (630 mm FL) from known (no scale sample as smolt) 2-ocean first-spawning spring immigrant adult steelhead trout tagged with PIT tag 44607D5148 at Sitkoh Creek weir, 5/29/06.

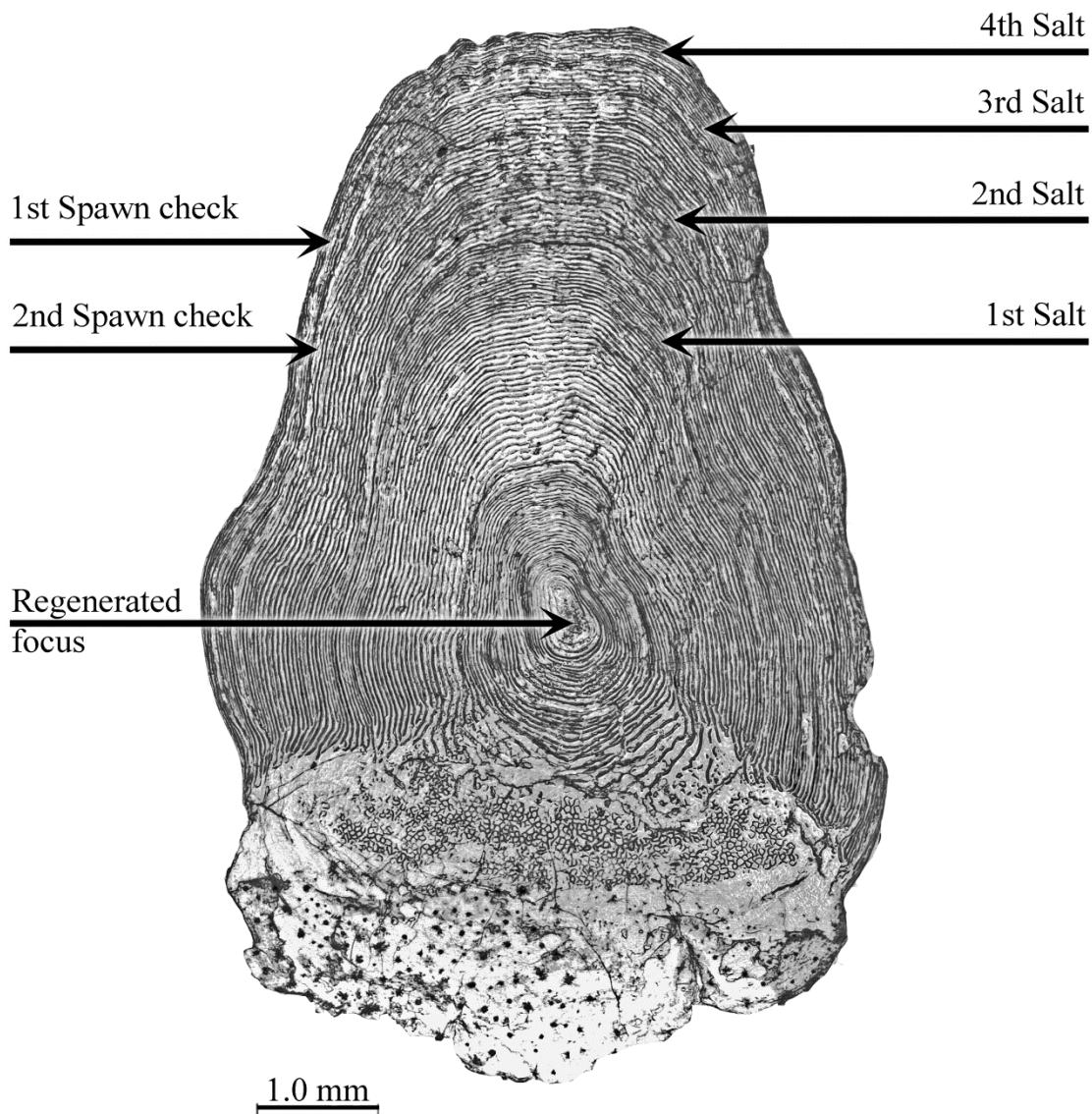
PIT 44607D5148
07sitSH011R_02



Appendix D8.—Steelhead trout aging example: scale image estimated as age-4.2S1 (670 mm FL), or 4-freshwater, known 3-ocean, 1-repeat, spring immigrant adult steelhead trout on second spawning migration that was recaptured at Sitkoh Creek weir, 5/19/07.

Note: Spawn check near outer margin of scale is evident.

