U. S. Gulf of Maine Regional Rainbow Smelt Collection and Ageing Protocol

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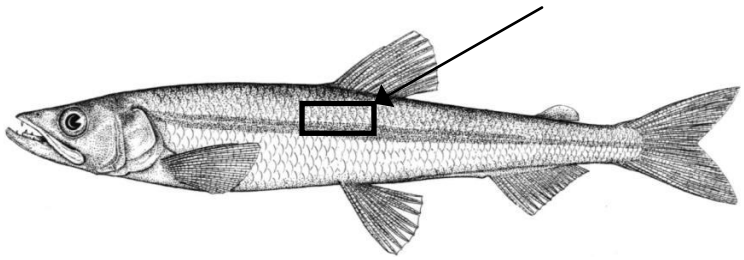
Sampling Procedure

Rainbow smelt are collected in fyke nets during the spring spawning run. To create sex specific age keys 300-400 individuals of each sex are needed. Given an average size range between 10cm and 25cm, twenty five fish of each sex should be collected for each centimeter increment. i.e. 50 fish total (25M 25F) between 100mm and 109mm. See table 1 for field tally sheet. In order to avoid bias caused by potential changes in length at age during the season, these samples should be spread out as evenly as possible over the entire spawning run.

Scales should be collected from just ventral of the dorsal fin (figure 1). A blade such as a scalpel should be used for the scale collection. The first movement should be from anterior to posterior in order to scrape away any debris such as the mucus coating and scales that may have fallen off other fish. The blade should then be scraped from posterior to anterior to remove a section of scales. At least 15-20 scales should be removed to be sure there are sufficient numbers for ageing. Scales should be stored in a folded strip of paper inside an envelope labeled with sample #, capture site, sex, total length and date.

Figure 1. Location of scale samples



Processing Scales

Scales should be removed from the paper with a pair of forceps (fine tip jewelers forceps work well) and placed into a small vial such as a micro-centrifuge tube with a 5% solution of pancreatin (Whaley, 1991). If enough scales are present, some should be left in the envelope in case no acceptable scales are found in the first batch. If working with batches of several fish, the vials should be labeled consecutively and corresponding numbers should be transferred to the envelopes. The vials are then floated in a sonic bath for 15 minutes. The vials are emptied one at a time into a small Petri dish and rinsed with clean water. Sixteen scales are selected, patted dry and placed on a glass microscope slide. If possible, lining up the scales and orienting them in the same direction will speed ageing. A glass cover slip is then placed over the scales and held down with tape on one end and a label on the other. The label should contain the sample number but no information regarding the fish size. To avoid bias when reading the scales, slides are stored in a slide box instead of in the envelope with the fish information written on it.

Reading Scales

Scales are viewed using the image analysis program Image Pro (version 6.2) which drives a digital video camera mounted atop a lens tube. The computer is calibrated to the zoom selected for reading the scales so that measurements of annuli can be made. All scales on a slide should be examined before selecting the individual scale to age. When making a selection avoid regenerated scales (figure 2). This is the time to look for contamination from other fish as well as to look for the differences between annuli and false annuli (checks). Annuli will appear on every scale and will have breakages of circuli on both sides of the scale. You should be

able to follow an annulus around the entire scale. False annuli often do not show on every scale and may only have breakages of circuli on one side (figure 3). Annuli will be accompanied by a "shiny line" scar as described by Mckenzie (1958). Fish in Massachusetts should have circuli forming inside the first annulus. Some fish have a small false annulus that is very hard to distinguish from the first annulus. This false annulus may or may not have circuli inside. Growth rates seen in the rest of the scale can provide information to aid in determining if the annulus is real or false. Circuli should appear as though they are crowded as they approach an annulus and then spread out after the annulus. The edge of the scale is assumed to be an annulus in fish captured during the spawning season. It is presumed that the annulus is being laid down during or just following the spawning season. Age three and older fish tend to show little growth between annuli and may have as few as one complete circulus between annuli (figure 4). These annuli can still be identified by the "shiny line".

Once the first reader has chosen a scale to age, a photograph should be taken and saved with the sample number. The Image Pro data collector tool should be set to record image name, lengths measured with the measurement tool and total feature count. The annuli distances should then be measured with the measurement tool as shown in figure 5. Annuli should be measured in the same order each time (i.e. first annulus, second annulus, third annulus…). The scale aged should then be marked with a fine point permanent marker. After being exported to excel, the data can be rearranged so that one row will accommodate all data from one fish and annuli measurements are aligned by column. A series of "if, than" statements and sorts can accomplish this.

A second reader should read all the scales in the same fashion as the first with the exception of measuring annuli distances. If the two readers disagree on an age, new annuli measurements can be taken later. The data from both readers should be compared using the precision template found on the National Marine Fisheries Service, Fishery Biology Program website (http://www.nefsc.noaa.gov/fbi/fbi.html). The two readers, or a quality assurance reader, should go over the scales from fish that age was not agreed upon, as well as fish that do not fit into the normal length distribution for a its given age. During this read length and sex data are available to the readers. If a consensus age is not reached the fish should be removed from the data set.

