3. AGE IN YEARS FROM OTOLITHS OF ADULT TROPICAL FISH

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1 Introduction

Otoliths of fish are hard, calcified internal structures that assist in orientation and sound perception (Popper et al. 2005, Green et al., Chapter 1, this volume). As otoliths grow inside each fish's head they record an extraordinary amount of information about the life of that fish and the environments that it experiences (Begg et al. 2005). The challenge to scientists is to retrospectively access this information, to interpret it accurately in terms of the biology and life history of the fish, and to use the data to appropriately understand and manage the natural resources of the fish population and the broader aquatic ecosystem. The greatest application of otoliths to date has been in providing information on the age and growth of fish in years, which has ultimately been used in the management of associated fisheries (Campana & Thorrold 2001, Campana 2005). Prior to the 1980s, and stretching back to the late 19th Century, most such ageing work was done in the temperate regions of the world and contributed to managing the enormous fisheries in the oceans of these regions (Beamish 1992, Beamish & McFarlane 1995). There was little attention paid to ageing fish from tropical regions (Longhurst & Pauly 1987). This imbalance has been redressed to some extent over the past couple of decades, however, as scientific effort in the tropics has increased, resulting in a substantial increase in the number of studies and publications on age-related subjects for tropical fishes (Morales-Nin & Panfili 2005).

This chapter is concerned with the use of growth increments that form each year (annuli) in otoliths to age tropical fish to a resolution of years. The information presented is based largely on the numerous population biology studies that have been done since the early 1990s. The first section of the chapter considers historic, geographic and taxonomic issues, followed by a discussion on the nature and characteristics of the otoliths of tropical fishes. Some of the population characteristics of tropical fish that have become apparent through ageing studies are examined, particularly with respect to their life histories, demography and ecology. Issues and uncertainties that remain about using otoliths for ageing tropical fish are also presented. The second section of the chapter is primarily concerned with the practical issues of working with and interpreting otoliths to provide estimates of fish age in years. A summary of how the methodological approach to ageing work has changed through the 20th Century is followed by a description of the sequential steps involved in a fish ageing study once a validated ageing protocol for that species has been established.

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1.1 HISTORIC, GEOGRAPHIC AND TAXONOMIC CONSIDERATIONS

1.1.1 Development of annual ageing for tropical fish using otoliths

The determination of fish ages in laboratories around the world for fishery stock assessment purposes constitutes the most significant application of direct ageing and age-structured analyses in any biological application (Campana & Thorrold 2001). Approximately 2 million fish are aged each year as part of this process. The resulting estimates of age provide the fundamental information for understanding the demography and life history of each fish species and for assessing the productivity of its fishery. These estimates of fish age are interpreted to provide insights into the population-specific characteristics of growth, longevity and mortality and to relate to other age-related population processes, such as reproduction and movement.

Otoliths are the preferred anatomical structure for direct ageing of many species of fish because they provide the most accurate and precise estimates of age over the broadest age ranges whilst being relatively easy to prepare and interpret (Secor et al. 1995, Campana & Thorrold 2001). The remarkable success of otoliths as chronometers relates to a number of their characteristics. Firstly, otoliths continue to grow, at least in some dimensions, throughout the lives of the fish, as daily increments are added to the growing surface of the otolith (Campana & Neilson 1985, Morales-Nin 2000). Secondly, otolith growth varies seasonally, which manifests optically as the banded macrostructure that is interpreted as annual increments to estimate fish age. Finally, otoliths are not subject to resorption and reworking, as happens with skeletal bone, and so represent a complete chronological record of the life of the fish (Campana 1999).

Ageing studies based on annuli in otoliths have been most successful to date for temperate species of fish. Ageing applications for tropical species have never attained the same level of significance for several reasons. Firstly, the much smaller and less valuable fisheries of the tropics have never attracted the level of research effort that has been directed towards the enormous temperate water fisheries (Munro & Williams 1985, Longhurst & Pauly 1987). This has been exacerbated by the limited research capability in many tropical countries (Longhurst & Pauly 1987). Secondly, there was an historical perspective that originated as early as the 1920s (Longhurst & Pauly 1987) that tropical fish would not manifest an incremental structure in their otoliths (Pannella 1974). This dogma was based on the conceptual impression of the tropical environment as one of constancy, i.e., lacking in seasonality, to which the fish supposedly responded by growing and spawning continuously throughout the year without the physiological variation in growth that was believed to be central to the formation of seasonal patterns in otolith structure (Longhurst & Pauly 1987). Accordingly, the potential usefulness of otoliths for ageing tropical fishes remained unexplored for most of the 20th Century.

In the late 1970s and early 1980s it was discovered that the otoliths from the adults of some tropical fish did in fact display a macrostructure of banding that may represent an annual pattern of growth increments (Loubens 1978, Brothers 1987, Brothers & Mathews 1987, Manooch 1987, Samuel et al. 1987), in addition to the microstructural banding that was hypothesised to represent daily increments. These early studies considered the generality of such macrostructure amongst different taxa and explored methods for preparing and examining the otoliths. There was a considerable focus on understanding the nature of the otolith macrostructure by exploring its relationship with the microstructure. This was done by counting the number of daily increments that constituted a complete sequence of an opaque and a translucent band in the macrostructure (Brothers & Mathews 1987, Hill & Radtke 1988, Morales-Nin 1989).

There were several focussed population studies in the early 1990s that were the first attempts to validate the periodicity of macroincrement formation for tropical species based on treating fish with tetracycline (Fowler 1990, Fowler & Doherty 1992). There has been considerable expansion in the number of age-based studies on the population biology of tropical species since the mid-1990s that is likely to be related, at least in part, to the considerable expansion in production of the tropical marine and inland fisheries that has taken place since 1990 (Morales-Nin & Panfili 2005). These studies generally have heeded the warnings from the 1980s about the requirement to validate the periodicity of increment formation and otolith interpretation (Beamish & McFarlane 1983). They have undertaken validation studies, developed ageing protocols and then provided age-related information such as age structures, estimates of longevity, and rates of growth and mortality. Such biological studies have become more sophisticated since 2000, describing spatial, temporal and age-based differences in population characteristics (Adams & Williams 2001, Gust et al. 2002, Kritzer 2002, Williams et al. 2003, Choat et al. 2003).

1.1.2 Geographic and taxonomic issues

The tropical and sub-tropical regions of the earth support an extraordinary diversity of aquatic environments that can be broadly classified as inland, estuarine and marine. Each broad category is represented by many different types of habitats ranging in diversity, for example, from high altitude mountain ponds, enormous rivers to the brackish waters where the rivers meet the sea and the great depths of the ocean. Such habitats would clearly provide an immense diversity of physicochemical environments. Not surprisingly, there is an extraordinary diversity of ichthyofauna distributed throughout these many tropical habitats (Sale 1980, Blaber 2000).

The formation of an annual macrostructure within an otolith reflects seasonal growth of the otolith, presumably related to seasonal variation in the life cycle and physiology of the fish in response to environmental change throughout the year (Wright et al. 2002). The two forcing physical environmental variables that would most likely influence the lives of tropical fishes are water temperature and hydrology (Morales-Nin & Panfili 2005). Tropical sea surface temperatures can show considerable seasonal variation that can even exceed that of some temperate oceanic waters (Morales-Nin & Panfili 2005). The forcing variable that is most likely to influence the biological cycles of living organisms in inland systems is seasonal rainfall, such as the monsoons of South-East Asia. Temperature variations can be superimposed on these wet and dry seasons and result in highly seasonal environmental conditions. The flood regimes associated with seasonal rains must also impose considerable seasonality on the physicochemical regimes and the natural trophic systems of estuarine environments. Many tropical fishes are adapted to exploit this seasonal variation in conditions, as manifested through their trophic relations, cycles of reproduction, growth and condition. Such seasonality may well also result in differential growth of fish otoliths, which becomes evident in the otolith macrostructure (Jepsen et al. 1999, Faunce et al. 2002, Panfili et al. 2004, Morales-Nin & Panfili 2005).

There is an uneven distribution of otolith-based ageing studies from the different tropical environments. The greatest success so far appears to have been for the demersal species that occupy the marine neritic zone, particularly those that are associated with tropical reefs and inter-reefal areas (Fowler 1995). These fishes come from the Order Perciformes, Suborder Percoidei and include families such as the Acanthuridae, Chaetodontidae, Haemulidae, Labridae, Lethrinidae, Lutjanidae, Scaridae, Scianidae and Serranidae. Some success has been achieved also for a number of pelagic, open water species from the Suborder Scombroidei, Family Scombridae, primarily the mackerel species (McPherson 1992, Begg & Sellin 1998, Tobin & Mapleston 2004). There has been considerable ageing work on the Clupeiforme fishes that have a pelagic existence in the tropical, neritic and oceanic waters. Such fishes have generally proven to be relatively short-lived, however, living only up to a maximum of about 2 years and so the ageing work for these species has generally been based on daily increments in their otoliths (Struhsaker & Uchiyama 1976, Gjosaeter et al. 1984, Milton et al. 1993, Hoedt 2002, Sponaugle, Chapter 4, this volume). There have been far fewer ageing studies reported for fish from tropical inland systems. Some notable success has been achieved for the family Cichlidae, for which studies have been done in West Africa (Panfili et al. 2004), the rivers of Venezuela (Jepsen et al. 1999), and in south-east Florida (Faunce et al. 2002). The lack of diversity for the taxa considered from tropical inland systems may reflect that the production of fisheries from this environment is approximately only one sixth of that from tropical marine environments (Morales-Nin & Panfili 2005).

1.2 CHARACTERISTICS OF OTOLITHS OF TROPICAL FISH

1.2.1 The nature of otolith structure

For the otoliths of tropical fish to be useful in providing estimates of fish age in years they must fulfil three criteria:

1. they must display an internal structure of increments that can be quantitatively resolved, optically or otherwise;
2. the formation of increments must conform to a regular and determinable time scale; and
3. the otoliths must continue to grow throughout the life span of the individual fish (Fowler & Doherty 1992).

It was assumed initially that the first of these criteria was the one that the otoliths of tropical species would fail. Once researchers began to examine such otoliths, however, they realised that a macrostructure often was apparent (Loubens 1978, Manooch 1987). The second criterion has also been fulfilled by many tropical species. Early attempts to validate the periodicity of increment formation involved determining the relationship between the macrostructure and microstructure of the otoliths by enumerating the number of daily increments that constituted a complete macro-increment (= one opaque and translucent band) using scanning electron microscopy (Brothers & Mathews 1987, Hill & Radtke 1988, Morales-Nin 1989, Morales-Nin & Ralston 1990). Most attempts since 1990 to determine the periodicity of increment formation have involved the use of oxytetracycline tagging (Fowler 1990, Newman et al. 1996, Cappo et al. 2000, Williams et al. 2005) or marginal increment analysis (Begg & Sellin 1998, Brown & Sumpton 1998, Pilling et al. 2000, Faunce et al. 2002, Panfili et al. 2004). The third criterion above generally has been assessed through considering the relationship between otolith weight and fish age. Such relationships often have proven to be approximately linear, suggesting that relatively consistent amounts of otolith material are added to the otolith surface annually, throughout each fish's life (Ferreira & Russ 1994, Worthington et al. 1995, Newman et al. 1996, Craig et al. 1997, Lou et al. 2005).