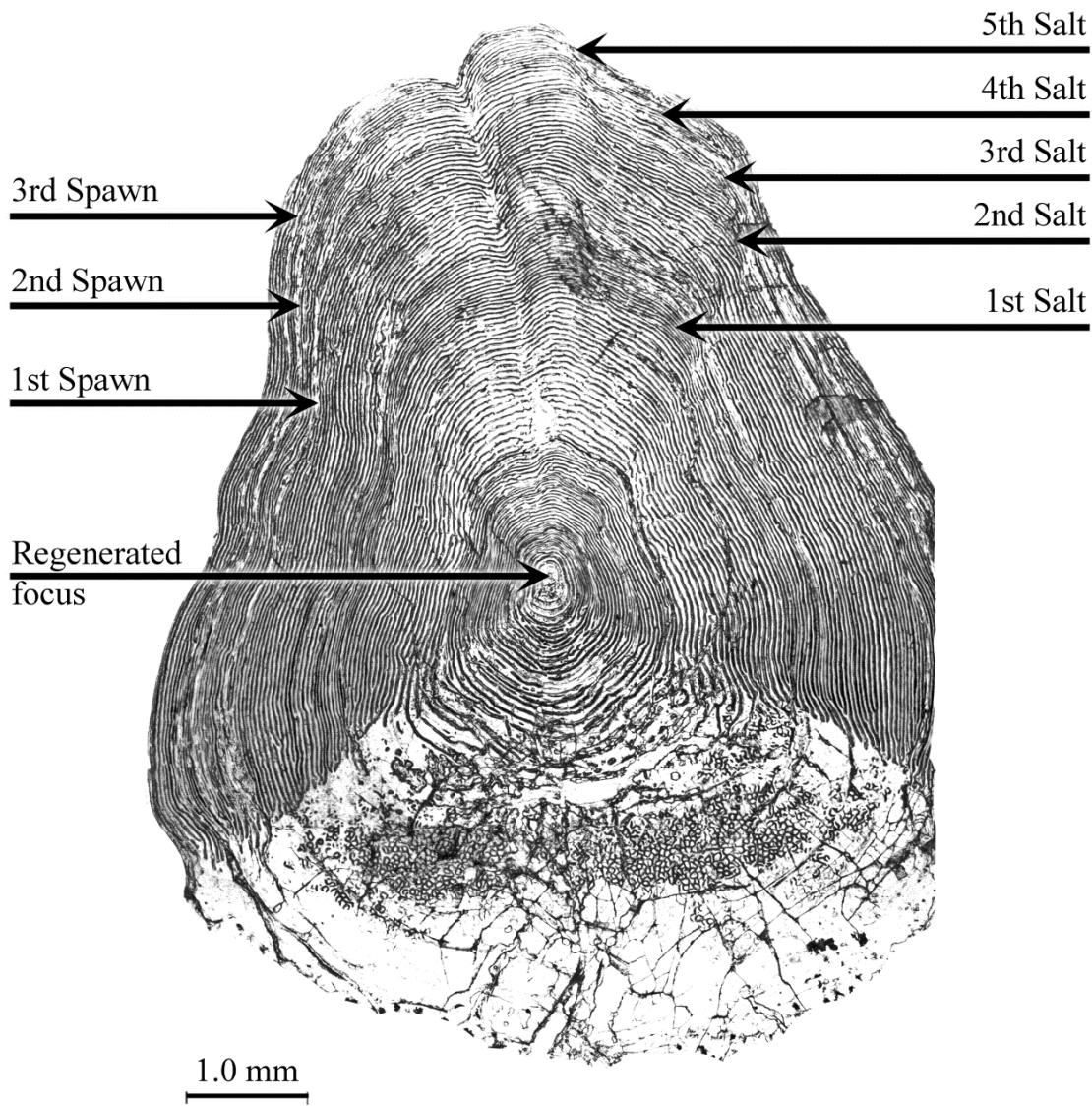
PIT 44607D5148
08sitSH008R_04



Appendix D9.—Steelhead trout aging example: scale image estimated as age-X.2S1S1 (690 mm FL), or unknown freshwater, 4-ocean, 2-repeat, spring immigrant adult steelhead trout on third spawning migration that was recaptured at Sitkoh Creek weir, 5/21/08.

Note: From the 2006 smolt sample and 2007 recapture, this is a known 4-freshwater fish. Note large, grainy, regenerated focus matching description in Erickson (1999).

