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Age determination protocol for orange roughy (*Hoplostethus atlanticus*)

New Zealand Fisheries Assessment Report 2016/03
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This report documents the age determination protocol for an important New Zealand deepwater finfish species: orange roughy (*Hoplostethus atlanticus*). It is based on a 2007 unpublished report where the most recent scientific methodologies used for otolith preparation and interpretation, age determination procedures, and investigations of ageing precision were developed as part of a collaborative workshop with participants from New Zealand, Australia, and Chile. In addition, an otolith reference collection of 158 preparations has been compiled and documented from previously prepared archived samples. Agreed readings and ages determined for the reference set are stored in a reference table in the *age* database. The reference set sample was a selection from the Chatham Rise fishstock and included comprehensive ranges of fish size and age.

Digital image examples of otolith preparations are presented and fully illustrate the zone interpretation used in determining fish age for orange roughy. Associated difficulties and idiosyncrasies related to ageing prepared otoliths are also documented.

1. INTRODUCTION

Determining an accurate estimate of age for a fish species is an integral part of fisheries science supporting the management of the fisheries resources in New Zealand. Knowing the age of a fish is critical for estimating growth, mortality rate, population age structure, and age-dependent fishing method selectivity - all important inputs for age-based stock assessments. Information on fish age is also essential for determining biological traits such as age at recruitment and sexual maturity, and longevity.

This report describes the age determination protocol developed for an important New Zealand deepwater finfish species: orange roughy (*Hoplostethus atlanticus*). Significant fishstocks of this species fall within Tier 1 of the National Fisheries Plan for Deepwater and Middle-depth Fisheries, with service strategies that promote regular stock assessment, thus utilising catch-at-age information. The purpose of the protocol is to provide a practical guide for age determination, describing methods and techniques for otolith preparation, viewing and interpreting visible zones within otoliths, and converting annual zone counts into fish age. This protocol will ensure that the best methods are used in determining as accurate an estimate of fish age as possible, and that consistency in age determination is maintained over time. It will also serve as a valuable training tool for new otolith readers. This document draws extensively on an unpublished report of an orange roughy age determination workshop conducted in 2007 and involving collaboration by New Zealand, Australian and Chilean researchers (Tracey et al. 2007).

No attempt has been made to document protocols related to daily increments in otoliths (usually associated with the age determination of larval or juvenile fish) or investigations into otolith ultrastructure or chemical composition. In addition, of the three otolith pairs present within the otic capsules of bony fishes (asteriscae, lapillae, sagittae), only the sagittus, generally the largest and most often used in age estimation (Panfili 2002), has been used to age orange roughy. Therefore, throughout this report, the use of 'otolith' will be synonymous with sagittal otolith. A glossary describing otolith terminologies and ageing definitions relevant to this species has also been included in this report for reference purposes (Appendix A).

Stock assessments used to define TACC levels depend upon estimates of biomass and on the species productivity, the latter often determined by age and growth analyses. While there is growing confidence in the current age interpretations of deepwater species, and proposed mechanisms for why deepwater fishes can attain a high longevity (Cailliet et al. 2001), most age estimates are based on growth zone counts from otoliths, and many remain unvalidated.

1.1 Previous orange roughy age determination studies

Numerous age estimation studies have been performed on New Zealand orange roughy (Kalish et al. *in* Annala 1993, Doonan 1994, Francis & Horn 1997, Horn et al. 1998, Paul et al. 2002, Ackerman & Green 2004a, 2004b, Tracey et al. 2004). There is general consensus that orange roughy are slow growing and long-lived with an age at maturity of about 30 years and maximum age approaching or exceeding 150 years.

Age determination studies of orange roughy were summarised by Tracey & Horn (1999) and Paul et al. (2002), and included references to size at age studies for juvenile orange roughy showing that a 5-year-old fish would have a standard length of approximately 12.4 cm (Mace et al. 1990), as well as to ageing studies to update estimates of *M* (Doonan 1994, Doonan & Tracey 1997). In the 1990s, age determination of New Zealand orange roughy was carried out at NIWA to enable age and growth estimates for the Northwest Chatham Rise (Doonan 1994) and Bay of Plenty stocks (Doonan & Tracey 1997). In addition, otoliths from several regions were aged by Francis & Horn (1997) to further study the transition zone and age at maturity, and by Horn et al. (1998) to look at regional differences in size at the age at maturity.

More recently, age determination work has been carried out at NIWA and the Central Ageing Facility (CAF), Victoria, Australia. Tracey et al. (2004) presented ages for additional Northeast Chatham Rise orange roughy otoliths. Several unpublished reports were presented at Deepwater Fisheries Working Group meetings on orange roughy growth data for the northwest and northeast Chatham Rise, and mid-east coast North Island (Green & Ackerman 2004; Robertson & Green 2004; Ackerman & Green 2004a, 2004b). Catch-at-age distributions have been estimated for orange roughy from various areas on the Challenger Plateau, Chatham Rise, and mid-east coast North Island using the age determination protocols described in this document (Doonan et al. 2013a,b, 2014a,b, 2015).

Along with the New Zealand and Australian studies, age estimates have yielded similar results for longevity for the North Atlantic Ocean orange roughy population (Allain & Lorance 2000), and for fisheries in Chilean (Gili et al. 2002) and Namibian (Butterworth & Brandão 2003) waters.

1.2 Orange roughy age validation studies

Age validation studies for orange roughy have used modal length analysis and otolith edge analysis (Mace et al. 1990, Doonan unpub. data), radiometric age determination (Fenton et al. 1991, Smith et al. 1991, Andrews & Tracey 2003, 2007, Andrews et al. 2009), and bomb radiocarbon (^{14}C) dating (Morison et al. 1999) (which was critiqued by Sparks (2000)). Reinterpretation and support for the early radiometric studies was provided by Francis (1995). Trials using the bomb radiocarbon technique for deepwater species found it to be unsuitable for orange roughy because they lack a pelagic phase in their life-cycle, a requirement if the timing of the influx of bomb radiocarbon is to provide a temporal marker (Kalish 2002). Andrews et al. (2009) used an improved lead–radium dating technique to provide independent age estimates from sagittal otoliths. The improvement allowed the use of smaller samples than previously possible; allowing the analysis of otolith cores, containing the first few years of otolith growth. The measurement of lead-radium ratios for a series of age group otolith cores, grouped based on growth-zone counts from thin sections, showed a high degree of correlation to the expected lead-radium ingrowth curve. This finding provided support for age estimation procedures using thin otolith sectioning. As independent estimates of age, the results indicated that fish in the oldest age group were at least 93 years old.

1.3 Development of an age determination protocol

Concerns about assumed orange roughy productivity were raised during the 2006 stock assessment for the East Chatham Rise; it was also acknowledged that orange roughy productivity, and in particular recruitment patterns, were poorly known. Recruitment patterns are usually estimated from age frequency distributions. Unfortunately, previous age determination had poor precision (Francis 2006, Hicks 2005), and, consequently, relative year class strengths were obscured. Even more importantly, Francis (2006) noted bias or "drift" in the ages estimated in the early 2000s. As a result of the age bias the Deepwater Fisheries Working Group had low confidence in the age frequency data for orange roughy.

As a consequence of Francis' (2006) finding, an age determination workshop was conducted in 2007 to determine whether orange roughy otoliths could be read with sufficient accuracy and precision for the data to be of use in stock assessments. Specifically, the aim of the workshop was to determine if it was possible to use orange roughy age data to provide reliable estimates of changes in age frequency, and recruitment patterns, and to help determine the scope of future research and sample collection protocols for orange roughy. A summary of the aims, processes and findings of the workshop is presented in Appendix B.

2. OTOLITH PREPARATION

Sagittal otoliths are chosen for age determination as these are the hard parts most likely to allow an accurate estimate of age for this species. Orange roughy otoliths are sectioned along a longitudinal plane that includes the primordium to the posterior arm on the longest path (Figure 1). Two laboratories have been involved in the routine age determination of orange roughy from New Zealand: the National Institute of Water and Atmospheric Research (NIWA) in New Zealand, and the Central Ageing Facility (CAF) [now Fish Ageing Services (FAS)] in Australia. Both laboratories have consensus on plane of sectioning and area sectioned, and the sectioning method is essentially similar between institutes. However, while NIWA prepare a single otolith section, FAS prepare multiple sections of the one otolith at a time. Methods of preparation for both institutes are described below.

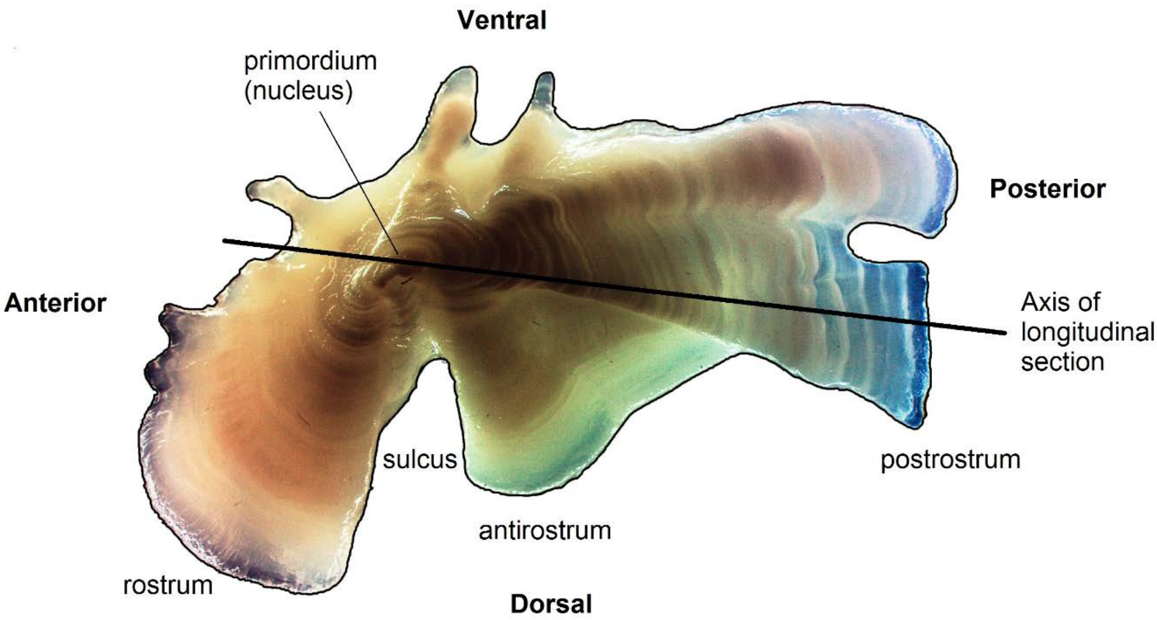

Figure 1: The proximal surface of a left whole orange roughy otolith from an adult fish, illuminated using polarised light, indicating the axis of longitudinal sectioning plane (thick line). (Photo by Caoimhghin Ó Maolagáin, NIWA).

