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Age determination protocol for freshwater eels (*Anguilla dieffenbachii, Anguilla australis*)

New Zealand Fisheries Assessment Report 2016/02
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This report documents the age determination protocol for two freshwater eel species, the longfinned (*Anguilla dieffenbachii*) and shortfinned (*Anguilla australis*), both important New Zealand species. The protocol describes current scientific methods used for otolith preparation and interpretation, ageing procedures, and the estimation of ageing precision, and also documents the changes in these methodologies over time. In addition, an otolith reference collection numbering approximately 130 preparations has been compiled for elver, juvenile and adult eels and agreed readings and ages determined for the reference set are stored in a reference table (*t_reference*) in the *age* database. The reference set sample was generally a random selection from collections made over the spring and summer months from a wide range of North and South Island freshwater habitats to account for spatio-temporal variations in otolith readability, however the selection process also ensured a range of fish size and age were included.

Digital image examples of otolith reference set preparations are presented to illustrate the zone interpretation used in determining age for elver, juvenile and adult eels. 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.

To maintain accuracy and consistency in ageing fish in New Zealand, the Ministry for Primary Industries (MPI) (previously Ministry of Fisheries) held a fish ageing workshop in Wellington (May 2011), producing a document "Guidelines for the development of fish age determination protocols" (Ministry of Fisheries Science Group, 2011) based on the workshop's results. From this, it was anticipated that age determination protocols would be developed for every species that was routinely aged through MPI funding.

This report describes the age determination protocols for glass eels, elvers, juveniles and adults of the two New Zealand species of freshwater eels: the longfinned (*Anguilla dieffenbachii*) and shortfinned (*Anguilla australis*). Eels form an important food source in customary Maori practices, and significant commercial fisheries operate throughout the New Zealand's accessible freshwaters (lakes, rivers, streams, farm ponds, tarns) and some estuarine and coastal waters. MPI has initiated research that monitors the relative abundance of elvers arriving at large in-stream barriers to provide an index of eel recruitment into New Zealand's freshwaters (MPI 2014), utilising routinely collected catch-at-age information. The purpose of this protocol is to describe methods used for otolith preparation and age determination to ensure accuracy and consistency over time, not only for elvers, but for freshwater eels of all ages.

Of the three otolith pairs occurring in bony fishes (asteriscae, lapillae, sagittae), only the largest, i.e., the sagitta, have been used to age eels. Therefore, throughout this report, the use of 'otolith' will be synonymous with sagitta. A glossary describing otolith terminologies and ageing definitions outlined in the "Guidelines for the development of fish age determination protocols" has also been included in this report for reference purposes (Appendix 1).

Overall objective

1. To develop age determination protocols for Inshore Finfish species.

Specific objective

1. To develop an age determination protocol for longfinned eel (*Anguilla dieffenbachii*), including the compilation of otolith reference collections.

2. To develop an age determination protocol for shortfinned eel (*Anguilla australis*), including the compilation of otolith reference collections.

2. AGE DETERMINATION PROTOCOL FOR FRESHWATER EELS

2.1 Background

Biology of eels

Freshwater eels have a life history unique among fishes that inhabit New Zealand waters. All *Anguilla* species are facilitative catadromous, living predominantly in freshwater and undertaking a spawning migration in late summer to early autumn to an oceanic spawning ground (MPI 2014). They spawn once and then die (i.e., are semelparous). The major part of the life-cycle is spent in freshwater or estuarine/coastal habitat. Spawning of New Zealand species is presumed to take place in the south-west Pacific. Progeny undertake a long oceanic migration to freshwater where they grow to maturity before migrating to the oceanic spawning grounds. The average larval life is 6 months for shortfins and 8 months for longfins.

The longfinned eel is endemic to New Zealand and is thought to spawn east of Tonga. The shortfinned eel is native to New Zealand and can be also found in South Australia, Tasmania, and New Caledonia. Shortfinned eels are thought to spawn northeast of Samoa. Larvae (*leptocephali*) of both species are transported to New Zealand largely passively on oceanic surface currents, and the metamorphosed juvenile “glass” eels enter freshwater from August to November. The subsequent upstream migration of elvers (pigmented juvenile eels) in summer distributes eels throughout the freshwater habitat. The two species are abundant throughout New Zealand and have overlapping habitat preferences, with shortfins predominating in lowland lakes and slow moving muddy rivers; and longfins preferring fast flowing stony rivers, penetrating further inland to high country lakes.

Ageing eels

The first attempt at ageing freshwater eels in New Zealand was undertaken by MacFarlane (1936) in a study that focused on parasitic trematodes in freshwater fish hosts in the Canterbury region. To determine age and growth estimates for a sample of 70 longfinned eels, MacFarlane investigated scales in conjunction with otoliths (presumed whole) and length frequency distributions, tabulating mean length-at-age estimates for fish between 3 and 8 years old, finding length modes to correlate with otolith annuli. This was based on an assumption that the nucleus of the otolith was complete at the time the eel larvae first reached freshwater, and assumes that the inner pair of dark rings (herein referred to as zones) in the otoliths indicate a two year period spent at sea, meaning that the youngest freshwater eels measuring 6 cm in spring are in their third year of life. Using scales to estimate age in large eels, MacFarlane postulated that longfin eels 70–80 cm migrate to sea at 12–14 years from hatching, and that an eel 100 cm in length would usually be 15–17 years old. Cairns (1941) states that MacFarlane was able to prove that dark (winter) and light (summer) zones in eel otoliths represent complete years of growth.

In a study titled “The life history of the two species of New Zealand freshwater eels”, undertaken during the late 1930s with collections made from all parts of the country, Cairns (1941) examined scales mounted in water and laterally ground[1] whole otoliths mounted in aniline oil, counting dark and light zones to determine age. Cairns presented age and growth data for 475 shortfinned and 574 longfinned eels, finding shortfin to grow more rapidly in length than longfin, but longfin attaining a larger size. The oldest recorded age by Cairns for shortfinned eels was fourteen years (92.5 cm) and for longfinned eels was nineteen years (145.5 cm). Although Cairns (1941) assumed that zones in scales and otoliths were annual, he did not attempt to validate this assumption.

After completing a thesis on the ecology of New Zealand freshwater eels in 1955, Burnet (1969) investigated the growth of both longfinned and shortfinned eels in three Canterbury streams during the late 1950s and early 1960s by tag and recapture, using otoliths to determine the age of small eels (up to about 20 cm) to fill the gap where data were lacking, and to confirm the adult growth curve. Although Burnet did not define the preparation method used in examining otoliths for age, it is unlikely he followed the same approach as Cairns (1941), achieving good agreement in the growth rates between tagged eels and otoliths, thus validating otoliths as an ageing tool. Burnett demonstrated much slower growth than the otolith results of MacFarlane (1936) and Cairns (1941), attaining ages of up to 30 years for shortfins and 60 years for longfins. As a result, ages derived for eels using whole otoliths in previous studies (i.e., MacFarlane 1936; Cairns 1941) are likely to have been underestimated, particularly for old fish, where the marginal annuli are usually crowded. Burnet (1969) found otoliths from the smallest eels easiest to read, and agreed with modes in length frequency data for eels up to two to three years, but stated that beyond this age, length data cannot be used because of the great individual variation in growth. Furthermore, he determined that females grow faster than males, and that otoliths from shortfins show more distinct zones than those from longfins. Jellyman (1974) records that Cairns (1941) and Burnet (1969) used 'age in years' rather than 'freshwater life' as a measure of age, assuming a sea-life of two years, and therefore may have overestimated age, as did MacFarlane (1936).

During the 1970s, a considerable volume of research in four university theses was dedicated to New Zealand freshwater eels, including age determination. These studies investigated the biology of juvenile eels (Jellyman 1974); the reproductive biology of eels (Todd 1974); sex, growth and distribution of longfinned eels (Harries 1974); and ecology of shortfinned eels (Ryan 1978). Along with subsequent papers on scale development and age determination in eels (Jellyman 1979b), and size and age of migrating eels (Todd 1980), a combination of this literature formed the basis of the methods used for ageing freshwater eel otoliths today.

Jellyman (1974, 1979b) examined both scales and otoliths collected from the Wellington region to determine age in eels. Scales were selected from various parts of the body to find those showing the maximum number of zones, and this number was found to be linear with fish length, but with no seasonal pattern in zone formation, it was concluded that scale zones were not indicative of fish age. Neither could age be determined from the length frequency data collected during the study due to the overlapping of adjacent year classes. Nevertheless, Jellyman (1974, 1979b) found that otoliths (whole, fish under 9 cm; whole and laterally ground, 8–25 cm; break and burn, over 25 cm using his own technique, but similar to Christensen (1964)[2] and Moriarty (1973)[3] burn and break methods), regardless of the preparation method, showed a pattern of broad opaque and narrow hyaline (herein referred to as translucent) zones, reflective of summer and winter growth. Jellyman (1974) found that laterally ground whole otoliths resulted in a loss in the margin, which removed zones and led to increased ageing error. In comparison, break and burn otoliths (embedded in plasticine) showed greater contrast and sharper boundaries between adjacent zones, with translucent zones depicted as distinct heavy lines. To demonstrate the level of difficulty associated with ageing eels, Jellyman (1974) developed a five point readability scale for ageing. Jellyman also determined that with

[1] Prepared method for laterally ground otoliths involves fitting the otolith on the end of the forefinger with the convex side outermost and grinding the otolith surface in a circular motion on a grinding base. This methodology can remove the obvious portion of marginal increments making them difficult to observe.
[2] Prepared eel sagittal otoliths by burning in a flame and cracking to reveal annuli on the broken face.
[3] Prepared eel otoliths in a similar manner to above but made permanent mounts of otolith fragments.

residence in freshwater at the glass eel stage, the outer margin of the otolith becomes differentiated into a definite translucent zone (colloquially recognised as the "freshwater check") with successive concentric opaque and translucent zones laid down around this, most clearly seen and well-spaced along the longest (ventral) axis of the otolith. Jellyman was the first to note the presence of secondary or subsidiary zones in New Zealand freshwater eel otoliths, describing them as: often narrow, incomplete, do not traverse a full circle, and show various degrees of opacity with the translucent boundary thin in comparison to annual opaque and translucent zones.

Similar to Jellyman (1974, 1979b), Todd (1974, 1980) also used the break and burn method for age determination with the modification of the preparation methods of Christensen (1964) and Moriarty (1973). Todd's method was to hold the otoliths in a bunsen flame and then embed the burned edge uppermost of both otolith halves into clear silastic silicone and view them in a bath of paraffin oil using reflected light. Todd collected otoliths from eels at three different regions (Wellington, Wairarapa and Canterbury) and using reflected light counted the number of black winter (translucent) zones along the otolith ventral radius to estimate fish age. However, to accurately determine the age of adult eels, Todd first examined whole otoliths from glass eels (52–62 mm) caught in September. These showed a black translucent centre (core) surrounded by a broad opaque white zone with a thin dark hyaline zone (freshwater check) present on the otolith edge; with subsequent opaque (summer) and translucent (winter) zones formed outside this check in older eels. Todd determined the calculation of age starts at the first black winter zone formed in freshwater (the next translucent zone outside the freshwater check), and the complete formation of this zone and the beginning of the second summer zone (in freshwater) represent the first complete year of freshwater life. Todd found most otoliths from adult eels to be difficult to read due to the crowding of zones, stating that many ages were only approximations, and for discrepant readings between otolith halves used an average to obtain age.

Harries (1974) initially trialled laterally ground whole otoliths to age longfinned eels from the Otago province, principally following the technique documented by Cairns (1941) and found the method to be slow and unsatisfactory, due to the grinding process removing marginal zones, or making them difficult to distinguish. Similar to Jellyman (1974) and Todd (1974), Harries adopted the break and burn technique of Christensen (1964) and found the procedure simple, and the clarity of the zone sequence in prepared otoliths superior to that produced by grinding whole otoliths, depicting a series of summer (white) and winter (dark) zones, counting fully formed winter (translucent) zones to determine fish age. Although non-annual 'intermediate' zones were evident among summer zones, zone patterns between otolith pairs were identical and therefore only one otolith of the pair was read. In ageing shortfinned eels from Te Waihora (Lake Ellesmere), Ryan (1978) followed the same techniques as Harries (1974), but found that the break and burn preparations using the Christensen (1964) method resulted in a ragged edge and proved difficult to read. Instead he adopted a modification of the method of Moriarty (1973), later developed by Hu & Todd (1981), where the otoliths split, usually across the short axis, of their own accord while in the bunsen flame, and once cooled are inserted face upwards in silicone rubber onto a glass microscope slide. Although recognising Jellyman's (1974, 1979b) description of zone deposition in eel otoliths, a five point readability scale, and an October birthday, Ryan (1978) incorrectly counted the first black translucent freshwater check as the first annulus and would have therefore overestimated age by one year. Furthermore, no attempt was made to establish whether the otolith margin represented summer or winter growth due to it being badly charred, which was thought to also underestimate age by one year. Both Harries (1974) and Ryan (1978) used the annual formations in break and burn eel otoliths to back-calculate fish lengths at earlier ages in order to estimate eel growth rates.

The periodicity of annual zone deposition in freshwater eel otoliths was first validated by Jellyman (1974, 1979b) using mean marginal increments (the reciprocal of the width of the outer otolith zone divided by the width of the penultimate zone) in break and burn shortfinned eel (over 15 cm) otoliths; and showed that translucent zones were deposited annually in early spring (September to October), and opaque zone formation commences from October. Direct validation that translucent zones were formed annually in otoliths was achieved by Chisnall & Kalish (1993) from recaptured tagged shortfinned and longfinned eels injected with oxytetracycline (OTC). The ageing methodology was supported by two further studies: a translocation experiment of tagged longfinned eels, where recaptured fish otoliths depicted nine years of completed growth following the observed growth inflection on the otolith from the time of release (Beentjes & Jellyman 2015); and ages of juvenile eels validated by trends in length frequency of different year classes over six consecutive years (Graynoth & Jellyman 2002).