PIT 44607D5148
09sitSH_011R_06

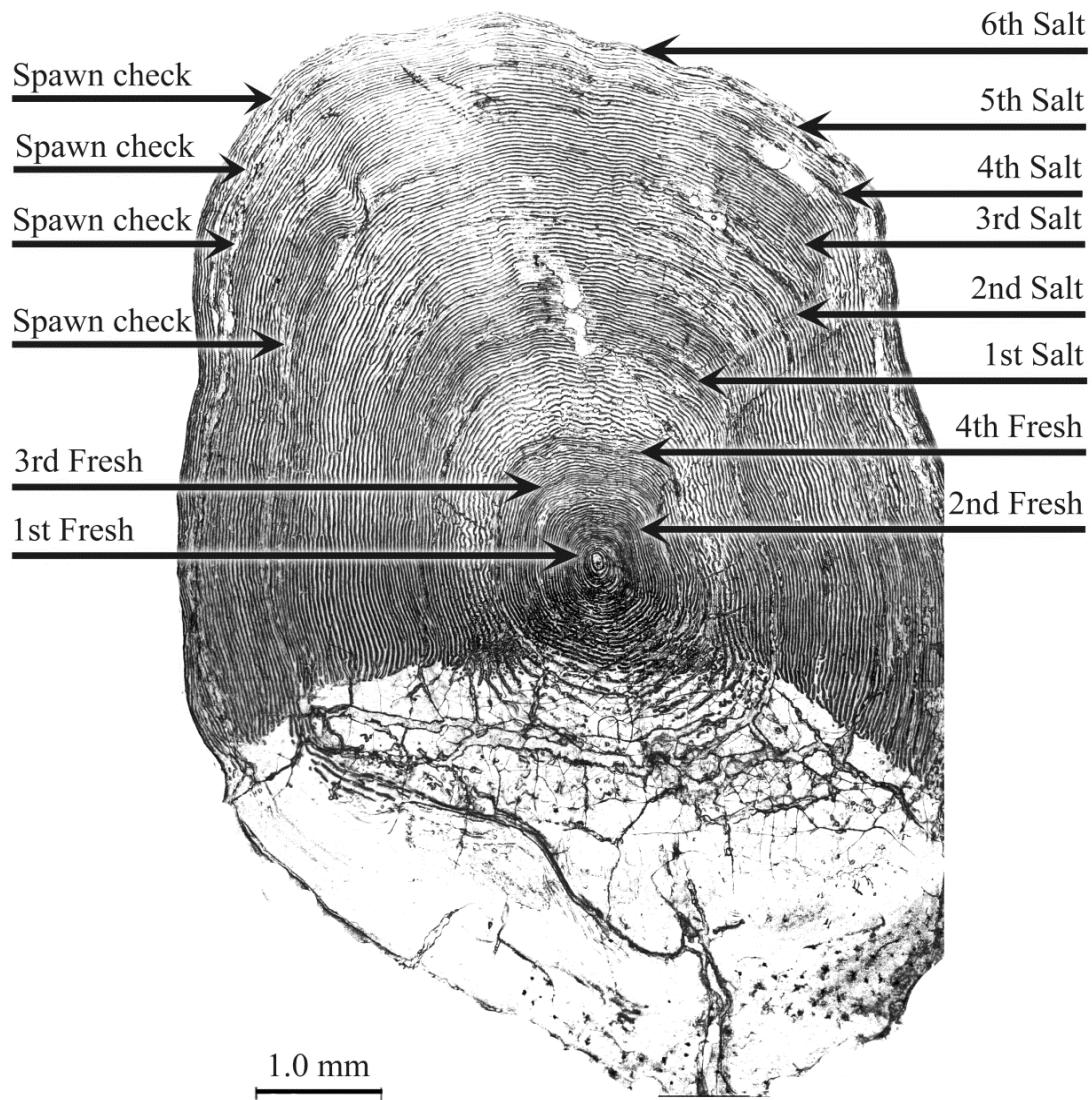


X.2S1S1S1

Appendix D10.—Steelhead trout aging example: scale image estimated as age-X.2S1S1S1 (728 mm FL), or unknown freshwater, 5-ocean, 3-repeat, spring immigrant adult steelhead trout on fourth spawning migration that was recaptured at Sitkoh Creek weir, 5/19/09.

Note: This is a known 4-freshwater fish.

