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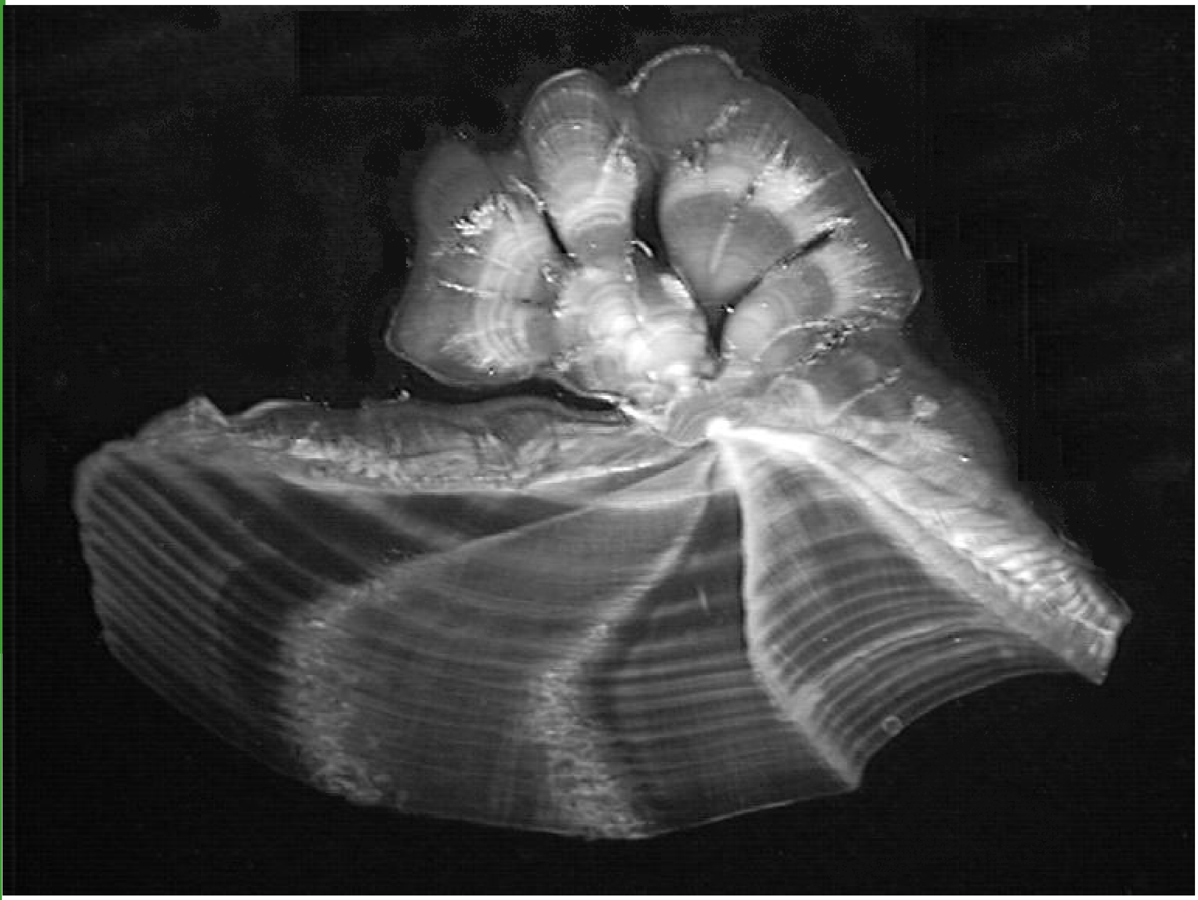
Fisheries Long Term Monitoring Program

Fish Age Estimation Review

April 2007



Queensland Government
Department of **Primary Industries and Fisheries**

Fisheries
Long Term
Monitoring Program

Fish Age Estimation Review

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Cover figure: Transverse section of a *Protonibea diacanthus* (black jew) otolith

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Acronyms

APE	average percent error
CV	coefficient of variation
DPI&F	Department of Primary Industries and Fisheries, Queensland
EFAN	European Fish Ageing Network
GSI	gonadosomatic index
IAPE	index of average percent error
LTMP	Long Term Monitoring Program, DPI&F
MIA	marginal increment analysis
MIR	marginal increment ratio
TACADAR	Towards Accreditation and Certification of Age Determination of Aquatic Resources
QC	quality control
QA	quality assurance

Introduction

Age data form the basis for calculations of growth rate, mortality rate and productivity; age is one of the most influential of all biological variables (Campana 2001). The quality of age data of fish populations plays a vital role in the sustainable management of fish populations as age data are the key component of most classic stock assessments (Appelberg *et al.* 2005). Errors in age estimation can be propagated through assessments and management decisions, possibly resulting in overfishing or overexploitation of stocks (Morison *et al.* 2005).

