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An Integrated System for Production Fish Aging: Image Analysis and Quality Assurance

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An Integrated System for Production Fish Aging: Image Analysis and Quality Assurance

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Abstract.—There is an increasing demand for aging data to provide inputs to stock assessment models for management of exploited fish populations. Image analysis software and computer hardware allow more rapid processing of samples and data. This paper describes a fully integrated system that has been in operation for 5 years and has been used to provide age estimates for more than 150 species. The system combines the requirements of high-quality ''production'' aging with the benefits of a customized image analysis system. The system improves the work environment, increases efficiency, aids data collection, and improves quality control. All aging studies require unbiased and precise age estimates; however, the ongoing process of production aging has particular requirements for quality assurance. A classification of aging studies is proposed based on objectives of the study, and the features and key procedural requirements of each study type are described.

Age-structured stock assessment methods are ideally suited to the rational management of fisheries resources and are the primary basis for providing management advice in many world fisheries (Megrey 1989). Age composition data provide fundamental insights into fish biology and stock productivity and allow the estimation of the basic parameters for describing growth, mortality rates, and recruitment (Smith 1992). Advances in computers have facilitated the wide use of sophisticated fishery models, particularly over the past 15 years (see Walters 1989; Allen 1994). This has in turn increased the demand for data on exploited fish populations, particularly aging data, as inputs to the models. This ongoing process of assessing age structures is ''production'' aging in the sense of Kimura and Lyons (1990).

A specialist unit, the Central Ageing Facility (CAF), was established at the Marine and Freshwater Resources Institute (then called the Victorian Fisheries Research Institute) in 1991. The main aim of this unit is to provide data on the age composition of the catch for key Australian fisheries. Such data are used in the annual assessments of the status of the main species in the Australian South East Fishery and as a performance measure of the effectiveness of programs contributing to the objectives set for each species (Chesson 1996).

Modern technology, as well as stimulating the demand for data, has provided new equipment to assist in the production aging process. This paper describes an integrated system that was developed by the CAF that and has been in operation for 5 years. The system is based on image analysis software for on-screen digitizing, accurate data capture, and automated file handling.

When the CAF was being established, it became apparent that there was a lack of published information about appropriate operating procedures for such a laboratory. This applied in particular to the issue of quality assurance as it applies to production aging. All age determination studies aim to produce precise and accurate age estimates. However, studies vary in their purposes and, therefore, also require different approaches both to the collection of samples and to the process of age estimation. The process of production aging has requirements that are additional to those of other studies that either validate an aging method for a particular species, describe the growth of a species, or apply an aging method in a short-term study. For example, providing estimates of the age composition of the catch from commercial fisheries over many years requires special attention to maintaining accuracy across years and often across readers, which a validation study may never have to address. By what procedures can such consistency be guaranteed, and what are the appropriate procedures for each type of study? What ancillary information, such as fish size or date of capture, will help readers produce more accurate ages, and what information has the potential to bias the results?

The procedural standards that are applied to the process of age determination affect the consistency and reliability of the data produced but have received little attention. There has been much written on the selection of appropriate aging methods

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(e.g., Bagenal and Tesch 1978; Jearld 1983; Brothers 1987; Smith 1992) and techniques for preparing and interpreting structures (Penttila and Dery 1988; Secor et al. 1991). More recently, the use of image analysis software to assist the age estimation process has received attention (Estep et al. 1995; Macy 1995; Welleman and Storbeck 1995). Others (Beamish and McFarlane 1983) have highlighted the need for greater rigor in the validation of aging methods. Increasingly, there has also been attention given to the analysis of the statistical properties of aging data, recognizing the potential errors (e.g., Kimura and Lyons 1991; Beamish and McFarlane 1995; Campana et al. 1995) and the need to account for these in subsequent analyses (e.g., Richards et al. 1992).

This paper classifies aging studies according to their objectives and describes the requirements of each type to highlight the issues of quality assurance that are unique to the process of production age estimation. The customized image analysis system developed by the CAF, and described in this paper, is an integral part of the CAF operations. We also describe the benefits and limitations of this image analysis system as it is applied to the process of production age estimation.

The CAF Image Analysis System

The CAF has used its system for providing age estimates for regular stock assessments on 11 species in Australia's South East Fishery (Chesson 1995; Morison 1996) and on 3 species in Victoria's Bay and Inlet Fisheries. Since the system was established, the CAF has used it to provide age estimates on over 150 species of fish.