The general conclusion from the numerous validation studies to date is that the otoliths of many tropical species do display a macrostructure that can be displayed optically. These increments consist of either opaque or translucent bands. The appearance of these bands depends on the method of illumination. Opaque bands are denser than the translucent bands and are relatively dark under transmitted light as they transmit less light. They appear lighter under reflected light because they are more reflective than the translucent bands (see Figure 1 in Green et al., Chaper 1, this volume). This is further described under Section 2.2.3 of this chapter, *Interpretation of otolith macrostructure to estimate fish age*. The terminology of opaque and translucent bands used in this chapter assumes transmitted light.

The periodicity of formation of the macrostructure has generally proven to be annual, regardless of the environment that the different species occupied (Fowler 1995, Choat & Robertson 2002, Faunce et al. 2002). One annulus involves the combination of one opaque band and its adjacent translucent band. For numerous marine species, the opaque band forms at some time throughout the spring and early summer (Fowler 1995, Choat & Axe 1996, Begg & Sellin 1998) but for inland fishes its formation depends on the timing of the local wet and dry seasons (Jepsen et al. 1999, Panfili et al. 2004). Furthermore, a relatively consistent mass appears to be deposited onto the growing surface of the otoliths during each year throughout the fish's life (Worthington et al. 1995, Lou et al. 2005). These latter two points indicate that the otoliths demonstrate a systematic pattern of growth that is manifested as an optical pattern that can provide an accurate estimate of age in years. That is, the macrostructure of otoliths of many tropical fishes so far examined do represent chronometers of the fishes' lives.

1.2.2 Optical characteristics

A short review in 1995 summarised the information that was available to that time on the ageing of coral reef fish species (Fowler 1995). Fowler had examined the sagittae, i.e. the largest pair of otoliths, from 28 species of percoid fishes from eight reef fish families and identified that the otoliths of most species had some characteristics in common. There have been many more studies on tropical percoid species since then and the characteristics of the otoliths, based on their descriptions and photographs in the literature, appear to be very consistent with the earlier descriptions. These characteristics are summarised below.

1. The whole otoliths of the percoid species have a glassy appearance and when immersed in oil transmit some light, which means that, to some extent, they are translucent, contrasting with the otoliths from some high latitude species whose otoliths are so opaque they do not transmit light.

2. Transverse sections of otoliths display a macrostructure consisting of narrow opaque and broad translucent bands (Figures 1a, b and 2). This also contrasts with the otoliths of some high latitude species, in which both bands are relatively similar in width (Blacker 1974, Williams & Bedford 1974, Beckman & Wilson 1995, Campana 2001, Campana & Thorrold 2001). This may reflect that the period of the year during which the opaque band is deposited is shorter for tropical than for temperate species, possibly accounting also for their differences in opacity.
3. The alternation between the opaque and translucent bands can be abrupt and distinct and therefore may not represent a gradient of opacity (Figure 1b).
4. The opaque band may be represented by a narrow, distinct check, or can be broader consisting of numerous closely spaced discontinuities (compare Figure 1b, c).
5. The different opaque bands within the same otolith are not necessarily of the same optical density. Specifically, the first one or two closest to the nucleus may be relatively broader but more diffuse and less distinct than those that are further from the nucleus in older fish (Figure 1a, b). Because of this, it can be easier procedurally to determine the ages of older fish than younger ones whose otoliths display only one or two diffuse opaque bands.
6. The opaque band has been related to a short period of fast growth during the year, and the translucent band associated with a longer period of slow growth (Brothers & Mathews 1987, Morales-Nin 1989, Morales-Nin & Ralston 1990). The evidence for this conclusion, however, was not made apparent in these earlier publications and the relationship between opaque and translucent bands and specific growth patterns remains equivocal for coral reef fish species. This is considered further below in Section 1.4.5.

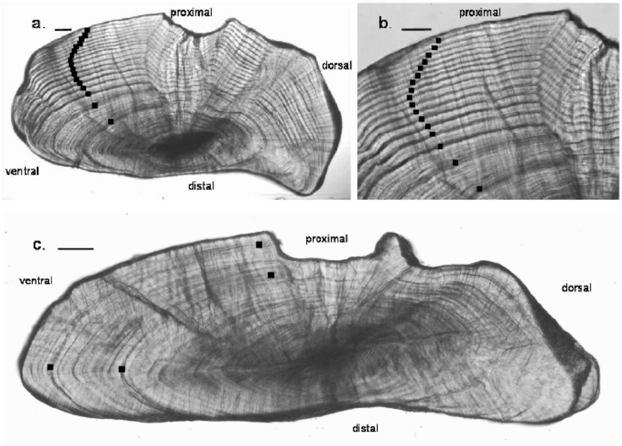

Figure 1. Transverse sections of sagittae from *Pomacentrus moluccensis* from the Great Barrier Reef. (**a**) View of transverse section with 17 opaque bands. (**b**) Higher magnified view of the same otolith showing the macrostructure of the ventral side of the sulcus. (**c**) View of a transverse section from an otolith with two relatively poorly defined opaque bands. *Dark dots* indicate the opaque bands on each image when viewed with transmitted light. Scale bars = 100 μm.

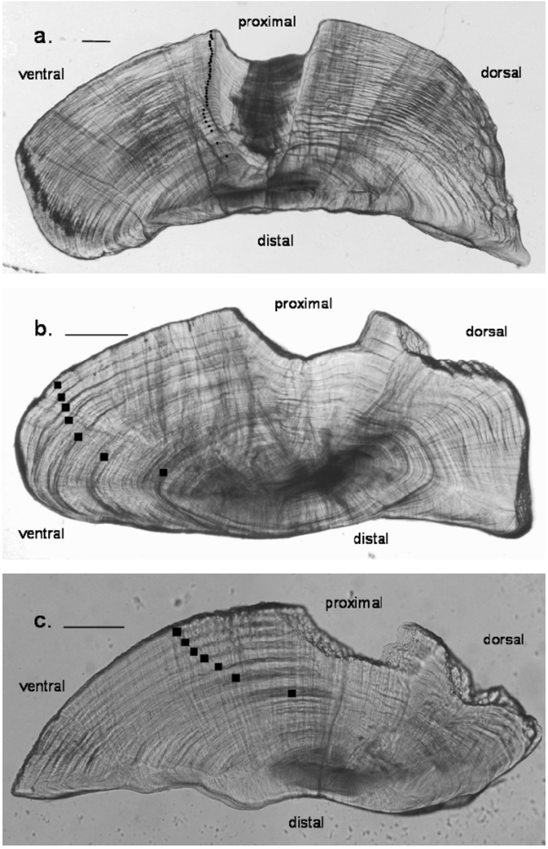

Figure 2. Transverse sections of sagittae from adult individuals from each of three species of fish from the central Great Barrier Reef. (**a**) *Acanthurus lineatus* with 37 opaque bands. (**b**) *Pomacentrus moluccensis* with seven opaque bands. (**c**) *Chaetodon aureofasciatus* with seven opaque bands. *Dark dots* indicate opaque bands when viewed with transmitted light. Scale bars = 100 µm.

The otoliths of cichlid species from inland systems display a number of the characteristics described above for marine reef fish (Jepsen et al. 1999, Faunce et al. 2002). The otoliths of scombrid fishes, however, show some interesting differences. Such otoliths are elongate and laterally compressed, which means that the translucent and opaque bands can be relatively clear in the whole otoliths (McPherson 1992, Begg & Sellin 1998, Tobin & Mapleston 2004). The first several translucent bands are characterised by radial striae that refract light and give the otoliths a crazed appearance (McPherson 1992, Buckworth 1998, Lewis & Mackie 2002). The first translucent band is broad, whilst subsequent ones become increasingly narrow. The first opaque band can be quite indistinct, after which the subsequent ones become more distinct. The later-formed opaque bands are relatively broader than the alternating translucent ones. This can make it easier to interpret the otoliths of older fish relative to younger ones. The interpretation of otolith structure also can be complicated by the presence of secondary opaque bands or sub-annuli. Such secondary features are apparent in the otoliths of some temperate fishes (Campana & Thorrold 2001), but are generally not significant structures in the otoliths of tropical percoid species (Figures 1, 2; Fowler 1995). The formation of the opaque band is more often associated with the spring–summer period (Schmidt et al. 1993, De Vries & Grimes 1997, Begg & Sellin 1998, Tobin & Mapleston 2004) but winter formation was recorded in one case (McPherson 1992).

1.3 USES OF FISH AGE ESTIMATED FROM ANNUAL INCREMENTS

Fish populations are dynamic biological entities that vary in size over time as influenced by several input and output processes (Hilborn & Walters 1992, King 1995). Population abundance and biomass increase through the natural processes of reproduction and recruitment of new individuals. The total biomass also increases through the accumulation of mass by individual fish as they grow over time. Simultaneously, the abundance and biomass are reduced as individuals die through predation, starvation and disease and, in some cases, harvest. Developing an understanding of these dynamic processes provides fundamental insights into the demography and population dynamics of a species. Understanding population characteristics is fundamental to the stock assessment procedures to determine stock status of fished species and to provide advice about potential yields and appropriate fishery management strategies (Hilborn & Walters 1992, King 1995).

Developing an understanding of the population dynamics and the controlling influences for non-exploited fish populations is also important. This indicates how natural populations are influenced by environmental variation, independent of the confounding influence of fishing mortality (Hilborn & Walters 1992). This may contribute to the better management of exploited fish stocks as well as providing valuable information for formulating ecosystem management plans. For example, the establishment and assessment of aquatic reserves and marine protected areas would benefit from detailed knowledge for key indicator species or rare species with high conservation value (Russ 2002).

It is essential to obtain estimates of growth, mortality and recruitment rates to understand the demographics and population dynamics of fish populations. Estimates of fish age provide the measures of elapsed time that are required for estimating these rates (Campana 2001). Fish ageing underpins the stock assessment procedures for many species of fishes from temperate regions of the world, which accounts for why approximately 2 million fish are aged annually around the world (Campana & Thorrold 2001). The earlier perception that direct ageing procedures would not work for fishes in tropical areas, however, (Longhurst & Pauly 1987) meant that such parameters were estimated using alternative methods such as length-based procedures (King 1995), whose applicability for tropical species is particularly concerning (Section 1.4.1). Consequently, the recent successes in using otoliths for direct ageing of tropical fish have been particularly revealing about the demography and life histories of such species and for addressing hypotheses about the factors that limit their abundances and influence their size and age structures (Thorrold & Hare 2002). These advances are evident in the following summary of some of the recent findings.