Fish age can be estimated by counting and interpreting structural patterns that occur on calcified structures (otoliths, scales, bones). Otoliths (which occur in the cranial cavity) grow throughout the lifetime of the fish and act as a permanent record of life history (Pontual *et al.* 2002).

In the past there has been a tendency to view fish age estimation as an art rather than a science. Nonetheless, fish age estimation is not a creative process but a diagnostic and analytical one; it is a transferable skill which increases and strengthens with practice, knowledge and experience (Morison *et al.* 2005).

One of the principal aims of the Department of Primary Industries and Fisheries (DPI&F) Long Term Monitoring Program (LTMP) is to collect and provide a reliable time series of length, sex and age structure data for quantitative stock assessments and management strategy evaluations. The LTMP routinely estimates age for a number of different species, including barramundi (*Lates calcarifer*), mullet (*Mugil cephalus*), spotted mackerel (*Scomberomorus munroi*), Spanish mackerel (*Scomberomorus commerson*), stout whiting (*Sillago robusta*), tailor (*Pomatomus saltatrix*), mangrove jack (*Lutjanus argentimaculatus*), saddletail snapper *(Lutjanus malabaricus*) and crimson snapper (*Lutjanus erythropterus*). Because the individual ageing components for each species have evolved at different times, at two regional centres and with different staff, variation has occurred within the ageing protocols, with little consistency in quality control.

Age estimation of fish for stock assessments is also conducted on behalf of DPI&F by external agencies. The collation of age data, from numerous sources for stock assessment, has emphasised the need for a standardised ‘best practice’ approach to documenting quality control of fish ageing data, both within the LTMP and for external agencies working on behalf of DPI&F.

In Europe, there has been a commitment to the standardisation of age estimation methodologies which has already been embraced through the establishment of the European Fish Ageing Network (EFAN). The main objective of EFAN was to develop, conduct and coordinate collaborative research and training and thereby ensure that age determinations become a reliable element of assessments. This opportunity enabled agencies to discuss age estimation problems on a common platform and set up guidelines comparing multiple readings between readers (Appelberg *et al*. 2005).

EFAN subsequently led to the development of a concerted action group called TACADAR (Towards Accreditation and Certification of Age Determination of Aquatic Resources). The main activities of TACADAR are to define a framework for the application of quality assurance (QA) and quality control (QC) mechanisms for fish ageing (Appelberg *et al.* 2005). A shift in otolith research towards acceptance of quality control procedures was recognised at the Third International Symposium on Fish Otolith Research and Application (Begg *et al.* 2005).

This literature review aims to give an overview of fish age estimation and the practice of the application of QA/QC to such estimations. This review does not consider otolith preparation or the development of species-specific age estimation methods. Rather, the focus is on the standardised data types required for age estimation and the current theory and practice of the application of QA/QC to each of these. Throughout this document, ‘increment’ refers to annual increment. All special terminology in this document is explained in the Glossary.

Not all literature reviewed is cited within the text. A complete list is available in the Bibliography.

Quality assurance and quality control issues in fish age estimation

There have been many instances where errors in age estimation have led to serious overexploitation of a population or species (Campana 2001). There are two main types of age estimation error that can occur:

1. Error inherent in the structure itself
2. Error in the interpretation of the structure.

The first type of error can be estimated but not controlled. The second type of error can be both estimated and controlled (Morison *et al*. 2005).

This review focuses on what QA and QC measures are needed to control and measure interpretation errors. QA is considered to cover areas that can have a standardised approach but are not species- or sample-specific (e.g. staff training, testing, and review of results). QA affects the way the work is performed. It can be described as a cyclical process including planning, execution, performance, control and revision (Appelberg *et al.* 2000). QC is considered to be the methods and procedures that are used in checking the results of individual samples (e.g. bias and precision tests) (Morison *et al.* 2005).

