

AGEING LABORATORY/FISHERIES MANAGEMENT DIVISION

PROTOCOL

PREPARATION OF SCALE IMPRESSIONS FOR AGE ESTIMATION

October 2, 2023

Contents

Introduction	1
Structure of Scales	1
Prepare Acetate Slides	2
Preheat Hydraulic Press	2
Select Scales	2
Clean Scales	2
Position Scales on Acetate Slide	3
Press Scales	4
Label and Store Scale Slides	5
References	6
Equipment and Supplies	7

Introduction

Aristotle may have been the first scientist to speculate on the use of hard parts of fishes to determine age, stating in *Historia 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.

Structure of Scales

In the VMRC Ageing Lab we age Striped Bass *Morone saxatilis* and Summer Flounder *Paralichthys dentatus* using the impressions of their scales. Even though the scale shapes of both species look different, Striped Bass's is more squared whereas Summer Flounder's is more round, both species have ctenoid scales with distinguishable anterior and posterior region (Figure 1 and 2). Therefore, we will introduce their scales together here.

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

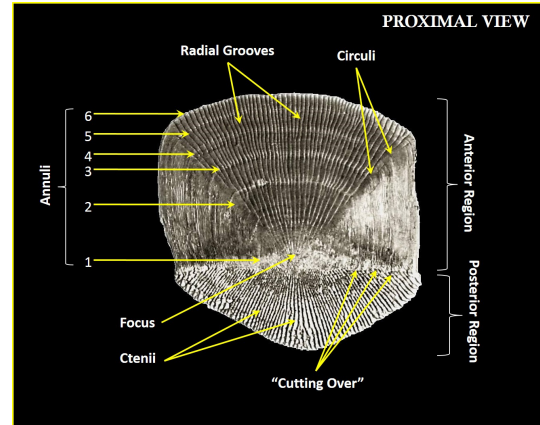


Figure 1: Striped Bass scale

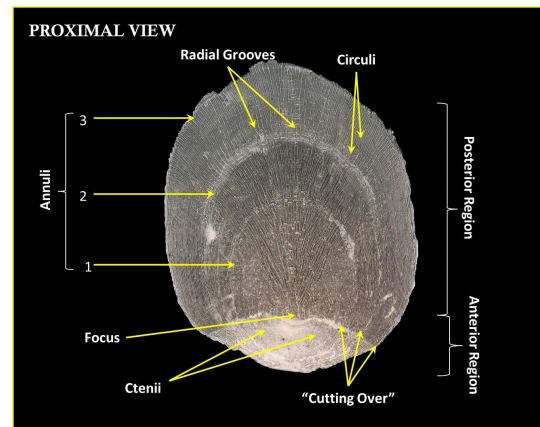


Figure 2: Summer flounder scale

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 (Murphy and Willis 1996).

Because the procedures to make scale impressions of Striped Bass and Summer Flounder are the same, we will use Striped Bass as an example to demonstrate how to make scale impressions in the VMRC Ageing Lab.

Prepare Acetate Slides

Striped Bass scales are prepared for age estimation by making acetate impressions of the scale microstructure. Cut down one 6"x5"x1" sheet to ten 3"x1" slides.

Preheat Hydraulic Press

We use a heated hydraulic Carver Laboratory press (Figure 3) to make scale impressions. To

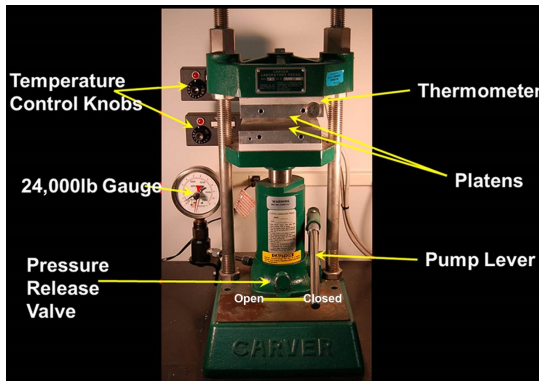


Figure 3: Carver Laboratory Press

preheat the press, rotate clockwise both the top and bottom temperature control knobs to the setting marked with White-Out. The top and bottom control knob sets the temperature for the top and bottom platen, respectively. The setting is species-specific and determined based on previous experience. The setting will heat the top and bottom platens 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.

