4.9 Hard parts

Many parameters are considered critical for the assessment and management of species (as already detailed in this chapter). Amongst these are the age and growth rate of fish.

Where hard parts show marks laid down at a regular time-interval, they can be used to age fish. Marks may be on a seasonal or annual scale (macroincrements), and comprise an opaque and translucent band that can be seen under a light microscope. Marks may also be laid down on a daily basis (microincrements). These require high-power microscopy or scanning electron microscopy to view. Microincrements can be particularly useful when ageing larvae and juveniles. The formation and biomineralization of these growth bands depends on many metabolic and environmental factors, including climate, migrations, nutrition etc.

It is recommended that the nomenclature detailed in Kalish *et al*. (1995) be used when reporting all studies.

Readers are also referred to the excellent manual prepared by CCSBT for southern bluefin, available at: http://www.ccsbt.org/docs/pdf/about_the_commission/age_determination_manual.pdf.

4.9.1 Validation

Before increments in hard parts can be used for routine ageing, they must be validated. This means proving a technique is accurate (Beamish and McFarlane, 1983).

The interaction of factors influencing band formation may result in the formation of bands once or twice a year (e.g. Ortiz de Zárate *et al*., 1996 for albacore). Therefore, the periodicity of formation of bands must be validated to ensure they can be used for accurate age reading. Failure to validate ages can lead to considerable errors in stock assessments.

There is a wide range of methods which can be used to attempt to validate bands in hard parts. These include back-calculation and marginal increment analysis. The most conclusive approach is the use of mark-recapture techniques, including the use of markers such as oxytetracycline (e.g. Ortiz de Zárate *et al*., 1996). Oxytetracycline can be introduced inter-muscularly to captured and tagged fish at dosage rates of approximately 70mg/kg body mass, administered by syringe in body area of the dorsal fin. The resulting mark can be seen as a yellow/gold fluorescence under UV light, and related to the elapsed time since release and the natural marks formed in the hard part. Strontium chloride ($SrCl_2$) can also be used where potential health concerns rule out the use of oxytetracycline. $SrCl_2$ marks in sectioned otoliths can be seen under scanning electron microscopes with a Robinson backscatter detector (Clear *et al*., 2000). Care must be taken to ensure there is no change in the pattern of growth following the marking procedure.

It **must** be stated whether validation was achieved in any study presented. There is also a need to specify the ages for which validation has been achieved.

4.9.2 Sampling for hard parts

A wide range of hard parts have been used to age tuna and billfish species. These include otoliths (sagittae, e.g. Atlantic bluefin tuna), the 1st dorsal fin spine/ray (where spines are generally hard and rays soft) or anal fin spines/rays (e.g. swordfish (spines), yellowfin tuna, bigeye tuna (rays)), vertebrae (generally from the caudal peduncle, vertebra no. 35, e.g. Atlantic bluefin tuna, bigeye tuna).

Some structures are more suitable for a particular species or age than others. There are further practical considerations. Spines can be removed without damage to the fish. This is of particular advantage where purchase of the fish in high value fisheries would otherwise be necessary to examine hard parts such as vertebrae. The caudal peduncle can also be accessed without affecting some commercial processing approaches.

Two strategies are usually used for sampling; random sampling or sampling by length (where a certain number of samples are collected from each length group) (see section 4.2.2). The sampling approach is defined by the purpose of the programme. Monthly samples may be required to validate the use of hard parts for ageing through marginal increment analysis (section 4.9.2), while less frequent sampling (up to the annual level) may be required to develop age-length keys (section 4.3), or even less frequently to estimate general growth parameters. Sampling from different sexes will be required to identify sex-specific growth rates.

A length-stratified sampling programme is preferable, as it will ensure adequate sampling of the whole length range, and improve the estimates of growth parameters (see Section 4.9.5) and the utility of ALKs. However, there are often problems in obtaining fish at the lower or upper end of the length range. In these cases, it may be necessary to form a lower or upper group and combine all the hard parts into this group.