Numerous studies involving the age and growth of freshwater eels in New Zealand were completed over subsequent decades (Chisnall, 1987, 1989, 1993, Chisnall & Hayes 1991, Chisnall & Hicks 1993, Graynoth & Jellyman 2002, Jellyman & Ryan 1983, Jellyman 1995, Jellyman et al. 1995, Jellyman & Sykes 1998, Jellyman & Cranwell 2007, Beentjes 1998, Beentjes & Jellyman 2015) including a few catch sampling programmes (1995–96 to 1998–99 and 2000–01) that estimated age-weight (i.e., age at the minimum legal size of 220 g) and age-length relationships and annual growth increments for eels caught from North and South Island commercial eel fisheries (Beentjes & Chisnall 1998, Beentjes 1999, Chisnall & Kemp 2000, Speed et al. 2000). The vast majority of age-related research on eels has focused almost entirely on break and burn otoliths as the preferred ageing method, following a combination of the techniques described by Jellyman (1979b), Todd (1980), Hu & Todd (1981), some with modifications by Graynoth (1999). Graynoth (1999) proposed alternative otolith preparation techniques for ageing eels to improve the accuracy of age estimation, which were implemented in some subsequent ageing studies (Graynoth & Taylor 2004, Graynoth et al. 2008), and also developed useful criteria to distinguish annuli (translucent zones) from opaque zones and non-seasonal secondary zones. In a few of the studies (Chisnall & Hicks 1993, Jellyman et al. 1995, Jellyman & Cranwell 2007) that estimate eel growth, only a proportion, usually about half, of otoliths were used, typically those read with confidence, reflecting the inherent difficulty researchers face in accurately ageing eels.

Jellyman (1974) was the first to check for consistency in otolith readings by independently re-reading a set of whole ground otoliths nine months after the initial reading, finding considerable differences in interpretation. Over two decades later, two readers were first used by Jellyman et al. (1995) to check on the consistency of reading a subset of break and burn otoliths, and in finding low agreement between readers, chose only high readability otoliths for analysis. Speed et al. (2000), on the other hand, found independent verification of a random sample of otoliths to be highly consistent between readers, achieving considerably higher reader agreement than Graynoth's (1999) preliminary trial, where over 80% of burnt otoliths were aged within ± 1 year by different readers.

Further support for validation of the ageing methodology may be contributed by year-to-year consistency in the relative strength of cohorts, or the existence of year class strength patterns within a fishery, as demonstrated by Graynoth & Jellyman (2002) for juvenile shortfinned eels in Te Waihora. Although estimating length-at-age using the break and burn methodology has been common to the vast majority of eel population stock status studies since the mid-1970s, albeit mainly within discrete lake or river systems throughout New Zealand, seldom have comparisons of patterns of relative year class strengths been reported, particularly for adult eels, either within (or by sex) or between surveys. Jellyman & Cranwell (2007) found, from age distributions, that there was evidence that years of "better than average recruitment" were the same for both species of eel in Wairewa (Lake Forsyth); while Jellyman & Sykes (1998) showed at least two-fold differences exist between some age classes of shortfinned eels in the Waihao fishery. The strength of recruitment indices may be affected by a number of factors, including the diel and seasonal timing of glass eel recruitment, delayed recruitment of ontogenetic life stages, anthropogenic (i.e., dams) or natural influences; notwithstanding the difficulty in accurately ageing eels (Jellyman et al. 1995, Jellyman 1995, Horn 1996, Graynoth et al. 2008, Graynoth & Jellyman 2002). Nevertheless, glass eel recruitment has been shown to vary between years (Jellyman 1979a, Jellyman et al. 2002), sometimes by a factor of 10 (Francis & Jellyman 1999), although recruitment into coastal lakes (i.e., Te Waihora) may not show the same natural variations as nearby regions, due the duration and timing of lake openings (Graynoth & Jellyman 2002).

The two species of New Zealand freshwater eels are long lived, particularly the longfinned eel (*Anguilla dieffenbachii*), although ages currently stored in the MPI *age* database tend to be predominantly from the commercial catch sampling programme with only 0.6% of the nearly 4000 ages over 50 years. The oldest recorded age in the *age* database determined for longfinned eel is 64 years for 97 cm and 100 cm females captured in the Waiau and Mataura Rivers, respectively. There are about 3500 ages of shortfinned eel (*Anguilla australis*) in the *age* database with only 0.1% over 50 years, the oldest a 54 year old female captured in the Hurunui River. Investigating the longevity of a virgin population of longfinned eels from a high country lake, Jellyman (1995) determined a maximum age of 106 years (from an approximately 115 cm female), finding the population to have the slowest recorded growth rate and highest maximum age of any *Anguilla* spp. to date. For shortfinned eels, Jellyman (1997) reported a maximum age of 60 years (about 70 cm) captured from a small lowland lake.

A third species of freshwater eel, the Australian longfinned (*Anguilla reinhardtii*) was first confirmed by eel fishers from the Waikato catchment of the North Island (Jellyman et al. 1996) and is native to New Guinea, eastern Australia (including Tasmania), Lord Howe Island, and New Caledonia. Evidence suggested that small numbers of *A. reinhardtii* had been present for at least 25 years, confirming a history of self-introduction to New Zealand waters (Jellyman et al. 1996, Chisnall 2000), so much so that *A. reinhardtii* is now a confirmed Australasian species and should be considered as a component of the New Zealand indigenous fauna (Chisnall 2000). Similar to the New Zealand longfinned eel (*Anguilla dieffenbachii*), *A. reinhardtii* is long-lived and attains a large size, but can grow at 2–3 times faster in length and four times faster in weight. It is distinguished by its unusual colouration and black mottled appearance and although considered relatively widespread in its distribution in North Island freshwaters, abundance is low and has mostly been encountered along the west coast (Jellyman et al. 1996, Chisnall 2000). It has also been postulated that *A. reinhardtii* glass eel recruitment to New Zealand is likely to be during autumn-winter, outside of that (spring-summer) of the two New Zealand species, and that fish size may be smaller, making length a useful differentiate between the species (Chisnall 2000), especially when sampling for elvers to determine relative year class strength estimates. Nonetheless, as the catch of the Australian longfinned (*A. reinhardtii*) is infrequent, the information presented within this age determination protocol relates only to the commonly caught New Zealand species of freshwater eel: *Anguilla dieffenbachii* and *Anguilla australis.*

Jellyman (1974, 1979b) determined, using marginal increment analysis, that the annual deposition of translucent zones in eel otoliths occurs at the end of winter and that 1 October provided an appropriate theoretical 'birthday' for both species, and is closely aligned to glass eel recruitment into freshwater (Jellyman 1977, 1979a, Todd 1974, 1980). This represents the agreed time from which the age of the eel begins, and is not inclusive of the *leptocephalus* larval marine phase or related to the spawning period for eels.

There are two published reviews that document age and growth for New Zealand freshwater eels. The first by Skrzynski (1974) reviews the biological knowledge of eels, but records only a paucity of literature dedicated to age and growth studies over previous decades. The second by Horn (1996) is a comprehensive review of published and unpublished age and growth studies and growth-related biology for eels, and presents a growth model to determine factors affecting growth.

2.2 Methods

Sagittal otoliths are acknowledged as the primary structure for ageing New Zealand freshwater eels. All scientific methodologies described in the following sections will be associated with ageing using break and burn (sometimes referred to as crack and burn) sagittal otoliths, following a modification of techniques described by Christensen (1964) and Moriarty (1973), and is currently the preferred preparation method used in National Institute of Water and Atmospheric Research (NIWA) and MPI eel ageing research and the traditional method used for ageing European and American eels (ICES

2009). In New Zealand, the methodology used for preparing and/or ageing eel otoliths was initially described by Jellyman (1974, 1979b) and was further modified and expanded upon by Todd (1974, 1980), Ryan (1978), Hu & Todd (1981), Graynoth (1999) and more recently in an internal unpublished NIWA report titled “Eel ageing and sexing manual”, contributing small but important additions over time. The following sections present additional information pertinent to freshwater eel ageing.

2.3 Otolith preparation

Post extraction, eel otoliths are cleaned of adhering tissue, rinsed in water, dried and stored in micro-centrifuge (commonly referred to as Eppendorf) tubes within paper envelopes labelled with sample details, including trip code (or landing number for catch samples), species code (SFE, LFE), (or landing number for catch samples), fish number, date, length and sex (Figure 1). If the otoliths are collected for an MPI project that does not have a trip code or landing number assigned (e.g., sampling elvers or similar one-off sampling event) then the MPI project code is included on the otolith envelope. The envelopes are stored in labelled boxfiles relating to the project code, fishstock and year of collection, and are archived in the MPI otolith collection, currently housed at NIWA, Wellington. We recommend that each project has its own well labelled boxfile to ensure that otolith envelopes and microscope slide boxes are not mixed with those from other projects and to facilitate ease of access. All otolith material that is archived in the otolith store at NIWA Wellington is also entered into the *age* database so that it can be cross-referenced.

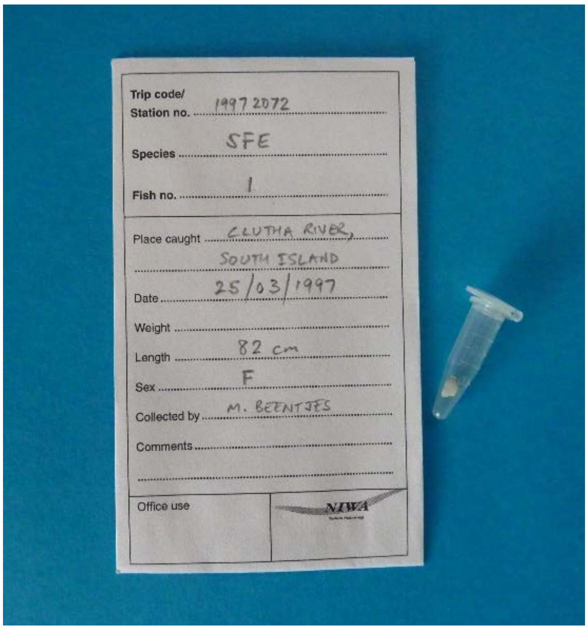

Figure 1: Images of a labelled envelope and microcentrifuge tube used to store otoliths for freshwater eels. If there is no trip code or landing number, project code is included.

Appendix 2 outlines the most recent methodology used for break and burn otolith preparations. In short, otoliths are sawn transversely into halves through the core along the dorso-ventral axis using a scalpel blade; placed on a thick scalpel blade and heated using a high temperature gas flame until both halves turn brown, and the sawn edges mounted in clear ‘Silastic’ silicone downward against a glass microscope slide. We recommend that as break and burn preparations are viewed through the glass slide (see Appendix 2) into the broken otolith half embedded in silicon, that they be labelled on the side of the glass that is uppermost whilst reading so that the fish number can be easily read. When the number written is on the underside it is difficult to read under the microscope.

2.4 Otolith interpretation

A standardised procedure for reading break and burn eel otolith sections generally follows Jellyman (1974, 1979b) and Todd (1974, 1980), but also includes some recent findings/additions, in particular, the identification of the margin state relative to the month of collection. Essentially, when viewed with a compound microscope, a series of opaque (light) and translucent (dark) growth zones are apparent under reflected light (Figure 2). One opaque and one translucent zone is laid down in freshwater eels otoliths each year (Jellyman 1974, 1979b, Todd 1974, 1980), reflective of fast growth over spring-autumn, and slow or no growth over winter. Initial viewing may be undertaken at low magnification (10× objective) to determine which of the preferred sites on the section are the clearest for reading, although high magnification (20–40× objectives) is recommended for accurate zone count and margin interpretation, especially for older fish (i.e., those 15 years of age and older). For young eels (elvers and juveniles) less than about ten years of age, both ventral and dorsal sides of the otolith may be read. For sub-adult and adult eels, counts should only be made along the long radial axis from the core toward the ventral tip and the number of complete translucent zones counted. Zones often coalesce into a single zone on the distal surface, and are concentrated and therefore less discernible along the dorsal axis. If a discrepancy in count occurs between the sectioned otolith halves, the reader rechecks the count until agreement is reached or chooses the highest count determined with confidence, unless given a grade 5 (unreadable), which is removed from the collection.

The main assumptions made when interpreting zones in break and burn eel otoliths are:

1. The dark translucent zone is laid down at the end of winter during a period of slow growth and becomes fully apparent on most otoliths in spring (during October) and the wide light opaque zone is laid down throughout the late spring–early autumn period of rapid growth. The first translucent zone outside the freshwater check is consistent with being the first annual increment.
2. The theoretical 'birthday' for all freshwater eels is 1 October.
3. Translucent zones are counted as being annual.

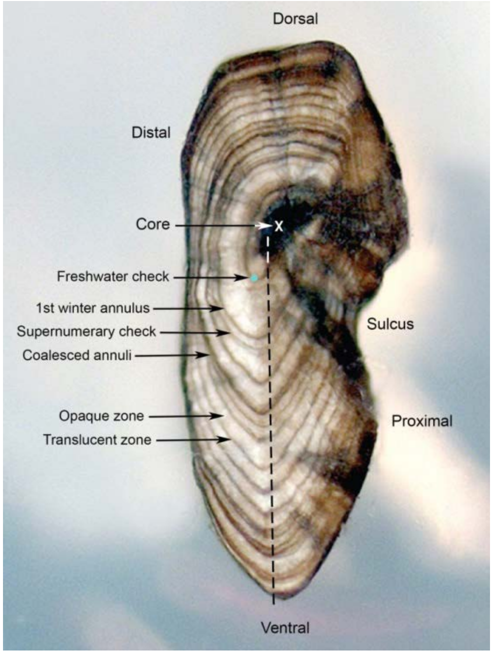

Figure 2: Image of a cut, scorched, and mounted transverse section of a longfinned eel otolith under reflected light, illustrating otolith terminology. From the otolith core to the freshwater check represents the marine phase of a larval freshwater eel. From the freshwater check to the otolith margin represents the eel's recruitment into freshwater and the agreed time from which the age of the eel begins. Counts are generally made along the long radial axis from the core toward the ventral tip (dashed line) and the number of complete translucent zones is counted. This otolith section was interpreted as 11 years old.

Break and burn otolith preparations for three life stages (glass eels, elvers, adults) of freshwater eels are presented to illustrate the differences in age and otolith structure (Figures 3–5).

Glass eels

Metamorphosed juvenile shortfinned and longfinned “glass eels” recruit into freshwater in late winter and spring (August–November, peaking in September) at about 6–8 months of age and 52–71 mm in length (Jellyman 1974, 1979b, Jellyman et al. 1999, Todd 1980), with shortfins slightly smaller than longfins and both species exhibiting greater mean lengths at higher latitudes (Jellyman 1974, Chisnall et al. 2002). Glass eel otoliths are about 0.32–0.40 mm in diameter and when viewed under reflected light comprise a black translucent nucleus (herein referred to as the core) sometimes containing a white spot, surrounded by a broad white opaque zone formed in the last summer of ocean life. A dark translucent zone is present at the otolith margin (Todd 1980), and is commonly referred to as a “freshwater check”, and appropriately named the “zero band” for ageing European and American eels (ICES 2009). Todd (1980) determined a radial distance of core-to-freshwater check of 0.16–0.20 mm (160–200 µm), which brackets Graynoth’s (1999) mean radial distance of 0.17 mm (170 µm). It is important to note that the time spent in seawater as *leptocephalus* larvae, represented by the otolith core to the freshwater check, is not included in the age determination of eels (Jellyman 1974, 1979b, Graynoth 1999) (Figure 3). As a result, the age of glass eels, or pigmented eels comprising only a freshwater check and no first annulus, will be of an age equal to 0 years, and classified as such.

