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STRUCTURE AND GROWTH OF PACIFIC HALIBUT OTOLITHS: IDENTIFYING SPATIAL AND TEMPORAL VARIATION

A

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By

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Juneau, Alaska

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STRUCTURE AND GROWTH OF PACIFIC HALIBUT OTOLITHS:
IDENTIFYING SPATIAL AND TEMPORAL VARIATION

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ABSTRACT

Otoliths are polycrystals of calcium carbonate and protein that grow through the process of biomineralization within the otic capsule of teleost fish. Otoliths of Pacific halibut (*Hippoglossus stenolepis*) are routinely collected to provide age information, but other information has not been examined in detail. The purpose of this study was to investigate whether otolith structural patterns reveal information about otolith growth, and by inference, about fish growth and habitat during its early life. Variation in the increment widths of the first five annuli of adult halibut otoliths over a 26 year period were partitioned in two ways: the year the growth took place and the year-class to which the fish belonged. The year of growth explained temporal variation in the youngest ages and was attributed to changes in temperature which may influence recruitment success, while the year-class of growth explained temporal variation in older juveniles, but could reflect sampling bias. An analysis of microstructure increments indicated that relative otolith growth rate was an indicator of larval somatic growth. Young halibut from the Gulf of Alaska exhibited similar larval growth histories, though individual and nursery area differences were apparent. Specimens from the Bering Sea had slower larval growth rates than halibut from the Gulf of Alaska. Trace levels of strontium within otoliths were associated with ontogenetic changes of larvae and winter annuli formation of adults. Levels of potassium and sodium varied by nursery area of capture suggesting some utility for stock separation, though there was indication of significant interannual variation. The shape of the larval crystal within the otolith microstructure of young halibut was found not to be associated with nursery area of capture, and thus is not a good candidate as a stock separation tool. The high variation within individuals suggests that the shape of the crystal is not determined by external events. Overall, several patterns preserved in otoliths can provide insight into processes that influence the growth of halibut and distribution of individuals and these patterns can be recovered from adult fish. However careful interpretation is still required to separate meaningful information from spurious data.

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Friends and colleagues at University of Alaska, NMFS's Auke Bay Lab, the IPHC, and ADFG provided council on innumerable occasions. I thank them for their help. The staff of the IPHC provided many of the otoliths used in the study, as well as helpful comments and suggestions on interpreting the data. Joan Forsberg (IPHC) took careful measurements of the otoliths that were analyzed in chapter 2. Jerry Pella, (Auke Bay Lab) provide the suggestion of a randomization test that was used extensively in chapter 2. Ken Severin (UAF) shared his expertise on the elemental analysis methods used in Chapter 4. My committee members, Lew Haldorson, Joe Niebauer, Ron Smith, Don McCaughran, and Ole Mathisen provided careful review of the thesis. Ole Mathisen, in particular, has served up engaging insights into the nature of fisheries science throughout my studies. I thank him for those discussions. My committee chairman, Terry Quinn, provided the guidance that initiated this thesis and the sound suggestions and feedback that brought it to completion. I thank him for this help, his editing of early drafts, and his collaboration in this research. Sara Hagen has followed this project from its inception, and for her enthusiasm, humor and patience, I am most appreciative. My parents, John and Dottie Hagen, tended to my early ontogeny which was ultimately critical to this work. I thank them for their continued inspiration and encouragement and to them I dedicate this thesis.

Chapter One | Introduction

Rationale

Fluctuations in fish population size are poorly understood and difficult to predict, though such variation can be of critical importance for fisheries management. For example, year-class strength of Pacific halibut (*Hippoglossus stenolepis)* is most likely determined during early life phases where the highest mortality rates occur and the least information is available. Only after several years of harvesting is the year-class size known with any precision through catch-age analysis, and halibut are generally not vulnerable to commercial harvest until eight years of age. Another concern in the management of many species, including halibut, is identifying component stocks of the population. Intermixing by different stocks makes it difficult to determine optimal harvest rates and to evaluate the impacts of bycatch and interception fisheries. Individual halibut can undergo extensive movements across broad areas in the Bering Sea and Gulf of Alaska (Trumble et al. 1990). Most of the movement takes place early in life, as a result of a long period of larval drift to distant nursery areas and compensatory movement by juveniles (Skud 1977). Tagging studies are currently the only means through which information on migration rates is available, but the data are expensive to collect and generally incomplete, in that nursery area origins are not identified. Genetic techniques, while useful for stock separation in many other species, has so far proven unsuccessful for identifying components of the halibut population, though new studies are being undertaken (R. J. Trumble, IPHC, pers. comm.).

