GUIDE FOR SAMPLING STRUCTURES USED IN AGE DETERMINATION OF PACIFIC SALMON

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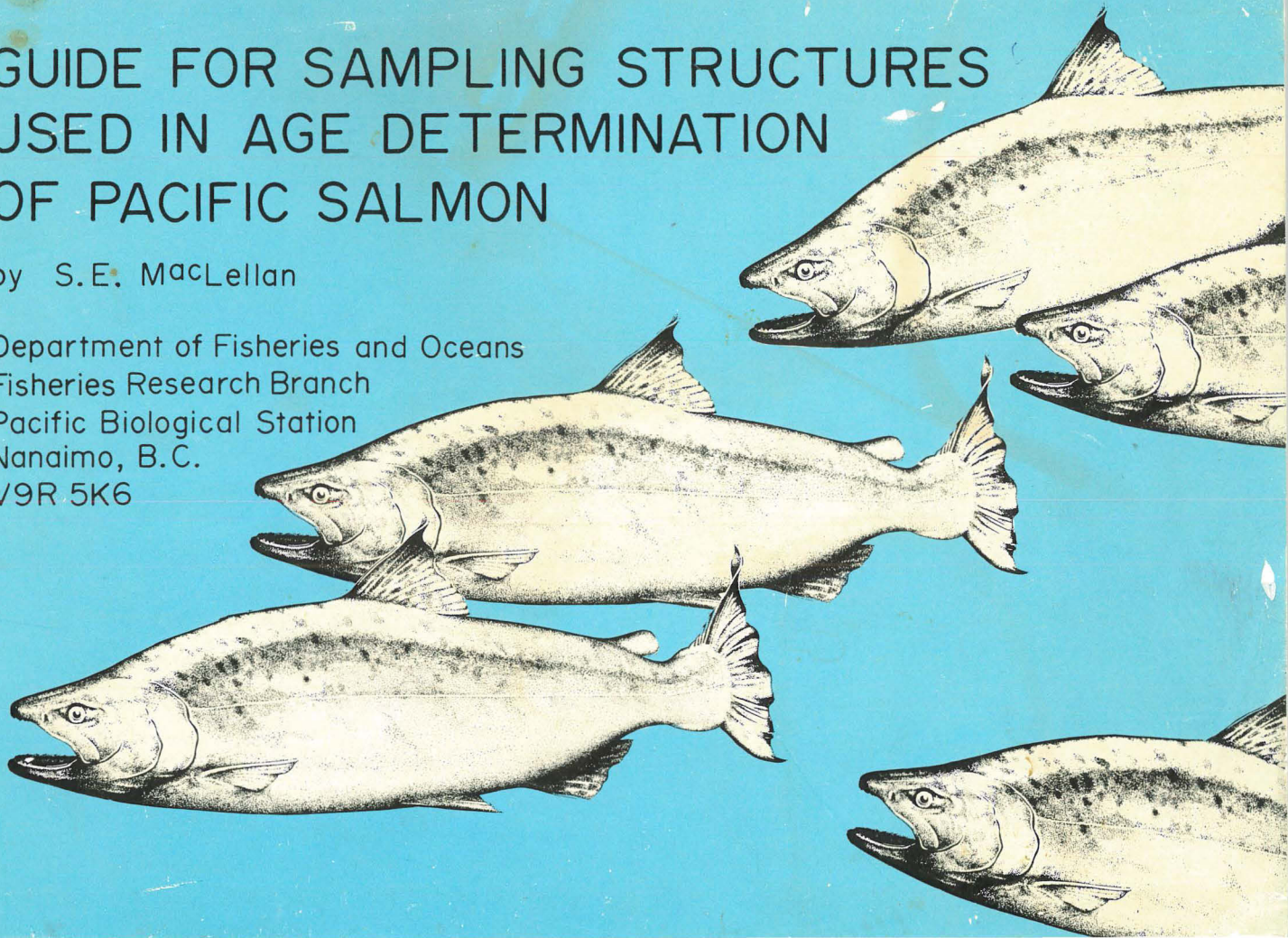

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INTRODUCTION

The purpose of this sampling guide is to provide samplers with brief, clear instructions on the collection of "Quality" samples of structures (e.g. scales, otoliths, fin-rays) used in age determination of fish. In work of this nature, quality is the key word. Sampling of salmon for age, size, and sex composition is the first step towards developing and establishing fisheries and environmental management strategies. The collection of poor quality samples could result in incorrect age determinations, leading to improper management of stocks. Therefore, it is critical that the importance of collecting Quality Samples be understood by the samplers.

This guide is designed for use by samplers on its own, but preferably in conjunction with a 1/2 - 1 day training session given by the Fish Age Determination Unit at the Pacific Biological Station, Nanaimo, British Columbia. New samplers are given practical demonstrations on how to take scales, fins and otoliths. The importance of taking Quality Samples is emphasized by showing samplers how these structures are processed in the

laboratory. By the end of the session, each person should have a basic understanding of the reasons behind specific instructions.

Knowledge of how the data collected is used, can make a considerable difference in understanding the need for good quality samples. Age-structures such as scales, fins, and otoliths are processed by age determination technicians, who interpret age from the annual growth pattern on such structures. The resulting ages are provided to biologists who use this information in a variety of ways. Ages provide information on various aspects of salmon life history, such as:

a. survival/mortality,
b. age at maturity/spawning,
c. growth rate,
d. reproductive capability,
e. extent of fresh water and marine residence.