Ruiz *et al*. (2005) have developed recommendations for the length-stratified sampling of hard parts, from which much of the following is taken (with permission). Every month a number of hard parts (e.g. spines) must be collected for each 5cm fork length range. Sampling should take place on different days throughout the month until sufficient hard parts have been collected to complete, as far as possible, the length range of landings. Samples should come from the different catch areas of the stock under study such that most catch areas are covered as well as possible. Therefore, sampling must also be spread amongst different vessels and landing ports.

Hard parts should be obtained from the same landings as the length samples when generating ALKs. If separate landings are used, efforts should be made to ensure samples are obtained from areas/gears that have previously been sampled for length measurements.

Information must be collected corresponding to the individual from which the hard parts were taken, and its capture. These include:

Data	**Notes**
Species	
Unique fish identification number	
Specimen size	The most commonly used measurement is Fork length (FL). (See section 4.3.3). Fork length is the straight line from the end of the upper jaw (end of the snout) to the posterior of the shortest caudal ray (fork of the caudal fin) (**Figure 4.9.1**). This can best be measured using a calliper. Alternatively, a tape measure can be used, although it **must** be kept straight while measuring. The fish should be placed on a flat surface in a horizontal position. In the case of very large specimens in which this measurement is very difficult to take, one of the following two lengths may be used to substitute it: - First dorsal length (LD1): this is the straight line from the end of the upper jaw (end of the snout) to the base of the first dorsal spine (the start of the first dorsal fin) (**Figure 4.9.1**). - Curved fork length (CFL): this is the length from the upper jaw (end of the snout) to the fork by an imaginary longitudinal line, with the corresponding fish curvature (**Figure 4.9.1**). The type of measurement being used must be clearly specified, with the measurement unit (cm). FL and CFL are measured to the lower centimetre (a specimen of 70.8 cm or 70.2 cm would correspond to the 70 cm range), LD1 is measured to the lower half centimetre (a specimen of 30.4 cm measures as 30 cm and one of 30.7 cm corresponds to 30.5 cm).
Date of capture of specimen	Day, month and year
Fishing area	This is the location of the catch from which the sample was extracted, and does not refer to the place where sampling took place. A precise geographical delimitation must be established. The most exact is the latitude and longitude where it was caught. As this information is not always available, in the case of sampled specimens captured in different fishing operations, the latitude and longitude of the area (between 44º - 45ºN and 5º - 7º W, for example), or at least a more or less defined geographical area such as the Bay of Biscay or the Alboran Sea, for example, should be noted.
Country	The country to which samples, organization and personnel responsible for sampling correspond.
Date of sampling	Day, month and year
Live and/or gutted specimen weight	kg
Sex	Male, female, unknown
Vessel type and fishing gear used	Purse seine, longline, baitboat etc.
School type	Free school, FAD associated
Vessel name	Name of vessel that caught the specimen and the port at which it was landed
Hard part	Otolith (left, right, both), vertebrae (and details of vertebrae number), or spine (and spine number)

