FISH LIFE HISTORY ANALYSIS PROJECT

STANDARD OPERATING PROCEDURES

FOR COLLECTION & PREPARATION OF FISH SCALES & DATA MANAGEMENT

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MANUAL OUTLINE

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- Standard Operating Procedures
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PURPOSE

The purpose of this manual is to provide guidelines for scale collection, preparation, and data management for new, supporting and collaborating staff. Information on a description of the work performed by the Fish Life History Analysis Project can be found in Clemens et al. 2013, and information on methods of scale analysis can be found in Borgerson et al. 2014.

The project maintains high standards of quality in estimation of life history parameters, based on reads of fish scales, including accuracy, precision and efficiency from sample and data handling through age estimations and other life history analyses and data dissemination.

STANDARD OPERATING PROCEDURES

Data Management

A critical aspect in the process of collecting, processing, analyzing and reporting data from fish scales is the management of the data associated with the scales. For example, information on **basin, location, survey date, fin clip status, Snout ID #, survey type (e.g., creel, hatchery broodstock, spawning ground survey, seine, etc.), fish length (units; MEPS or FL?) is essential**. Many projects have established data collection procedures that usually include all of this information along with the fish scales.

Please submit the fish scale intake form (Appendix 1) electronically when submitting scale samples.

Because data management is critical, it is imperative that protocols be in place for data collection before the scale samples are collected. The protocols must include a method to keep data for each fish clearly associated with its scale sample, from collection in the field through scale analyses and data dissemination.

- Assign a **Scale ID Number** to each sample prior to OR at the time of scale collection. The Scale ID Number should be written on the scale sample envelope and be included in any field data collected at the time the scale is taken.

- Data for each scale sample includes associated location and biological data. **Follow the protocol of your project.**

- **Follow FLHAP protocol for scale collection (below)**

Scale Collection

Scale collection is often done in less than ideal field conditions. Most salmon species are coming in river during cold and wet weather. Even with difficult conditions, the FLHAP is better able to serve the needs of the project providing the scales if care is taken in collecting scales.

- The "key area" or anatomical location for cool water fish like salmonids is based upon where sockeye 1st generate their scales during development (Figure 1).
- The correct 5-step procedure for obtaining scale samples is as follows:

1. **Locate key area (Figure 1).**
2. **Scrape slime off with non-serrated portion of knife.**
3. **Pluck 4-5 scales with forceps and place in between the fold of the paper insert in the scale envelope, using care to not stack scales.**
4. **Repeat on other side of fish.**
5. **Allow scale envelopes to dry and keep them organized in a well-ventilated container**