<u>National Institute of Water and Atmospheric Research, New Zealand</u>

The left otolith was consistently chosen for sectioning, unless it was chipped, broken or 'crystalline' (i.e. comprised partially or completely of vaterite). Using a fine pencil, a line extending from the primordium to the leading postero-dorsal growth edge is inscribed on the lateral face of the otolith, while viewed under transmitted light. The optimal sectioning plane was longitudinal through the otolith ensuring that it went through the primordium and along the most uniform postereo-dorsal axis (Figure 1). This was generally oriented close to the dorsal edge of the postrostrum. Each otolith is then individually embedded in a mould with epoxy resin (Araldite K142), cured at about 50°C for a minimum of 4 hours and left to completely harden overnight. Thin sections are taken using a high precision Struers Secotom sectioning saw at a thickness of 400 μm (±20 μm). The inscribed surface pencil line is used as a visual sectioning guide orientation. The sections are cleaned in detergent and water to remove any residual particulates. Air dried sections are then mounted on a glass microscope slide with epoxy resin under a glass cover slip.

<u>Fish Ageing Services, Australia</u>

Clean dry otoliths (either left or right) are arranged in two columns of five otoliths and embedded in clear casting polyester resin (methyl-ethyl ketone peroxide is used as hardening agent/catalyst). The block of 10 embedded otoliths was cut into two strips of five. A maximum of four sections approximately 250–400 μm thick are cut from each embedded otolith using a modified Gemasta™ lapidary diamond cutting saw fitted with a high-speed 200 μm wide diamond impregnated blade lubricated by water. The sections are taken longitudinally through the otolith approximately along an axis between the primordium and the postrostrum (Figure 1). Sections are then cleaned in water, rinsed with alcohol and dried before being mounted on numbered microscope slides using further polyester resin and covered with glass coverslips.

Some advantages and disadvantages of the two preparation techniques were identified.

Multiple section technique (FAS)

- at least one section will generally include the primordium, but sometimes it can be missed
- a lower level of cutting precision is required (i.e., it is a quicker method)
- the section thickness can vary (note that this can be either good or bad)
- it relies on a specialised high speed cutting machine
- sometimes it is necessary to use two part-sections to derive a reading for a single otolith (e.g., inside the transition zone on one section, and the transition zone to the margin on another) if a single section has not been taken along the ideal plane.

Single section technique (NIWA)

- the sectioning transect is carefully marked on the otolith, ensuring that the primordium is included in almost all preparations
- it produces a high proportion of successful preparations
- only one section is taken — if it is not clear then there is no alternative
- it is slightly more time-consuming than fast multiple sectioning.

3. OTOLITH INTERPRETATION

A sectioned orange roughy otolith can exhibit several distinct regions of zone types. These are described below as juvenile, adolescent, pre-transition, transition (TZ), and post-transition (Figure 2) and are viewed using transmitted light. The juvenile section (about the first three years of growth) comprises very dark opaque annual zones (often comprised of multiple fine sub-zones), and the margins between annual increments may be very difficult to differentiate. Consequently, a measurement of about 1.7 mm from the primordium is sometimes used to approximate the outer edge of the third zone (a measurement derived from Mace et al. (1990) who validated the first three annual zones). The adolescent section of the otolith comprises a set of relatively clear and moderately wide opaque zones between the juvenile section and the pre-transition zone (pre-TZ). The zones in the pre-transition region are generally dark, but may not be regular in width (e.g., they may range from wide to narrow and then back to wide). Individual zones can also be clearly comprised of a small number of sub-zones. Because of the accepted variation in zone width, the sub-zone characteristics can cause complications with counts when it is not clear if a zone is split or actually two true annual zones. The pre-transition region terminates at the transition zone — ideally, a single zone where zone width markedly changes from relatively wide to very narrow. However, the change is often more gradual. This generally manifests as a relatively clear transition zone followed by a gradual, but distinct, reduction in the widths of subsequent zones. Sometimes the transition zone cannot be defined because there is a gradual reduction in zone width from the moderately wide adolescent and pre-TZ zones to the very narrow post mature zones, i.e., there is no single point on the otolith when a clear change in zone width, zone colour, or otolith curvature is indicative of a lifestyle change. Finally, there is a section (post-transition) of the otolith that is clearly formed during the post-mature stage where zones are very narrow and can be quite difficult to distinguish.

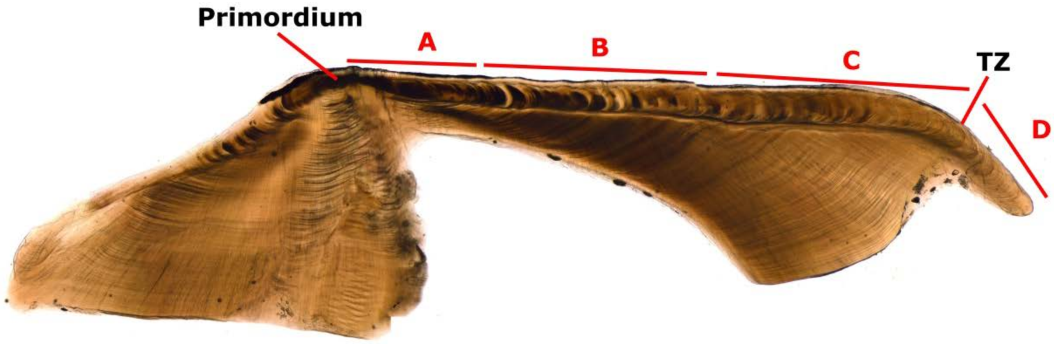

Figure 2: Transversely sectioned orange roughy otolith viewed under transmitted light, indicating morphological features and section of zone counts; (A) juvenile, (B) adolescent, (C) pre-transition and (D) post-transition. TZ denotes the position of the transition zone.

Based on the information derived from the discussions and reading trials at the 2007 age determination workshop (Appendix B), the following set of otolith reading protocols was determined to try to decrease the variability in the age estimates, particularly in the pre-TZ area of the otolith section.

3.1 Overall approach

- View preparations under transmitted light
- Use the longest path along the posterior arm of the otolith for age determination (as it is believed that this area will provide the most reliable age estimate)
- Count dark (opaque) growth zones
- View under 36–40× magnification for counts out to the transition zone (TZ)
- View under 36–40× magnification for counts immediately post-TZ
- View under 64–100× magnification for fine zones post-TZ to otolith margin
- Before making final readings, view sufficient otoliths to become familiar with the otolith pattern and reading protocol (perhaps about 50 preparations)

3.2 Initial examination

- Determine light intensity and focus
- View otolith section under low magnification to increase field of view
- Determine if the sample is young, medium-aged, or old taking into account:
    1. Relative size of the otolith
    2. Relative thickness of the otolith
    3. Presence of fine zones near margin
    4. Curvature of the anterior arm
    5. Presence of a TZ
- Use different filters (e.g., polariser) to potentially increase clarity

3.3 Zone counts

3.3.1 Region A – Juvenile section

- Identify primordium and determine likely positions of the first three zones
- Check with measurement (distance to completion of third zone is about 1.7 mm, Mace et al. 1990, Figure 3)
    1. Individual zones may be unclear (Figure 4) or could consist of multiple fine bands that should be grouped
    2. If the zone count at 1.7 mm is 3 ± 1 and zone structure is clear then use the actual count

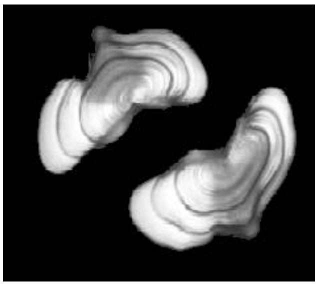

Figure 3: Image of whole otoliths from a juvenile orange roughy (7 cm standard length) estimated to be 2+ years old.

Figure 4: Orange roughy otolith section showing how the 1.7 mm measurement from the primordium is used to identify the likely location of the first three zones.

3.3.2 Region B – Adolescent section

- Dark broad zones are counted out to a count of about 10
- Dark zones are generally quite broad (and sometimes similar in width to the first three zones) and may consist of finer dark sub-zones.
- Use widths of adjacent clear zones to assist in the interpretation of zones that appear split or have multiple banding structure

3.3.3 Region C – Pre TZ

- Dark zones are counted out to the TZ
- Count all strong and distinct zones, generally regardless of spacing (i.e., zone width can vary markedly in this area, both within and between otoliths (Figures 5 and 6))
- Use widths of adjacent clear zones to assist in the interpretation of zones that appear split or have multiple banding structure

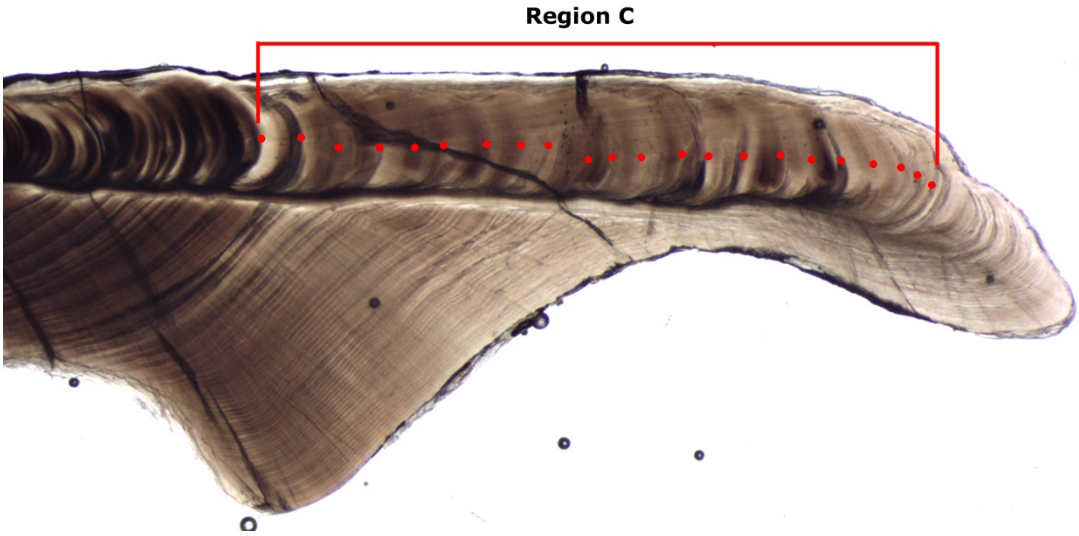

Figure 5: Orange roughy otolith section showing the pre-transition Region 'C'. Discrete zones are marked with red dots.

