

**Special Publication No. 98-2**

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# **Rainbow Trout Sampling and Aging Protocol**

by

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and

**Jason E. Dye**

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March 1998

Alaska Department of Fish and Game

Division of Sport Fish



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<b>Weights and measures (metric)</b>		<b>General</b>		<b>Mathematics, statistics, fisheries</b>	
centimeter	cm	All commonly accepted abbreviations.	e.g., Mr., Mrs., a.m., p.m., etc.	alternate hypothesis	H <sub>A</sub>
deciliter	dL			base of natural logarithm	e
gram	g	All commonly accepted professional titles.	e.g., Dr., Ph.D., R.N., etc.	catch per unit effort	CPUE
hectare	ha			coefficient of variation	CV
kilogram	kg	and	&	common test statistics	F, t, $\chi^2$ , etc.
kilometer	km	at	@	confidence interval	C.I.
liter	L	Compass directions:		correlation coefficient	R (multiple)
meter	m	east	E	correlation coefficient	r (simple)
metric ton	mt	north	N	covariance	cov
milliliter	ml	south	S	degree (angular or temperature)	°
millimeter	mm	west	W	degrees of freedom	df
		Copyright	©	divided by	÷ or / (in equations)
		Corporate suffixes:		equals	=
		Company	Co.	expected value	E
		Corporation	Corp.	fork length	FL
		Incorporated	Inc.	greater than	>
		Limited	Ltd.	greater than or equal to	≥
		et alii (and other people)	et al.	harvest per unit effort	HPUE
		et cetera (and so forth)	etc.	less than	<
		exempli gratia (for example)	e.g.,	less than or equal to	≤
		id est (that is)	i.e.,	logarithm (natural)	ln
		latitude or longitude	lat. or long.	logarithm (base 10)	log
		monetary symbols (U.S.)	\$, ¢	logarithm (specify base)	log <sub>2</sub> , etc.
		months (tables and figures): first three letters	Jan,...,Dec	mideye-to-fork	MEF
		number (before a number)	# (e.g., #10)	minute (angular)	'
		pounds (after a number)	# (e.g., 10#)	multiplied by	x
		registered trademark	®	not significant	NS
		trademark	™	null hypothesis	H <sub>0</sub>
		United States (adjective)	U.S.	percent	%
		United States of America (noun)	USA	probability	P
		U.S. state and District of Columbia abbreviations	use two-letter abbreviations (e.g., AK, DC)	probability of a type I error (rejection of the null hypothesis when true)	α
				probability of a type II error (acceptance of the null hypothesis when false)	β
				second (angular)	"
				standard deviation	SD
				standard error	SE
				standard length	SL
				total length	TL
				variance	Var
<b>Weights and measures (English)</b>					
cubic feet per second	ft <sup>3</sup> /s				
foot	ft				
gallon	gal				
inch	in				
mile	mi				
ounce	oz				
pound	lb				
quart	qt				
yard	yd				
Spell out acre and ton.					
<b>Time and temperature</b>					
day	d				
degrees Celsius	°C				
degrees Fahrenheit	°F				
hour (spell out for 24-hour clock)	h				
minute	min				
second	s				
Spell out year, month, and week.					
<b>Physics and chemistry</b>					
all atomic symbols					
alternating current	AC				
ampere	A				
calorie	cal				
direct current	DC				
hertz	Hz				
horsepower	hp				
hydrogen ion activity	pH				
parts per million	ppm				
parts per thousand	ppt, ‰				
volts	V				
watts	W				

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March 1998

This investigation was partially financed by the Federal Aid in Sport Fish Restoration Act (16 U.S.C. 777-777K) under Project F-10-12, Job No. R-2-1.

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*This document should be cited as:*

*Minard, R. Eric and Jason E. Dye. 1997. Rainbow trout sampling and aging protocol. Alaska Department of Fish and Game, Special Publication No. 98-2, Anchorage.*

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## ABSTRACT

This paper presents a protocol adopted by the Alaska Department of Fish and Game for sampling and aging rainbow trout *Oncorhynchus mykiss*, and describes the process of scale sampling, sorting, cleaning, mounting, and pressing, as well as a standardized method for training scale readers. Aging criteria for recognizing annuli, regenerated scales, false "checks," plus growth, and spawning marks are examined for rainbow trout. The training process establishes methods for testing within-reader precision and between-reader accuracy. Standardized training of scale readers helps to ensure that age estimations made by future rainbow trout scale readers will remain consistent with the historical database.

Key words: rainbow trout, *Oncorhynchus mykiss*, sampling, scales, age, annuli, precision, accuracy.

## INTRODUCTION

Scales are routinely used to estimate age composition. Scales can be collected quickly and in large numbers without killing or severely injuring fish. Although age determinations from rainbow trout scales may not be a valid indicator of absolute age, they do offer a good index of relative age (Alvord 1954). In addition to being nonlethal, preparation and estimation of approximate age from scales is relatively easy and inexpensive compared to aging other bony structures.

Although collection and preparation of scales is relatively simple, it is extremely important that it be done carefully and consistently. Therefore, a standardized collection and preparation protocol is needed to outline procedures which minimize mortality from sample collection and maximize the number of ageable scales in a sample.

Since age composition is often essential to proper management of an area's rainbow trout stocks, estimates of precision and accuracy associated with age estimation from scales are important. Therefore, a protocol was created to train personnel to age rainbow trout scales precisely and accurately. This protocol is necessary because personnel associated with the aging of rainbow trout will change over time which, without standardized training, may alter the age composition estimates of rainbow trout populations.

To counterbalance the possible effects of changes in personnel on age estimates, a standardized procedure for age estimation was developed for rainbow trout scales. This was accomplished by minimizing within-reader age estimation variability and between-reader age estimation variability. Coggins (1994) stated that minimizing within-reader variability can be accomplished with multiple readings of each scale to obtain modal ages. Minimizing between-reader variability can be accomplished with a training program that establishes a standard set of scales and ages and teaches scale readers standard criteria for age estimation of rainbow trout scales. This ensures that age estimations made by future rainbow trout scale readers are consistent with the historical database.

## OBJECTIVES

1. Establish a protocol for collection of age and size information from rainbow trout in the field.
2. Establish a protocol for preparation of scales for aging.
3. Establish a protocol for estimating age from scales for rainbow trout.

# PROTOCOL

## SAMPLE COLLECTION

Following the capture of a rainbow trout, the fish should be immediately placed in a water-filled tote, or left in the river in either a net or pen. In order to minimize mortality, it is important to keep the fish in the water as much as possible and to minimize handling. Additionally, any necessary handling of the fish must be done with wet hands. An efficient way of collecting scales and taking length with minimal handling is to place a measuring board across the top of the tote and, while the fish is on the measuring board, quickly take the length to the nearest millimeter and collect the scales. Ideally, a smear of 10 to 15 scales should be removed and stored for later age estimation. Due to high regeneration rates, it is extremely important that a minimum of 10 scales be removed from each fish. This will allow aging of a higher percentage of the samples which will increase the precision of estimated age compositions. Scales may be removed with a knife or forceps (tweezers) from an area on a diagonal line between the posterior insertion of the dorsal fin and the anterior insertion of the anal fin, two rows above the lateral line. Rainbow trout scales develop first along the lateral line and spread most rapidly in the middle and posterior part of the body (Paget 1920). Thus, the annulus marking the first year of growth is most likely to be visible on scales from this area. Scales in this area also tend to be oval-shaped and symmetrical (Maher and Larkin 1955) and therefore relatively easy to read. If scales in this area appear irregular or regenerated, sample the fish from the same area on the opposite side. Also, if it appears that there is excessive mucus in this area, use the back edge of the knife or forceps to gently clear away some of the mucus before scale removal. Following sampling, all rainbow trout not kept by anglers are to be released unharmed at the approximate site of capture.

After removal, scales should be immediately stored in a coin envelope. Information such as sampling date, sample number, species, location, length, tag number (if tagged), and weight (if taken) for that particular sample should be recorded on the envelope. Storing scales in coin envelopes saves time in the field, protects scale loss from wind or rain, and allows several scales to be removed to ensure a sufficient amount of legible scales for aging.

Following a day of sampling, all data recorded on scale envelopes should be transferred to an Age-Weight-Length Form (AWL) Version 1.1 (Appendix B1) as described by Heineman (1991); (Appendix B2). The AWLs are then reviewed for any errors or stray marks and stored in chronological order for later analysis. All scale envelopes should then be numbered with the corresponding AWL page and line number and put in chronological order. The scale envelopes should remain together and arranged in order until it is time to remove the scales for aging. In addition to the information recorded on AWLs, a daily tally of the total number of trout sampled and tagged (if tagging) along with any additional information pertinent to that particular project, should be kept.