The European Fish Ageing Network (EFAN) has devised guidelines for providing quality assurance for age estimations (McCurdy *et al.* 2000):

1. Written procedures designed to implement best practice
2. Application of these procedures to the age reading process
3. Inspection of the process and its outputs to ensure that an acceptable level of quality is being achieved
4. Revision of the process to improve the procedures where the required quality standard is not attained.

From reviewing current QA/QC protocols it is evident that large differences exist between fish ageing programs. The same result was apparent in responses to a questionnaire that was sent to 53 laboratories in 23 countries (Morison *et al*. 2005). Morison *et al.* (2005) found that reference collections are mostly used for the initial training of an inexperienced reader and not as a routine part of the fish ageing programs.

Reference collections

Reference collections are an important quality assurance tool (Campana 2001; Morison *et al.* 2005). A reference collection can be defined as a collection of prepared otoliths, representative of all factors which might reasonably be expected to influence the appearance or relative size of increments (CRC In Prep.). These collections are often made up of otoliths and digitised annotated images of the otoliths. The annotations indicate exactly how the otoliths were read and remove any ambiguity as to how the otoliths were interpreted.

The primary role of the reference collection is to ensure that the age estimation method remains consistent over both the short and the long term. The way this is achieved is through (1) training of inexperienced readers, (2) ongoing familiarisation for experienced readers, and (3) quality control (Campana 2001).

A reference collection provides a stable reference point required for comparisons, even in instances where multiple readers gradually shift their ageing criteria in tandem. It is believed that the development and regular use of reference collections is probably the most important single measure that laboratories should implement (Campana *et al.* 1995; Gröger 1999; Morison *et al.* 2005).

A questionnaire completed by 53 laboratories in 23 countries revealed that ~ 48% used reference collections for training only and ~ 40% used reference collections for both quality control and training purposes. Of those that used reference collections for quality control, only a low proportion (~ 10%) used them as a standard procedure. The numbers of otoliths that are in such reference collections varied from < 100 (~ 55%) to > 1000 (~ 12%) (Morison *et al.* 2005).

EFAN has devised guidelines for the requirements of a reference collection. Some of these guidelines are that the reference collection should be from age 0 to the maximum age possible, that it should have at least 10 otoliths within each age group, and that those included should be representative of all edge classifications (Eltink *et al.* 2000). However, a review of the literature revealed a lack of information regarding the logistics of reference collections—with regard to how large the reference collection should be, and the frequency with which new material should be added to prevent readers from memorising the ages of individual fish (Campana 2001; Morison *et al.* 2005).

Protocols

Much variation appears to exist in the application of QA and QC measures to fish age estimation. In many age estimation programs, re-reads of a subsample of otoliths are carried out to provide QA (Wischniowski and Bobko 1998; Eltink *et al.* 2000; Campana 2001; DPI&F 2005; Duarte *et al.* 2005; Kimura and Anderl 2005; CRC In Prep.). This re-read approach has been designed for practicality and cost effectiveness. In some cases the subsample used is proportional to the original sample (25% of each age class), with samples being randomly selected from each age class.

Some laboratories have more rigorous QA/QC protocols in place if sample numbers are low. For example, in some protocols two independent readers estimate the age for all the otoliths, and if the increment counts from both readers are in agreement then the result is accepted. If there are differences between the first two readers, a third reader estimates the age of the otolith. If the increment count from the third reader is in agreement with either of the first two readers, then that result is accepted. If there is no consensus between any two of the three readers, the otolith is rejected (e.g. CRC In Prep.). Another variation on this approach is to accept a median or average age when there is an acceptable amount of variation in the three estimates. Another option employed involves one reader performing two complete reads of all otoliths and a second reader performing one complete read of all otoliths combined with a 25% re-read (e.g. DPI&F 2005). Many programs stipulate two independent readers (Wischniowski and Bobko 1998; CARE 2000; DPI&F 2005; Gaughan *et al.* 2006).

The results of the Morison *et al.* (2005) questionnaire showed that 74% of respondents carried out a subsample re-read, 30% carried out re-reads regularly and 25% of respondents did not re-read otoliths. In a critical review of such QA/QC protocols, Campana (2001) suggested that, if a QA/QC protocol is based around a reference collection, there is no need for a secondary reader.

Quality control measures

The quality control measures that are applied to increment counts test for bias and precision.