The CAF aging protocols and system were designed to efficiently handle the large sample sizes of otoliths characteristic of production age determination. Typically, in a 12-month period, 20,000 otoliths are received, from which three readers make up to 25,000 individual age estimates, including data analysis. For the high numbers processed, it was necessary to design a system that reduced user strain and minimized possible sources of error. Modern image analysis software provided the tool to build an integrated package that is simple to operate, efficient, and very flexible and that operates with common graphic user interface software.

Hardware and Software

Details of the equipment used are provided in the Appendix. The CAF has four workstations comprising a stereomicroscope, a video camera, and a computer. A customized image analysis system based on Bioscan Optimas image analysis software (version 5.2) is installed on each computer. Programs written in the Optimas language run a selected subset of functions and purpose-built features on each computer using Optimate. These workstations are connected through a local area network to a larger central workstation for data storage and processing (Figure 1).

Frame-grabber boards installed in the computers allow the video signal to be displayed on the computer screen. The video image, controlling menus, screen objects, and other applications are all displayed on the monitor. This arrangement was selected as the most efficient method in terms of cost, space, and system operation. The video signal has dimensions of 640 $\times$ 484 pixels and is acquired at a rate of 30 frames/s. This rate allows for real-time acquisition and focusing of images. The image size is 640 $\times$ 480 pixels at 8 bits/pixel (255 levels of grey). The black and white video cameras (surveillance cameras fitted with a C-mount) capture images on a charged couple device (CCD) square sensor (12.7 mm $\times$ 12.7 mm). One color system is also currently in operation, but the black and white system is suitable for most applications. The color system is used for on-screen digitizing of shark vertebrae (used for aging) that are stained with a calcium stain (alizarin red S) and shows calcium-rich zones in shades of red.

A series of programs provide all the necessary image analysis and data capture facilities in a customized application. Customized features include pull-down menus for selection of species and readers' names, magnifications, batch numbers, and type of samples being aged. Programs for performing different functions, such as image capture, recalibration, age estimation, measurement of otolith dimensions, or simple line measurement, are activated from these pull-down menus (Figure 2).

After testing that all the relevant header information has been supplied, one program invokes the spreadsheet program and establishes a dynamic data exchange (DDE) link for data export. Automatic file building and handling procedures use the DDE link to automatically transfer species, batch, readers' names, specimen details, counts, and measurements to a spreadsheet. File names are automatically constructed to reflect details of species, batch, and reader information previously selected. The destination subdirectory of the file is also controlled by the species code. Files are saved automatically by the spreadsheet application after each specimen is aged.