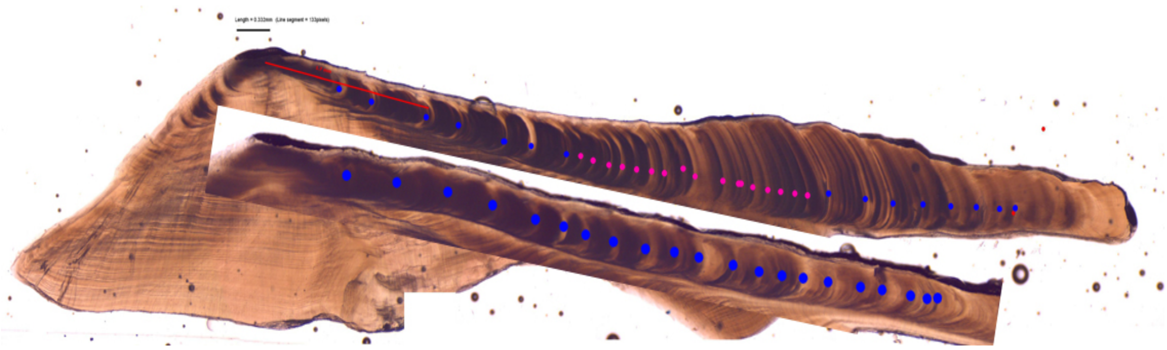

Figure 6: Orange roughy otolith section showing zone interpretations and different growth patterns in the pre-TZ area on two different otoliths photographed at the same magnification.

3.3.4 Transition zone

- The transition zone (TZ) is identified using the following three criteria:
  1. Clear reduction in zone width
  2. Marked change in optical density from dark to light
  3. Change in curvature of the posterior arm

At least two of these rules must be met to define a TZ. If only one or no criterion is met, but it is apparent from the narrow widths of zones near the otolith margin that the fish must be post-TZ but with an undefinable TZ position, then this should be recorded.

See Figure 2 for an example where all three TZ criteria are met. Figure 7 shows the transition zone area from several otolith sections.

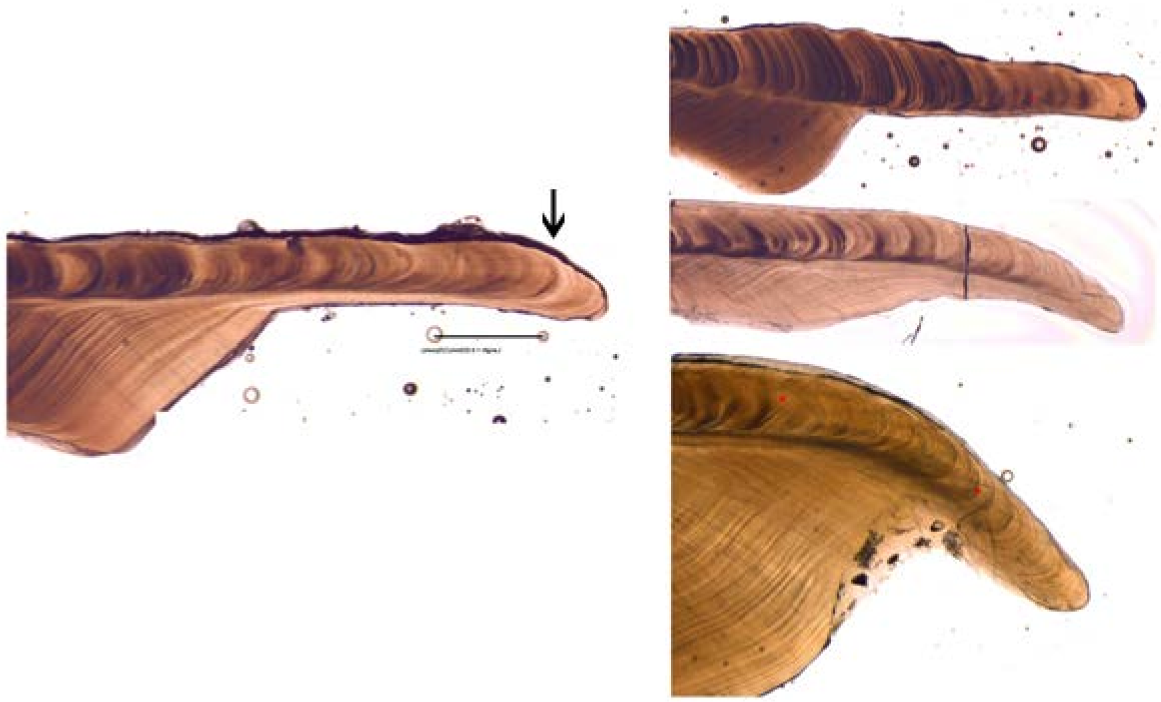

Figure 7: Various forms of transition zone. The example on the left has a clear TZ where all three criteria are met (see arrow). The examples on the right most likely have transition zones, but their positions are difficult to determine.

3.3.5 Region D – Post Transition

- Count all zones visible along the line of the curve apex (Figure 8)
- Zones immediately after the TZ may not be as narrow as those near the otolith margin
- There may be a gradual reduction in post-TZ zone width until very narrow (fine) structure becomes apparent
- Use 40× magnification immediately after the TZ until very narrow zones are apparent, then use 64–100× magnification for the narrow fine zones

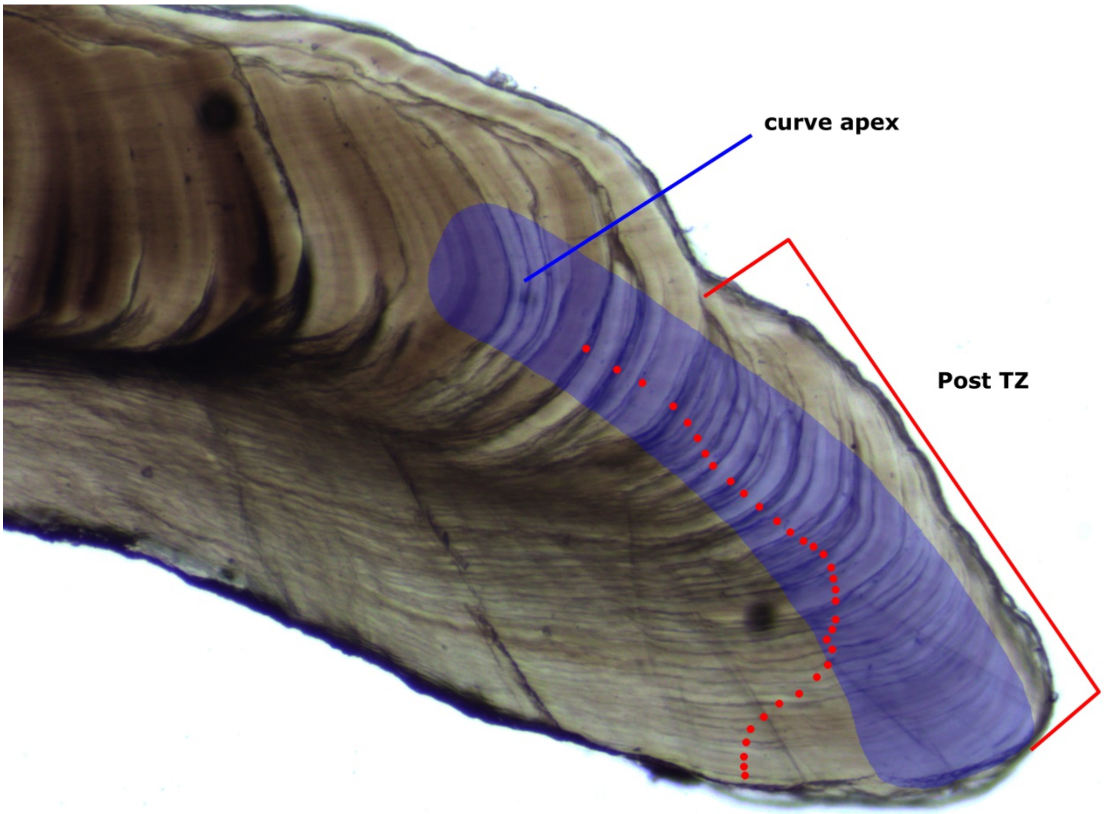

Figure 8: Otolith section of the post-TZ area at 60× magnification showing the fine zones near the otolith margin. Curve apex region is marked in blue. Discrete zones are marked with red dots. The initial 24 zones (approximately) have been marked along the curve apex, but in this example the most recently formed zones were clearest away from the curve apex.

3.4 Scales of readability

For each otolith section that is read, the reader will assign readability categories as defined below.