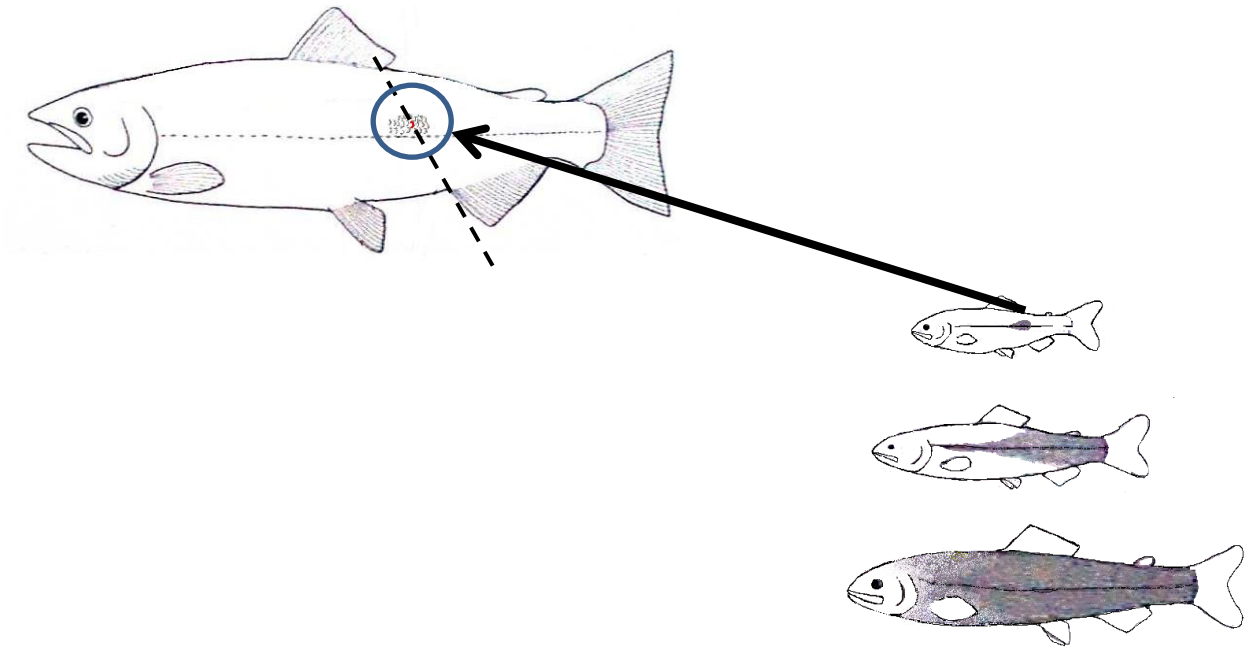


Figure 1. The key area is where scales first form on juvenile fish. These scales contain the most complete life history information. The key area is located above the lateral line, on a trajectory transcribed by an imaginary line passing between the posterior insertion of the dorsal fin and the anterior insertion of the anal fin.

Variations:

- **If scales are absent from the key area on one side of the fish, sample from twice as many scales from the key area on the other side of the fish.**

- **<u>Live fish</u>: If you want to be able to sample the fish again at a later date, only sample from one side of the body, so that key area scales will be available if the fish is encountered again.**

- **<u>Steelhead and cutthroat</u>: Tend to have high scale regeneration rates (i.e. lose and regenerate scales frequently), so a few more scales should be taken to ensure we get quality scales.**

- **<u>Warm water fish</u>: Key area is just under the distal tip of the pectoral fin.**

- Make sure that the biological data associated with the fish scales is clearly written down on the scale envelope and on data sheets or entered into a data entry device. There must be a scale ID # written on the envelope and contained in the data that is collected for the scale sample.
- Scale samples are most easily processed by the FLHAP if there is a scale ID # and an electronic data file.
- Allow scale envelopes to dry and store them in a well-ventilated container.

- Scale envelopes will develop mold and mildew if stored in closed containers.

Submitting Scale Collections

Please only submit complete scale collections.

If submitting scales in envelopes (not mounted), ensure that the envelopes are grouped appropriately by year, collection method, basin, and species, and then by the scale ID number within each collection. For example, scales collected during creel surveys for fall Chinook Salmon in the Elk River basin would be organized by scale ID numbers in one group, while scales collected during spawning ground surveys for fall Chinook Salmon in the South Umpqua River basin would be organized by scale ID number in another group.

An electronic version of the biological data must be submitted with the scale collection. Biological data should be checked to ensure that they are complete, accurate, and sorted by scale envelope number. Please ensure that the electronic biological data has been cross-referenced to the scale envelopes and any differences have been resolved; the people who collected the data have the best understanding of how to reconcile differences.

* Without prior planning, different projects working in the same basin could end up with the same basin codes *and* sample number ranges. This could lead to confusion over duplicity of mount ID #s. To avoid this, the Fish Life History Analysis Project will block a range of numbers for samples for each project working within the same basin. For example, if the Willamette spring Chinook Project collects samples from the North Santiam and submits those first, and the Willamette Reservoir Project collects samples from Detroit Reservoir (same basin) and submits those, then the numbering scheme would be:

12116/5001-6000: Juvenile samples 5001-6000 for the 2012 sample year, from the North Santiam (basin "116") from the Willamette spring Chinook Project

12116/6001-7000: Juvenile samples 6,000-6,999 for the same year and basin, from the Willamette Reservoir Project

The above system is tailored to scale samples from CCRMP, OASIS, and new for 2012, Willamette RM&E and District Biologists.

Caveats include samples from the Hood River, juvenile scales mounted on glass slides from the Deschutes River and Project CROOS. Samples from Hood River and juvenile scales mounted on slides from the Deschutes River already have particular numbering practices that are easier to adopt than to change. And, for Project CROOS samples, it is easiest and most intuitive to label these samples directly by name, location and year.

Scale Mounting

Scales are mounted onto gum cards that contain 20 cells for scale samples from 20 fish. Mounting requires that the scale samples are sorted, cleaned and oriented on the gum card in a specific way. The scale samples need to be linked to the data by a particular numbering scheme. Scales that are collected in the same year, by the same method, from the same basin and from the same species are considered one collection and are mounted within the same numbering scheme.