Select Scales

While the press is heating, identify a sample (coin envelope containing its scales) to be processed in the Hardpart Processing Log. Locate the coin envelope with the fish ID within

the Striped Bass hardpart storage box. The fish IDs are found on the lower right-hand corner of each envelope. Empty the scales from the selected Striped Bass 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 readings. 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 4);

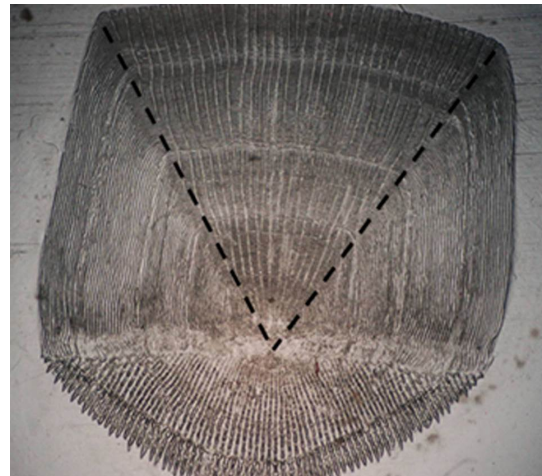


Figure 4: Striped Bass scale with a clear focus and V-shaped radii marked with black Sharpie.

3. The scale is not a regenerated scale (Figure 5);
4. There are no visible deformations on the edges or within the field of the scale.

Select 2 to 6 scales depending on their sizes for cleaning and pressing, and put the remaining scales back into the coin envelope.

Clean Scales

Fill the sonicator (Figure 6) halfway with plain water. Then place the selected scales in the

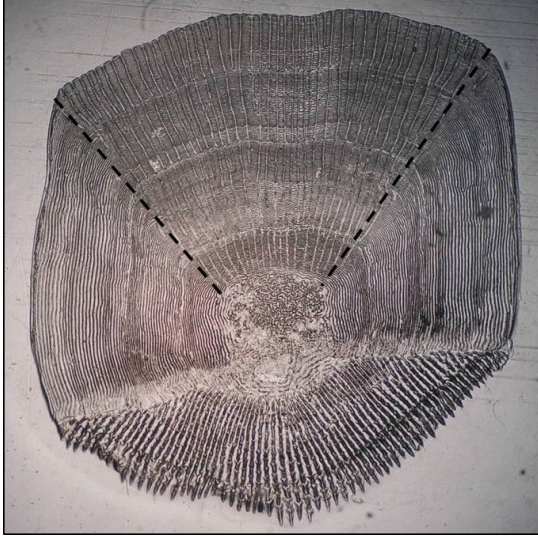


Figure 5: Striped Bass regenerated scale. Focus is not clear and radii do not form a true V-shape.



Figure 6: Ultrasonic cleaner is used to clean Striped Bass scales before pressed.

sonicator and turn it on. The sonicator will run for 3 minutes and then turn off (While the sonicator is running, begin selecting scales for the next selected sample). When the sonicator turns off, remove the scales and wipe with a Kimwipe[®]. If there is any remaining dirt or tissue, use a toothbrush to gently remove it.

On the external/proximal (rough) side of the scale, where the anterior and posterior regions meet, there is strong evidence of circuli formation. It 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.

Position Scales on Acetate Slide

After cleaning all the selected scales, position them on an 3"x1" acetate slide for pressing using two 6"x6" steel warming plates (one has 0.1" thickness (hereafter referred to as "top plate") and another has 0.5" thickness (hereafter referred to as "bottom plate")) and a 5"x5" transparency film. More specifically:

1. Center the 5"x5" transparency film on the bottom plate;
2. Center a 3"x1" acetate sheet on the transparency film;
3. Start at the left and begin positioning individual scales with the rough-side down and the shiny-side up onto the 3"x1" acetate sheet (Figure 7). Make sure that the scales



Figure 7: Placement of the acetate slide with scales on the bottom plate, and the rough-side of scales facing down with posterior regions pointing away from the technician.