Primordium to TZ Readability
1 – Otolith structure is exceptionally clear with unambiguous zones
2 – Sample may be ± one year from determined count
3 – Sample is difficult to interpret and subject to small interpretational differences
4 – Sample is difficult to interpret and subject to large interpretational differences
5 – Sample is unreadable due to failed preparation or unreadable structure

TZ Readability
0 – TZ not believed to have yet been formed
1 – Clear and unambiguous, meets all three criteria. TZ count recorded
2 – Gradual transition, however at least two criteria met. TZ count recorded
3 – Gradual transition, none or one of the criteria met. TZ count not recorded

TZ to Margin Readability
1 – Otolith structure is exceptionally clear with unambiguous zones
2 – Sample may be ± one year from determined count
3 – Sample is difficult to interpret and subject to small interpretational differences
4 – Sample is difficult to interpret and subject to large interpretational differences
5 – Sample is unreadable due to failed preparation or unreadable structure

If an otolith has a TZ readability of 3, then a single zone count is recorded (primordium to margin) with a single readability using the 5-stage scale above.

3.5 Use of digital images in age estimation

Using digital images for age determination of this species would be difficult because images do not always capture the fine scale detail required for ageing. However, annotated images of a reference set of otoliths could be beneficial for re-calibration for experienced readers and initial calibration (i.e., training) of new readers.

<u>Advantages of direct microscopic examination</u>

- Greater ability to perceive variations in intensity, density and colour of otolith zones
- Greater ability to interpret three-dimensional structure by altering the focal plane and magnification
- Greater resolution and ability to alter light intensity and direction (e.g., with filters)
- Faster for a simple zone count (but slower if measurements are required)

<u>Advantages of digital images</u>

- Ability to mark, count and display growth zones on the image for later examination, discussion, and dissemination
- Reduced chance of counting same zone twice, or missing zones (if zones are marked on-screen while counting)
- Facilitate and speed up measurement of distances, and reduce likelihood of transcription errors

4. PRECISION and ACCURACY

In age estimation studies, 'accuracy' refers to the closeness of an age estimate to the true age whereas 'precision' is a measure of the variability between individual readings, either within or between readers (Campana 2001).

The aim of the age determination workshop (Appendix B) was to increase precision and accuracy for orange roughy ageing studies so that the age estimates may be useful in future stock assessment processes. However, age estimates can still be useful where precision is low but where the mean age reflects the true age. Problems arise when there is bias in age estimates, as seen in earlier age estimates derived by CAF and NIWA (Francis 2006, Hicks 2005).

Direct age validation of this species has proven problematic, so testing for accuracy is difficult. Andrews et al. (2009) used a lead-radium dating technique to provide independent age estimates from groups of sagittal otolith cores, with the otoliths in each group judged (by zone counts) to be of similar ages. The lead-radium ratios for each age group showed a high degree of correlation to the expected lead-radium ingrowth curve. While this process did not confirm the age of individual fish, it did indicate strongly that the otolith interpretation method described above using thin otolith sectioning does produce relatively accurate estimates of age. The results indicated that fish in the oldest age group were at least 93 years old.

Indices of precision are a necessary tool for comparing between and within readers. They can indicate bias, provide an indication of "uncertainty" surrounding the age estimates, and indicate whether age estimates from a particular species are reliable for stock assessment purposes. An analysis of between-reader and between-institution precision for orange roughy age determination, following the development of the age determination protocol described above, is presented in Appendix C. Although it is not strictly part of an age validation protocol, it is included here as it demonstrates that the outcome of the workshop to develop a more consistent otolith interpretation method did, indeed, result in more consistent between-reader comparisons.

5. FORMAT FOR DATA SUBMISSION TO *age* DATABASE

NIWA (Wellington) currently undertake the role of Data Manager and Custodian for fisheries research data owned by the Ministry for Primary Industries (MPI). This includes storing physical age data (i.e., otolith, spine and vertebral samples) and the management of electronic data in the *age* database. A document guide for users and administrators of the *age* database exists (Mackay & George 1993). This database contains several tables, outlined in an Entity Relationship Diagram (ERD) which physically shows how all tables relate to each other, and to other databases.

When research has been completed, NIWA receives the documented age data (usually in an Excel spreadsheet format) from the research provider and performs data audit and validation checks prior to loading these data to the *age* database (Table 1). Additional information that should be recorded include the readability scores, TZ classification, MPI project code, reader identifier, date of reading, and preparation method.

Table 1: An example of orange roughy age data from a research survey submitted for loading onto the *age* database. Data recorded for each otolith are: total zone count, result1; count from the primordium to the TZ, result2; count from the TZ to the margin, result3; readability grade for the pre-TZ zones, error1; readability grade for the post-TZ zones, error2; readability grade for the TZ, error3.

origin	yr	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	reading_no	reading_date	reader	lgth	sex	result1	result2	result3	error1	error2	error3	age	proj_code
DWG	2014	thh1401	2	-1	ORH7A	ORH	25	81	1	16-Apr-15	10	36.5	2	40		40		3	3	40	MID2010-01E
DWG	2014	thh1401	2	-1	ORH7A	ORH	26	82	1	16-Apr-15	10	34.9	2	34	25	9	4	3	2	34	MID2010-01E
DWG	2014	thh1401	2	-1	ORH7A	ORH	27	83	1	16-Apr-15	10	33.3	1	36	25	11	4	4	2	36	MID2010-01E
DWG	2014	thh1401	2	-1	ORH7A	ORH	28	84	1	16-Apr-15	10	29.6	1	25	25		4		0	25	MID2010-01E
DWG	2014	thh1401	2	-1	ORH7A	ORH	29	85	1	16-Apr-15	10	36.4	2	50	24	26	3	3	1	50	MID2010-01E

6. REFERENCE COLLECTION

The reference collection for orange roughy comprises the 158 otolith sections produced by NIWA for the post-workshop analysis of between-reader precision (see Appendix C, Table C2). The agreed ages for these otoliths were taken as the readings by NIWA Reader 1. They have been stored in a table (*t_reference*) in the *age* database (administered by NIWA for MPI). The reference set can be used for training new readers as well as monitoring their progress as they gain experience in age determination. Any new readings of the reference set are also stored in the *age* database (in table *t_ref_reading*). New readings of the reference set are stored in this separate table to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

7. ACKNOWLEDGMENTS

This work was funded by the Ministry for Primary Industries under project SAP200716. We acknowledge the contributions of other participants at the 2007 orange roughy age determination workshop: Peter Marriott (NIWA), Simon Robertson and Corey Green (Central Ageing Facility, Department of Primary Industries, Victoria, Australia), Raul Gili (Centro de Estudios Pesqueros, Valparaiso, Chile), and Luis Cid Mieres (Instituto de Fomento Pesquero, Valparaiso, Chile). We thank Caoimhghin Ó Maolagáin for selecting and preparing otolith sections. We acknowledge the inclusion of a glossary of otolith terminology and images that have been reproduced within this report from a

number of fish ageing publications, and are most grateful to those authors for this, particularly Kalish et al. (1995) and Marriott & Manning (2011). Useful comments on a draft of this report were provided by Kevin Sullivan, Marc Griffiths and Cameron Walsh.

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APPENDIX A: Glossary of otolith terminology and ageing definitions

Based on Kalish et al. (1995) "Glossary for otolith studies" but with some added items to describe New Zealand practice.

Accuracy – the closeness of a measured or computed value to its true value.
Age estimation, age determination – these terms are preferred when discussing the process of assigning ages to fish. The term ageing should not be used as it refers to time-related processes and the alteration of an organism's composition, structure, and function over time. The term age estimation is preferred.
Age frequency – the frequency distribution of fish by age group in a particular sample.
Age group – the cohort of fish that have a given age (e.g., the 5 year old age group). The term is not synonymous with year class or day class.
Age class – same as age group.
Annulus (pl. Annuli) – one of a series of concentric zones on a structure that may be interpreted in terms of age. The annulus is defined as either a continuous translucent or opaque zone that can be seen along the entire structure or as a ridge or a groove in or on the structure. In some cases, an annulus may not be continuous nor obviously concentric. The optical appearance of these marks depends on the otolith structure and the species and should be defined in terms of specific characteristics on the structure. This term has traditionally been used to designate year marks even though the term is derived from the Latin "anus" meaning ring, not from "annus", which means year. The variations in microstructure that make an annulus a distinctive region of an otolith are not well understood.
Antirostrum – anterior and dorsal projection of the sagitta (see Figure 1).
Asteriscus (pl. Asteriscii) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes.
Bias – The systematic over- or underestimation of age.
Birth date – A nominal date at which age class increases, generally based on spawning season.
Check – a discontinuity (e.g., a stress induced mark) in a zone, or in a pattern of opaque and translucent zones. Sometimes referred to as a false check.
Cohort – group of fish of a similar age that were spawned during the same time interval. Used with both age group, year class and day class.
Core – the area or areas surrounding one or more primordia and bounded by the first prominent D-zone. Some fishes (e.g., salmonids) possess multiple primordia and multiple cores.
Corroboration – a measure of the consistency or repeatability of an age determination method. For example, if two different readers agree on the number of zones present in a hard part, or if two different age estimation structures are interpreted as having the same number of zones, corroboration (but not validation) has been accomplished. The term verification has been used in a similar sense; however, the term corroboration is preferred as verification implies that the age estimates were confirmed as true.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.
Hyaline zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone.
Increment – a reference to the region between similar zones on a structure used for age estimation. The term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g., subdaily, daily, annual). Depending on the portion of the otolith considered, the dimensions, chemistry, and period of formation can vary widely. A daily increment consists of a D-zone and an L-zone, whereas an annual increment comprises an opaque zone and a translucent zone. Both daily and annual increments can be complex structures, comprising multiple D-zones and L-zones or opaque and translucent zones, respectively.
Lapillus (pl. Lapilli) – one of three otolith pairs found in the membranous labyrinth of osteichthyan fishes. The most dorsal of the otoliths, it lies within the utriculus ("little pouch") of the pars superior. In most fishes, this otolith is shaped like an oblate sphere and it is smaller than the sagitta.