Annual monitoring of the age, size, and sex compositions of stocks, as well as catch abundance and escapement, helps in assessing fishing impact and hatchery strategies.

COLLECTION OF STRUCTURES USED IN AGE-DETERMINATION, AND RELATED DATA

IDENTIFYING SAMPLES

First, it is important to write clearly when recording information.

Always identify samples completely by:

1. Species.
2. Location caught and sampled. Be specific.
3. Date caught and sampled. Day/month/year (it can be a time range, but not more than 2 weeks).
4. Sample identification--book number, tag number, individual fish number, etc. Be sure to identify clearly each fish on data sheets, trays, books, or envelopes. If more than one structure is taken, e.g., scales and fins, make sure their I.D. numbers match up.
5. Gear.
6. Sampler(s).

OTHER INFORMATION

Length

The Age Determination Unit is concerned mainly with the quality and identification of samples collected for them. Instructions for other data to be gathered, such as length, weight, maturity, etc., should be supplied by the requesting biologist. The unit usually requests that fish length be recorded. Figure 1 shows how to take the 2 types of length measurements, hypural and fork.

1. Fork length - in millimeters (mm)
   - measure from tip of snout to tail fork
2. Hypural length - in millimeters (mm)
   - measure from posterior margin of eye orbit to end of hypural plate (last vertebra)
   - the hypural plate is found by flexing the tail up, then measuring to the resulting fold

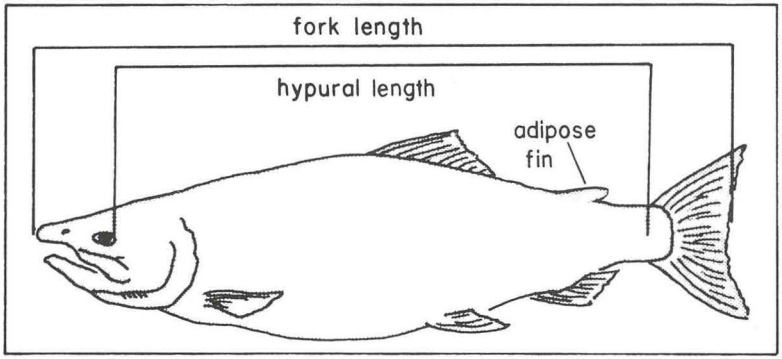

Fig. 1. Shows left side of salmon and where to take fork and hypural lengths. Samplers should look for missing adipose fin on all salmon.

Marked fish

A missing adipose fin (Fig. 1) often indicates that a fish contains a coded-wire-tag (CWT) in its nose. Record the information and remove the head. The head should be frozen and individually identified to correspond to other sampling data. The data gathered from the removed CWT is very important. The Age Determination Unit uses this information to develop and validate ageing criteria.

SCALE SAMPLING

COLLECTION

1. Place the fish on its right side to sample the left side.
2. Locate and wipe the preferred area (Fig. 2) clear of water and slime. Figure 2 shows INPFC "rated" areas for scale removal (INPFC 1958). Take scales from area A, before area B. Area C is not preferred. In each area, take scales from above the lateral line before trying below the lateral line.
3. Remove the preferred scale (Fig. 3a) by grasping its exposed posterior edge with forceps and pulling (Fig. 3b). Clean the scale of dirt and skin by rubbing it between thumb and fingers.

4. Hold the scale up to a light source to check for deformation or regeneration (Fig. 4). Scales with these features cannot be aged. Discard the scale and try again in the preferred area. Take alternate scale samples from the next rated area if necessary (Fig. 2). Try the right side of the fish if there are no suitable scales on the left side. Indicate on the sampling sheet the location of the alternate scale, using the INPFC sampling code in Fig. 2 (INPFC 1958).
5. Center the scale on the numbered square so that the "rough" surface that faces "outwards" from the fish remains facing up on the scale book. See Fig. 3b. The outward surface contains the growth pattern. Orient all scales in the same direction (Fig. 5a), not as seen in Fig. 5b. To check if the correct side is facing up, scrape the surface of the scale with forceps/fingernail. It should feel rough.
6. Be sure the scale firmly adheres to the scale book. A slightly moist scale will adhere well. Press down with dry finger or pencil end.
7. The scale book surfaces must be kept dry. Excessive moisture will dissolve the book's adhesive coating and cover or wash away the scale.

Collection of scale smears from small, juvenile fish

Collect single scales from small fish as long as individual scales are visible. Otherwise, scale smears can be taken:

1. Using a scalpel, gently scrape the blade in a posterior to anterior direction over the preferred area.

2. It is important to take care that scales adhere to the card, rough side up. Therefore, without turning the scalpel over, push the scales onto the gummed card with a forefinger. Exert just enough pressure to spread and smooth the scales evenly onto one square.