Immediately after returning from the field, AWLs should be checked for any obvious errors or stray marks and sent to Research and Technical Services (RTS) in Anchorage for optical scanning. Scanned forms and their corresponding data files are then sent back to Dillingham for final editing and addition of age information.

## **SAMPLE PREPARATION**

### **Scale Selection**

Prior to aging, scales are removed from the coin envelope, placed in a dry petri dish, examined under a microscope at 30X magnification for legible scales, sorted, moistened, cleaned, and mounted on gummed cards. Legible scales are those which are not regenerated beyond the fourth or fifth circuli (Appendix A2), are not resorbed around the edges, and have circuli which can be clearly seen by the reader. If no legible scales are found, a regenerated or illegible scale should be mounted on the gum card as a place marker. If no scales were sampled from a fish, a line should be drawn through the appropriate space on the gummed card. This helps the reader keep his/her place when aging.

### **Scale Cleaning**

Care must be taken to thoroughly clean the legible scales, often a poorly cleaned scale will have a line of mucus embedded between two circuli. This causes a problem when the mucus is located between two circuli in the anterior portion of a period of winter growth. The mucus could appear as a period of faster growth, simulating the appearance of a spawning check. An effective way of cleaning the scales is, after sorting out the scales that are to be mounted, place them in a petri dish filled with water. Then press down firmly on the scale with an index finger, rub in small circles, remove from the petri dish with forceps, and dab dry on a paper towel. This should remove the skin and mucus from the scale.

### **Scale Mounting**

If possible, three or four clean, legible scales should then be placed on a labeled, gummed card. Gummed cards can be made by cutting packing tape into 4.5 inch by 2.5 inch strips and stamping the gummed side with sample numbers and the opposite side with blanks for sample information. Packing tape that must be wetted before use is the only type of tape that will work (ADF&G - Dillingham currently uses Central Brand Gummed Kraft Sealing Tape Item No. UFS-2799 from Northern Office Supply in Anchorage - phone 907/344-6200). Scales should be mounted with the rough side up and with the forward or anterior end (end with complete circuli) of the scale toward the top of the card. A probe can be used to gently scrape each scale to determine which is the rough side. This should be done carefully to avoid scratching the scale. Care should also be taken when mounting to make sure that the scale is wet enough to stick to the card but not so wet that glue flows over the scale. Finally, each gummed card should be labeled with sampling location, sampling date, species, and AWL page and line number. The label ensures that each scale is referenced to the fish from which it was sampled.

### **Scale Pressing**

After the scales are mounted on gummed cards they are then ready to be pressed. There are several guidelines which should be followed when pressing scales. An acetate is placed on top of each gummed card (scale side up), which should then be placed between two pieces of 22 or 24 gauge brushed stainless steel, which are in turn placed between two sheets of heat resistant rubber. The rubber sheets help to even out the pressure exerted on the scales. The performance of scale presses is highly variable and therefore each press must be experimented with to find the ideal combination of temperature, pressure, and pressing time. It is important to examine the first few pressed acetates with a microfiche reader to determine if the pressings are satisfactory. The press parameters can vary depending on the type of acetate used; acetates with high plastic

content require lower temperatures than do those with high cellulose content. If, after pressing, the acetates behave like taffy, reduce temperature. On the other hand, if the acetates don't have a frosted appearance around the scales or if the scales aren't leaving a good impression, try increasing temperature and pressure. Also, certain areas of the press may not press well and therefore these areas must be identified and avoided. Immediately after the scales are removed from the press, a weight should be placed on top of the acetates (while they are still between the steel sheets) for approximately 1 minute. This helps to flatten the acetates which makes for easier handling when aging. Then, using a knife, the acetates should be carefully peeled away from the steel sheets. After the acetates have cooled, they should be labeled and put in order. The labels should have information such as location, date, species, card number, and electronic file number on them and should be placed at the top of the acetate on the same side as the scale impressions. Placement of the label is extremely important so the reader can correspond each age to the correct fish.

Specifics for using the press at the Dillingham office of the Department of Fish and Game are as follows:

1. Begin pressing at 250°F at 25,000 lb of pressure for 3 minutes. This combination generally works best, and any necessary adjustments can be made from this starting point.
2. Do not exceed 325°F; at this point the rubber sheets may begin to burn.
3. Do not exceed 30,000 lb of pressure; continual use above this limit will quickly wear out the hydraulic components.
4. Be careful that part of the plates are not hanging out the back of the press, this part of the press does not exert any pressure.
5. The rubber sheets used for pressing should be 1/8 to 1/4 inch thick. These sheets can be ordered from Interior Rubber and Supply in Fairbanks (phone 907/451-0200). The rubber comes in rolls that are 36 inches wide. Request that the company cut two 15 inch pieces. This is enough for 3 pairs of 12 inch by 15 inch sheets.
6. Acetates should be 4.5 inches by 2.5 inches and .020 inches thick. They can be ordered from Cadillac Plastic & Chemical Co. in Anchorage (phone 907/563-4761) and interleaves should be requested to prevent scratching and sticking. The .020 inch thick acetates generally give the best results, although thicker or thinner acetates will work when no others are available (temperature, pressure, and pressing time will have to be adjusted).

Following pressing, scales are then ready to be aged. Scales should be read using a microfiche projector under 40X magnification. It is important that aging be done in a relatively dark room with a quality projector; this will make annuli easier to identify.

### **DETERMINING AGE**

Seasonal variability in growth of rainbow trout results in seasonal patterns of growth being exhibited on their scales. Ages of rainbow trout may be estimated by interpreting characteristics of these patterns of growth. Some general observations of fish scale growth and development followed by specific interpretations of characteristics found in Southwest Alaska rainbow trout scales were combined by Coggins (1994) to form the following set of criteria.

Scale growth begins with the formation of the focus, or area enclosed by the first circulus (Mosher 1969). The scale grows outward from the focus with the greatest growth occurring toward the forward or anterior margin of the scale. Fine ridges called circuli are laid down in a circular pattern around the focus as growth continues. Multiple circuli are added to the scale each year and those circuli which are closely spaced appear darker than those which are more widely spaced. The first few circuli completely surround the focus. Beyond the first few circuli, the others appear as arcs that tend to end abruptly at the junction with the exterior (posterior) portion of the scale (Mosher 1969). In some species of salmon and trout, the bases of the circuli may not end abruptly, but may extend posteriorly for varying distances, or the circuli may be broken or enlarged in this area (Mosher 1969; Lux 1971).

Scale growth reflects fish growth. Generally, circuli are widely spaced in warm seasons when rainbow trout growth tends to increase and closely spaced in cold seasons when growth tends to be slower. The growth of a fish during one year is reflected on its scale as a series of widely spaced spring and summer circuli followed by a series of closely spaced fall and winter circuli. This pattern is repeated each year as fish continue to grow throughout their life. The outer edge of a series of closely spaced circuli is accepted as the end of growth for that year, and this point is referred to as the year-mark or annulus (Appendix A1). The age of a fish is determined by counting the number of annuli (Lux 1971). Annuli should be counted on the anterior portion of the scale from the focus to the margin along a line approximately 30 degrees to the anterior-posterior axis of the scale (Appendix A1).

Several factors and potential problems should be considered when reading a scale. These include:

1. **FORMATION OF THE FIRST ANNULUS (Appendix A3):** In some cases rainbow trout do not lay down an annulus during their first year of life. Therefore, a number of age 0 and age 1 juvenile rainbows should be captured and a range of circuli to the first annulus should be determined. If this is not possible, it should be assumed that the first annulus is somewhere between the seventh and fourteenth circuli (Dan Bosch, Alaska Department of Fish and Game, Anchorage, personal communication).
2. **SPAWNING CHECKS (Appendix A4):** A spawning check occurs when, during spawning, the ventral and dorsal portions of a scale are resorbed quickly, while the anterior portion is slowly resorbed. This may result in the appearance of a single annulus on the ventral and dorsal margins and two annuli that, together, form a crescent shape on the anterior margin. In some cases the crescent shape may appear on the anterior portion, but no annuli will be visible on the dorsal and ventral portions. Either way, a spawning check is to be interpreted as 2 years.
3. **INTERPRETATION OF PLUS GROWTH (Appendix A5):** It appears that trout that are not spawning in the spring lay down an annulus sooner than do trout which will spawn in the spring. This is because fish which are not spawning are able to put more energy into growth than a fish that is producing gametic tissue. This accounts for the differences observed in plus growth on scales collected during the summer and fall. Rainbow trout which are not spawning will lay down an annulus starting in April, while a spawning rainbow may not lay down an annulus until late June (Dan Bosch, Alaska Department of Fish and Game, Anchorage, personal communication). In general, when aging a fish sampled before June 30,

plus growth should be counted as an extra year on all fish. For fish sampled between June 30 and July 31, fish younger than 5 should have laid down an annulus and therefore plus growth is not counted as an extra year, while in older fish, plus growth may be counted as an extra year. After July 31, plus growth is considered to be that summer's growth and is not counted as an extra year.