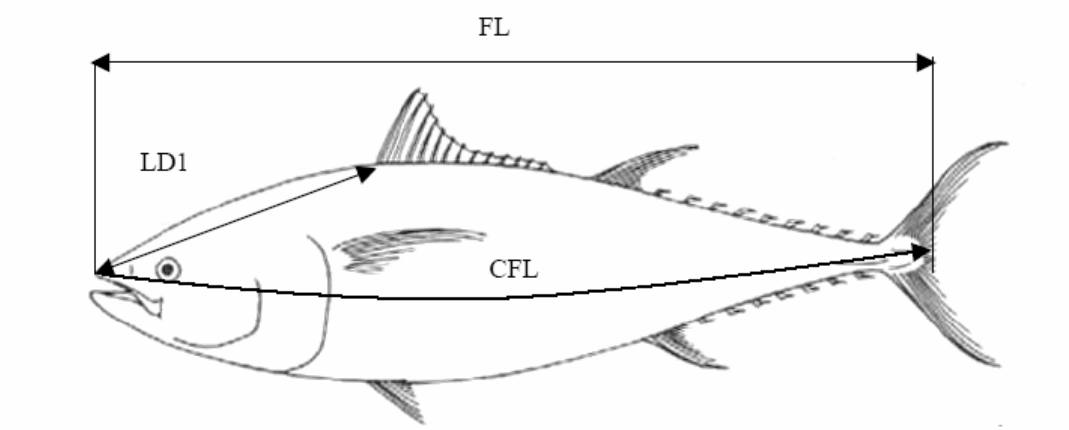

Figure 4.9.1. Types of measurements of bluefin tuna: Fork length (FL), First dorsal length (LD1), Curved fork length (CFL) (from Ruiz *et al*., 2005, used with permission).

The details should be noted on the relevant statistical sheet to ensure the sampling regime is completed.

Spine sampling

The first spine of the first dorsal fin should be collected from each specimen of the appropriate species. The spine must be pulled out whole from the base.

Using a knife, cut the membrane joining the 1st and 2nd dorsal fin rays (**Figure 4.9.2**). Push the spine forward progressively **(Figure 4.9.3B)** until the ligament breaks (**Figure 4.9.3C**). Twist the spine left and right alternatively until it comes loose and pull to finally extract it (**Figure 4.9.3D**).

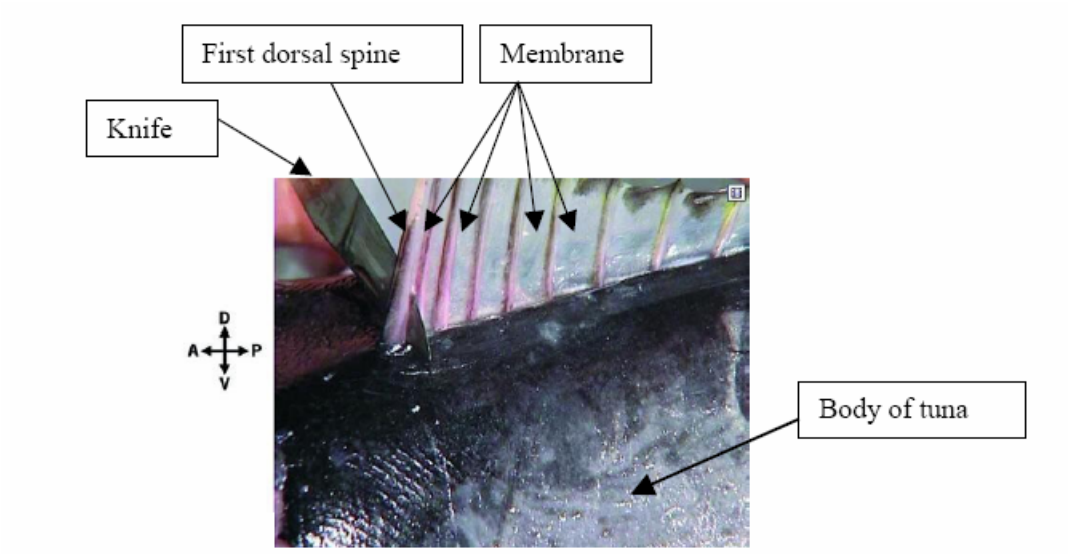

Figure 4.9.2. Insertion of the knife into the membrane separating the first two spines of the 1st dorsal fin. (Figure taken from Panfili *et al*., 2002).