- Sort the scale envelopes by year, method, basin and species; then sort by scale ID #.

- Mounting supplies:
  - Fine point forceps
  - Small dish of water
  - 3" X 5" piece of card board
  - Dissecting microscope
  - Gum cards
  - Pen
  - Paper towels

- Attach the gum card to the card board using tacks or pushpins to keep it flat.

- Fill out the data requested on the top of the gum card.

- Location should include the basin name.

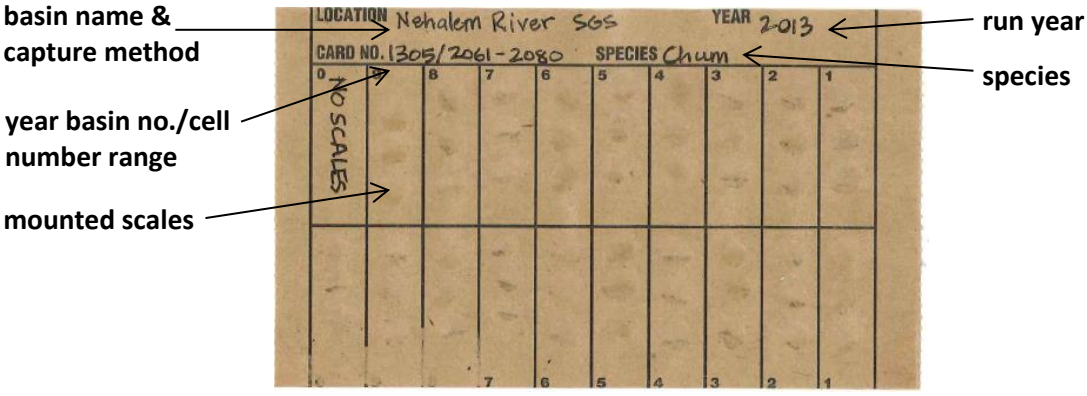
- The FLHAP uses a systematic numbering system for the gum cards. It includes the year, basin code, and cell numbers.

- The gum card number includes a range of cell numbers. The envelopes of scale samples mounted on that gum card will have numbers that correspond to the exact cell where the sample is mounted. Along with the year and basin code, those cell numbers are the Mount ID#.

    - A Mount ID # is not a random number like a scale ID #. A Mount ID # refers to a specific cell and gum card where a scale sample is mounted.

- The first number of the range of cell numbers on a gum card always have a one as the last integer and are multiples of 20. For example,-1, 21, 41, 61…..181…..2421.

Example of a gum card



❖ **An example of a Mount ID # range to be labeled at the top of a gum card would be: 1149/1-20, where "*1149*" is the year (2011) and basin number (49- Coquille), and the number range is the samples.** *See Appendix 2 for a list of basin numbers.*

- To help prevent duplication of the Mount ID#, common survey types are assigned Mount ID#s within a specific range of numbers. <u>New for 2012 (and some 2011 samples), samples from the Willamette RM&E and from District Biologists will be fit into this system.</u>

Sample # range	Survey or other use
1, 2, 3,…..1000	**CREEL**
1001-2000	***Adults:*** **TAGGING/TRAPPING/SEINING/TRAP & HAUL/DAM OR WEIR COLLECTION/ELECTROFISHING/PEDIGREE**
2001-4000	**SPAWNING GROUND SURVEYS**
4001-5000	**HATCHERY BROODSTOCK & WILD FISH USED FOR BROODSTOCK AT THE HATCHERY**
5001-?*	***Juveniles:*** **TAGGING/TRAPPING/SEINING/TRAP & HAUL/DAM OR WEIR COLLECTION/ELECTROFISHING/PEDIGREE**
9001-?	**Scales mounted offsite by others**

- Print a hard paper copy of the data sorted by the scale ID #; this is called the Mount Sheet. Write the actual Mount ID# for each scale as you go. This allows the FLHAP to track any discrepencies back through data versions if needed. Send the paper mount sheet with the mounted scales.

- To begin mounting, select the scale envelope you are going to mount and write the Mount ID # on the top of the envelope and on the mount sheet next to the corresponding data for that envelope.