A record of the early life stages of halibut is contained in their otoliths, which grow by accretion of new layers in a manner closely associated with physiological development of the fish. Otolith microstructures contain a record of these layers, which can reflect an individual's age and growth and mark transitional periods during early life history development. Otoliths may also incorporate in their microstructure a natural chemical tag indicating the halibut's area of origin or marking other transitional changes.

The purpose of this thesis is to investigate the structural and chemical patterns in the otoliths of halibut and to evaluate the feasibility of using that information to predict or estimate year-class strength and to identify subcomponents of the population. Pacific halibut is an ideal candidate for these investigations because there is a long history of information on halibut recruitment and an extensive collection of otoliths is maintained that can be used in the investigations.

In this chapter, background information is provided on otoliths in general and the history of halibut otolith research in particular. The general approach used to evaluate various types of patterns is given and the specific questions addressed by the remaining chapters are summarized.

Otolith background

Otoliths are crystalline aggregates of calcium carbonate interspersed with small amounts of protein (0.2% to 10%) that reside in the auditory capsule of teleost fish (Degens et al. 1969). Part of the fish's vestibular apparatus, they are used in sound reception in addition to having a function as a balance receptor (Popper and Combs 1980). Functionally, otoliths are analogous to otoconia found in the inner ears of other vertebrates but their crystalline structure is more complex. There are three pairs of otoliths: the asteriscus, the lapillus and the sagitta. The sagitta is generally the largest. It is embedded in a gelatinous membrane and resides within an endolymphatic sac as part of the *pars inferior* which sits beneath the semicircular canals. For most species, the sagittae appear to grow continuously throughout the life of the fish. In this discussion, the term otolith will refer to the sagitta unless stated otherwise.

Otoliths grow acellularly by the precipitation of calcium and carbonate ions in the aragonite crystal morphology. Several types of protein may be incorporated into the otolith during its formation but their composition and role are not well understood. Protein fractions containing both acidic and polar amino acid side branches have been observed in otoliths and some appear to have calcium-binding properties (Asano and Mugiya 1993, Gauldie et al. 1990). If similar to other biogenic structures, different types of protein can both inhibit and promote mineralization along particular crystal axes and this may help

guide the overall shape of the otolith (Lowenstam and Weiner 1989). In many species the concentration of protein varies seasonally. In addition aragonite crystals become long and narrow at higher temperatures and short and wide at colder temperatures. This can give rise to differential optical characteristics that appear as light and dark banding. Since Reibisch first observed this phenomenon in the otoliths of plaice in 1899 (as reported in Ricker 1975), fisheries biologists have routinely utilized this characteristic of otoliths to provide age estimates for many commercially important marine species.

One of the major discoveries in regard to otoliths in the last 25 years was the finding of Pannella (1971) that otoliths contain increments in the microstructure which appear to form daily. Investigations of these increments have yielded insights into the circadian-based processes that regulate otolith formation. Counts and measurements of these increments, which are most apparent in young fish, have been used in a wide variety of studies to estimate growth and the timing of physiological changes in larvae and juveniles of many species (Campana and Neilson 1985). Many of these studies are directed towards understanding the factors that may determine the growth and mortality of young fish for the purpose of determining recruitment success (Jones 1992). In addition to daily increment patterns, other microstructure features, which appear to be common to many species and have been used as reference points, include check rings, which appear to be deep discontinuities in the microstructure; the central primordia, which are initial sites of nucleation; and accessory primordia which are nucleation sites that appear on the growing surface of the otolith during advanced larval development.

Microstructure patterns in adult fish of many species, particularly temperate species, are more difficult to interpret. There is generally an increase in the frequency of check rings with age, which in some cases are associated with winter growth (Gauldie 1987). The check rings, though, are not continuous and may appear more often along some growth axes than others. Daily ring formation, if it occurs, becomes more compressed and below the resolution of light microscopy (Campana 1992). The layering of aragonite crystals may also change with age when less protein is laid down (Hoff and Fuiman 1993), making it more difficult to interpret the microstructure patterns as part of a subyearly temporal scale.