PIT 135119510A
09sitSH16R_01

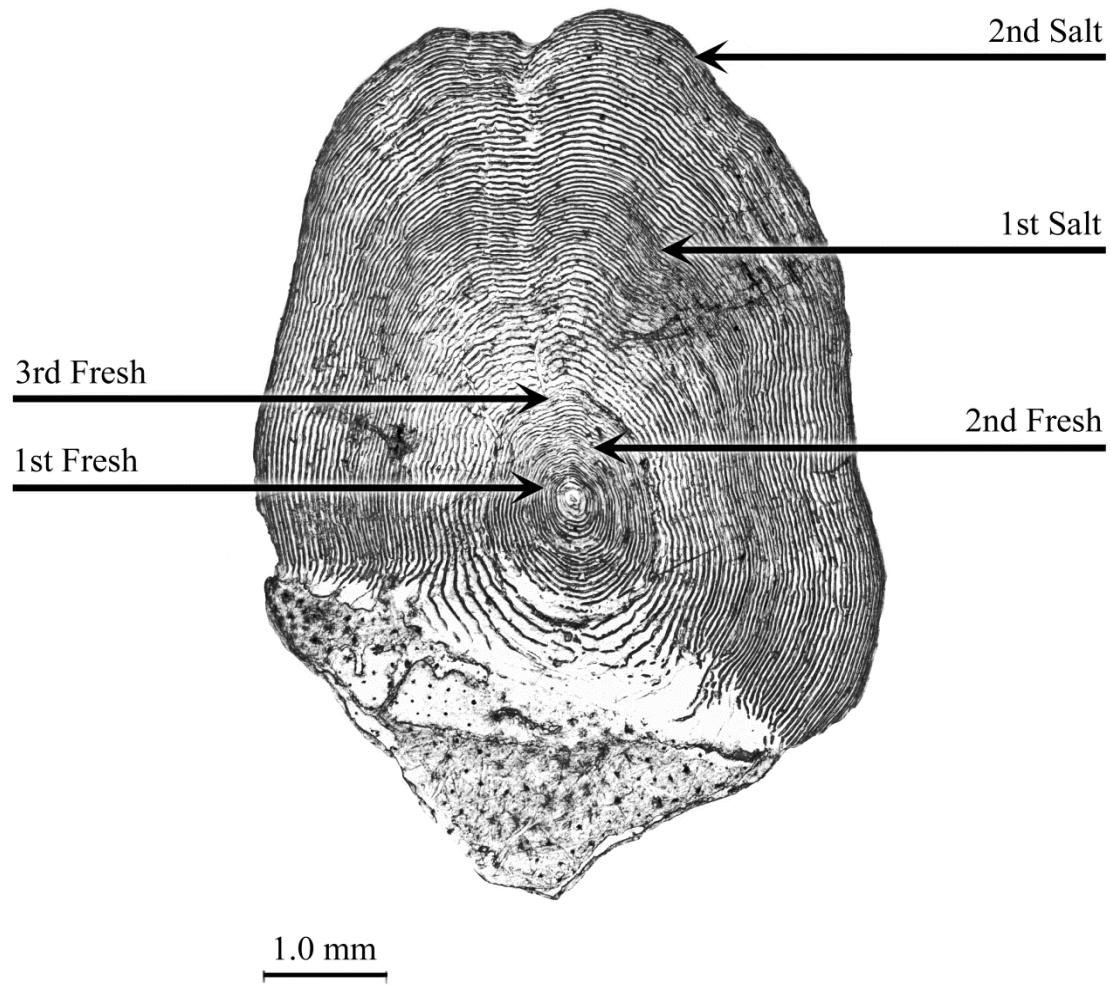


4.2S1S1S1S1

Appendix D11.—Steelhead trout aging example: scale image estimated as age-4.2S1S1S1S1, or 4 freshwater, 6-ocean, 4-repeat, spring immigrant adult steelhead trout on fifth spawning migration that was recaptured at Sitkoh Creek weir, on 5/31/09.

Note: Initially PIT-tagged as emigrant smolt in 2003, this repeat-spawning female (850 mm FL) was the oldest known-aged adult at an estimated total age of 10 years when the project ended in 2009. Not randomly selected for scale-sampling as an emigrant smolt, so no smolt scale.

PIT 134519292A
04sitSH013_5



3.2

Appendix D12.—Steelhead trout aging example: scale image of a steelhead trout scale estimated as age-3.2 (601 mm FL), or 3-freshwater, known 2-ocean spring immigrant adult steelhead trout on first spawning migration sampled at Sitkoh Creek weir, 5/4/04.

**APPENDIX E: LISTS OF PRACTICE IMAGES FOR SMOLT
AGED USING STANDARDIZED METHODS, VERIFIED
AGES OF SMOLT BASED ON REPLICATE SCALE AND
OTOLITH SAMPLES, AND KNOWN OCEAN-AGE ADULT
STEELHEAD SCALES FROM PIT-TAGGED RECAPTURES**

Appendix E1.—Scale image identification numbers, total scale circuli counts, matching scale ages, and number of equivalent reads for steelhead smolt of various freshwater ages collected at Sitkoh Creek from 2003 and 2008.

Scale card No.	Total circuli count	Overall scale age	No. matching reads
03sitSH2539	27	3	6
03sitSH2692	34	3	6
03sitSH3068	31	3	8
03sitSH3071	32	3	8
03sitSH3130	30	3	8
03sitSH3150	29	3	8
03sitSH3151	27	3	8
03sitSH3165	30	3	8
04sitSH0003	30	3	5
04sitSH0013	34	3	5
04sitSH0027	32	3	5
04sitSH0038	31	3	6
04sitSH0041	32	3	6
04sitSH0046	31	3	5
04sitSH0066	34	3	6
04sitSH0068	32	3	5
04sitSH0154	30	3	5
04sitSH0170	30	3	5
04sitSH0230	32	3	5
04sitSH0243	27	3	6
04sitSH0273	28	3	5
05sitSHJ0025	34	3	5
05sitSHJ0035	36	3	5
05sitSHJ0046	33	3	5
05sitSHJ0058	33	3	5
05sitSHJ0086	34	3	5
05sitSHJ0090	30	3	5
05sitSHJ0093	36	3	5
05sitSHJ0120	29	3	5
05sitSHJ0163	34	3	5
06sitSHJ0182	29	3	6
06sitSHJ0250	28	3	6
06sitSHJ0307	28	3	6
06sitSHJ0309	31	3	6
07sitSHJ0075	33	3	5
07sitSHJ0077	27	3	5

-continued-

Appendix E1.–Page 2 of 4.

