AN ABSTRACT OF THE THESIS OF

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David B. Sampson

This thesis evaluates the current break-and-burn ageing method for the southern stock (U.S. west coast) of sablefish (*Anoplopoma fimbria*). Differences in growth rates between the northern (north of Vancouver Island, BC) and southern stocks (south of Vancouver Island, BC) and results from a radiometric study conducted on fish from the southern stock suggest that the ageing method, developed from fish from the northern stock, may not be appropriate for fish in the southern stock. An edge analysis, using fish landed in Oregon, confirmed the formation of one hyaline zone annually. The timing of the deposition of the hyaline zone, May through September, is unique for fish from temperate waters. Sablefish tagged and injected with oxytetracycline (OTC) did not indicate ageing error significant enough to warrant the development of a new ageing method but some amendments to the current ageing method are recommended: do not excessively split marks in the transition zone (the area on the otolith that corresponds to the change from early fast growth to older slow growth); if multiple marks originate from the same spot on the ventral edge of the otolith, concentrate on the narrowing opaque zones when determining which marks to count as annuli; and assign age ranges or discard otoliths that display odd growth patterns. These amendments may increase the accuracy and precision of sablefish age estimates. Neither final age nor any of the auxiliary catch data (sex, size, depth, and

latitude at recapture) contributed significantly to the ageing error. It is also recommended that the thin section method of preparing otoliths be used in future sablefish OTC studies.

A Critical Examination of the Ageing Method for Sablefish (*Anoplopoma fimbria*) From the U.S. West Coast Using Edge Analysis and Oxytetracycline.

by

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Chapter 1

INTRODUCTION

1.1 Sablefish Life History

Sablefish or blackcod (*Anoplopoma fimbria*, Pallas) range throughout the Pacific Ocean; from southern Baja California to northern Japan. The largest concentration is found in the Gulf of Alaska (Low et al., 1976). Depth distribution ranges from the continental shelf (100 m) to slope and abyssal plain habitats (2560 m) (Pearcy et al., 1982).

Sablefish off the U.S. west coast spawn from October through March, with the peak occurring in January and February (Philips and Imamura, 1954; Fujiwara and Hankin, 1988; Hunter et al., 1989). Off California, the primary spawning habitat is the continental slope at depths of about 800 m (Hunter et al., 1989). Larval and juvenile fish are found in surface waters for the first 6-9 months before settling on the shelf. Sablefish grow at incredibly fast rates during their first few years but upon reaching maturity growth becomes considerably slower (McFarlane and Beamish, 1983; Rutecki and Varosi, 1997). Length at 50% maturity for males and females is between 55-62 cm, and is achieved by age 8 (Mason et al., 1983; Parks and Shaw, 1987; Fujiwara and Hankin, 1988; Hunter et al., 1989; Macewicz and Hunter, 1994). However, size at 50% maturity decreases substantially with increasing depth (Fujiwara and Hankin, 1988) and increasing age (Methot, 1995). Adult sablefish off the U.S. west coast reach a maximum size of 102 cm and an age of 73 years old, although most fish caught in the commercial fishery are under 85 cm and under 60 years old (Crone et al., 1997). Females generally reach larger sizes and ages than male sablefish (Fujiwara and Hankin, 1988; National Marine Fisheries Service, 1998).

As sablefish increase in both size and age they move to deeper water (>500 m) and spend the rest of their lives at these greater depths (Saunders et al., 1997; Sigler et al., 1997; Caillet et at., 1988; Fujiwara and Hankin, 1988). Methot (1994, 1995) speculated that this movement is more closely related to age than size. What triggers this migration is still unknown, but maturation is commonly believed to be the determining factor. Small sablefish feed primarily on small crustaceans (euphausiids), heteropods, and small fish; adults have a wider diet but mainly feed on fish (*Sebastes* and *Sebastolobus* spp.) and cephalopods (Laidig et al., 1997).

Due to variation in size and growth rates, it has been hypothesized that two sablefish 'stocks' exist within the northeast Pacific Ocean. A stock found north of Vancouver Island, BC exhibits faster growth rates and larger maximum size relative to the second stock found south of Vancouver Island, BC (Mason et al., 1983; Fujiwara and Hankin, 1988; Methot, 1994, 1995). Mark/recapture data also supports the two stock idea (Kimura et al., 1998). The northern stock of sablefish shows a proclivity to greater latitudinal migrations than their southern counterparts (Kimura et al., 1998). Because these fish tend to move southward there is a mixing of the two stocks south of Vancouver Island into Oregon. The southern sablefish stock may be split into two 'morphs', with a smaller, slower growing morph to the south of Monterey Bay (Phillips and Imamura, 1954; Caillet et al., 1988; Fujiwara and Hankin, 1988; Methot, 1994, 1995).

1.2 Commercial Fishery History and Management

The first recorded harvesters of sablefish were the Makah Indians of Cape Flattery and the Haida Indians of Queen Charlotte Islands (Ketchen and Forrester, 1954). Sablefish exploitation off the U.S. west coast did not begin in earnest till the early 1970s, when the foreign fishery substantially increased sablefish landings. When the Magnuson Fishery Conservation and Management Act was enacted in 1977, the domestic fleet filled the void left by the foreign fleet and landed record catches until 1982 (18,548 mt), when the first sablefish regulations were established. Since then, the annual landings have declined to approximately 8,000 mt, where it has remained since 1993 (NMFS, 1998).

Today sablefish are an important component of the U.S. west coast groundfish fishery. Landings in 1997 (7,844 metric tons, worth 27.5 million dollars) accounted for 31% of the total ex-vessel value of all groundfish caught off California, Oregon, and Washington (PFMC, 1998). Sixty percent of the annual catch is allocated to the trawl fishery, with the remaining going to the fixed gear fishery (pot and longline).

Since 1988, sablefish management advice has been based on age-structured stock assessment models (Methot and Hightower, 1988; Crone et al., 1997; National Marine Fisheries Service, 1998). The ability of these models to accurately estimate sablefish biomass, and hence catch quotas, hinges on accurate and precise age information. Due to data uncertainties, including ageing information, the results of these assessments have been highly contested. In fact, the 1998 assessment arose because the Pacific Fisheries Management Council decided in 1997 that sablefish needed to be reassessed with additional data.

1.3 Sablefish Ageing

Initially, sablefish were aged using scales and otolith surfaces (Pruter, 1954; Kennedy and Pletcher, 1968; Webb and Lockner, 1973). These studies showed that sablefish were a relatively fast-growing and short-lived species (max. 12 yr.). But a new break-and-burn technique applied to otoliths (Chilton and Beamish, 1982; C.A.R.E., 1997 (Appendix A)) indicated that sablefish are slower growing and longer lived (60 yr.) than previously thought. This ageing method was validated to show that each narrow zone is deposited annually by an intensive mark/recapture study, including the use of oxytetracycline (OTC) (Beamish and Chilton, 1982; Beamish et al., 1983; McFarlane and Beamish, 1995) and by growth analysis (McFarlane and Beamish, 1983; Rutecki and Varosi, 1997). A recent study using known-age sablefish from southeast Alaska validated the ageing methodology for fish 2-9 years old (Heifetz et al., 1999). The break-and-burn ageing method was also evaluated using radiometric dating techniques applied to sablefish otoliths from California (Kastelle et al., 1994). Because this type of validation methodology requires a large amount of sample material, this study was conducted with fish in four age categories, 1 year, 9-11 year, 14-23 year, and 24-34 year old fish. The authors found that in each age category the radiometric ages were lower than the break-and-burn ages. For example, the results for one age reader was as follows: category 1 - radiometric age = -0.09, break-and-burn age = 1; category 2 – radiometric age = 5.2, break-and-burn age = 9.8; category 3 – radiometric ages = 17.8, break-and-burn ages 18.9; category 4 - radiometric ages = 22.7, break-and-burn ages = 28.6. The authors concluded that, although lower, "the radiometric study generally confirms the ageing criteria used to interpret the otoliths burnt cross section".

Sablefish are difficult to age. An examination of fish species aged from the Pacific Ocean showed that sablefish ages have greater between-reader variability than any other species (Kimura and Lyons, 1991). The most recent stock assessment of sablefish from the U.S. west coast states that "in general, the percent agreement (between readers) was 54% at age 1, 39% at age 3, to below 10% for fish >10 years old" (National Marine Fisheries Service, 1998). This large variability is due to the large abundance of checks and the variations in growth patterns displayed on sablefish otoliths. These can cause reader-specific interpretation of the sablefish ageing method (Kastelle et al., 1994; C.A.R.E., 1997). The poor agreement can only be corrected by extensive training in the sablefish ageing method and periodic testing among experienced age readers.

Poor precision (reproducibility of a given age estimate) among age readers is one component of the poor accuracy (the proximity of the estimated age to the true age) of the sablefish ageing method. The development of the current ageing method and its corresponding OTC validation study were conducted on sablefish from the northern stock. Given that the two stocks of sablefish exhibit different growth patterns, it is possible that differences may also be exhibited in the growth patterns of their otoliths.

If this is the case, then the ageing technique developed for fish in the northern stock may not be applicable to fish in the southern stock. The difference between radiometric ages and break-and-burn ages, may already indicate this to be true.

The Cooperative Ageing Project and the National Marine Fisheries Service, Tiburon Laboratory are currently using the Chilton and Beamish (1982) break-and-burn method to produce ages for sablefish caught off California, Oregon, and Washington- the southern stock. The combination of poor precision, the results of the radiometric validation study, and differences in the growth rates between the two stocks indicate a need for a critical evaluation of the current method used to age sablefish from the U.S. west coast.

The objective of this thesis research is to critically evaluate the current ageing method for sablefish from the U.S. west coast. Chapter 2 describes an edge analysis performed on sablefish from Oregon in order to determine if more than one annuli is being deposited annually. The edge analysis also determines the seasonality of the deposition of the hyaline zone (commonly referred to as the annuli), which allows age readers to be as accurate as possible while estimating the final age of each fish. Chapter 3 examines otoliths from sablefish that were time marked with oxytetracycline (OTC) in 1991, released, and then recaptured by the commercial fishery. The difference between the number of years the fish was at liberty and the number of marks counted as annuli is a direct measurement of the accuracy of the current ageing method. Areas of significant ageing error are examined and revisions to the ageing method are recommended. The final chapter of this thesis summarizes the results of the edge analysis and the OTC study.

Chapter 2

SEASONALITY OF GROWTH ZONE DEPOSITION ON SABLEFISH, *ANOPLOPOMA FIMBRIA*, OTOLITHS FROM THE U.S. WEST COAST

2.1 Introduction

The basic assumption of fish ageing is the formation each year of one hyaline and one opaque zone on the ageing structure (Appendix B). The combination of these two zones equals one year's growth with the hyaline zone commonly referred to as an annuli. In fish from the temperate Pacific Ocean the period of slow growth (hyaline zone) occurs in the winter, and fast growth (opaque zone) occurs in the summer (Beckman and Wilson, 1995). This was verified by examination of otoliths from petrale sole, *Eopsetta jordani* by Gregory and Jow (1976), English sole, *Pleuronectes vetulus* by MacLellan and Fargo (1995), yellowtail rockfish, *Sebastes flavidus* by Kimura et al. (1979), and widow rockfish, *Sebastes entomelas* by Pearson (1996). The changes in yearly growth rates, and the subsequent changes in otolith growth zones, is commonly attributed to changes in water temperature (Schramm, 1989). Although the formation of the hyaline zone has also been ascribed to spawning (Johnson et al., 1983; MacLellan and Fargo, 1995), physiological stress (Liew, 1974), and movement between different environments (Lowerre-Barbieri et al., 1994).

The timing of hyaline zone formation is important to age readers for two reasons: 1) verification that one hyaline zone forms per year (validation of the ageing method), and 2) knowledge of the timing of hyaline zone formation helps interpret the final year of growth. The latter enables fish ages to be estimated accurately to the year;

misinterpretation of the final year of growth may cause age estimations to be incorrect by one year.

Today sablefish are an important component of the U.S. west coast groundfish fishery. Landings in 1997 (7,844 metric tons, worth 27.5 million dollars) accounted for 31% of the total ex-vessel value of all groundfish caught off California, Oregon, and Washington (PFMC, 1998). Since 1988, sablefish management advice has been based on stock assessment models that are partially derived from data on age composition and growth (Methot and Hightower, 1988; Crone, 1997; National Marine Fisheries Service, 1998). Sablefish ages for these assessments have been provided by the Cooperative Ageing Project and the National Marine Fisheries Service (NMFS), Tiburon Laboratory. Both of these laboratories use the break-and-burn sablefish ageing methodology developed by the Canadian Department of Fisheries and Oceans (Chilton and Beamish, 1982; McFarlane and Beamish, 1995; C.A.R.E., 1997 (Appendix A)).

Included in the break-and-burn ageing methodology is the assumption that hyaline zone formation takes place from fall through the early spring months, the period of reduced growth. Rutecki and Varosi (1997), examining monthly growth rates of juvenile sablefish in southeast Alaskan waters, found that age-1 and age-2 fish increased in length from May to October with peak growth occurring in June through August. McFarlane and Beamish (1983) report the 'growing season' to be June to September for sablefish off British Columbia. If this is so, then the edge of the otolith should be opaque during the summer months and hyaline during the winter. To date, there has been no edge analysis performed on sablefish otoliths to determine if the type of otolith deposition corresponds to the type of somatic growth.

The goal of this study is to examine sablefish otoliths from the U.S. west coast, using edge analysis techniques and time-marked sablefish otoliths, in order to determine the seasonality of the type of growth zone deposition.

2.2 Material and Methods

2.2.1 Edge Analysis

A maximum of ten sablefish sagittal otoliths from fish caught in Oregon were selected from each month from 1991, 1994, and 1997 (Table 2.1). Fish were previously aged by the Cooperative Ageing Project, Newport, OR using the current sablefish break-and-burn ageing criteria (Chilton and Beamish, 1982; McFarlane and Beamish, 1995; C.A.R.E., 1997). Only fish under the age of seven were used in the edge analysis, because growth increments are widest during this relatively fast growth period of the fishes' lives. There were some months where ten otoliths meeting these criteria were unavailable. Otoliths were coated with mineral oil to enhance the viewing of the growth zone and examined under a dissecting microscope using reflected light. When examined under reflected light the burned otoliths' hyaline zones appear dark and the opaque zones appear white (Appendix B).

