

Center for Quantitative Fisheries Ecology

PROTOCOL

PREPARATION OF SCALES FOR AGE ESTIMATION OF

SUMMER FLOUNDER

Paralichthys dentatus

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Equipment and supplies

Item/Model	Number/Use
Carver Laboratory Press Model-C	1
Bell and Howell Microfiche Reader	1
020 Acetate Sheets 6 in. x 5 in. x .5 mm (cut down to ten 3 in. x 1 in x .5 mm slides)	1 per 3-5 scales
Extruded Clear Acetate Backing Sheet 5 in x 5 in x 1 mm	2
Steel Warming Plate 6 in x 6 in x .4 in	1
Steel Warming Plate 6 in x 6 in x .1 in	1
Micro Slide Storage Box	1 per 100 Scales
Stender dish	1
Toothbrush with medium bristle	1
Tweezers	1
Oven Hot Mitts	1 pair
Safety Goggles	1 pair
Desk Lamp	1
Timer that displays minutes and seconds	1
Kimwipes [®] Delicate Task Wipers or VWR Light-Duty Tissue Wipers	1 box per work station for any spilled water
Sharpie [®] Ultra Fine Point Permanent Marker	1
Hard-Part Processing Log	lists selected hard-parts to be processed, categorized alphabetically by species
Transparency film for laser printer (5x5 in.)	1

Introduction

Aristotle may have been the first scientist to speculate on the use of hard parts of fishes to determine age, stating in *Historica Animalium* that the age of a scaly fish may be told by the size and hardness of its scales. Scales are the most widely used ageing structure in North America because of their non-lethal ease of collection. The number of annuli (rings) on a scale represents the fish age, and the spacing between rings is proportional to the growth of the fish. The ease and economy of ageing scales is not without its trade-offs. Specifically, the scale age tends to be biased, i.e., under- and over-estimation of older and younger fish, respectively.

It is now generally agreed that otoliths age provides a more accurate estimate of fish age. However, due to the historical use, easy collection, and preparation of scales, they are still used for some species, including summer flounder.

Structure of scales from summer flounder

Ctenoid scales display concentric rings (circuli) crossed by radial grooves (Figure 1). The scales are divided into two regions of

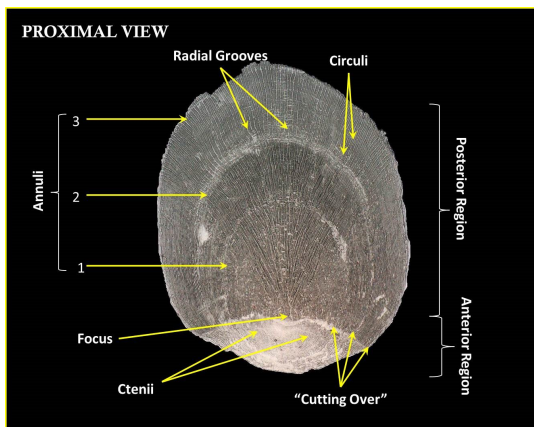


Figure 1: Summer flounder scale

growth, the anterior and the posterior. The radii, in the anterior region of the scale, serve as the attachment site to the dermal layer of the fish's skin. The posterior region is the part of the scale exposed to the environment. The scales serve to protect the fish and to aid in its hydrodynamic functions. As scales are lost, the fish has the ability to regenerate new scales in their place if the scale pocket remains undamaged. The regenerated scales are typically not used for age estimation due to the gap in morphological features representing the life history of the fish. Cyclic events such as spawning and seasonal metabolic slowing induce modifications in the growth patterns of the circuli. The circuli tend to become narrow and overlap in the direction where the anterior and posterior margins meet. These cyclic rings are termed annuli and are used to estimate the age of the fish.

Preparing scale acetate slides and preheating the Carver laboratory press

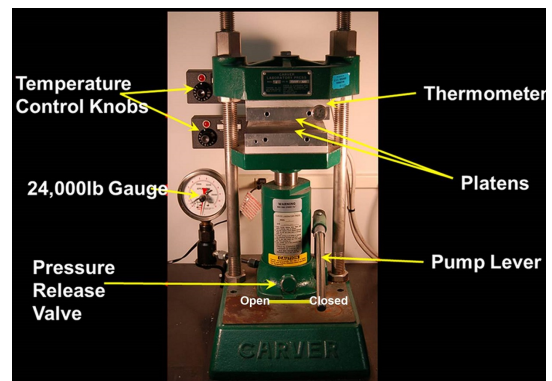


Figure 2: Carver Laboratory Press

Summer flounder scales are prepared for age determination by making acetate impressions of the scale microstructure. Cut down one 6x5 inch sheet to ten 3x1 inch slides.