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 in the press;

4. Cover the scales with the top plate.

Now it is ready to put this set (hereafter referred to as "scale set") of the bottom plate, transparency film, acetate slide, a group of arranged scales, and top plate on the bottom platen of the press.

Press Scales

Refer to Figure 3. 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. **Before attempting to use the hydraulic unit, make sure that the pressure release valve screw has been closed and hand tightened.**

Slowly put the scale set on the bottom platen, making sure that the top plate is aligned with the bottom plate and both plates are aligned with the bottom platen. Any misalignment among the three parts may result in uneven pressure and heat on the scales during the pressing process.

Place the extender pole onto the pump lever to increase leverage, and begin pumping the hydraulic unit so that the bottom platen with the scale set slowly move up until the top plate contacts with the top platen. Continue pumping until 22,000 pounds of pressure is observed on the gauge. Note that the gauge on the Carver Laboratory Press shows the total force exerted by the press, not the amount of pressure on the scales. In order to determine the pressure on the scales use the following equation:

$$P = F/A,$$

where P, F, and A stand for pressure (psi) on the scales, reading on gauge, and the area of the scales, 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, pump the lever for a couple of times to

bring it back up to the correct level.

Upon completion of the pressing time, turn the pressure valve 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 bottom platen with the scale set will drop slowly. Once the bottom platen has dropped enough to remove the scale set, close the pressure valve by turning it to the right until finger tight.

Carefully remove the scale set from the bottom platen, and leave it 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 8 and 9).



Figure 8: Scales on acetate slide after pressing



Figure 9: Scale impressions with scales removed

There will be impressions of the scale surface left in the acetate sheet. Put the sheet with the impressions into a microfiche reader to ensure that the impressions are readily ageable by viewing in the microfiche reader (Figure 10).

Make sure that the impressions have no regeneration, scratches, holes, missing/broken edges, and evidence of tissue remaining on the scale during the pressing process. The scale needs to be repressed if:

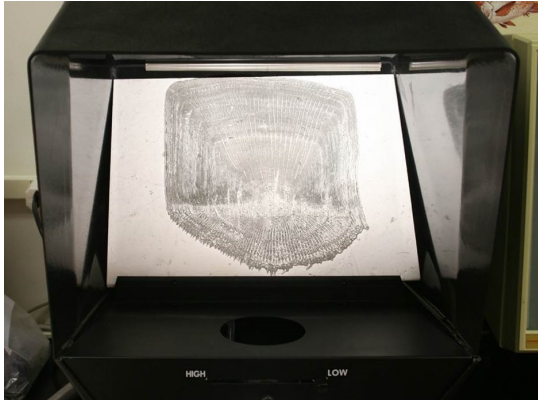


Figure 10: Reading an acetate Striped Bass impression in the microfiche reader.

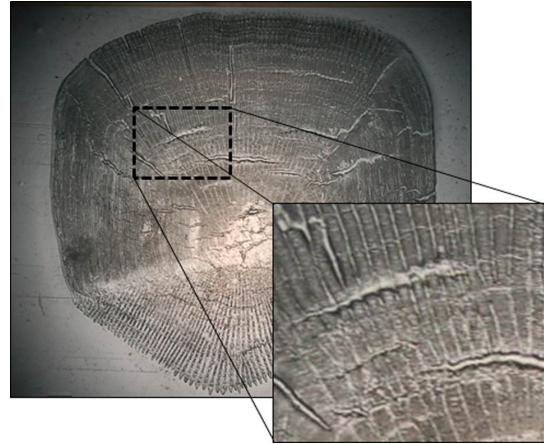


Figure 12: Striped Bass scale impression damaged from too much heat during pressing.