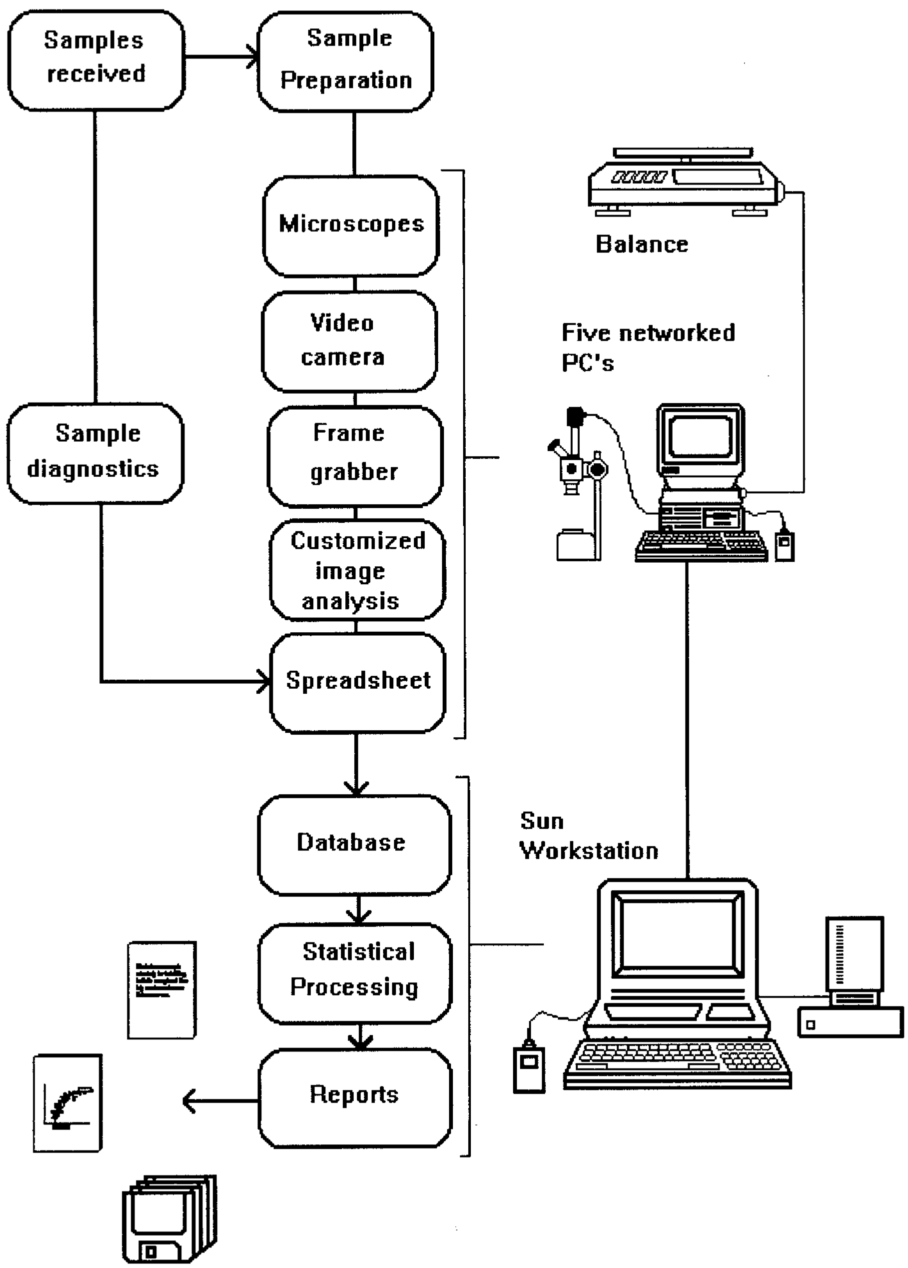

FIGURE 1.—Schematic diagram of the equipment used at the Central Ageing Facility.

The software initialization file also adjusts for the distortion of the image caused by the projection of an image captured by a square CCD chip onto an oblong video display. Without adjustment for these different aspect ratios, correct calibration and measurements are only possible in one direction at a time (either horizontally or vertically).

The stereomicroscopes have fixed magnifications with a range of 6.4–40×. This range can be increased or decreased by changing the primary objective (the lens immediately before the video camera) or the secondary objective (the lens immediately above the specimen), giving a range of magnifications of 2.5–80×. Fixed magnification steps are an important feature because different configuration files to recalibrate the system are au-

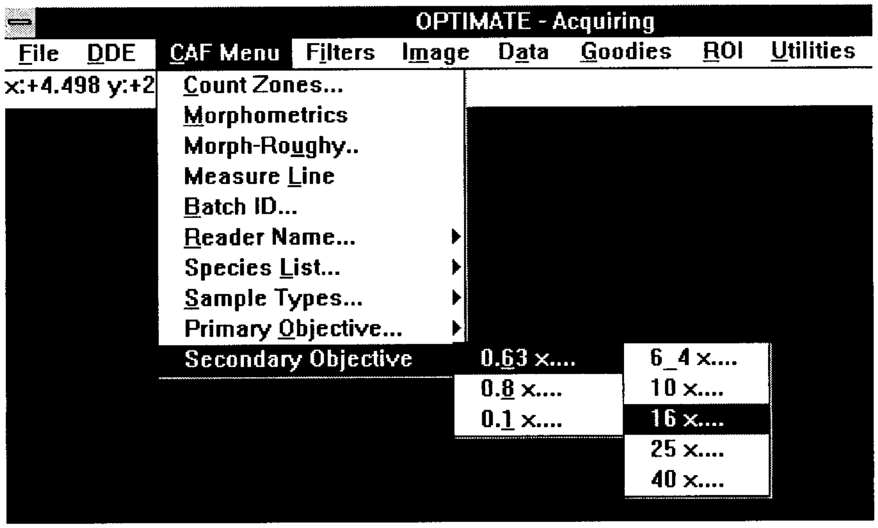

FIGURE 2.—Example of customized pull-down menus for correct file handling and system configuration.

tomatically loaded when the desired magnifications are selected. The compound microscope has a fixed magnification range of 25–1,000×. All microscopes have photo tubes or, in the case of the compound microscope, a trinocular head for attachment of the video camera. Stereomicroscopes are also fitted with a sliding mount to allow positioning of the objective serving the video camera vertically above the stage to remove any parallax error during measurements. One workstation also has a compound microscope with epifluorescence capabilities for examination of samples marked with fluorochrome dyes, such as oxytetracycline.