4. FALSE CHECKS (Appendix A6): False checks occur during a period of reduced growth, injury, or shock (Lux 1971). False checks are thinner than normal annuli and many times are visible to only one side of the anterior-posterior axis of the scale (Coggins 1994).
5. LIFE HISTORY: It may be helpful to know something of the life history of the fish being aged. For example, trout which come from fairly sterile waters would exhibit slow growth and closely spaced annuli, while fish from fertile waters would exhibit fast growth and widely spaced annuli.

Ages will be determined three times for each sampled rainbow trout. All scales in a sample will be read once, the order of the samples altered, and all scales are then read a second time. This procedure will then be repeated for the third reading. Because Coggins (1994) found modal ages among replicate readings to more likely be accurate ages, only those fish with modal ages will be used to estimate age composition. A t-test will be used to test the hypothesis that size (mean length) is the same for rainbow trout with and without modal ages. When this hypothesis is rejected ( $p=0.10$ ), scales from these fish without modal ages after three readings will be read a fourth time. If a modal age is produced for a fish with this fourth reading, that fish is used to estimate age composition. This procedure is repeated with more readings until the hypothesis of equal mean lengths is no longer rejected on less than 5% of the sample lacking modal ages.

After aging of a particular sample is complete, the modal ages are recorded on the corresponding AWL (Heineman 1991; Appendix B2) and in the electronic data file created for that sample. The AWLs and electronic data files then go through final editing and copies of the data files are made and sent, along with the original edited AWLs, to RTS for archiving.

## **TRAINING PROCEDURES**

After review of the aging criteria, training can begin. Random selections are read from a standard set of 60 scales developed for training. The 60-fish training data set was formed by merging lacustrine and riverine data sets as described by Coggins (1994). Scales from two populations of lacustrine rainbow trout were merged, sorted by fork-length, and divided into six equal length intervals to form the lacustrine data set. Ten samples (fish) were randomly drawn from each of the six length intervals to create a 60-fish lacustrine experimental data set. An additional five samples randomly selected from each of the six length intervals formed the lacustrine half of the training data set. In this manner, the lacustrine data set samples formed the lacustrine experimental data set (60 fish), and the lacustrine half (30 fish) of the training data set. These procedures were repeated to select the 60-fish riverine experimental data set, and the riverine half of the training data set. Any samples exhibiting extreme regeneration were removed from the experimental data sets and alternates randomly selected from the appropriate length interval (Coggins 1994). Also, each sample is labeled with the date the fish was sampled, allowing more accurate interpretation of plus growth. The complete 60-fish training data set is available for use by management agencies from the Sport Fish Division at the Dillingham office of the Department of Fish and Game.

The age assigned to each scale of the training set is based on a consensus of several highly experienced readers. While this standard age may not be the actual age of the fish, the goal is to use the standard ages to assess consistency from one reader to the next.

After practice, all 60 of the standard scales are read three times as a test of repeatability and “accuracy.” Before each replicate reading, the scales are randomly shuffled to avoid memorization by order. Age estimates for each scale are compared among replicates for repeatability and, if present, modal age is compared to the standard age for accuracy. Because the use of modal ages eliminates imprecise readings and undesirable scales from the sample and because random error in aging is reduced, Coggins (1994) recommends using modal age as the correct estimate. Mean length of standards with modal estimates should then be compared to mean length of standards without modal estimates using an analysis of variance (ANOVA) test to see if there is any significant difference. Also, a Kolmogorov-Smirnov (K-S) test can be used to test the null hypothesis of equal length distributions for these two groups of standards (Sokal and Rohlf 1981). A well trained reader should not produce modal ages for predominantly larger or smaller standards.

Replicate and modal ages should be recorded as shown in Table 1. The last column, age matches, represents the number of matching ages between replication one, two, and three. This column is later used in the estimation of within-reader precision.

### **Within-Reader Precision**

The within-reader precision is estimated by measuring repeatability, the probability of equal age distributions, and the probability of assigning a modal age for the three replications. Random error in assigning ages for rainbow trout is measured through blind replication where error is estimated as the probability of any two replicated ages matching (repeatability). A good reader has a high repeatability and a poor reader has a low repeatability. Repeatability is measured as (Coggins 1994),

$$\hat{p}_r = \frac{\sum_{i=1}^n y_{ri}}{3n} \quad , \quad (1)$$

where  $\hat{p}_r$  is the estimated probability of any two replicate estimates by a reader matching (repeatability),  $y_{ri}$  is the number of successful matches in three replicates from the  $i$ th fish by the  $r$ th reader, and  $n$  is the number of fish in the study. Division by  $3n$  is included in Equation (1) because, for each fish, there are three possible matching pairs in the three replicates. Essentially, the ‘age matches’ column in Table 1 is summed and divided by three times the number of fish in the sample.

To minimize within-reader age estimation variability, Coggins (1994) recommends a repeatability estimate of at least 60%. Using equation (2), a reader with 60% repeatability will, on average, fail to assign a mode to only 6% of fish given three replications (probability of .94 of assigning a modal age). Additionally, readers with a high estimated repeatability assign modal ages that more accurately describe the central tendency of a distribution of estimated ages for a particular fish (Coggins 1994). Finally, readers that eliminate low numbers of scales are less likely to eliminate good samples, which not only increases sample size, but also reduces costs

**Table 1.-Example showing the standard ages, estimated age for each replication, modal ages, and number of age matches.**

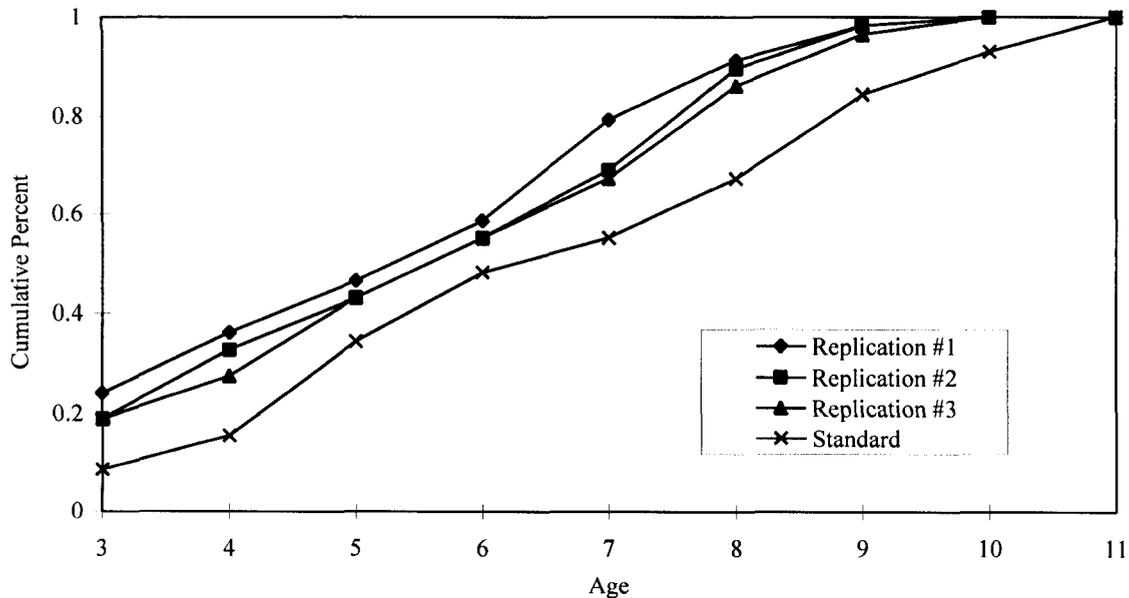
Scale Number	Standard Age	Reading 1 Age	Reading 2 Age	Reading 3 Age	Modal Age	Age Matches
1	9	9	9	9	9	3
2	9	8	8	8	8	3
3	9	7	8	8	8	1
4	9	9	9	9	9	3
5	10	8	8	9	8	1
6	9	7	8	8	8	1
7	3	3	3	5	3	1
8	9	8	8	8	8	3
9	7	7	6	6	6	1
10	R	R	R	R	R	3
11	9	7	7	7	7	3
12	7	10	9	9	9	1
13	8	9	8	8	8	1
14	5	5	4	4	4	1
15	8	7	8	8	8	1
16	8	9	9	10	9	1
17	4	3	5	3	3	1
18	5	4	6	5	NM	0
19	5	5	4	5	5	1
20	7	8	8	9	8	1
21	8	8	8	7	8	1
22	7	7	9	8	NM	0
23	5	4	4	5	4	1
24	6	6	6	8	6	1
25	5	3	4	3	3	1
26	4	4	4	4	4	3
27	4	3	3	3	3	3
28	4	3	4	4	4	1
29	7	6	8	8	8	1
30	10	8	10	10	10	1
31	3	2	2	2	2	3
32	6	7	7	7	7	3
33	8	7	7	7	7	3
34	3	2	2	2	2	3
35	R	R	R	R	R	3
36	3	2	3	3	3	1
37	4	6	6	6	6	3
38	6	6	7	6	6	1
39	7	7	7	9	7	1
40	6	5	6	6	6	1
41	3	3	3	3	3	3
42	7	6	7	6	6	1
43	2	3	3	3	3	3
44	7	7	7	7	7	3
45	2	3	2	3	3	1
46	3	5	5	5	5	3
47	6	5	5	5	5	3
48	6	4	5	5	5	1
49	2	2	3	3	3	1
50	4	3	3	3	3	3
51	7	7	8	8	8	1
52	7	6	6	6	6	3
53	4	4	4	5	4	1
54	6	6	5	7	NM	0
55	7	8	8	8	8	3
56	8	7	7	7	7	3
57	2	3	3	4	3	1
58	5	4	5	4	4	1
59	3	4	4	5	4	1
60	6	5	6	6	6	1

