

## Lab 10: Fish Biology

### Introduction

The effective management of fish populations requires knowledge of the growth rate of the fish. This requires determination of the age of fish to develop a relationship between the size and age of fish. For an inventory, this information provides insights to evaluate the potential effect of harvesting on the population and to monitor the health of a population that may be affected by developments that affect fish habitat. Age can be determined directly or indirectly from the population of interest. Age and associated length or weight can be measured empirically from individuals reared in captivity or from fish specially marked at a known age and size, and recaptured at some later date. However, the cost and space required to rear fish often precludes the use of this as a practical method. As well, it can be argued that captive or marked individuals do not demonstrate growth typical of unmolested animals in the natural environment.

For many species analyzing hard body structures can make a direct measure of age. In temperate-zone waters, both fish and shellfish species exhibit seasonal growth patterns indicative of age. Generally, growth is rapid during warm "summer" months, and slow during cold "winter" months. As fish grow, they deposit minerals in their skeletal tissues, producing characteristic growth patterns. One year of growth consists of one summer zone plus one winter zone. In bones, these patterns are called annuli and in scales they are called circuli. The annulus is usually defined as the winter zone. Summer and winter growth zones differ in appearance, thus providing the basis for age determinations. Length proportional to the growth of the age structure is used as a basis for empirical relationships. Different periods of growth can be determined by counting the light and dark bands typical of annuli or by observing the differences in spacing of the circuli. By assessing these patterns the age of the fish can be determined. By convention, a 1 January birth date is assigned to nearly all species in the Northern Hemisphere (exceptions to this rule are given in the individual species descriptions). This means that a winter growth zone forming on the edge of the age structure is designated as an annulus on 1 January, even though the zone may not complete.

Otoliths, scales, fin rays, cleithrum, or the operculum are some of the typical structures collected for age information. The structure that is collected should depend on the needs of the inventory and the characteristics of the species collected. For example, scales cannot be used to age fish that are very long lived, such as lake trout, because the circuli near the center of the scale become very compressed and difficult to read accurately. Also, an ageing method that requires sacrificing the animal may not be desirable when studying sensitive populations.

Scales are one of the most common and convenient methods for determining the age of a fish. Scale removal is relatively quick and easy, requires only simple dissecting tools, and has minimal impact on live fish when properly done. However, ageing fish with scales does have disadvantages. Many fish have the ability to re-absorb scales or produce new scales to replace lost ones resulting in growth patterns that do not accurately reflect the age of the fish. As well, scales from older fish, such as lake trout, Dolly Varden and bull trout are very difficult to read and interpret, and thus fin rays and otoliths (in special cases) are the preferred structures for ageing these fish.

Depending on the species, scale samples are taken from different locations on the body. Figure 1 illustrates several typical areas where scales can be removed. Scales must be taken from an area the fish known to exhibit complete and clear growth patterns. For salmonids and trout, scales are usually removed from area between posterior edge of dorsal fin and the lateral line, approximately two scale rows above the lateral line on the left side of the fish. For gadids (cod) and flounders, this area is on either side of the lateral line anterior to the caudal, the area where the first and largest scales develop. For other species, such as bluefish (*Pomatomus saltatrix*), black sea bass (*Centropristis striata*), weakfish (*Cynoscion regalis*) and scup (*Stenotomus chrysops*), scales are from the area behind the pectoral fin where the largest scales are.

Before sampling scales, clear away dirt and excess mucilage from the area to be sampled. Scales are removed by gently scraping against the grain of the scales with the blade of a clean scalpel or knife. If the scales are large they may also be removed with small forceps. Since any one scale may not accurately represent the true age of the fish, several scales (~5) should be collected from the fish. Removed scales are deposited onto a glass microscope slide, into a scale book, or envelope. The slide or envelope is then clearly labeled. No special solutions are used to preserve the scales, as air-drying is sufficient for preserva-

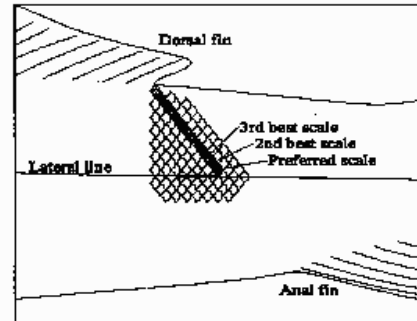
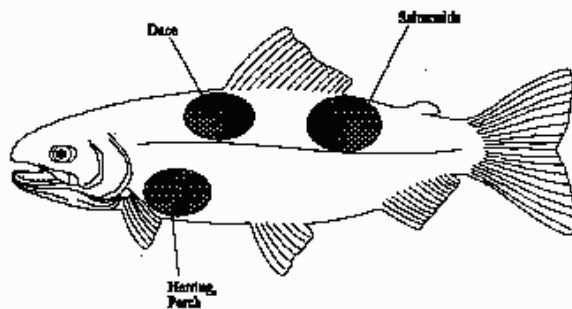


Fig. 1: left - preferred locations on body for collecting scales for different species; right - preferred scales to be taken from salmonids for ageing

tion, especially if the scales will be analyzed soon after collection. However, if left too long, scales can turn cloudy, obscuring circuli. For long-term preservation, scales can be frozen.