Bias is the systematic over- or underestimation of age. It can be measured in several ways. When assessing the reference collection reads, there are several statistical and graphical tests available for bias testing, including tests of symmetry (Hoenig *et al.* 1995) and a range of matched pair tests (Kimura and Lyons 1991; Campana *et al.* 1995). These tests are effective in detecting systematic bias, which occurs across all ages (e.g. when a reader constantly misses the first annual increment). However, when bias varies across ages the statistical tests become less sensitive.

Campana *et al.* (1995) recommended the age bias plot as the best graphical measure of bias. The age bias plot provides an age-by-age measure of deviation away from the accepted value or reference value. It clearly shows under-ageing or over-ageing, even if this error is restricted to the youngest or oldest fish.

Precision is defined as the reproducibility of repeated measurements on a given otolith, whether or not measurements are accurate (Chilton and Beamish 1982). Traditionally, percentage of agreement has been used to measure precision. This is the percentage that are aged the same on two different occasions by the same or different readers (Kimura and Anderl 2005). Many authors have pointed out the inadequacies of this method—that it is dependent on age, and that it varies widely between species (Beamish and Fournier 1981; Chang 1982; Kimura and Lyons 1991; Campana *et al.* 1995). Beamish and Fournier (1981) recommended the use of average percent error (APE):

$$APE_j = 100 \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - X_j\right|}{X_j}$$

where R is the number of times the fish are aged, X_{ij} is the i^{th} age determination of the j^{th} fish, and X_j is the mean age estimate for the j^{th} fish. When APE is averaged across many fish it becomes an index of average percent error (IAPE) (Campana *et al.* 1995).

Chang (1982) suggested the use of coefficient of variation (CV) as a standard deviation rather than the absolute deviation from the mean age:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^{R}\frac{\left(X_{ij} - X_j\right)^2}{R-1}}}{X_j}$$

where R is the number of times the fish are aged, X_{ij} is the i^{th} age determination of the j^{th} fish and X_j is the mean age estimate for the j^{th} fish. As with APE, the CV is averaged across the range of fish.

For both APE and CV, the lower the measures the more precise the set of age estimations (Beamish and Fournier 1981).

Despite the obvious deficiencies associated with percentage of agreement, Morison *et al.* (2005) found that percentage of agreement was still the most commonly used measure of precision (49%), followed by CV and APE.

Chang (1982) argued that CV is more statistically robust when compared with APE; however, the superiority of CV rests on the assumption of normally distributed error. He also derived an equation showing that CV will always exceed APE by an appreciable amount. Campana (2001) demonstrated that CV is readily estimated from APE, CV tends to be 40% higher than APE and there is a close relationship between the two measures. Campana also stated that it is not self-evident that one measure is to be preferred over the other.

The acceptable level of precision for each species varies—there is no set target level that can be applied (Campana 2001). The measure of precision varies with the complexity of the species involved. The Central Ageing Facility recommends that APE should be less than 5%, stating that higher levels may indicate that material is very difficult to interpret (Morison *et al.* 1998b). The regular undertaking of QC checks should enable the development of benchmark levels for each species (Morison *et al.* 2005).

An additional QC measure incorporated into fish ageing protocols is the use of a qualitative index of readability (CARE 2000; Lewis and Mackie 2002; Green and Krusic-Golub 2004). This index of readability is essentially a measure of confidence assigned to each otolith reading. In some protocols the ease of readability for an otolith reading is ranked as one of three levels (Good, Fair, Poor) (CARE 2000). In other protocols it is ranked in five levels: (1) Unreadable, (2) Interpretable not confident, (3) Multiple interpretations possible, (4) Readable not totally confident, and (5) Confident (GSMFC 2003; Green and Krusic-Golub 2004).

This literature review found little documentation available concerning the logistics of QC monitoring. The Morison *et al.* (2005) questionnaire found that many laboratories that have developed reference collections tended to use them more for training of inexperienced readers than as QA/QC.

Age estimation

The LTMP collects samples and data suitable for estimating the age of fish.

The two main steps are:

(a) Collecting enough fish to be representative of either the population or the harvested portion of the population (i.e. the catch)

(b) Collecting enough data from individual fish to estimate their age.