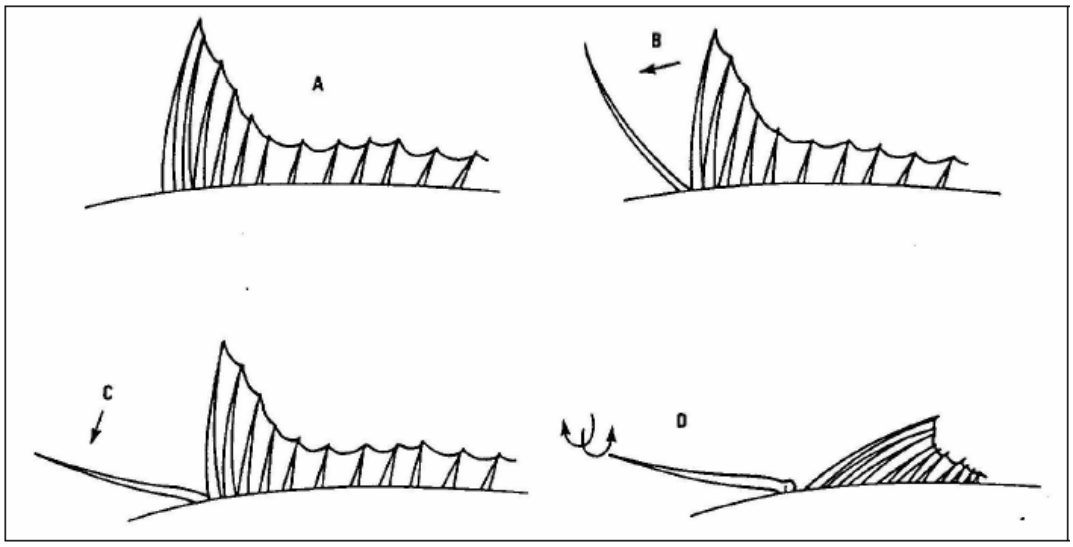

Figure 4.9.3. Technique of extraction of the first spine of the bluefin tuna dorsal fin. (Figures taken from Compeán-Jiménez, 1980).

Spines are ideally preserved dry in a paper envelope, which should be kept in a cool place (refrigerated). If the spine collected is too large to fit in the envelope, it can be cut in half or even in three pieces and kept in the envelope, remembering that the piece forming the base of the spine is the most important since it is the part used for age interpretation. The data of the specimen sampled or its corresponding code must appear on the envelope.

Otolith sampling

Sagittal otoliths are small, calcified structures found in the semicircular cavities of the inner ear, situated at the base of the brain. They are formed by the accumulation of calcium carbonate and protein. The sagittal otolith is the largest of the three otoliths found in each inner ear of the bluefin tuna.

There are two main techniques of removal: transverse head section and frontal head section. In the second, a frontal section of the superior part of the cranium is made, passing above the eye and parallel to the major axis of the fish. The first technique is detailed here.

The transverse head section approach consists of making a cut in the upper part or back of the head at the level of an imaginary line: trace an imaginary line perpendicular to the horizontal fish, which passes through the mid-point between the corner of the mouth and the preoperculum (**Figure 4.9.4A**). For this purpose, the use of a ruler is recommended for dividing this distance in two, and afterwards making a cut in the upper part of the fish which follows this imaginary line. Once the point has been marked to make the cut, use a metal saw and cut down through the head perpendicular to the horizontal axis of the fish.

The sectioned part of the head contains the otoliths. If the above described cut has been made properly, the cavities below the brain in the upper part of the head (**Figure 4.9.4B**) should be searched to find the otoliths. If they are not found here, it may be that they are in the other part of the sectioned fish. Using fine forceps and with great delicacy to avoid breaking these fragile pieces, extract each otolith. They must be taken out of a very fine transparent capsule, which covers them. The otoliths are between 7 and 20 mm in size approximately, and both otoliths must be collected from each specimen. If the otolith has broken, try to recover the pieces and keep them all together. Once extracted, rinse them in water or diluted alcohol and leave them out to dry.