1.3.1 Demographic and life history characteristics

Longevity

Perhaps one of the biggest surprises from studies to date is that the estimates of life spans of a broad range of tropical fish have been in decades rather than just a few years. This finding is contrary to the notion that tropical systems are characterised by species with fast growth, high productivity and high turn-over (i.e., short lives) (Pannella 1974, Sale 1980). In fact, many species have maximum ages that exceed 30 years of age. Such unexpected longevity is true not only for some species that attain a large maximum size for which it might be expected that older individuals attain some protection from predation because of their size, but also applies to the small reef-associated species. One species of pomacentrid on the Great Barrier Reef, for example, had maximum ages of approximately 20 years (Doherty & Fowler 1994a). Several other species from the Galapagos Islands even reached >30 years of age, despite barely attaining 100 mm in length (Meekan et al. 2001). Several species of acanthurids had some of the highest estimates of maximum age with *Acanthurus lineatus* living up to 42 years, despite attaining only 183 mm in length (Choat & Axe 1996, Choat & Robertson 2002). Similarly, the small serranid *Cephalopholis cyanostigma* that grows to 350 mm in length reached up to 46 years of age (Mosse et al. 2002), whilst the tropical snapper *Lutjanus bohar* attained a maximum age of 54 years (Marriott 2002). Indeed, there is no apparent relationship between maximum age and asymptotic size when numerous tropical percoid species from several families are considered, since even relatively small species attain considerable ages (Figure 3a). Alternatively, there is a significant linear relationship between maximum age and asymptotic size for the scarids, indicating that those species that live longer generally attain larger sizes than the shorter lived scarid species (Figure 3b). Most estimates of maximum age for the scombrids exceeded 10 years, with the highest estimate attained so far being 22 years for the Spanish mackerel (*Scomberomorus commerson*) (Mackie et al. 2003).

Growth

The best way to determine the pattern of growth of fish is to sample a broad size range of individuals from the population, measure their size and obtain an estimate of age in years from their otolith macrostructure. This approach has been applied in numerous recent studies for tropical fishes, which have described growth in terms of the von Bertalanffy equation, i.e., $L_t = L_\infty[1 - e^{-K(t - t_0)}]$, where L_t = length at age t, L_∞ = theoretical maximum or asymptotic length that fish would reach if they lived indefinitely, K = rate at which maximum size is reached, and t_0 = the theoretical age at zero length (King 1995). This equation to describe growth has a strong physiological basis (Longhurst & Pauly 1987) and has become a standard in fishery studies.

The uniform approach of describing growth using the von Bertalanffy equation has provided a basis for comparing growth characteristics amongst species and between populations of the same species from different places (e.g. Newman et al. 2000a,b; Kritzer 2002). This has revealed that tropical species display different types of growth patterns, which relate, to some extent, to their longevities. Some species display the typical indeterminate growth pattern of fish from higher latitudes, where somatic growth is continuous throughout the life of the fish, albeit at a decreasing rate, ultimately resulting in a relationship between fish size and age (Figure 4a). Such growth has been described for species of coral trout (*Plectropomus leopardus* and *P. maculatus*) from the Great Barrier Reef (Ferreira & Russ 1992, Russ et al. 1996), as well as some of the species of the Scaridae family from the same region (Figure 4a) (Choat et al. 1996). Both the spotted mackerel (*Scomberomorus munroi*) (Begg & Sellin 1998) and Spanish mackerel (*Scomberomorus commerson*) (Tobin & Mapleston 2004), which occur in the offshore, open, tropical waters of Queensland, also exhibit this type of growth. Studies of cichlid species from different inland systems have also demonstrated such continuous growth throughout cichlids' relatively short lives (Jepsen et al. 1999, Faunce et al. 2002, Panfili et al. 2004).

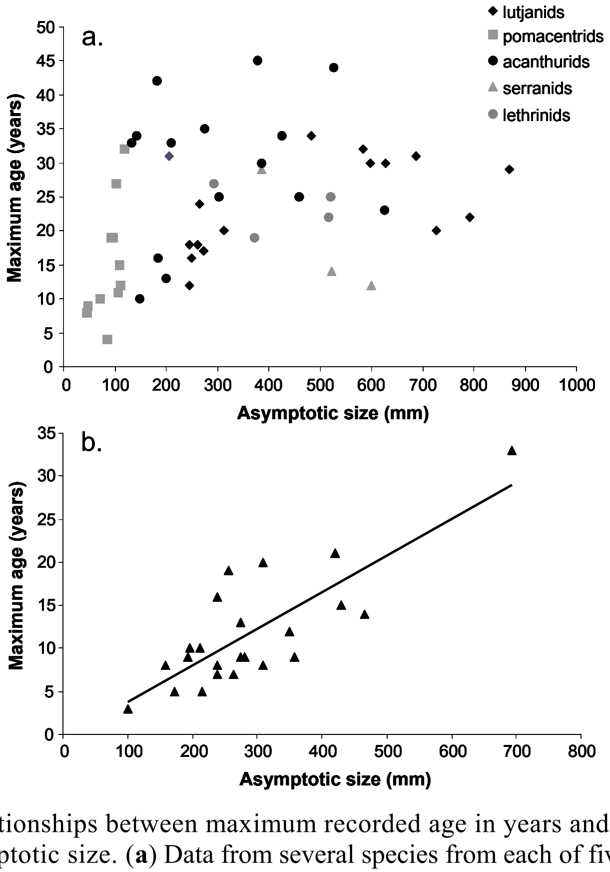

Figure 3. Relationships between maximum recorded age in years and the asymptotic size. (**a**) Data from several species from each of five reef fish families; (**b**) Data for a number of species from the Scaridae family with the line of best fit also indicated. Data extracted from the following sources: Fowler (1990), Ferreira and Russ (1992), Fowler and Doherty (1992), Ferreira and Russ (1994), Choat et al. (1996), Newman et al. (1996), Brown and Sumpton (1998), Newman et al. (2000a,b), Meekan et al. (2001), Burton (2002), Choat and Robertson (2002), Grandcourt (2002), Gust et al. (2002), Kritzer (2002), Newman (2002), Newman and Dunk (2002), Schwamborn and Ferreira (2002), Choat et al. (2003), Newman and Dunk (2003).

Other tropical fishes grow very fast over a relatively short part of the early life and quickly reach asymptotic size, after which there is virtually no further growth in length over the remainder of a fish's life. Such determinate growth results in a characteristic flat-topped growth curve (Figure 4b). Some species from the lutjanid, acanthurid, pomacentrid and serranid families demonstrate this type of growth. For example, *Acanthurus lineatus* from the Great Barrier Reef, which has a maximum age of 42 years, achieves 95% of its asymptotic size in only 6 years (Choat & Robertson 2002), meaning that individuals might experience no further growth in length for up to 85% of their life span (Figure 4b). The pomacentrid *Stegastes acapulcoensis* from the Galapagos Islands lives for up to 32 years but requires only 1 year to attain 80% and 3 years to reach 95% of its asymptotic size (Meekan et al. 2001). Thus, individuals can remain at approximately the same size for up to 29 years.

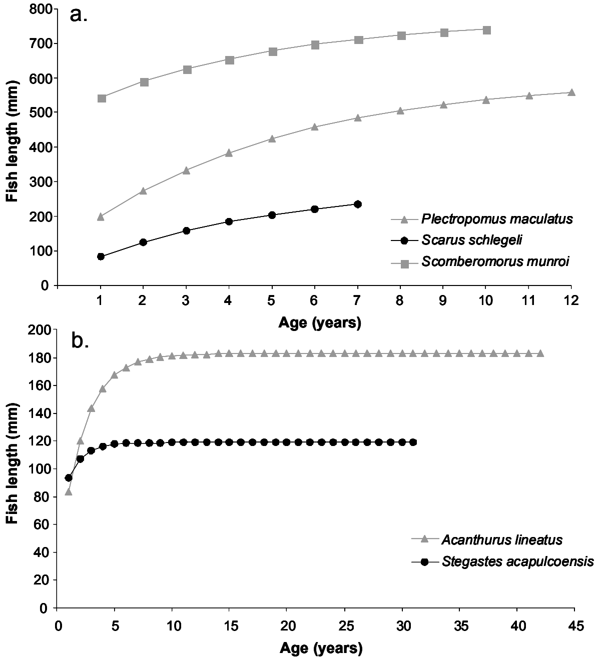

Figure 4. Von Bertalanffy growth curves for a number of tropical species. (**a**) Three species of fish with indeterminate growth, where size is related to age; (**b**) Two species for which growth is determinate and size and age are decoupled through much of a fish's life. Data extracted from Ferreira and Russ (1994), Begg and Sellin (1998), Meekan et al. (2001), Choat and Robertson (2002).

The species of reef fish with indeterminate growth tend to be relatively short-lived, whilst many long-lived species conform to the flat-topped growth curve. Thus, tropical species differ with respect to the extent to which size is related to age. For example, the largest size class of many long-lived species will be composed of many age classes, which means that for most of their lives their size and age will be decoupled (Choat & Robertson 2002). Some calculations were done to estimate what proportion of the maximum age would be required to attain 80% of the asymptotic size based on the von Bertalanffy growth parameters that are reported in the literature (Figure 5). These estimates demonstrated a clear decreasing spread of datapoints as maximum age increased. Some short-lived species, mainly the scarids, required a greater proportion of their lives to approach their asymptotic size, indicating that they conformed to the indeterminate growth pattern. The lutjanid species generally required 10–40% of their lifespan to attain 80% of the asymptotic size, whilst most acanthurid species required <20% to achieve this size. The pomacentrids had the fastest relative growth in this sense, generally requiring <10% of their life span to attain 80% of their asymptotic size.

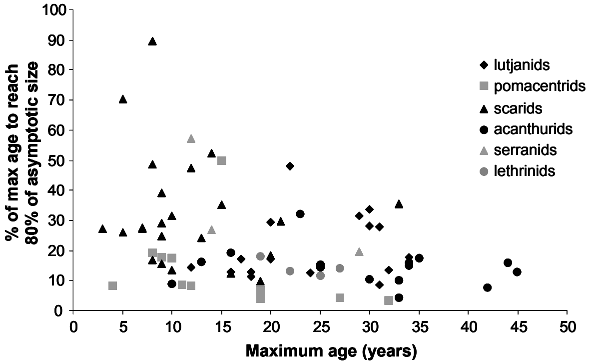

Figure 5. The percentage of maximum age required to attain 80% of the asymptotic size plotted against maximum age for several species from each of six reef fish families. Data were calculated from von Bertalanffy growth parameters provided in references listed for Figure 3.