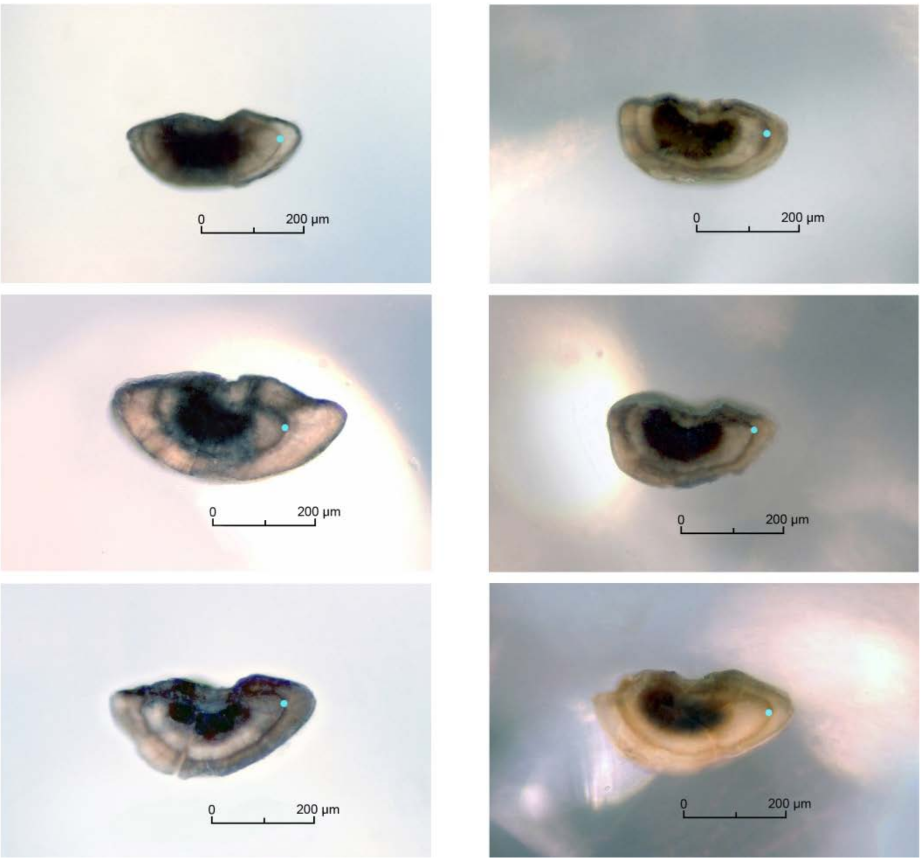

Figure 3: Images of break and burn transverse section preparations of glass eel otoliths under reflected light illustrating the radial measurement (using the scale bar) from the core centre to the freshwater check (blue dot). Samples were collected from the Wairua and Patea Rivers between November and December 2013.

Elver, juvenile and adult eels

The subsequent stage of “elver” (pigmented juvenile) eels in their first year following recruitment from the sea as a glass eel occurs during the following summer when eels distribute throughout the freshwater habitat. However, the term ‘elver’ in New Zealand may also include eels ranging in size from about 6 to 20 cm and up to an age of five or more years (Jellyman & Ryan 1983), therefore comprising fish from a number of age classes. In young elver otoliths, the width of the opaque zone outside the freshwater check broadens over the summer and autumn period and is usually the widest of all zones, with the first annulus deposited during late winter (Jellyman 1974, 1979b) (Figure 4). This represents the first complete year of freshwater life, with subsequent dark translucent zones counted as annuli in older elver (Figure 4), juvenile and adult eels (Figure 5). It is also difficult to distinguish when the last translucent zone is laid down in large (inferring old) eels if growth is slow and the zones become crowded (Jellyman 1974). The distance between the freshwater check and first annual translucent zone in eel ‘elver’ otoliths, reflecting one full year of freshwater growth, is most often greater than that between successive zones. Late recruits and poor transverse sectioning of otoliths (i.e., not through the core or the shortest dorso-ventral diameter) may result in the first annulus being closer to the core. Using marginal increment values in shortfinned eel otoliths to infer somatic growth, Jellyman (1974, 1979b) found seasonal growth rates to vary considerably, with growth over spring and summer rather slow, growth fastest in March, and little or no growth taking place between April and September.

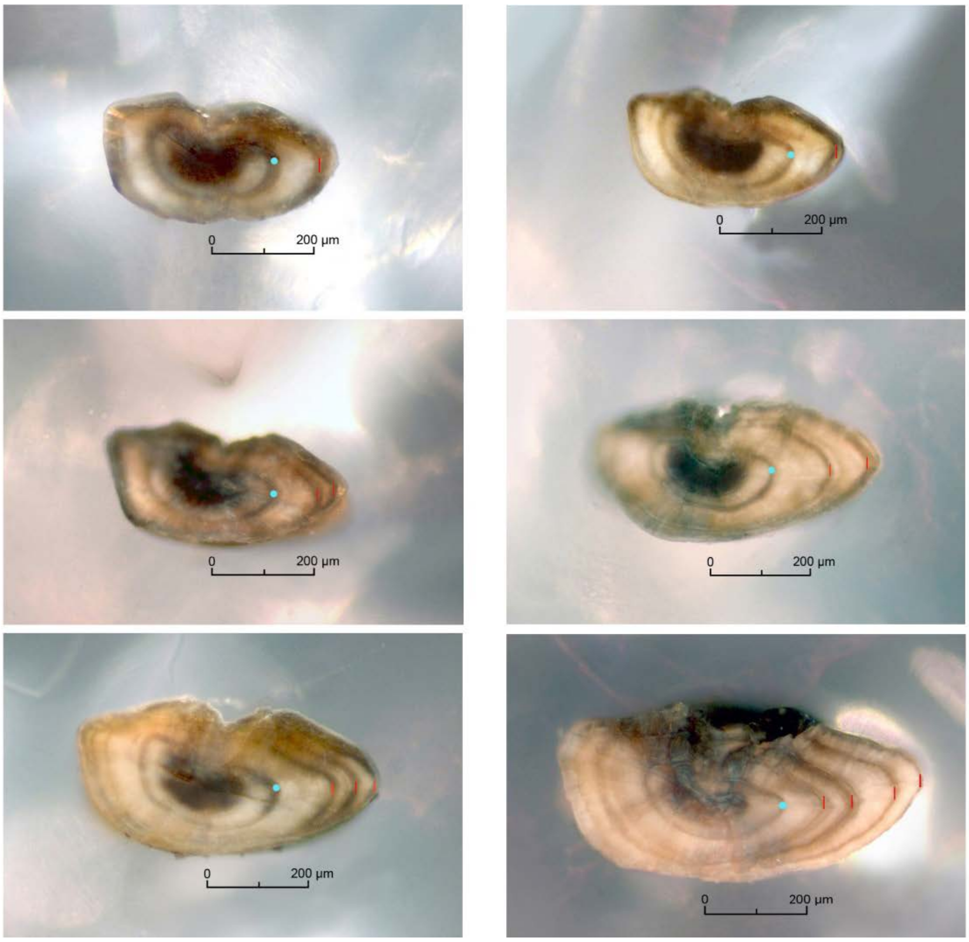

Figure 4: Images of break and burn transverse section preparations of elver otoliths under reflected light illustrating the radial measurement (using the scale bar) from the core centre to the freshwater check (blue dot) and location of subsequent annuli (red bars). Samples were collected from the Patea and Waikato Rivers between December 2013 and February 2014.

The use of a graticule to accurately determine the radial distance and position of the freshwater check, and therefore the first translucent zone, and the count of successive translucent zones for the sectioned otolith, is seen as essential for ageing eels, particularly when zone formation is unclear or secondary zones are present. Furthermore, inter-annual somatic growth of freshwater eels may vary due to density-independent factors such as water temperature and change in diet, or density-dependent factors such as intraspecific and interspecific density and interaction effects for limited area of suitable cover, and the quality and quantity of food available (Burnet 1969, Beentjes & Jellyman 2003, 2015, Chisnall, 1987, 1989, Chisnall & Hayes 1991, Chisnall & Hicks 1993, Graynoth & Jellyman 2002, Graynoth & Taylor 2004, Graynoth et al. 2008, Jellyman 1989, 1991, 1997, 2001, Horn 1996). Such factors will undoubtedly influence the deposition width of opaque zones throughout the fish’s life, which have been found to vary up to fourfold in some instances (Graynoth 1999). As a result, the distance between the subsequent narrow dark (translucent) winter zones used to determine fish age is seldom uniform across the reading plane of the ventral axis (see Figure 5) and the reader must have a good understanding and appreciation of what zones should be counted as annual and those which are not.

Figure 5: Examples of images of break and burn transverse section preparations of juvenile and adult eel otoliths under reflected light. Samples were collected from the Oreti, Mataura, Aparima Rivers and Te Waihora.

Supernumerary checks

The identification of non-seasonal secondary zones (i.e., false or supernumerary checks) can be a major cause of age-reading errors (Panfili et al. 2002) and was seen as a problematic issue in the MPI freshwater eel ageing workshop in March 2015. False checks are common in the first year of growth in many New Zealand inshore finfish species and may be confused with the first annual increment (Walsh et al. 2014a,b,c). Although the incidence of secondary zones in New Zealand freshwater eels may vary between waters, a high proportion of eels have been found to have ‘checks’ close to the core in the first summer of life (Graynoth 1999). Overseas research on secondary zones in eel otoliths suggests that they are common and imply that growth has stopped due to either starvation, low water temperatures, sudden temperature changes, low oxygen concentrations or other factors (Liew 1974, Deelder 1976, Guan et al. 1994, Tzeng et al. 1994, Oliveira 1995, Domingos et al. 2006). For New Zealand eel ageing, secondary zones have been referred to as subsidiary zones (Jellyman 1974, 1979b), false rings (Chisnall & Hayes 1991) or supernumerary checks (Graynoth 1999), the latter following “supernumary or false winter” of Deelder (1976) and considered an appropriate terminology in eel ageing. Supernumerary checks may form within a zone, but show different opacity, colour, width, structure, continuity and spacing compared to ‘true’ zones (Jellyman 1979b, Graynoth 1999). Nevertheless, it has been postulated that supernumerary zones may have led to an overestimation of the number of annuli in freshwater eel ageing studies in New Zealand (Graynoth 1999) and overseas (Deelder 1976, Moriarty & Steinmetz 1979), therefore questioning the accuracy in age estimation of the break and burn method. Improved otolith preparation techniques (i.e., ground acid-etched whole otoliths viewed with light and scanning electron microscope, sawing and burning, acid-etched and stained thin sections) as alternatives to more easily investigate annulus discrimination have been proposed (Tzeng et al. 1994, Graynoth 1999) but some are costly and take longer to prepare.

Criteria for distinguishing between annual growth zones and supernumerary checks in break and burn otolith preparations on the basis of colour, width, structure, continuity and spacing for juvenile and adult eels was documented by Graynoth (1999) and a summary is outlined in Table 1.

Table 1: Criteria for separating translucent zones (winter annuli) from opaque zones (summer growth) and supernumerary checks on break and burn shortfinned eel otolith preparations viewed with reflected light (reproduced in an abbreviated form from Graynoth 1999). 10 µm = 0.01 mm.

Feature	Opaque zone	Translucent zone	Supernumerary check
Colour	Usually white, but may be light brown in narrow zones	Black or dark brown	Light brown
Width	Wider than translucent annuli, always >5 µm, usually >15 µm, mean 61 µm	Usually 4–18 µm, median 10 µm	Narrow <40% of translucent zone width, usually <4 µm and always <10 µm
Fine structure	Thin dark lines or striations occasionally seen	Not apparent	Usually one or two thin lines
Continuity	Continuous band	Continuous band	Sometimes broken and not visible on dorsal axis
Position	Uniform spacing	Uniform spacing	Often present adjacent to core or annulus

To derive an accurate zone count from ageing freshwater eel break and burn otoliths, readings are typically made along the long radial axis from the core toward the ventral tip, designated the primary reading axis, where clear, distinctive alternating translucent and opaque zones are generally visible (Figure 2). Outside the core and freshwater check, the number of complete translucent zones should be counted. Comparison readings should be made from the other otolith half to confirm initial reads, although multiple readings may be required for otoliths without clear definition. If discrepancies occur between counts of the sectioned halves, the default read is to choose the highest count determined with confidence, unless given a grade 5 (unreadable), in which case it is removed from the collection (see readability scale at end of Section 2.4). Discrepancies may occur where readers are unable to accurately identify poorly defined zones, sometimes the first or second annuli, or due to misidentification of the freshwater check, which is not counted when ageing eels. Discrepancies in zone counts may also be due to the break not going through the otolith core (Moriarty 1983), but can also be attributed to the age of the fish, growth rate, collection date, clarity of the deposited structure, and the quality of the preparation.

The conversion of a zone count to an age estimate involves considering the relationship between the date of the increment formation, the date of capture, and the nominal birthdate (Panfili et al. 2002). However, compared to most MPI catch-at-age studies that are undertaken over extended periods (i.e., New Zealand fishing year, October to September), most freshwater adult eel age-related surveys are normally completed within discrete fixed time periods (i.e., between a few days and up to four months; Chisnall & Hayes 1991, Chisnall & Hicks 1993, Jellyman 1995, Beentjes & Jellyman 2015). Furthermore, there appears to have been no requirement historically to determine the otolith margin type (i.e., wide, line, narrow) in the month in which the fish was sampled, unlike in other fish ageing studies (i.e., snapper, trevally, kahawai). Instead, all eel age estimates within a survey have been assumed to be equal to that of the translucent zone count. As the period of the survey is short, the otolith margin type for recruited eels is likely to be roughly the same for all fish captured within the survey period and should not affect the estimate of true age, although very young fish may display changes in zone deposition slightly earlier than adults (i.e., a newly formed opaque zone may be more visibly present in young fish than older fish).