- Using the dissecting microscope and forceps, select the 3 best scales and drop them into the small water dish. *See Appendix 3 for juvenile scale mounting instructions.*
    - The best scales are the largest and roundest and are the least regenerated or resorbed [1].
    - *If* the scales are in poor condition, select up to 5 scales.
    - If the largest scales are all regenerated then include in the 3-5 scales a few smaller scales that are not regenerated when possible.

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[1] *Regenerated*: New scales are grown quickly when lost. When the new "blank" scale reaches the size of surrounding scales, it starts growing circuli again. *Resorbed*: When a fish is stressed, it pulls calcium out of the scales. The scales look dissolved from the edge inward and the posterior field will wear off (Figure 3).

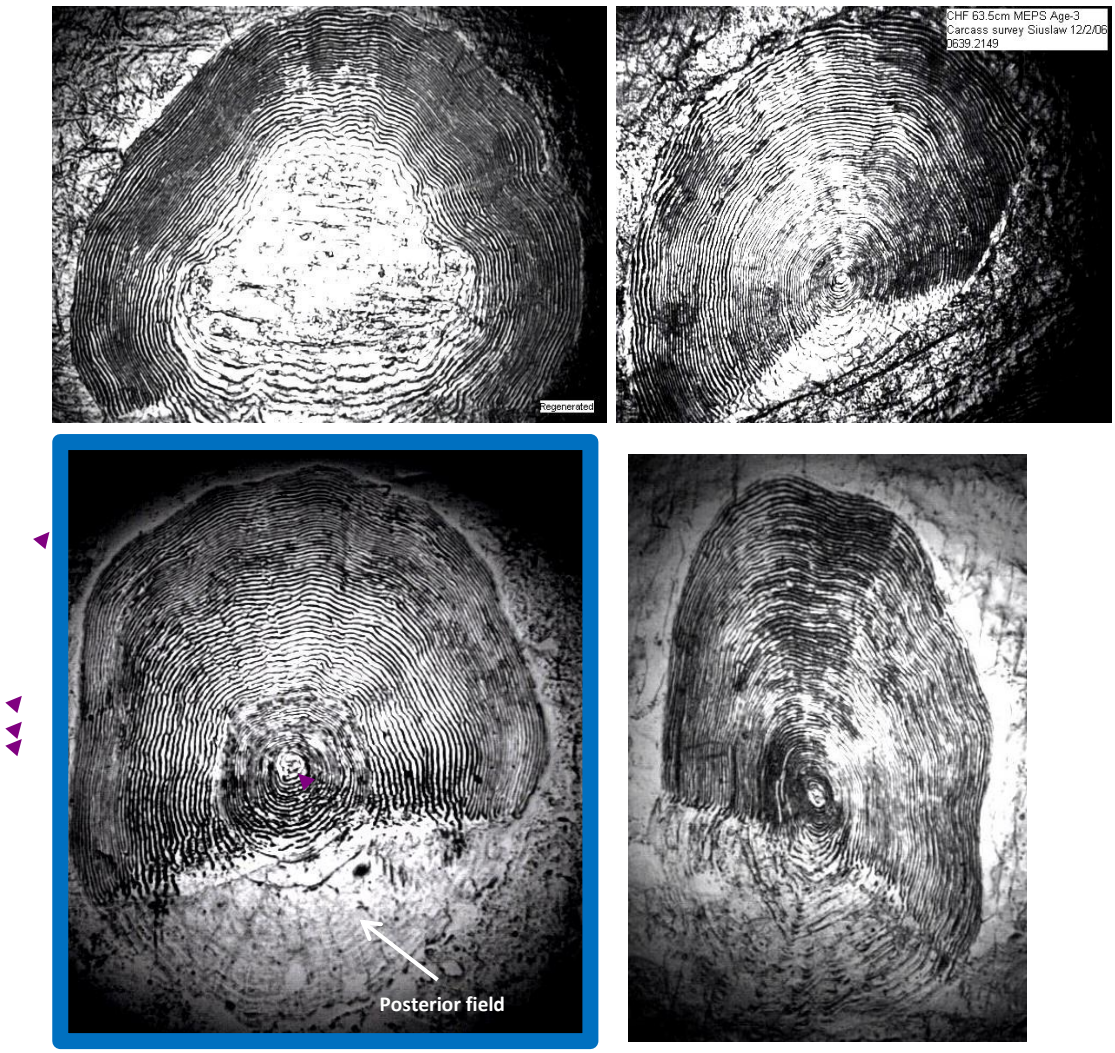

Figure 3. Images of scales taken from a camera mounted onto a light microscope (they won't be this magnified in a dissecting microscope). Clockwise, from top left: regenerated scale; resorbed scale; scale from the non-key area (non-key scale); textbook example of a scale that should be mounted. Regenerated, resorbed and non-key scales should be the last choices for scales to mount. Note the clearly visible posterior field in the textbook example.