Clearly, there are differences in growth patterns amongst the different fish taxa related to their longevities but such differences appear to not be reflected in the von Bertalanffy growth parameters of K, L_∞ and t_0 (Choat & Robertson 2002). In general, the reef fishes tend to lie somewhere along a continuum of small fish with fast growth rates and large fish with slower rates of growth (Figure 6). The species differ not so much in how they grow but rather in the number of years they persist without further growth. Such information is not well encapsulated in the von Bertalanffy parameters of K and L_∞ (Choat & Robertson 2002), indicating that it is necessary to simultaneously report the von Bertalanffy growth parameters along with estimates of maximum age to describe adequately the pattern of growth of such species.

Some understanding of the factors that influence the growth patterns of tropical fish have emerged from numerous population studies. For example, there were sex-based differences in growth for some species such as *Lutjanus sebae* (Newman & Dunk 2002) and *Lutjanus carponotatus* (Newman et al. 2000b), where in each case the males attained a larger L_∞ but grew at a slower rate than the females. Furthermore, size-at-age differed with sexual identity for sex-changing species such as the scarids *Scarus schlegeli* and *S. rivulatus*, where the terminal phase males were larger than similar-aged initial phase males and females (Choat et al. 1996). Males of the protogynous coral trout *Plectropomus maculatus* had larger mean size at age throughout life compared to individuals that remained females (Adams & Williams 2001).

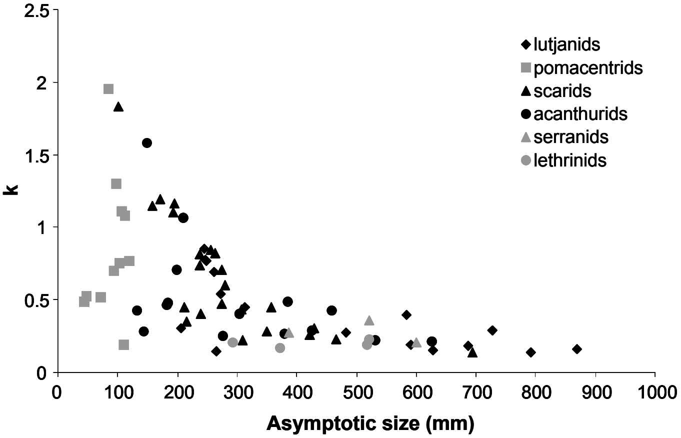

Figure 6. The relationship between the von Bertalanffy growth parameters '*k*' and 'L_∞' (asymptotic size) for several species from each of six reef fish families. Data from the references listed for Figure 3.

Some studies have used hierarchical sampling designs and begun to explore the issues of spatial variation in growth under different environmental conditions, and identified considerable variation at different spatial scales (Kritzer 2002, Gust et al. 2002, Williams et al. 2003). There is a trend for populations to have a shorter longevity, reduced asymptotic size but faster growth rate as mean water temperature increases over a large geographic scale (Choat & Robertson 2002). This may be reflected as a latitudinal effect, but local environmental influences can also complicate such broad-scale patterns. For example, several species of scarids and acanthurids on the northern Great Barrier Reef showed variations in growth across the continental shelf at the same latitude over distances of 10s of kilometres. The patterns were consistent amongst several species and were thought to relate to different resource levels and population abundances among the cross-shelf locations (Gust et al. 2002). Significant small-scale variation in growth patterns has been identified for *Lutjanus carponotatus* amongst small, localised reefs (Kritzer 2002). Growth of *Lethrinus miniatus* was relatively uniform amongst reefs separated by distances of up to 10s of kilometres but differed significantly among regions separated by 100s of kilometres (Williams et al. 2003). The pattern of variation was not consistent with latitude, however, which suggests that water temperature was not the single controlling factor. Again, patterns of growth may have been influenced by the distribution and abundance of food resources.

Mortality. The construction of age structure(s) for a population, based on ageing individual fish, provides the basic data from which an estimate of total mortality rate (Z) can be derived. This 'catch curve' analysis can be done either with a single sample of fish and based on the relative abundances of the different age classes collected at the same time, or with several age structures collected in different years, allowing cohorts of individuals to be followed through time (King 1995). Estimates of mortality rates in most tropical studies to date have been based on a single age structure. The samples of fish for several such studies on important fishery species were collected from broad areas across wide ranges in latitude, in each case producing a single age frequency distribution from the numerous estimates of age (e.g. Newman & Dunk 2002, Grandcourt 2002). The slope of the line of best fit through the natural logarithm-transformed frequencies for the different age classes from the modal class to the oldest age recorded provides the estimate of mortality (Z). This can then be easily converted to the % survival (S) of each age class, where $S = 100 \times e^{-z}$ and to the % mortality for each age class per year, where $M = 100(1 - e^{-Z})$.

The historic perception of tropical fish having high rates of turn-over and poor survivorship (Pannella 1974, Sale 1980) reflects a preconception that natural mortality rates must be very high. In contrast, however, the surprisingly high estimates of longevity for some species, and the fact that some older age classes are well represented in the age structures, indicate that the rates of mortality for many tropical species are not as high as previously expected (Doherty & Fowler 1994a,b, Meekan et al. 2001, Patterson et al. 2001, Newman & Dunk 2002). The estimates of total annual mortality for a diversity of taxa presented in the literature were in the range of 0.1–0.5, which translate into relatively high estimates of annual survivorship (90.5–60.7% respectively). There was a negative curvilinear relationship between maximum age and mortality rate, where those species with the low mortality rates of around 0.1 had the highest estimates of maximum age (Figure 7). Several outlier points are evident on Figure 7, which relate to several species of lethrinid, lutjanid and serranid that had high rates of fishing mortality. Concern was expressed for several of these that the estimated levels of fishing mortality were excessive, and that some management action was required.

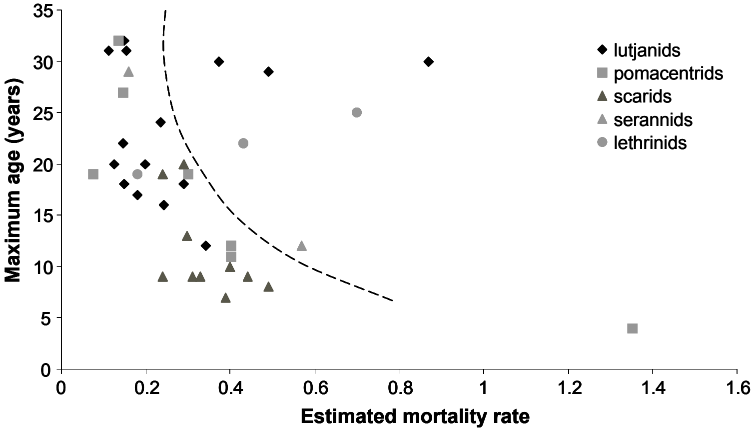

Figure 7. Relationship between maximum age and mortality rate for a variety of species from five families of reef fish species. The high estimates of mortality for the species to the right of the dashed line were related to high levels of fishing mortality. Data extracted from Ferreira and Russ (1992), Newman et al. (1996), Brown and Sumpton (1998), Newman et al. (2000a,b), Meekan et al. (2001), Burton (2002), Choat and Robertson (2002), Grandcourt (2002), Gust et al. (2002), Kritzer (2002), Newman (2002), Newman and Dunk (2002), Choat et al. (2003), Newman and Dunk (2003).

1.3.2 Ecological processes

The ability to determine the ages of adult tropical fish through interpretation of the macrostructure of otoliths has played an important role in understanding the factors that influence population abundance and structure. A significant example was in assessment of the influence of temporally variable recruitment on populations (Doherty & Fowler 1994a,b) and the debate surrounding the relative significance of pre- and post-settlement processes for the ecology of coral reef fishes (Doherty & Williams 1988, Mapstone & Fowler 1988). The issue was whether variable recruitment of new individuals to coral reef fish populations was the dominant influence over post-settlement population dynamics and demography, or whether post-recruitment events substantially modified the patterns of distribution and abundance established at the time of settlement. These alternative hypotheses have very different implications for assemblage structure and fisheries management (Doherty & Williams 1988, Mapstone & Fowler 1988). If post-recruitment events dominated, then the assemblage would likely be resource-limited and stable in structure with strong influences of intra-specific competition and density-dependent mortality. The alternative hypothesis was that reef fish populations were constrained mainly by limited numbers of larvae available to recruit to the post-settlement population, meaning that recruitment was inadequate to bring populations up to levels where compensatory, density dependent processes become important.

The study of Doherty and Fowler (1994a,b) was based on a time-series of recruitment rates for different species of fish that had been collected annually for the 9 year period of 1981–1989 for seven coral reefs at the southern Great Barrier Reef, Australia. A validated ageing protocol had been established by treatment of fish with tetracycline for two species of fish, *Pomacentrus moluccensis* and *P. wardi* (Fowler 1990, Fowler & Doherty 1992). In September 1989, the complete assemblages of fish associated with numerous patch reefs from within the lagoons of the seven coral reefs were collected, including the populations of *P. moluccensis* and *P. wardi.* Samples for these two species were aged from their sectioned otoliths and the resulting population age structures were compared with their 9-year recruitment history. The average annual rates of recruitment were good predictors of the differences in mean abundance amongst the seven reefs. Furthermore, the population age structures preserved the patterns of year-to-year variation in recruitment, with particularly strong age classes being relatable to years of exceptional settlement. It was thus apparent that the abundances and population structures for the two small species of fish were the consequence of variable recruitment and different rates of density independent mortality (Doherty & Fowler 1994a,b). This important ecological work was only possible due to the ability to age the adult fish in years based on interpreting the macrostructure of their otoliths using a validated ageing protocol.

A further conclusion can now be drawn from the work of Doherty and Fowler (1994a,b), taking into consideration the summary of demographic characteristics presented in Section 1.3.1 above. It is now reasonable to conclude that at the southern end of the Great Barrier Reef, where the Doherty and Fowler study was done, both *Pomacentrus moluccensis* and *P. wardi* were slower growing, attained larger maximum sizes and on average lived longer than the populations at lower latitudes further north on the Great Barrier Reef. The estimates of longevity for *P. moluccensis* of 17 years and 9 years for the southern and central parts of the Great Barrier Reef respectively, are consistent with this inference (Doherty & Fowler 1994a,b, Fowler 1990). Thus, such geographic variation in demographics for individual species along a latitudinal gradient cautions against applying the conclusion of recruitment limitation to the populations outside those that were directly considered.

Another age-based study that was done on the central Great Barrier Reef (18º30′S), and which involved the important fishery species the coral trout (*Plectropomus leopardus*), also identified the likely significance of recruitment variation to population dynamics and structure (Russ et al. 1996). Population age structures were developed in this study from samples of fish collected from two reefs that had been closed to fishing for a number of years. These age structures indicated the existence of a single strong year class of fish that had settled to the two reefs in early 1984. This strong year class was then tracked through the age structures from samples that were collected annually through the period of 1990–1993, which was the first time that temporal tracking of a cohort of fish had been achieved for a species of coral reef fish. It also provided the rare opportunity to estimate the rate of natural mortality for such an important fishery species (Russ et al. 1998). The most tractable explanation for the existence of this strong year class was inter-annual variation in recruitment, which ultimately influenced population abundances and age structures more than post-settlement, density-compensating processes. This significant finding was made only due to the availability of population age structures, determined from the macrostructure of the otoliths of the samples collected (Ferreira & Russ 1994).