R = regenerated scale

NM = no mode

associated with not repeating an observation. In other words, the time and money spent collecting those samples was not wasted.

A chi-squared statistic estimates the probability of equal age distributions between the three replicates. When conducting a chi-square test, it is usually recommended that all expected cell counts equal or exceed five. To accomplish this, pooling the upper and/or lower ages into one group may be necessary. The null hypothesis that the three age frequency distributions are equal is tested at  $\alpha = 0.05$ . Failure to reject the null hypothesis is required for the new reader to be considered acceptably precise. Because managers are attempting to maintain “historical age and size composition” (ADF&G 1990) and because changes in frequency of specific age and length classes may not affect the mean age, the age frequency distribution is a much more useful management tool than is the mean age. This relationship can be viewed by plotting the cumulative age frequencies for all three replications (Figure 1).



**Figure 1.-Example of cumulative age frequencies for the three training replications and the standard age set.**

The last method of assessing within-reader precision is by tallying the number of fish for which a reader has assigned a modal estimate of age (Coggins 1994). In three replicates per fish, any match between two replicates creates a mode; no matches mean no modes. The relationship between the probability of assigning a fish a modal age and the probability of matching replicated age (repeatability) is estimated as:

$$\hat{p}_r^* = 1 - (1 - \hat{p}_r)^3, \quad (2)$$

where  $\hat{p}_r^*$  is the estimated probability of a reader assigning a fish a modal age. The results of Coggins (1994) work suggest that the probability of a reader assigning a fish a modal age should be at least 90%. This would be comparable to the estimates produced by experienced scale readers in Coggins' study and would keep the number of samples that are eliminated to a level of 10% or less, maximizing sample size and minimizing costs associated with eliminated samples.

### **Between-Reader Accuracy**

Next, accuracy is measured between estimated ages and standard ages in two ways. First, the mean modal age for the three replicates is compared to the mean standard age of the scales to test if they are statistically similar. This is done with an analysis of variance (ANOVA) test between standard ages and modal ages. Second, the age frequency distribution of the trainee's modal ages is compared to the standard age frequency distribution. A chi-squared statistic tests the null hypothesis that the standard age frequency is equal to the modal age frequency distribution at  $\alpha = 0.05$ . Failure to reject the null hypothesis is required for the new reader to be considered acceptably accurate. These tests do not test a reader's ability to obtain actual ages, but rather they test a reader's ability to age accurately with respect to a set of standard ages. This relationship can be viewed by plotting the cumulative age frequencies for each of the three replications with the cumulative age frequency for the standard ages (Figure 1).

## **ACKNOWLEDGMENTS**

Many fishery professionals contributed to the development and review of procedures in this paper. Alaska Department of Fish and Game staff included: Dave Bernard, Susie Hayes, Dan Bosch, Lew Coggins, Robert Clark, Mike Jaenicke, Renate Riffe, Lowell Fair, Scott Meyer and Bob Lafferty. U.S. Fish and Wildlife Service staff included: Mark Lisac, Jim Larson, Steve Klosiewski, and Jeff Adams. Many thanks to all of them for their assistance and review.

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## LITERATURE CITED (Continued)

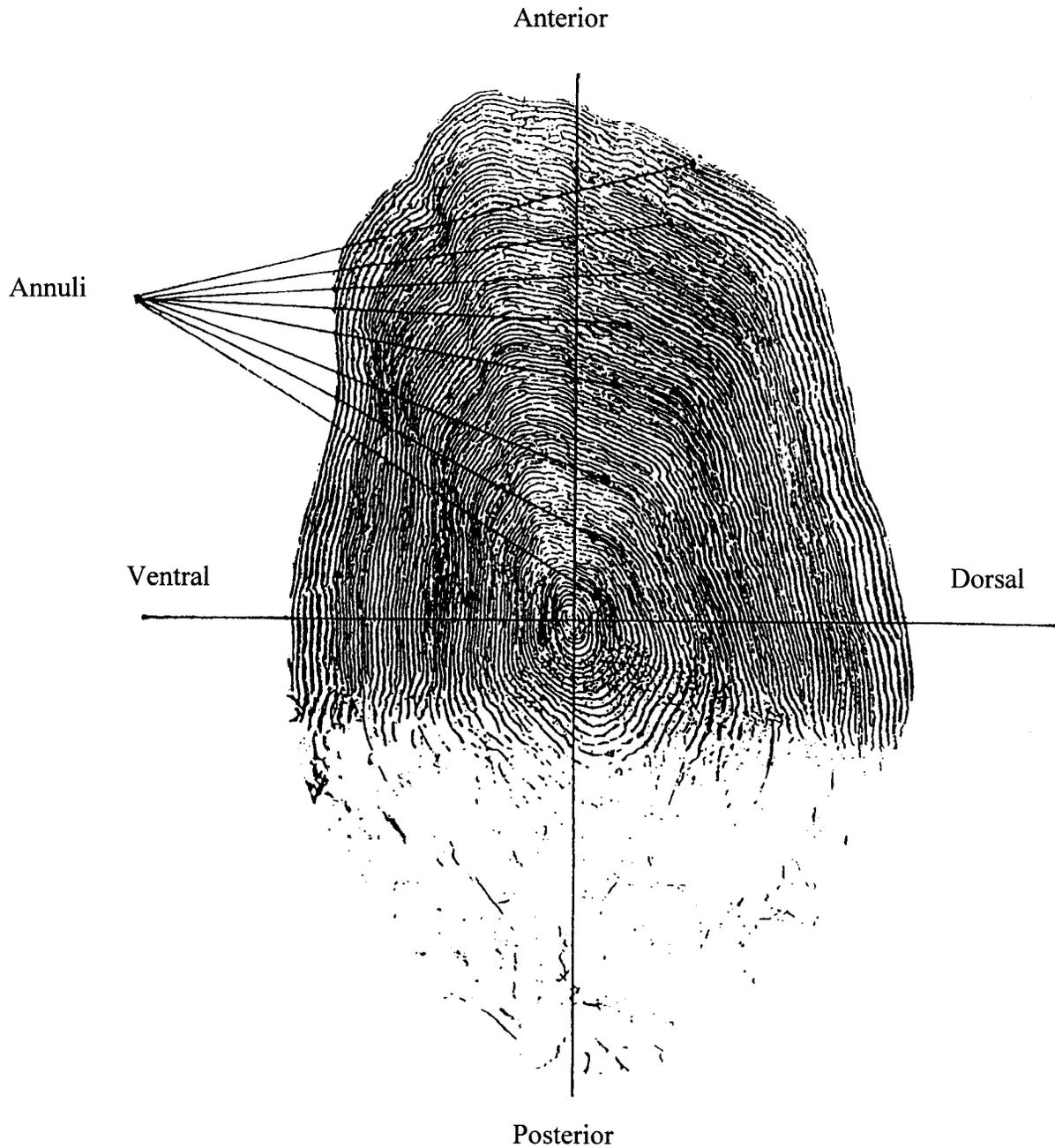
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## **APPENDIX A. SCALE ILLUSTRATIONS**

**Appendix A1.-Annual mark or "check."**

An annulus is defined as a concentrated group of broken circuli running forward from the posterior margin on one side of a scale around to the posterior margin on the other side.