- Use the forceps to remove one of the selected scales from the water dish and rub it between your thumb and forefinger to clean it.

- Use your thumb nail to feel both sides of the scale after it is clean. The side that feels rough, like fine sandpaper, should face up when you press the scale onto the gum card. This is the side with the circuli and annuli features that enables the scales to be "read" for life history characteristics (age, origin, life history diversity). If the scale is mounted with this rough side facing down, then the scale cannot be read.

- Dip the scale in water once more, touch it to a paper towel to remove excess water, and orient the scale with the posterior field (Figure 3) toward the bottom of the gum card and the rough side up. Press the scale into the cell to which you assigned it when you gave it a Mount ID #.

- Repeat the cleaning, orienting and pressing until all of the selected scales are mounted into that cell.
    - The cells on the gum card are numbered with 2 rows of 1-10, from left to right.
    - The first cell will be in the upper right hand side of the gum card.
    - Fill the top row first, working *right* → *left*, and then begin the bottom row, working *right* → *left*.
    - There should never be scales from more than 20 fish (scale samples) on a gum card. Often less than 20 scale samples are on a card.

- Continue to the next sample and repeat the mounting process with each of the scale samples. Keep the Mount ID #s running consecutively as you mount all of the scales in the collection.
    - If there are no scales in an envelope, write "*No scales*" on the envelope, the data sheet, and in the assigned cell of the gum card.
    - If there is scale sample listed on the data sheet, but no scale sample is found, write "*No sample*" on the data sheet and skip it. Do not assign it a cell on the gum card.
    - If there is a scale sample, but no data found for it, place this sample at the end of the collection, and mount it last. Write down any data from the envelope onto the data sheet and include the Mount ID# that sample is assigned.

- A hydraulic heat press is used to transfer the scale impressions from the gum card to a plastic "Vivak" card. You must receive training from FLHAP staff prior to using the press. *See Appendix 4 for heat press instructions.*

- After pressing, the plastic cards with the scale impressions should be labeled with a ***black*** or ***blue*** permanent marker only. The labeling should be redundant to minimize chances of a single label fading with time to the point of becoming unreadable.

Data Codes for Scales

Sometimes the status of the scales to be analyzed is extremely subjective at best. These codes denote particular problems prohibiting age estimations:

94	**Other (non-target) species**
95	**Not mounted**
96	**No scales**
97	**Lateral line or other non-key scales**
98	**Unreadable; damaged**
99	**Unreadable; regenerated (see Figure 3)**

REFERENCES

Clemens, B., K. Bowden, and L. Borgerson. 2013. Fish life history analysis project: Project description. Oregon Department of Fish and Wildlife.

Borgerson, L., B. Clemens, K. Bowden, & S. Gunckel. 2014. Fish life history analysis project: Methods for scale analysis. Information Report 2014-10. Oregon Department of Fish and Wildlife.

APPENDIX 1

Scale Intake Form- please send electronic version only

Fish Life History Analysis Project (FLHAP) Scale Intake Form
Please send to: scalelab@oregonstate.edu

Scales will not be processed unless you have made arrangements with us, including completing the top part of this form, which must accompany your samples.

Project dropping off scales: Click here to enter text.

Person dropping off scales, with contact info: Name: Click here to enter text.

Phone: Click here to enter text. **Email:** Click here to enter text.

Project requesting data, including contact information: Project: Click here to enter text.

Phone: Click here to enter text. **Email:** Click here to enter text.

Basin: Click here to enter text.

Subbasin: Click here to enter text.

Species: Choose an item. **If other, please describe:** Click here to enter text.

Return Year, if applicable: Click here to enter text.

Known origin? ☐Hatchery ☐Wild ☐N/A

Is there associated CWT data? ☐Yes ☐No ☐Unknown

Collection method: Choose an item. **If other, describe:** Click here to enter text.