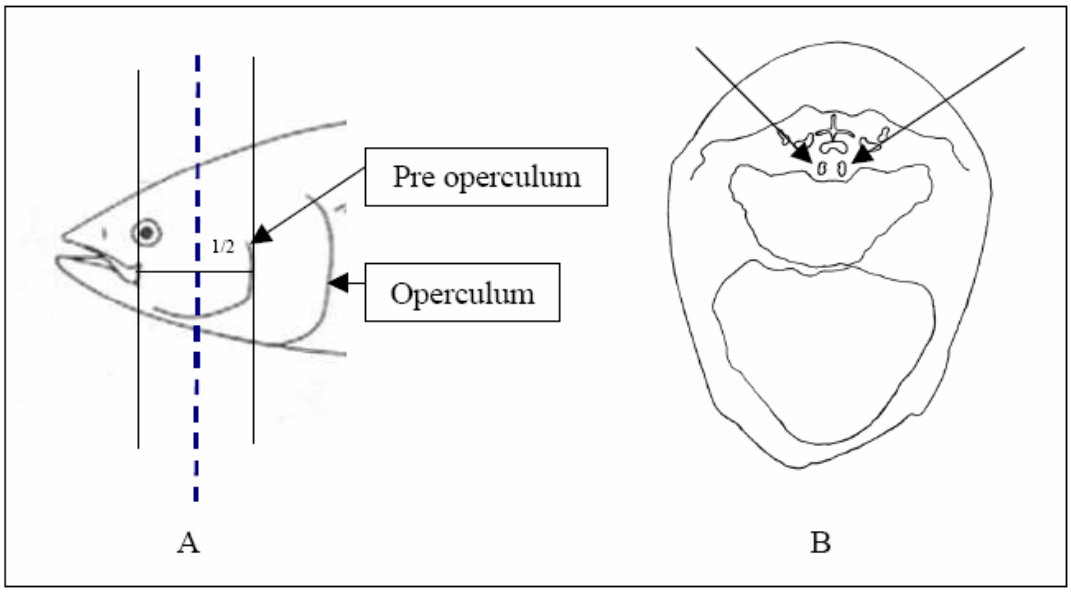

Figure 4.9.4. A. Tracing the imaginary line (dotted) along which to make the cut. B. View of the cavities where the pair of otoliths are found in the back of the head. (from Ruiz *et al*., 2005, used with permission)

Otoliths are best stored dry in a tube or in an envelope. If using an envelope avoid applying pressure that might break them. The data of the specimen sampled or its corresponding code should appear on the envelope or the tube.

Caudal vertebrae

Vertebra 35 is used for the study of growth (Farber and Lee 1981). However, it is better to collect vertebra 35 and 36 without separating them. Collecting both gives the opportunity of comparing the "whole vertebra" and the "vertebra section" methods. Also, storing vertebrae 35 and 36 attached preserves the quality of the inner surface preventing dehydration caused by refrigeration. As the surface comes in contact with air, it dries and becomes more difficult to read.

To find vertebra 35, a transversal cut is made in the caudal area between the 4th and the 5th finlet (counting from the end of the tail forwards, i.e. there must be 4 more finlets behind the one indicated). On making the cut vertebra 35 should be exposed. The cut should coincide with the intervertebral space and the tail can be cut easily. If not, the intervertebral space must be found further forward in the fish. Vertebra 35 is the first vertebra found in the part sectioned, and can be separated together with vertebra 36 from the rest of the caudal vertebrae, cleaned and peeled, with any flesh attached to it removed.

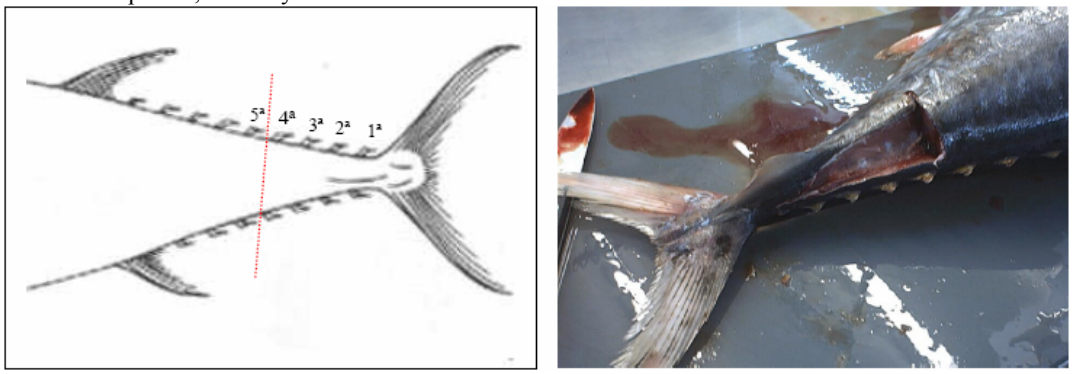

Figure 4.9.5. Cutting line to find vertebra 35. The photograph shows the transversal cut and the tail has been peeled to uncover the vertebrae (white marks). (from Ruiz *et al*., 2005, used with permission)