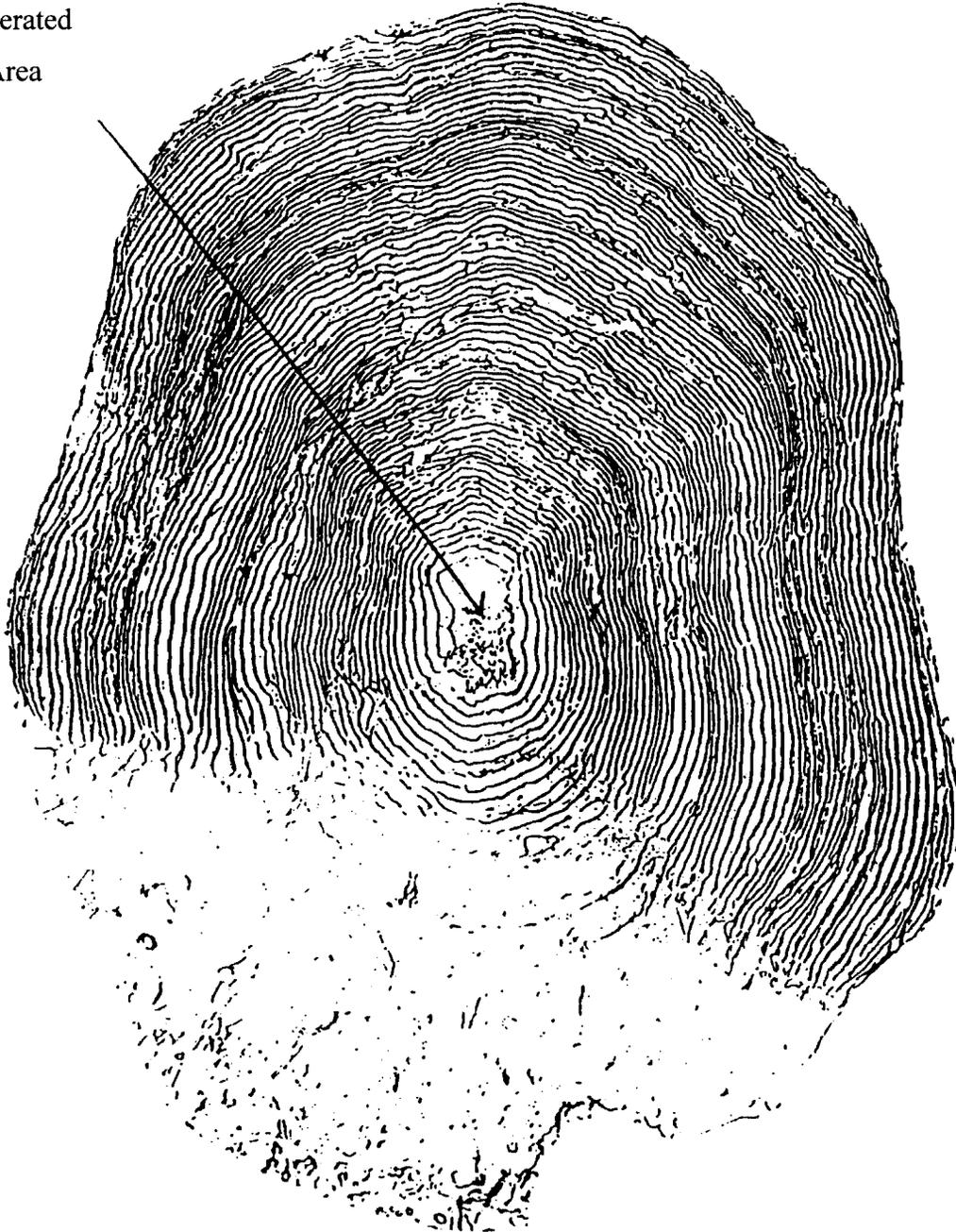


Modified from: Coggins 1994.

**Appendix A2.-Scale regeneration.**

When a scale is lost, a replacement (regenerated) scale grows rapidly to reach the size of the original. Regenerated scales do not form circuli during this period of rapid growth. Therefore, scales regenerated beyond the fourth or fifth circuli should not be used for age estimation.

Regenerated  
Area



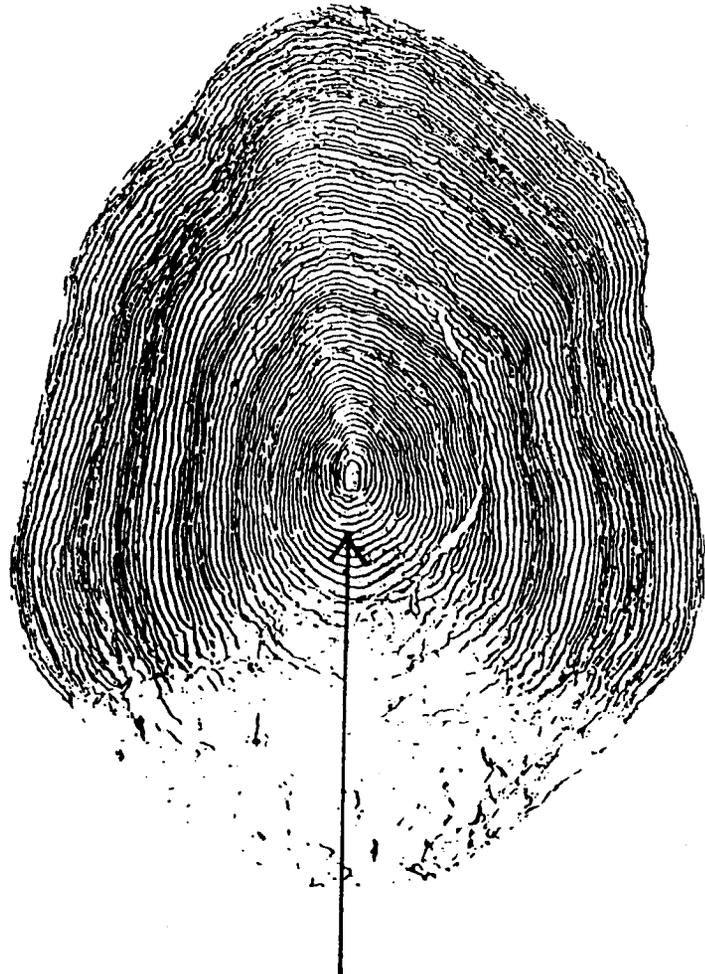
Modified from: Coggins 1994.

**Appendix A3.-First annual mark.**

Rainbow trout may not produce an annual mark their first year of life (Lentsch and Griffith 1987). If there is no distinct first year annulus within the first 20 circuli, an annulus is assumed to be within the seventh to fourteenth circuli.