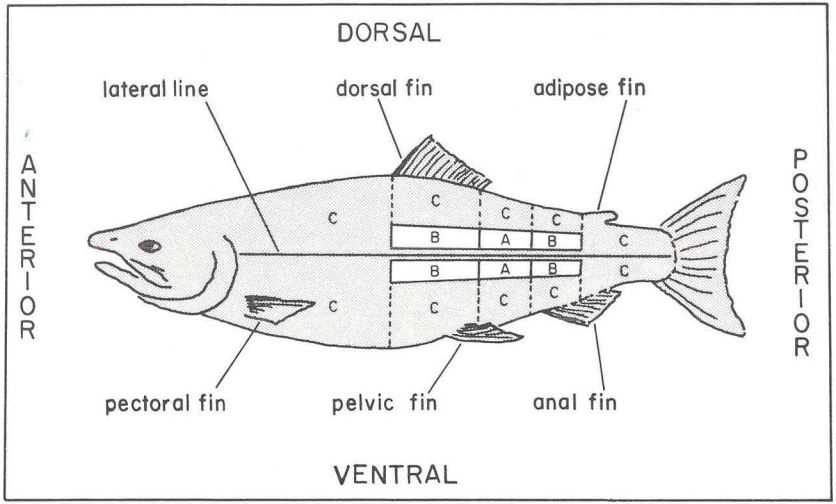

Fig. 2. INPFC rated areas for scale removal. Area A is the preferred area. B is the second choice if there are no scales in A. C designates nonpreferred areas. If scales on the left side of the fish are not good, try the right side.

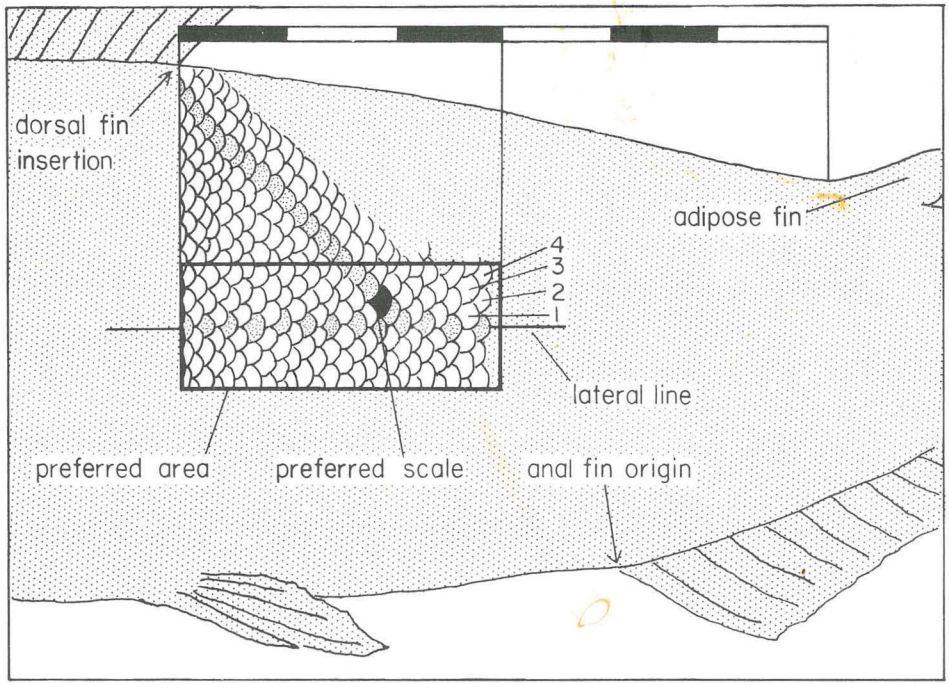

Fig. 3a. (Unpublished, Bilton 1967). The preferred scale is solid black. It is located 2 rows up from the lateral line, on a diagonal from the insertion (posterior) of the dorsal fin "back" towards the origin of the anal fin.

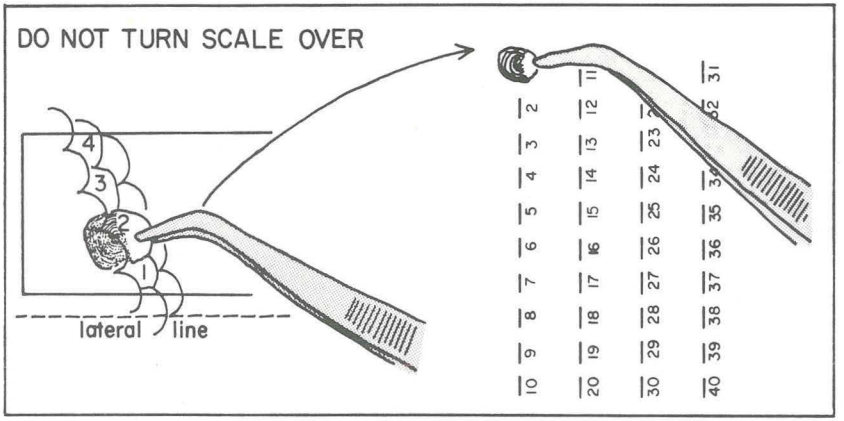

Fig. 3b. (Unknown source). Shows how to remove the scale from its pocket. Place the scale, rough side up, on the card without turning it over.

Fig. 4. Noticeably regenerate scale, about 1/3 is regenerate. When a fish regrows a lost scale, there is an absence of early growth zones in the scale centre.