Refer to Figure 2. Rotate (clockwise) the pressure release valve until it is tight. In this position, the press will hold pressure when pressure is applied by the pump lever. With two warming plates (hereafter referred to as "plates"), a thinner top one (hereafter referred to as "top plate") and a thicker bottom one (hereafter referred to as "bottom plate"), still between the platens, apply a small amount of pressure so that both platens are touching the plates. This will ensure that the plates are preheated along with the platens when heat is applied to the system. Rotate clockwise the temperature control knobs to the setting marked with White-Out. The setting is species-specific and determined based on previous experience. The setting will heat the press to approximately 170 °F/77 °C. This is the approximate temperature required to soften the acetate slides enough to leave an impression of the scale. The red lights above the temperature control knobs will turn off as the press reaches its proper temperature. This takes approximately 3-5 minutes and is an ideal time to begin selecting scales for pressing.

Selecting and cleaning the scales

In the Hard-Part Processing Log, identify the next hard-part (coin envelope containing scales) to be processed. Locate the coin envelope with the Age and Growth ID number (AGID) within the CQFE Summer flounder hard-part storage box. The AGIDs are found on the lower right-hand corner of each envelope. Empty the scales from the selected summer flounder coin envelope onto a clean area of workspace. Due to a wide range of life experience of individual fish, the size, shape, and quality of its scales may vary dramatically. Please make sure to select the best scales which may provide most reliable annulus read-

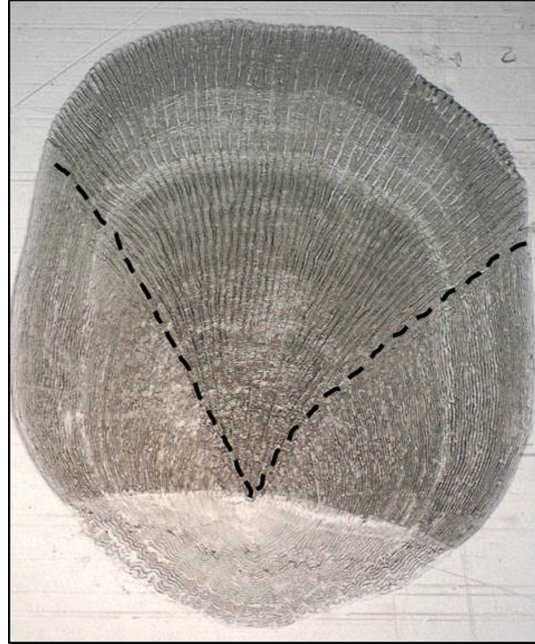


Figure 3: Summer flounder scale with a clear focus and V-shaped radii marked with black Sharpie.

ings. Following are 4 criteria for selecting the best scales for ageing:

1. The scale is average in size compared to other scales in the coin envelope (based on a visual inspection);
2. The focus is a clear point, the radii form a true V-shape (Figure 3);
3. The scale is not a regenerated scale (Figure 4);
4. There are no visible deformations on the edges or within the field of the scale.

Put the remaining scales back into the coin envelope.

Fill the Stender dish with water. On a clean area of the laboratory workspace hydrate the first of the selected scales with some water. Use a toothbrush to gently remove any residual dirt or tissue found on the scale (Figure 5). On the external/proximal (rough) side of the scale, where the anterior and posterior regions meet, there is strong evidence of circuli formation. It

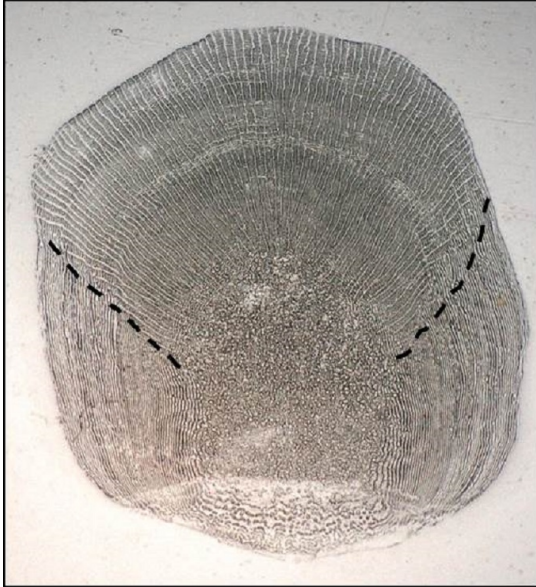


Figure 4: Summer flounder regenerated scale. Focus is not clear and radii do not form a true V-shape.

is important to dislodge any dirt or tissue remaining in these grooves so that the morphological characteristics of the scale are revealed for accurate age estimation.