Definite first year annulus

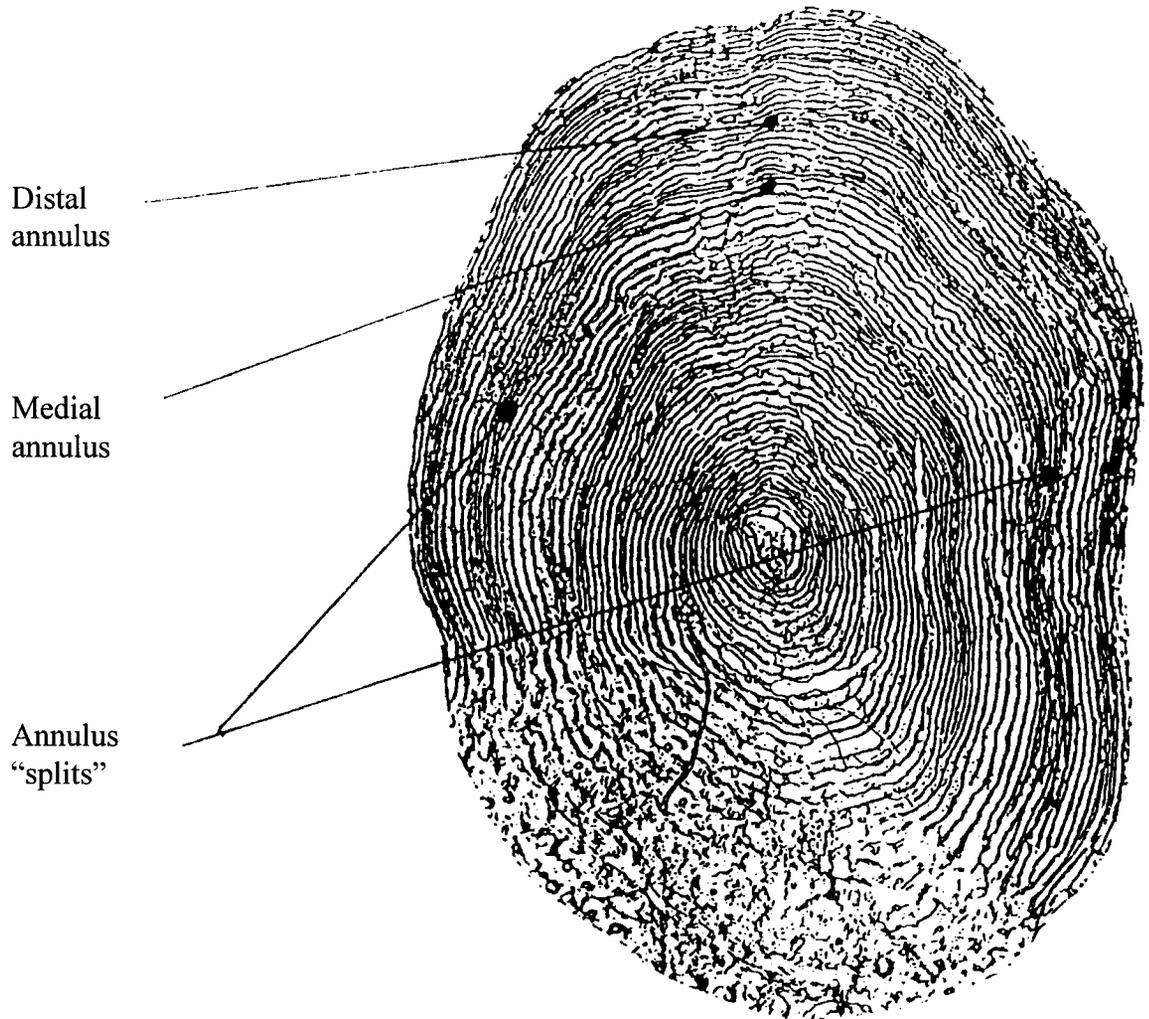


No definite first year annulus

Modified from: Coggins 1994.

#### Appendix A4.-Spawning mark or “check.”

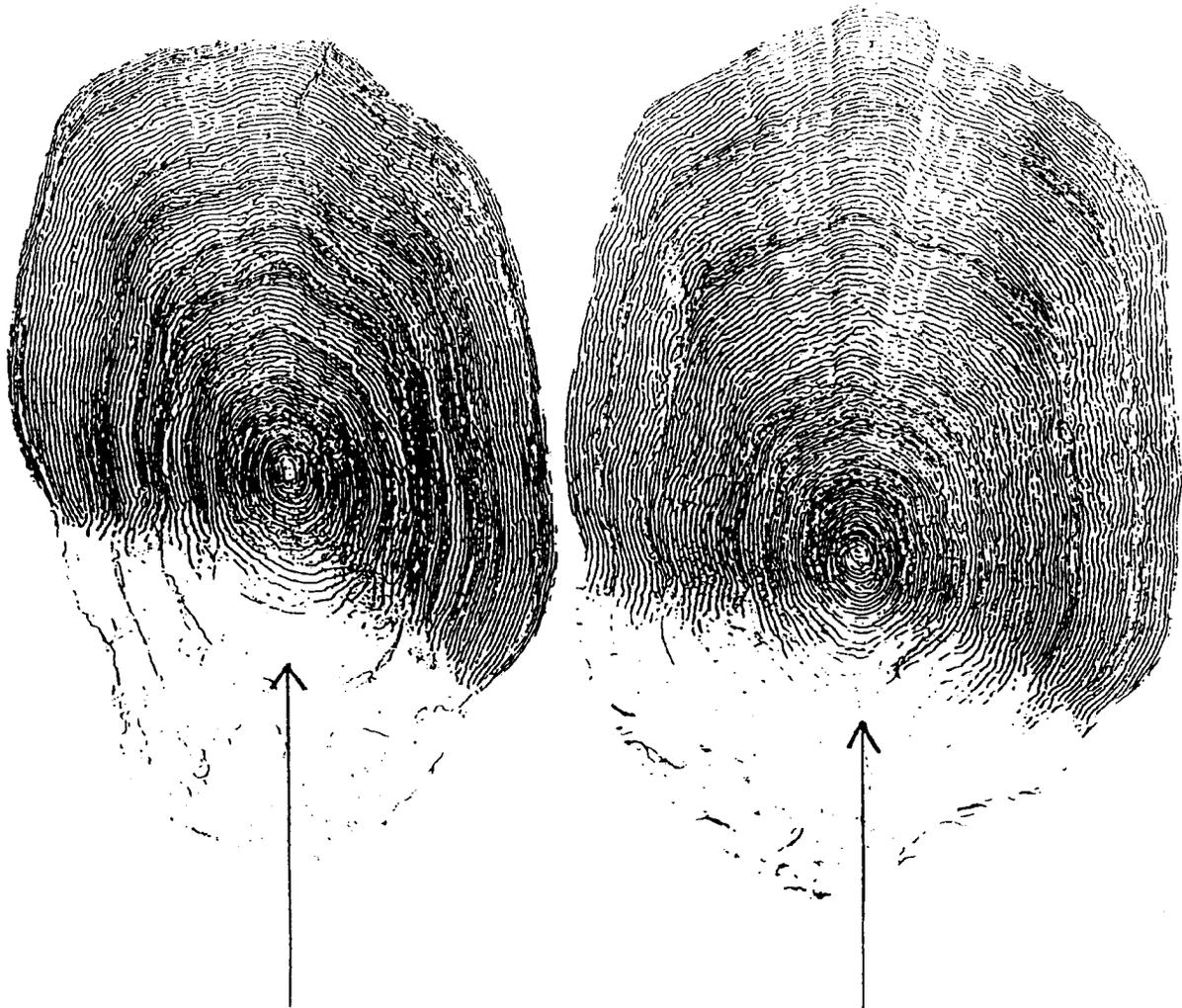
During spawning, rainbow trout tend to resorb the margin of their scales. Resorption may consume the scale through zero, one, or two annuli. This results in a structure called a spawning check and is interpreted as 2 years.



Modified from: Coggins 1994.

**Appendix A5.-Plus growth.**

The growth occurring after the production of the last annulus until the collection of the sample is called "plus" growth. A reader must be aware of when samples were collected to accurately assess plus growth.



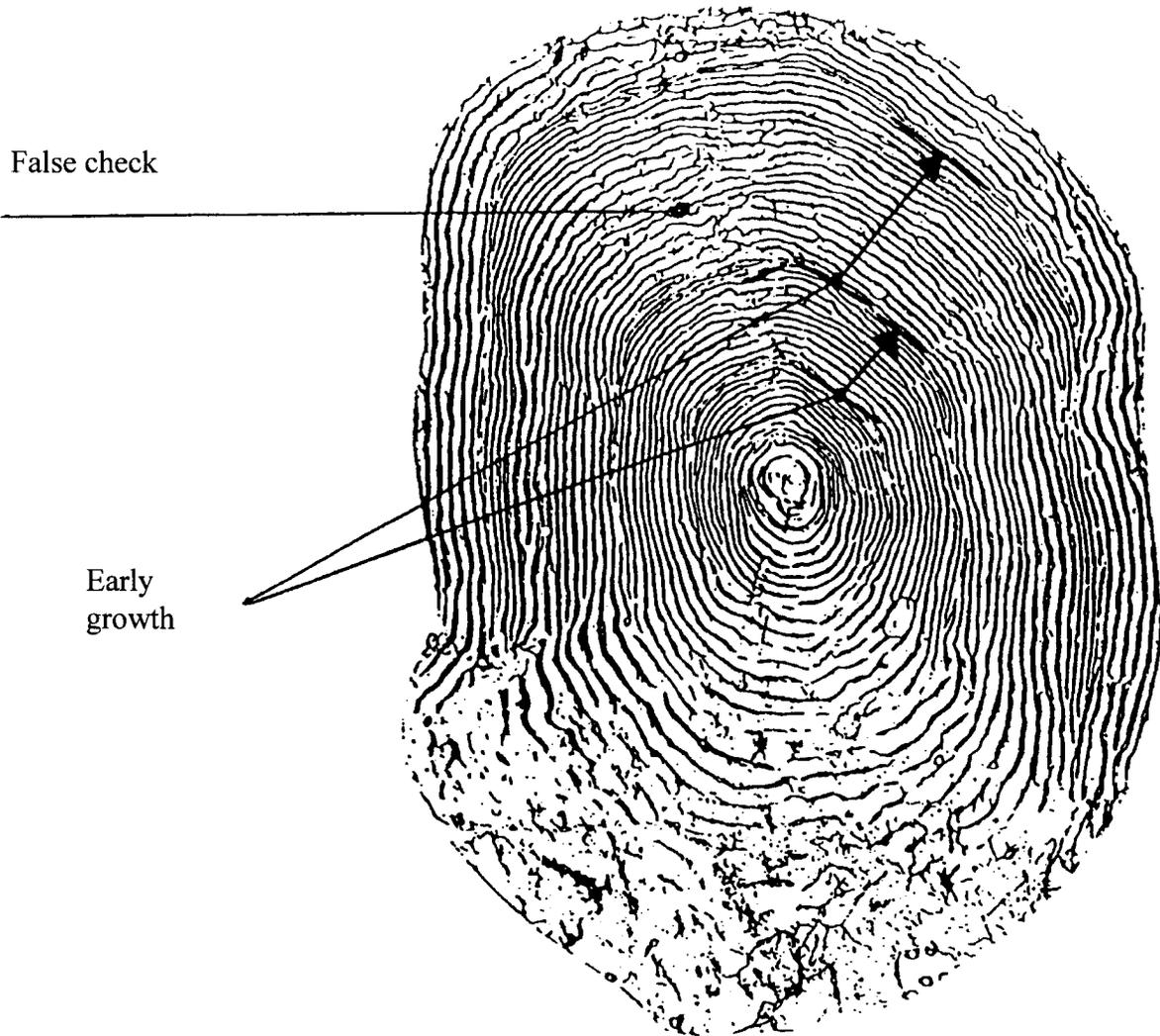
Spring sample with a check on the margin