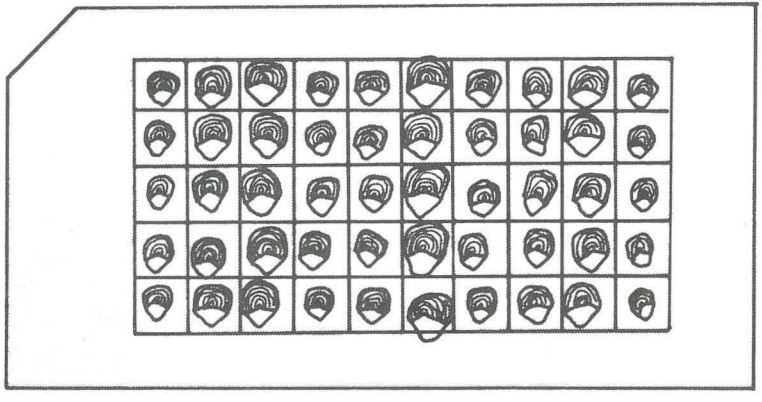

Fig. 5a. The scales are all correctly oriented on the card in the same direction.

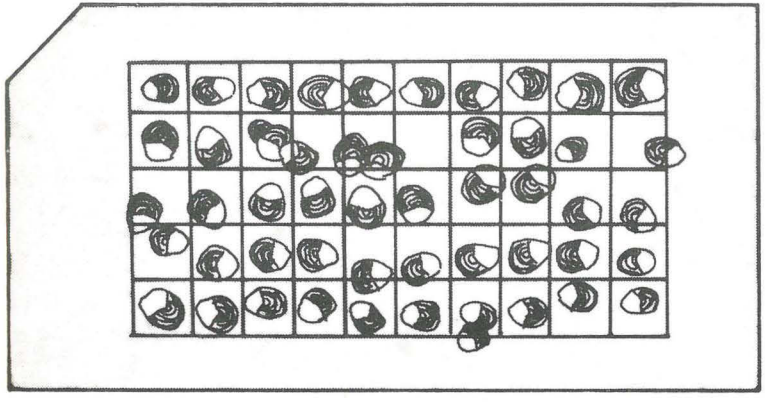

Fig. 5b. The scales are incorrectly oriented in different directions. This increases the time spent to age the sample.

Note: The inside brown cover of the scale book is important. Include the information marked in Figure 6. Do not write important data on the outside covers. These are discarded. Do not write code numbers for species, location, gear, etc. or abbreviate proper names. Codes change over time, and raw data such as scale card information can be lost. It may take a little longer to write it out, but it is safer. Fill out scale cards ahead of time if need be. Also, do not mix species or catch dates of more than 2 weeks on the same scale book. Have two books ready if it is anticipated that 2 species will be sampled at the same time.

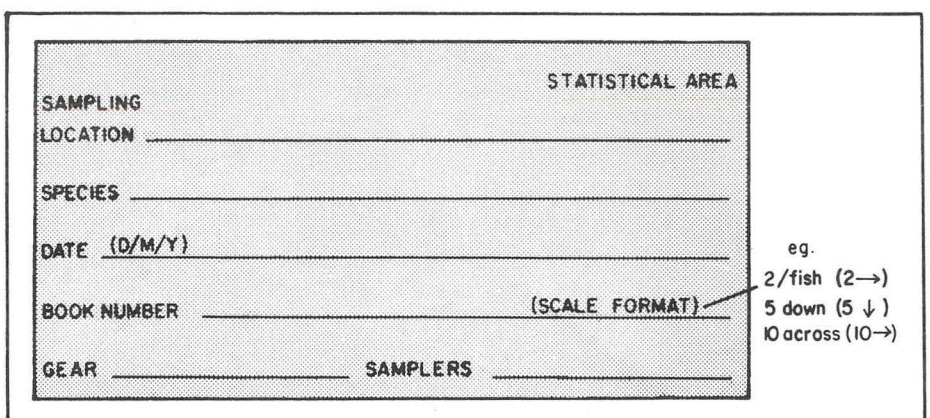

Fig. 6. Sample information requested on scale book cover.

HOW MANY SCALES ARE NECESSARY?

The number of scales per fish generally recommended to meet the current needs of salmon sampling at the Pacific Biological Station is presented in Table 1. Always check to verify the number of scales per fish required.

Table 1. Number of scales to be collected and book type to use depending on species, life stage and capture location.

Species	Life stage	Location of capture	No. of scales	Book type
Sockeye	adult	fishery	one	single: 1-50, 1 across (1 →)
Chum	"	"	one	" " "
Pink	"	"	one	" " "
Coho	"	"	two	double: 1-25, 2 across (2 →)
Chinook	"	"	two	" " "
Sockeye	adult	spawners	two	double: 1-25, 2 across (2 →)
Chum	"	"	two	" " "
Pink	"	"	two	" " "
Coho	"	"	five	single: 1-50, 10 across (10 →)
Chinook	"	"	five	" " "
All species	juveniles	migrants	smear	double: 1-25, 2 across (2 →)

Scales are aged by examining their magnified plastic impressions. Scale pressing involves a combination of very high temperature and pressure. The method is described by Clutter and Whitesel (1956). Figures 7-12 illustrate what correctly/incorrectly sampled and mounted chinook scale impressions look like.

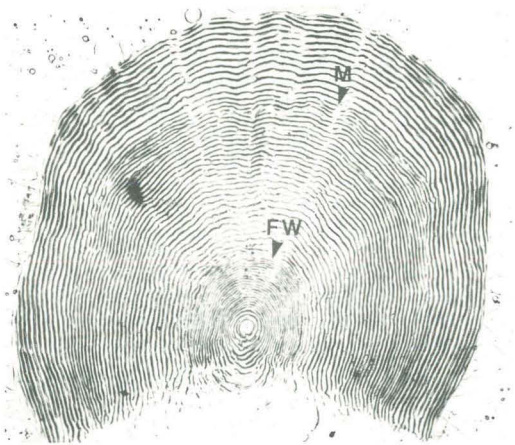

Fig. 7a. Preferred scale. Age 1.1 = 1 freshwater (FW) annulus + 1 marine (M) annulus.