Number of scale envelopes: Click here to enter text.

Are scales already mounted? Choose an item. **If yes, number of gum cards:** Click here to enter text.

What kind of information would you like FLHAP to obtain: Choose an item.

If other, please describe: Click here to enter text.

Absolute deadline for receiving data: Click here to enter a date.

Electronic data files are required. Are hard copies also included? Choose an item.

FLHAP records:

Date scales dropped off: Click here to enter a date. **Contact:** Choose an item.

Deadline approved: Choose an item. **If modified, new deadline:** Click here to enter a date.

Specific location of where samples are being held: Click here to enter text.

APPENDIX 1

LIST OF BASIN CODES

Coastal Basins

(From OASIS basin codes – adopted by FLHAP for run year 2000 and subsequent run years. *See* OASIS project codes for more complete listing.)

Basin No.	Basin
1	Necanicum
5	Nehalem
6	Miami
7	Kilchis
8	Wilson
9	Trask
10	Tillamook
13	Nestucca
16	Salmon
20	Siletz
25	Yaquina
28	Alsea
30	Yachats
33	Tenmile
39	Siuslaw
40	Siltcoos
41	Tahkenitch
43	Umpqua
45	Coos
49	Coquille
*53	Floras; New River
54	Sixes
55	Elk
59	Euchre
60	Rogue
62	Pistol
63	Chetco
64	Winchuck

*Different from OASIS coding: 52 New River, 53 Floras.

Columbia, Snake, and Willamette Basins

(Assigned and adopted by FLHAP for run year 2000 and subsequent run years. The SGS Survey Code Manual for FLHAP [black binder] has a more complete listing.)

Basin No.	Basin
101	Columbia River, from mouth to Willamette
102	Columbia River, from Willamette to John Day
103	Columbia River above John Day
105	Youngs Bay
106	Lewis and Clark
107	Youngs River
108	Klaskanine
110	Willamette River, mainstem
111	Clackamas
122	Sandy
116	North Santiam
116	South Santiam
119	McKenzie
120	Middle Fork Willamette, N. Fork Middle Fork
120	Middle Fork Willamette, Fall Creek
121	Coastal Fork Willamette
124	Deschutes, mouth to Round Butte
125	LBC – headwaters
126	John Day, mouth to N. Fork
127	John Day, N. Fork
128	John Day, above N. Fork
129	Willow
130	Umatilla
131	Walla Walla
132	Snake River, mainstem
133	Grand Ronde
134	Imnaha
135	Powder
136	Malhuer

APPENDIX 3

Mounting Juvenile Scales. ***Please do not begin to mount without some hands-on training from FLHAP personnel.***

- Put a large drop of water onto the paper insert from the scale envelope that contains the scales.
- Using the dissecting microscope and fine point forceps, select 8 good scales - following the same criteria as for adult scales - and place them in the drop of water. Dry the forceps frequently throughout the mounting process to avoid unintentionally wicking scales and prevent scales from sticking to the forceps. If a scale is stuck to the forceps, use a dissecting pin or probe to push the scale onto the paper insert.
- Using the forceps, gently swirl the scales in the water drop to clean them.
- Remove the scales from the water drop and line them up on the paper insert to dry.
- Note the direction that the scales curl as they dry. The concave surface of the dry scale is the smooth surface, with no circuli. This side will be pressed down onto the gum card.
- Put a second, clean water drop onto the microscope stage
- Pick up a dry scale by the posterior field and dip it into the clean water drop to re-wet, then press the scale onto the gum card with the concave side down, rolling the forceps to the side as you press. Repeat until all scales are mounted into the cell.
- Mounting juvenile scales accurately requires a lot of time, patience, and practice.

APPENDIX 4

Pressing Scales Using the Hydraulic Heat Press (Figure 4)