Scale card No.	Total circuli count	Overall scale age	No. matching reads
07sitSHJ0085	30	3	5
07sitSHJ0115	38	3	5
07sitSHJ0123	36	3	5
07sitSHJ0140	29	3	5
07sitSHJ0147	30	3	5
07sitSHJ0155	31	3	5
07sitSHJ0160	30	3	5
07sitSHJ0161	28	3	5
07sitSHJ0165	32	3	5
07sitSHJ0171	31	3	5
07sitSHJ0172	30	3	5
03sitSH0028	36	4	6
03sitSH2383	38	4	6
03sitSH2538	35	4	6
03sitSH2558	35	4	6
03sitSH2581	37	4	6
03sitSH2679	35	4	6
03sitSH3035	46	4	8
03sitSH3037	36	4	8
03sitSH3056	40	4	8
03sitSH3058	43	4	8
04sitSH0006	38	4	5
04sitSH0007	47	4	5
04sitSH0014	40	4	5
04sitSH0017	44	4	5
04sitSH0020	35	4	5
04sitSH0021	40	4	5
04sitSH0025	42	4	5
04sitSH0026	37	4	5
04sitSH0040	46	4	6
04sitSH0044	41	4	5
05sitSHJ0002	36	4	5
05sitSHJ0006	39	4	5
05sitSHJ0018	41	4	5
05sitSHJ0019	42	4	5

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Scale card No.	Total circuli count	Overall scale age	No. matching reads
05sitSHJ0022	43	4	5
05sitSHJ0023	41	4	5
05sitSHJ0024	34	4	5
05sitSHJ0026	47	4	5
05sitSHJ0037	39	4	5
05sitSHJ0044	36	4	5
06sitSHJ0026	42	4	6
06sitSHJ0033	45	4	6
06sitSHJ0046	42	4	6
06sitSHJ0054	45	4	6
06sitSHJ0056	41	4	6
06sitSHJ0059	45	4	6
06sitSHJ0060	43	4	6
06sitSHJ0061	40	4	6
06sitSHJ0063	41	4	6
06sitSHJ0090	38	4	6
07sitSHJ0010	37	4	5
07sitSHJ0020	37	4	5
07sitSHJ0021	41	4	5
07sitSHJ0032	36	4	5
07sitSHJ0034	43	4	5
07sitSHJ0037	42	4	5
07sitSHJ0039	39	4	5
07sitSHJ0046	38	4	5
07sitSHJ0050	42	4	5
07sitSHJ0051	36	4	5
04sitSH0024	52	5	5
04sitSH0047	47	5	6
04sitSH0051	50	5	5
04sitSH0059	50	5	5
04sitSH0084	49	5	5
04sitSH0294	49	5	5
04sitSH0315	53	5	6
05sitSHJ0053	52	5	5
05sitSHJ0113	47	5	5
05sitSHJ0134	47	5	5
05sitSHJ0143	42	5	5

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Scale card No.	Total circuli count	Overall scale age	No. matching reads
05sitSHJ0182	51	5	5
05sitSHJ0197	48	5	5
06sitSHJ0086	50	5	6
06sitSHJ0100	45	5	6
06sitSHJ0197	52	5	6
06sitSHJ0291	53	5	6
07sitSHJ0016	55	5	5
07sitSHJ0024	48	5	5
07sitSHJ0040	56	5	5
07sitSHJ0048	50	5	5
07sitSHJ0055	44	5	5
07sitSHJ0088	47	5	5
07sitSHJ0095	48	5	5
07sitSHJ0132	47	5	5
08sitSHJ0002	NC	5	5
08sitSHJ0004	NC	5	5
08sitSHJ0079	NC	5	5
08sitSHJ0090	NC	5	5
08sitSHJ0099	NC	5	5
08sitSHJ0182	NC	5	5
08sitSHJ0184	NC	5	5
07sitSHJ0012	305	6	6
07sitSHJ0015	271	6	6
08sitSHJ0094	258	6	6
08sitSHJ0094	NC	6	5
08sitSHJ0014	NC	NGS	5

Note: “NC” indicates circuli counts were not counted. “NGS” means “no good scales.”

Appendix E2.—Scale and otolith image identification numbers, total scale circuli counts, and verified matching scale/otolith samples ($n = 88$) from various freshwater parr and smolt steelhead trout ages collected at Sitkoh Creek in 2003 and 2007.