Future versions of this protocol will include a reference collection which is currently being compiled.

Literature Cited

McKenzie, R. A. 1958. Age and Growth of Smelt, *Osmerus mordax* (Mitchill), of the Miramichi River, New Brunswick. Journal of the Fisheries Research Board of Canada, 15(6), pp. 1313-1327

Whaley, R. A. 1991. An Improved Technique for Cleaning Fish Scales. North American Journal of Fisheries Management, 11, pp. 234-236

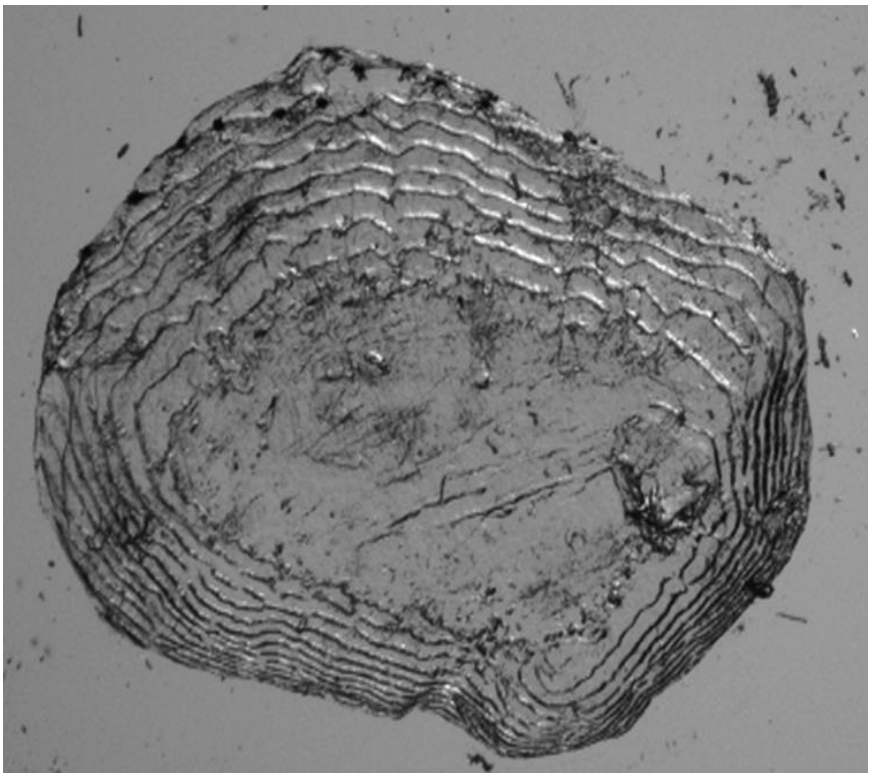


Figure 2. Regenerated scale from a 214mm smelt. Note the lack of circuli near the origin of the scale.

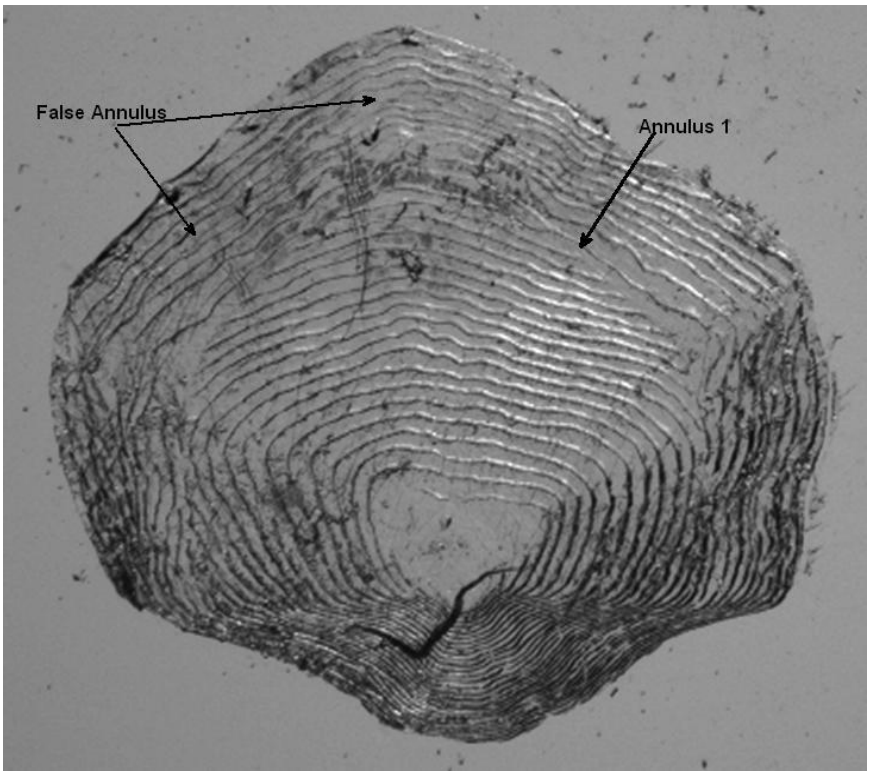


Figure 3. False annulus between the first annulus and the edge of the scale in an age 2, 214mm smelt.