Spring sample without a check on the margin

Modified from: Coggins 1994.

**Appendix A6.-False “check.”**

False annual marks may occur during a period of reduced growth, injury, or shock (Lux 1971). False checks can occur in rainbow trout and readers should be aware of their existence.



Modified from: Coggins 1994.



**APPENDIX B. INSTRUCTIONS FOR RECORDING DATA ON  
AGE-WEIGHT-LENGTH FORM VERSION 1.1.**



ALASKA DEPARTMENT OF FISH & GAME  
STANDARD AGE WEIGHT LENGTH  
FORM VERSION 1.1

DESCRIPTION: \_\_\_\_\_

COLLECTED BY: \_\_\_\_\_

AGE STRUCTURE TYPE:  1  2  3  4  5  6  7  8  9

TYPE OF TAG:  1  2  3  4  5  6  7  8  9  0  1  2  3  4  5  6  7  8  9

DO NOT WRITE IN THIS MARGIN

1826322

#	AGE OF FISH		AGE CRK	T A G E	R E P E T	FIN CLIP	TAG COLOR	- TAG NUMBER -						
	10's	1's						100,000's	10,000's	1000's	100's	10's	1's	
1	1	2	3	4	0	1	2	3	4	5	6	7	8	9
2	1	2	3	4	0	1	2	3	4	5	6	7	8	9
3	1	2	3	4	0	1	2	3	4	5	6	7	8	9
4	1	2	3	4	0	1	2	3	4	5	6	7	8	9
5	1	2	3	4	0	1	2	3	4	5	6	7	8	9
6	1	2	3	4	0	1	2	3	4	5	6	7	8	9
7	1	2	3	4	0	1	2	3	4	5	6	7	8	9
8	1	2	3	4	0	1	2	3	4	5	6	7	8	9
9	1	2	3	4	0	1	2	3	4	5	6	7	8	9
10	1	2	3	4	0	1	2	3	4	5	6	7	8	9
11	1	2	3	4	0	1	2	3	4	5	6	7	8	9
12	1	2	3	4	0	1	2	3	4	5	6	7	8	9
13	1	2	3	4	0	1	2	3	4	5	6	7	8	9
14	1	2	3	4	0	1	2	3	4	5	6	7	8	9
15	1	2	3	4	0	1	2	3	4	5	6	7	8	9
16	1	2	3	4	0	1	2	3	4	5	6	7	8	9
17	1	2	3	4	0	1	2	3	4	5	6	7	8	9
18	1	2	3	4	0	1	2	3	4	5	6	7	8	9
19	1	2	3	4	0	1	2	3	4	5	6	7	8	9
20	1	2	3	4	0	1	2	3	4	5	6	7	8	9
21	1	2	3	4	0	1	2	3	4	5	6	7	8	9
22	1	2	3	4	0	1	2	3	4	5	6	7	8	9
23	1	2	3	4	0	1	2	3	4	5	6	7	8	9
24	1	2	3	4	0	1	2	3	4	5	6	7	8	9
25	1	2	3	4	0	1	2	3	4	5	6	7	8	9
26	1	2	3	4	0	1	2	3	4	5	6	7	8	9
27	1	2	3	4	0	1	2	3	4	5	6	7	8	9
28	1	2	3	4	0	1	2	3	4	5	6	7	8	9
29	1	2	3	4	0	1	2	3	4	5	6	7	8	9
30	1	2	3	4	0	1	2	3	4	5	6	7	8	9

DO NOT WRITE IN THIS MARGIN

VARIABLE	0	1	2	3	4	5	6	7	8	9
----------	---	---	---	---	---	---	---	---	---	---

VARIABLE	0	1	2	3	4	5	6	7	8	9
----------	---	---	---	---	---	---	---	---	---	---

VARIABLE	0	1	2	3	4	5	6	7	8	9
----------	---	---	---	---	---	---	---	---	---	---

VARIABLE	0	1	2	3	4	5	6	7	8	9
----------	---	---	---	---	---	---	---	---	---	---

## **Appendix B2.-Instructions for completing Age-Weight-Length Form version 1.1.**

### **BIOLOGICAL (AGE-WEIGHT-LENGTH) FORM**

#### **6.1 Introduction**

The Biological Form is used to record biological information about sampled fish. Each sheet is intended for the recording of fish caught on one date. Each line of the form is intended for the measurement of a single fish. Information in the header blocks at the top of the sheet must apply to all fish on the sheet. The form is shown in Figure 6.1.

The back of the page is a continuation of the front of the page. Line 1 on the front is the same fish as line 1 on the back.

We highly recommend that you record the page number, because the data file will be sorted by date, page, litho-code, and line number before it is sent to you. You may write the numeral for the page number in the right margin or next to the "PAGE NUMBER" text if you wish.

#### **6.2 Header Fields**

The Location, Sublocation and Date fields are used to identify to which study the data belong. It is very important that these fields be used consistently between the forms.

##### **Location**

The location field is in the upper right-hand corner on the front of each form. The first row of the location block will be used to designate the general survey area. These survey areas correspond to the areas used by the Statewide Harvest Survey. Number codes for these areas are given in Appendix C.

Generally, the third digit of the first row will be left blank. However, the Kenai Peninsula Area has been subdivided and uses three digits to denote the survey area.

If you are not sure what survey area your study is in, either contact RTS or consult the latest Statewide Harvest Survey Federal Aid report.

The second row of the location block will be used for the specific site code within the area. This is a unique three digit code assigned to your survey location (refer to Appendix D). Consult RTS for the correct site code for your survey location if it is not listed in Appendix D.

The third row of the location block will not be used.

### Sublocation

Sublocation codes are used to separate portions of a fishery. You will need to do this if you want to generate separate estimates for subsections of a site. You may, for instance, want an estimate of not only the Kenai River as a whole, but also for the Lower Kenai River and the Upper Kenai River. The Lower and Upper Kenai would be sublocations.

Different optional variables may be recorded for different sublocations of a fishery; however, if they are to be combined to generate an estimate for the entire fishery, then the combined estimates will be limited to those variables common to all sublocations. For example, if you record boat type on the Lower Kenai River sublocation but not on the Upper Kenai River, then an estimate of the number of canoes on the entire Kenai River can not be generated.

Sublocation codes shall be numbers between 01 and 26. These codes cannot be numbers higher than 26. The numbers are converted to letter codes (01 will be A, 04 will be D, etc.) when the data are processed. Within that framework, sublocation codes can be assigned by the project staff involved in the creel survey. Once sublocation codes are assigned they must be used consistently throughout the study. These assigned values should be recorded on the Field Specification form shown in Figure 6.2.

Sublocation codes are entered in the first row of the sublocation block. The second row of the sublocation block will be blank.

### Date

The date entered in this field is the date that the data are collected.

Data for only one date may be put on a single mark-sense form.

It is important to record the date correctly because this information is used to determine the time strata (weekend or weekday for instance).