Scale card No.	Total circuli count	Overall scale age	Otolith No.	Overall otolith age
SHM003	11	1	07sitSHminnows4X_03	1
SHM006	10	1	07sitSHminnows4X_06	1
SHM008	12	1	07sitSHminnows4X_08	1
SHM012	11	1	07sitSHminnows4X_12	1
SHM018	10	1	07sitSHminnows4X_18	1
SHM020	10	1	07sitSHminnows4X_20	1
SHM021	7	1	07sitSHminnows4X_21	1
SHM022	9	1	07sitSHminnows4X_22	1
SHM023	7	1	07sitSHminnows4X_23	1
SHM025	8	1	07sitSHminnows4X_25	1
SHM035	12	1	07sitSHminnows4X_35	1
SHM036	11	1	07sitSHminnows4X_36	1
SHM015	17	2	07sitSHminnows4X_15	2
SHM026	12	2	07sitSHminnows4X_26	2
SHM027	16	2	07sitSHminnows4X_27	2
SHM037	16	2	07sitSHminnows2X_37	2
03sitSH3013	27	3	03sitSHoto3013	3
03sitSH3018	33	3	03sitSHoto3018	3
03sitSH3046	29	3	03sitSHoto3046	3
03sitSH3066	34	3	03sitSHoto3066	3
03sitSH3068	31	3	03sitSHoto3068	3
03sitSH3071	32	3	03sitSHoto3071	3
03sitSH3076	35	3	03sitSHoto3076	3
03sitSH3077	33	3	03sitSHoto3077	3
03sitSH3115	33	3	03sitSHoto3115	3
03sitSH3117	32	3	03sitSHoto3117	3
03sitSH3119	31	3	03sitSHoto3119	3
03sitSH3122	32	3	03sitSHoto3122	3
03sitSH3127	32	3	03sitSHoto3127	3
03sitSH3130	30	3	03sitSHoto3130	3
03sitSH3131	33	3	03sitSHoto3131	3
03sitSH3133	30	3	03sitSHoto3133	3
03sitSH3137	33	3	03sitSHoto3137	3
03sitSH3138	30	3	03sitSHoto3138	3
03sitSH3150	29	3	03sitSHoto3150	3
03sitSH3151	27	3	03sitSHoto3151	3

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Scale card No.	Total circuli count	Overall scale age	Otolith No.	Overall otolith age
03sitSH3154	32	3	03sitSHoto3154	3
03sitSH3161	32	3	03sitSH003161	3
03sitSH3165	30	3	03sitSH003165	3
03sitSH3173	29	3	03sitSH003173	3
03sitSH3174	35	3	03sitSH003174	3
07sitSHJ0031	30	3	07sitSHsmolt2X_11	3
07sitSHJ0058	32	3	07sitSHsmolt2X_20	3
07sitSHJ0085	30	3	07sitSHsmolt2X_29	3
07sitSHJ0091	33	3	07sitSHsmolt2X_31	3
07sitSHJ0103	33	3	07sitSHsmolt2X_35	3
07sitSHJ0118	35	3	07sitSHsmolt2X_40	3
07sitSHJ0137	32	3	07sitSHsmolt2X_46	3
07sitSHJ0140	29	3	07sitSHsmolt2X_47	3
07sitSHJ0155	31	3	07sitSHsmolt2X_52	3
07sitSHJ0161	28	3	07sitSHsmolt2X_54	3
SHM044	19	3	07sitSHminnows2X_44	3
SHM046	21	3	07sitSHminnows2X_46	3
SHM052	19	3	07sitSHminnows2X_52	3
03sitSH2822	38	4	03sitSHoto0038	4
03sitSH3020	46	4	03sitSHoto3020	4
03sitSH3021	43	4	03sitSHoto3021	4
03sitSH3023	49	4	03sitSHoto3023	4
03sitSH3030	40	4	03sitSHoto3030	4
03sitSH3047	50	4	03sitSHoto3047	4
03sitSH3050	46	4	03sitSHoto3050	4
03sitSH3056	40	4	03sitSHoto3056	4
03sitSH3064	35	4	03sitSHoto3064	4
03sitSH3072	40	4	03sitSHoto3072	4
03sitSH3079	44	4	03sitSHoto3079	4
03sitSH3083	46	4	03sitSHoto3083	4
03sitSH3086	50	4	03sitSHoto3086	4
03sitSH3087	44	4	03sitSHoto3087	4
03sitSH3088	40	4	03sitSHoto3088	4
03sitSH3090	40	4	03sitSHoto3090	4
03sitSH3094	41	4	03sitSHoto3094	4
03sitSH3135	39	4	03sitSHoto3135	4
03sitSH3136	39	4	03sitSHoto3136	4
03sitSH3149	42	4	03sitSHoto3149	4