It is still possible in older otoliths to recover patterns that were laid down during the earlier developmental stages of the fish, where the pattern of increment formation is much clearer. Otoliths grow by accretion, and resorption of calcium appears to be a rare event (Mugiya and Uchimura 1989). Removing the overlaying material in older otoliths can reveal the patterns formed early in the development of the fish. Campana (1984) first suggested that adult otoliths of commercially valuable species could be examined for a record of early growth and that such an examination may reveal correlations with indices of year-class strength. Campana and Neilson (1985) expanded on this idea when discussing future applications of otolith microstructure research. They emphasized its potential use as a predictor of year-class success prior to recruitment to the fishery by examining the otoliths of pre-recruit fish. They also indicated such a study may reveal at what age year-class strength is determined. Despite these suggestions, retrospective studies using adult otoliths, with some exceptions (e.g. Neilson and Geen 1986, Pereira et al. 1995), have generally not been conducted.

Backcalculation studies have been the primary means of reconstructing past growth history from otoliths and they have a long history in ageing studies (Francis 1990). The method however has been found to be unreliable in some cases. In particular there is evidence that the assumption of proportionality of otolith growth and fish growth is not inviolate, and faster growing fish may have smaller otoliths at any given size (Reznick et al. 1989). As a result, traditional backcalculation methods can underestimate previous size at age. This can be partially avoided by establishing a common point early in life in which the fish-otolith size relationship is known to be constant regardless of growth rate (Campana 1990). The method however still assumes a linear or proportional relationship between otolith and fish size. In general, any application in which inferences are drawn about somatic growth from otolith patterns needs to be conducted carefully.

A recent development in otolith research has been the availability of methods for detecting and measuring trace elements incorporated into the otolith microstructures. These methods take advantage of technological advances in microchemistry detection and in some cases are able to target specific

locations in the otoliths. Elemental analysis has been used to identify stock composition (e.g. Thresher et al. 1994), to determine ambient environmental conditions at the time of otolith formation (e.g. Secor 1992), and to provide an indication of physiological condition (Kalish 1991). Despite some promising advances in these approaches, more work is necessary to refine the technologies and to help determine the limitations of the methodology (Jones 1995).

Halibut background

The Pacific halibut supports one of the oldest continuously managed fisheries in the eastern Pacific Ocean. Established by a convention between Canada and the United States in 1924, the International Fisheries Commission (which was later renamed the International Pacific Halibut Commission, IPHC), was given responsibility for stock assessment and determining harvest levels. Early in the establishment of the IPHC, the importance of age estimates of halibut was recognized. Procedures were developed for the routine sampling of otoliths from the commercial catches, and a collection series was established which to date contains over 250,000 otoliths (C. Blood, IPHC, pers. comm.).

Early on in the investigations of halibut, both otoliths and scales were initially considered as ageing structures and different approaches for preparing otoliths were examined. Scales were rejected as unreliable for older fish (Thompson 1915) and the preferred method of viewing otoliths was determined to be surface age readings of whole otoliths after soaking them in a glycerin solution (Dunlap 1934). The glycerin method is still in use today as the primary means of counting annuli. The temporal nature of the annulus has been validated through an analysis of its seasonal formation (Dunlap 1934) and more recently through tetracycline marking (Gilroy et al. 1995). The "break and burn" method - snapping the otolith along the proximal distal plane and charring the exposed surface with an alcohol flame (Chilton and Beamish 1982) - has been shown to provide maximum ages older than those seen with surface readings (C. Blood, IPHC, pers. comm. 1995).