### Project Number Optional Valid: 4 digits or blank

This is intended to be your project budget code, but you may use it as needed. The first 3 digits are entered in the top row, and the last 3 digits in the second row.

Page Number Required Valid: 001-999 or blank

This field is required. You should instruct your field crews whether you want to start the page numbers at 1 each day, or start at 1 the first day of the study and go up from there throughout the season. You can decide for yourself whether or not you want leading zeros marked, but be consistent for a given study.

Species Code Optional Valid: Sport Fish Division codes (see Appendix E)

This is the species of fish recorded on this sheet. Information on only one species of fish can be recorded on a page. Enter one digit of the Biological Sport Fish Division code in each field of this block. See Appendix E for the Sport Fish Division species codes.

Type of Measurement - Length & Weight Optional Valid: one type for each measurement or blank

Only one type of measurement can be marked for length and weight. See the code listing in Figure 6.3 for the possible types of measurements.

Type of Measurement - Fishery Optional Valid: one type or blank

This is the type of fishery (test, sport, commercial, or subsistence) in which the fish were caught. Only one type can be marked.

Gear Code Optional Valid: Sport Fish Division gear codes or blank

This is the type of gear used to catch the fish. Valid codes are those used in the Sport Fish Division system (see Figure 6.3). The top row is for the tens digit, the bottom row is for the ones digit.

Mesh Size - Inches and Eighth's Optional Valid: 0-4 in, 0-7 eighth's or blank

This is the mesh size of the net (if the fish were taken with a net).

### 6.3 Biological Data

Sex Optional Valid: M or F or blank

Maturity Index Optional Valid: Sport Fish Division codes or blank

This is a binary field. Enter the combination of numbers that adds up to the appropriate Sport Fish Division code. See Figure 6.3 for the appropriate Sport Fish Division codes.

Length Optional Valid: length in mm (up to 1999) or blank

Trailing zeros must be entered, but leading zeros need not be. Note that there is a single "1" to left of the hundreds for the thousandths place. You can, therefore, record a fish length up to 1999 mm.

Weight Optional Valid: weight in units specified (up to 9999) or blank

This is the weight in the units specified under TYPE OF MEASUREMENT. You must enter trailing zeros, but need not enter leading zeros.

#### 6.4 Back of Page Header Fields

Variable Fields Optional Valid: varies

These are variable fields that can be used to record information unique to your study. The meaning of these fields must be defined before your study begins, and the fields must be used consistently throughout the study. Record the meaning of codes in these fields on your Field Specifications Form.

Age Structure Type Optional Valid: one type or blank

This is the structure used to age the fish (such as scale or otolith). Only one type can be entered. There are 5 variable cells in this field which can be used to record an age structure that is unique to your study. Be sure to use these variable fields consistently, and record the meaning of these codes on your Field Specifications Form.

See Figure 6.3 for the possible age structure types.

Type of Tag Optional Valid: two digit code for type of tag or blank

See Figure 6.3 for the codes for different types of tags.

## 6.5 Back of Page Biological Data

Age Optional Valid: Age of fish or blank

Use the European method to record age of salmon. For all other species record the total age in years.

Example of the European method: A salmon has lived 3 years in fresh water and 2 in salt and is 6 years of age. The European age would be 3.2. You would enter a 3 in the tens digit and a 2 in the ones digit. For salmon that spent 0 years in fresh water, enter a 0 in the tens digit.

Age Error Optional Valid: R or I or blank

Mark R for a regenerated scale, I for illegible, or leave blank. Only one of these cells may be marked.

Fate Optional Valid: K or blank

Mark this box if the fish was killed.

Recp. Optional Valid: marked or blank

Mark this box if this is a recaptured fish.

Fin Clip Optional Valid: one or more marked or blank

Mark each fin that has been clipped. This is one of the rare fields where you may mark more than one response. See Figure 6.3 for the meaning of the fin clip codes.

Tag Color Optional Valid: one or more marked or blank

This form has space for marking black, white, the primary colors, and clear. If your tag is one of these colors, mark only one.

If you have a tag that is not one of these colors, mark the two colors that would combine to make your color. For example, if you have a green tag, mark yellow and blue. See Figure 6.3 for the appropriate combinations.

If you have a multi-color tag, mark three colors. This way we can differentiate between the blend colors and the multi-color tags. If you have a color that cannot be recorded by this system, contact RTS.

Tag Number Optional Valid: 000000-999999 or blank

The upper digits of tag number are binary fields. They have the choices 0,1,2,4 and 7. Mark these to sum to the total you want. For example, if your tag number is 8000 you would mark the 7 and the 1 under the 1,000's, and zero under the 100's, 10's and 1's. (Normally a binary field would have the choices 1,2,4,and 8. Due to a printing error we have 1,2,4,and 7. Because the forms are printed that way, that is the way we will use them. (Do not use the last cell as an 8!)

You must enter trailing zeros, but you don't need to enter leading zeros.

## 6.6 Litho-code

Litho-code Valid: DO NOT PUT ANY MARKS IN THIS AREA

The left side of the page has a special field called a litho-code. This is the machine-readable form of the number printed on the front of the form. Do not mark any of these litho-code boxes. Do not mark in the blank litho-code boxes on the back of the form. When your data sheets have been read you will receive a listing of your data in which each line will be associated with this litho-code.

## 6.7 Scale Numbers

### Scale Numbers

There is no space designated on this form for scale numbers. Scale numbers have been used in some studies to match scale envelopes with fish entries. If you need to associate scales taken in the field with data recorded on these forms, record the litho-code number and the line or entry number from your AWL form on to your scale envelope.

Suppose the litho-code number is 68345 and the entry number is 6, then the scale number will be 68345-06. The dash to separate the numbers, and the leading zero in front of the 6 are required.

If you use gum cards instead of envelopes, a slightly different approach should be used. A typical gum card will hold 30 or 40 scales (10 rows of 4 scales).

Method 1: Since the biological mark-sense form has rows for 30 fish, then 1 scale from each fish could be put on the gum card in positions 1 through 30. There would be a one gum card to one mark-sense form relationship. Simply use the litho-code to match gum cards and mark-sense forms.

Method 2: In method 2, up to 4 scales are collected from each fish. Each row on a gum card would be one fish or line on the mark-sense form. This method would have 3 gum cards per mark-sense form. Under this method, not only is the litho-code required, but it is also important to record on the gum card which set of 10's (1-10, 11-20, 21-30) that it matches.

When the ages have been determined, you will mark the age information on the same sheets you have marked the length and weight information. RTS will then feed these forms through the optical scanner. If length-weight data reports are needed before the scales have been read, a two step process can be used.

## 6.8 Example

### Example 6.1

You are recording King salmon from survey area A, site 23, sublocation Z. Today is April 22, 1991, and you are recording on page 1. Your project leader has decided not to record gear codes. You measure, using mid-eye to fork of tail, an immature female king at 620 mm. It weighs 10 pounds. This fish was caught in a commercial fishery. You take a scale, and mark the line number and litho-code on the scale envelope. The fish has no tag, but has an adipose clip. Later on, you read the scale and determine that the fish is a 1.4.

Figure 6.3 Biological (AWL) Form Code Definitions

FIELD NAME	CODE	MEANING
GEAR CODE	See Appendix F for gear codes.	
TYPE OF MEASUREMENT: LENGTH	SL	Standard length
	FL	Fork length
	TL	Total length
	EF	Mid-eye to fork
WEIGHT	G	Grams
	KG	Kilograms
	LB	Pounds
	OZ	Ounces
FISHERY	TE	Test fishery
	SPF	Sport fishery
	CF	Commercial fishery
	SUF	Subsistence fishery or Personal use fishery
SEX	M	Male
	F	Female
MATURITY INDEX	0	Not checked
	1	Immature
	2	Developing
	3	Spawning
	4	Post-spawning
	5	Redeveloping (consecutive)
	6	Redeveloping (non-consecutive)
7	Unknown	
AGE STRUCTURE TYPE	SC	Scale
	OT	Otolith
	CL	Cleithrum
	CE	Centrum
	FR	Fin rays
	1	Determined by Project leader
	2	Determined by Project leader
	3	Determined by Project leader
	4	Determined by Project leader
	5	Determined by Project leader

Figure 6.3 Biological (AWL) Form Code Definitions (continued)

TAG COLOR:

Single color tags:	W	White
	BK	Black
	R	Red
	Y	Yellow
	B	Blue
	C	Clear

Tags with a single color not listed on form: Mark 2 colors which when blended together would be the color of the tag. Some examples are:

W and BK	Gray
R and Y	Orange
Y and B	Green
B and R	Purple

If you have a multi-color tag, mark three colors. This way we can differentiate between the blend colors and the multi-color tags.

From: Heineman 1991.