1. The focus is unclear or the impression is of a regenerated scale (Figure 5);
2. There is debris imbedded in the scale impression (Figure 11);



Figure 11: Striped Bass scale impression showing results of insufficient scale and/or acetate slide cleaning.

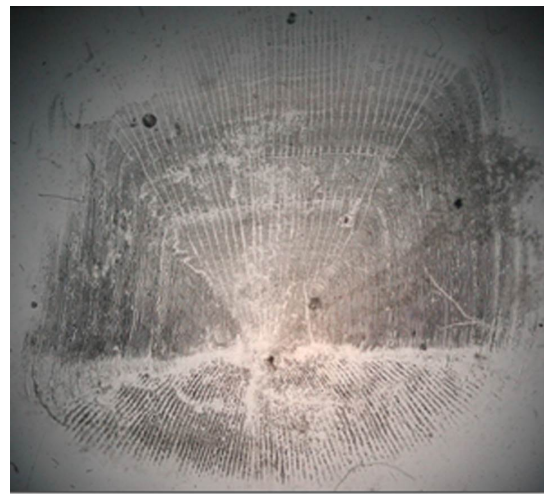


Figure 13: Striped Bass scale impression lacks definition from low heat and/or insufficient pressure.

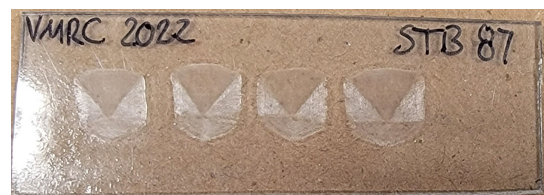


Figure 14: Completed scale impression slide with correct labels.

3. The scale is deteriorated by excessive heat and/or pressure(Figure 12);
4. The scale's impression is unclear due to insufficient heat and/or pressure (Figure 13).

Label and Store Scale Slides

When good impressions are acquired, use a fine point black Sharpie® to write VMRC and the current year (2022) in the upper left-hand cor-

ner of the acetate slide. In the upper right-hand corner, write the species code (STB) and the fish ID (e.g., 87) (Figure 14). Store the acetate slides in a labeled micro-slide box with the fish ID number facing up for easy identification during age estimation (Figure 15).

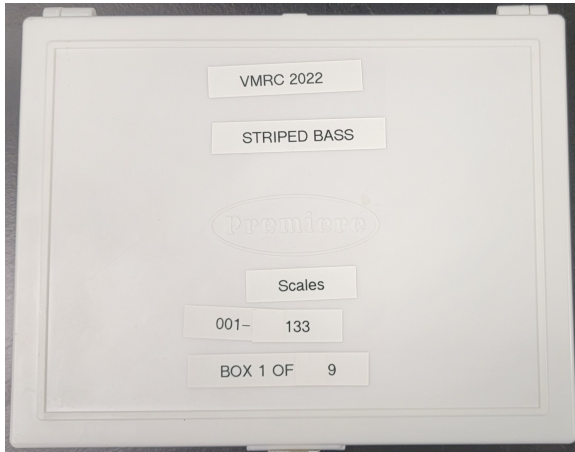


Figure 15: Striped Bass slide box

References

Murphy, B. R. and D. W. Willis
1996. *Fisheries techniques (2nd ed.)*.
Bethesda, Maryland: American fisheries society.

Equipment and Supplies

Item	Specification
Carver Laboratory Press	Model-C
Steel Warming Top Plate	6"x6"x0.1"
Steel Warming Bottom Plate	6"x6"x0.4"
Mini Sonicator	Grainger 0.16 gallon capacity
Microfiche Reader	Bell and Howell
020 Acetate Sheets	6"x5"x0.06"
Transparent Film	5"x5"
Micro Slide Storage Box	VWR 28511-012, 100 slides per box
Timer	Displaying minutes and seconds
Wipers	Kimwipes [®] Delicate-Task or VWR Light-Duty
Ultra Fine Point Permanent Marker	Sharpie [®]
Stender Dish	
Toothbrush with Medium Bristle	
Tweezers	
Oven Hot Mitts	
Safety Goggles	
Desk Lamp	