The primary use of otoliths by the IPHC has been to provide age estimates. In the 1950's, however, the widths of the annual growth zones of otoliths from the historical collection were measured to provide a long term time series of halibut growth (Southward 1967). The purpose of these investigations was to determine if changes in the growth rates of halibut were the result of fishing pressure or environmental conditions. The analyses relied on a backcalculated relationship between otolith size and fish size and presumed the relationship was largely invariant from year to year. Studies were also undertaken in the 1960's to determine if a predictive relationship between otolith size and fish size (Southward 1962) could provide a more efficient means of sampling the commercial catch landings by eliminating the need to measure the fish in the field. Measuring otoliths, first by length, then weight, became a routine procedure for determining the size of halibut in the commercial catches (Quinn et al. 1983). The predictive relationships were refined over time as an awareness arose that differences in growth rates could result in a difference in the relationship of otolith size to fish size (Southward and Hardman 1973) and these differences could require an adjustment in the annual stock assessment (Quinn et al. 1985). In light of significant changes in apparent growth rates of halibut in the late 1980's, the relationship between otolith size and fish size was reexamined, and it was decided to discontinue the practice of predicting fish size from otolith size and to measure the size of the fish directly (Clark 1992).

Other studies conducted with otoliths of adult fish have included a method for automatically ageing otoliths with image processing techniques (Neal 1987) and determining if otolith shape could be used to identify male and female halibut (Forsberg and Neal 1993). Despite some limited success, neither method has proved useful in the routine examination of otoliths. Currently the information extracted from adult halibut otoliths is limited to ages.

Microstructure patterns in the otoliths of larval halibut were investigated by Hagen (1986) using specimens obtained from a halibut larval survey in 1985 (St-Pierre 1989). The purpose of the study was to relate otolith size, microstructure features, and increment counts to the size and developmental stages of larvae from two different areas. The investigation found two check rings appearing constantly at 23 ±

3 μm and 57 ± 4 μm in diameter. Based on their constant appearance and their locations in the otolith, the prominent outer check was hypothesized as being associated with first feeding, while the diffuse inner ring was thought to be associated with hatching. Regularly spaced increments appeared outside the outer check ring and accessory primordia were observed to form in larval halibut in the late stages of development. Under the assumption of daily formation, the increment counts were used to provide an estimate of growth rates and to infer the timing of spawning. Slight differences in growth were found that matched differences in sea surface temperature.

An example that illustrates both how otoliths of adult halibut can reveal microstructure patterns corresponding to early life history events as well as demonstrating the utility of the IPHC historical collection can be seen in Figure 1-1. This otolith comes from a collection made in 1914 by William F. Thompson (who later helped establish the International Fisheries Commission and became its first director), while participating in commercial halibut fishing expeditions under the auspices of Fisheries Department of the Province of British Columbia, Canada (Thompson 1915). The purpose of the trip was to collect biological information from halibut fishing grounds in Canada and Alaska and to provide estimates of size at age of male and female halibut from different areas. The otolith in Figure 1-1a had apparently been used as part of a series to evaluate alternate ageing methods and had been cut as a thin-section along the transverse axis and polished and mounted with balsam resin on a glass slide. The annuli are not clearly visible in this otolith, perhaps due to the effects of time. However without additional preparation, a series of fine increments can be observed at higher magnification in an area likely corresponding to the first summer's growth as a juvenile (Fig 1-1b). The line in Figure 1-1b is 350 μm long and the average width of the increments (indicated by the letter A) is 7.4 ± 1.4 μm. The periodicity of the increments is suggestive of daily formation based on visual criteria discussed by Campana (1992). Increment widths have been used in many studies as an indication of relative growth rates (e.g. Wilson and Larkin 1982), though most studies use increment widths as part of a backcalculated approach for estimating growth history (Jones 1992). For this particular otolith there was