Otolith Preparation and Age Estimation

Samples are generally received in batches of up to 500 pairs of otoliths with associated biological data. The samples are referenced by a species code number and a sequential batch number in order of receipt. Individual fish are given arbitrary but sequential numbers. Batches that have been submitted already sorted (e.g., by length) are mixed randomly before numbering as knowledge of even relative fish size may potentially bias the subsequent age estimates.

Each batch is registered in a database that includes information about the batch and progress in sample processing and age estimation. After they are dried, one undamaged otolith from each pair is weighed to an accuracy of 0.001 g on a digital balance linked to a computer for automatic recording of weights. These data, along with biological information, are transferred to spreadsheets for compilation after age estimation is completed.

The CAF works mostly with otoliths, and for the majority of the samples, thin sections (0.3–0.5 mm) are cut and mounted on slides. Scales are known to often lead to an underestimate of age of many species (e.g., Beamish and Chilton 1982). Otoliths have a number for advantages for aging including a greater reliability in aging older fish (Jearld, 1983). Also, Beamish (1979) and Bedford (1983) suggest that otoliths be sectioned, particularly for fish species that have thick otoliths, to reveal increments that have been deposited only on the proximal surface. Break and burn techniques and thin sectioning both reveal such increments. Our system is applicable to a variety of structures commonly used for age estimation and has been used for broken and burnt otoliths, shark vertebrae (Walker et al., in press), whole otoliths (Morison 1992), and scales.

Otoliths are embedded in blocks of clear polyester casting resin in custom-made Teflon or latex moulds, sectioned, and mounted as described in Anderson et al. (1992). Slides are placed on the stage of the stereomicroscope, and an image is acquired. The aging program is then selected. The reader draws a transect line from the primordia to the edge of the section using a mouse. This line is drawn in the same relative position whenever possible to give measurements that are comparable among specimens. The position of each increment is marked manually by the reader along the transect, together with false or supernumary zones

TABLE 1.—Examples of values of the average percent error (APE) obtained with repeated age determinations by staff at the Central Ageing Facility (CAF). Preparation method is by thin sections of sagittal otoliths, except whole otoliths for *Rexea solandri* and *Zeus faber* and vertebrae for *Mutelus antarctus.*

Species	Family	Habitat[a]	Maximum age in sample (years)	APE (%)	Source[b]
Macquaria australasica	Percichthyidae	Freshwater	16	2.2	CAF
Acanthopagrus butcheri	Sparidae	Estuarine—M	29	0.39	CAF
Pagrus auratus	Sparidae	M: temperate	26	0.34	CAF
Rexea solandri	Gempylidae	M: temperate	17	4.4	CAF
Hoplostethus atlanticus	Trachichthyidae	M: deepwater	125	3.5	Smith et al. (1995)
Pseudocyttus maculatus	Oreosomatidae	M: deepwater	78	4.2	Smith and Stewart (1994)
Allocyttus verrucosus	Oreosomatidae	M: deepwater	130	3.3	Stewart et al. (1995)
Zeus faber	Zeidae	M: temperate	12	5.7	Smith and Stewart (1994)
Cyttus traversi	Zeidae	M: temperate	38	3.7	Smith and Stewart (1994)
Macruronus sp.	Merluciidae	M: temperate	25	4.6	CAF
Sillaginodes punctata	Sillaginidae	M: temperate	7	0.5	CAF
Hyperoglyphe antarctica	Centrolophidae	M: temperate	42	5.1	Morison and Robertson (1995)
Centroberyx affinis	Berycidae	M: temperate	44	3.8	CAF
Centroberyx gerardi	Berycidae	M: temperate	64	4.3	CAF
Neoplatycephalus	Platycephalidae	M: temperate	33	3.3	CAF
Mustelus antarcticus	Triakidae	M: temperate	16	7.8	CAF
Plectropomus leopardus	Serranidae	M; tropical	<1[c]	1.05	CAF

[a] M = marine.
[b] CAF is unpublished data from the Central Ageing Facility.
[c] 310 days.