Nevertheless, should surveys be conducted several months apart before and after the birthdate of 1 October, for example during May and then in January, then fish age for the same cohort should theoretically differ by 1 year, as a fully formed translucent zone near the margin will be present only in otoliths collected in January. Knowing increment formation is therefore fundamental in accurately determining fish age and making valid comparisons in catch-at-age compositions, not only within studies conducted over an extended period (i.e., 6–12 months), but also for comparisons made between years, as occurs for long-term MPI catch sampling programmes. Furthermore, anticipating increment formation also allows otolith readers to more accurately assess the otolith margin state in a given month or season, especially where the margin may be unclear due to the burning process inhibiting zone recognition. It is proposed that margin state become a mandatory requirement for all MPI freshwater adult eel ageing studies which will allow for alignment of age with the birthdate (1 October) and in relation to time of capture, and no change in the estimation of same age eels should occur with a change in calendar year (i.e., in samples collected between December and January).

If 1 October is assumed to be the ‘birthday’ of all eels, marking the time at which freshwater life begins (Jellyman 1974, 1979b) and disassociated with spawning (unlike that for most other fish species), then the first translucent zone (annulus) is formed during winter and early spring after about 12 months of freshwater life, and the first opaque zone completed the following autumn (usually about May), with all subsequent zones being laid down annually. Fortuitously, 1 October also marks the beginning of the MPI fishing year, meaning that the age of a freshwater eel within a sampling programme will remain the same throughout the period October to September, with only the margin state changing through time. Therefore, an otolith with four translucent zones collected in December will be approximately 4.17 years old, and one with four zones collected in May will be about 4.58 years old. Based on a calendar year, these fish will belong to the same age class (age group), 4 (Table 2).

Table 2: Diagrammatic representation of the age assignment for freshwater eel in relation to each month of the New Zealand fishing year, October–September. The birthdate for eel is 1 October and the forced margin states used are: L = line, N = narrow, W = wide.

Month	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Age class	4	4	4	4	4	4	4	4	4	4	4	4
Age group	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Decimalised age	4.00	4.08	4.17	4.25	4.33	4.42	4.50	4.58	4.67	4.75	4.83	4.92
Forced margin	L	N	N	N	W	W	W	W	W	W	W	W

Forced margin method

To provide the reader with guidance and improve accuracy and precision in age estimations, a forced margin was implemented to anticipate the otolith margin relative to the month in which the otolith was collected (Table 2), as occurs in other fish ageing studies (i.e., snapper, trevally, kahawai). For ageing freshwater eels in New Zealand, this is dependent upon the position of the outermost translucent zone and is as follows: '**L**ine' (dark translucent zone in the process of being laid down or fully formed on the margin), October; '**N**arrow' (a narrow to moderate light (opaque) zone present on the margin), November–January; '**W**ide' (a moderate to wide light (opaque) zone present on the margin), February–September. Although the timing of the deposition of the newly formed zones is influenced temporally and may vary slightly between individual fish, geographic locality and years, readers are able to anticipate the expected temporal change to the otolith margin in comparison to what they visually see by using the forced margin method, and at the same time allowing for minor variations in zone deposition between otoliths in the collection they are reading. This is particularly important for eels sampled in the later months of the fishing year (i.e., July to September) where a partially deposited newly formed translucent zone may appear on the otolith margin, but must not be counted or will result in an increase in age by 1 year. Similarly, for old eels, the zones close to the otolith margin, although most often regularly spaced, can be narrow and difficult to interpret, occasionally showing variation in incremental deposition (see Figure 5). Although the clarity of the margin appears reasonably clear under low magnification in break and burn preparations, viewing under high magnification can also be problematic with poor preparation (i.e., over burnt, eroded margin), or the presence of residual endolymphatic sac tissue, resulting in reader uncertainty.

To demonstrate the application of the forced margin to ageing eels, consider an otolith sampled in August that has four completed translucent zones and a narrow translucent margin. Using the forced margin method (Table 2), the translucent margin is ignored, and the otolith interpreted as 4W (wide referring to a wide opaque margin). When determining age, however, the sampling date and assumed birthdate are taken into account to assign an age of 4.83 years (Table 2). Ignoring the translucent margin, which may be present from July to September, in some, but not all otoliths of fish from a particular cohort, does not compromise the age determination. In fact the forced margin method results in consistent ageing of fish in a given cohort. By way of example, if the forced margin method was not used, 4.75–4.92 year old eels sampled in July to September could be assigned ages of either 4 or 5, depending on whether a translucent margin was visible, and deemed to be complete (see Table 2).

It is prudent that prepared otoliths are presented to the reader in the same chronological order that the otoliths were sampled, making interpretation of the margin much easier, and reducing the potential for error. We believe that using the forced margin method obviates the need for algorithms that convert a reader zone count to an age estimate, which may increase unnecessary error in age should reader interpretation of the margin state vary. This is especially important when ageing a species with a broad age range, such as eels, and where samples may be collected over an extended time period (i.e., six months to year-round), for example, from freshwater eel catch sampling programmes (Beentjes & Chisnall 1998, Beentjes 1999, Chisnall & Kemp 2000, Speed et al. 2000). Although "fishing year age class" is commonly used in ageing other New Zealand marine finfish species caught year-round (i.e., snapper, tarakihi, trevally), determining "fishing year age class" for freshwater eels was deemed unnecessary as no formal stock assessments are undertaken. To determine the age of an eel using the forced margin, 'line', 'narrow' and 'wide' readings remain the same as the zone count (e.g., 4L, 4N or 4W and are aged as a 4 year old) (see Table 2). As age in eels is represented only by the time spent in freshwater, age estimates may begin from 0 years.

As growth rates for both New Zealand freshwater eel species are known to be highly variable within and between geographic locations and within an individual eel's life (Burnet 1969, Beentjes 1999, Beentjes & Chisnall 1998, Beentjes & Jellyman 2003, 2015, Chisnall, 1987, 1989, 1993, Chisnall & Hayes 1991, Chisnall & Hicks 1993, Chisnall & Kemp 2000, Graynoth & Jellyman 2002, Graynoth & Taylor 2004, Graynoth et al. 2008, Jellyman 1989, 1991, 1995, 1997, 2001, Horn 1996, Ryan 1978, Speed et al. 2000), the resulting irregular spacing of translucent zones will undoubtedly pose problems for some readers leading to otoliths being under or over-aged. Otolith images from elver, juvenile and adult eel otoliths within this report should assist the reader in understanding the level of variability present and provide some confidence when determining age.

Readability scale

A readability scale ranked 1–5 for ageing freshwater eels in New Zealand was first developed by Jellyman (1974) and mainly categorised otolith readability by zone contrast and irregularity of zone widths. Although the scale is not mandatory, its use (or that of a very similar scale used by Graynoth 1999), has been reported in a number of New Zealand freshwater eel age-related publications (Jellyman 1995, Jellyman et al. 1995, Jellyman & Sykes 1998, Beentjes 1998, Jellyman & Cranwell 2007) and is documented in a revised form in an unpublished NIWA eel ageing and sexing manual. The scale demonstrates the level of difficulty associated with ageing this species. For example, Jellyman & Cranwell (2007) and Jellyman et al. (1995) only used those otoliths with a readability of "very good" or "excellent" in their final age selections for catch-at-age analysis (using an age-length key) or when determining growth rates. However, for most other studies it is not clear which otoliths were used in the final age selection. Chisnall (1989) and Chisnall & Hayes (1991) made use of all otoliths except those categorised "unreadable" which amounted to 3–4% of the total collection.

It is worth noting that the readability scale for freshwater eels used extensively in the past (where 1 = unreadable and 5 = excellent), is in fact, the reverse of the convention for readability scales used in ageing other New Zealand marine finfish species and stored on the MPI *age* database (C. Sutton pers. comm.). The standard scale (where 1 = excellent and 5 = unreadable) has been used in at least one eel ageing study (Beentjes 1998), and will become the standard for ageing freshwater eel otoliths in this report and future MPI eel ageing research. The descriptions used for the five point readability scale, presented below, are a combination of those determined by Jellyman (1974) and those listed in the unpublished NIWA eel ageing and sexing manual, and are reflective of both the quality of the preparation and the reader's confidence in achieving an accurate zone count. Nevertheless, it should be noted that the majority of scores already within the MPI *age* database for freshwater eels may be in the opposite order of the scale below and unique to freshwater eel ageing in the past and will not have been adjusted to the convention used here (pers. comm. Manager of MPI research database, NIWA).

1 Excellent. Clear demarcation of annuli and high confidence in zone count.
2 Very good. Confidence within ± 1 zone of true zone count.
3 Good. Confidence within ± 2–3 zones of the true zone count.
4 Fair. Only moderate confidence in zone count.
5 Unreadable. Very poor demarcation of annuli and low confidence in zone count.

2.5 Ageing procedures

In his review, Horn (1996) summarised the available data for both shortfin and longfin eels reporting, among other details, an otolith reader number (for five different readers) who aged a total of almost 8000 eels sampled from 63 different geographic locations in New Zealand, spanning four decades from Burnet (1969) to Jellyman et al. (1995).

Although the reader is often not recorded, the information by Horn (1996) suggests that in most of the ageing studies on New Zealand freshwater eels a single reader has been used to age break and burn otolith preparations. In the MPI *age* database virtually all ages have been assigned by a single reader. Two different readers were used by Graynoth & Taylor (2004) who discussed either photographs or video images of the otoliths in an attempt to resolve differences in age estimates. Two readers were also recorded in projects which checked for consistency in readings by re-ageing a subsample of otoliths (Jellyman et al. 1995, Speed et al. 2000) and in a preliminary trial on the reproducibility (precision) of age estimates (Graynoth 1999), but no attempt to resolve differences in age estimates between the readers was made.

The MPI ageing workshop in March 2015, to review the ageing of freshwater eels, made several recommendations for ageing future otolith collections. In summary, it was agreed that age determination of all otoliths should be undertaken independently by two experienced readers without prior knowledge of counts obtained by the other reader or of the fish length or sex, knowing only the collection date. Where sections have differing counts, they should be viewed jointly in an effort to establish an agreed age (as is the current practise for other MPI studies where catch-at-age is regularly determined i.e., snapper, tarakihi, trevally and kahawai). It is anticipated that by using two experienced eel otolith readers and the ageing techniques outlined in this age determination protocol, that improvement in age estimation and consistency will be achieved.

It is recommended that vaterite (calcium carbonate polymorph in abnormal otoliths) otoliths in eel age determination be avoided as growth checks do not appear, or at least appear only partially (ICES 2009) and are unlikely to derive an accurate estimate of age.

2.6 Estimation of ageing precision

Shortfinned and longfinned freshwater eels are long-lived, slow growing and have very small otoliths (0.3–3.0 mm in diameter) comprising a large number of condensed zones, which may vary in width, making them difficult to age (Jellyman 1974, 1979b, 1995, Todd 1974, 1980, Chisnall 1987, 1989, Chisnall & Hicks 1993, Graynoth 1999). For example, Todd (1980) aged 178 longfinned (12–60 years) and 1619 shortfinned (6–35 years) adult eels and estimated that only 10% of break and burn otoliths derived accurate age estimates, 20% could not be aged because of multiple zones and excessive variation between readings of each otolith half, and 70% with an accuracy of ± 1 year, stating that many ages were only approximations. Furthermore, Jellyman (1995) found that it was not uncommon for differences of 5 years to be recorded between the halves of the same otolith when investigating the longevity of a virgin population of longfinned eels from a high country lake.

In presenting a growth model for New Zealand freshwater eels to determine which factors most affect growth, Horn (1996) found that “otolith reader” explained a significant proportion of the variation, inferring that the true determinants of growth are confounded by apparent difficulties in otolith interpretation. Horn (1996) further qualified this by stating that although current readers agree on how otolith zone patterns should be interpreted, it is not clear whether all studies were interpreted similarly. To date, only a handful of eel ageing studies, using break and burn otoliths, have documented within- or between-reader agreement (Jellyman 1974, Jellyman et al. 1995, Speed et al. 2000), or to a level within one or more years (Graynoth 1999).

Within-reader comparison

The first (and only) within-reader comparison test was undertaken by Jellyman (1974) who independently re-read a set of 108 longfinned and 57 shortfinned eel (less than 26 cm long) whole ground otoliths (0–8 age groups) nine months after the initial reading, finding considerable differences in interpretation between the two readings, attaining 65% agreement.

Between-reader comparison

The first between-reader comparison of two readers was undertaken by Jellyman et al. (1995) who plotted only the “good” otoliths (i.e., those with a readability of 1 (excellent) or 2 (very good)) from Te Waihora, reporting 51% agreement (compared with 29% when all 296 otoliths were incorporated) with others often giving ages that varied by ± 2 or more years, but with little evidence of bias present (Figure 6). Graynoth (1999) suggested that by using his modified break and burn preparation technique of sawing otoliths consistently through the core, otoliths were easier to age, and undertook preliminary trials on reproducibility (precision) of age estimates, determining that 80% of burnt otoliths were aged ± 1 year by different readers. In an independent verification of a random sample of 100 otoliths, Speed et al. (2000) found readings to be highly consistent, with a correlation coefficient of 0.94 between readers.

Age: 1st reading

Age: 2nd reading	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
6																					
7		4		1																	5
8	1	4	8	2																	15
9		1		3	2																6
10			1	1		4	2														8
11					2	13	4	4													23
12						1	13	2													16
13							1	10		1											12
14							1	3	9	2		1									16
15								1	6	7	3	2		1							20
16									1	4	3	1	1								10
17									1	1	4	8			1						15
18												1	3	3							7
19																	1				1
20														1			1				2
21														1	1		1				3
22																	2				2
23																	3	2		1	6
24																					
25																					
	1	9	9	7	4	18	21	20	17	15	10	13	4	6	2		8	2		1	N = 167

Figure 6: Between-reader comparison plot results of reading a sample of “good” eel otolith preparations (readability scores 1 and 2 only) from a Te Waihora (Lake Ellesmere) status of the stock study in 1994–95. Reproduced from Jellyman et al. (1995).

Horn (1996) reported in his review that an examination of within-reader variance (for the primary reader of most of the age-length data sets), indicated that 80% of second readings were within ± 1 year of the first (B. Chisnall, pers. comm.) and that between-readers, was 95% within ± 3years. In order to maintain consistency in age related studies, Horn (1996) stated that it is important that one worker reads, or at least validates the readings of, all otoliths used in the study, or better still, that a detailed reading protocol be established.