1.4 ISSUES AND UNCERTAINTIES

1.4.1 Length-based methods for parameter estimation

Estimation of demographic parameters for stock assessment work on tropical fish was done largely using length-based analytical procedures prior to the relatively recent discoveries about the usefulness of otoliths for ageing tropical fish. The conceptual basis here was that discrete spawning events produced different size classes of fish that formed a polymodal length frequency distribution, where the different modes represented the normally distributed sizes of fish derived from the different spawning events. The different modes were relatable to time by making assumptions about the age of the first mode and the time periods between subsequent modes, thus making it possible to estimate parameters for growth and mortality (King 1995). There are two main analytical procedures for achieving this, based on single-sample and multiple-sample methods. In the former case, a single length frequency distribution is divided into separate modes. In the latter, multiple length distributions that are collected over time are arranged sequentially and the modes of the cohorts are traced as the fish increase in size over time.

There are several serious problems with using length-based methods of analysis, regardless of whether for tropical or temperate species. The first problem is that attaining length samples of fish that are representative of wild populations is extremely difficult and costly (Hilborn & Walters 1992). One reason for this is that fishing gear is always selective of fish size to some extent. Size selectivity is an interactive process between the method of capture and the behaviour of fish of different sizes. Furthermore, the life histories of many species of fish include ontogenetic migration amongst habitats and locations. As such, sampling the sizes of fish at particular places must select for particular size classes, which means that it would be necessary to sample the different size classes in different places, possibly using different fishing gears, in order to reconstruct representative size distributions for such populations. Finally, Hilborn and Walters (1992) also indicate that it is difficult to define a field-sampling regime and to train field workers to properly take representative samples of the size distributions from fishery catches.

There are also problems with length-based analyses that relate to the biology of fish species. Clearly, length-based methods will work best for those species that have discrete modes in their size distributions. Such species would be likely to have a discrete spawning season that leads to recruitment over a relatively short time period, after which growth is relatively fast and continuous. Alternatively, if the spawning period is extended or growth is slow or ceases at some point in life, then the normally-distributed sizes of fish originating from the different spawning events will overlap to such a degree that it will not be possible to distinguish separate modes. Furthermore, the rate of growth of fish slows as they get bigger, which means that the older age classes will ‘bunch’ together and form a mode in which the numerous age classes are indistinguishable.

The account of the demographics of tropical fish species presented above suggests that many species would be unsuitable for analysis using length-based analytical techniques. Many species are relatively long-lived, with relatively low rates of mortality, meaning it is likely that their size distributions will not display discrete modes that relate to individual age classes. This is likely to be the case for those species with indeterminate growth, but will be exacerbated for those species with determinate growth. The tropical snapper *Lutjanus carponotatus* lives for up to 18 years on the coral reefs of the central Great Barrier Reef but the size distributions on five different reefs were normally distributed, not displaying modes that were relatable to age classes (Kritzer 2002). Similarly, the males and females of *Lutjanus sebae* from North-western Australia, that live to 30 and 34 years respectively, each had length frequency distributions that were normally distributed without strong modes being apparent (Newman & Dunk 2002). Even the size distribution of the first 6 year classes for the black grouper, *Mycteroperca bonaci*, from Florida waters, which can live to 32 years of age, did not show modes that were relatable to age classes (Crabtree & Bullock 1998). Finally, despite the presence of a very strong year class in the age structures of coral trout (*Plectropomus leopardus*) collected annually over 4 years, there was no obvious modal progression in length distributions from year-to-year and the modal lengths of catches for the 4 years overlapped substantially, reflecting the large variability in size-at-age for this species (Russ et al. 1996).

1.4.2 Sampling for fish ages

Some consideration must be given to the process of sample collection and to the number of fish to be aged to develop a population age structure. There are two general ways to sample fishes for developing age structures. The simplest method is simple random sampling where a sample of fish is removed from a population and all or as many as possible are aged using a validated ageing protocol. It is assumed that the fish are sampled independently and have the same probability of being sampled, meaning that cohorts will be represented in the sample in proportion to their abundances in the wild population. Such sampling is not usually characteristic of fishery studies (for example the sequence of individual fish captures from line fishing is almost certainly non-independent), and many tropical studies have sought to address this problem. For example, researchers have produced combined samples of fish accessed from multiple sources, such as commercial and recreational fishers, and research sources. Such sampling also has been used in ecological studies involving hierarchical sampling. For example, Kritzer (2002) collected samples of *Lutjanus carponotatus* by spear fishing, where fish were targeted as sighted, without preference based on body size, so as to ensure that the sample was as representative as possible. The coral trout *Plectropomus leopardus* were sampled on different coral reefs and occasions using a standardised approach using recreational line fishers (Russ et al. 1996). Doherty and Fowler (1994a,b) used rotenone to collect all individuals of *Pomacentrus moluccensis* and *P. wardi* from patch reefs in the lagoons of coral reefs. In each of these examples, as many fish as possible from the samples were aged for development of age structures. The study of Kritzer et al. (2001) is an example of investigating the optimal sample numbers required to estimate demographic parameters for populations of tropical reef fish.

An alternative form of sampling has been called two-stage random sampling (Quinn & Deriso 1999). This method, which appears to not have been commonly applied in tropical applications, is more likely to be used in fishery applications where there is greater accessibility to samples. Here, the first stage of sampling is where a simple random sample is removed from the catch and each individual is measured for generation of a size frequency distribution. The second stage of sampling is where a sub-sample of the first sample is then selected for ageing work based on the interpretation of otolith structure. The age frequencies for each length class of fish are used to generate an age-length key, which is then used to convert the larger length sample into estimates of age. Clearly the main efficiency here is that not all fish are aged, thus representing a cost saving in labour time and material costs.

There are two well-known methods for selecting those fish to be aged from the length sample. The first is called ‘fixed allocation’, where a constant number of fish are aged from each length class. The alternative is ‘proportional allocation’ where the number in the age sample is proportional to the number of fish in the different length categories. Such proportional allocation of samples results in the self-weighting of data across these length categories and is likely to constitute the best default allocation in any new application (Kimura 1977, Quinn & Deriso 1999).

The application of the age-length key provides an age structure based on the frequencies of age classes in each length category. The estimated variance for each age class comes from the theory of two-stage sampling and involves both a within-length and between-length component of variance (Quinn & Deriso 1999). The numbers of fish that are required for measurement and ageing can be determined statistically in order to achieve a pre-determined level of precision in the age structure.

1.4.3 Accuracy and precision of fish ageing

The process of determining fish age in years from the structure of otoliths involves several possible sources of error that can result in erroneous estimates of fish age. This is true regardless of whether it is a tropical or temperate species and is independent of the size or significance of the ageing study. Such errors, if undetected or uncorrected, would propagate through the subsequent quantitative processes, resulting in incorrect frequency distributions, erroneous estimates of growth rates, mortality rates, yield estimates and the misunderstanding of the potential productivity of the fishery (Lai & Gunderson 1987, McFarlane & Beamish 1995, Eklund et al. 2000). There are two major sources of such error: the first has been called 'process' error and relates directly to the otolith structure being interpreted; the second relates to errors in the interpretation of the otolith structure (Campana 2001, Morison et al. 2005).

As indicated earlier, otoliths must satisfy three criteria to be useful for ageing work (Section 1.2.1, Fowler & Doherty 1992). Otoliths used to estimate fish age when any of the three criteria is not satisfied will result in counts that do not accurately represent the fish age in years. This is the 'process' error identified above. Such errors were common in many studies on temperate fish species done prior to the 1980s based on interpreting either the structure of fish scales or whole otoliths. The errors resulted from using procedures that had not been validated to estimate fish age. A landmark publication in 1983 highlighted the lack of attention that scientists had paid, to that time, to appropriate validation procedures (Beamish & McFarlane 1983). The authors also pointed out that such inattention was likely to have resulted in serious misunderstandings of the biology and population dynamics of the important commercial fish species that were involved, with serious economic consequences.

One advantage of the relatively late start to tropical fish ageing is that researchers have learned from the earlier mistakes and heeded warnings from their temperate fish counterparts to ensure that fish ageing methods are appropriately validated. There are a number of methods that can be used for such validation work that vary in their scientific value, as well as their applicability, advantages and disadvantages (Campana 2001). Tropical fish researchers to date have paid considerable attention to ensuring that their ageing protocols have been validated appropriately (see also Choat et al. in Chapter 2 of this volume).

It is inevitable that some ageing errors related to the preparation and interpretation of the incremental structure of the otoliths will occur even when a validated ageing protocol is used (Morison et al. 2005). The structure of otoliths is complex, displaying a variety of increments and discontinuities (Pannella 1974). The annual macrostructure can vary considerably in clarity and interpretability both amongst individuals from the same population and among populations from different places (Fowler 1995). Reading and interpreting such complex structures, therefore, must itself be a complex process. Correctly interpreting otolith structure is an analytical process that must be learned and for which skill level increases with practise and experience, but also declines with lack of practise (Morison et al. 2005). Not surprisingly, there is considerable variation in the aptitude of different personnel for this task.

Differences in aptitude of fish agers and variation over time in their skill levels should be taken into consideration in ageing studies. This requires the implementation of appropriate quality assurance and control procedures. Many fish ageing laboratories around the world have established quality control protocols that are aimed at detecting and redressing errors in otolith interpretation to minimise the influence of incorrect age estimates on stock assessments (Campana 2001, Kimura & Anderl 2005, Morison et al. 2005). Such quality assurance and control measures are based on monitoring the consistency of the ageing work over time, particularly between years, to ensure that the way the age readers interpret the otoliths does not drift through time. 'Drift' would introduce bias to the estimates relative to those from earlier age determinations. Using appropriate quality measures also helps to ensure that age interpretations are comparable between readers. This process will detect systematic or biased errors, which are of greatest concern because of their consequences for the calculation of age-based parameters that relate to the population dynamics (Campana 2001). Thus, using appropriate quality control monitoring is an important part of the data collection process that underpins stock assessment and fishery management procedures. The appropriate implementation of such procedures will likely provide confidence to the otolith readers, as well as those who use the age data in quantitative processing, and to the fishery managers whose decision-making depends on the outcomes of the stock assessments.