The sagittal otolith bones (sagittae) from the head of the fish are another structure used for ageing fish. 'Otolith' is a generic term used for small calcareous particles that are present in fluid filled sacs in the fish's middle ear. The paired middle ears are located latero-posteriorly (behind and to the sides) to the brain. Otoliths possess a white center surrounded by alternating concentric opaque and clear (hyaline deposits) rings. These structures assist in giving the animal its position with respect to gravity and allowing it to balance. Collecting otoliths require killing the fish and, hence, should only be performed when other, non-lethal methods cannot be employed. The removal procedure is easily learned, relatively quick, and requires only basic dissecting tools. Otolith bones collection is the preferred method for determining age in species that do not produce reliable scale readings, or grow to great ages. Usually only sagittal otoliths, the largest of the three pairs found in the sacculi of the inner ear located posterior to the brain, are removed for examination.

To remove the sagittal otolith bones, hold the killed fish upside down and cut through the gill arches and isthmus to expose the roof of the mouth. Cut  $\frac{3}{4}$  through the roof of the mouth (parashenoid bone) where the first gill arches join the roof of the mouth. Holding the head of the fish, break the backbone downwards where the cut was made in the roof of the mouth. This will expose the otolith bones within membranous sacs on either side of the mid-line at the posterior ventral portion of the brain cavity. The otoliths should be extracted unbroken and as clean as possible, using small forceps. Both bones should be removed. Once removed from fish, any residual tissue, gelatinous membrane or blood should be rinsed from the otolith with fresh water.

Generally otoliths can be preserved by air-drying or by freezing. If stored dry, otoliths may become brittle and easily damaged by rough handling. A solution of glycerin, glycerin/water, or glycerin/alcohol can be used to preserve otoliths and prevent them from becoming dry and fragile. Glycerin has also been

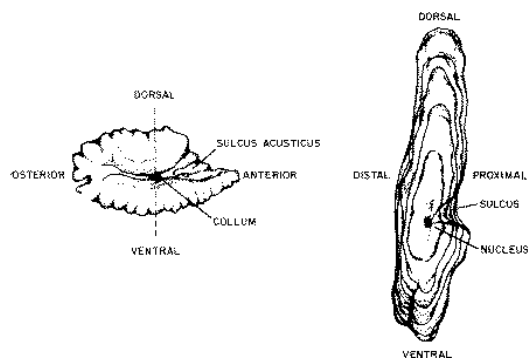
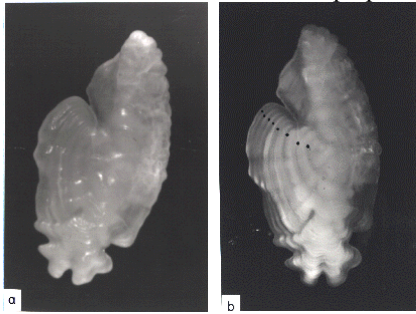


Fig. 2: Otolith and cross section with descriptive terms and direction of cut (dashed lines) for removing a thin section. Proximal side of the whole otolith, with sulcus acusticus and collum is shown

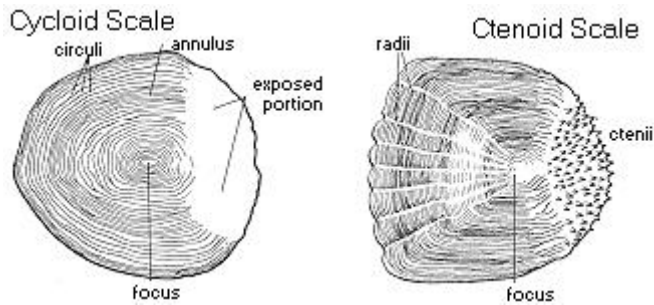
known to have a mild clearing effect on the otoliths making them easier to read. Formalin should not be used to preserve otoliths or fish from which otoliths may later be taken. The formalin tends to de-calcify bone resulting in chalky otoliths where the annuli are obscured. Otoliths are generally stored in small, labeled envelopes. If preserved with a liquid, the bones should be kept in a small, sealed container with a label placed inside the container with the bones.

In some fish, otoliths become too thick and calcified to distinguish annuli clearly. Such otoliths are studied by thin-sections, half-sections (one half of the otolith is ground away, revealing the view on a cross-section), or otoliths are "baked" or "burned". In the latter method, otoliths are exposed to 275°C (525°F) for 3-6 minutes. Properly baked otoliths are

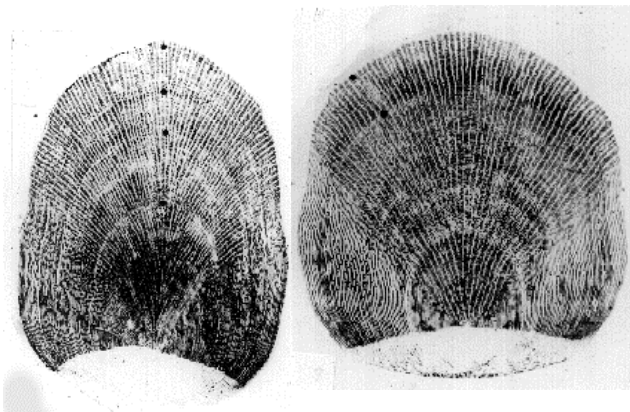
a caramel color; a gray or ashen color is an indication of overbaking, which may cause the otolith to crumble when broken at the nucleus. Baking enhances visibility of the annuli, since the hyaline zones turn brown in contrast to the white opaque zones.