Historically the number of visible increments on otoliths was used as the estimate of fish age. However, this procedure has been refined over time to acknowledge variability in the timing of increment formation within a species, as well as when fish are caught, and also the species' birth date. For example, differences occur, between fish, in processes that affect increment formation (e.g. feeding and reproduction), meaning that the latest or newest opaque zone is not laid down (or visible) at the same time for all individuals in a population. Consequently, if age estimation is based only on the number of visible opaque zones, there is a risk that fish belonging to the same cohort could be separated (DPI&F 2005). This is particularly the case during the months when increments are forming for a species (i.e. become visible on the otolith edge), and it means that the number of increments may need to be adjusted to take into account 'late-forming' increments. As stock assessment uses cohort data, it is important to keep fish belonging to the same cohort together.

Adjustment of the number of increments to estimate age of individuals may also be necessary around the nominal birth date for that species. For example, a fish with two visible increments cannot be two years old earlier than its second birthday.

The data required to estimate age from interpreting the incremental macrostructure of otoliths are:

1. Increment count, i.e. number of visible increments
2. Edge classification
3. Periodicity and timing of opaque zone formation for the species
4. Date of capture
5. Nominated birth date for the species.

This review deals with each of the data types mentioned above, and quality assurance and quality control measures associated with each.

Increment counts

The age estimation of fish depends on visible changes in otolith growth. These visible changes occur at various levels of resolution: daily increments, seasonal increments, annual increments and growth checks (Wright *et al.* 2002).

The LTMP estimates the age of fish using annual increments combined with ancillary data. An annual increment comprises an opaque zone and a translucent zone, and represents one year of growth (Kalish *et al.* 1995; Panfili *et al*. 2002).

Under transmitted light, the opaque zone appears dark and the translucent zone appears light. Under reflected light, the opaque zone appears light and the translucent zone is dark (Wright *et al.* 2002).

The processes that control the development of increments are not well understood. However, it is believed that seasonal zones are related to seasonality in somatic growth and environmental factors. One suggestion is that seasonal variation in otolith formation is related to spawning (Fowler 1990). Another hypothesis is that increment formation is independent of any physiological processes that are occurring in the fish and is related to environmental variation (Fowler and Doherty 1992).

The season of formation of opaque and translucent zones can change during development of the fish and with geographical distribution (Vianet *et al.* 1989). The timing of translucent zone formation in rockfish, *Sebastes entomelas*, can vary with sex, geographical area and year (Pearson 1996). In this species a definite link was found to exist between temperature and zone formation.

The first essential step in age estimation is to count the number of increments. Increment counts can be used to identify cohorts (year classes) or to estimate age (in months or in whole years).

Edge classification

Edge classification refers to the classification of the relative stage of increment formation present on the edge of the otolith. Edge classification is an important and necessary data type which needs to be recorded to estimate age.

Because of differences in growth rates, the newest or most recently formed increment is not always laid down (or visible) at the same time for all individuals in a population. Consequently, if age estimation is only based on the counting of visible increments there is a risk that fish belonging to the same cohort would be put into different age classes (DPI&F 2005). Without interpretation of the edge of otoliths, fish that belong to the same age class could be assigned to different age classes based on the number of visible opaque zones.

Researchers have recommended that errors in assigning age classes due to edge classification can be avoided by not sampling fish during the months in which opaque material is deposited (Francis *et al.* 1992; Smith and Deguara 2003). LTMP age data are used as input for stock assessments; analysis must be taken from samples that are representative of the recreational and commercial catches. Consequently, sampling fish during the period of increment formation cannot always be avoided.

Edge can be described based on:

- appearance—as opaque or translucent (Francis *et al.* 1992; Fowler and Short 1998; Stewart *et al.* 1999; Smith and Deguara 2003), or
- the amount of translucent material present—narrow, intermediate or wide (Morison *et al.* 1998a; Ewing *et al.* 2003; Green and Krusic-Golub 2004), or
- a combination of both—opaque zone on margin; translucent zone forming to 1/3 complete on edge; translucent zone 1/3 to 2/3 complete on edge; translucent zone 2/3 to fully complete on edge (Vanderkooy and Guindon-Tisdel 2003).

Translucent and opaque zones become progressively narrower further away from the nucleus (Vanderkooy and Guindon-Tisdel 2003). This information can be used to help with edge classification—for example, (1) opaque zone at margin, (2) marginal translucent zone is 1–50% of the previous translucent zone, (3) marginal translucent zone is 51–100% of the previous translucent zone (Lewis and Mackie 2002).