The two vertebrae should be stored attached, and not separated until they are analyzed. They should be stored dry in an envelope and refrigerated (some flesh always remains attached). The vertebrae can be stored together with the spine in the same envelope.

4.9.3 How to prepare hard parts for reading

Spines are prepared by taking a cross-section through the basal portion of the spine (where the spine is approximately half the maximum width of the condyle base). These sections are mounted in resin and cut with a low-speed saw to obtain a thin section (e.g. 0.5 mm thickness). These thin sections can then be mounted on a slide in resin, and can be cleared with 95% ethanol for 5mins, if required. The sections can then be examined under the microscope.

Otoliths have been read whole, but it can be difficult to age otoliths of older individuals and ages may be under-estimated. Sectioning is recommended. Otoliths should be embedded in polyester resin and a transverse section taken through the primordium using a low-speed saw. The slice can be attached to a microscope slide using resin and polished with appropriate polishing grit if necessary. The otolith can then be read under a binocular microscope.

Vertebrae can be thin-sectioned in the sagittal (dorso-ventral) plane, using a low-speed saw. Following mounting on a microscope slide, they can be stained for additional clarity using silver nitrate or polished. Stained sections can then be read under a binocular microscope.

4.9.4 Reading

Reading of prepared hard parts develops an integer age for an individual fish, and by reference to the length of that individual, a length-at-age.

Ages are developed relative to an assigned 'birthday' for a species. The birthday is usually related to the spawning period for a species. When ageing relative to a birthday, a complete annual ring will not be counted until this date is passed. For example, if the birthday were 1st June, a fish with a third annual ring that was just completed would be counted as a two-year-old until caught on or after the 1st June.

Generally, readers should not be provided with additional information on the fish (e.g. length, date of capture) to avoid bias. Date of capture may be important when assigning ages around the birthday of the species.

Care must be taken when reading spines, as the central portion of the spine can become vascularised in older fish. This obliterates the age bands formed when young. These bands have been accounted for in ageing by back-calculating the likely number of annuli suspected (e.g. Lee and Yeh, 1993) or through the use of values provided in previous studies (e.g. in Cort (1991) for bluefin tuna).

A simple measure of the precision hard part age estimates from multiple readers is the individual average percentage error (IAPE, Beamish and Fournier, 1981). This can be calculated as:

$$IAPE = \frac{100}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}\right]$$

where N is the number of fish aged, R is the number of readings, X_{ij} is the ith age determination of the jth fish, and X_j is the mean age calculated for the jth fish.

4.9.5 Growth parameter estimation

Length-at-age data can be fitted to growth equations to estimate parameters important for stock assessment and management. Generally, a von Bertalanffy growth equation is fitted to the data. This equation satisfies two important criteria, fitting most of the observed data of fish growth, and being readily incorporated into stock assessment models. The formula is:

$$L_t = L_\infty\left[1 - e^{-K(t-t_o)}\right]$$

where L_t is the length at age *t*, L_∞ is the asymptotic length, K is the coefficient of growth, and t_0 the theoretical age for length at zero.

Gascuel *et al*. (1992) proposed a five-parameter growth function to model the two stanza growth curves in Atlantic yellowfin tuna. This model combined a linear function and generalized von Bertalanffy model:

$$L_t = L_0 + bt + \left[L_\infty - (L_0 + bt)\right]\left[1 - e^{-Kt}\right]^m$$

where L_t is the length at age t, L_0 is the length at age 0, L_∞ is the asymptotic length, K is the coefficient of growth, *b* is the initial growth rate, and *m* is an estimated parameter.