- Turn both dials to just below “1” on the dial setting, and let the press heat up. When the press is ready, the indicator light will turn off.
- Moisten the corners of a gum card and stick it onto a precut Vivak card. It is easiest to follow the curl of the Vivak and stick the gum card to the concave surface.
- Two gum cards can be pressed simultaneously. Position the tops of the gum cards to the outside of the metal plate, so as much of the gum card with scales on them is in the middle of the press, and therefore under the hottest, highest pressure.
- Place the prepared cards onto the metal plate labeled “Bottom”, positioning the cards in line with the marks on the plate. Be sure to leave enough space between the two cards on the plate so that they will not melt together in the press. Place the metal plate labeled “Top” on top of the lined up cards. Note that the metal plates are also labeled “inside” and “outside”, due to stippling on the surface that can interfere with the scale impressions. Position the plates accordingly.
- Carefully place the metal plates in between the plates of the heat press, lining up the marks on the metal plates with the edges of heat press plates.
- Using the lever, pump the press until the plates clamp together tightly, between 15,000 and 20,000 ppsi.
- Leave the metal plates in the press for two minutes, not less, and not more.
- Turn the knurled release valve at the bottom of the press to release the pressure and open the press. Tighten the valve when the plates are open just wide enough to easily remove the metal plates from the press.
- As the cards begin to cool, they will loosen from the metal plates. Gently remove the cards from the metal plates and carefully peel the gum cards away from the Vivak. Peel the gum cards from the Vivak while it is still somewhat warm, otherwise they become increasingly difficult to peel. Place the gum cards and Vivak cards between the pages of a heavy book to keep them flat while you finish pressing the other scales.
- Using a fine tip, permanent marker with black or blue ink, transfer *all* of the label information from the gum cards to the corresponding Vivak cards.
- PLEASE INSURE YOU HAVE TURNED THE HEAT DIALS TO THE “OFF” POSITION WHEN YOU ARE DONE.

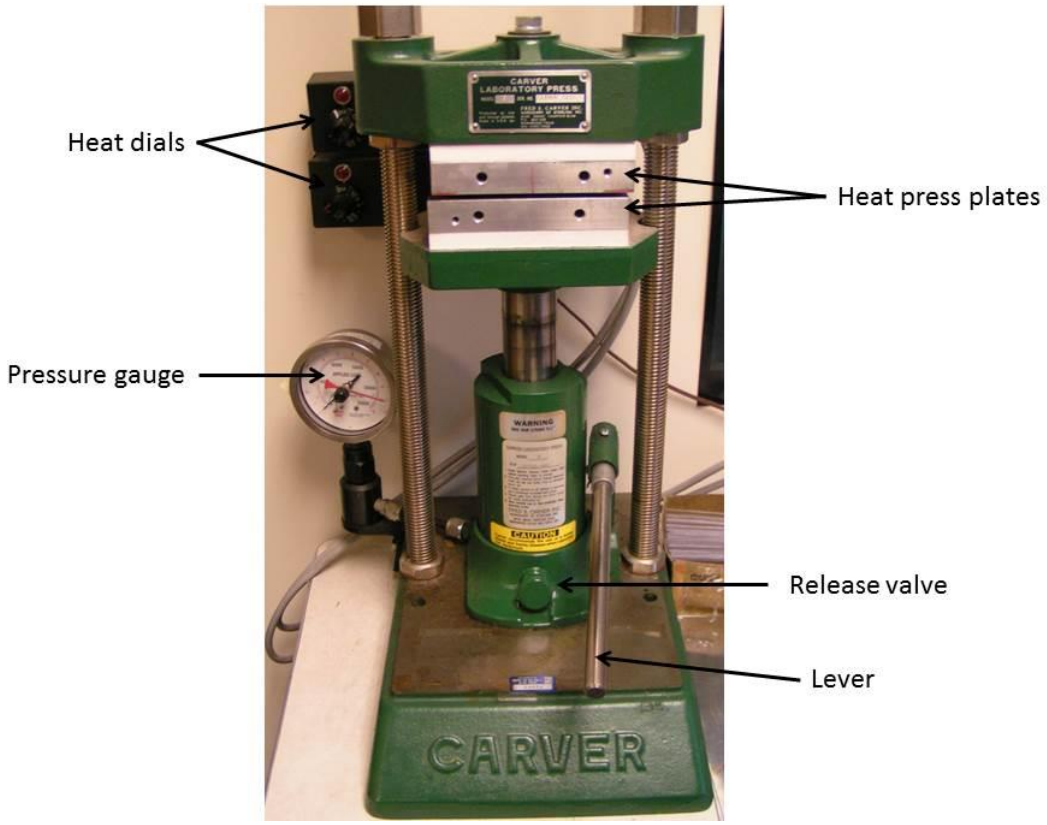

Figure 4. Carver Hydraulic Heat Press used for creating scale impressions in plastic Vivak cards.