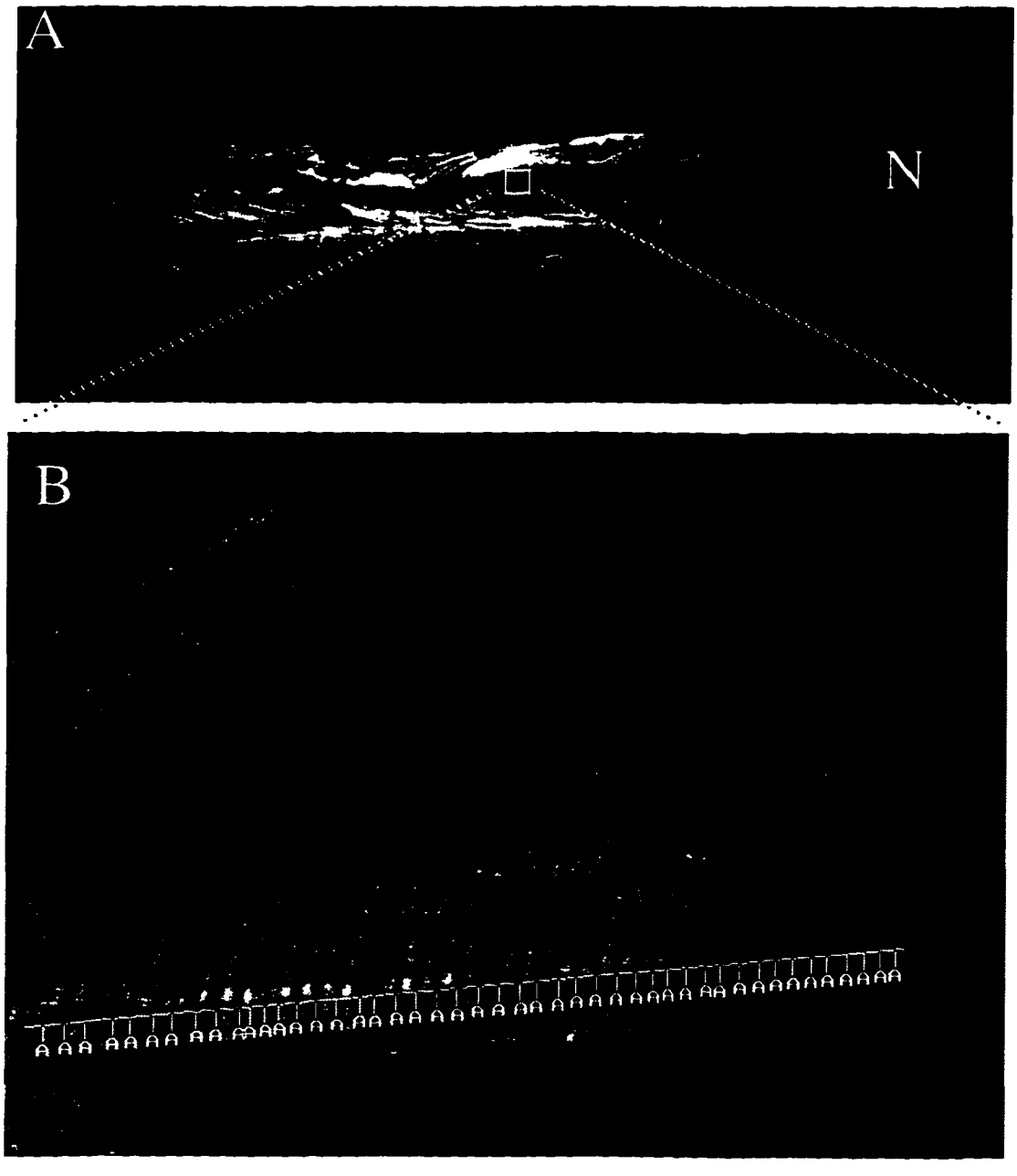

Figure 1-1 a). Transverse cross-section of an adult halibut otolith processed circa 1915. Viewed with transmitted light at 10x. N denotes the location of the otolith nucleus. Distance from N to the location of the box is 1.34 mm. b). Expanded view of otolith microstructure at the location of the box (157x). Increment spacing, denoted by letter A, averages 7.35 ± 1.4 μm

no record indicating its location of capture, the year-class the fish belonged to, or the orientation on which the cut was made, which limits the utility of the increment width data. The sample illustrates however one type of information that could be extracted from otoliths of adults and from the historical collection.

Approach

It is against this background of a long history of Pacific halibut research, an extensive otolith collection maintained by the IPHC, and new developments in the examination of otolith patterns; that this investigation was undertaken. Each chapter is devoted to a different feature or measure of halibut otoliths and its relationship to the biology of the halibut and its environment. The common approach taken in these chapters is to measure pattern variation and to then determine how that variation is explained by different factor levels for the purpose of evaluating the patterns as natural tags or for providing insight into the process that may determine year-class strength. An important consideration in addressing the sources of variation is to also measure variation within the fish and within otoliths where feasible. Through this means it is possible to separate extrinsic sources of variation from those that are due to intrinsic reasons and thus provide some understanding of what may give rise to the patterns in the first place.