(stress marks, reproductive marks or other discontinuities) and the edge of the otolith. An additional separate marker type is used to distinguish annual increments whose position cannot be precisely identified on the reading transect or those marked on a nonstandard transect. This allows these measurements to be excluded from later analyses. Data collected with the aging program include the number of annual increments marked, the distances from the primordia to the first 10 annual increments, the distance to the edge, and the number of supernumerary zones. Once all required features are marked along the reading transect, a dialogue box is displayed that contains the header information and DDE options. These data can then be exported to the spreadsheet, or the sample can be reprocessed. Once the aging work has been completed, the data are merged with data on fish length, sex, and otolith weights for inspection and analysis.

Laboratory Protocols and Quality Assurance

Maintaining accuracy and precision in production aging is not a simple matter, and the quality assurance procedures and protocols needed to establish and verify this consistency are not obvious or straightforward. A variety of measures are employed at the CAF to detect any loss of accuracy or precision.

One of the main sources of error is the potential for a change in the way that the otolith structure is interpreted by the reader. This may be either systematic, such as a shift to increasing or decreasing counts (drift) leading to increasing bias, or it may be random, leading to a loss of precision. While differences between successive batches in the resulting outputs (such as mean lengths at age or growth curves) may be easily detected, it is not easy to discover whether such shifts are real or the result of reading errors. At the CAF, when a new reader is employed or there has been a substantial time interval between aging of batches, rereadings of previously aged material are used to check for consistency.

Repeated readings are made on a subsample of 25% of all batches, and the index of average percent error (APE; Beamish and Fournier 1981) is calculated as a standard measure of the precision of age estimates. There have been no critical levels determined for what constitutes an acceptable APE. However, from repeated application to a range of species (Table 1) and other published values, we expect the APE to be generally less than 5%. Higher values may indicate material that is very difficult to interpret, but more commonly they indicate insufficient training of readers. To allow comparisons of APE values (for example to compare within and between reader values), the CAF employs bootstrap techniques (Efron and Tibshirani 1993) to calculate bias-corrected means and confidence intervals of APEs.

Scatter plots of first versus second readings are also used to give an initial indication of biases between readings that are not revealed by a single APE value or by other single statistics (e.g., Chang 1982; Sharp and Bernard 1988; Baker and Timmons 1991; Kimura and Lyons 1991). If there is no bias, the slope and intercept of a regression line fitted to such plots will not differ significantly from unity and zero, respectively. However, as both variables have measurement error, the estimated slope of the line will be biased if a normal regression equation is used, and a functional relationship regression line is a more appropriate option (Ricker 1975). Campana et al. (1995) have proposed an age bias plot as a refinement of this use of scatter plots. Inspection of cross tabulations of the frequency of differences between readings by age are used to highlight any biases and also show the density of points in each cell, which scatter plots do not. When data are presented in this form the chi-squared test of symmetry (Hoenig et al. 1995) is used to test for differences between two sets of age estimates for the one sample.

Ages are estimated without the use of auxiliary information, such as fish size or sex, that may bias the reader's interpretation. There is an important use for such information in the error-checking process after reading, and it is important that it is used at a stage that will help identify and reduce errors rather than potentially increase them. This point is addressed further in the discussion.

Once data on sex, length, and otolith weights have been combined with the age estimates, scatter plots of age versus length and age–length keys are produced and inspected for outliers (suggested aging errors), sudden shifts, or unusual trends. Changes will be expected over time, particularly when recruitment is variable or fishing pressure is increasing, but large or sudden changes are indicators of possible errors.

In addition, we use relationship between age and otolith weight to help identify possible errors. This relationship is usually linear and frequently quite tight (Figure 3). However, there may be an inflection point about the age of maturity, as can be seen clearly for *Centroberyx affinis* (Figure 3) and which has also been reported for *Hoplostethus atlanticus* (Smith et al. 1995). Outliers on scatter plots of pairs of the variables age, otolith weight, and fish length indicate possible errors, allowing for rechecking of age estimates or measurements.

The distributions of the radii measurements of the first two or three increments is used as an indicator of consistency in identification of the early increments. However, after the first three or four increments, such distributions usually overlap to such a degree that errors would not be readily detectable. The distribution of otolith weights is also used in a similar way. For fish length, otolith weight, and increment radii, distributions within age-classes are usually unimodal and show mean values that increase with age.

Postreading procedures employed include not only the incorporation of auxiliary sample information, such as fish lengths and otolith weights, but information gained solely from the age estimation process, such as age distributions and radii of measured increments. They also include both the examination of data from a single sample and the comparison with previously aged material. In undertaking such exercises, it must be recognized that fish growth is highly variable and outliers do not necessarily indicate errors. Both fast- and slow-growing fish are relatively common in many fish populations.