Fig. 7b. Nonpreferred scales. These scales do not show symmetrical growth. They appear elongated or "lopsided" when compared to the preferred scale shape.

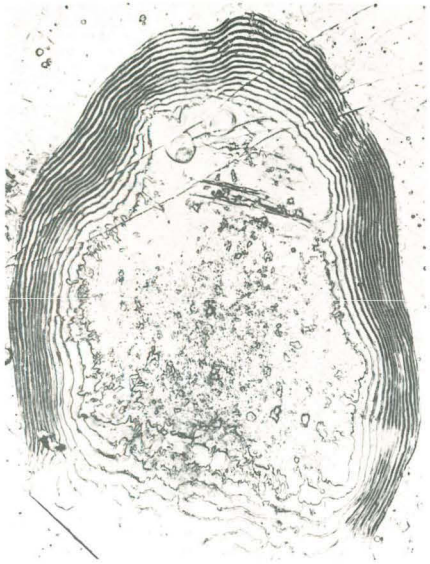

Fig. 8. Very noticeably regenerate scale. When held up to light, the scale centre will appear smooth with no lines.

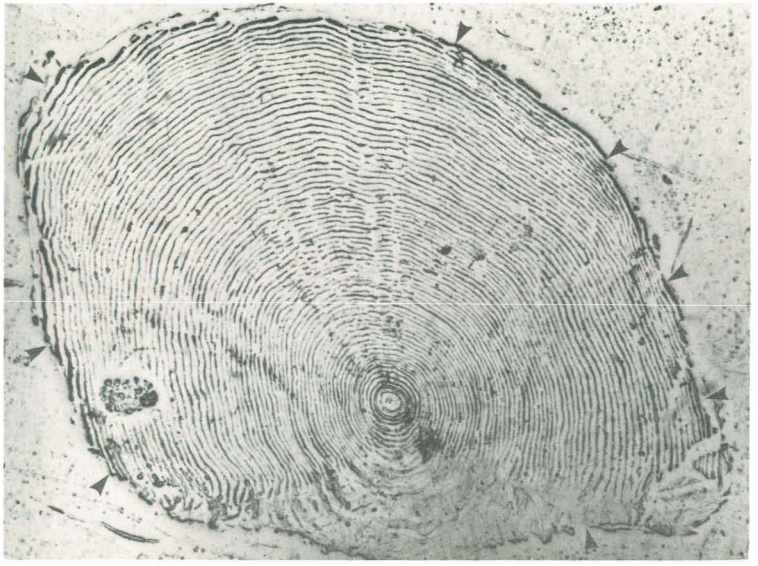

Fig. 9. Scale from spawning chinook. The edges are resorbed. See arrows.

Fig. 10. Upsidedown scale. Note the absence of a growth pattern.

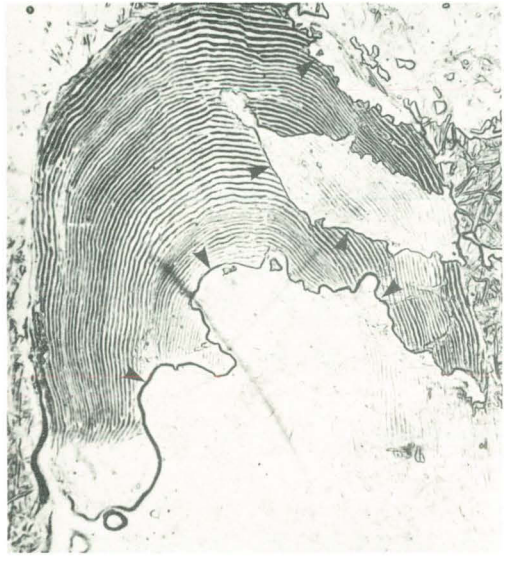

Fig. 11. Wet scale. Too much water has caused glue (arrows) to fill in between circuli. This produces a smooth impression which cannot be aged.

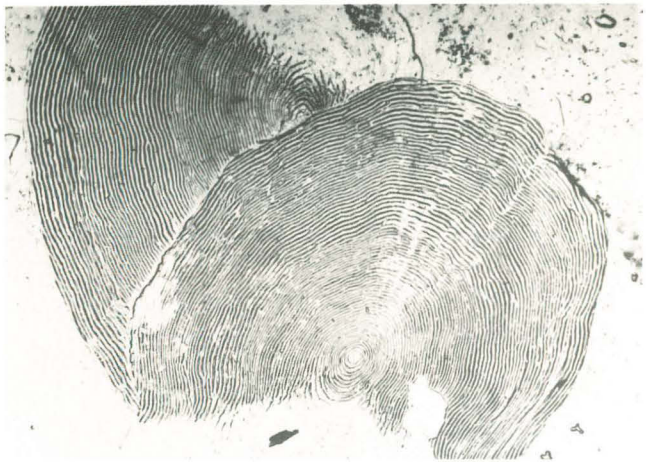

Fig. 12. Be sure only 1 scale is mounted in each square.