Table 2.1. Number of sablefish otoliths selected for edge analysis study.

	Year Landed		
Month Landed	**1991**	**1994**	**1997**
January	10	9	8
February	10	-	10
March	10	10	10
April	9	-	10
May	10	10	10
June	10	7	10
July	3	-	10
August	3	10	10
September	10	3	10
October	10	10	10
November	3	10	10

Each otolith was assigned an edge type, hyaline, narrow opaque, or wide opaque, based on the amount of growth between the last hyaline zone and the edge of the otolith compared to the width of the previous opaque zone. The edge types were correlated with the date of capture to determine the time periods when each edge type is formed. The results of the edge analysis were examined for each year and as a combination of all years.

2.2.2 OTC Injected Sablefish

Fish from the NMFS mark/recapture sablefish age validation study were examined to determine seasonality of hyaline zone deposition. Sablefish were captured by NMFS personal aboard the 1991 NMFS pot survey in the southern INPFC area (Eureka, Monterey, and Conception). Ten baited conical traps, equipped with time-release devices to close the trap after 24 hours of fishing, were attached by gangions at 91 m intervals on a 1,001 m groundline. Sampling occurred from 9/7/91 to 10/7/91. Sablefish were caught at sites between Coos Bay, OR and San Diego, CA. Fish were randomly selected for tagging and injection with oxytetracycline (OTC), based on available time and number of fish caught, but emphasis was placed on fish captured at all survey areas and depths. Fish were injected with 50 mg of OTC/kg of body weight, tagged with an external floy tag, measured to the nearest centimeter, and then released. A total of 2,574 fish were floy tagged, of which 1,930 (75%) were injected with OTC.

As of 10/13/97, 415 (16%) of the tagged sablefish had been recovered by the commercial fishing fleet and returned to NMFS. Sagittal otoliths were collected from 350 of these returns, along with some recapture information (date, location, depth, sex, size). Fish recaptures ranged from northern Washington to San Diego, California. Otoliths were cleaned of tissue and stored in dark containers because the OTC mark is photolabile.

Otoliths were prepared and examined using the bake-and-slab method developed by Pearson (1997). The otoliths were embedded in casting resin and allowed to dry. The mounting ensures that the otolith will always be viewed on the same plane. The embedded otoliths were then cut in half, along the dorso-ventral axis, using a diamond saw. The mounted otolith half was placed under a compound microscope (magnification 120x), coated with mineral oil (to enhance the OTC mark), oriented in such a way as to provide the best view of the otolith, and viewed under a UV light of 360 angstroms. The UV light excites an OTC mark and causes it to fluoresce blue. If there was a visible OTC mark on the otolith, the image was captured and saved using Scion Image, an imaging program based on National Institute of Health public domain software.

Otoliths with a visible OTC mark were removed from the microscope, wiped clean, and heated in a toaster oven until the otoliths turned dark brown (550 degrees for approximately 10 to 20 minutes). After cooling, mineral oil was applied to the otolith surface (to enhance the growth zones), and placed under the microscope for viewing under white light. This image was also captured and saved. The otoliths were viewed under UV light first because heating the otoliths, in order to distinguish the growth zones, destroys the OTC mark.

A section of the UV image of the otolith, which contained distinct 'landmarks' and the OTC mark, was cut out of the image and overlaid in precisely the same location on the image of the baked image by realigning 'landmarks'. In some cases the OTC mark (white line) and the edge of the otoliths (black line) was illustrated using imaging software in order to highlight these areas of interest. The final result was one image with growth zones and an OTC mark. The type of growth zone, hyaline or opaque, that the OTC mark appeared in was recorded. Additionally, fish were visually examined to determine the type of growth zones that had occurred between the OTC tagging and the recapture date.

2.3 Results

2.3.1 Edge Analysis

The deposition of the hyaline zone on sablefish otoliths appears to occur from late spring into early fall. The hyaline zone was the dominant edge type in 1991 from May through August (Figure 2.1); in 1994 from May through July, but persisting through October (Figure 2.2); and in 1997 from May through October (Figure 2.3). When all years were combined the hyaline zone was the dominant edge type from May through July and in September (Figure 2.4).

These graphs (Figures 2.1, 2.2, 2.3, 2.4) also display only one hyaline zone peak each year. This validates the current ageing method by establishing that the hyaline zone can be considered an annuli.

2.3.2 OTC Injected Otoliths

Fish from the OTC study displayed seasonal timing of growth zones similar to the edge analysis results, opaque edges during the winter and hyaline edges during the summer. All fish that were injected with OTC in September and October 1991 showed the OTC mark on the outside edge of a hyaline zone. For example, fish number 10539 (Figure 2.5), injected with OTC on 9/14/91 and recaptured on 9/19/92, shows the OTC on the outside edge of a hyaline zone, displays wide opaque growth and then substantial hyaline growth near the edge of the otolith. Fish number 10209 (Figure 2.6), injected with OTC on 9/7/91 and recaptured on 5/25/92, shows the OTC mark on the outside edge of a hyaline zone and displays only opaque growth until the edge of the otolith, where the next hyaline zone is beginning. There is no visible hyaline zone between the OTC mark and the edge of the otolith.

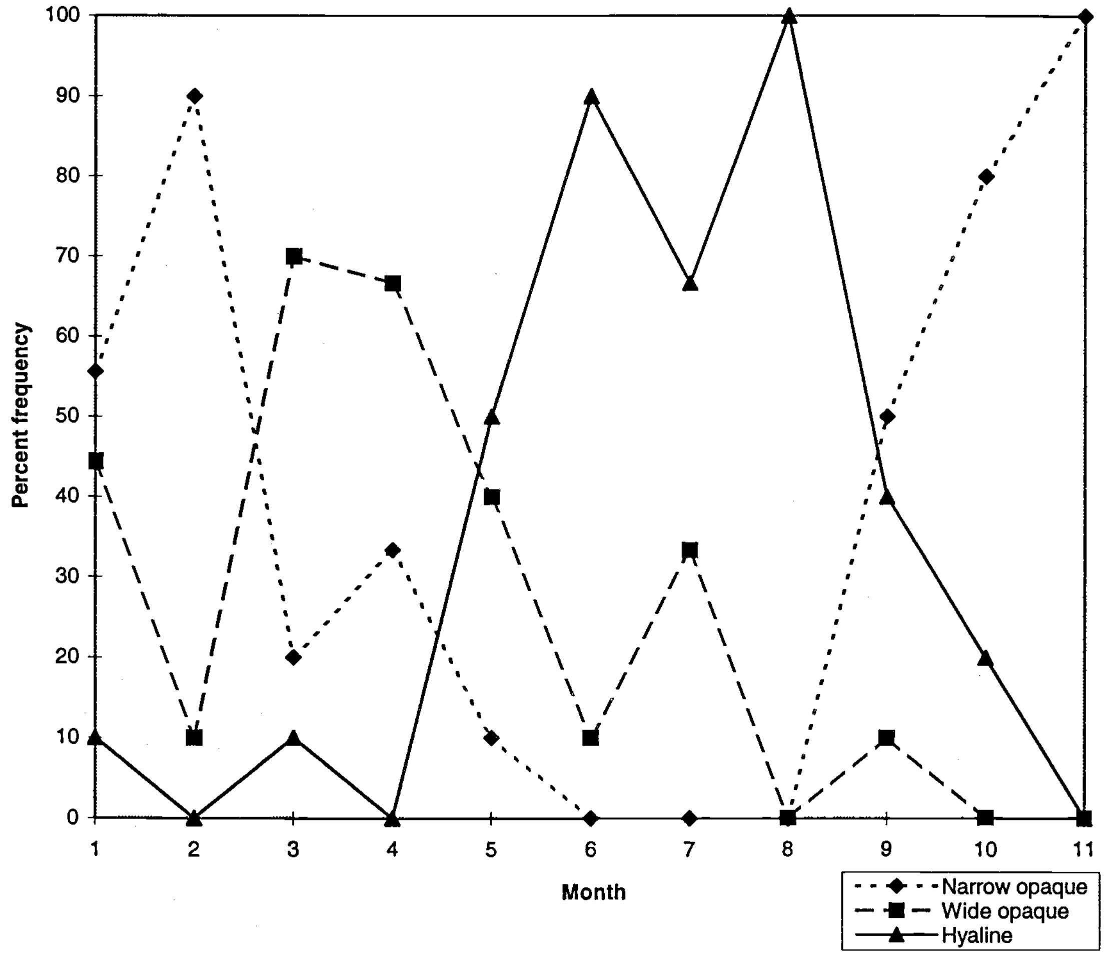

Figure 2.1. Edge analysis of Oregon sablefish landed in 1991. See methods for description of edge type.

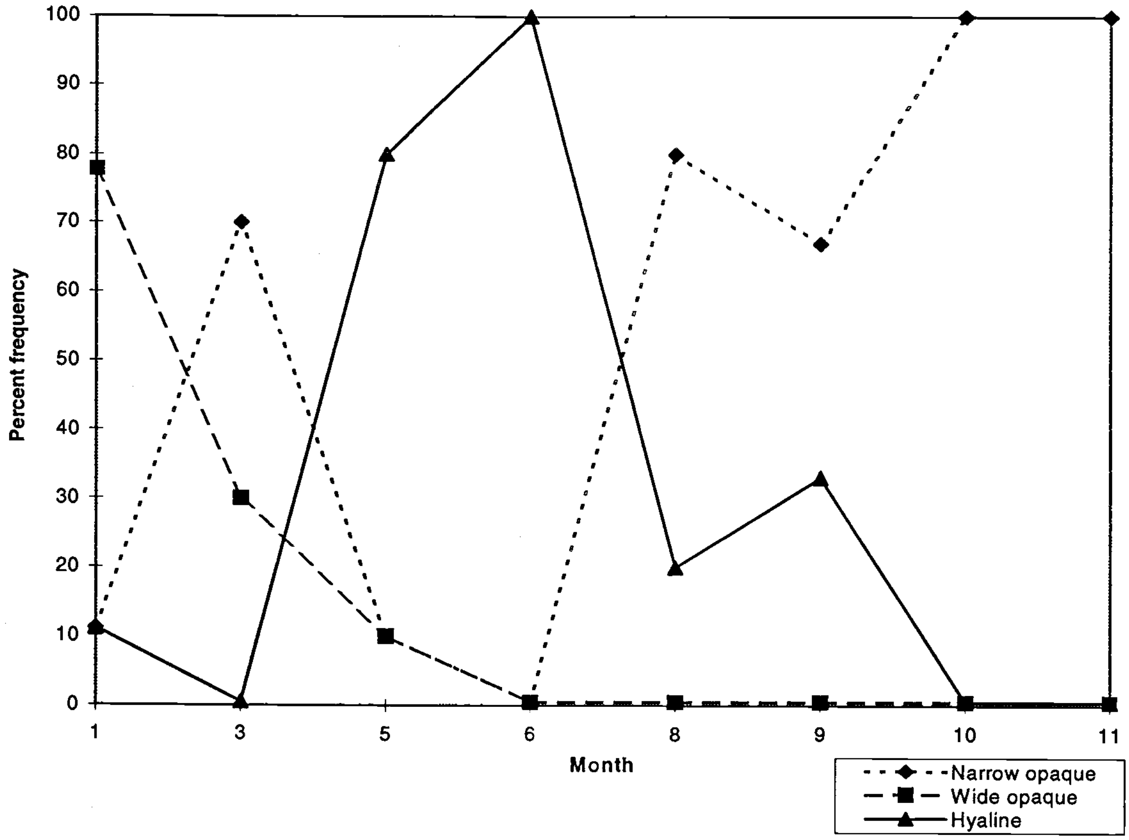

Figure 2.2. Edge analysis of Oregon sablefish landed in 1994. See methods for description of edge type.

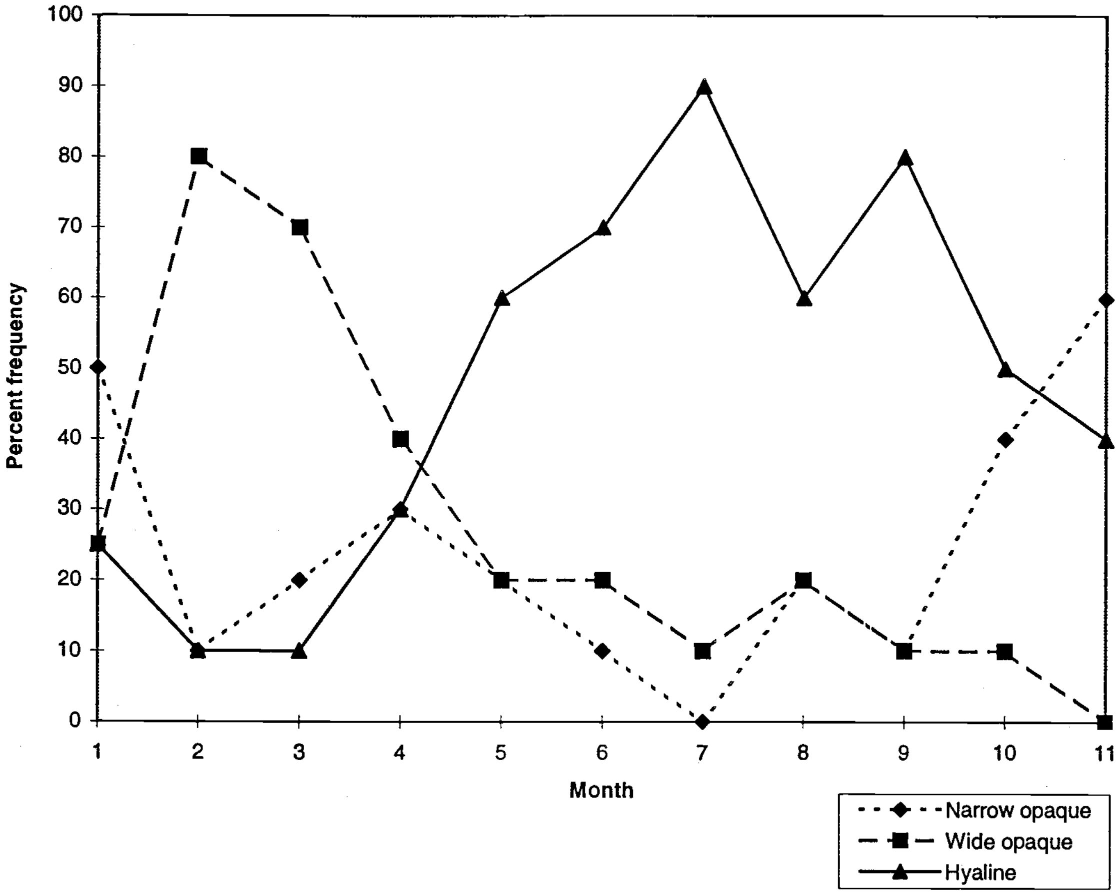

Figure 2.3. Edge analysis of Oregon sablefish landed in 1997. See methods for description of edge type.