Rinse the cleaned scale with water and set it aside on a Kimwipe[®] to dry. Complete the cleaning process for the remaining scales that were selected for pressing.

Pressing the scales

After the press and the plates are heated, take the plates down from the press on the table and remove the top plate aside. Center a 5x5 inch transparency film on top of the bottom plate. Center a 3x1 inch acetate sheet on top of the transparency film. Start at the left and begin positioning individual scales with the rough-side down and the shiny-side up onto the 3x1 inch acetate sheet (Figure 6). Make sure that the scales are all facing the same direction with the anterior and posterior regions aligned with the long side of the sheet. Do not press the scales if they are still wet or they will crack



Figure 5: Cleaning summer flounder scale

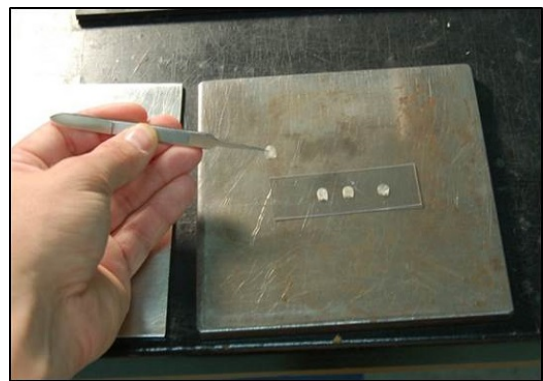


Figure 6: Placement of scales, rough-side down with posterior regions pointing away for the technician.

in the press. Cover the scales with the top plate. Place the plates into the Hydraulic press between the heated platens. Align the plates so that the pressure and heat is distributed evenly during the pressing process.

Before attempting to use the hydraulic unit, make sure that the pressure release valve screw has been closed and hand tightened.

Place the extender pole onto the pump lever to increase leverage, and begin pumping the hydraulic unit so that the plates come into contact with each other.

Continue pumping until 22,000 pounds of pressure on the plates is observed on the gauge. Note that the gauge on the Carver Laboratory Press shows the total force ex-

erted by the press, not the amount of pressure on the sample. In order to determine the pressure on the sample use the following equation:

$$P = F/A,$$

where P, F, and A stand for pressure (psi) on the specimen, reading on gauge, and specimen area, respectively.

Safety goggles should be worn whenever the press is in use.

Allow the press to maintain this level of force for about 3 minutes. Set the timer to keep track of the time. If the pressure begins to drop, stroke the pump a couple of times to bring it back up to the correct level.

Upon completion of the pressing time, turn the release valve screw to the left about a half turn to release the pressure (**Do not overturn the valve or the oil will leak out**). At this time the lower platen will drop slowly to its starting position.

Using oven mitts, carefully remove both of the plates from the press and leave them on a heat resistance surface. Remove the top plate so that the acetate slide with the scales can be retrieved. The scales should be somewhat embedded in the acetate slide and are easily removed by using tweezers (Figures 7 and 8).



Figure 7: Scales on acetate after pressing

There will be impressions of the scale surface left in the acetate slide. Put the impression slide into a microfiche reader to ensure that the impressions are readily ageable by viewing in the microfiche reader (Figure 9).

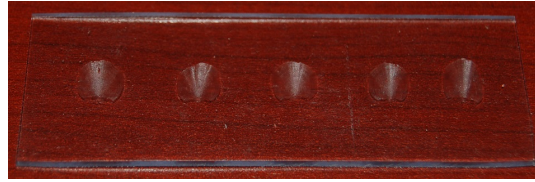


Figure 8: Scale impressions with scales removed

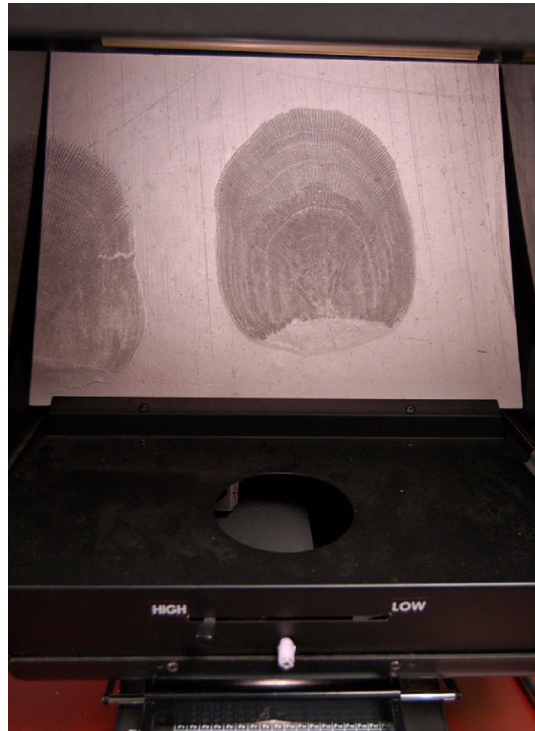


Figure 9: Reading an acetate summer flounder impression in the microfiche reader.