FIN SAMPLING

Fins have become important as a means of ageing salmon spawners because of their scale resorption (Chilton and Bilton 1986). Fin-rays can also be taken without killing the fish, a useful advantage for many research studies.

The most important thing to remember when cutting fins from a fish, is to ensure that the fin-ray bases are included. This means the area where the two ray elements separate to attach to the backbone or other supportive

bones. It is this basal area that contains the first few years of growth zones. When fin-rays are cross-sectioned, early growth zones do not appear in sections cut towards the fin tip. See Figures 13a and 13b.

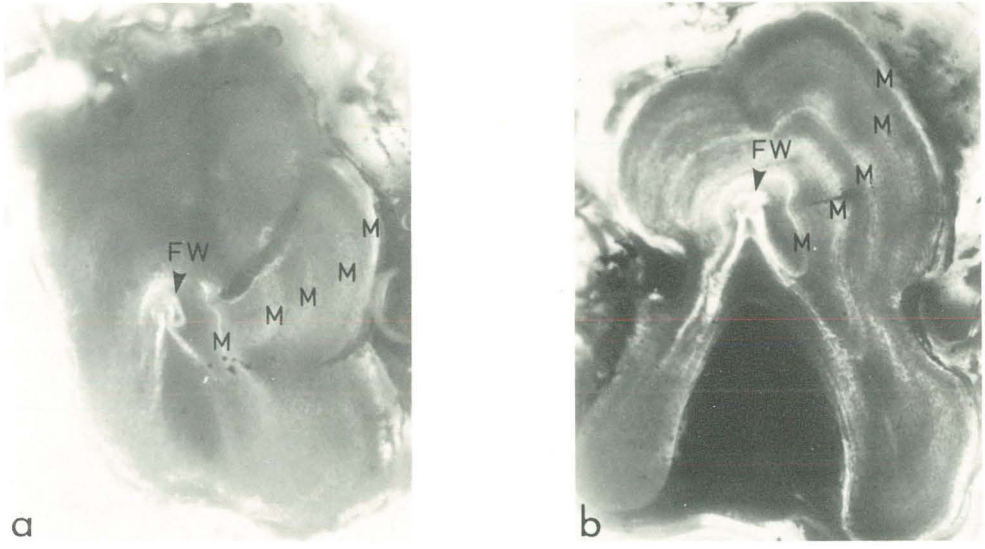

Fig. 13a, b. Photos of chinook fin-ray cross-sections taken from the same fin. a. is cut close to the fin base and shows a very clear fresh water (FW) annulus. b. is from further "up" the same ray. Note that the FW zone is not present/clear in b, but the marine (M) zones are clearer than in a.

The sampler should know, before going into the field, which fins and rays are required. A thin, sharp knife is best to use for removing fins.

Removal of the dorsal fin

Make the first cut downwards to the fish's backbone, just ahead of the first ray of the dorsal fin (Fig. 14a.). Holding on to the fin, cut posteriorly (towards the tail), moving the knife edge along the backbone. It is helpful to pull up on the fin while cutting. Trim off any excess flesh

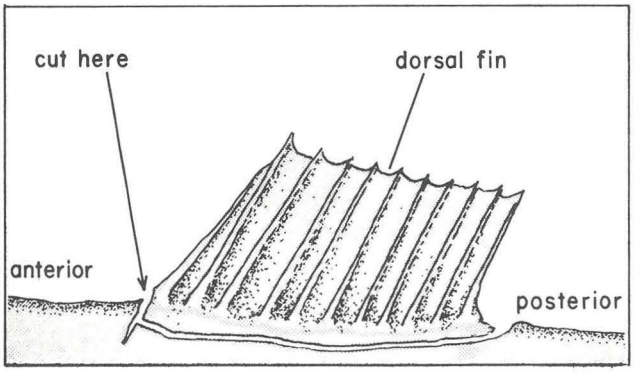

Fig. 14a. How to cut off the dorsal fin.

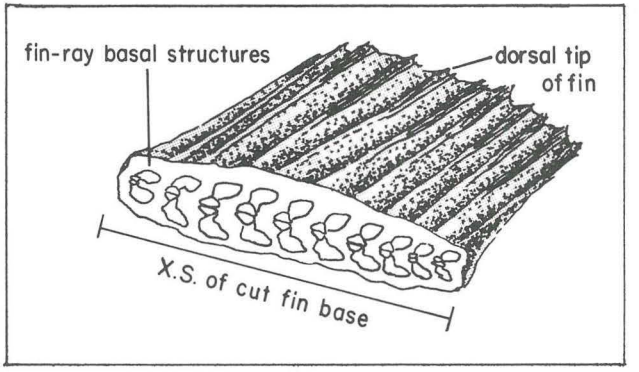

Fig. 14b. Ventral x.s. view of dorsal fin base cut. Removal of the pectoral fin.

along the fin base. Be careful not to cut off bone. A double row of white fin-ray bases will be evident if the cut is deep enough (Fig. 14b).

Removal of the pectoral fin

Cut through the basal structure where the fin-rays flare out to attach to the fish's body (Fig. 15). It is easier to start the cut through the leading (thickest) ray.