Margin/marginal increment – the region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.
Nucleus, Kernel – collective terms originally used to indicate the primordia and core of the otolith. These collective terms are considered ambiguous and should not be used. The preferred terms are primordium and core (see definitions).
Opaque zone – a zone that restricts the passage of light when compared with a translucent zone. The term is a relative one because a zone is determined to be opaque on the basis of the appearance of adjacent zones in the otolith (see translucent zone). In untreated otoliths under transmitted light, the opaque zone appears dark and the translucent zone appears bright. Under reflected light the opaque zone appears bright and the translucent zone appears dark. An absolute value for the optical density of such a zone is not implied. See translucent zone.
Postrostrum – one of the two posterior extensions of the otolith. The preferred sectioning plane is from the primordium to the postrostrum.
Precision – the closeness of repeated measurements of the same quantity. For a measurement technique that is free of bias, precision implies accuracy.
Primordial granule – the primary or initial components of the primordium. There may be one or more primordial granules in each primordium. In sagittae the granules may be composed of vaterite, whereas the rest of the primordium is typically aragonite.
Primordium (pl. Primordia) – the initial complex structure of an otolith, it consists of granular or fibrillar material surrounding one or more optically dense nuclei from 0.5 um to 1.0 µm in diameter. In the early stages of otolith growth, if several primordia are present, they generally fuse to form the otolith core.
Rostrum – anterior projection of the sagitta (see Figure 1).
Sagitta (pl. Sagittae) – one of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes. It lies within the sacculus ("little sack") of the pars inferior. It is usually compressed laterally and is elliptical in shape; however, the shape of the sagitta varies considerably among species. In non-ostariophysan fishes, the sagitta is much larger than the asteriscus and lapillus. The sagitta is the otolith used most frequently in otolith studies.
Sulcus acusticus (commonly shortened to 'sulcus') – a groove along the medial surface of the sagitta (see Figure 1). A thickened portion of the otolithic membrane lies within the sulcus acusticus. The sulcus acusticus is frequently referred to in otolith studies because of the clarity of increments near the sulcus in transverse sections of sagittae.
Transition zone – a region of change in otolith structure between two similar or dissimilar regions. In some cases, a transition zone is recognised due to its lack of structure or increments, or it may be recognised as a region of abrupt change in the form (e.g., width or contrast) of the increments. Transition zones can be formed in otoliths 1) during metamorphosis from larval to juvenile stages, 2) at the onset of sexual maturity, 3) when growth rate changes markedly, or 4) during significant habitat changes such as the movement from a pelagic to a demersal habitat or a marine to freshwater habitat. If the term is used, it requires precise definition.
Translucent zone – a zone that allows the passage of greater quantities of light than an opaque zone. The term is a relative one because a zone is determined to be translucent on the basis of the appearance of adjacent zones in the otolith (see opaque zone). An absolute value for the optical density of such a zone is not implied. In untreated otoliths under transmitted light, the translucent zone appears bright and the opaque zone appears dark. Under reflected light the translucent zone appears dark and the opaque zone appears bright. The term hyaline has been used, but translucent is the preferred term.
Validation – the process of estimating the accuracy of an age estimation method. The concept of validation is one of degree and should not be considered in absolute terms. If the method involves counting zones, then part of the validation process involves confirming the temporal meaning of the zones being counted. Validation of an age estimation procedure indicates that the method is sound and based on fact.
Vaterite – a polymorph of calcium carbonate that is glassy in appearance. Most asteriscii are made of vaterite, and vaterite is also the principal component of many aberrant 'crystalline' sagittal otoliths.

Verification – the process of establishing that something is true. Individual age estimates can be verified if a validated age estimation method has been employed. Verification implies the testing of something, such as a hypothesis, that can be determined in absolute terms to be either true or false.
Year class – the cohort of fish that were spawned or hatched in a given year (e.g., the 1990 year class). Whether this term is used to refer to the date of spawning or hatching must be specified as some high latitude fish species have long developmental times prior to hatching.
Zone – region of similar structure or optical density. Synonymous with ring, band and mark. The term zone is preferred.

APPENDIX B: Orange roughy Age Determination Workshop, 2007

The workshop was convened at NIWA Greta Point, Wellington, New Zealand, from 7 to 9 February 2007 (Tracey et al. 2007). The participants involved with the interpretation of orange roughy otolith sections were: Di Tracey and Peter Horn (NIWA), Kyne Krusic-Golub and Corey Green (Central Ageing Facility, Department of Primary Industries, Victoria, Australia [subsequently Fish Ageing Services]), Raul Gili (Centro de Estudios Pesqueros, Valparaiso, Chile), and Luis Cid Mieres (Instituto de Fomento Pesquero, Valparaiso, Chile). The terms of reference for the workshop were:

1. To review ageing protocols and ageing precision, and thereby determine best practice for this species.
2. To age a sample of orange roughy otoliths, using experienced readers and the best practice identified in 1 above, and to estimate the accuracy and precision of the age estimates.
3. To produce a report to the Deepwater Fisheries Working Group on the outcome of the workshop.

The workshop began with a discussion of the problems with the available age data (i.e., reader drift, Francis 2006). Representatives from each institute described their otolith preparation and interpretation practices. It was suggested that between-reader difference in the chosen position of the TZ is the greatest source of error in the age interpretation of orange roughy. To ascertain where the differences were occurring in the pre-TZ and post-TZ regions of the otolith the following trial was conducted.

Prior to any discussion, seven prepared sections were selected and read by the six different readers. The following data were recorded by all readers independently, and compared.

- The zone count from the primordium to a pre-determined point on the otolith section clearly before the TZ
- The zone count at TZ
- The zone count age from a pre-determined point on the otolith clearly after the TZ out to the otolith margin.

Results of the comparison showed that readers from CAF were counting fewer zones in the pre-TZ area than readers from NIWA and IFOP (Figure B1). Estimates of age were highest for readers from IFOP in all samples examined.

The position at which TZ was recorded was not the largest source of variation in the age reading comparisons. In this series of samples the interpretation of the pre-TZ zones was the major source of variation observed (Table B1).

The pre-TZ area consisted of generally dark zones sometimes irregular in width. Some individual zones were also comprised of a small number of sub-zones (Figure B2). Because of the accepted variation in zone width, the sub-zone characteristics can cause complications with counts when it is not clear if a zone is split or actually two true annual zones. The readers from NIWA and IFOP tended to count the finer sub-zones in this area, while the CAF readers were grouping several assumed sub-zones into one zone. In two of the samples one of the IFOP age readers counted significantly more zones. In these cases the reader counted the fine zones within the adolescent region (region B of Figure 2).

Based on the information from the reading trial described above, a set of otolith reading protocols were determined to try to decrease the variability in the age estimates, particularly in the pre-TZ area of the otolith section. These protocols are described in Section 3.3.

Table B1: Zone counts on seven samples of orange roughy by six readers from three institutions. CAF readers = C & K; NIWA readers = D & P; IFOP readers = R & L.

Otolith		D	C	L	K	P	R
15_2	0 – pre-TZ count	20	18	32	19	20	25
	post-TZ - edge count	3–5	–	6	5	4	–
	count sum	25	18	38	24	24	25
26_9	0 – pre-TZ count	35	23	33	23–24	29	39
	post-TZ - edge count	49–53	57	57	52	60	59
	count sum	88	80	90	76	89	98
48_17	0 – pre-TZ count	36	21	42	23–24	32	32
	post-TZ - edge count	–	–	–	–	–	–
	count sum	36	21	42	24	32	32
COR9002_225_12	0 – pre-TZ count	21	16	31	16	20	41
	post-TZ - edge count	–	–	–	–	–	–
	count sum	21	16	31	16	20	41
707_163_120	0 – pre-TZ count	27	16	34	15	19	37
	post-TZ - edge count	18	14	17	7	20	–
	count sum	45	30	51	22	39	–
707_163_87	0 – pre-TZ count	36	18	49	17	35	41
	post-TZ - edge count	–	–	–	–	–	–
	count sum	36	18	49	17	35	41
ORH_0207654	0 – pre-TZ count	33	21	29	19	24	33
	post-TZ - edge count	85	98	75	68	93	75
	count sum	118	119	104	87	117	108

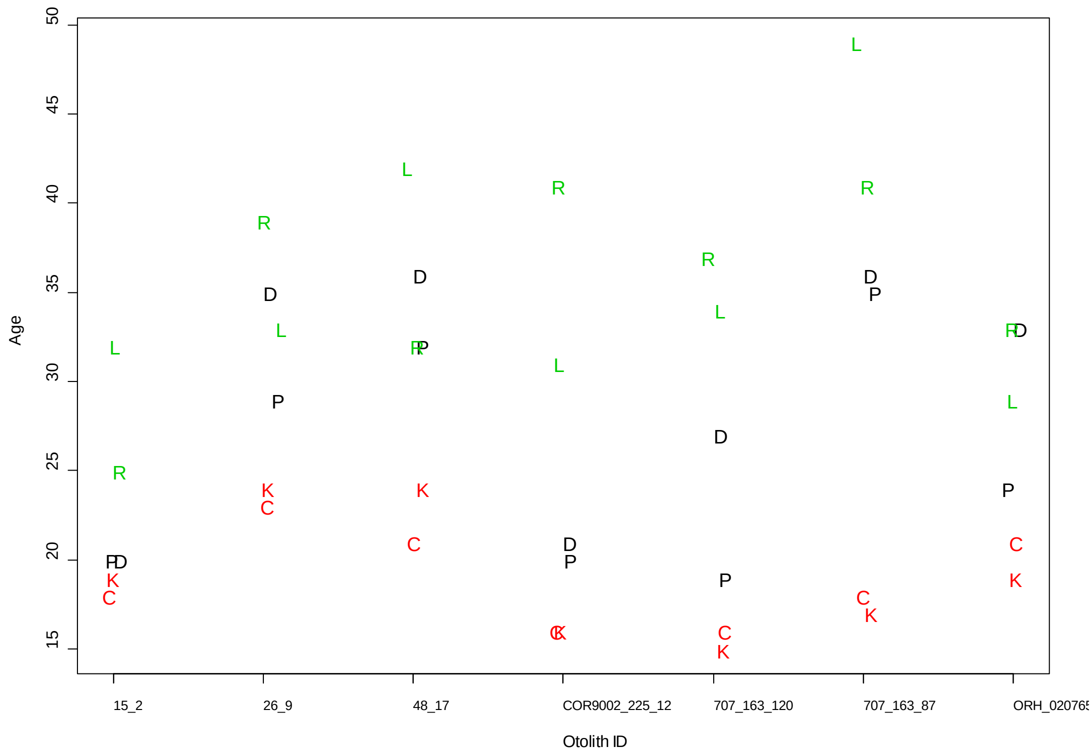

Figure B1: Zone counts from the primordium to a pre-determined point on the otolith section clearly before the TZ on seven samples of orange roughy by six readers from three institutions. CAF readers = C & K; NIWA readers = D & P; IFOP readers = R & L.