Geographic variation was examined by looking at otoliths of juvenile halibut from known nursery areas. These samples were obtained from surveys conducted by United States Mineral Management Service as part of the Outer Continental Shelf Environmental Assessment Projects (OCSEAP) in the Bering Sea and Bristol Bay, as well as from IPHC juvenile surveys in the Gulf of Alaska and Southeast Alaska.

Otolith specimens from the IPHC's general series collection were used to examine temporal variation in Chapter 2. A 26 year time series of annual growth increments was measured covering a more recent time period than that examined by Southward (1967). The approach for examining historical changes in

otolith growth is to consider two ways that growth data can be partitioned, either by the year the growth took place or by the year-class to which the fish belonged. By this means it is possible to separate environmental effects on growth from those due to intrinsic sources that are attributed to the year-class of the fish. The time series of growth is then considered in relationship to environmental effects and year-class success.

In Chapter 3 an examination of larval increment patterns is undertaken. The approach treats increment patterns as an expression of otolith growth and examines differences between individuals and areas in terms of changes in velocity and relative growth. Information is presented on the formation of structural patterns through the use of scanning electron microscopy. The chapter extends the information presented in Hagen (1986), by examining the coupling between otolith growth and larval growth and serves as a basis for reconstructing larval growth histories of juveniles and adult fish.

Chapter 4 considers variation of trace elements in otoliths. Variation of element distributions is considered within otoliths and analysis of variance models are used to partition variation among nursery areas, individuals within nursery area, and otoliths within individuals. Various trace elements are evaluated for their ability to serve as natural tags, and to provide insights into physiological changes in halibut.

Chapter 5 presents an analysis of the shapes of the larval core of otoliths. The larval core is defined as crystal growth that originates from the central primordia, and the shape is determined by the number and placement of the accessory primordia. The larval core is one of the more prominent features in the otolith microstructures, yet there is little information about what gives rise to variability in its shape. An analysis of its various shape parameters within and between individuals and nursery areas is used to evaluate the utility of larval core shape as a natural tag.

Chapter 6 concludes with a summary of the major findings presented in chapters 2 through 5. The discussion then ties the results from the previous chapters along with additional observations to describe ontogenetic changes in halibut otoliths in relationship to somatic growth and development from

prehatch to sexual maturation. The utility of these patterns is discussed in relationship to those factors that may affect the recruitment of halibut. Throughout this chapter I also provide suggestions on additional lines of research that may help solve remaining questions concerning the meaning of patterns contained in otoliths and their relationship to halibut ontogeny. I also specifically consider how extracting information that is contained in IPHC's extensive historical collection of halibut otoliths can be useful in understanding and anticipating future changes in abundance.

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Chapter Two [1] Long-term growth dynamics of young Pacific halibut: evidence of temperature-induced variation

Abstract

The first five otolith growth zones of 745 Pacific halibut from 26 year-classes (1953-1978) were analyzed to identify patterns of annual growth and sources of temporal variation. Differences in male and female growth appeared at an early age, and a difference in the size selective property of capture gear was shown to influence the perception of past growth. I adjusted the growth record for these two effects by removing variation in annual otolith growth that was linearly related to the size of the fish at capture. Correlation patterns of the otolith zones at the youngest ages suggested a general uncoupling of individual growth from one year to the next. Temporal variation was examined by developing a linear model to partition growth at different ages into both year and year-class effects. Randomization tests indicated that both effects are significant when all five ages are included, but when subsets of the data are examined, the year effect is strongest for youngest juveniles, while the year-class effect is significant for older juveniles. The year effect is likely attributable to interannual temperature changes as evidenced by a strong linear relationship between sea surface temperature (SST) and otolith growth from ages 0 to 2. However, analysis of residuals indicated that there was a remaining year effect, suggesting that SST is an imperfect measure of the actual environment that regulates juvenile growth. The year-class effect observed in older juveniles was not present in the data adjusted for size selectivity, suggesting that this effect could be influenced by sampling bias, though intrinsic influences cannot be ruled out. Density-dependent growth did not appear to be a factor accounting for growth variation; instead there appeared to be a slight positive correlation between otolith growth at ages 1 and 2 and estimated year-class abundance (at age 8), suggesting that early growth is a factor in determining year-class strength.