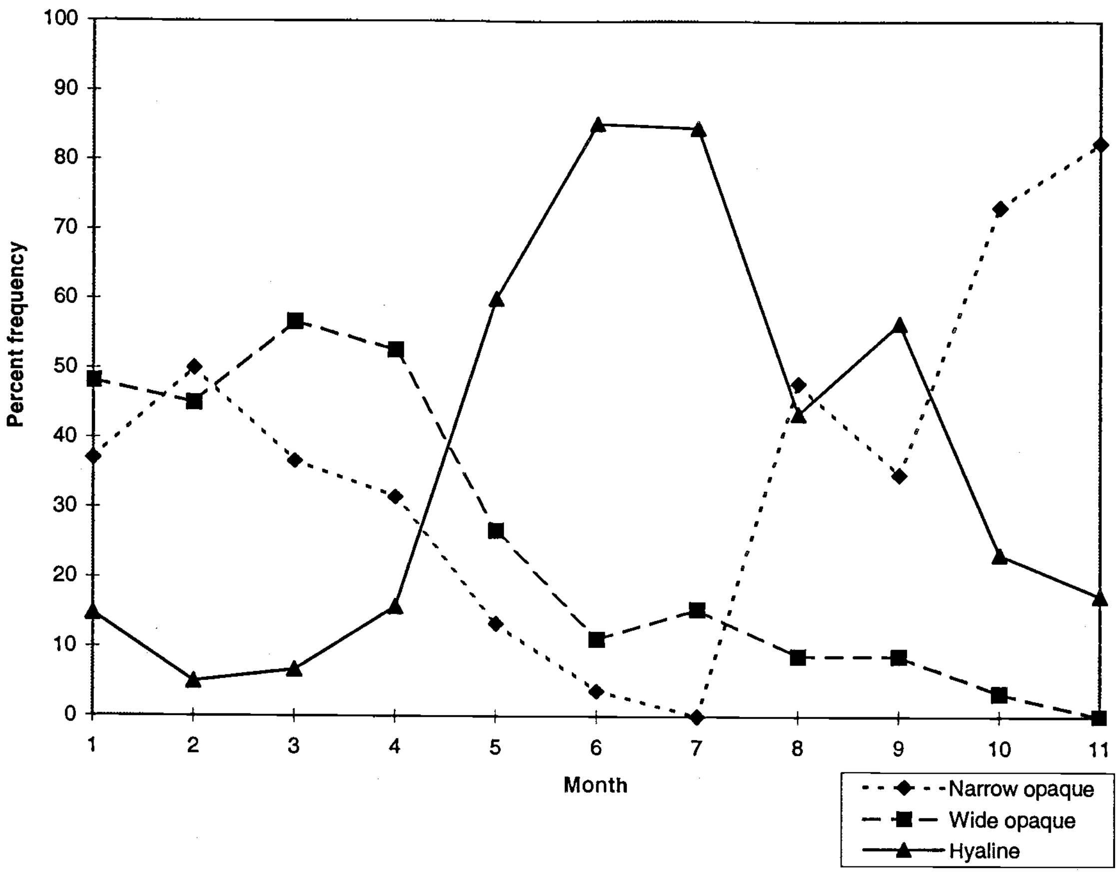

Figure 2.4. Combined edge analysis of Oregon sablefish landed in 1991, 1994, 1997. See methods for description of edge type.

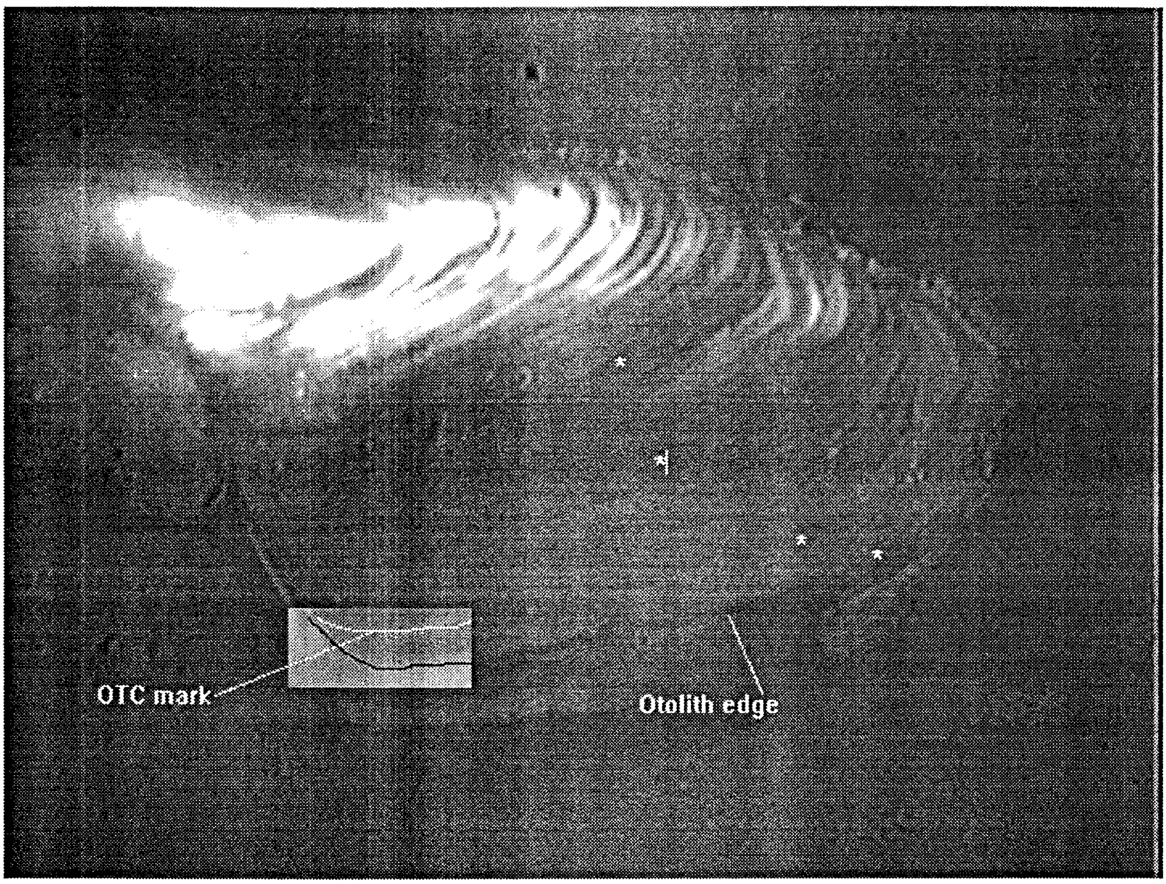

Figure 2.5. Final image of a bake-and-slab sablefish otolith, injected with OTC on 9/14/91 and recaptured 9/19/92. Stars (*) indicate hyaline zones counted as annuli.

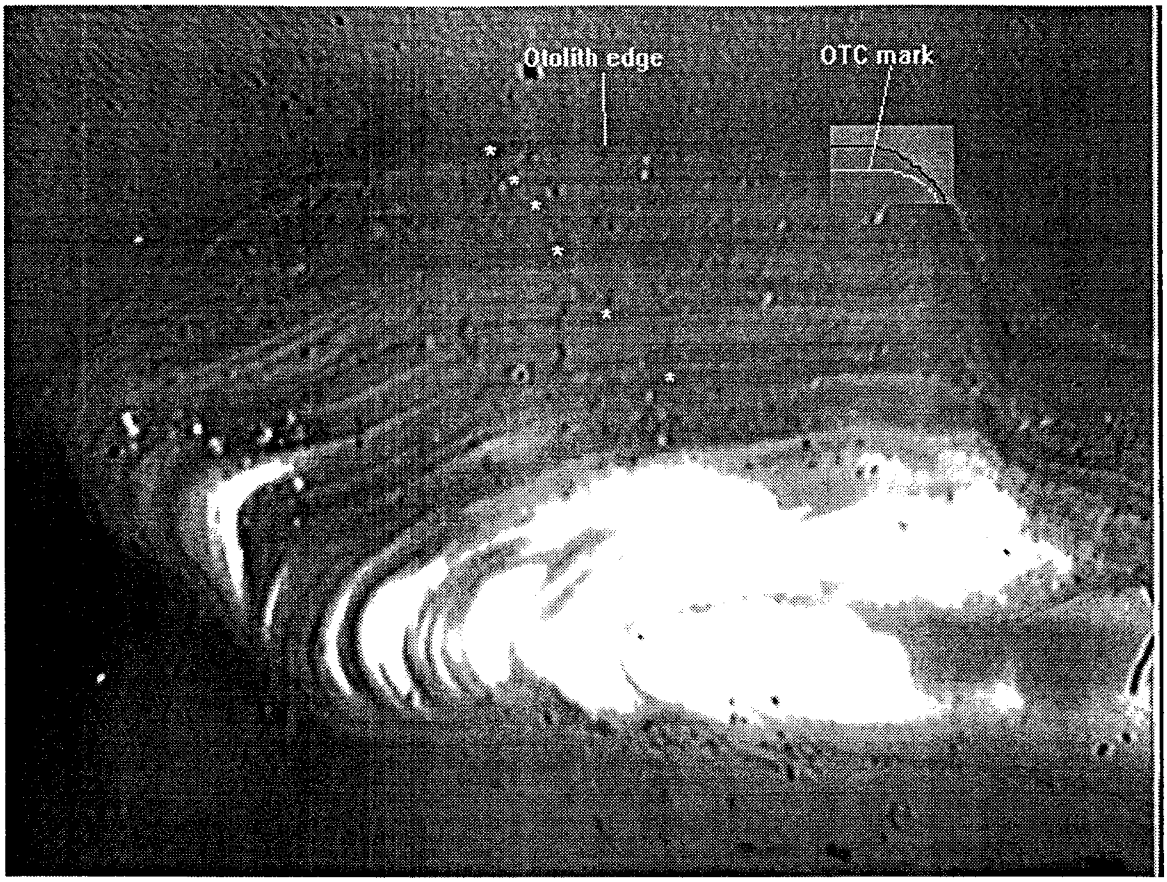

Figure 2.6. Final image of a bake-and-slab sablefish otolith, injected with OTC on 9/7/91 and recaptured on 5/25/92. Stars (*) indicate hyaline zones counted as annuli.

2.4 Discussion

This is the first example, that I am aware of, of a fish species from temperate waters which displays hyaline zone formation in the late spring and summer, the period of traditional fast growth. However it is important to note that, while studies have shown that juvenile sablefish experience faster growth rates in the summer (Rutecki and Varosi, 1997), no formal studies have been conducted to determine the seasonality of growth rates of adult sablefish from the U.S. west coast. Some species from the temperate Atlantic Ocean (weakfish) display a hyaline zone in late spring, determined by marginal increment analysis, although it follows months of slow growth (Lowerre-Barbieri et al., 1994). In addition, the weakfish hyaline zone forms quickly, within two months. Sablefish from this study showed a protracted hyaline zone formation, starting in late spring and lasting till the early fall.

The timing of the hyaline zone deposition appears to form during a similar, although a bit earlier, time period on sablefish otoliths from the northern stock. In a study using known-age sablefish from the Gulf of Alaska, Heifetz et al. (1999) confirmed this by stating that "many experienced sablefish age readers have concluded that the annulus usually forms in spring, but some annuli may form in summer".

Sablefish from the edge analysis portion of this study showed hyaline zone formation generally from May through September. The fish from the OTC study, tagged after September, showed the OTC mark inside a hyaline zone that apparently was still forming into October. This extended hyaline zone formation is likely due to the stress of capture and handling. MacLellan and Fargo (1995) noted a similar observation in English sole. They found that fish that were injected with OTC started their hyaline zone formation earlier compared to fish that were not injected with OTC.

There are few biological explanations to this apparent 'flip-flop' in growth zones seen on sablefish otoliths. The results of juvenile sablefish growth studies (McFarlane and Beamish, 1983; Rutecki and Varosi, 1997) makes it hard to state that sablefish are not experiencing maximum growth in the summer months, as the existence of the hyaline zone during this time period would suggest. Species from the NW temperate region that show hyaline zone formation in the wintertime are from the shelf regions (e.g., petrale sole, English sole, yellowtail rockfish, and widow rockfish). Although sablefish migrate to deep water at older ages, the fish from this edge analysis were relatively young and were caught on the shelf. It is unlikely that the formation of the hyaline zone is caused by spawning because sablefish spawn from October through March, and the formation of the opaque zone during the spawning period has been reported in other species (Beckman et al., 1990). The summer formation of the hyaline zone in sablefish may be due to a lag in otolith material deposition compared to the timing of somatic growth, but this delay would be on the order of four to six months and thus seems unlikely.