Growth equations can be fitted to hard-part derived length-at-age data through least squares methods, or likelihood approaches (Kimura, 1980). In either case, the standard errors of the parameters should be presented.

Care should be taken when interpreting growth parameter estimates, as they are strongly affected by the quality and quantity of data available. Issues arise due to a lack of smaller younger individuals due to gear selectivity, and larger individuals due to historical fishing pressure. Failure to include larger, older individuals reduces the information on the L_∞ parameter of the von Bertalanffy growth equation, while a lack of younger individuals reduces the information on the K parameter. Considerable uncertainty can result, which is transferred to stock assessments when these growth parameters are used.

4.9.6 Age-length keys (ALKs)

Age and growth data are required for the development of age-length keys. The construction of these keys has been described in Section 4.3.6.

4.9.7 Microconstituent analyses

Microconstituent analysis refers to the examination of trace elements occurring in otoliths (Secor and Chesney, 1998). The approach relies on two properties of otoliths: that they grow throughout the life of the fish, and unlike bone, otoliths are metabolically inert; the calcium carbonate and trace elements that make up over 90% of the otolith structure are derived mainly from sea water, as modified by ambient temperature (Humphreys *et al*., 2005). Particular elements are incorporated into otoliths in direct proportion to their availability in ambient water or food. Therefore, individuals from different locations may incorporate different mixtures of elements in their otoliths, forming an elemental fingerprint unique to the area/stock. Analysis of otolith microconstituents therefore has the potential to measure a number of life history characteristics. They may be used for validation, and to study homing fidelity, nursery origins (where juvenile otoliths are examined; Rooker *et al*., 2003), stock structure, migration rates etc.

Magnesium (Mg), calcium (Ca), strontium (Sr) and Barium (Ba) are incorporated and retained in the inorganic lattice structure of otoliths, and can therefore be used to examine environmental histories. Other elements such as sodium (Na), sulphur (S), potassium (K) and chlorine (Cl) are associated with organic material or interstitial spaces, and are likely to be less stable.

Appropriate decontamination and handling procedures are required to prevent leaching of elements post-extraction. Contamination can occur during dissection, handling, storage or cleaning procedures. Typically, highly purified water (e.g. doubly de-ionized water) is used to soak the otoliths to hydrate and aid removal of remaining biological tissue. 3% hydrogen perozide can then be used to soak the otolith for 5 minutes to dissolve remaining tissue. Otoliths can then be immersed for 5 minutes in 1% nitric acid to remove surface contamination, and then the otolith can be flooded with doubly de-ionized water for 5 minutes to remove the acid. The otolith should then be dried under a laminar flow hood. This decontamination procedure has been shown to be effective in removing Mg, Mn and Ba contaminations, without affecting the original composition of the otolith.

The main technique used to study otolith microconstituents is ICP-MS – Inductively Coupled Plasma Mass Spectrometry. The technique is capable of simultaneously assaying multiple elements at very high sensitivity (at sub parts per million detection limits). Solution-based ICPSM requires the material to be introduced in solution after dissolving in acid.

The stable oxygen isotope ratios ($\delta^{18}O$:$\delta^{16}O$) in otoliths can be used as a proxy of the ambient water temperature. At higher temperatures, otoliths contain more of the lighter $\delta^{16}O$ isotope. Carbon isotope ratios ($\delta^{13}C$) can be related to metabolism. However, factors controlling the stable carbon isotope composition ($\delta^{13}C$) in otoliths are more complex than those controlling oxygen isotopes, since 13C is also influenced by fish metabolism and feeding pattern. Micro-scale sampling techniques in otoliths using micromill or laser ablation techniques allow the evaluation of environmental information in high temporal resolution, with the limitation now the constraints of the mass spectrometer. Accurate sampling of material from otoliths using micromill or laser ablation techniques therefore allows temperature patterns to be related to the age and hence life history of the fish.

4.9.8 Further reading

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