Other Uses of the System

Apart from production aging, the integrated system has been adapted to perform a range of other functions. Programs have been developed to allow a similarly rapid collection of data on otolith dimensions, for otolith shape analysis by Fourier analysis, and measurement of shark vertebrae. Measurements of increment widths for analyzing growth history and marginal increments for identifying periodicity of increment formation have been obtained from the image analysis system. In addition, automatic identification and counting of increments is possible on very clear specimens, and work is continuing on refining this system. For example, procedures for scale pattern recognition that have been used for stock identification (e.g., Cook and Lord 1978; Ross and Pickard 1990) would be straightforward to implement.

Discussion

The integrated system in use at the Central Ageing Facility uses modern technological advances to provide an efficient system for the production aging of fish from otoliths. The system is flexible and not only reduces the likelihood of errors or biases but also provides simple outputs as means to check for a bias or other errors.

Major benefits of this integrated system of onscreen viewing and digitizing of samples are that

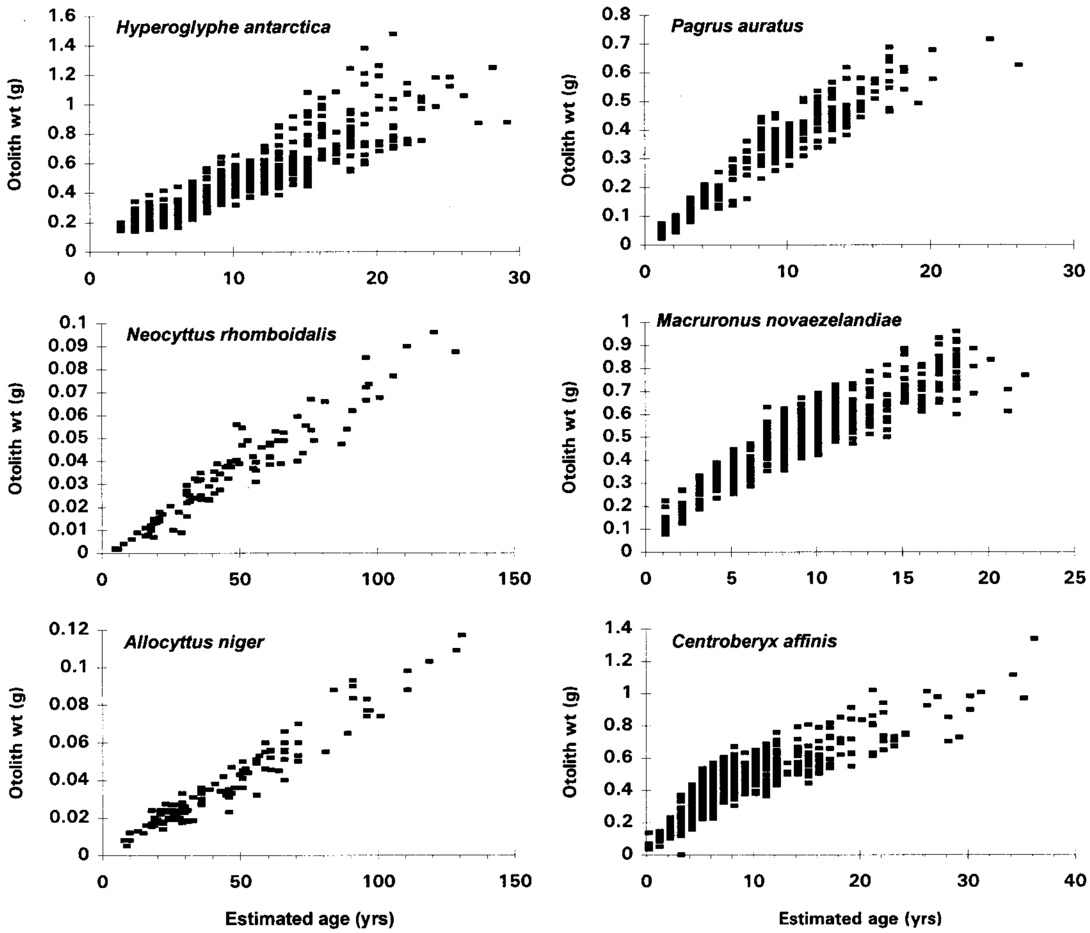

FIGURE 3.—Examples of relationships between otolith weight and fish age (years). The relationships are usually linear, as shown by the three species on the left, but there may be an inflection point and a decreased slope after the age at maturity, as shown clearly by *C. affinis.*

the user can assume a more comfortable posture, the data can be rapidly and accurately acquired, data collected automatically are not subject to any transcription errors, and data are immediately available in a standard format for analysis and reporting. The automatic building of files and selecting of file destinations by the programs reduce the possibility of users overwriting or misplacing data. The automatic calibration of the system reduces the potential for user calibration error. These factors all contribute to a very efficient system, as evidenced by the volume of samples processed in a 12-month period.