Make sure that the impressions have no regeneration, scratches, holes, missing/broken edges, evidence of tissue remaining on the scale, and been damaged during the pressing process. (Figure 10, 11, and 12).

The scale needs to be repressed if:

1. The focus is unclear or the impression is of a regenerated scale (Figure 4);
2. There is debris imbedded in the scale impression (Figure 10);
3. The scale is deteriorated by excessive heat and/or pressure(Figure 11);



Figure 10: Summer flounder scale impression showing results of insufficient scale and/or acetate slide cleaning.

4. The scale's impression is unclear due to insufficient heat and/or pressure (Figure 12).

Labeling and storing scale slides

When good impressions are acquired, use a fine point black Sharpie® to write VMRC and the current year in the upper left-hand corner of the acetate slide. In the upper right-hand corner, write the species code (SMF) and the AGID (e.g., 594) (Figure 13). Store the acetate slides in a labeled micro-slide box with the AGID number facing up for easy identification during age determination (Figure 14).

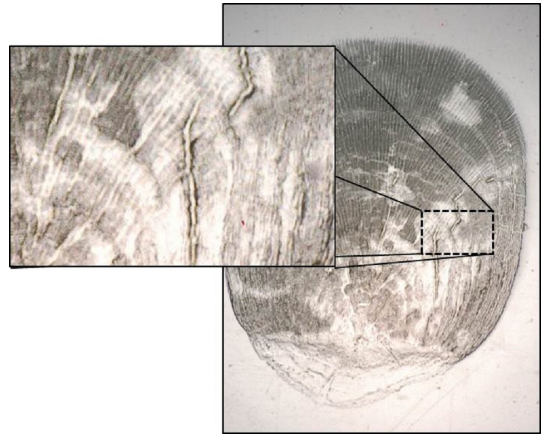


Figure 11: Summer flounder scale impression damaged from too much heat and/or pressure during pressing.

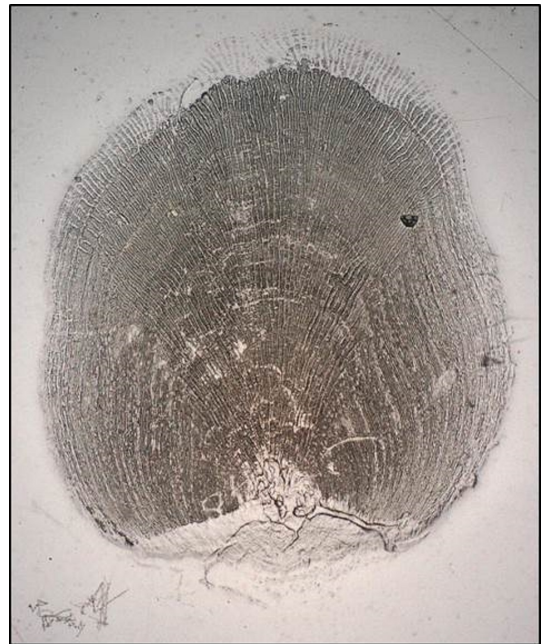


Figure 12: Summer flounder scale impression lacks definition from low heat and/or insufficient pressure.



Figure 13: Completed scale impression slide with correct labels.

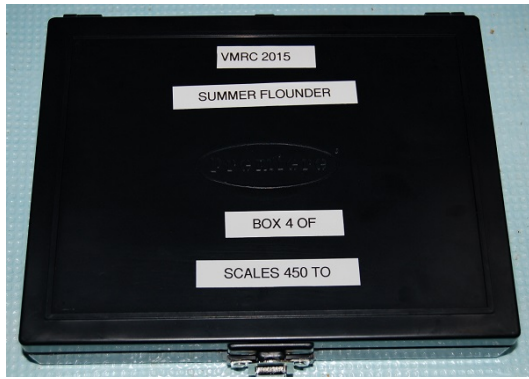


Figure 14: Summer flounder slide box

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