Each of the indicators of the seasonality of hyaline zone formation (edge analysis and visual examination of the OTC marked fish) are subject to criticism for numerous reasons. The break-and-burn method for preparing otoliths for ageing carbonizes the edge of the otolith. This carbonization causes the edge of the otolith to appear dark, similar to the hyaline zone. The edge analysis techniques used in this study took this into consideration by classifying the edge primarily on the amount of recent opaque deposition compared to the amount of the previous year's opaque deposition, not on the actual 'color' of the edge. The OTC marked otoliths were prepared using the bake-and-slab methodology (Pearson, 1997). While this method does not carbonize the edge of the otolith, it generally does not bake the edge of the otolith dark enough to clearly distinguish its edge type. This was considered when examining the OTC marked sablefish otoliths and resulted in the same edge type analysis techniques as those used in the break-and-burn. Some sablefish are thought to have an abrupt transition from the younger, fast growth stage to older, slow growth stage. A fish in this transition will show little opaque growth compared to the previous year. This would call for a narrow opaque zone classification, when in reality it may be either a wide or even a hyaline classification. Additionally sablefish otoliths have an abundance of accessory marks, especially younger fish that are in the rapid growth phase. The current ageing criteria takes this into consideration by grouping together several marks that appear in very close proximity to each other. Each grouping is considered one annuli. Within each grouping, the otolith material between each of these marks is opaque. A rapidly growing sablefish may have considerable distance between each mark of the hyaline zone. A fish caught between these checks may be incorrectly classified as having opaque zone formation. An experienced age reader may be able to determine true opaque growth from the opaque growth that occurs within a hyaline zone, but not in all cases. This alone may make edge analysis inapplicable to sablefish otoliths.

Interesting enough, annual rings can appear on inanimate objects as well. There are stacked, thin, transparent layers on glass jars from the late Roman and Islamic periods found in the desert and on a wine bottle found in the ocean off Jamaica (Brill, 1963). The thought is that these are annual rings caused by seasonal variations in temperature and rainfall. By counting these rings they have able to date the bottles in a fashion similar to ageing fish.

Nevertheless, it appears that the hyaline zone is deposited on sablefish otoliths during the summer and early fall months. More importantly, only one hyaline zone is being deposited each year, thus validating the current ageing method. To further investigate this phenomenon I recommend a detailed marginal increment analysis, where the distance between the last hyaline zone and the proximal edge of the otolith is measured and correlated to the catch date (MacLellan and Fargo, 1995; Lowerre-Barbieri et al., 1994), be conducted on sablefish from Washington to California.

Chapter 3

EVALUATION OF THE AGEING METHOD FOR SABLEFISH (*ANOPLOPOMA FIMBRIA*) FROM THE U.S. WEST COAST USING OXYTETRACYCLINE

3.1 Introduction

Sablefish are important component of the U.S. west coast groundfish fishery. Landings in 1997 (7,844 metric tons, worth 27.5 million dollars) accounted for 31% of the total ex-vessel value of all groundfish caught off California, Oregon, and Washington (PFMC, 1998). Since 1988, sablefish management advice has been based on stock assessment models that are partially derived from data on age composition and growth (Methot and Hightower, 1988; Crone, 1997; National Marine Fisheries Service, 1998). The current sablefish break-and-burn ageing method was validated (each narrow (hyaline) growth zone is deposited annually) by an intensive mark/recapture study, including the use of oxytetracycline (OTC) (Beamish and Chilton, 1982; Beamish et al., 1983; McFarlane and Beamish, 1995; C.A.R.E., 1997) and by growth analysis of juvenile sablefish (McFarlane and Beamish, 1983; Rutecki and Varosi, 1997). A recent study using known-age sablefish (determined by tagging studies) from southeast Alaska validated the ageing methodology for two to nine year old fish (Heifetz et al., 1999). The break-and-burn ageing method was also evaluated using radiometric dating techniques applied to sablefish otoliths from California (Kastelle et al., 1994). Because this type of validation methodology requires a large amount of sample material, this study was conducted with fish in four age categories, 1 year, 9-11 year, 14-23 year, and 24-34 year old fish.). The authors found that in each age category the radiometric ages were lower than the break-and-burn ages. For example the results for one age reader were as follows: category 1 - radiometric age = -0.09, break-and-burn age = 1; category 2 – radiometric age = 5.2, break-and-burn age = 9.8; category 3 – radiometric ages = 17.8, break-and-burn ages 18.9; category 4 - radiometric ages = 22.7, break-and-burn ages = 28.6. The authors concluded that, although the radiometric ages were lower, "the radiometric study generally confirmed the ageing criteria used to interpret the otoliths burnt cross section". Although validated, sablefish age readers experience poor agreement between readers, primarily due to the large abundance of accessory marks and the variations in growth patterns displayed on sablefish otoliths.

Due to variation in size and growth rates, and indications from mark and recapture data, it has been hypothesized that two sablefish 'stocks' exist within the northeast Pacific Ocean. A stock found north of Vancouver Island, BC exhibits faster growth rates and larger maximum size relative to the second stock found south of Vancouver Island, BC (Mason et al., 1983; Fujiwara and Hankin, 1988; Methot, 1994, 1995; Kimura et al., 1998). The development of the current ageing method and its corresponding OTC validation study were conducted on sablefish from the northern stock. Given that the two stocks of sablefish exhibit different growth patterns, it is possible that differences may also be exhibited in the growth patterns of their otoliths. If this is the case, then the ageing technique developed for fish in the northern stock may not be applicable to fish in the southern stock. The differences between radiometric ages and break-and-burn ages in fish from the southern stock may already indicate this to be true.

The goal of this study is to verify that the sablefish ageing criteria developed for sablefish from the northern stock is applicable to fish from the southern stock. Additionally weak areas of the current ageing method are identified and advice given to age readers that could improve accuracy and precision. These goals are accomplished by applying the current ageing method to OTC tagged sablefish from the U.S. west coast.

3.2 Materials and Methods

3.2.1 Otolith Preparation

Sablefish were captured by NMFS personal aboard the 1991 NMFS pot survey in the southern INPFC area (Eureka, Monterey, and Conception). Ten baited conical traps, equipped with time-release devices to close the trap after 24 hours of fishing, were attached by gangions at 91 m intervals on a 1,001 m groundline. Sampling occurred from 9/7/91 to 10/7/91. Sablefish were caught at sites between Coos Bay, OR and San Diego, CA. Fish were randomly selected for tagging and injection with oxytetracycline (OTC), based on available time and number of fish caught, but emphasis was placed on fish captured at all survey areas and depths. Fish were injected with 50 mg of OTC/kg of body weight, tagged with an external floy tag, measured to the nearest centimeter, and then released. A total of 2,574 fish were floy tagged, of which 1,930 (75%) were injected with OTC.

As of 10/13/97, 415 (16%) of the tagged sablefish had been recovered by the commercial fishing fleet and returned to NMFS. Sagittal otoliths were collected from 350 of these returns, along with some recapture information (date, location, depth, sex, size). Fish recaptures ranged from northern Washington to San Diego, California. Otoliths were cleaned of tissue and stored in dark containers because the OTC mark is photolabile.

Otoliths were prepared and examined using the bake-and-slab method developed by Pearson (1997). The otoliths were embedded in casting resin and allowed to dry. The mounting ensures that the otolith will always be viewed on the same plane. The embedded otoliths were then cut in half, along the dorso-ventral axis, using a diamond saw. The author and one age reader from the NMFS, SWFSC Tiburon Laboratory each viewed one half of each otolith. Reader one examined the otoliths without previously seeing any tagging information in order to accurately assess the current ageing method. Reader two periodically examined the tagging information, specifically the recapture date, in order to try to develop a new ageing method while assessing the current method.

The mounted otolith half was placed under a compound microscope (magnification 120x), coated with mineral oil (to enhance the OTC mark), oriented in such a way as to provide the best view of the otolith, and viewed under a UV light of 360 angstroms. The UV light excites an OTC mark and causes it to fluoresce blue. If there was a visible OTC mark on the otolith, the image was captured and saved using Scion Image, an imaging program based on National Institute of Health public domain software.

Otoliths with a visible OTC mark were removed from the microscope, wiped clean, and heated in a toaster oven until the otoliths turned dark brown (550 degrees for approximately 10 to 20 minutes). After cooling, mineral oil was applied to the otolith surface (to enhance the growth zones), and placed under the microscope for viewing under white light. This image was captured and saved. The otoliths were viewed under UV light first because heating the otoliths, in order to distinguish the growth zones, destroys the OTC mark.

A section of the UV image of the otolith, which contained distinct 'landmarks' and the OTC mark, was cut out of the image and overlaid in precisely the same location on the image of the baked image by realigning 'landmarks'. In some cases the OTC mark (white line) and the edge of the otoliths (black line) was illustrated using imaging software in order to highlight these areas of interest. The final result was one image with growth zones and an OTC mark.

After the final image was produced, the fish was aged, the number of annuli between the OTC mark and the edge of the otoliths was counted, the edge type was recorded, and any distinguishing features were noted.

3.2.2 Expected count calculation

For each otolith I calculated an expected count of the number of hyaline zones that should have formed during the time the fish was at liberty given the number of years at liberty, the month of capture, the timing of hyaline zone formation and the assumption that one hyaline zone was formed per year. The hyaline zone is thought to form on sablefish otoliths from the U.S. west coast from May through September (Chapter 2). For example, a fish tagged and injected with OTC on 9/8/91 and recaptured on 9/15/95 has an expected count of 4 including the edge; a fish tagged on 9/7/91 and recaptured on 5/25/92 has an expected count of 1 including the edge even though it was at liberty for less than a year, but a fish tagged on the same day but recaptured on 4/5/92 has an expected count of 0 because no hyaline zone will have formed by April. This method allows a more precise estimate of the true number of hyaline zones that should have formed during the fish's time at liberty rather than using just the number of years at liberty. The difference between the expected count and the reader's estimated number of annuli after the OTC mark is now referred to as the ageing error.

3.2.3 Accuracy measurement

The accuracy, a measure of proximity of the estimated age to the true age, of the current ageing method was tested using various techniques:

1) Percent agreement (percentage of otoliths with no ageing error), and percent agreement within one year of the expected count. This was done for all fish, fish with an expected count ≥ 1, ≥ 2, and ≥ 3 years. Using only fish with an expected count ≥ 1 removes fish where there is no annuli expected to be formed because these fish can artificially inflate the accuracy results. Using only fish with higher expected counts is one way to test for increasing error with increasing time at liberty.

2) Hoenig's measure of systematic bias, which utilizes a χ^2 test to look for evidence of systematic disagreement (Hoenig et al, 1994).
3) Paired t-test to look for significant differences between estimated and expected number of hyaline zones after OTC mark.
4) Simple linear regression model used to test for increasing ageing error with increasing time at liberty. An increase in ageing error as time at liberty increase is an indication that the current ageing method is not accurate; it means that the ageing error increases as your chance of error increases due to an increase in the number of marks between the OTC mark and the edge of the otolith.

3.2.4 Precision measurement

Each reader's estimates of final age was compared using percent agreement (percentage of otoliths in which both age readers estimated the same final age) and percent agreement within 1 year. The number of estimated hyaline zones after the OTC mark was also compared for each reader using percent agreement (percentage of otoliths in which both age readers estimated the same number of hyaline zones after the OTC mark) and percent agreement within 1 hyaline zone for all fish and for fish with an expected count ≥ 1, ≥ 2, and ≥ 3 years.

3.3 Results

3.3.1 Reader to Reader Precision

The final age percent agreement (percentage of otoliths in which both age readers estimated the same final age) and percent agreement within 1, 2 and 3 years between reader one and reader two were 14%, 32%, 53%, and 64% respectively. Percent agreement (percentage of otoliths in which both age readers estimated the same number of hyaline zones after the OTC mark) and percent agreement for fish with an expected count ≥ 1, ≥ 2, and ≥ 3 between the two readers number of estimated annuli after the OTC mark was 47%, 42%, 24%, and 19% respectively. Percent agreement within one year between the two readers estimated number of annuli after the OTC mark was 78% for all fish, 72% for fish with an expected count ≥ 1, 54% for fish with an expected count ≥ 2, and 57% for fish with an expected count ≥ 3. It should be noted that agreement between readers is a measure of precision and does not necessarily mean any agreement with the expected number of annuli. These results are lower than expected, even for sablefish. Reader one assigns lower final ages to younger fish and higher final ages to older fish compared to reader two (Fig. 3.1). This same general pattern holds for the estimated number of annuli after the OTC mark; reader one estimates less annuli for fish with lower expected counts and more annuli to fish with higher expected counts compared to reader two (Fig. 3.2). This poor precision is likely due to the fact that the two readers had different objectives while examining the otoliths. Because of this no further comparisons between the readers are made in this report and each readers results are examined separately.

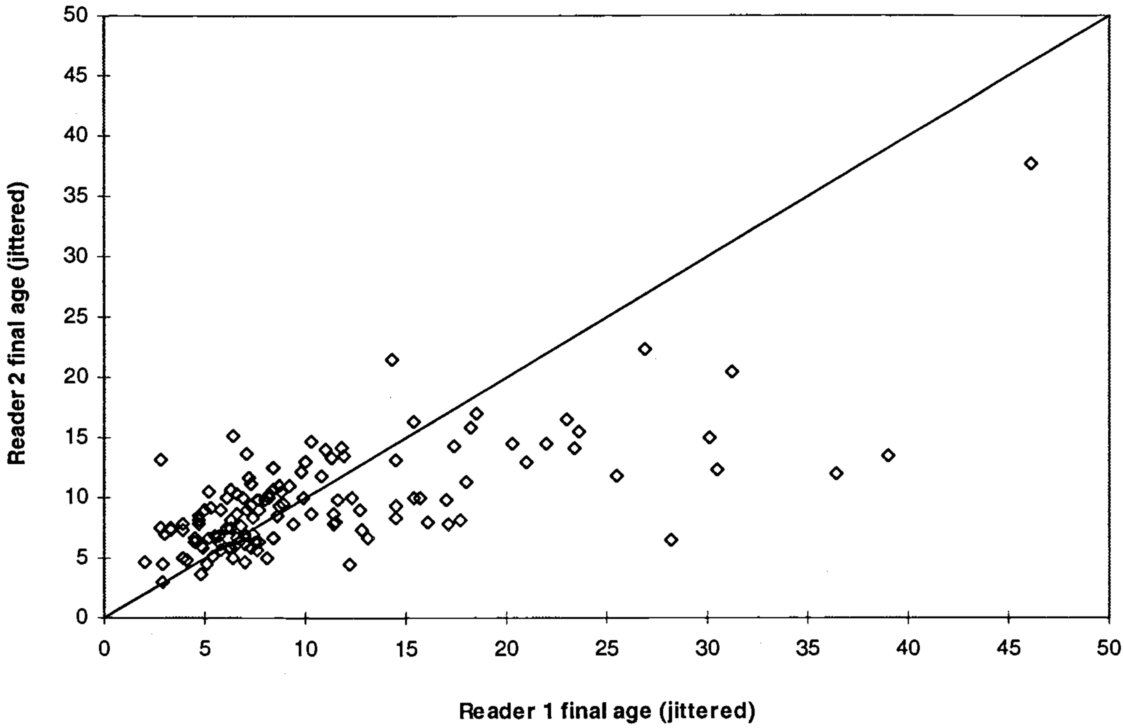

Figure 3.1. Reader to reader comparison of final age estimates of OTC tagged sablefish. Forty-five degree line represents perfect agreement.