1.4.4 Geographic variation in otolith clarity and interpretability

A further issue for tropical fish otoliths, compared to those from temperate fish, is that there is considerable variation in the clarity and interpretability of the otolith structure. Such variation is apparent amongst individuals within the same population, but is more apparent among populations of the same species from different places (Fowler 1995). There can be considerable differences in the usefulness of otoliths among confamilial and even congeneric species (Fowler 1995). These taxonomic and geographic differences have been demonstrated most clearly for species from the pomacentrid family of coral reef fishes. There were notable differences in the clarity of the incremental macrostructure of otoliths from each of *Pomacentrus moluccensis* and *P. wardi* between locations on the central and southern Great Barrier Reef, which differed in latitude between 19° and 23°S (Fowler & Doherty 1992). Furthermore, the otoliths from the former species were easier to interpret than those from the latter at each location. A similar phenomenon was described for the congeneric species *Stegastes planifrons* and *S. partitus* collected from five locations throughout the tropical western Atlantic, whose latitudes ranged from about 9° to 28°N (Caldow & Wellington 2003). In both examples, the fish collected from the higher latitudes had the clearer otoliths. Those locations with broader temperature ranges had clearer otoliths, but an incremental structure was still apparent in otoliths of fish from locations with a seasonal temperature range of only 3°C in the latter study. This suggests that seasonal water temperature variation is to some extent implicated in otolith increment formation in tropical marine fishes. The situation is more complex than this, however, since there were clarity differences between otoliths of two *Stegastes* species collected from places that experienced similar seasonal temperature regimes (Caldow & Wellington 2003).

There was also considerable geographic variation in the clarity of the macrostructure of the opaque and translucent bands for several species of peacock cichlids sampled from a number of tropical rivers and reservoirs in Venezuela (Jepsen et al. 1999). Otolith clarity was more pronounced for fishes from fluvial ecosystems, whilst those from reservoirs were mostly diffuse and difficult to interpret. The formation of opaque bands in the clear cichlid otoliths corresponded with periods of slow somatic growth that were associated with the heaviest rainfall period of the year.

Thus, there was a correlation between hydrological seasonality and otolith macrostructure. Alternatively, the populations living in the reservoir environment, which is a relatively benign and aseasonal environment compared to that of the rivers, had otoliths that were so diffuse and unclear that their macrostructure could not be interpreted with confidence.

1.4.5 Otolith macrostructure – causes and need for validation

It is now apparent for a range of taxa from a diversity of tropical systems around the world that fish otoliths have proven extraordinarily useful for ageing. Nevertheless, there remains a lack of understanding of the process of otolith growth and the nature of what is perceived optically as an otolith macrostructure. In fact, there remains a general lack of fundamental understanding of the relationship between otolith macrostructure, the physiological processes of the fish and the environmental conditions in which the fish has grown (Wright et al. 2002). This may not necessarily impede the use of otoliths for direct ageing, but it does mean that there must remain a level of caution and some limitations to their use. For example, there remains the requirement to undertake validation studies in new applications of otoliths for ageing work for tropical fish species. This requirement might not be so stringent, however, if our fundamental understanding about otolith macrostructure was more advanced.

There are two issues related to understanding the formation of annuli in fish otoliths: understanding the basis of what is perceived as an otolith macrostructure – i.e., the sequence of bands of alternating optical density; and identifying the processes of physiological control over otolith formation and how this is influenced by exogenous factors (Fowler 1995). There was little understanding of either of these factors in 1995 and it was recommended that this be a key focus for future research. This challenge appears to not have been met in the interim (Wright et al. 2002). Otolith-based studies in the tropics since then have focussed primarily on the use of otoliths as chronometers, with little attention to the nature of otoliths themselves. Few papers have provided comprehensive descriptions of otolith structure and there has been a lack of consideration of the physiology of macrostructure formation.

The studies from the 1980s that related otolith macrostructure to the incremental microstructure were not all in agreement with regard to the nature of the variation in optical density at the macroscopic level. Some suggested that the opaque bands reflected wide daily increments indicative of fast growth in spring and early summer, and the translucent bands related to thin daily increments (Brothers & Mathews 1987, Morales-Nin 1989). Alternatively, a study of a pomacentrid species from Midway Island in the Pacific Ocean suggested that the opaque band reflected a period of slow growth (Hill & Radtke 1988). This latter finding is similar to the more recent findings for the cichlid species for which it has been determined that the opaque bands correspond to periods of slow somatic growth in response to environmental stress (Jepsen et al. 1999, Panfili et al. 2004).

There has been little recent work on tropical species to resolve the ambiguity about the nature of the macrostructure of the otoliths. Some work for a suite of South African species from the Sparidae family from the latitude range of 33–34°S (Mann-Lang & Buxton 1996), however, may be useful here because sparid otoliths share a number of characteristics with those described earlier for tropical percoid fishes. Their otoliths have a glassy appearance and, when sectioned, show an alternating sequence of narrow opaque and broad translucent bands, which occasionally display discontinuities (Mann-Lang & Buxton 1996, Fowler personal observation). The microstructure of the different bands in the otoliths of these species was examined using scanning electron microscopy (Mann-Lang & Buxton 1996). The translucent bands were found to be composed of daily increments each with a wide calcium carbonate incremental phase, which meant that the proteinaceous discontinuities were broadly spaced. Conversely, the opaque bands were characterised by narrow incremental structures, which meant that the proteinaceous discontinuities of daily increments were closely spaced. This finding is intuitively appealing as it sensibly accounts for the optical characteristics of the macrostructure, i.e., that the opaque band is optically dense because it consists of numerous thin increments with closely aligned proteinaceous discontinuities, whilst the translucent band transmits light because of the broad crystalline incremental part of the daily microincrements. This suggests that the opaque bands represent slow otolith growth and the translucent bands relate to periods of fast growth. This finding is somewhat counterintuitive since for 9 of the 12 species of sparids the opaque bands formed in spring–summer and the translucent bands related more to autumn–winter, indicating that otolith growth was faster during the cooler part of the year and slower during the warmer part of the year. The thin opaque band also forms during spring–summer in numerous species of tropical reef fish (Fowler 1995). Maybe for these fish the opaque band also represents a period of slow otolith growth, and the broader translucent band is from the faster growth period of the year. This suggestion is contrary to the findings of early studies that explored this issue (Brothers & Mathews 1987, Morales-Nin 1989, Morales-Nin & Ralston 1990).

The sparid study also developed a model that related fish physiology to otolith growth (Mann-Lang & Buxton 1996). The timing of formation of opaque bands, which generally incorporated spring and summer, corresponded with the time of reproduction for most of the 12 species considered. Thus, the period of slow otolith growth at the warm time of the year may reflect the impact of calcium metabolism in relation to reproductive activity. This implies that otolith growth in mature fish is strongly influenced by endogenous processes, rather than being directly or predominantly driven by exogenous factors such as water temperature. Mann-Lang and Buxton (1996) proposed that water temperature had a stronger influence on otolith growth in immature fish. This model is complicated because it suggests that otolith growth and macrostructure formation are controlled by a combination of endogenous and exogenous factors throughout the lifetime of the fish, whose influence varies at different ages and life history stages. This model remains equivocal because it has been suggested that although reproducing females have elevated plasma calcium concentrations, this takes the form of protein-bound calcium, which might not affect the calcium levels in the endolymph (Wright et al. 2002). Nevertheless, if the model is correct, it suggests that there could be a shift in the timing of formation of opaque bands with the onset of maturity, from temperature driven periods of slow otolith growth in winter to physiologically driven periods of slow growth in spring–summer.

The poor understanding of the formation of otolith macrostructure for tropical fish has consequences for the on-going requirement for validation processes. Two main procedures have been used for validation in the tropical studies since 1990: the treatment of fish with oxytetracycline to form a time marker in the otolith structure; and marginal increment analysis. Both validation procedures can be demanding of time, labour and resources, particularly if validation is to be achieved for the total range of age classes (Beamish & McFarlane 1983), which can be up to approximately 50 years for some tropical species (Section 1.3.1). Because of the poor understanding of the nature of otolith growth and the controlling influences, however, such validation procedures remain necessary in new applications. This may be particularly so for the scombrid species that may display a secondary macrostructure that is formed sub-annually (Lewis & Mackie 2002). It might not remain essential to complete validation for each new ageing application if in the future there is improvement in our understanding of the relationships amongst otolith growth, environmental seasonality and physiological processes.

2 Methodological approaches to fish ageing studies

2.1 HISTORICAL APPROACHES FOR ESTIMATING FISH AGE FROM OTOLITHS

2.1.1 Whole versus sectioned otoliths prior to 1980

Many ageing studies prior to the 1980s, even those related to the huge fisheries in the cold water regions of the world, were based on interpreting the structure of whole otoliths (Beamish 1992). It was finally realised, however, that it may be impossible to obtain accurate estimates of age from the optical characteristics of whole otoliths for the entire size and age range of fish in a population because of the way otoliths grow in three dimensions throughout the lives of the fish. Otoliths generally do not grow consistently in length, breadth and thickness throughout life, with growth in length and breadth slowing and, in many cases, ceasing whilst they continue to accumulate thickness. This results in the ‘stacking’ of growth increments in the sagittal plane between the otolith core and the proximal surface, which is evident in Figures 1 and 2 (Beamish 1979a, Mann-Lang & Buxton 1996). Such a pattern of otolith growth clearly means that the alternating sequence of opaque and translucent bands cannot be observed when whole otoliths are examined from either the proximal or distal surfaces, but most otoliths are too thick for light to pass through sufficiently to show the incremental structure when examined side-on.

It became apparent that it was possible to determine ages accurately only by exposing the incremental macrostructure within the otoliths of older fish, which required exposing the otolith structure perpendicular to the plane of maximum growth. This generally has been achieved by either: (a) removing a transverse section through the otolith centre using a diamond saw; or (b) breaking the otolith through the centre to expose the transverse face and then burning the otolith to help differentiate the internal bands. Burning highlights the different bands within each annual growth increment because of their relative differences in protein (which discolours with burning) and calcium carbonate (which does not) (Christensen 1964, McFarlane & Beamish 1995, Fowler & Short 1998). It has become apparent from these more recent methods that many ageing studies from the first 80 years of the 20th century, even for some species that supported huge and highly valuable fisheries, produced estimates of age that consistently underestimated the true age of the fish. For example, the sablefish (*Anoplopomia fimbria*) was considered to be a fast-growing, short-lived species with longevity of up to 12 years until early results from broken and burnt otoliths indicated that some fish in fact lived longer than 60 years (Beamish & Chilton 1982, McFarlane & Beamish 1995). Similarly, estimates of age from sectioned otoliths of walleye Pollock (*Theragra chalcogramma*), which supports the largest fishery in the world, indicated that longevity was twice that indicated by counts from whole otoliths (Beamish 1992). Similar differences in age estimates between whole and sectioned otoliths have been identified for other important fishery species, such as the Pacific Ocean perch (*Sebastes alutus*) and Pacific hake (*Merluccius productus*), with some estimates of longevity differing by up to a factor of three (Beamish 1979a,b).