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Scale card No.	Total circuli count	Overall scale age	Otolith No.	Overall otolith age
03sitSH3157	41	4	03sitSHoto3157	4
03sitSH3168	33	4	03sitSH003168	4
07sitSHJ0025	43	4	07sitSHsmolt2X_09	4
07sitSHJ0034	43	4	07sitSHsmolt2X_12	4
07sitSHJ0049	37	4	07sitSHsmolt2X_17	4
07sitSHJ0067	38	4	07sitSHsmolt2X_23	4
07sitSHJ0073	42	4	07sitSHsmolt2X_25	4
07sitSHJ0109	48	4	07sitSHsmolt2X_37	4
07sitSHJ0112	38	4	07sitSHsmolt2X_38	4
07sitSHJ0128	43	4	07sitSHsmolt2X_43	4
03sitSH3034	46	5	03sitSHoto3034	5
03sitSH3065	60	5	03sitSHoto3065	5
07sitSHJ0016	55	5	07sitSHsmolt2X_06	5
07sitSHJ0055	44	5	07sitSHsmolt2X_19	5

Appendix E3.—Scale image identification numbers for a variety of known ocean-age adults collected at Sitkoh Creek from 2005–2009.

Scale card No.	Age: all reads matched	No. of reads
05sitSH001R_07	3.2	3
05sitSH002R_03	3.2	3
05sitSH007R_04	3.2	3
05sitSH007R_09	3.2	3
05sitSH007R_10	3.2	3
05sitSH011_05	3.2	3
06sitSH007R_05	3.2	5
07sitSH003R_08	3.2	6
07sitSH005R_07	3.2	6
07sitSH007R_05	3.2	6
07sitSH008R_09	3.2	6
07sitSH009R_09	3.2	6
07sitSH010R_03	3.2	6
07sitSH011R_04	3.2	6
07sitSH011R_09	3.2	6
07sitSH011R_10	3.2	6
07sitSH014R_09	3.2	6
07sitSH015R_03	3.2	6
07sitSH019R_04	3.2	6
08sitSH0006R_01	3.2	5
08sitSH0009R_07	3.2	5
09sitSH11R_05	3.2	3
09sitSH13R_01	3.2	3
09sitSH13R_02	3.2	3
09sitSH13R_03	3.2	3
09sitSH13R_07	3.2	3
09sitSH13R_09	3.2	3
09sitSH16R_04	3.2	3
09sitSH2R_08	3.2	3
09sitSH2R_09	3.2	3
09sitSH4R_06	3.2	3
09sitSH5R_03	3.2	3
09sitSH5R_04	3.2	3
09sitSH7R_02	3.2	3
09sitSH7R_05	3.2	3
09sitSH8R_09	3.2	3
09sitSH9R_08	3.2	3
06sitSH006R_03	3.3	5
06sitSH011R_09	3.3	5
07sitSH004R_07	3.3	6

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Scale card No.	Age: all reads matched	No. of reads
07sitSH005R_05	3.3	6
07sitSH007R_03	3.3	6
07sitSH010R_09	3.3	6
07sitSH013R_04	3.3	6
07sitSH014R_10	3.3	6
07sitSH017R_10	3.3	6
07sitSH018R_02	3.3	6
07sitSH018R_05	3.3	6
07sitSH018R_07	3.3	6
07sitSH018R_08	3.3	6
07sitSH019R_09	3.3	6
09sitSH10R_04	3.3	3
09sitSH12R_02	3.3	3
09sitSH12R_07	3.3	3
09sitSH13R_10	3.3	3
09sitSH17R_05	3.3	3
09sitSH1R_06	3.3	3
09sitSH1R_08	3.3	3
09sitSH5R_06	3.3	3
09sitSH6R_01	3.3	3
06sitSH005R_04	4.2	5
06sitSH008R_09	4.2	5
06sitSH009R_01	4.2	5
06sitSH011R_01	4.2	5
06sitSH014R_05	4.2	5
07sitSH001R_01	4.2	6
07sitSH001R_10	4.2	6
07sitSH005R_08	4.2	6
07sitSH014R_04	4.2	6
07sitSH017R_02	4.2	6
09sitSH15R_07	4.2	3
09sitSH16R_05	4.2	3
09sitSH16R_08	4.2	3
09sitSH3R_02	4.2	3
09sitSH3R_09	4.2	3
06sitSH001R_01	4.3	5
06sitSH007R_06	4.3	5
06sitSH008R_02	4.3	5
06sitSH010R_03	4.3	5

-continued-

Appendix E3.–Page 3 of 5.