Aside from the paucity of information for reader comparisons in ageing New Zealand freshwater eels, no precision estimates have yet been calculated using Average Percentage Error (APE) (Beamish & Fournier 1981) or mean coefficient of variation (CV) (Chang 1982), nor any age-bias plots (Campana et al. 1995) presented, as are currently required in MPI catch-at-age research (see Figure 7 example). Furthermore, few studies have attempted to resolve reader disagreements by jointly rereading eel otoliths (Graynoth & Taylor 2004), which is standard practise for other species (i.e., snapper, trevally, tarakihi, kahawai, kingfish) to improve the accuracy and precision of the final agreed age, and which should become mandatory for MPI eel ageing in the future, unless the otolith is discarded from the dataset as unreadable. Recommendations for quantifying precision and bias in future MPI freshwater eel ageing studies have been summarised in Appendix 3.

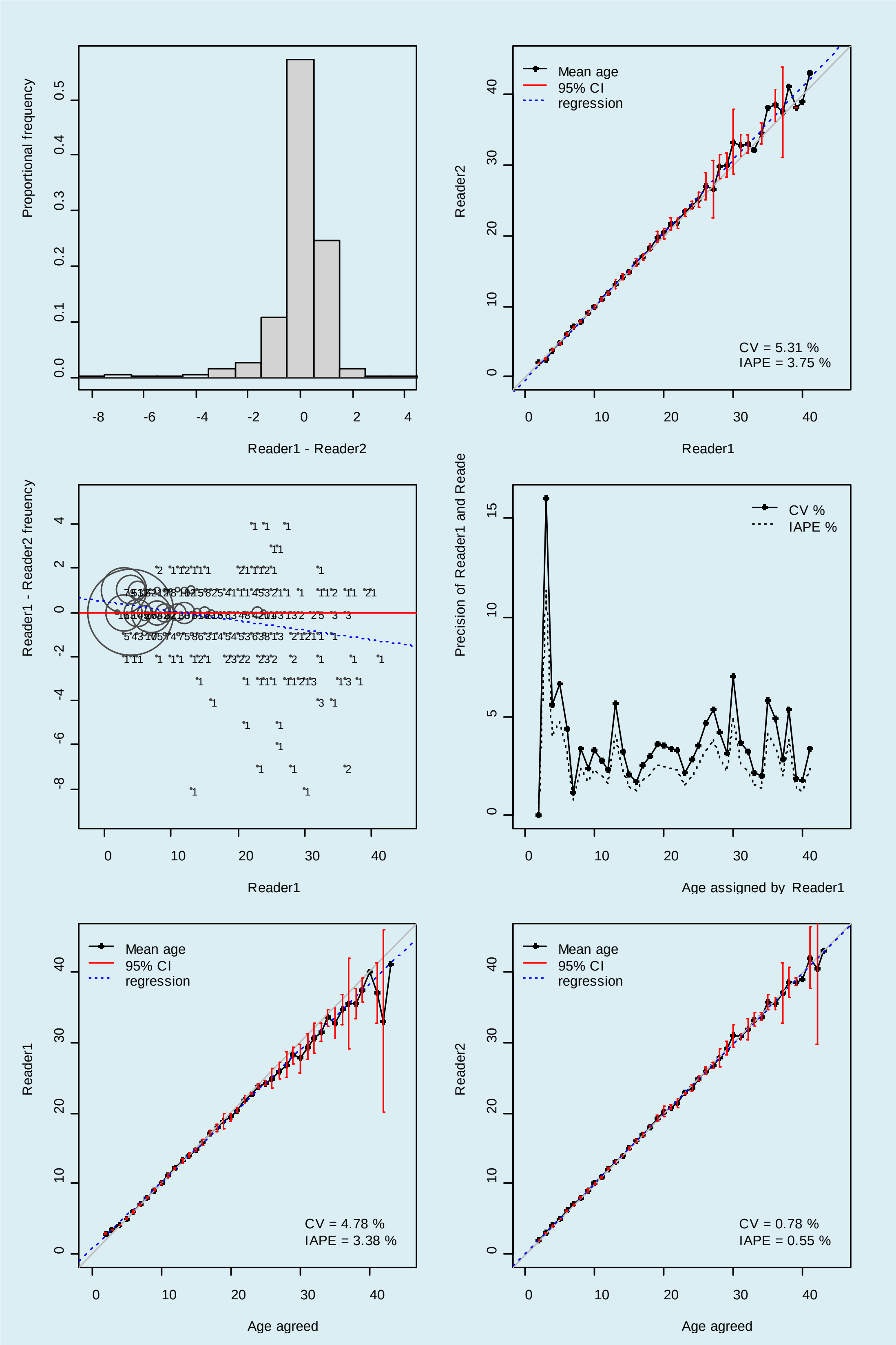

Figure 7: Reader disagreement and age-bias diagnostic plots with precision estimates for trevally sampled from the TRE 7 fishery in 2009–10. Reproduced from Walsh et al. (2012).

2.7 Reference collection

As freshwater elver, juvenile and adult eel break and burn otolith preparations are most often mounted in sets of about 10 on each microscope slide, 43 slides have been assembled for the reference collection, rather than about 400 individual preparations. This is expected to be sufficient for quality control monitoring in assessing reader performance, and may also be added to over time. The primary role of the reference set is to monitor ageing consistency (and accuracy) over both the short and long term, particularly for testing long-term drift, as well as consistency among age readers (Campana 2001). The initial reference collection was assembled in 2015 from 230 longfin and 213 shortfin otolith samples for elver, juvenile and adult eels (some of which were archived at NIWA, Wellington) collected from a wide range of North and South Island New Zealand rivers, lakes and estuaries between 1995 and 2014.

Of the full collection of 443 otoliths, only a subset of 135 has been assigned a new age as part of the ageing protocol (Appendix 4, Tables A4.1 and A4.2). Not all 443 otoliths could be assigned ages (as was initially intended) because of the time required to assign an agreed age by three readers and photograph/annotate each aged otolith. The 68 longfin and 67 shortfin otoliths were aged by three eel otolith readers in 2015.

Although previous ages exist for some of the remaining 308 otoliths in the MPI *age* database, ages may be affected by ageing error, the use of only one reader, slight differences in the preparation method, and without recognition of the margin type. We recommend that these ages be ignored until they are re-aged by readers who have first undergone training commensurate with the ageing protocol standards described in this document. Over time, the selection process for the eel reference set will ensure that otolith samples are aged by key readers, be well represented by samples selected from throughout New Zealand's accessible freshwaters (lakes, rivers, streams, farm ponds, tarns) and some estuaries, and that the full length range is covered. Nevertheless, as the main emphasis for eel ageing in New Zealand by MPI has been research that monitors the relative abundance of elvers arriving at large in-stream barriers to provide an index of eel recruitment, the selection process for the reference set should be strongly dominated by length classes representative of elvers (Figure 8). The size and age range of eels used in the reference collection, thus far, is outlined in Figure 8 and preparation examples presented in Section 2.7.1 (Figures 9–11). As freshwater eels are considered a species of considerable longevity, a reference collection of 500 otolith preparations is expected to be more than sufficient for quality control monitoring purposes.

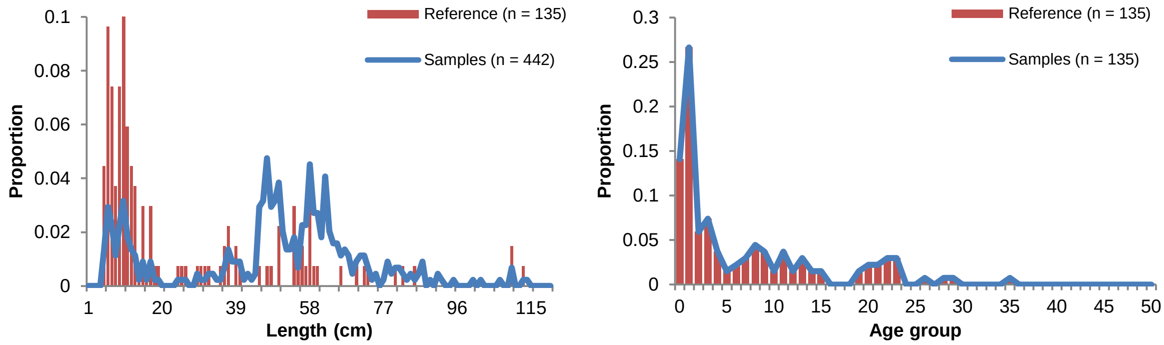

Figure 8: Length and age proportions (lines) of freshwater eels sampled for otoliths from throughout New Zealand freshwaters and estuaries and selected for the reference set (histograms).

Growth rates for New Zealand freshwater eels can vary considerably both within and between locations and by sex (Burnet 1969, Beentjes 1999, Beentjes & Chisnall 1998, Beentjes & Jellyman 2003, 2015, Chisnall, 1987, 1989, 1993, Chisnall & Hayes 1991, Chisnall & Hicks 1993, Graynoth & Jellyman 2002, Chisnall & Kemp 2000, Graynoth & Taylor 2004, Graynoth et al. 2008, Jellyman 1989, 1991, 1995, 1997, 2001, Horn 1996, Ryan 1978, Speed et al. 2000). Nevertheless, although the interpretation of eel otoliths may be affected by where the samples came from, the collation of area-specific reference collections is deemed unnecessary. When new age samples for eels become available from other locations, and are aged following those protocols outlined within this document, then subsamples of these should be included within the current reference collection.

The agreed ages for some of the otoliths selected for the reference set already exist on the *age* database (administered by NIWA for MPI), and have been stored in a new table, *t_reference*, created within this database along with another table, *t_reference_reading*, comprising any new readings of the reference set collection. As these preparations have been aged following the guidelines of the MPI freshwater eel ageing workshop (March 2015), and those outlined in this age determination protocol, they may be treated with a reasonable level of confidence, despite both eel species often being difficult to age, and some preparations being less than optimal in quality and clarity. The reference set may also be used for training new readers as well as monitoring their progress as they gain experience in ageing.

2.7.1 Examples of break and burn preparations of longfinned and shortfinned eel otoliths and agreed age estimates for a range of fish size and age

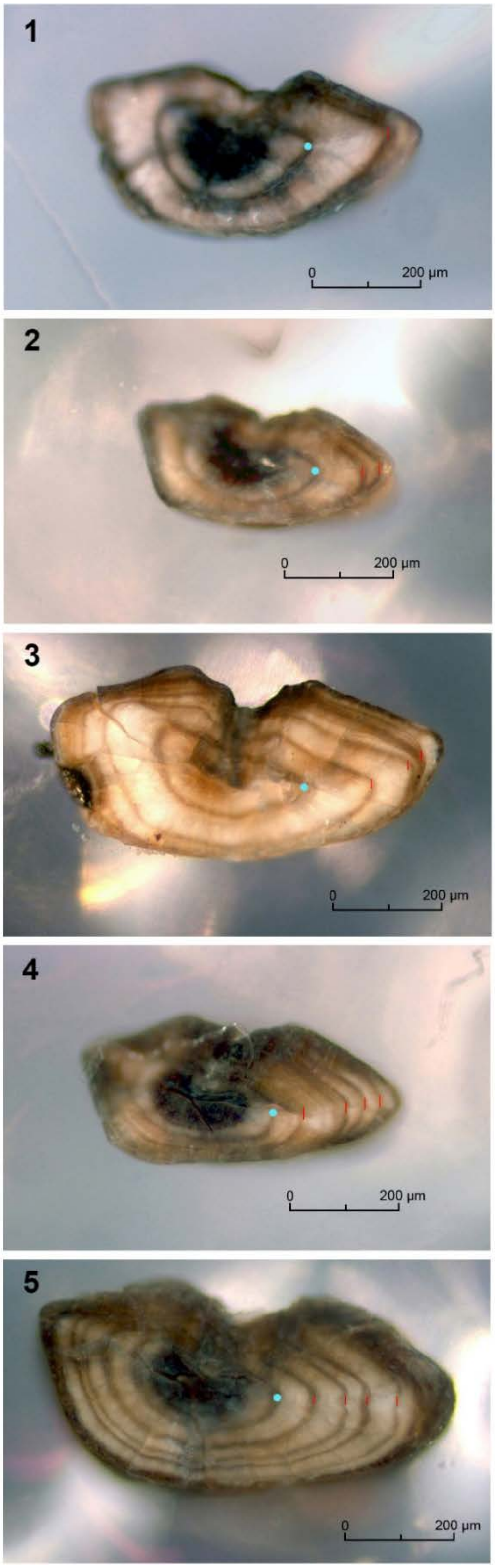

Figure 9: Aged elver eel otoliths (transverse section) from the reference set: fish#1 January (Arnold River, SFE, slide 2-5, 10 cm, agreed reading 1N, agreed age 1); fish#2 Jan (Lake Karapiro, LFE, slide 4-3, 9 cm, 2N, 2); fish#3 Jan (Arnold River, SFE, slide 1-6, 12 cm, 3N, 3); fish#4 Dec (Lake Karapiro, LFE, slide 3-7, 11 cm, 4N, 4), and fish#5 Jan (Arnold River, LFE, slide 1-2, 16 cm, 4N, 4). Blue dot signifies freshwater check, red bars, annuli.