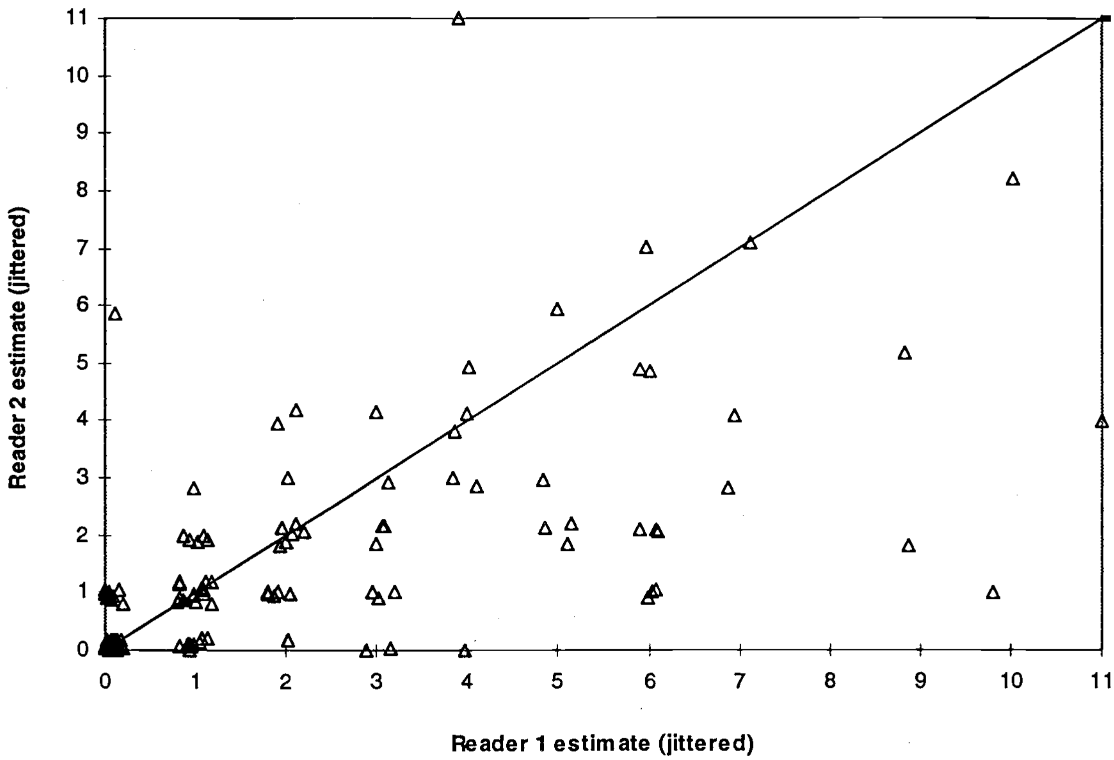

Figure 3.2. Reader to reader comparison of estimated number of annuli after the OTC mark. Forty-five degree line represents perfect agreement.

3.3.2 Accuracy

Reader one examined 157 (45%) otoliths that had a visible OTC mark and were baked adequately so the growth zones were easily seen. Percent agreement with the expected count drops from 39% for all fish to 13% for fish with an expected count $\geq$3, and percent agreement within one year drops from 77% for all fish to 40% for fish with an expected count $\geq$3 (Table 3.1; Fig. 3.3). There was a significant difference between the expected and the estimated number of hyaline zones after the OTC mark (paired t-test, $p = 0.004$, two tailed, negative difference) and Hoenig's measure of systematic bias indicated systematic differences between the expected and the estimated number of hyaline zones after the OTC mark (χ^2 test, $p = 0.002$). A simple linear regression model indicated increasing error with increasing time at liberty ($p = 0.002$). But this is likely due to a decrease in the number of otoliths with zero ageing error rather than an increase in the range of ageing error. The average absolute error for fish with an expected count $\geq$1 was 1.3 years (SD=1.6). Closer examination of these indices of ageing error indicated that reader one has a tendency to systematically overage sablefish otoliths using the current ageing methods (Fig. 3.4). Fish with an expected count $\geq$1 that had ageing error were overaged 60% of the time.

Reader two examined 168 (48%) otoliths that had a visible OTC mark and were baked adequately so the growth zones were easily seen. Percent agreement with the expected count drops from 45% for all fish to 14% for fish with an expected count $\geq$3, and percent agreement within one year drops from 81% for all fish to 44% for fish with an expected count $\geq$3 (Fig. 3.5, Table 3.2). There was no significant difference (paired t-test, $p = 0.36$, two tailed) between the estimated and the expected number of hyaline zones, the Hoenig measure of systematic bias indicated no systematic ageing error (χ^2 test, $p = 0.07$) and a simple linear regression indicated no significant increase in error with increasing time at liberty ($p = 0.2$). The average absolute error for fish at liberty >1 year was 1.0 year (SD = 1.1). But, in general, reader two underages 70% of the fish with ageing error that had an expected count $\geq$1 (Fig. 3.6).

Table 3.1. Reader 1 - estimated number of annuli versus the expected number of annuli after OTC mark.

Expected # of annuli	**n**	**Estimated # of annuli after OTC mark**									**Age range at recapture**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**>7**	
0	41	**33**	5	2	1	-	-	-	-	-	2-17
1	69	25	**26**	11	3	1	2	-	-	-	3-40
2	20	3	2	**5**	5	5	-	-	-	-	4-46
3	11	1	3	-	**1**	1	2	2	-	1	3-45
4	11	1	-	1	2	**3**	-	1	2	2	7-27
5	4	-	-	-	-	2	-	1	-	1	8-24
6	3	-	-	-	-	-	1	-	1	1	11-39

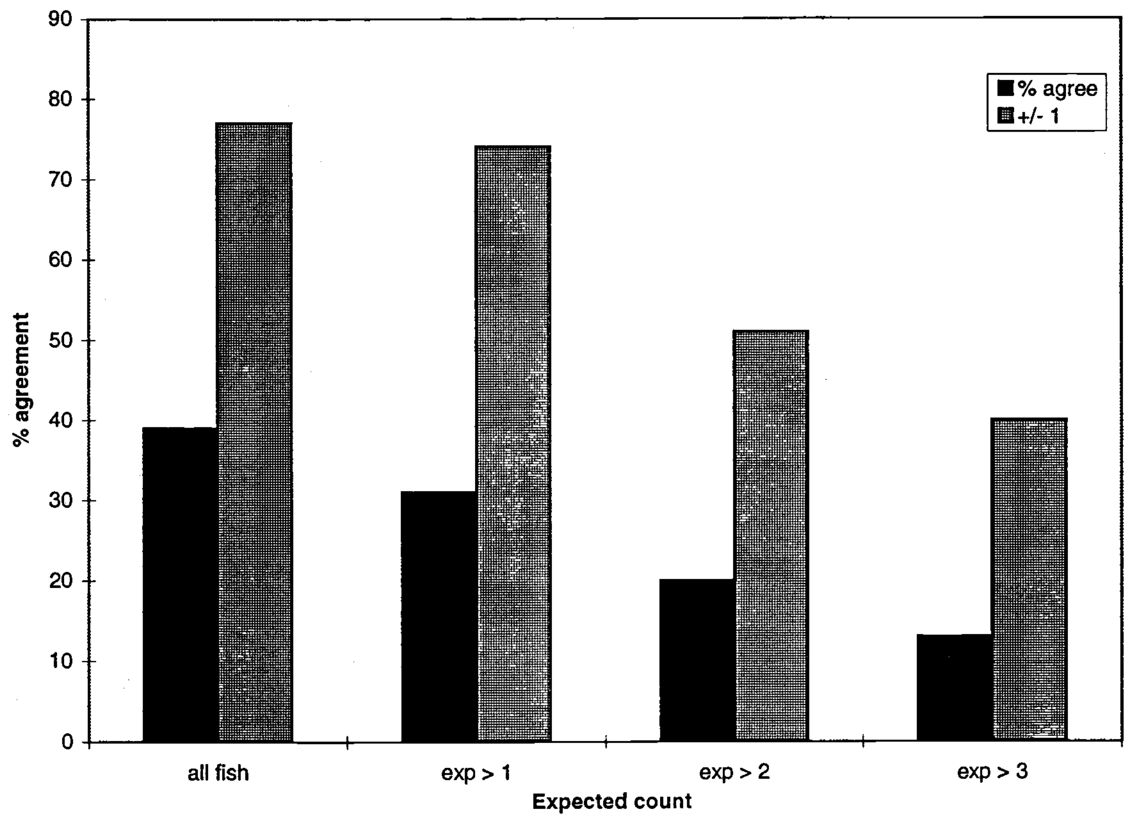

Figure 3.3. Reader 1 accuracy of estimated number of annuli after the OTC mark. Percent agreement is number of fish with no ageing error, +/- 1 is percent agreement within one year of the expected number of annuli.

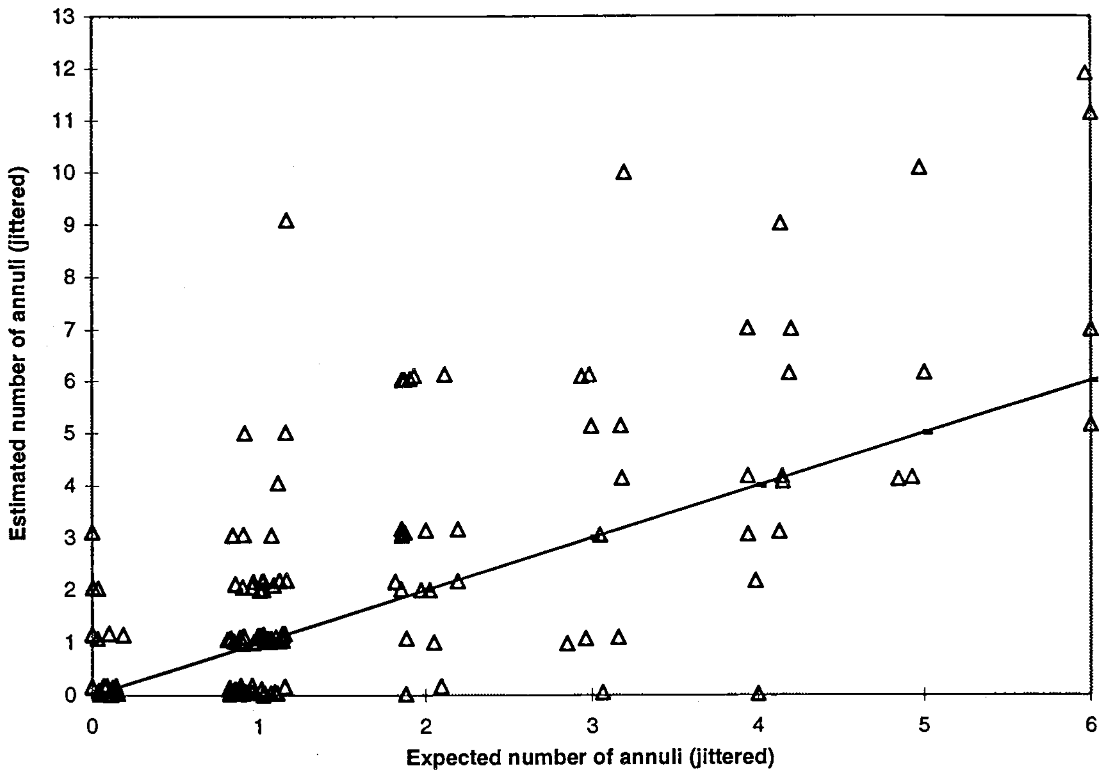

Figure 3.4. Reader 1- expected vs. estimated number of annuli after OTC mark. Line represents perfect agreement.

Table 3.2. Reader 2- estimated number of annuli versus the expected number of annuli after OTC mark.

Expected # of annuli	**n**	**Estimated # of annuli after OTC mark**									**Age range at recapture**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**>7**	
0	42	**34**	7	1	-	-	-	-	-	-	4-28
1	69	27	**28**	10	3	-	-	-	-	-	3-45
2	20	2	5	**10**	-	3	-	-	-	-	6-38
3	15	2	5	3	**1**	2	1	1	-	-	7-14
4	13	1	-	2	3	**2**	2	1	2	1	6-22
5	4	-	-	-	-	1	**2**	-	-	1	10-17
6	4	-	-	1	1	1	-	-	1	-	8-14

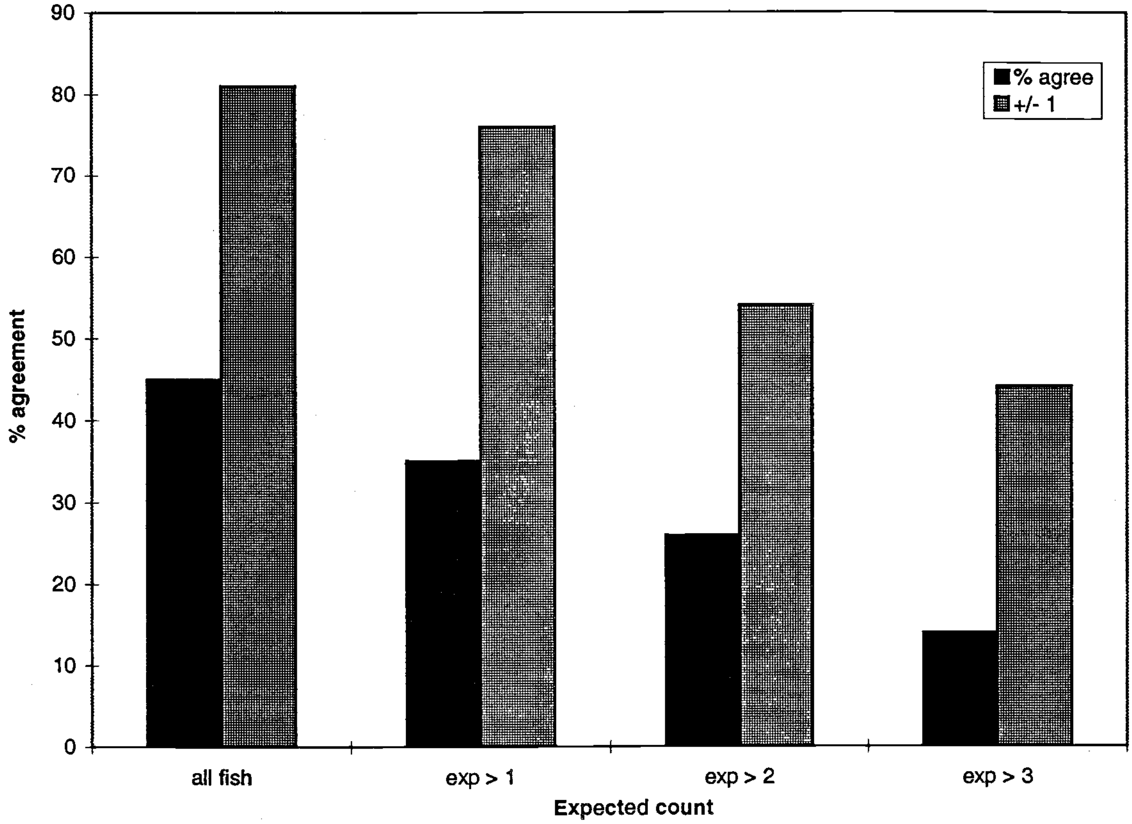

Figure 3.5. Reader 2 accuracy of estimated number of annuli after the OTC mark. Percent agreement is number of fish with no ageing error, +/- 1 is percent agreement within one year of the expected number of annuli.