Scale card No.	Age: all reads matched	No. of reads
06sitSH012R_07	4.3	5
07sitSH002R_04	4.3	6
07sitSH002R_09	4.3	6
07sitSH003R_01	4.3	6
07sitSH003R_05	4.3	6
07sitSH004R_05	4.3	6
07sitSH004R_10	4.3	6
07sitSH006R_09	4.3	6
07sitSH015R_06	4.3	6
07sitSH016R_07	4.3	6
07sitSH018R_03	4.3	6
09sitSH11R_04	4.3	3
09sitSH15R_05	4.3	3
09sitSH1R_01	4.3	3
09sitSH1R_04	4.3	3
09sitSH2R_07	4.3	3
09sitSH12R_05	5.2	3
05sitSH001R_06	3.2s1	3
05sitSH001R_08	3.2s1	3
05sitSH004R_08	3.2s1	3
05sitSH010R_03	3.2s1	3
05sitSH010R_08	3.2s1	3
07sitSH002R_03	3.2s1	6
07sitSH005R_03	3.2s1	6
07sitSH006R_02	3.2s1	6
07sitSH008R_10	3.2s1	6
07sitSH013R_01	3.2s1	6
07sitSH013R_09	3.2s1	6
07sitSH015R_09	3.2s1	6
07sitSH016R_03	3.2s1	6
09sitSH14R_04	3.2s1	3
09sitSH14R_07	3.2s1	3
09sitSH14R_10	3.2s1	3
09sitSH1R_09	3.2s1	3
09sitSH3R_06	3.2s1	3
09sitSH3R_10	3.2s1	3
09sitSH5R_05	3.2s1	3
09sitSH6R_07	3.2s1	3
09sitSH8R_06	3.2s1	3
05sitSH004R_01	3.3s1	3

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Scale card No.	Age: all reads matched	No. of reads
05sitSH009R_02	3.3s1	3
07sitSH006R_08	3.3s1	6
07sitSH008R_07	3.3s1	6
07sitSH014R_05	3.3s1	6
07sitSH015R_01	3.3s1	6
07sitSH019R_06	3.3s1	6
06sitSH007R_09	4.2s1	5
06sitSH012R_06	4.2s1	5
07sitSH010R_05	4.2s1	6
07sitSH011R_02	4.2s1	6
07sitSH012R_04	4.2s1	6
07sitSH014R_03	4.2s1	6
07sitSH018R_09	4.2s1	6
08sitSH007R_07	4.2s1	5
09sitSH11R_02	4.2s1	3
09sitSH14R_01	4.2s1	3
09sitSH15R_01	4.2s1	3
09sitSH15R_09	4.2s1	3
09sitSH1R_02	4.2s1	3
09sitSH3R_04	4.2s1	3
09sitSH8R_03	4.2s1	3
09sitSH8R_04	4.2s1	3
07sitSH008R_02	4.3s1	6
07sitSH011R_03	4.3s1	6
09sitSH2R_03	5.2s1	3
09sitSH1R_03	5.3s1	3
05sitSH009R_05	x.3s1	3
07sitSH001R_05	x.3s1	6
07sitSH005R_09	x.3s1	6
07sitSH007R_10	x.3s1	6
07sitSH009R_06	x.3s1	6
07sitSH011R_06	x.3s1	6
07sitSH012R_07	x.3s1	6
07sitSH014R_07	x.3s1	6
07sitSH017R_04	x.3s1	6
07sitSH017R_09	x.3s1	6
08sitSH0009R_09	x.3s1	5
09sitSH13R_04	x.3s1	3
09sitSH15R_08	x.3s1	3

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Scale card No.	Age: all reads matched	No. of reads
07sitSH008R_01	3.2s1s1	6
07sitSH018R_04	3.2s1s1	6
08sitSH0008R_08	3.2s1s1	5
09sitSH1R_07	3.2s1s1	3
09sitSH6R_05	3.2s1s1	3
09sitSH9R_04	3.2s1s1	3
09sitSH3R_03	3.3s1s1	3
09sitSH12R_03	x.3s1s1	3
09sitSH7R_09	x.3s1s1	3
06sitSH005R_03	4.2s1s1	5
06sitSH012R_10	4.2s1s1	5
07sitSH001R_02	4.2s1s1	6
07sitSH019R_02	4.2s1s1	6
07sitSH019R_05	4.2s1s1	6
08sitSH0009R_05	4.2s1s1	5
09sitSH10R_05	4.2s1s1	3
09sitSH16R_09	4.2s1s1	3
05sitSH003R_08	x.2s1s1	3
06sitSH005R_05	x.2s1s1	5
08sitSH0005R_03	x.2s1s1	5
08sitSH0008R_04	x.2s1s1	5
07sitSH003R_10	3.2s1s1s1	6
06sitSH003R_05	x.2s1s1s1	5
09sitSH12R_01	x.2s1s1s1	3
06sitSH012R_05	NGS	5

Note: Images represented are those with 3 to 6 equivalent reads made by 1 to 2 scale aging technicians.

APPENDIX F: ELECTRONIC FILE DESCRIPTION

Appendix F1.—Electronic data files used in this project.

File name	Software	Contents
Steelhead SCALE AGE FDS tables_figures.xlsx	Excel 2010	Figures 1 and 2, tables 1 and 3 and associated data used to generate them for this report
SitkohAdultRecaps03to09_table 2.xlsx	Excel 2010	Table 2 and associated data used to generate it for this report