The use of on-screen viewing of specimens facilitates group discussion of interpretation for comparisons between readers and for training. In addition, images showing which increments have been counted are saved as digital files or printed as records of interpretations. This assists in training or retraining readers to reduce drift in interpretation over time. Images are also enhanced, if necessary, by using filtering algorithms to increase definition or improve the contrast between zones.

A disadvantage of the system is a slightly reduced quality in the viewed image. This is partly because the level of resolution on the screen is lower than that obtained directly from the microscope. However, it is also a result of working with a ''frozen'' image (i.e., captured video image). Active manipulation of lighting direction, focal plane, or specimen orientation can increase visibility of increments, but these options are not available with a frozen image. Francis et al. (1992) reported similar problems in using photographs of otolith sections. As a result, direct viewing of samples with the microscope is necessary for speci-

TABLE 2.—A classification of aging studies showing how requirements and features vary depending on the objective

Attribute	Validation study	Population study	Production aging
Objective	To validate a method	To describe the age composition, recruitment events, and life history parameters	Estimate catch at age to model a population and make predictions (e.g., about recruitment or effects of fishing)
Requirements	Structures from marked or known age fish, or other means to validate a method	A validated aging method	A validated aging method consistently applied (i.e., quality assurance needed)
	A sample of all age-classes (need not be representative of the population)	A representative sample of the population	Representative samples of the catch
Features	Samples usually collected over several years	A cross-section (snapshot view) of a population	A longitudinal (time series) view of recruited cohorts Large sample sizes

mens or preparations that are not clear on the monitor.

The system requires improvement for dealing with specimens that require a high magnification or high resolution over a large field of view, such as when counting daily increments or determining age of long-lived species such as *H. atlanticus* from otolith sections (Smith et al. 1995). In such cases it is not possible with the existing system to view the entire area of interest on the monitor at the necessary magnification. Successive images can be acquired to cover the area of interest, but the system presently requires the operator to mentally note reference points at which counting ceases to enable the images and the counts from the sequence of images to be accurately linked together. Newer versions of the image analysis software provide functions to overcome this problem.

A Classification of Aging Studies

Estimates of age are used in many fisheries research and monitoring studies. These studies vary in their requirements and features and can be broadly grouped according to their objectives: (1) validation studies, (2) population studies, and (3) production aging (Table 2).

For validation studies, the purpose is self-evident; the requirements have been clearly described by Beamish and MacFarlane (1983). An attribute of such studies is that the samples are usually collected over several years (e.g., when tagged or captive fish are used or when marginal increment analysis is undertaken). However, it is worth restating the point made by Francis et al. (1992) that aging procedures cannot simply be categorized as validated or unvalidated and that any claim that a procedure is validated should be qualified by some measure of accuracy.

Population studies aim to describe population characteristics, such as age composition, growth, or mortality, and often make comparisons between stocks or habitats (e.g., Barbour and Einarsson 1987; Chisnall and Hicks 1993). These studies require that a validated aging method has been developed, and that representative samples of populations are obtained. An attribute of such studies is that they are typically a cross-section (snapshot view) of a population at one point in time. Thus, all individuals of the population sample can be aged as a single batch.

For production aging, the aim is to estimate the catch at age for a fishery. Again a validated aging method is needed, and a representative sample of the catch is also required. An attribute of such studies is that samples are collected over a sequence of years (i.e., they are a longitudinal study, and thus the age estimation may also take place over several years). The consistent application of the aging method over years, among readers, and even among laboratories, is therefore a particular requirement of such studies. Quality assurance in the aging process to guarantee this consistency is uniquely important to this type of aging work. However, the results of such routine monitoring work are seldom published in scientific journals, and the methods and procedures are therefore not well documented.

Production aging, as practiced by the CAF, has three distinct stages, each with different protocols. First, when readers are training or are ''calibrating'' themselves, they are given information on fish size and date of capture. Second, when reading new material or testing for consistency between readings, information on fish size is not available to readers. Third, when assigned ages are being checked for errors, ancillary information is used as an important diagnostic tool.