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Introduction

The body size of fish appears to be a more important determinant than age of such demographic parameters as birth and death rates (Kirkpatrick 1984, Hughes 1984), the timing of ontogenetic shifts (Osenberg et al. 1988), fishing mortalities, and time of recruitment to the fishery (Ricker 1969, Deriso and Parma 1988). Since a fish's size is the cumulative result of previous growth events, understanding the factors which determine size requires a consideration of the growth events themselves and factors which may result in observation bias such as size-selective fishing mortality. Otoliths from many temperate fishes can provide a unique measure of individual annual growth, and there is a long history of using otoliths to backcalculate body size (Francis 1990). The relationship, however, between otolith size and fish size may not be as invariant as once suspected (Reznick et al. 1989, Secor and Dean 1989, Clark 1992) and inferences drawn from otoliths should be considered carefully. Nonetheless, it seems reasonable to postulate that factors which cause variation in otolith growth are the same factors which influence body growth. Thus, a first step toward understanding body growth is to directly consider variation in otolith growth.

In this paper I analyze factors influencing variation in otolith growth of juvenile Pacific halibut (*Hippoglossus stenolepis*). The Pacific halibut is a long-lived, commercially important flatfish whose catch, age and growth records extend back to the 1920's and before. Halibut are generally not targeted by the commercial longline fishery until 8 years of age. Since 1962, the size of adult fish has been determined indirectly through a predictive relationship with otolith size (Quinn et al. 1983). Information on juvenile growth is obtained primarily from otoliths of adult fish through a back-calculated relationship (Southward 1967). Previous studies of growth have speculated that changes in size-at-age of halibut stem from a density-dependent mechanism along with unknown environmental changes (Southward 1967, Schmitt and Skud 1978). There are also indications that historically, the production of halibut is controlled by density-dependence (Deriso 1985). However, McCaughran (1987), in an

analysis of tag return data, found no indication of density-dependent growth for halibut. More recently, there has been concern that the predictive relationship used to determine adult size has been unable to account for possible changes in sex ratio of the catch or for shifts in gear selectivity (IPHC 1990).

To analyze the factors which may influence otolith growth of juvenile halibut, I first consider those factors which can obscure the perception of growth variation, such as sexual differences and the effects of gear selectivity. In addition, I consider the degree to which growth variation is determined by intrinsic factors. Intrinsic growth, referring to growth that is predetermined perhaps through a genetic factor, may be shown through an individual's pattern of correlated growth from one year to the next (Kirkpatrick 1984) and may have important consequences for management (Parma and Deriso 1990).

I examine temporal variation in growth by first considering whether the growth record can be characterized by either year or year-class effects. Year effects occur if trends in the annual growth of different cohorts show similarities within years. Broad-based environmental factors that operate across different ages may result in year effects. In contrast, year-class effects occur if annual growth at different ages show similarities within year-classes. Density-dependent growth and population-wide genetic response are two mechanisms which may result in year-class effects. After developing a test to measure the influence of these two effects and considering possible sources of bias, I then consider two candidates that may explain the different aspects of growth variation: an environmental time series and a measure of year-class strength.

Data

Otoliths from research charter surveys conducted by the International Pacific Halibut Commission (IPHC) (Hoag et al. 1980) were used for analyzing otolith annuli patterns. Most of these surveys used longline gear. I restricted the selection of otoliths to those taken in the central Gulf of Alaska to avoid inter-regional differences. I targeted on eight-year-old fish because that is the youngest age that is generally well represented in the catches and older fish are more subject to ageing errors. I randomly

selected otoliths from fish that fit these criteria. However, in some cases I had to take older and younger fish caught in adjacent years to establish a long-term record because research charters were not conducted every year in the Gulf of Alaska. For this same reason I also included specimens that were obtained from trawl surveys. In the early 1960's in particular there were very few longline surveys in the Gulf of Alaska.