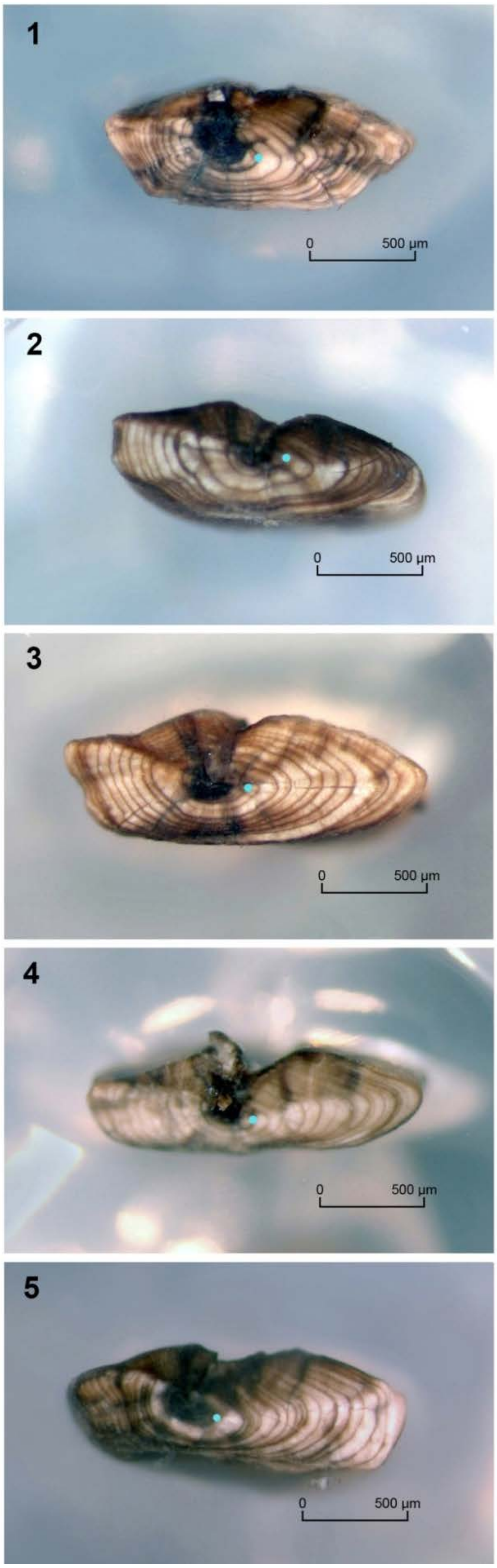

Figure 10: Aged juvenile eel otoliths (transverse section) from the reference set: fish#1 March (Mataura River, LFE, slide 2-3, 29 cm, agreed reading 13W, agreed age 13); fish#2 Mar (Te Waihora, SFE, slide 2-2, 32 cm, 9W, 9); fish#3 Mar (Te Waihora, SFE, slide 2-5, 36 cm, 8W, 8); fish#4 Mar (Te Waihora, SFE, slide 3-7, 37 cm, 9W, 9) and fish#5 Mar (Aparima River, LFE, slide 2-1, 35 cm, 14W, 14). Blue dot signifies freshwater check.

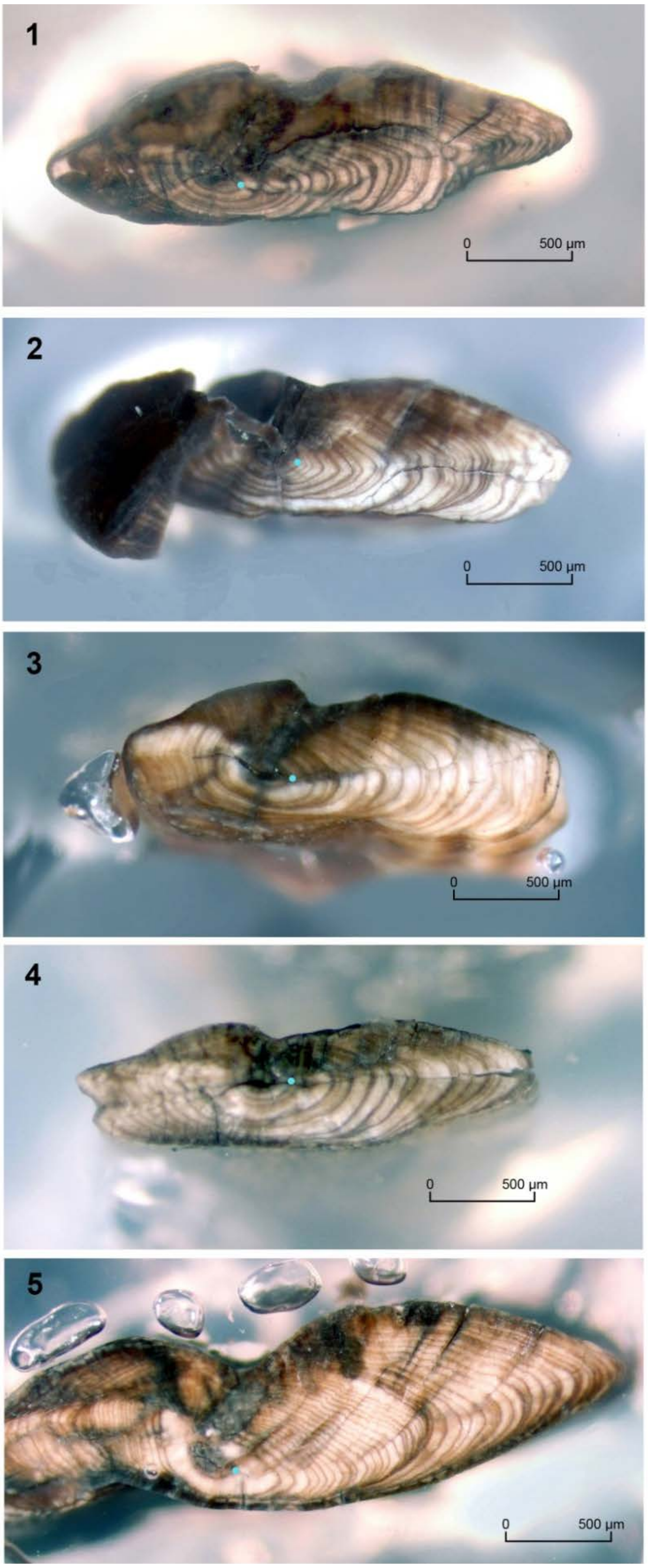

Figure 11: Aged adult eel otoliths (transverse section) from the reference set: fish#1 January (Waikato, LFE, slide 6-502, 60 cm, agreed reading 22N, agreed age 22); fish#2 Mar (Northland, LFE, slide 8-32, 45 cm, 21W, 21); fish#3 Mar (Mataura River, LFE, slide 15-6, 54 cm, 19W, 19); fish#4 Nov (Northland, SFE, slide 8-51, 47 cm, 11N, 11) and fish#5 Mar (Clutha River, SFE, slide 3-1, 82 cm, 35W, 35). Blue dot signifies freshwater check.

2.8 Format for data submission to *age* database

NIWA (Wellington) currently undertake the role of Data Manager and Custodian for fisheries research data owned by 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 3). Additional information that should be recorded include the MPI project code, reader(s) name or number(s), date of reading, preparation method, and a description of how the agreed ages were derived from zone counts. A readability score from 1 (excellent) to 5 (unreadable), although not mandatory, should be recorded for all freshwater eel readings on the *age* database. Note that the majority of scores already within the MPI *age* database for freshwater eels may be in the opposite order of this scale and unique to freshwater eel ageing in the past and will not have been adjusted to the convention of that used here (pers. comm. Manager of MPI research database, NIWA).

Table 3: A scientific research example of freshwater eel age data submitted for loading onto the *age* database. Note that only a single reader has been used to age eel otoliths in this example.

Species = SFE
Stock = ANG14
Material = Otolith
Method = 25 (Otoliths prepared following methods of Hu & Todd (1981) - modified 'crack & burn' method.)
Reader = 71
Project code = EEL2009-01
Sampling period = 8-16 February 2010

origin	trip_code	sample_no	sub_sample_no	area	species	fish_no	prep_no	collection_date	lgth	sex	reader1	count1	reading_date	reader2	count2	reading_date	agreed_count	margin	agreed_age	proj_code	comments
SCR*	20102802	1	2	ANG14	SFE	1		10/02/10	52	3	71	18	10/06/11				18		18	EEL2009-01	
SCR	20102802	1	2	ANG14	SFE	2		10/02/10	53	3	71	20	10/06/11				20		20	EEL2009-01	
SCR	20102802	1	2	ANG14	SFE	4		10/02/10	27	3	71	10	10/06/11				10		10	EEL2009-01	
SCR	20102802	1	2	ANG14	SFE	5		10/02/10	11	3	71	2	10/06/11				2		2	EEL2009-01	

*Scientific research

For reference sets, a new table, *t_reference*, has been developed within the *age* database to include record counts and accepted ages. Readings of the reference set, prior to embarking on reading a new otolith collection, are stored on a second new table, *t_reference_reading*, to distinguish each calibration or training reading from those used to estimate catch-at-age distributions or growth parameters.

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APPENDIX 1: Glossary of otolith terminology and ageing definitions.

Reprinted from the MPI "Guidelines for the development of fish age determination protocols". These were based on Kalish et al. (1995) "Glossary for otolith studies", with modifications and addition of items including definitions for "fishing year age class" and "forced margin" 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 aging (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 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, but see "Fishing year age class".
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. Generally shorter than the rostrum (see Figure A1.1).
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 primordial 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.
D-zone – that portion of a microincrement that appears <u>dark</u> when viewed with transmitted light, and appears as a <u>depressed</u> region when acid-etched and viewed with a scanning electron microscope. This component of a microincrement contains a greater amount of organic matrix and a lesser amount of calcium carbonate than the L-zone. Referred to as discontinuous zone in earlier works on daily increments; D-zone is the preferred term. See L-zone.
Daily increment – an increment formed over a 24 hour period. In its general form, a daily increment consists of a D-zone and an L-zone. The term is synonymous with "daily growth increment" and "daily ring". The term daily ring is misleading and inaccurate and should not be used. The term daily increment is preferred. See increment.
Drift – Shift with time in the interpretation of otolith macrostructure for the purposes of age determination.

Forced margin or Fixed margin – Otolith margin description (Line, Narrow, Medium, Wide) is determined according to the margin type anticipated *a priori* for the season/month in which the fish was sampled. The otolith is then interpreted and age determined based on the forced margin. The forced margin method is usually used in situations where fish are sampled throughout the year and otolith readers have difficulty correctly interpreting otolith margins.

Fishing year age class – The age of an age group at the beginning of the New Zealand fishing year (1 October). It does not change if the fish have a birthday during the fishing season. This is not the same as age group/age class.

Hatch date – the date a fish hatched; typically ascertained by counting daily increments from a presumed hatching check (see check) to the otolith edge.

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.

L-zone – that portion of a microincrement that appears light when viewed with transmitted light, and appears as an elevated region when acid etched and viewed with a scanning electron microscope. The component of a microincrement that contains a lesser amount of organic matrix and a greater amount of calcium carbonate than the D-zone. Referred to as an incremental zone in earlier works on daily increments; L-zone is the preferred term. See D-zone.

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.

Microincrement – increments that are typically less than 50 µm in width; with the prefix “micro” serving to indicate that the object denoted is of relatively small size and that it may be observed only with a microscope. Often used to describe daily and subdaily increments. See increment.

Microstructural growth interruption – a discontinuity in crystallite growth marked by the deposition of an organic zone. It may be localized or a complete concentric feature. See check.

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.

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 µm 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 and ventral projection of the sagitta. Generally longer than the antirostrum (Figure A1.1).
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.
Subdaily increment – an increment formed over a period of less than 24 hours. See increment.
Sulcus acusticus (commonly shortened to 'sulcus') – a groove along the medial surface of the sagitta (Figure A1.2). 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 are often formed in otoliths during metamorphosis from larval to juvenile stages or 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.

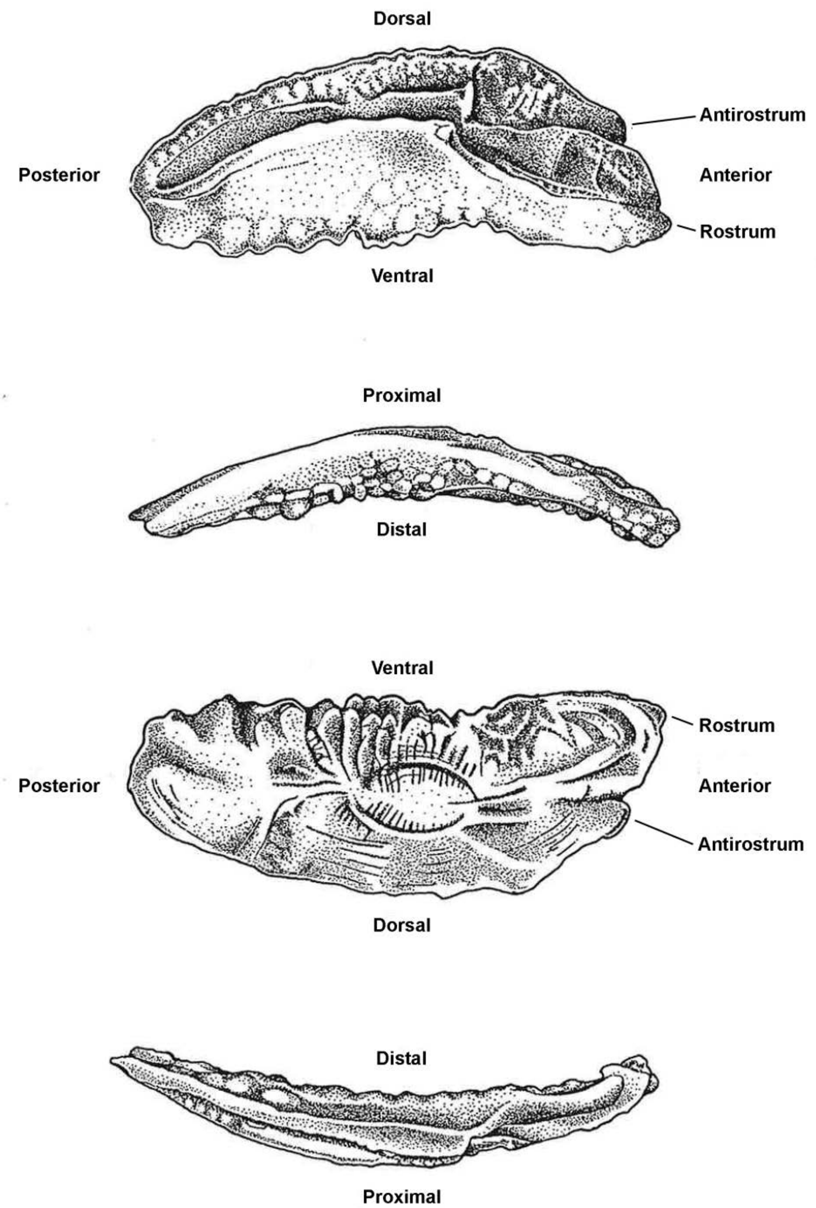


Figure A1.1: Views of a left sagittal otolith from *Arripis trutta* illustrating orientation and basic structure. A) the proximal surface, B) the ventral edge, C) the dorsal edge. (Drawing by Darren Stevens, NIWA).

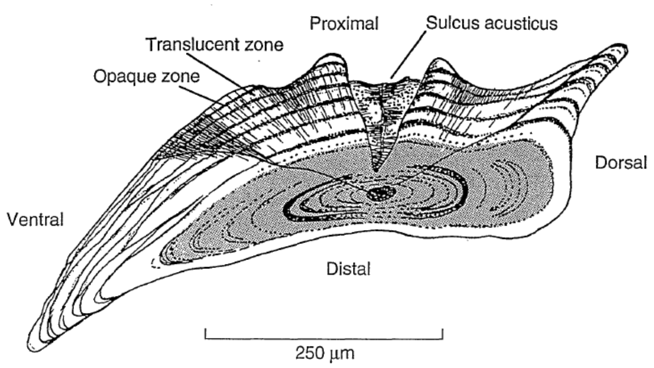


Figure A1.2: Transverse thin section through a sagittal otolith from *Arripis trutta* viewed with transmitted light illumination. The section is taken through the core. (Drawing by Darren Stevens, NIWA).