Because of the decrease in distance between zones, it can be difficult to classify edge for older fishes (Kimura and Anderl 2005). When classifying edge, a reader needs to make a subjective decision, yet an informed judgment, with regard to the expected changes in otolith growth with age. A reader needs to have a basic understanding of otolith growth and needs to understand that accretion rates in otoliths decrease with increasing age (Vanderkooy and Guindon-Tisdel 2003).

Periodicity and timing of opaque zone formation

In general, there are stock-wide patterns in the formation of opaque zones; this is because the factors that affect one fish usually affect the majority of the ‘local’ population. The timing and the periodicity of increment (or opaque zone) formation refer to the time of year (or season) and the frequency with which it occurs, and can vary with each species.

When estimating age from otoliths we assume that (1) the increments are laid down with periodicity that is relatable to a regular time scale, (2) increments display a consistent interpretable pattern, and (3) otoliths continue to grow at a measurable rate throughout the lifetime of the fish (Beamish and McFarlane 1983).

In assessing the timing and periodicity of opaque zone formation, the age estimation method is validated. Validation of an age estimation procedure indicates that the method is sound and based on fact (Kalish *et al.* 1995). This is a fundamental factor in age estimation. Between-fish variability (e.g. in physiology or behaviour) means that an opaque zone is not always visible on the edge of all otoliths from a single cohort at the same time.

Validation methods available can be divided into two main categories: (1) direct validation and (2) indirect validation. The lapse of time between two determined events is known as direct validation, allowing accurate estimates of absolute age. There are various methods for directly validating the age of fish: captive rearing (Beckman and Wilson 1995), chemically tagged fish (McFarlane and Beamish 1995; Fowler 1990), bomb radiocarbon (Andrews *et al.* 2005; Piner *et al.* 2006), and radiochemical dating (Bennett *et al.* 1982; Campana *et al.* 1990).

Indirect validation can qualitatively and/or quantitatively assess the evolution of the otolith over time. Marginal increment analysis (MIA) provides a quantitative description of opaque zone formation. This is the most commonly used validation method. It is popular because of its modest sampling requirements and low cost. It is also the most likely to be abused (Campana 2001). MIA is calculated as a proportional state of completion. The marginal increment (defined as the distance between the margin of the otolith and the distal edge of the last opaque zone) is expressed as a proportion of the previous increment (the distance between the distal edges of the two outermost opaque zones) (Vilizzi and Walker 1999; Williams *et al.* 2005). When plotted as a function per month or season, the mean marginal increment should describe a sinusoidal cycle with a frequency of one year if increments are truly formed annually (Campana 2001). The marginal increment ratio reaches a maximum before it is formed and a minimum shortly after it has been completed (Williams *et al.* 2005).

In the validation of the formation of opaque zones in the sagittal otoliths of mullet (*Mugil cephalus*), marginal increment analysis was carried out. This analysis involved expressing the mean marginal width for each increment number as a proportion of the mean width of the previous increment (Smith and Deguara 2003). For red throat emperor (*Lethrinus miniatus*), marginal increment analysis was carried out for age classes 2–17 years (Williams *et al.* 2005). However, such analysis for fish over 10 years of age should be treated with caution, as in these cases the opaque zones form closer together. At this age, growth rate (body length and depth) in many species decreases dramatically and there is a corresponding reduction in the rate of otolith growth, as in the case of red snapper (GSMFC 2003). The accuracy of the estimated age, and thus the strength of validation, declines with increasing age (Francis *et al.* 1992).

If samples are collected throughout the year, analysis of the edge classifications can confirm the frequency of opaque zone formation. To perform edge type analysis, edge classifications recorded from each otolith are pooled and the frequency is plotted as a function of month or season (Haas and Reckshiek 1995; Labropoulou and Papaconstantinou 2000). Edge type analysis does not assign a state of completion to the edge (e.g. narrow or wide), but records its presence as either translucent or opaque (Campana 2001). Despite the problems inherent in its use, edge type analysis is the easiest and least time-consuming method of determining the month or season of opaque zone formation.

Other work has involved a two-step procedure consisting of marginal increment ratio analysis and edge analysis (Vilizzi and Walker 1999). In this method the marginal increment ratio (MIR) value was used to classify edge into one of three categories—if the MIR was less than or equal to 25%, C (close); between 25 and 75%, Z (other); or greater than or equal to 75%, N (now due).