Williams and Bedford (1974) recommended that fish length remain unknown to readers to minimize bias. This practice has reportedly been followed (Boehlert 1985; Sharp and Bernard 1988; Lowerre-Barbieri 1993) in the belief that such knowledge may bias the estimated age towards the average age for fish of a particular size. However, any measure which is correlated with age is capable of both increasing bias and improving the age determination. Similarly, if readers are recording measurements while aging, they may unintentionally bias their interpretations towards the perceived norm. With the image analysis system described above, it is possible to eliminate nearly all auxiliary information that may bias a reader's interpretation. A reader may still know the relative size of the otolith being examined, but the reader does not know the distances to the increments that are marked until after the reading process is finished for each otolith. If it is considered to be a particular problem, then the data can be exported to a file that cannot be viewed until after readings are completed.

The potential for reader bias to result from having auxiliary information depends on the particular information, the reason for the aging being undertaken, and the way the information is used. When an aging technique is being validated, for example through the progression of strong year-classes, information on the size fish or the date of capture may significantly, if subconsciously, bias assigned age. Similarly, if marginal increments are being used for validation, knowing the date of capture may lead readers to include or exclude increments according to an expected pattern of increment deposition, leading to circularity in the argument. Also, when markers, such as tetracycline, are being used to validate ages, knowledge of the time at liberty may bias the number of increments counted outside a tagging mark, unless appropriate reading procedures are adopted (see Francis et al. 1992). In the process of converting increment counts to ages, the date of collection is used in conjunction with an assigned birthday and information on marginal growth. If marginal increments are being used for validation, this step should occur after age estimation is completed, but the timing of this step is not important for production ageing.

When production aging is being undertaken, there is a balance to be struck between achieving unbiased age estimates and reducing aging errors. In New Zealand, for example, knowledge of the year of recapture of tagged *Pagrus auratus* increased the accuracy of assigned ages because of known annual variation in growth rates related to variation in water temperature and its effect on increment widths (Francis et al. 1992). If samples are being collected and read routinely each year, then the year of capture will be known to the reader, but this is unlikely to bias the age estimates (and may reduce aging errors). However, Kimura et al. (1992) have shown that readers with knowledge of strong year-classes in a sample will tend to age toward those year-classes more than will readers without such knowledge. This is the opposite effect of ''smearing'' of strong year-classes across adjacent weaker ones referred to by Beamish and McFarlane (1995). However, if a validation study is being undertaken and knowing the year of capture also conveys other information about the specimen, it may be necessary to mix samples collected over several years before reading begins. Protocols must be established and followed that allow the objectives of the study to be attained. Failure to do so compromises the integrity of the data obtained.

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Appendix follows

Appendix: Production Aging System

TABLE A1.—Equipment and software used by the Central Ageing Facility.

Item	Description	Features
Stereomicroscope	Wild Leitz MC3	Phototube, fixed magnification stops, transmitted and reflected light
Compound microscopes	Wild Leitz LaborLux-S	Epifluorescence, trinocular head
	Wild Leitz Orthoplan	Trinocular head
Computer	Compaq 5133	Pentium 133 Mhz, 32 megabytes of random access memory
Frame grabber	Coreco TCI Ultra II, PCI	
Cameras	Oscar OS-25 CCD	Monochrome
	Pulnix TM6CN CCD	Monochrome (low light)
	Pulnix TMC-76 CCD	Color, single chip
Software	Optimas, version 5.2	Menus and functions customized with macros
	Optimate, version 5.21	Runtime version, runs customized macros developed with Optimas
	Excel 5.0	
Sectioning saw	Gemmasta lapidary saw	Modified chuck to accept resin blocks with embedded otoliths; lubricated with water; single cut in <5 s
Cutting blades	LaserLight	100 mm diameter, 0.1 mm thick; stabilized with 75-mm-diameter flanges
Embedding moulds	Teflon or latex	Custom fabricated for 64×68-mm blocks of resin, 5–8 mm thick; no release agents needed
Embedding and mounting medium	Clear polyester casting resin	For normal sections; hardened with MEKP catalyst; sections mounted on 50×75-mm glass slides
	Crysalbond, thermoplastic resin	For grinding thin sections of otoliths individually embedded on standard microscope slides (for examination of daily growth increments)
Orbital disk grinder	Custom built	Variable speed, two disks, for coarse and fine grind.