APPENDIX 2: Protocol for break and burn otolith preparation.

A protocol for preparing New Zealand freshwater eel otoliths using modified excerpts from an unpublished NIWA Christchurch “Eel ageing and sexing manual” compiled by Don Jellyman, Marty Bonnett and Greg Kelly.

Otolith storage

When collected, all eel otoliths need to be first cleaned and dried, and then stored in 0.5 ml plastic microcentrifuge tubes to protect them, as they are very small and fragile. Each tube can then be placed in a standard otolith envelope which is appropriately labelled with trip code (or landing number for catch samples), species code (SFE, LFE), fish number, place caught, date, weight, length, sex, and the name of the sampler (see Figure 1). The envelopes are stored in labelled boxfiles relating to the project code, fishstock and year of collection, and are archived in the MPI otolith collection, currently housed at NIWA, Wellington. It is recommended that all future otolith collections are permanently stored in this manner and not cello-taped to sheets of paper as has been implemented in the past (Figure A2.1).

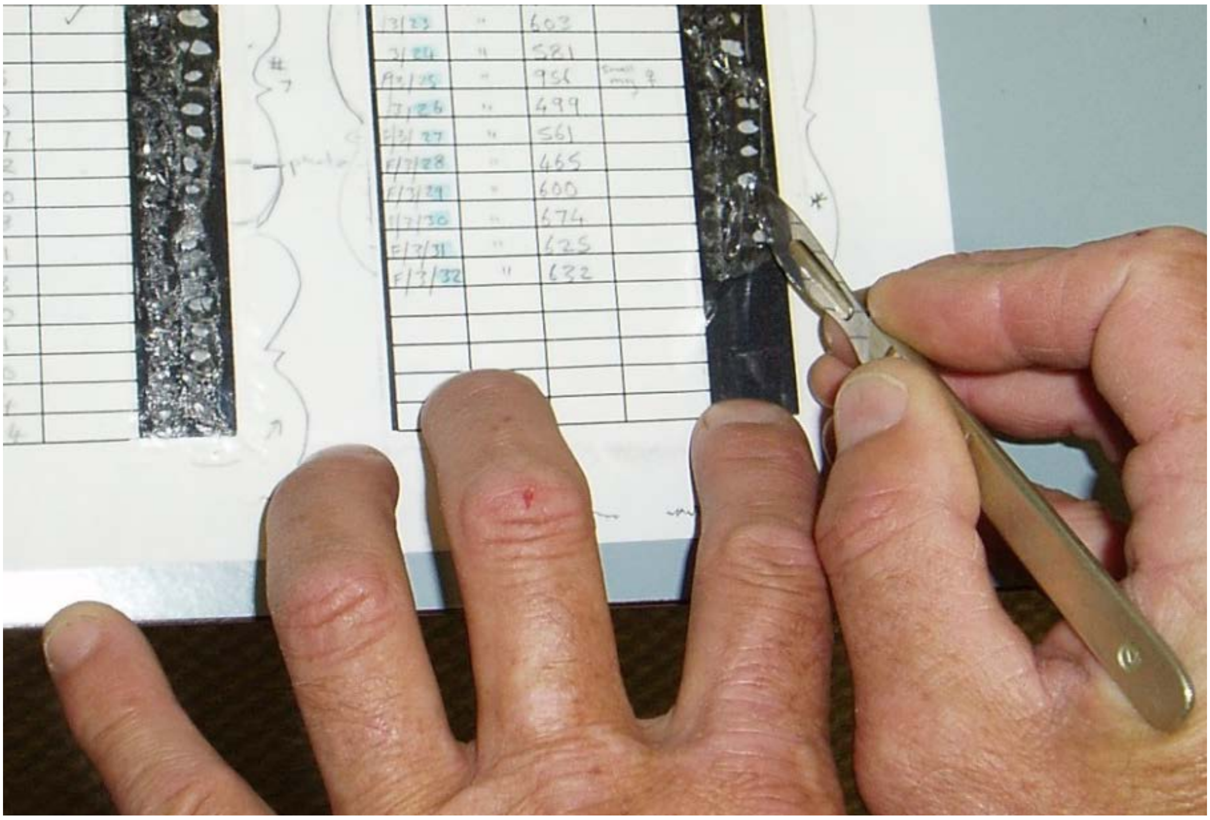


Figure A2.1: Example of an eel otolith collection ‘sheet’ recording date, species and fish length (mm) with whole otoliths cello-taped to a black background. It is recommended that long term storage of eel otolith samples should be kept in microcentrifuge tubes within well labelled otolith envelopes (see Figure 1, Page 11).

Otolith preparation

Before they can be prepared for ageing, otoliths must be dry. This is best achieved by leaving them for two to three weeks at room temperature before preparation. If they are not completely dry, they may shatter when heated or pieces may jump off the scalpel blade. Ideally, it is best to work over a sheet of black paper, so any pieces of misplaced or lost otoliths can be easily found.

There are a number of ways to prepare otoliths for reading, but the simplest involves sawing each otolith transversely through the core along the dorso-ventral axis using a scalpel blade (see Figure

A2.1), scorching the otolith halves for a few seconds at a high temperature to enhance the visibility of the growth zones (Figure A2.2), and then mounting the otolith pieces on a glass slide for viewing under a compound microscope (Figures A2.3 and A2.4).

It is important to place the otolith on a firm surface and cover with adhesive tape to prevent parts of otolith flying off after cutting similar to that demonstrated in Figure A2.1. Sectioning the otolith into halves is best done with a fine scalpel blade. A sawing action with the scalpel usually produces a cleaner and more readable surface on the sectioned otolith than simply fracturing the otolith with direct downward pressure. It is important that the cut passes through the core of the otolith along the dorso-ventral axis (i.e., the part that represents the eel when it was very young) so that growth zones covering the whole life of the eel can be observed.

To scorch the cut half of otolith, place it on a scalpel blade in a high temperature (400–450 °C) gas flame for about 10 to 15 seconds (Figure A2.2). Judging how long to expose the otolith to heat requires experience and practice, but for most otoliths the “rule of thumb” is leave the scalpel blade in the flame until the blade becomes “cherry red”. Sometimes the heat will cause the otolith pieces to “jump” off the blade – which usually results in the piece being lost. This can be prevented by lying a second (loose) scalpel bade on top of the otolith piece. It is very important that care be taken during the burning process and that appropriate safety equipment (i.e., safety glasses) is used.

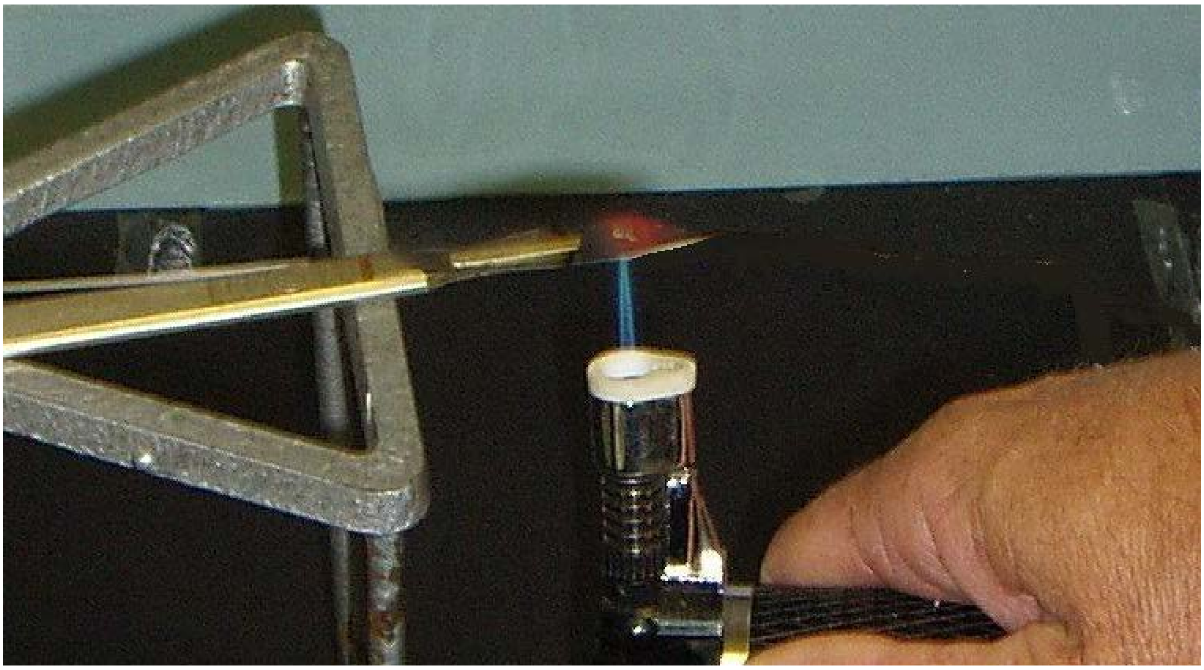
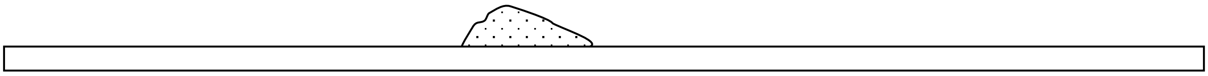


Figure A2.2: Example of the process used for burning eel otoliths.

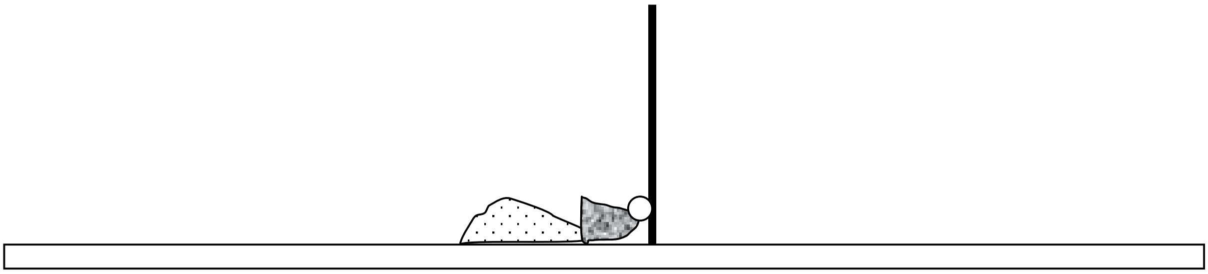
Once successfully "scorched", the otolith is then mounted, with the cut face downwards against a labelled glass microscope slide with a small "blob" of clear ‘Silastic’ silicone sealant following the directions outlined in Figure A2.3. When the sealant is dry, the slide will be viewed "upside down" with the otoliths stuck on the underside of the glass. Use a needle with a speck of sealant on the tip to pick up and place the otolith half. It is best to have the blob of sealant on the slide and place the otolith half into the sealant. However, if you push the cut surface straight down through the sealant, air bubbles may be permanently trapped between the glass and the otolith surface and make the preparation difficult to read. To get around this, use the needle to push the otolith half sideways into the sealant and then tilt it upright, which should squeeze any air bubbles to the side. The other half of the same otolith can be processed and mounted alongside the first piece in the same blob of sealant.

- First place a “blob” of sealant on a clean glass slide.

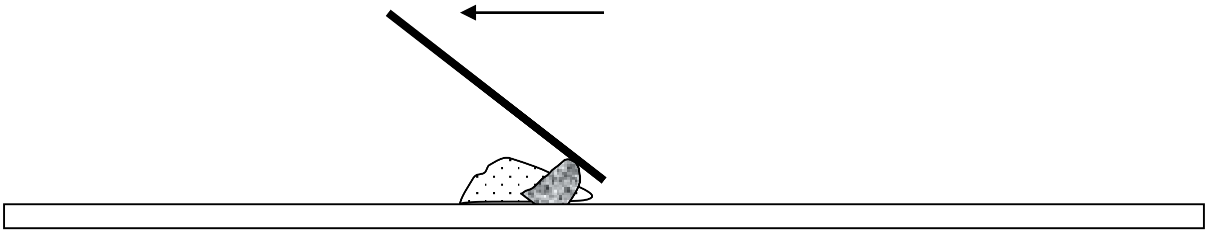


- Pick up an otolith half (from the “uncut” end) using a speck of sealant on the end of a needle.

- Place the otolith half onto the glass and slide it sideways (cut side first) into the sealant.



- Tilt or rotate the otolith half so that the cut face is pressed against the glass and any air bubbles are pushed out.



- When the otolith half is neatly aligned in an upright position on the slide, the needle is taken away and the sealant left to cure.

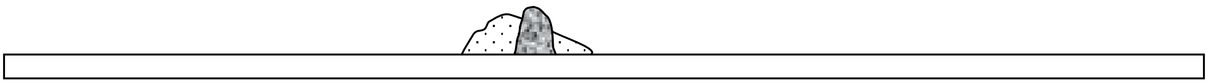


Figure A2.3: Schematic diagram to demonstrate the methodology used in mounting break and burn eel otolith transverse sections onto the underside of a glass microscope slide using clear ‘Silastic’ silicone sealant as the adhesive.

Many otoliths can be mounted onto a single slide – typically 10 (i.e., 20 halves), preferably in the same orientation for the readers benefit (Figure A2.4). Clear, accurate and relevant labelling of the slide with reference to which sampled eel the pair of otolith halves relate to is very important. Should the first otolith be lost, broken or "over-burnt" during the preparation process, the remaining otolith, in a labelled envelope, is available to re-prepare. To ensure prepared slides of break and burn freshwater eel otolith samples remain secure, they are to be stored in microscope slide boxes (appropriately labelled with project code, fishstock and year of collection) and also archived in the MPI otolith collection at NIWA, Wellington.