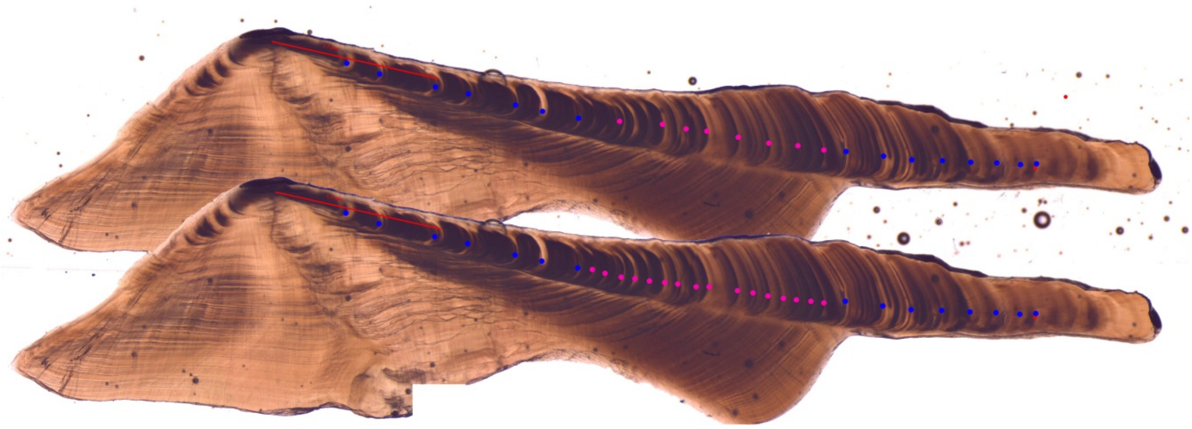

Figure B2: Orange roughy otolith section showing two differing zone interpretations in the pre-TZ area on the same otolith.

Four additional otoliths were then read to determine if using the designated age determination protocols (described in Section 3.3 above) had increased precision. Zone counts were recorded from the primordium to the TZ and from the TZ to the otolith margin. Analysis of these readings indicated that greater levels of precision were achieved than with the initially aged samples (Figure B3).

Results also indicated that the position of the TZ may not always have as big an influence on the final age as previously thought. In the case of sample COR9002-223-13, one reader counted 28 zones to the TZ and 6 from the TZ to the otolith margin; another reader counted 30 zones to the TZ plus 4 to the otolith margin. For this sample the readers were obviously interpreting the same zone pattern regardless of position of the TZ.

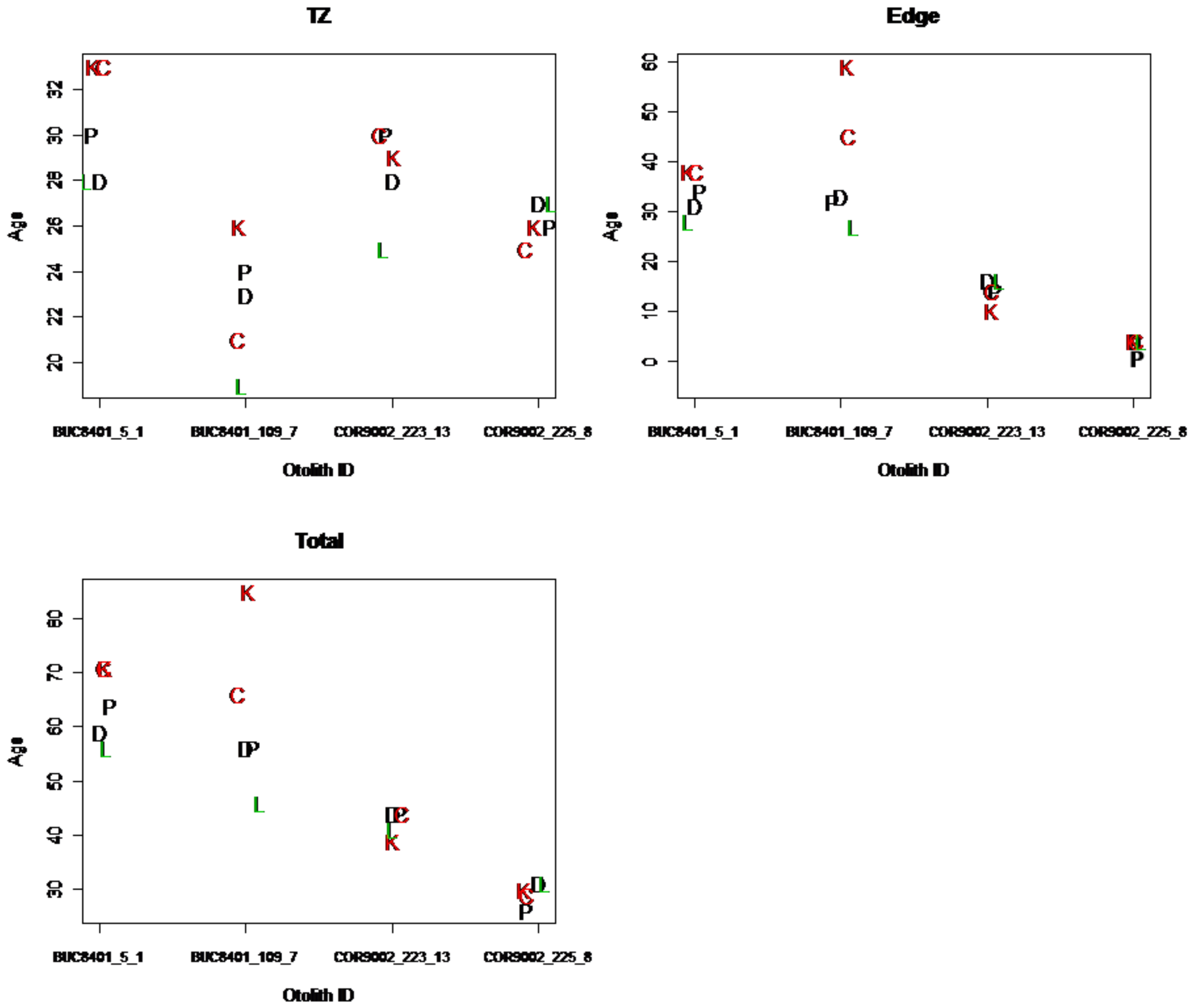

Figure B3: Counts by five readers to the transition zone (TZ), from the transition zone to the otolith margin (Edge), and the sum of these (Total).

Workshop summary

The workshop participants produced a revised protocol that they believed should provide more reliable consistent readings. They were unsure of the effect this would have on previously age readings, and it was agreed that the significance of the new protocol on re-readings should be tested.

There was a need to:

- complete a larger scale cross-ageing study to determine accuracy and precision between and within readers and institutions.
- re-age some previously aged samples using the newly defined protocols to determine potential differences.

APPENDIX C: Testing the developed ageing protocol

A revised orange roughy age determination protocol was agreed upon during a workshop held in February 2007 (Section 3, Appendix B). The Deepwater Fisheries Working Group discussed the outcome of the workshop and agreed that the next steps required were as follows:

- the different agencies should further develop and test the new protocol;
- the Working Group needs to decide what the age data will be used for, as this will affect the required level of accuracy;
- the Working Group should decide what is an acceptable level of difference in precision between readings from different agencies. This should be investigated using the ratio difference of readings, not significance tests; and
- the Working Group should define a benchmark ratio to measure how good the new protocol is, and a 5% difference (ratio of 1.05) was suggested.

In 2008, a cross-ageing study was funded by Ministry of Fisheries to apply and test the revised age determination protocols and determine if precision between and within readers and institutions had improved. Orange roughy age estimates were obtained by two NIWA readers and two CAF readers, from otolith pairs from 160 fish, in which one otolith set of each pair was prepared by CAF and the other "sister" otolith was prepared by NIWA. Samples were from the largest stock on the Chatham Rise, in the Spawning Plume region, and covered the size range of orange roughy in this area.

The study produced between-reader ageing results and subsequent analyses of precision of age estimates, and potential biases between readers (drift), institutes, and preparation methods. This section is based on a report by Tracey et al. (2009).

Methods

Age data

Otoliths were collected during random trawl surveys on the north eastern Chatham Rise in 1984 (survey BUC8401) and in 2000 (survey COR9002). The otoliths were prepared following methods described in Tracey & Horn (1999) and Robertson & Green (2004).

Otolith interpretation followed that described in Section 3.3 above. All readers read the NIWA prepared otolith samples first, then the CAF prepared sample. CAF Reader 1 was not present at the previous orange roughy age determination workshop but had considerable prior experience in reading otoliths of this species. For each otolith section, zone counts and a readability category were obtained for the primordium to TZ, and from the TZ to the edge.

Estimating drift, precision and bias

Francis (2006) used the mean ratio of estimated ages to investigate drift in reading otoliths. However, when fish ages are estimated with error between 5 and 10%, the mean ratio can have some undesirable properties. For example, comparing NIWA's reader 1 and 2 for the NIWA preparations, gave a mean ratio for reader 1/reader 2 of 1.003, but a ratio of 1.022 for reader 2/reader 1. Comparing CAF's readers 1 and 2 on the CAF preparations, gave a mean ratio for reader 1/reader 2 of 1.022, but a ratio of 1.043 for reader 2/reader 1. The median ratios were all 1.000. The higher the reading error, the more pronounced this effect. Median ratios and bootstrapping were used to estimate the 95% confidence intervals. For the regression (see below), a log transformation was used which gives results similar to a median ratio when parameters are back-transformed.