It is important to remember that, if a method is not validated for all age classes, then some age classes could have some error. Several studies have demonstrated significant variation in the timing of opaque zone formation between ages (Hyndes *et al.* 1992; Fowler and Short 1998; Stewart *et al.* 1999; Vilizzi and Walker 1999; Cappo *et al.* 2000) and between locations (Fowler 1995; Pearson 1996; Smith and Deguara 2003; Williams *et al.* 2005).

It is important to note that determining the timing and periodicity of increment formation (validation) defines accuracy of the method and not the individual ages (Beamish and McFarlane 1983).

Determining the timing and periodicity of opaque zone formation for a population usually indicates a period of several months when this can occur. During these months, we consider that all fish in the population should have a visible opaque zone on the otolith edge. When fish sampled during this period do not exhibit a newly formed but wide translucent zone on the edge, it is assumed that the opaque zone is present and not yet visible, or that it has not yet been formed. It is assumed that the fish is ‘late’ in forming an opaque zone, so it is necessary to add one to the number of visible increments. This adjusting of increments helps to keep fish from the same cohort together. When an otolith displays a narrow edge (new opaque zone) during the period of opaque zone formation, then there is no adjustment of the increment count. Essentially, it is necessary to determine the periodicity of increment formation to enable cohorts to be kept together.

Capture date

The capture date of a fish is required for age estimation.

As explained previously, it is necessary to keep fish from the same cohort together. Therefore the capture date for each fish allows us to assess whether the individual fish is predicted to have an opaque zone visible on the edge or not. Essentially, age cannot be estimated properly without knowing when the fish was captured.

The date of capture is recorded either in the field or in the laboratory immediately after collection.

Birth date

The true birth date of an individual fish is rarely known. For many fish species, standard birth dates are assigned to a population based on a period of peak spawning. As the spawning season for a population can extend over several months, the time of peak spawning can be inferred as the annual peak in gonadosomatic index (GSI) values (Ewing *et al.* 2003). If the spawning season of a species is unknown, the standard birth date in the northern hemisphere is 1 January and in the southern hemisphere it is 1 July (Morales-Nin 1992).

The relationships between increment formation, edge classification, date of capture and birth date (in relation to timing of opaque zone formation) are needed for age estimation. On the birth date each year, the fish in each cohort become one age group and one age class older (Morales-Nin and Panfili 2002).

Increment counts may need to be adjusted before and after the designated birth date to keep fish from the same cohort together. For example, a fish with two visible increments cannot be two years old before its second birthday.

Outcomes

This fish age estimation review was necessary in the development of the LTMP Sampling Protocol—Fish Ageing (DPI&F 2007).

The fish ageing protocol documents a standardised approach for:

- routine fish age estimation
- quality control for age estimates (Figure 1).

The LTMP sampling protocol sets the standards for all fish ageing by the Queensland DPI&F LTMP and any external agencies contracted to work on behalf of the LTMP.