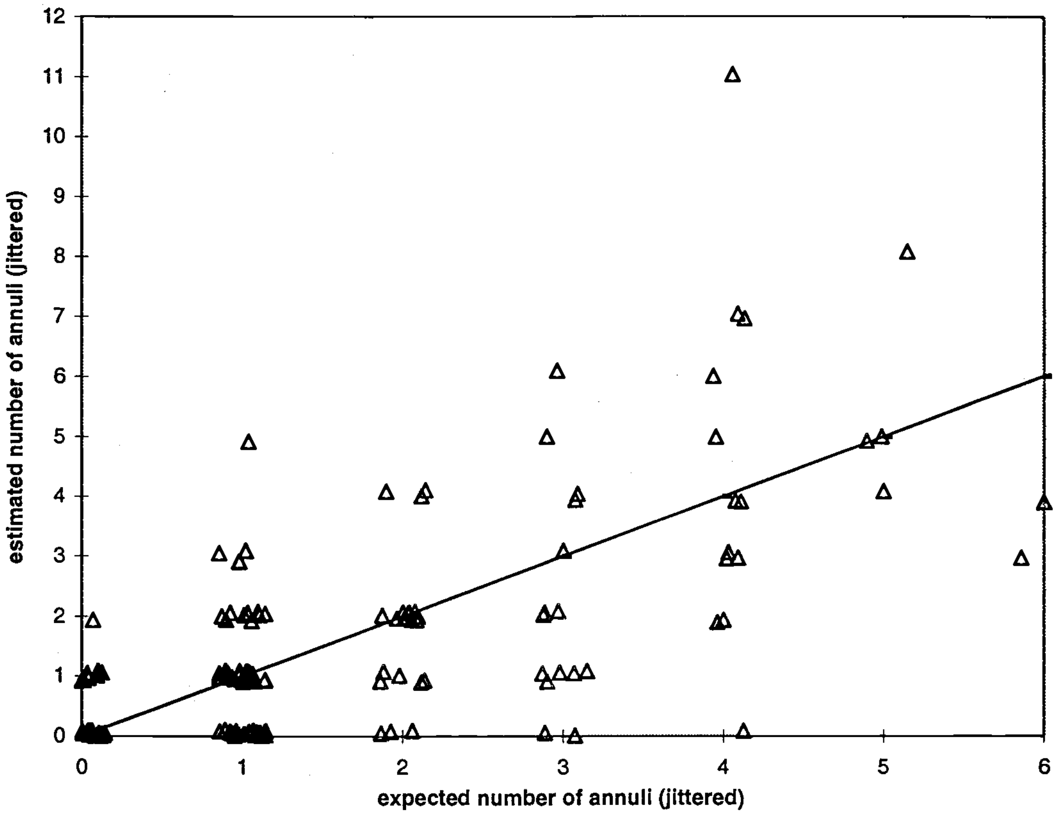

Figure 3.6. Reader 2- expected vs. estimated number of annuli after OTC mark. Line represents perfect agreement.

3.3.3 Examination of Auxiliary Catch Data for Patterns of Ageing Error

The estimates of final age and the available auxiliary catch data (recovery depth, recovery latitude, recovery size, and sex) that could have an effect on ageing error due to changes in physical habitats and life history traits were examined to identify factors that might influence ageing error. Two multiple linear regression models with different dependent variables were applied in a backward stepwise manner: 1) error model- the ageing error was calculated as described above and then compared to the auxiliary catch data. The error model with all catch variables was

$$Error = final\ age + recovery\ depth + recovery\ latitude + recovery\ size + sex. \quad (3.1)$$

2) count model- the number of estimated annuli after the OTC mark was the dependent variable and the expected number of annuli and the auxiliary catch data were the independent variables. In this model the amount of ageing error was measured by the model in terms of the deviation from a one-to-one slope coefficient. The advantages of the count model over the error model are the ability to transform dependent and independent variables and to provide an estimate for the amount of ageing error. The count model with all catch variables was

$$Number\ of\ estimated\ annuli = expected\ number\ of\ annuli + final\ age + recovery\ depth + recovery\ latitude + recovery\ size + sex. \quad (3.2)$$

Recovery latitude was the only variable that had a significant impact on the ageing error of reader one (error model $p = 0.005$; count model $p = 0.003$). The latitude of recaptured fish ranged from 32.19 to 47.46 degrees. Both models indicated that ageing error increased by 0.11 for each degree of increase in latitude. In the count model the coefficient for the expected count variable was 1.3 with a standard error of 0.09. Although the coefficient for expected count was not significantly different than 1 (t = 3.18, p = 0.8) the estimate indicates that only slight increases in error occur as expected count increases. For instance a fish with an expected count of 3 would, on average, have an estimated count of 4 with 95% falling between 3.3 and 4.5; and a fish with an expected count of 10 would, on average, have an estimated count of 13 with 95% falling between 11 and 15. Due to the low size of the coefficients for recovery latitude and expected count, the effect of these variables on ageing error is believed to be inconsequential.

None of the catch variables or the final age estimate significantly contributed to the ageing error of reader two.

3.3.4 Impact of Ageing Error on Final Age Estimates

The previous two sablefish assessment (Crone, 1997; National Marine Fisheries Service, 1998) created 17 age bins for model purposes; 1-yr bins for ages 1-14; 1 age bin for fish 15-19; 1 age bin for fish 20-24; and 1 bin for all ages $\geq$ 25 years. In order to assess the impact of the ageing error on the estimated final ages of the OTC marked sablefish, the fish were distributed into similar age bins as the previous assessments. Once the fish were sorted into the bins each fish's ageing error was added to its final age. The final ages, after accounting for the ageing error, was examined to determine if the ageing error caused them to move into a different age bin. Of the fish that reader one examined, 33% of the fish did not have enough ageing error to move them to another age bin, and 81% percent of the fish were within one ageing bin. Fifty percent of the fish that reader two examined did not have enough ageing error to move them to another age bin, and 82% of the fish were within one ageing bin. It should be noted that the ageing error added to the final age of each fish is only the ageing error that occurred after the OTC mark. It does not take into account any ageing error that occurs prior to the mark/recapture and is therefore a conservative estimate of the ageing error.

3.3.5 Visual Examination of OTC Marked Otoliths

Most otoliths that did not have any ageing error had fairly distinct annuli and growth patterns and adhered to the current ageing methodology. For example sablefish number 10246 was tagged and injected with OTC on 9/11/91 and recaptured on 4/15/93. This fish has an expected count of one plus growth on the edge. There is an opaque zone and one continuous hyaline mark, representing 1992, immediately after the OTC and edge is opaque representing part of 1993 (Fig. 3.7).

Otoliths with significant ageing error were visually examined to determine the possible source of the error. A common dilemma in estimating sablefish ages is whether to 'lump', count multiple marks as one annuli, or 'split', count each mark as an individual annuli. The current ageing method instructs readers to lump during the fast growth period and to split once the ventral edge of the otolith turns 'in', an indication of an older, slower growing fish. The first area on the otolith an age reader is faced with whether to lump or split is within the transition zone (area between early fast growth and older slow growth). The transition zone often contains numerous marks, which can tempt age readers to split and count each mark as an annuli, and it burns a slightly darker color compared to the rest of the otolith. It became apparent that no excessive splitting should take place in the transition zone. Fish number 10679 was marked with OTC during the transition zone phase and was at liberty for a total of six years (Fig. 3.8). A reader who split each mark would estimate seven annuli after the OTC mark, a lumper would estimate three. A correct reader would not count the first mark after the OTC mark as a separate annuli because it is still in the transition zone and the previous opaque zone is still wide, but each mark afterwards should be counted. Reader one split each annuli and overaged, reader two lumped and underaged.

A common questionable growth pattern is when multiple marks originate from the same spot on the ventral edge of the otolith. For example sablefish number 10629 was tagged and injected with OTC on 9/15/91 and recaptured on 11/2/95. This fish has an expected count of four plus growth on the edge (Fig. 3.9). The first three marks after the OTC mark originate from the same spot on the ventral edge, although they are distinct and the narrowing opaque zones call for splitting these into separate annuli. An age reader is faced with either counting these three marks as one (lumping) or as three (splitting) annuli. In this case the reader should clearly split these marks. Reader 1 split, correctly estimating four annuli after the OTC mark, while reader 2 lumped the first two, estimating a total of three, therefore underageing. Fish number 10137 is another example of how multiple marks from the same spot can lead to confusion (Fig. 3.10). In this fish the first band contains the OTC mark. The second band, which originates from the same spot as the previous mark, could also be split into three distinct annuli or lumped as one. Because this fish has an expected count of two and there is a distinct hyaline zone on the edge, the band in question should be counted as just one annuli. Reader one split the band into separate annuli and thus overestimated, while reader two lumped the marks together and correctly estimated the number of annuli after the OTC. The width of the previous opaque zone gives an indication to age readers to lump in these instances. The opaque zones after the OTC mark are narrower than the opaque zones before the mark, but not drastically narrower. Additionally the ventral edge of the otolith has not turned in yet. The combination of the narrowing size of the opaque zones and the edge of the otolith should be a clue to lump the marks because this fish has not concluded its fast growth period.

Otoliths with unique growth patterns are particularly problematic. The current ageing method does not address how to treat these fish. Fish number 11256 (Fig. 3.11) is unique because the last two marks are on a section of the otolith that appears to only have developed on the ventral and not the sulcus side of the otolith. Thus these marks do not completely extend from the sulcus groove to the ventral side of the otoliths. The current ageing method instructs readers to not count any marks as annuli if they do not fully extend across the otoliths, such as these. So this fish would automatically be underaged by two years. Because it is a unique otolith, age readers commonly 'do as they see fit'. Both age readers lumped two of the marks together and underaged this fish by one year. Many of the fish that display odd growth patterns do

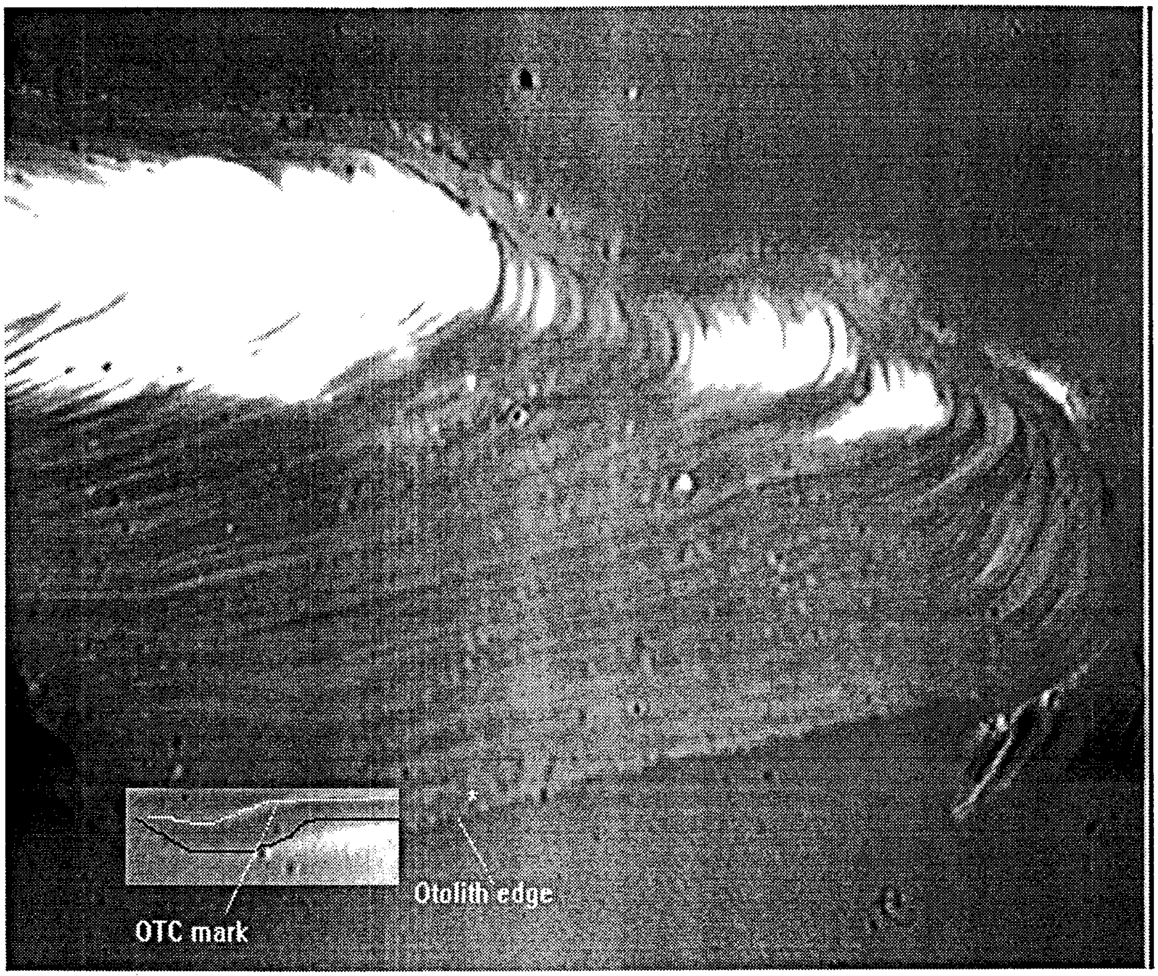

Figure 3.7. Final image of bake-and-slab sablefish otolith10246, injected with OTC on 9/11/91 and recaptured on 4/15/93. Stars (*) indicate hyaline zones counted as annuli.