Intra- and inter-reader variability (consistency) between otolith readings can be quantified using several methods. The most commonly used methods are: the index of average percentage error (IAPE), age bias graphs, and age difference plots. Details of these methods can be found in Campana et al. (1995) and Campana (2001).

The IAPE method was applied here and was based on data for each fish. For individual readers, the otolith pair data was used. For each institute, up to four readings from the two readers were used.

Another estimate of error obtained was from the residual error in the log regression. This error is equivalent to a CV and it can be converted into an IAPE by CV/1.41 (Campana 2001). A log regression (equivalent to a median ratio if the log data distribution is symmetric) was used to investigate drift and bias. The terms are given in Table C1 and the model used was:

log(y) ~
-1 + fish + prep:reader:institute + reader:institute + institute + TZR:reader:institute

Table C1: Variables used in the regression, log(y) ~ -1 + fish + prep:reader:institute + reader:institute + institute + TZR:reader:institute. The ":" denotes a nested effect, e.g., "reader:institute" is the reader effect nested within institute, and since there are two codes for reader (r1 and r2) this means that there is one parameter for each institute (in this case for r2, as the effect for r1 is set to zero in log space). The "n" in brackets is the number of parameters estimated in the term.

Code/term	Variable	Comment or parameter interpretation
y	Estimated age in years	For each fish, there are 8 estimates from the 4 readers and the two otoliths.
-1		Do not estimate an overall mean; needed for the "fish" term.
fish	Fish identifier, 1 to 160 (n=160)	Mean log age for the NIWA prepared otolith read by r1 at NIWA, as read when a TZ code of 0 is recorded.
prep	Preparation site; codes are NIWA or CAF	
reader	Reader code within an institute; codes are r1 or r2	
institute	Institute code, NIWA or CAF (n=1)	Parameter relative to NIWA and "r1" in both institutes; CAF_r1/NIWA_r1 in age space.
TZR	Transition zone (TZ) variable; codes are no_TZ (code=0) or TZrecorded (codes 1, 2, and 3)	See text above for the meaning of TZ codes.
prep:reader:institute	Preparation site effect, one for each reader (n=4)	Parameters relative to the NIWA prepared otolith for the same reader; CAF/NIWA in age space.
reader:institute	Within-institute reader effect, one parameter for r2 in each institute (n=2)	Parameter relative to r1 within the same institute; r2/r1 in age space.
TZR:reader:institute	Effect of recording a TZ, one effect for each reader (n=4)	Parameter relative to no_TZ seen for the same reader. Needed since there are 48 fish where there was at least one reading (from 8) with a TZ code of 0 and others with a TZ code of 1, 2, or 3.

The regression was bootstrapped to obtain the standard errors since those for the "TZR:reader:institute" parameters were under-estimated in the regression. Parameters are reported back-transformed into the age scale.

A QQ-norm plot of the standardized residuals was used to compare the residual distribution with the standard normal distribution.

Maturity ogive

The maturity ogive was estimated by fitting a logistic curve to presence/absence of a TZ in the otolith data. A separate curve was fitted to each reader's data. When Francis (2006) carried out this analysis, the CAF data curves were very flat and did not reach a proportion of 'one' since a TZ was often not routinely recorded. Francis (2006) also found that the non-recorded TZ by CAF readers resulted in a large bias between NIWA and CAF ages.

Results

Age data

Two readers from NIWA and CAF re-aged orange roughy sister otoliths taken from the plume region, Chatham Rise, from 160 paired sections of 320 otoliths (left otolith NIWA prepared, right otolith CAF prepared). Since there were two otolith sections per fish and four readers, there were up to 8 values for each section. Total age counts by reader for the NIWA and CAF prepared samples are shown in Table C2. Total counts varied between readers due to otolith section readability.

Table C2: Summary of readings and readability by of both NIWA and CAF prepared otolith sections.

	No. read	No. unreadable	Total counts
NIWA prepared sample			
Reader 1 NIWA	158	4	154
Reader 2 NIWA	158	2	156
Reader 1 CAF	158	2	156
Reader 2 CAF	158	5	153
CAF prepared sample			
Reader 1 NIWA	159	15	144
Reader 2 NIWA	159	14	145
Reader 1 CAF	159	21	138
Reader 2 CAF	159	27	132

The rate of unreadable otoliths was lower for NIWA prepared otoliths than for the CAF samples. There appears to be some individual variability between readers as to what constitutes an unreadable otolith (Table C3). When considering the results from all four readers, at least one reading was obtained for 94% of the CAF prepared otoliths and 100% of the NIWA prepared otoliths. For at least one out of four readers, 20% of the CAF prepared sections and 6% of the NIWA prepared sections were unreadable.

Table C3: Number of otoliths reported to be unreadable by readers 1–4 by preparation, and numbers where an otolith was reported unreadable by just one reader.

Type	Preparation	NIWA, R1	NIWA, R2	CAF, R1	CAF, R2	Total	Number where all 4 readers concurred
Only occurrence	NIWA	2	0	0	3	5	–
Total	NIWA	6	4	4	7	11	2†
Only occurrence	CAF	0	0	4	8	12	–
Total	CAF	16	15	22	28	33	11*

† 2 otoliths were missing
* 1 otolith was missing

There were some between-reader differences in the identification of a TZ, with a higher proportion of the CAF readings having no TZ recorded. The difficulty in interpreting the TZ was described above (Section 3.3.4, Figure 7).

Of the aged samples, about 27% (44 sections) had a mixture of ‘no TZ’ or ‘TZ present’ recorded. Of those 44 otolith sections, 15 had one reader record a TZ for one of the sister otoliths when all other readers did not, and of these 15 recorded TZs, 12 were made by NIWA readers. At the other extreme, 8 otolith sections had one instance of no TZ recorded by one reader when all the other readers had recorded a TZ, and of these 8 samples, 6 recorded TZs were made by the two CAF readers.

When identifying the transition zone, no clear bias was noted with either the preparation method or the institute.

Precision

IAPEs were estimated to be 7.9% (NIWA reader 1), 10.3% (NIWA reader 2), 7.8% (CAF reader 1), and 6.0% (CAF reader 2). Combining data for each otolith by institute gave IAPEs of 10.1% for NIWA and 8.9% for CAF, and, for all data combined, an IAPE of 11.0%. In comparison, New Zealand age and growth studies of the deepsea species smooth oreo and black cardinalfish found ageing precision estimates of 6% and 12%, respectively. Campana (2001) reported a median IAPE from 117 published fish ageing studies of 5.4% with a mode at 3.6% and an extreme upper limit of 19%. The orange roughy data in this study, therefore, are at the higher end of ageing precision estimates.

From the log regression (see below), the residual standard error from the regression was 0.1351 on 1005 degrees of freedom. This is equivalent to a CV of 13.5 % for an estimated age from one reading by one reader, and is equivalent to an IAPE of 9.6%.

Regression

The estimated effects expressed in years and their bootstrapped 95% CI are shown in Table C4, with the parameter interpretation in Table C5. About 95% of the residuals were normal (Figure C1), with a standard deviation of 0.875 of the regression-estimated standard deviation. About 5% of the residuals had a bootstrapped standard deviation greater than the regression-estimated standard deviation.

In the setup of the parameters (see Table C4), effects are made relative to one another, i.e., one implied parameter is set to 0 in log space. Hence, the “institute_caf” parameter (=0.916) is for CAF reader 1 relative to NIWA reader 1, so CAF reader 1 has ages about 8% lower than NIWA reader 1 for the NIWA prepared otoliths. For the parameter reader_r2:institute_niwa (=0.982) we see that NIWA’s reader 2 has ages 0.982 times that for NIWA’s reader 1 for the NIWA prepared otoliths, and for the parameter reader_r2:institute_caf (=1.06), the CAF reader 2 has ages 1.06 times that for CAF’s reader 1 for the NIWA prepared otoliths, and so on.

Table C4: Parameter estimates, CV of estimates, and the 95% confidence intervals (CI). **, parameters statistically different from 1 (which denotes no difference) at the 5% level. See Table C1 for the term definitions. For the parameter "reader_r2:institute_niwa", the term is "reader:institute" and the parameter is for reader 2 (r2) in the NIWA institute.

Parameter	Estimate (log scale)	Estimate Age scale	CV (%)	95% CI	
institute_caf	-0.088	0.916	1.9	0.883–0.953	**
reader_r2:institute_niwa	-0.018	0.982	1.3	0.957–1.006	
reader_r2:institute_caf	0.059	1.061	1.7	1.021–1.096	**
prep_caf:reader_r1:institute_niwa	0.022	1.022	1.5	0.992–1.054	
prep_caf:reader_r2:institute_niwa	-0.047	0.954	1.9	0.917–0.990	**
prep_caf:reader_r1:institute_caf	0.012	1.012	1.7	0.976–1.045	
prep_caf:reader_r2:institute_caf	-0.003	0.997	1.2	0.971–1.019	
reader_r1:institute_.niwa:TZR_TZrecorded	0.244	1.276	2.8	1.208–1.343	**
reader_r2:institute_.niwa:TZR_TZrecorded	0.287	1.332	3.1	1.256–1.408	**
reader_r1:institute_caf:TZR_TZrecorded	0.319	1.376	2.8	1.300–1.449	**
reader_r2:institute_caf:TZR_TZrecorded	0.215	1.240	2.7	1.171–1.306	**

Table C5. Interpretation of parameters shown in Table C4.