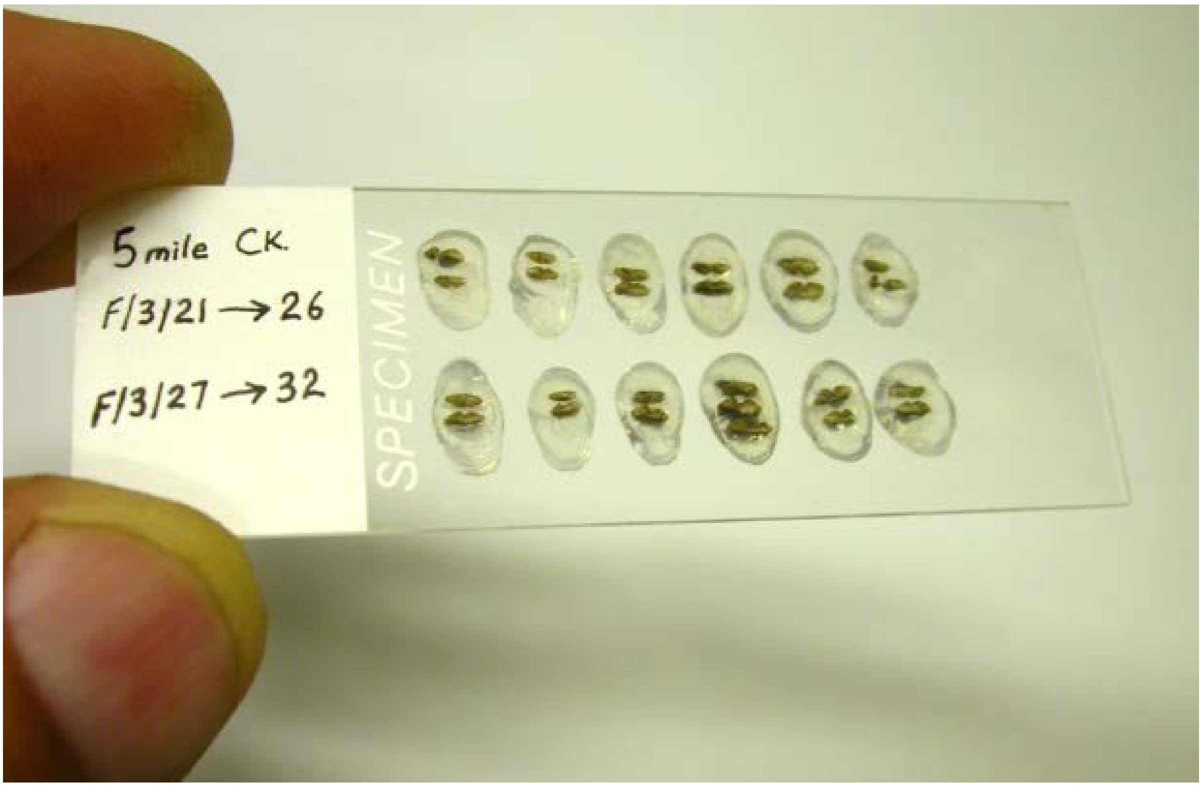


Figure A2.4: An example of mounted break and burn eel otolith transverse sections on the underside of a glass microscope slide, labelled and ready for ageing.

APPENDIX 3: Summary of precision estimates in ageing New Zealand freshwater eels.

Previously reported within- and between-reader agreement estimates determined from ageing eels in New Zealand are presented in Table A3.1. Uncertainty in age estimation arises when independent readers do not initially agree on their interpretation of otolith structures, and these may vary greatly between fishstocks due to specific growth characteristics and differences in population age structure (Davies et al. 2003).

Table A3.1: Within-reader* and between-reader# agreement estimates documented in ageing studies for freshwater eels in New Zealand (BT = Bottom trawl; EF = Electric fishing; GN = Glass-eel net).

Locality	Method	Collection period	No. of readers	Percent agreement	APE	CV	No. aged	Age range	Publication
Wellington region	GN, EF	1970–72	1*	63%	–	–	165	1–9	Jellyman (1974)
Te Waihora and tributaries	BT, EF	1994–95	2#	29%	–	–	296	6–25	Jellyman et al. (1995)

Although percent agreement is considered an inferior method of determining ageing precision compared to Average Percentage Error (APE) (Beamish & Fournier 1981) and mean coefficient of variation (CV) (Chang 1982) as it varies so widely among species and among ages within a species, all measures of precision may be artificially inflated by any bias which exists between readers (Campana 2001). It is therefore difficult to make firm conclusions when comparing between-reader precision estimates for a particular species as reader experience and ageing ability may vary. A CV estimate of 5% (APE 3.5%) may serve as a reference point for fishes of moderate longevity and reading complexity (Campana 2001), and suitable for ageing adult freshwater eels. However, in ageing eel elvers, we suggest that with a high level of reader competency and the guidance of the revised age determination protocol in this document, a CV of well below 5% should easily be attainable.

Furthermore, although error associated with initial readings may imply uncertainty in final age estimates, the process that we now implement in ageing eels, of independent identification and re-reading of otoliths where disagreements occur (when two readers are used), by and large resolves most disagreements. We feel that individual reader age-bias plots (Campana et al. 1995) and precision estimates (APE and CV) between each reader and the agreed age should become the mandatory requirement for reporting ageing results for new otolith collections, and will provide an additional quality control measure by identifying individual reader consistency and accuracy in ageing over time. We suggest that a minimum of two readers always read all otoliths once and resolve all disagreements to ensure accuracy in age estimation is maintained. Individual reader age-bias plots and precision estimates should also be used in setting target reference points and evaluating reader competencies against the reference collection, therefore making reader selection relatively straightforward and unequivocal. The target reference APE and CV estimates for individual readers in the ageing of adult freshwater eel in future studies that require fish age to be determined have initially been set at 5.00% and 7.07% respectively, and for ageing eel elvers, at 2.50% and 3.54%. At the time of writing this document, it is not fully clear whether these target APE and CV are suitable estimates and they may require revision (higher or lower) dependent on the outcome of reader's initial attempts at ageing eels and following the guidance of this age determination protocol. Investigations into the accuracy and precision of age estimation for the European eel (*Anguilla anguilla*) determined deviations from the correct age to be large and precision low (Svedang et al. 1998) further indicating the difficulties associated with accurately ageing eels. No comparison should be made with target reference APE and CV estimates for individual readers and those determined from ageing complete otolith collections, as target reference readings are likely to comprise a higher proportion of old fish, making them more difficult to accurately age, therefore resulting in inflated reader APE and CV estimates. Note: When two sets of readings are being compared (e.g., initial age from readings for reader 1 and the final agreed age), the relationship between APE and CV is an exact one, where the CV equals the APE multiplied by the square root of two.

APPENDIX 4: Summary of assembled longfin and shortfin eel reference collection as of 2015.

Table A4.1: Summary of assembled longfin eel reference collection. Of the 230 individual eels in the collection, 68 (8 juveniles, 17 adults and 43 elvers) have been assigned ages as part of the ageing protocol project and are to be used as ageing checks by readers.

Size	Species	Area_code	Region	Location	Year	Source	Landing_no	Slide label	No. of otoliths on slide	Fish_no/prep
Adults	LFE	WAIT	Otago	Waitaki River	1995/96	comm. catch sampling	962058	1	2	58 and 78
Adults	LFE	WAIT	Otago	Waitaki River	1995/96	comm. catch sampling	962044	1	2	90 and 91
Adults	LFE	WAIT	Otago	Waitaki River	1995/96	comm. catch sampling	962075	1	3	1, 2 and 42
Adults	LFE	WAIT	Otago	Waitaki River	1995/96	comm. catch sampling	962029	2	14	4 to 52
Adults	LFE	AWAT	Marlborough	Awatere River	1996/97	comm. catch sampling	972033	3	9	30 to 56
Adults	LFE	APAR	Southland	Aparima River	1997/98	comm. catch sampling	982006	4	12	10 to 94
Adults	LFE	CLUT	Southland	Clutha River	1997/98	comm. catch sampling	982002	5	14	7 to 45
Adults	LFE	WAIK	Waikato	Mokau River	1996/97	comm. catch sampling	972501	6	10	494 to 504
Adults	LFE	WAIK	Waikato	Mangataphiri River	1996/97	comm. catch sampling	972506	7	8	19 to 36
Adults	LFE	NORT	Northland	Puhoi River	1997/98	comm. catch sampling	983106	8	8	29 to 65
Adults	LFE	AUCK	Auckland	Stream/river	1997/98	comm. catch sampling	983102	9	4	1 to 4
Adults	LFE	WAIK	Waikato	hydro impoundments	1997/98	comm. catch sampling	982501	10	12	1 to 28
Adults	LFE	NORT	Northland	Rotokakahi River	1997/98	comm. catch sampling	983202	11	12	64 to 75
Adults	LFE	NORT	Northland	Wairoa River/ Waiutu swamp	1997/98	comm. catch sampling	983215	12	10	63 to 72
Adults	LFE	POMA	Southland	Pomahaka River	1997/98	comm. catch sampling	972100	13	14	7 to 68
Adults	LFE	ORET	Southland	Oreti River	1997/98	comm. catch sampling	972097	14	13	1 to 92
Adults	LFE	MATA	Southland	Mataura River	1996/97	comm. catch sampling	972052	15	15	1 to 16
Adults	LFE	MOK	Southland	Mokoreta River	1996/97	comm. catch sampling	972004	16	5	5 to 91
Juveniles	LFE	ORET	Southland	Oreti River	Mar-98	NIWA	–	Juv.slide 1	11	1 to 11
Juveniles	LFE	APAR	Southland	Aparima River	Mar-98	NIWA	–	Juv.slide 2	3	1 to 3
Juveniles	LFE	CLUT	Southland	Clutha River	Mar-98	NIWA	–	Juv.slide 2	3	1 to 3
Juveniles	LFE	MATA	Southland	Mataura River	Mar-98	NIWA	–	Juv.slide 2	3	1 to 3
Elvers	LFE	ARNA	Westland	Arnold River Dam	2013/14	Recruitment monitoring	–	ARNA slide 1	5	Prep 1 to 5
Elvers	LFE	ARNA	Westland	Arnold River Dam	2013/14	Recruitment monitoring	–	ARNA slide 2	5	prep 6 to 10
Elvers	LFE	KARA	Waikato	Karapiro Dam	2013/14	Recruitment monitoring	–	KARA slide 3	5	prep 6 to 10
Elvers	LFE	KARA	Waikato	Karapiro Dam	2013/14	Recruitment monitoring	–	KARA slide 4	10	1 to 10
Elvers	LFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 1	5	prep 5 to 10
Elvers	LFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 2	5	prep 1 to 5
Elvers	LFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 5	5	prep 5 to 8, and 10
Elvers	LFE	WAIR	Northland	Wairua Falls	2013/14	Recruitment monitoring	–	Wairua Falls 2013	3	prep 1 to 3

Table A4.2: Summary of assembled shortfin eel reference collection. Of the 213 individual eels in the collection, 67 (9 juveniles, 13 adults and 45 elvers) have been assigned ages as part of the ageing protocol project and are to be used as ageing checks by readers.

Size	Species	Area_code	Region	Location	Year	Source	Landing_no	Slide label	No. of otoliths on slide	Fish_no/prep
Adults	SFE	GREY	Westland	Grey River	1996/97	comm. catch sampling	972047	1	14	54 to 80
Adults	SFE	ELLE	Te Waihora	Te Waihora	1996/97	comm. catch sampling	972053	2	7	6 to 105
Adults	SFE	CLUT	Southland	Clutha River	1996/97	comm. catch sampling	972010	3	2	1 and 2
Adults	SFE	CLUT	Southland	Clutha River	1996/97	comm. catch sampling	972017	3	7	1 to 7
Adults	SFE	CLUT	Southland	Clutha River	1996/97	comm. catch sampling	972072	3	1	1
Adults	SFE	APAR	Southland	Aparima River	1996/97	comm. catch sampling	972030	4	1	1
Adults	SFE	APAR	Southland	Aparima River	1996/97	comm. catch sampling	972039	4	5	1 to 5
Adults	SFE	APAR	Southland	Aparima River	1996/97	comm. catch sampling	972056	4	9	1 to 9
Adults	SFE	CLUT	Southland	Clutha River	1997/98	comm. catch sampling	982040	5	1	2
Adults	SFE	CLUT	Southland	Clutha River	1997/98	comm. catch sampling	982002	5	6	6 to 24
Adults	SFE	WAIK	Waikato	Mokau River	1996/97	comm. catch sampling	972501	6	10	1 to 12
Adults	SFE	HAUR	Hauraki	Piako/Waitoa Rivers	1996/97	comm. catch sampling	972507	7	10	1 to 12
Adults	SFE	NORT	Northland	Kaipara Harbour	1997/98	comm. catch sampling	973101	8	12	46 to 92
Adults	SFE	AUCK	Auckland	Manukau Harbour	1997/98	comm. catch sampling	983110	9	12	1 to 63
Adults	SFE	NORT	Northland	?	1997/98	comm. catch sampling	983218	10	12	1 to 63
Adults	SFE	TAIE	Otago	Taieri River	1997/98	comm. catch sampling	982021	11	10	5 to 39
Adults	SFE	MOKO	Southland	Mokoreta River	1996/97	comm. catch sampling	972004	12	5	15 to 42
Adults	SFE	ELLE	Te Waihora	Te Waihora	1997/98	comm. catch sampling	972076	13	14	9 to 87
Adults	SFE	GREY	Westland	Grey River	1997/98	comm. catch sampling	972089	14	15	2 to 63
Juveniles	SFE	ELLE	Canterbury	Te Waihora	1998	NIWA	–	GPW-140325	4	2, 3, 5, 6
Juveniles	SFE	ELLE	Canterbury	Te Waihora	1998	NIWA	–	GPE-140325	5	4, 5, 7, 11, 14
Juveniles	SFE	ELLE	Canterbury	Te Waihora	1998	NIWA	–	FMP140328	5	1, 2, 3, 7, 10
Elvers	SFE	ARNA	Westland	Arnold River Dam	2013/14	Recruitment monitoring	–	ARNA slide 1	5	Prep 6 to 10
Elvers	SFE	ARNA	Westland	Arnold River Dam	2013/14	Recruitment monitoring	–	ARNA slide 2	5	Prep 1 to 5
Elvers	SFE	KARA	Waikato	Karapiro Dam	2013/14	Recruitment monitoring	–	KARA slide 3	5	Prep 1 to 5
Elvers	SFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 1	4	Prep 1 to 4
Elvers	SFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 2	5	Prep 6 to 10
Elvers	SFE	PATE	Patea River	Patea Dam	2013/14	Recruitment monitoring	–	PATA slide 5	5	Prep 1 to 4 and 9
Elvers	SFE	WAIR	Northland	Wairua Falls	2013/14	Recruitment monitoring	–	Wairua Falls 2013	17	Prep 4 to 10, 12 to 20