The examples presented above indicate that during the latter part of the 20th Century it was realised that many species of fish had been inaccurately aged from their scales and whole otoliths, because of insufficient attention to validation procedures (Beamish & McFarlane 1983). Such errors have highly significant implications for the estimates of population parameters such as growth and mortality rates, and associated estimates of fishery productivity and sustainable rates of harvest (Beamish & McFarlane 1995). More accurate estimates of age highlighted that many species of fish grew more slowly, lived longer and had lower rates of natural mortality than was originally estimated. It was concluded that these species of fish were far less productive and more vulnerable to overfishing than previously thought.

2.1.2 Age based on otolith measurements

Some studies in the latter part of the 20th Century explored the possibility that otolith size and weight might provide sufficiently accurate and precise estimates of fish age to by-pass the requirement for the visual interpretation of otolith structure. This was considered beneficial as it might provide savings in time and labour costs associated with preparing and interpreting the transverse sections of otoliths. Boehlert (1985) used multiple regression models to integrate data on otolith weight, length and width from individual fish to estimate their age for two species of rockfish, *Sebastes diploproa* and *S. pinniger*, from the west coast of the USA. The models typically accounted for 70–92% of the variability in age, depending on species, sex and method of age analysis.

A study of the pilchard (*Sardinops neopilchardus*) used otolith weight as an indicator of fish age, with the resulting age estimates being used to develop age frequency distributions from which population parameters were calculated (Fletcher 1991, 1995). A similar study done in the tropics assessed the utility of otolith weight to estimate fish age, and identified two sources of error in the estimates of age (Worthington et al. 1995). Firstly, the considerable overlap in otolith weight amongst fish of different age classes resulted in normally distributed errors in the estimates of age from otolith weights. Secondly, there was spatial variation in the otolith weight–age relationships that could bias subsequent age determinations. It was nevertheless concluded that otolith weight could provide an objective and economic method for age determination with the same reliability as counting annuli in sectioned otoliths provided the relationship between otolith weight and age was frequently calibrated.

2.1.3 Transverse sections of otoliths from tropical species

The majority of population studies for tropical fish species that have been based on direct ageing have used the transverse sections of otoliths and have enumerated the opaque and translucent bands towards the proximal surface in the vicinity of the sulcus. There appears to have been little consideration paid to the supposed potentially labour-saving methods of estimating fish age from otolith weight or size. Perhaps this preference for using transverse sections reflects that the labour involved in producing such preparations is less onerous than was thought would be the case. It has probably become evident to researchers that the laboratory work involved in preparing and interpreting transverse sections of otoliths is far lower and less demanding of resources than the field collection of the specimens to be aged. Using the preparation methods described in technical Box 2 (later in this chapter), it is quite tractable for a technician to produce several hundred transverse sections of sagittal otoliths within a week. Lou et al. 2004 have made a comparison of efficiencies of otolith processing of fishes from both tropical and temperate marine environments. With the notable exception of the Effects of Line Fishing experiment on the Great Barrier Reef (Mapstone et al. 2004), many tropical fish studies to date have been based on sample sizes of only hundreds rather than thousands of fish and the laboratory time and cost of producing and interpreting the otolith sections have been relatively low.

Researchers generally have been wary of using whole otoliths, due to the temperate fish experience, but several studies have combined the use of transverse sections with reading of whole otoliths. This introduces efficiencies into the protocol as it avoids having to section the otoliths of young fish that are effectively interpreted whole. For example, Ferreira and Russ (1994) first read whole all the otoliths of the common coral trout (*Plectropomus leopardus*) and when the increment count exceeded six or the otolith was considered difficult to interpret, it was sectioned and then reconsidered. For *Lutjanus carponotatus,* the first annulus in sectioned otoliths was often faint and difficult to discern, which meant that the counts deviated between whole and sectioned otoliths for the older age classes. Consequently, whole otoliths were used to age fish that were up to 10 years old, whilst sectioned otoliths were required for the older age classes from 10 to 18 years of age (Kritzer 2002). Williams et al. (2003, 2005) found for *Lethrinus miniatus* that fish of all ages up to the maximum of 19 years could be aged reliably from whole otoliths following a thorough comparison of readings between whole and sectioned otoliths.

2.2 PRACTICAL STEPS IN A FISH AGEING STUDY

There are a number of sequential steps involved in completing a fish ageing study once the validated ageing protocol to provide estimates of fish age has been established. These steps include: (1) collecting the specimens; (2) removing and preparing the otoliths; (3) interpreting the otolith structure to provide the count of the macro-increments that is used in deriving the estimate of fish age; and, finally, (4) completing some quality control assessment to ensure that the age estimates have an acceptably low error rate. Some notes on these steps are provided in the following text.

2.2.1 Collecting samples

The sampling strategy used for accessing samples of fish will be determined by the nature, aims and funds available to the study. There are two broad categories of sampling methods: fishery dependent and fishery independent. Clearly, the first of these relates to sub-sampling the catch of the fishery to obtain biological samples and size information. Such sampling may be through an on-board observer program or subsequently on land through port or market sampling. This approach has the advantage of being relatively cheap but can result in problems in achieving spatial coverage of the target population since fishers choose where to fish based on their knowledge, experience, and habit. Thus, samples may be dominated by those from accessible areas or places where the fish are more abundant, which could introduce biases into the estimates of age-based parameters. The spatial distribution over which the samples are collected and combined will determine the applicability and variability in the estimates of population parameters. A study of several important reef fish species in the artisinal fishery of the Republic of the Seychelles used traditional fish traps, spears and handlines to access the fish samples (Grandcourt 2002). Newman and Dunk (2002) accessed landings from the commercial Northern Demersal Scalefish Fishery of North-western Australia for a study on *Lutjanus sebae*, where samples were collected between July 1995 and December 1999 from latitudes 12º–20ºS using fish traps in water depths of 60–150 m. Begg and Sellin (1998) collected samples of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) from commercial net fishers and recreational anglers from June 1992 and January 1995, which provided 1,172 otoliths of the former species and 1,385 from the latter.

The alternative approach is to undertake fishery independent sampling, giving the scientist more control over the spatial and temporal aspects of the sampling and allowing standardisation of sampling equipment and techniques. It is possible to obtain further information that may not be possible from landed fishes, such as tissue samples for genetic studies and stomach contents for information about the interactions amongst species. The disadvantages of fishery independent sampling are the expense, particularly if a charter or research vessel is involved, and lower sample sizes than are accessible through the fishery. Fishery independent sampling has been used in numerous ecological studies on the Great Barrier Reef (notably Mapstone et al. 2004), which have aimed to provide a more refined understanding of population characteristics at several spatial scales. In each case, hierarchical sampling regimes were used to target particular species, which provided representative samples of fish from relatively small areas and occasions (Kritzer 2002, Gust et al. 2002, Williams et al. 2003), or over a specific geographic range (Williams et al. 2003, Lou et al. 2005). Such sampling clearly allows for the comparison of population characteristics over a range of spatial scales. Targeted, regular monthly sampling using dropnets, cast nets and line fishing was used to access samples of cichlids in South-eastern United States of America and Western Africa (Faunce et al. 2002, Panfili et al. 2004).

2.2.2 Preparation of otoliths for interpretation

After collection, fish are dissected and their otoliths (most commonly the sagittae) are removed. The otoliths are then cleaned to remove remnant tissue, blood and endolymph by bathing them in alcohol or dilute bleach, or by wiping them on paper towel. They are then dried, stored and labelled. Note that some cleaning procedures may preclude use of the sample in future studies such as otolith chemistry analysis (see Chapter 8 by Thorrold & Swearer in this volume) and population genetic analysis that rely on biological material adhering to archival otolith samples. Otoliths should be weighed after cleaning and prior to further processing if otolith weight might be used as a proxy for age in later work.

There are several methods by which the otoliths can be prepared for examination, depending on the chosen ageing protocol. Some researchers have examined some or all otoliths whole (Ferreira & Russ 1994, Kritzer 2002, Williams et al. 2003, 2005). This technique would now only be considered acceptable if it had been demonstrated previously that whole otoliths provided similar counts to those from sectioned otoliths (Fowler & Short 1998, Williams et al. 2003). Whole otoliths are immersed in a liquid or oil to clear them so that they transmit light, which more clearly exposes their internal structure (Morales-Nin & Panfili 2002b). Such liquids and oils include water, alcohol, glycerine, immersion oil, clove oil or aniseed oil. The otoliths are then examined using either transmitted light through a transparent container or reflected light against a black background.

It has been necessary in most studies of longer-lived tropical fish species to expose the otolith structure perpendicular to the plane of the growth layers to provide accurate counts of the annuli. There are two approaches to achieve this: by ‘breaking and burning’ the otoliths; or by preparing transverse sections through the otolith centres. These methods are broadly described here and detailed instructions are provided in Boxes 1 and 2 respectively.

In the ‘breaking and burning’ process (Box 1), the otolith is snapped in two, perpendicular to the long axis through the centre, thus providing two halves each with a freshly exposed transverse face. One half is then slowly heated in a flame, which gradually cooks the half otolith, turning it brown as the protein is denatured, and accentuating the difference between the opaque and translucent bands. The cooked half is then mounted so that the transverse face can be smeared with immersion oil and examined using reflected light and low power microscopy.

Box 1. Recipe for breaking and burning an otolith

1. Dissect fish and remove otoliths.
2. Clean otoliths.
3. Weigh one otolith.
4. Examine the otolith using a dissecting microscope and transmitted light – examine the distal surface with the otolith resting on its proximal surface.
5. Locate the core region of the otolith and mark with a single line across the otolith between the two edges through the centre, using a sharp pencil.
6. Orientate the otolith with the distal surface facing upwards, push downwards on the two ends of the otolith using the thumb and index finger of one hand, whilst simultaneously scoring the otolith across the pencil line with a scalpel. This should cause the otolith to snap into two pieces approximately through the centre to expose the transverse faces of both halves.
7. Hold one half of the otolith with a pair of forceps, heat it slowly in an alcohol flame or the blue flame of a Bunsen burner. Orientate the otolith piece so that its bottom end receives the heat of the flame and the otolith gradually cooks and turns brown from the end towards the centre. It might be necessary to repeatedly move the otolith through the flame for a number of seconds to control the rate of heating.
8. Allow the cooked otolith half to cool and then mount it in plasticine with the transverse face orientated upwards and ready for examination.
9. Smear the transverse face with a little immersion oil, illuminate with light from above, examine the surface and interpret the alternating sequence of thin, whitish, opaque bands and broader, brown translucent bands.

The preparation of transverse sections of otoliths (Box 2) is the most used method in studies ageing tropical percoid fishes to date, regardless of their taxonomic classification or the environment they inhabited. There have been many methods developed for preparing transverse sections but there are several fundamental requirements that must be taken into consideration. The section must be cut at the appropriate angle, ideally on the plane normal to the growing surface, to achieve maximum discrimination between the different bands. The section must also be sufficiently thin to allow enough light to pass through to highlight the optical characteristics of the bands, whilst minimising artefacts that relate to viewing too thick a section. There must also be appropriate preparation of the surface of the section to minimise the interference effects of scratches that may result from the cutting process that would affect the resolution of the internal structure of the otolith.