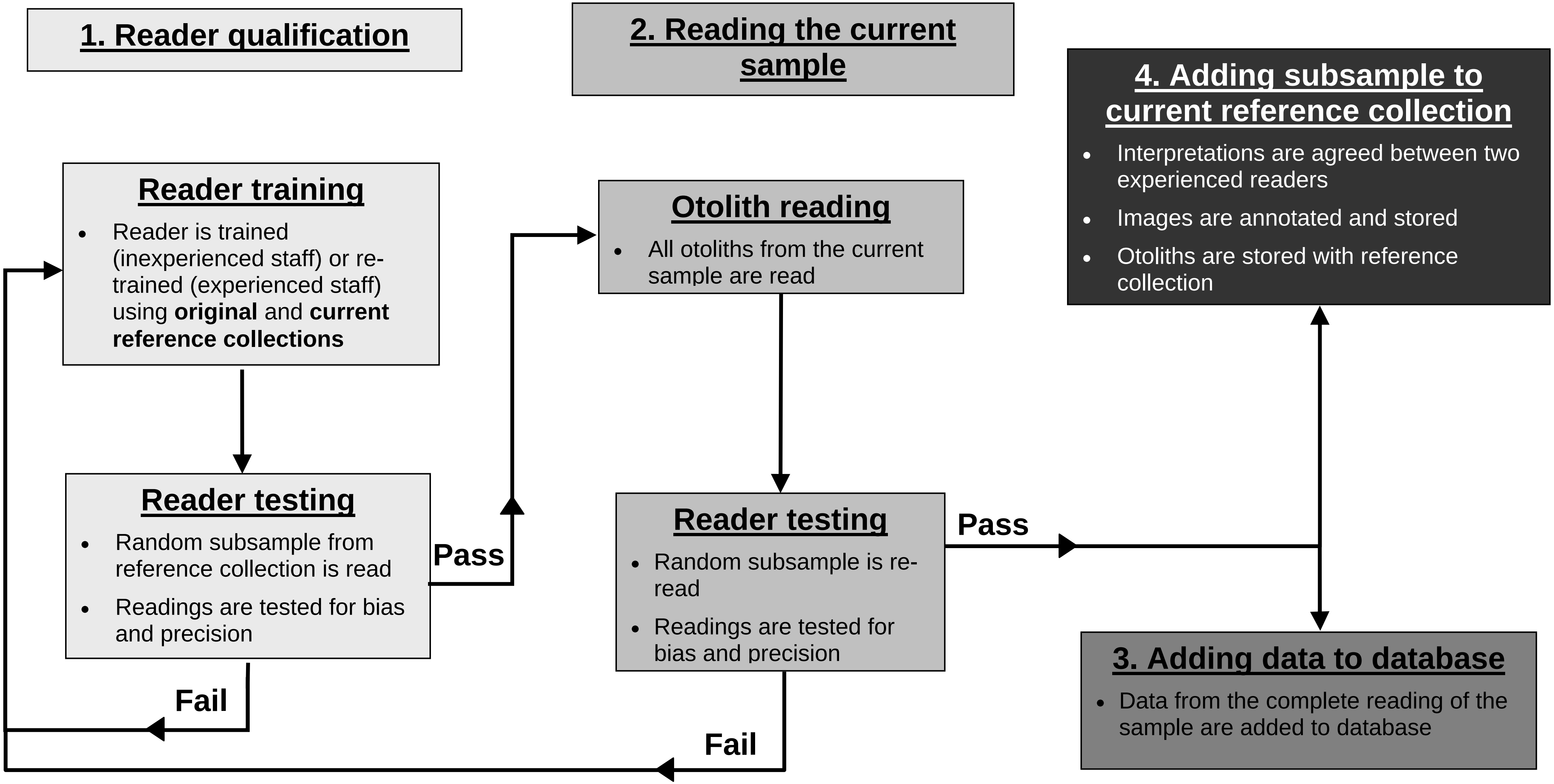

Figure 1. Quality assurance and quality control measures in the LTMP fish ageing program

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Glossary

Age class: a cohort of fish that has a given age (e.g. the 5-year-old age class).

Bias: the systematic over- or underestimation of age. Bias is regarded as a serious error because fish are allocated to the incorrect cohort.

Cohort: group of fish of a similar age that were spawned during the time period.

Distal edge: the external edge or external margin of the otolith.

Drift: consistent change over time in the interpretation of incremental macrostructure. Long-term drift refers to change in interpretations in a time period of greater than a year.

Edge: refers to the relative stage of increment formation present on the edge of the otolith.

Increment: refers to a region in the otolith. An annual increment comprises an opaque zone and a translucent zone.

Opaque zone: an area or band on the otolith that restricts the passage of light. Under transmitted light, the opaque zone appears dark. Under reflected light, the opaque zone appears bright.

Otoliths: paired calcareous structures found in the inner ear. In this document, ‘otolith’ refers to the sagittae, the largest of the otolith pairs.

Precision: the reproducibility of repeated measurements on a given structure.

Quality assurance: is considered to cover areas that can have a standardised approach (staff training, testing, and review of results). These procedural matters are not species- or sample-specific.

Quality control: is considered to be the methods and procedures that are used in checking the results of individual samples.

Read: to estimate the age from the calcified structure; this involves observation and interpretation.

Reader: the person who interprets the incremental macrostructure of the otolith.

Re-read: the second reading or second interpretation of the otolith.

Translucent zone: an area or band on the otolith that allows the passage of light. Under transmitted light, the translucent zone appears bright. Under reflected light, the translucent zone appears dark.

Validation: the process of estimating the accuracy of an age estimation method. Validation of an age estimation procedure indicates that the method is sound and based on fact.