Otolith-NIWA reader	Mean calculated by
NIWA prepared otolith, reader 1	fish_i + reader_r1:institute_.niwa:TZR_TZrecorded
NIWA prepared otolith, reader 2	fish_i + reader_r2:institute_niwa + reader_r2:institute_.niwa:TZR_TZrecorded
CAF prepared otolith, reader 1	fish_i + prep_caf:reader_r1:institute_niwa + reader_r1:institute_.niwa:TZR_TZrecorded
CAF prepared otolith, reader 2	fish_i + reader_r2:institute_niwa + prep_caf:reader_r2:institute_niwa + reader_r2:institute_.niwa:TZR_TZrecorded

The largest effect is the "reader:institute:TZR" effect, whereby regardless of the institute or the reader, ages are estimated to be about 30% higher when a TZ is recorded as being present than is the case when no TZ is recorded (Table C4, last four rows). Figure C2 shows the effect when both a TZ and no TZ are recorded for the same fish. Thus, the approximate value of these parameters is related to the latter data. There are relative differences in these parameters between the readers which would show up in the median ratios for data restricted to when a TZ was recorded. For the NIWA readers' data from the NIWA prepared otoliths, the regression would predict a median ratio when TZ was recorded, reader 1/reader 2, of exp(0.244 –(-0.018 + 0.287)) = exp(-0.025) = 0.975. From the actual data, this median ratio was estimated to be 0.933. For CAF readers, the same median ratio is predicted to be exp(-0.088 + 0.319 –(-0.088 + 0.059 + 0.215) = 1.046, compared to that from actual data of 1.050.

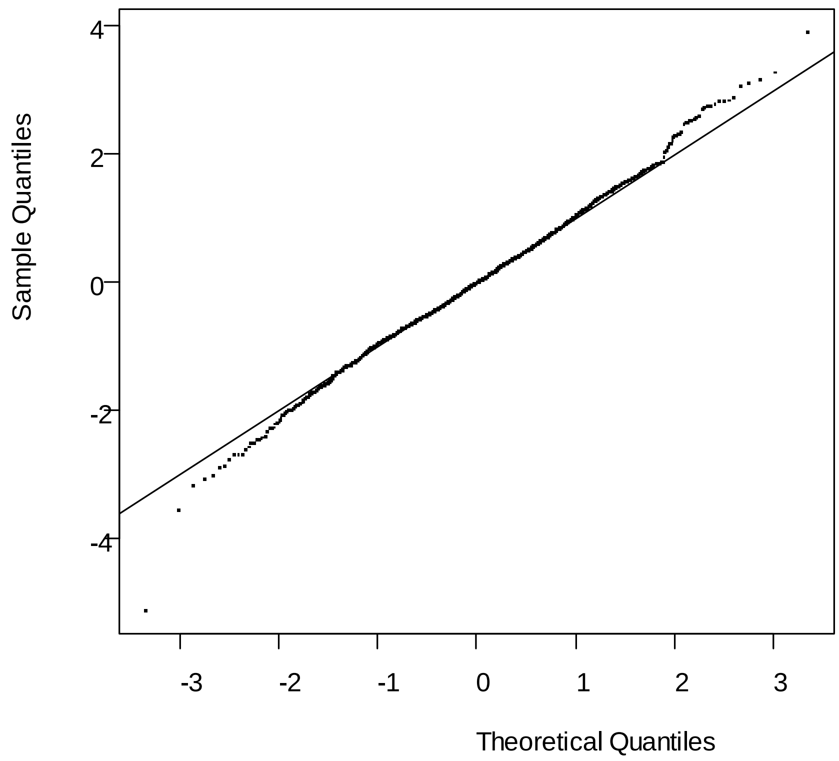

Figure C1: QQ-norm plot of the standardized residuals from the log regression.

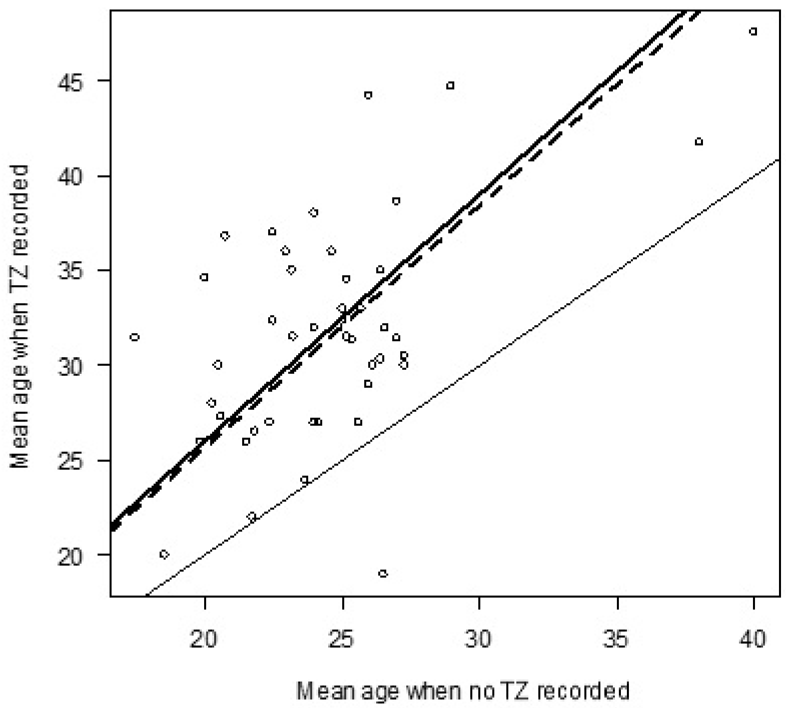

Figure C2: Mean age for a fish when no TZ was recorded compared to when a TZ was recorded. Data are from the two sister otoliths and 4 readers when both no TZ and a TZ were recorded for the same fish (44 fish), i.e. up to 8 values. The thin line is the 1:1 line. The thick solid line has a slope of 1.3, which is approximately equivalent to that estimated in the regression above, and the dotted thick line is a regression through these data with no intercept.

The next largest effect is the institute parameter, which has the CAF ages being about 8% lower than those for NIWA. However, this parameter is relative to the reader 1s and the NIWA prepared otoliths. The institute effect can be made relative to the reader 2s and the CAF prepared otoliths. In log terms this is given by:

log(y_CAF.r2_prep_CAF) – log(y_NIWA.r2_prep_CAF), i.e.,
(institute_CAF + fish_i + reader_r2:institute_caf + prep_caf:reader_r2:institute_caf) –
(fish_i + reader_r2:institute_niwa + prep_caf:reader_r2:institute_niwa)

The fish_i parameters cancel out, so the effect is given by:
(-0.088 + 0.059 – 0.003) – (-0.018 – 0.047) = 0.033, or 1.034 in the age scale, i.e., CAF has ages about 3% higher than NIWA. Consequently, perhaps a more direct way to estimate this effect is to use the median ages over the CAF readers to that for the NIWA readers. The result is 0.947 (95% CI, 0.925–0.963), or -0.054 in the log scale.

The other significant effects are CAF's reader 2 relative to their reader 1 and for NIWA's reader 2 reading the CAF prepared otolith relative to the NIWA prepared otoliths (both by about 6%).

There was no systematic difference in ages between the NIWA and CAF prepared otoliths, but the CAF prepared otoliths did have a higher proportion of unreadable otolith sections.

Maturity ogive

The maturity ogives (assuming age at TZ is age at maturity) are shown in Figure C3; these show none of the problems seen by Francis (2006). However, at the lower ages (i.e., 20–26 years) there were some interpretational differences. NIWA reader 2 recorded the highest proportion of mature fish (TZ present) in this lower age group, in contrast to CAF reader 1 who recorded no mature fish (i.e., no TZs) in this age group. The range of the estimated age at 50% maturity was from 28.2 to 29.8 years. These values encompass the estimated age at maturity range currently used in assessments of Chatham Rise orange roughy (i.e., 28.5–29 years, Ministry of Fisheries 2008).

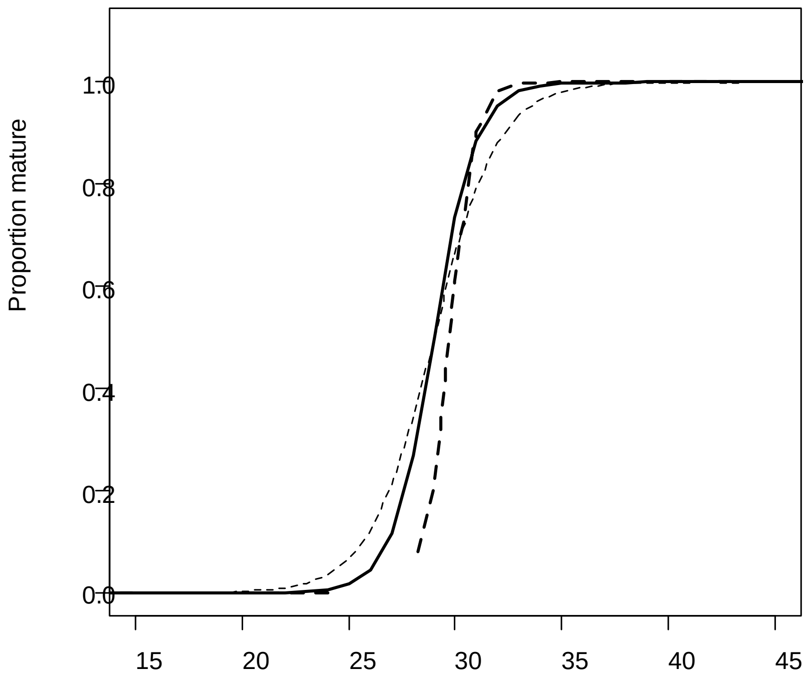

Figure C3: Maturity ogive estimated from the presence of a TZ by the four readers. Thick line, NIWA reader 1; thin line, NIWA reader 2; thick dashed line, CAF reader 1; thin dashed line, CAF reader 2.

Summary and Recommendations

Previous work had highlighted many problems with age determination of orange roughy otoliths, resulting in the age data being excluded from stock assessments. This work reported here has improved otolith reading protocols and the consistency of otolith reading, with repeated reading of the same sets of otoliths by NIWA and CAF (FAS) showing better consistency between institute and no bias. Nevertheless, the ageing imprecision remains relatively high compared to other species, particularly around the transition zone, and there are still some intrinsic problems with age determination of orange roughy.

The study found that identification of a TZ or otherwise still has a 30% effect on the perceived age of the fish. Therefore the reading protocol for the TZ and its readability criteria should ideally be strengthened, but there are no obvious ways to do this.