The clean, dry otoliths should be embedded in resin prior to being sectioned, to protect them during the sectioning process. The resin block encasing the otolith is then cut using a low speed diamond saw to produce sections of 100–500 μm thick, depending on the species. The section might then be polished using either diamond paste or lapping film, and smeared with immersion oil to clear any remaining surface scratches. Alternatively, appropriate gluing of the section to a glass slide may remove any need for polishing. The section can then be examined under low power microscopy using either transmitted or reflected light. Histological staining, although not often needed, may improve contrast between opaque and translucent bands in the section, which apparently reveals similar chromophilic increments to the burning process

(McCurdy et al. 2002). Good results have been obtained using a sequence of acid etching followed by staining with aniline blue and toluidine blue (Richter & McDermott 1990), whilst the use of just toluidine blue after acid etching has also been successful (McCurdy et al. 2002).

Box 2. Protocol for preparation of a transverse section of an otolith

1. Dissect fish and remove otoliths.
2. Clean and weigh the otoliths.
3. Embed one otolith in resin using an appropriately sized mould so that the whole structure is encased in resin. First set some resin in the mould, then place the otolith on the cured resin before topping up to completely encase it. It is possible to embed a number of samples per resin block, but special care must be taken so that otoliths are aligned for simultaneous cutting, and to preserve sample identity.
4. Examine the resin block containing the otolith under a dissecting microscope illuminated with transmitted light. Score the block with a scalpel to identify where to cut the block to provide the section of desired thickness.
5. Securely clamp the resin block containing the otolith in the cutting saw jig and align the block with the saw to produce the desired cut.
6. Use a diamond saw to remove one end of the resin block and otolith, thus exposing the transverse face of the otolith.
7. Make a second cut to remove the slice of otolith of the desired thickness. Several slices of the otolith can be removed in this way to obtain multiple sections. This is particularly advisable when multiple otoliths have been set in the same block because of the difficulties in aligning multiple otoliths. Note that if a double-bladed saw is being used then steps 6 & 7 represent a single step.
8. Retrieve the slice(s) of resin that incorporates the desired otolith section(s), dry, and mount on a glass slide by carefully lowering onto a drop of liquid glue such as pre-heated Crystalbond. Since this glue has approximately the same refractive properties as the resin, any scratches in the cut surface will be filled and there will be no requirement for time-consuming polishing.
9. Examine the section and check for appropriate thickness based on the clarity of the increment structure. If the section is too think it can be ground using wet and dry paper and polished with lapping film.
10. Smear the otolith surface with immersion oil and then examine under a dissecting microscope using either reflected or transmitted light.

2.2.3 Interpretation of otolith macrostructure to estimate fish age

The whole otolith, broken and burnt half otolith or the transverse section is then examined under low power microscopy. The structure is illuminated either by transmitted or reflected light. Transmitted light is directed from below through the slide or container and through the otolith. Reflected light is provided from a cold light source, with the light directed onto the otolith plane from above and oblique to the otolith. The lighting affects the appearance of the incremental structure and thus its interpretability, and some experimentation is required to achieve the optimal arrangement that maximises the contrast between the opaque and translucent bands. The opaque bands are more optically dense than the translucent ones and thus more effectively block the transmittance of light. Therefore, under illumination by transmitted light the opaque bands appear dark, whilst the translucent ones are light. Alternatively, under reflected light the opaque bands appear bright whilst the translucent bands are dull (see also Figure 1 in Chapter 1 of this volume).

The otolith structure is then interpreted by counting either the opaque or translucent bands. This is the difficult interpretative process that was discussed in Section 1.4.3, which the age reader must learn and practise to achieve proficiency. The interpretation of the structure is complicated by such difficulties as: the need to identify the first increment; recognising annuli that may vary in optical density across the otolith; distinguishing true annuli from false checks and discontinuities; and distinguishing the type of increment on the otolith edge and the relative width of this marginal increment.

The resulting count of the number of annuli does not necessarily translate into a direct estimate of fish age. Rather, this count must then be interpreted using other information to provide the best estimate of the fish's age. This further required information is rather complex and depends on having at least a basic understanding of the life history of the species and the growth pattern of the otolith. It is necessary to know the reproductive season of the species as this is used to nominate the standard birth date to be assigned to every fish (Morison et al. 2005). It is also necessary to know the time of year when the opaque and translucent bands form in the otolith. Finally, it is also necessary to know the time of year that the fish was captured. Then, the number of annuli in the otolith, the standard birth date, the time of year of capture and the interpretation of the marginal increment, with respect to the timing of increment formation, can be combined to provide the estimate of fish age (Francis et al. 1992, Fowler & Short 1998, Morales-Nin & Panfili 2002a).

2.2.4 Quality control and precision of age estimates

Clearly, attaining accurate estimates of fish age depends on achieving accurate counts of the annuli in the otoliths. As discussed in Sections 1.4.3 and 2.2.3, however, the interpretation process is complex, involving decisions on interpretation where many factors can influence the judgement call of the reader (Campana 2001, Morison et al. 2005). Ultimately, it is inevitable that there will be some errors in the otolith interpretation that lead to incorrect estimates of age. Thus, the implementation of an appropriate quality control and assurance program is important to detect inconsistencies in the ageing process and ageing errors and to ensure consistent and accurate interpretation of otolith structure. Such a protocol would assess both the relative accuracy of the counts and their precision. Whilst identifying ageing errors is important in any ageing study, such quality control monitoring is most relevant in high-volume, production ageing where consistency in otolith interpretation from year-to-year is essential (Campana 2001).

There is considerable variation in quality assurance and control processes amongst ageing labs around the world (Morison et al. 2005). They differ with respect to: whether reference collections of otoliths are retained and how such collections are used; whether the practise of assigning common birth dates for particular species is used; the number of otoliths that should be read to appropriately train an otolith reader for a new species; the level of information about a fish that is provided to the reader when the otolith is being interpreted; and the method used for comparing repeated age estimates from the same sample of fish (Morison et al. 2005). Some laboratories have a long history of quality control and have established sophisticated, computerised routines for estimating precision and identifying outliers in the age estimates (Kimura & Anderl 2005). The appropriate use of well-designed databases as well as digital images of otoliths from image analysis systems can contribute significantly to effective quality assurance and control in age estimation (Campana 2001, Morison et al. 2005).

Campana (2001) proposed a generic quality control protocol that is recognised as having considerable merit (Morison et al. 2005). The first step in this protocol is to develop a reference collection of otoliths for the species of interest. Ideally, this collection would involve otoliths from known-age fish or by second choice some for which there is agreement on the ages by expert readers. This reference collection provides the stable base for assessing the performance of fish agers over time. The recommended way to do this is to take a random, blindly-labelled sub-sample of otoliths from the reference collection, inter-mix these otoliths with a sub-sample of recently aged ones, and read this mixed set of otoliths. Then, the new counts for the otoliths from the reference collection can be compared and assessed against the original accepted ages. This is done using age bias graphs to test for ageing consistency and estimates of coefficient of variation (CV) to quantitatively describe the precision of the estimates (Campana et al. 1995, Campana 2001). Accurate counts from the otoliths in the reference collection will strongly suggest that counts from the recently aged sample of fish will also be accurate. Alternatively, evidence of bias between the earlier and recent counts for otoliths from the reference collection would suggest that the recently aged fish will be biased, indicating that some remedial action is required to address those errors. This may require some retraining for the otolith reader and possibly re-reading all otoliths from the recent sample once the reader was (re)trained sufficiently to be unbiased.

The discussion above has been concerned primarily with minimising ageing errors to ensure that estimates of age are as accurate as possible. There is also some value in considering the precision or repeatability of age estimates. Measuring precision provides a means of assessing the relative ease of determining the age from the otoliths, for assessing the reproducibility of an individual reader's determinations and comparing the counts between different agers. The percent agreement statistic has been used often as a measure of precision in such applications, but it is not particularly robust. Alternatively, both the index of average percent error (Beamish & Fournier 1981) and coefficient of variation (CV=SD/mean) (Campana et al. 1995) have been demonstrated to be robust measures of precision. Furthermore, it has been shown that they are functionally equivalent and it is possible to convert between the two measures (Campana 2001). These measures can be used to estimate precision at different hierarchical levels, e.g., between counts by an individual reader, between readers, and between laboratories where multiple readers have been used at each lab.

The equations presented by Beamish and Fournier (1981) to calculate average percent error are as follows:

$$X_j = \frac{1}{R}\sum_{i=1}^{R} X_{ij}$$

where X_j is the average age calculated for the *j*th fish, X_{ij} is the *i*th age determination of the *j*th fish and R is the number of times that each fish is aged;

$$\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}$$

is the average error in ageing the *j*th fish; and

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

is the index of average error which, when multiplied by 100, becomes the average percent error.

3 Conclusion

The dogma about the inability to age tropical fish from their otoliths was rejected in the late 1970s and early 1980s. Discovery of the usefulness of otoliths for ageing tropical fish meant that it was possible, for the first time, to collect samples of fish from tropical species and to use their otoliths to retrospectively reconstruct how their populations worked by providing insights into their demography, life history and population dynamics. There was a considerable period, however, before any real momentum developed in establishing new research programs based on ageing tropical fish from their otoliths. In fact, it wasn't until the 1990s and early 2000s that there was a significant increase in such studies.

The findings from the early ageing studies were profound. Many tropical species were found to be remarkably long-lived, contrary to previous belief that tropical fish species were short-lived. Mortality rates were found to be lower than expected, leading to the rejection of the paradigm about tropical species having fast turnover rates. Some species demonstrated growth patterns where initial growth was fast over a relatively short period, after which there was no further growth throughout the remainder of the relatively long life. Clearly, such new knowledge revolutionised the understanding of the functionality of these populations. Otolith ageing has now been established as a fundamental and essential technique for tropical fish scientists that will continue to provide invaluable insights into the biology of species at particular places and times. The on-going development of tropical fisheries will rely on the data from such studies, whilst the process of ecosystem management will also benefit.

The call for fundamental research into the nature of tropical fish otoliths that was made by Fowler (1995) is repeated here. It is true that otoliths can be used in ageing applications, so long as appropriate validation procedures have been completed, despite the lack of understanding about the relationship between otolith macrostructure, fish physiology and environmental seasonality. It is difficult, however, to ignore the implications of this poor understanding. There may be further valuable life history information in the otoliths that is currently being missed because of such poor understanding. There is a need to explore the relationships between otolith growth at the microscopic level, the calcium metabolism of the fish, particularly in relation to reproductive activity, and somatic growth, all of which are influenced by environmental seasonality. The scientific challenge here is significant, but the rewards for assisting in otolith interpretation in future studies may make facing the challenge worthwhile.

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