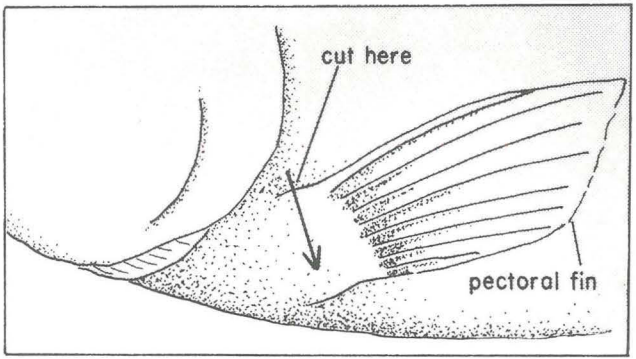

Fig. 15. How to cut off the pectoral fin.

Removal of fin ray(s) without killing fish

Some studies require ageing without sacrificing the fish. To do so, one or two rays can be taken by cutting them off as close to the fin base as

possible, (Mills and Beamish 1980). The number and location of rays to be sampled should be designated by biologists before samplers go into the field.

The size and condition of a fin/fish will dictate how it is to be sampled:

Small fish: If the fins are less than 5 cm (2 in) in length, take the whole fin.

Large fish: If the fin is too large to fit into an envelope, take only the first 6 rays. In this case, before cutting the dorsal fin off the back, cut the flesh behind the 6th ray. If the fin is still too large, divide it in half, i.e. slice down the flesh between the 3rd and 4th rays. Then store each half in its own labelled envelope. The dorsal tips (tapered end) can be cut off large fins to make them fit more easily into the envelope. Do <u>not</u> cut them shorter than 7.5 cm (3 in) from the base.

Broken fins: As long as there is 1 cm of fin left from the base structure it can be processed for sectioning. It takes extra time to do this, but can be done when necessary.

No dorsal fin: If there is no dorsal fin take the left pectoral, and if that is missing, take the right pectoral.

Storing the fin

Fins are stored in heavy paper envelopes. It is most important that the fin-rays are positioned as parallel as possible and all in one plane (untwisted and flat) (Fig. 16). Before inserting the fin, fold the envelope flap in and mark the fish number with a dark pencil. Put the identification on the bottom of the envelope. Proper identification of fins is necessary to relate to scale, otolith, and/or tag data. The basal portion of the fin should stick out, free of the envelope (Fig. 16). The natural moisture of the fin will adhere it to the paper. Spread and flatten the rays as the fin is inserted by pushing it up against one side of the envelope.

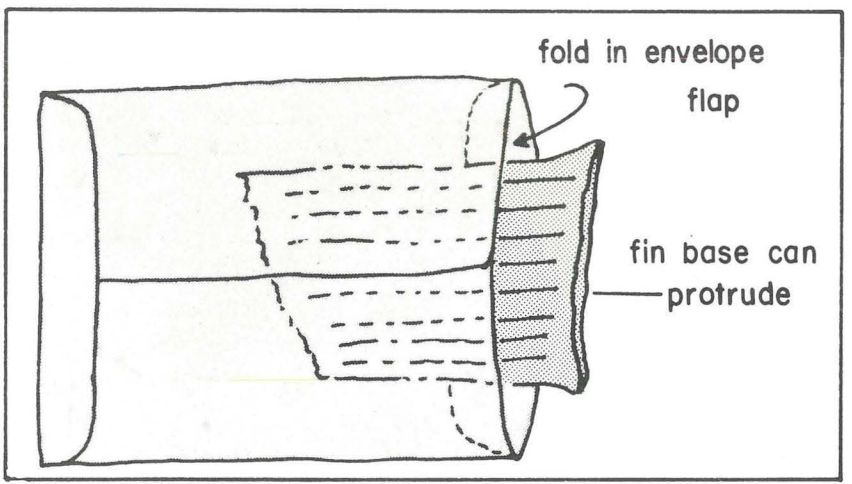

Fig. 16. When inserting the fin into the envelope, press it flat against one side and leave the base end protruding.

Fins may be stored in small plastic bags as long as the rays are "robust" enough to remain straight and untwisted. Otherwise, envelopes must be used to support "flimsy" rays of young/juvenile fish. Waterproof labels marked with pencil must be used for plastic bag storage.

The lab prefers that fin samples be frozen immediately. Alternatively, they can be spread out in envelopes to air-dry (1-3 weeks).

OTOLITH SAMPLING

Otoliths (Fig. 17) are also sampled. They are a bony deposition formed in the fish's middle ear. Otoliths function to help maintain balance. They are surrounded by a membranous sac and are located in shallow cranial grooves under the brain, just behind the eyes, (Fig. 18).

There are several types of cuts used to remove otoliths from a fish's head. See Fig. 19a for the more common ones. Each sampler may find one cut to be more efficient than another. A sharp, thin knife and fine-tipped forceps (Fig. 19b) are required. With some experience, samplers should attain skill at locating the cranial otolith pockets with very little difficulty (Fig. 19c). It is important to collect both otoliths, unbroken, and as clean as possible. All membranes and blood must be removed before

storing. If they are left on the otolith, bacterial and fungal growth results. This biological action can cause obliteration of the growth pattern. The membranes can be removed very easily by rubbing the otolith along the back of a hand or cotton glove.