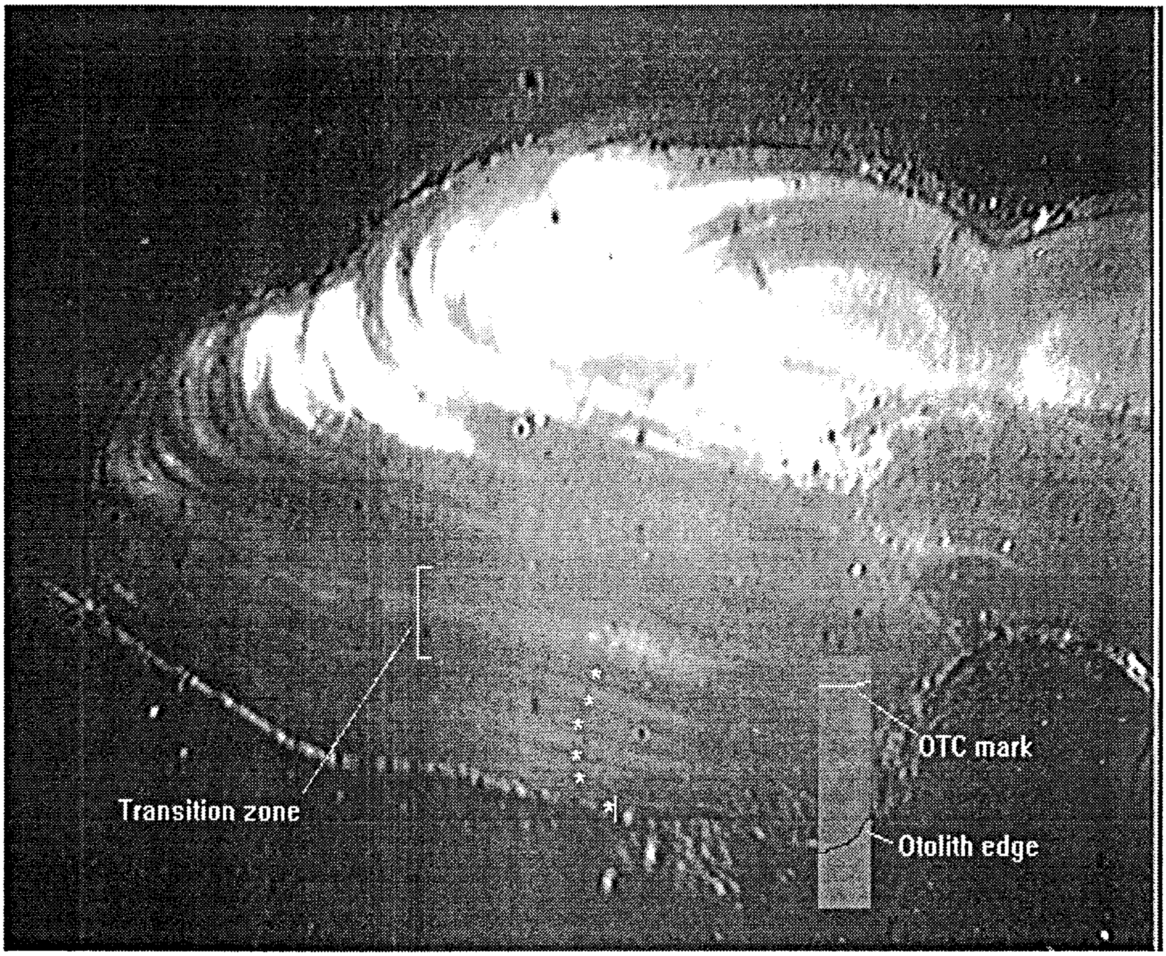

Figure 3.8. Final image of bake-and-slab sablefish otolith 10679, injected with OTC on 9/15/91 and recaptured on 8/2/97. Stars (*) indicate hyaline zones counted as annuli.

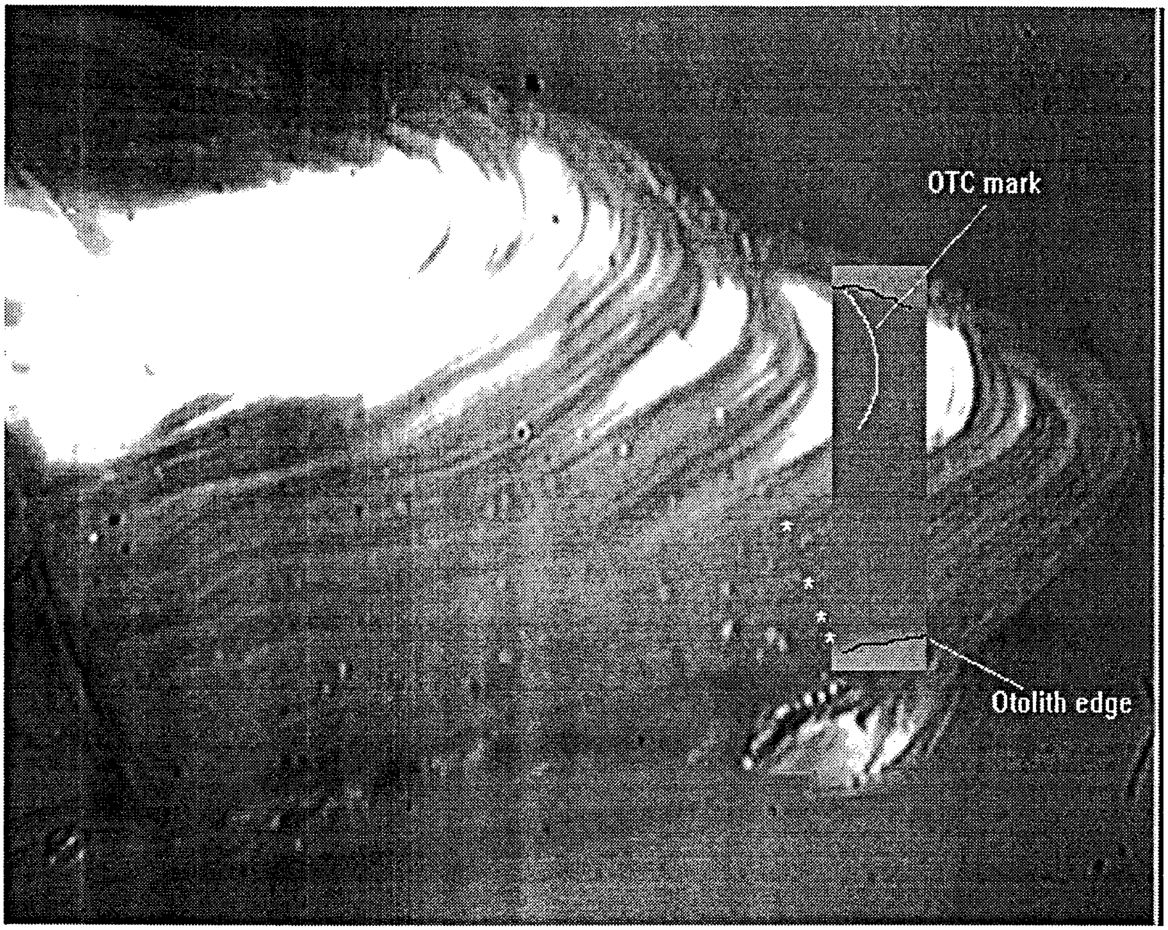

Figure 3.9. Final image of bake-and-slab sablefish otolith 10629, injected with OTC on 9/15/91 and recaptured on 11/2/95. Stars (*) indicate hyaline zones counted as annuli.

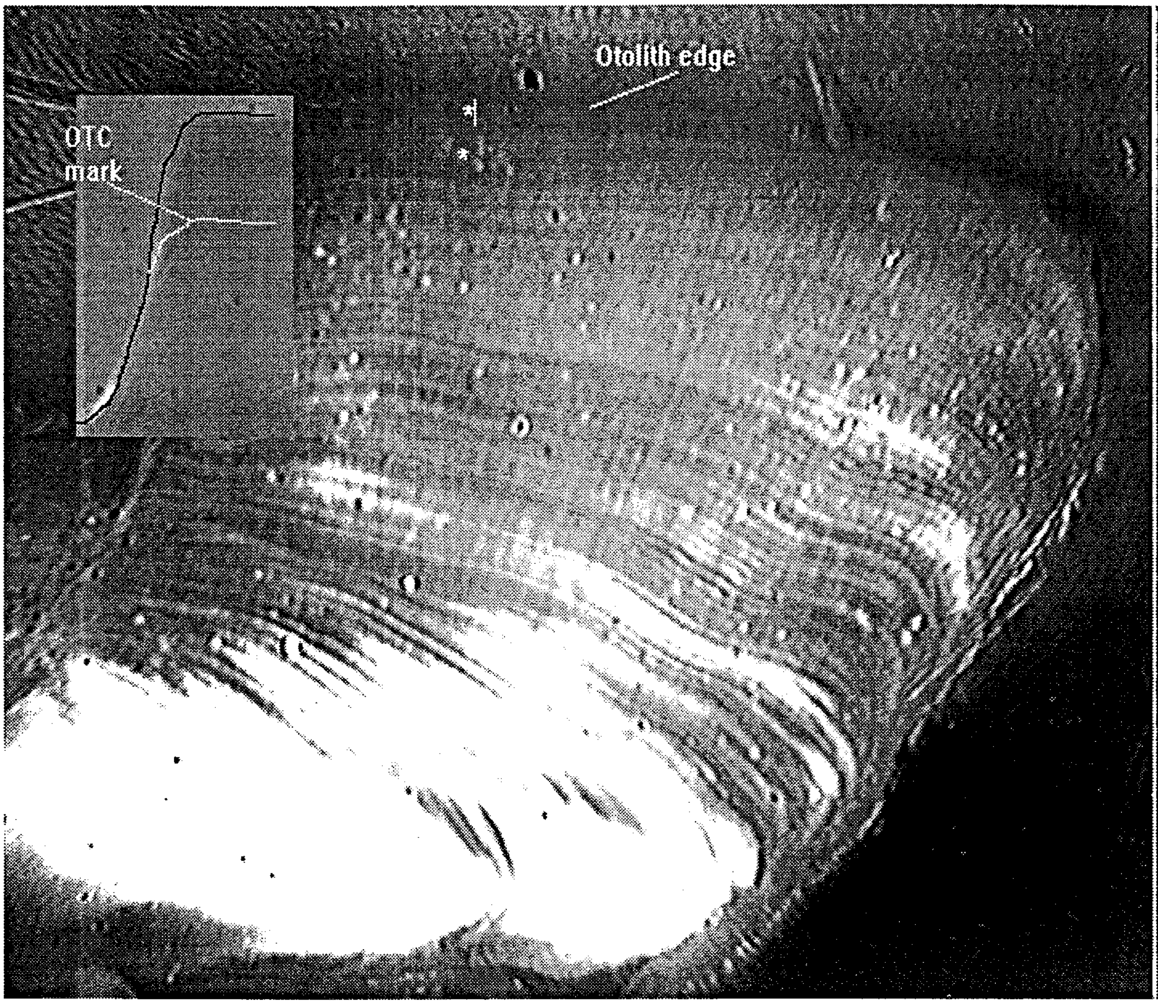

Figure 3.10. Final image of bake-and-slab sablefish otolith 10137, injected with OTC on 9/7/91 and recaptured on 9/16/93. Stars (*) indicate hyaline zones counted as annuli.