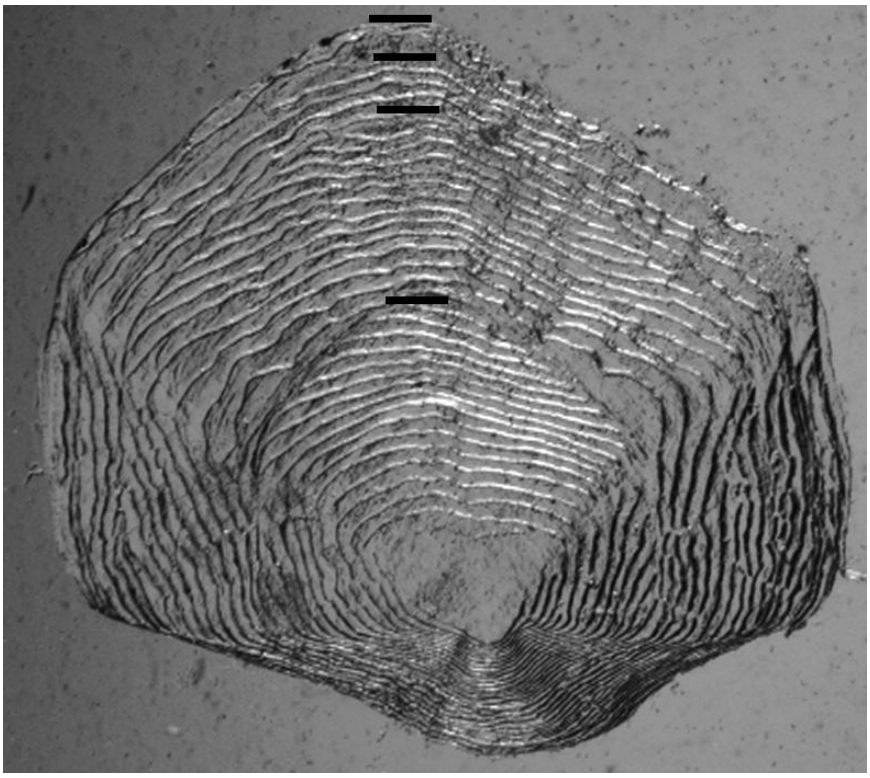


Figure 4. Age 4, 241mm smelt. Note how little growth there is between annuli 2, 3 and 4 as denoted by the black bars.

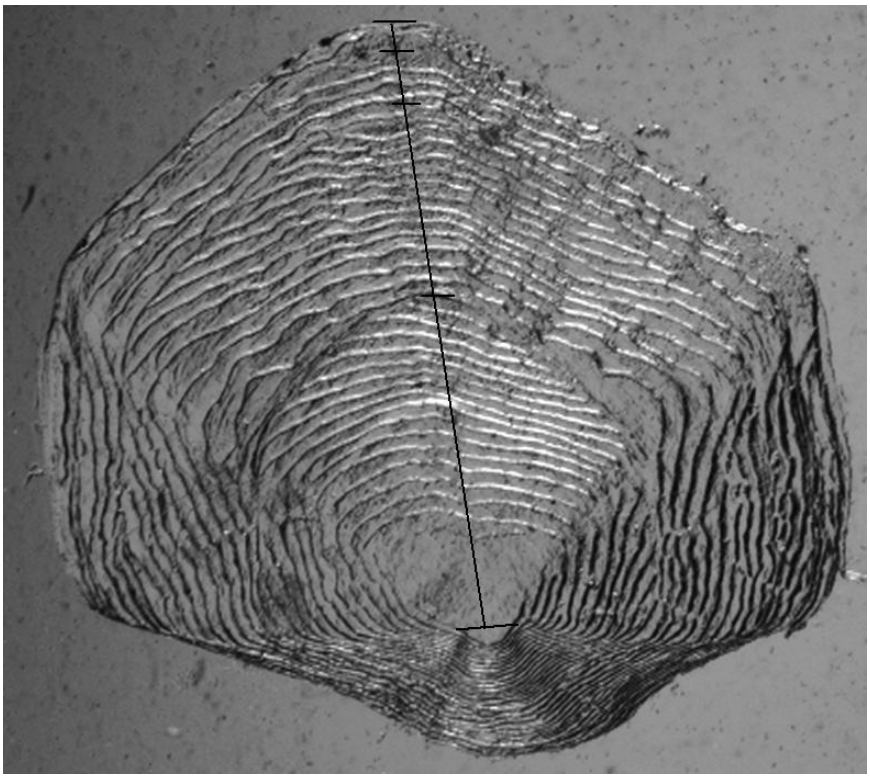


Figure 5. Age 4, 241mm smelt. Annuli measurements are taken from the origin of the scale to each annulus as depicted. The measurements should be taken along the plain from the origin to the farthest point of the scale. Annuli should be measured in order. i.e. annulus 1, 2, 3, edge.

Table 1. Field data sheet for keeping track of the number of smelt sampled over the spawning season.

Age Sample Check-Sheet

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