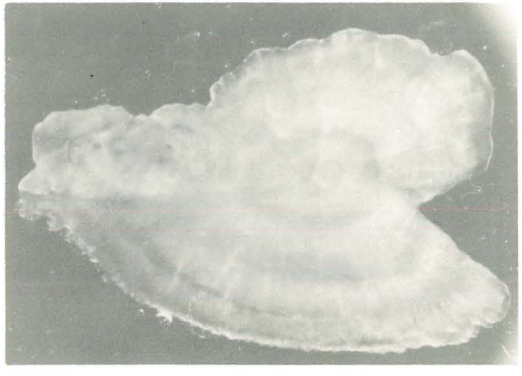

Fig. 17. Sockeye otolith. The dark zones are formed in winter, the light in summer.

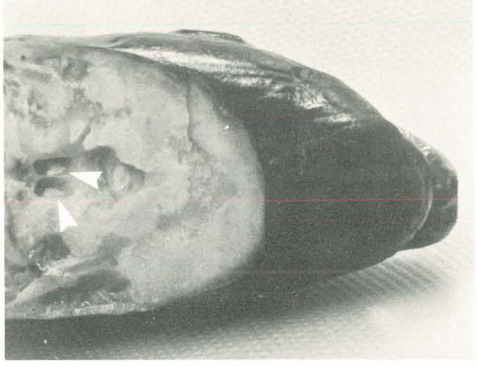

Fig. 18. Pair of otoliths located in cranial grooves (found under the brain). See arrows.

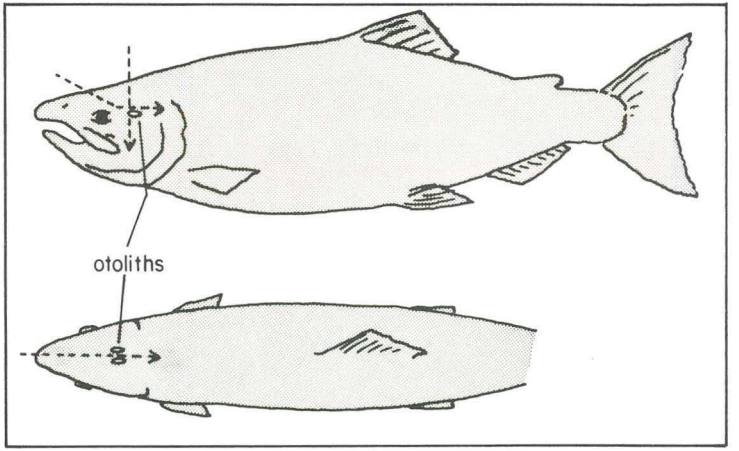

Fig. 19a. The three most common cuts (arrows) used to remove otoliths. Both illustrations show the approximate position of the otoliths. It may differ slightly between individuals.

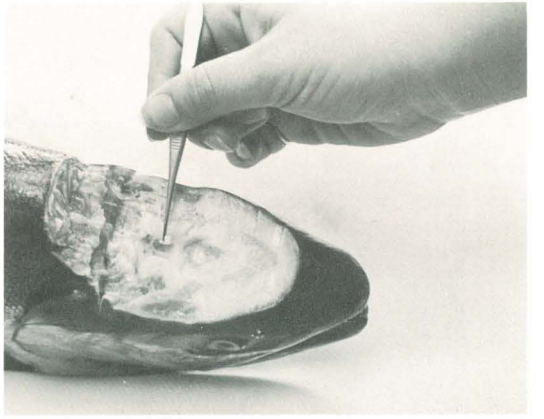

Fig. 19b. Serrated, fine-tipped forceps are best for removing the relatively small salmon otoliths.

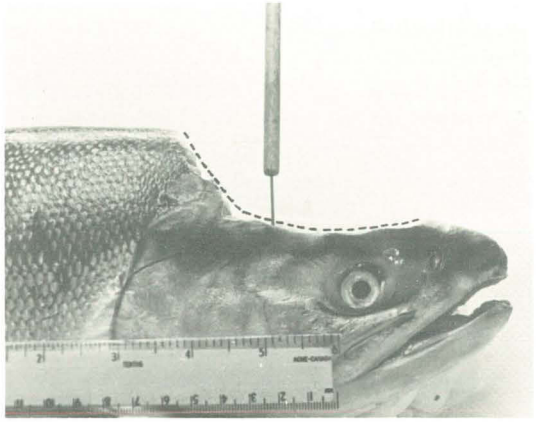

Fig. 19c. The dashed line indicates approximately the depth of a dorsal-ventral cut required to reach the level of the otolith cranial grooves. The probe and ruler show approximate location of the grooves behind the eye.

Otoliths should be stored in vials or trays. A 60:40, glycerin:water solution is used to preserve the otolith's inherent moisture and growth pattern clarity. Thymol is added to prevent fungal, algal, and bacterial growth. Proper identification of otoliths is necessary to relate to scale, fin, and/or tag data.

SUMMARY

Samplers must keep in mind that their job is important. The quality of their work will affect not only the time and effort required by technicians to process data, but also the accuracy of the results they achieve. These, in turn, could have an important influence on decisions made by biologists, scientists, and managers.

Should any questions arise with regard to salmon sampling procedures, samplers or supervisors can contact the Fish Age Determination Unit, phone 756-7179/7178, at the Pacific Biological Station in Nanaimo. The lab will be happy to clarify or expand on instructions pertaining to this manual.

ACKNOWLEDGMENTS

I would like to thank Doris Chilton for her helpful comments and Tom Bilton for his contributions and suggestions. Darlene Dodds assembled scale samples for photography and provided sampling knowledge. I would also

like to thank the numerous people who have reviewed this manual.

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