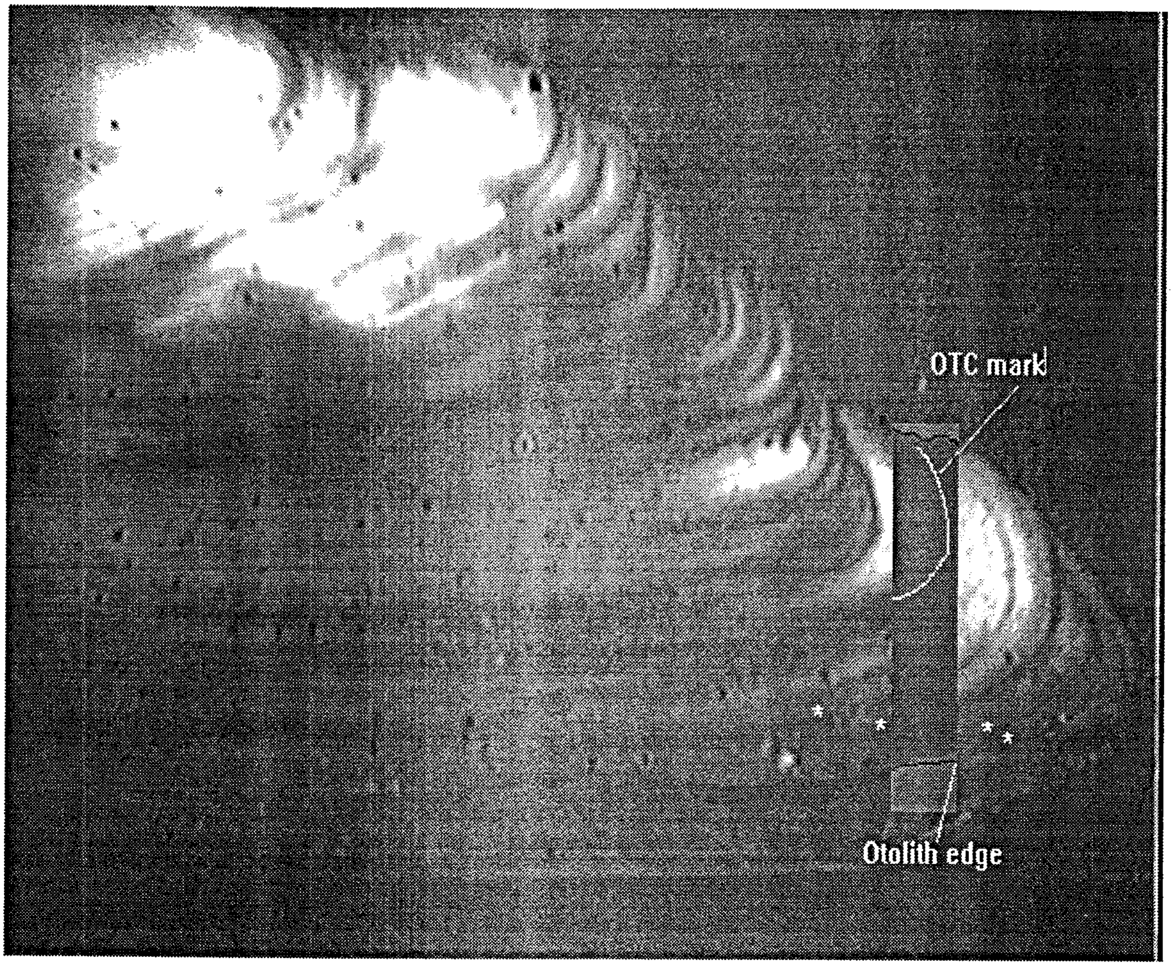

Figure 3.11. Final image of bake-and-slab sablefish otolith 11256, injected with OTC on 9/30/91 and recaptured on 4/2/96. Stars (*) indicate hyaline zones counted as annuli.

so in a unique manner, where there is no correct ageing method. Because of this, it is recommended that age readers either assign an age range, where the true age is most likely within a certain range, or these fish be eliminated from any sablefish age analysis.

3.4 Discussion

Despite generally poor results, this OTC study establishes that the current break-and-burn ageing method is applicable to sablefish from the U.S. west coast. These results are extremely similar to the known age sablefish study from the Gulf of Alaska (percent agreement 35% and 39% for the two age readers, of the misaged fish 81% and 70% were within one year) (Heifetz et al., 1998) and only slightly different from the original OTC study (percent agreement 43%, 82% within one year) (McFarlane and Beamish, 1995). These results also agree with the radiometric study that concluded that the ageing method is fairly accurate but age readers are possibly slightly overaging sablefish from the U.S. west coast. I conclude that the results of this study do not warrant development of a new ageing method. Nor is the ageing error sufficient to cause serious problems for researchers that use sablefish age data.

It is important to validate the ageing methods for all age ranges due to possible changes in the otolith growth patterns as changes occur in the fishes life history (Beamish and McFarlane, 1983). Because the final age estimates of the OTC marked fish range from 2 to 46 years old, it is safe to say that the ageing method is fairly accurate for all sablefish from the U.S. west coast that are caught in the commercial fishery.

Because sometimes sablefish deposit multiple marks annually on their otoliths, the current ageing method advises age readers how to interpret these marks into distinct annuli. The poor accuracy is a result of misinterpretation of the ageing method, and the lack of methodology for the transition zone, marks originating from the same spot on the ventral edge, and otoliths that display unique growth patterns. Poor precision is due to the reader specific approach to these problem areas of the current ageing method. Identification of those areas and development of recommendations on how to deal with those areas will result in increased precision and accuracy. Results from this study suggest that age reading accuracy could be improved if age readers: 1) do not excessively split marks in the transition zone; 2) follow the general ageing practice of identifying and focusing on narrowing opaque zones when multiple bands originate from a single location on the ventral edge of the otolith; 3) assign age ranges or discard otoliths that show unique growth patterns that are difficult to interpret. An increase in precision will result if age readers follow the above advice, regardless of the accuracy of the recommendations.

It is not recommended that future sablefish OTC studies employ the bake-and-slab method of preparing time-marked otoliths for examination. The baking does not provide significant contrast between the opaque and hyaline zones. This alone may have contributed to some of the ageing error in this study. In some cases the resin surrounding the otolith cracked during the heating. These cracked areas have a similar color as the opaque zones therefore making it difficult to discern the edge of the otolith if the edge type was opaque. Thin-slicing time-marked otoliths may be an acceptable alternative to the bake-and-slab method.

Before being incorporated into the current ageing method it is recommended that other sablefish age readers test these proposed amendments. A sample of OTC marked sablefish should be read by various sablefish age readers using the current ageing method. This same sample should be reread by the same age readers using the current ageing method plus the new amendments developed by this study. The accuracy of both reads can be assessed to determine if the new techniques result in improved accuracy and precision.

Chapter 4

SUMMARY

The goal of this study was to critically evaluate the current ageing method for sablefish from the U.S. west coast. The current method was developed in northern waters where sablefish are known to have different growth rates than fish in the south. There was growing concern among sablefish age data users that the current ageing method may not be appropriate for fish in the south due to these differences.

The current ageing method was evaluated using two methods. An edge analysis was conducted to determine the seasonality of the formation of the hyaline zone, which is commonly referred to as the annuli. The results indicate that only one hyaline zone is deposited yearly and it is formed on the otolith generally from May through September. This timing of the hyaline zone formation on sablefish otoliths may be unique to fish from temperate waters, which usually deposit their hyaline zone during periods of slow growth, October through May. It is recommended that a detailed marginal increment analysis be conducted on sablefish from the U.S. west coast to explore this phenomenon in detail.

The second method used to evaluate the current sablefish ageing method was a time-marked otolith study using oxytetracycline (OTC). Fish were injected with OTC, which leaves a fluorescent mark on the otoliths, and released to be recaptured by the commercial fishery. For each year the fish was at liberty one hyaline and one opaque zone should have been formed between the OTC mark and the edge of the otolith. The difference between the number of marks that should have formed and the number of marks counted by the age reader is a direct measure of the accuracy of the current ageing method. The results, which are similar to other sablefish age validation studies, indicate that the current ageing method is fairly accurate but, in general, a large portion of sablefish examined by reader one seems to be slightly overaged. Neither final age

nor any of the auxiliary catch data (e.g., recovery depth, recovery latitude, recovery size, and sex) contributed significantly to the ageing error. The average absolute ageing error for reader one was 1.3 (SD = 1.6) years and reader two was 1.0 (SD = 1.1) years. This error is not significant enough to warrant the development of a new ageing method but some amendments to the current ageing method are recommended: 1) do not excessively split marks in the transition zone; 2) if multiple marks originate from the same spot on the ventral edge of the otolith concentrate on the narrowing opaque zones when determining which marks to count as annuli; and 3) assign age ranges or discard otoliths that display odd growth patterns. It was also recommended that the bake-and-slab method not be used in future sablefish OTC studies. Thin-sectioning methods, although slightly more time consuming, should be adequate. Suggested further work is to test the new amendments to the ageing method by sablefish otolith exchanges that compare results before and after the employment of the new amendments to the current ageing method.

The results of this study will result in increased accuracy and precision of sablefish age data on the U.S. west coast. This will result in greater confidence in the reliability of sablefish ages and therefore a greater confidence in the research that depends upon this data.

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APPENDICES

APPENDIX A

Sablefish (*A. fimbria*) Ageing Procedures

Excerpt from: Manual on Generalized Age Determination Procedures for Groundfish. Prepared by: C.A.R.E. (Committee of Age Reading Experts), Pacific Coast Groundfish Ageing Technicians. Under the sponsorship of: Pacific States Marine Fisheries Commission. For: The Technical Subcommittee of the Canada/U.S. Groundfish Committee. April, 1997.

The sagittal otolith is the recommended structure for aging sablefish and the burnt section is the preferred method for age determination. The surface is an important aid which may be used without reference to a burnt section for clear, young otoliths. The surface is also a useful age range-finding tool for older specimens.

Sablefish grow very rapidly during the early years of their life and their otoliths can show a rather dramatic slowing in growth after 3-4 years. No generalized growth pattern can be applied to all sablefish because many factors appear to affect both the extent and age at which drastic slowing occurs. Whether the slowing of otolith growth is related to environmental circumstances, sexual maturity or other factors, has not been conclusively demonstrated.

Three general otolith section growth patterns are commonly seen in collections:

1. Otoliths of fast growing fish which do not show a marked decrease in growth rate at any point.

2. Otoliths showing transitional growth zones between the zone of fast growth (typically 3-4 years) and the areas of extremely slow growth.

3. Otoliths showing a dramatic growth rate decrease after the years of fast growth.

Because of the high variation in sablefish growth, readers are faced with many interpretive options during the age determination process. For this reason, high precision between readers and between agencies is difficult to achieve, and important to discuss and document. Interpretive problems which affect precision may be solved through calibration exchanges.

Interpretive problems include the following:

1. The "transition years" are particularly difficult to interpret. They follow the early years of fast growth and precede the zone of slow growth.

2. "Incomplete" deposition of annual zones along the proximal edge of the section occurs on the otoliths of some older fish (Fig. 13 a, b). Annual zones appear to form only in the sulcus area and do not extend to the ventral tip. Occasionally, growth zones may accumulate at the ventral edge rather than at the sulcus.

C.A.R.E. recommends that anyone planning to begin working with sablefish should arrange a preliminary training session with experienced sablefish reader. It is only with hand-on experience and between-agency calibration exercises that precision can be maximized.

The general preparation procedures developed for burnt section ageing of rockfish otoliths (see Rockfish Ageing section) may be applied to sablefish, with the following exceptions:

1. Whereas rockfish may be aged using less than 100X magnification, it is recommended that at least 200X be available for use with sablefish otoliths. If possible, acquire higher powered optics (20X eyepieces). Fiber optic illumination must be used.

2. As the first year is typically quite large in sablefish, and the otoliths are relatively thin, it is possible to snap the otolith with the fingers, producing a relatively clean fracture plane through the first year. Use of the Isomet saw to produce a usable surface for preparation of a burnt section is not necessary.

3. In order to identify the first 3-4 annuli, it is necessary to "tip' the burnt section to trace the prominent grooves containing annuli on the distal surface onto the cross-section surface of the otolith. This procedure enables the reader to avoid identifying prominent checks that are often present in the first few years, as annuli. Reading a burnt section without reference to the surface usually results in overageing.

4. It is important to try to trace annuli from the ventral tip to the sulcus in order to identify checks that split away from annuli.

5. The preferred axis used in ageing sablefish burnt sections includes the area between the ventral tip and the sulcus. Annuli are often more apparent near the sulcus and should be traced from the ventral tip to the sulcus.

APPENDIX B

OTOLITHS AND FISH AGEING

Otoliths, or ear stones, are located inside the base of the neurocranium of fish. Each inner ear contains three pairs of otoliths, which are found in sac-like pockets, the sagitta, in the sacculus, the lapillus, in the utriculus, and the asteriscus, in the lagena. The sagitta, which is generally the largest, is commonly referred to as the 'otolith', and used in fish ageing. Otoliths play an integral part in both hearing and balance. The body of a fish will move in response to a sound vibration or motion while the otoliths, which are up to three times denser than the fish, momentarily remain stationary. Because the otoliths are suspended in fluid and surrounded by sensory hair cells, the movement of the fish causes the otolith to brush against and bend some of these hair cells. The bending of these cells results in neural transmissions to the auditory center of the brain and thus the fish 'hears' or maintains balance.

The otoliths of teleost fishes are composed mainly of the aragonite morph of calcium carbonate and protein (otolin). Otolith growth occurs as a daily deposition of material on their surface (accretion). Seasonal variation in the ratio of the organic and inorganic components of the otolith is responsible for the banding pattern used in determining the age of the fish. The hyaline (translucent) zone is mainly comprised of otolin with a reduced amount of short, thin aragonite needles. The hyaline zone is commonly associated with the reduction in growth that occurs in the winter. Under reflected light this zone appears dark, while with transmitted light it appears light. The opaque zone is primarily composed of aragonite whose needles are longer and thicker compared to the hyaline zone. This zone appears bright under reflected light and dark under transmitted light. The opaque zone usually forms during periods of fast growth that usually occurs in the summer.

Because new material is deposited on the surface and the materials that comprise fish otoliths are not reabsorbed during periods of starvation, otoliths 'record' the past events of the fish's lives. The banding pattern exhibited by the alternating opaque and hyaline zones are similar to those exhibited by trees. One year's growth is represented by one opaque zone followed by one hyaline zone. The hyaline zone is commonly referred to as the annuli and is presumed to form once per year. By counting these annuli the final age of the fish may be estimated. Complicating the process of age determination is the formation of accessory marks, or checks, on the otolith. These false annuli may be deposited during times of physiological stress, such as spawning and starvation, or due to abrupt changes in temperature or salinity. False annuli are optically similar to hyaline zones and thus can cause confusion when determining which marks to count as annuli. When age readers incorrectly estimate the age of a fish it is known as ageing error. There are two components to ageing error, accuracy (the proximity of the estimated age to the true age) and precision (the reproducibility of a given age estimate, either between two readers or within the same reader). Validation studies determine the accuracy of a given ageing method. Only extensive training by accurate age readers and regular double reads, where two age readers exchange otoliths and compare age estimate, can ensure precision.

Otoliths have been used to age fish since the late 1800, when J. Rebisch used otoliths to age plaice. Today many fish species are aged using fish otoliths, but due to the variability of the banding pattern seen in each species and the lack of validation of the ageing methods otolith reading is not an exact science. Williams and Bedford (1973) put it best when they stated "It is the author's view, however, that otolith reading remains, for the present at least, as much an art as a science…".

Williams, T. and B.C. Bedford. 1973. The use of otoliths for age determination. *In*: T.B. Bagenal (ed.) Ageing of fish. Unwin Brothers, Old Woking, England. p. 124-136.