*Fig. 3: Distal surface of a dry (a) and water-submerged (b) otolith of rockfish (*Sebastes alutus*). Note that the annuli are much clearer when the otolith is submerged in water. Annuli are marked by dots in (b).*



*Fig. 4: Types of fish scales. Left – cycloid scale have smooth edges. Right – ctenoid scales have a toothed edge. Due to faster growth in summer than in winter, the circuli become closer to each other during winter, forming an annulus. An annulus is, thus, a cluster of dense circuli.*



*Fig. 5: Left - Scale impression of a 51 cm age 4 summer flounder collected in June showing clear annuli and "cutting over" just inside the scale edge. Right - Scale impression of a 31 cm age 2 summer flounder collected in March*

## Lab Work

Each student will be provided with one fish. The fish species will depend on availability. This lab class shall familiarize with the general techniques of fish aging. The ability to establish fish age from otoliths and scales will, however, depend on the species of fish available for the class and the age (size) of the available fish. If only fish of less than 1 year of age are available, growth rings (annuli) on otoliths and scales will not be visible but fish length to otolith size can be established.

All data on fish length and otolith size (and annuli, if possible) will be reported to the lab blackboard. After conclusion of the lab, you need to copy these data for the length to size relationships to be included in your lab report.

## Work Steps

1. Fish length: With a ruler, measure the length of your fish from the mouth tip to the center of the tail fin and note your fish length in your lab journal. Report your fish length to the lab class blackboard.
2. Take a few scales from the side of the fish, just behind the end of the dorsal fin and close to the central line of the fish (see Fig. 1). Use forceps to strike against the scale orientation to lift scales, pick them carefully with the forceps and place them on a microscope slide. Cover with glycerin and a cover slip and observe under the microscope. Draw the general shape and characteristics of your scale. Is it a cycloid or ctenoid scale? You will see multiple circuli and, in the case of ctenoid scales, radii. Indicate in your drawing the region in which you find complete circuli and the number and direction of radii. Can you discern annuli in your scales? Remember that annuli present regions of dense versus wide-spaced circuli (like growth rings in trees). If you discern annuli, document them in your scale drawing. Count the number of annuli, not the number in your lab journal, and report the number to the blackboard next to your fish length.
3. Following the instructions given in the Introduction, place your fish in the dissecting pan and cut  $\frac{3}{4}$  through the roof of the mouth (parashenoid bone) where the first gill arches join the roof of the mouth. Holding the head of the fish, break the backbone downwards where the cut was made in the roof of the mouth. This will expose the otolith bones within membranous sacs on either side of the mid-line at the posterior ventral portion of the brain cavity. The otoliths should be extracted unbroken and as clean as possible, using small forceps. Both bones should be removed. Once removed from fish, any residual tissue, gelatinous membrane or blood should be rinsed from the otolith with fresh water.
4. Place your otoliths on a microscope slide, cover with glycerin and cover slip and observe under the microscope. Draw the shape of your otolith and morphological characteristics. Depending on the size of your otolith, decide if you use the dissecting microscope or the compound microscope to get best information. Can you see annuli in the otoliths? Depending on the fish species and age, annuli may be visible but not in species that have thick and crystallized otoliths. If you can see annuli, document them in your otolith drawing. Count the number of annuli, note in your lab journal, and report the number to the blackboard next to your fish length.
5. Place a sheet of millimeter paper on the stage of the dissecting microscope. Place your microscope slide with your otoliths on the millimeter paper. Use the dissecting microscope and the millimeter paper to measure the size (length) of both otoliths. Note the otoliths' length in your lab journal. Calculate the average of the otolith size from both measurements and report the average otolith size to the blackboard next to your fish length.
6. Copy the blackboard table into your lab journal. For your final lab report, prepare a graph of otolith lengths versus fish length. Is there a (linear) relationship between the otolith size and the fish length? If so, express this relationship in a mathematical formula. Prepare similar plots for scale annuli and otolith annuli versus fish length and examine for relationships. Finally, prepare a frequency plot of fish lengths; round each fish length up to the next full cm and count the number of fish in each size class. Prepare a bar plot with the size classes on the x-axis and the number in each size class on the y-axis. In your lab report, discuss the length distribution of the examined fish population; what is the variability in fish length, which size class is the most abundant, what is the average and what is the median fish length? Refer to your statistics class and textbook for these analyses.