I determined the target sample size for each year-class by a preliminary examination of 50 halibut otoliths obtained from port sampling in Sitka, Alaska in 1987. I found that a 5.0% difference in mean annuli widths would be detectable with a sample size of 25 by calculating the coefficient of variation for each set of measurements (Cochran 1977). A total of 840 otoliths obtained from the IPHC were reexamined by an experienced age reader (Joan Forsberg, IPHC) to determine if there was agreement with the recorded age. The reader disagreed with 11% of the recorded ages, but most questioned ages (83%) differed by one year and were evenly split between older and younger estimates. Because these otoliths were examined more thoroughly for this study and were aged using current technology, I feel the revised ages are more accurate. Some of the otoliths selected were not used because they were either broken, or otherwise unsuitable for accurate ageing or measuring. This left a total of 744 otoliths that comprised the sample from 26 year-classes (1953 through 1978), for an average of 28.6 individuals in each year-class.

On the distal face of each otolith a reference line was identified extending from the nucleus to the closest point between the rostrum and antirostrum along the anterior edge. Surface measurements were made along the rostrum through a radial line at approximately 25 degrees counter-clockwise from the reference line. The measurements included the distance from the nucleus to the midpoints of the first five annuli. The radial line was chosen because it represented the greatest axis of otolith growth up to age five. No measurements were made beyond the fifth annulus because after that point the axis of greatest growth appeared to deviate from the radial line. Each otolith was placed under a dissecting microscope with a video camera attached to a monitor and microcomputer. A commercially available

image analysis system (Optical Pattern Recognition System (OPRS), Biosonics Inc. 3670 Stone Way North, Seattle WA 98103) was used to digitize the image for data collection. Distances between the nucleus and the first annulus and then between successive annuli were the variables of interest. I refer to these as zones 0 through 4, corresponding to the age at which the zones were laid down.

I sought a broad-based environmental measurement to relate to otolith growth, because halibut can undergo extensive migrations as prerecruit juveniles (Skud 1977) and the location of any individual when its otolith zones were laid down is uncertain. The environmental time series I used is the yearly average sea surface temperature (SST) at 55° N between 145° and 155° W (J. Namias, Scripps Institution of Oceanography, La Jolla, California). The data are compiled from a monthly temperature record extending back to 1947. Royer (1986) found a strong correlation between trends in surface temperature and temperatures at a depth of 150 m ($r = .607$) over a 14 year period at a station in the Gulf of Alaska. The changes were generally in phase suggesting that they are a result of horizontal advection. In addition, there appears to be a good areal coherence of sea surface temperatures in the Gulf of Alaska (0-100 m) with that of the temperatures over the coastal shelf (0-250 m) (Royer 1986). This suggests the SST data averaged across a wide area may be an appropriate temperature record to use, though it may not reflect subsurface warming that could be associated with El Nino events (Royer 1986). While other environmental time series have been compiled in relationship to halibut (Parker 1989), temperature is the only one with an obviously direct biological relationship with growth, and was the only environmental time series examined for this study.

Estimates of annual year-class strength (number of age 8 fish) were obtained from Dr. P. Sullivan (IPHC) and were based upon an analysis of coastwide halibut port sampling data from longline gear (Quinn et al. 1983, 1985) adjusted for incidental catches of halibut from other gear types. Though I use it as a measure of population size, year-class strength is not necessarily an indication of density, because the population range may expand or contract based on population size. In addition, there is a three to eight year lag between the time the growth takes place and when year-class strength is measured.

Variation in natural mortality or unaccounted removal of young halibut by other fisheries in the intervening years means, at best, year-class strength can only be an imperfect measure of previous population size. Nonetheless, the same measure of year-class strength has been used by Deriso (1985) to examine the production of halibut in a spawner-recruit context and by Parker (1989) to identify environmental factors influencing year-class strength during the larval stage.

Analytical approach

Each set of five otolith zone measurements for each fish was associated with sex, gear type, body length, age at capture, year-class, and year in which otolith growth took place. I used graphical methods to examine the relationship between growth zones within individual fish and in relationship to the external factors: year-class strength and SST. I was unable to incorporate all factors associated with each measurement simultaneously in one model. I therefore developed a series of linear models to account for variation in zones that may be a result of sex and gear types, temporal variation, and the external factors.

Details on models and the rationale for their development are described separately in each section. In general, the linear models are of the form

$$Y = \beta_0 + \sum \beta_i X_i + \varepsilon$$

where Y is the zone measurement indexed appropriately, the $\{X_i\}$ are independent factors or covariates that are examined, the $\{\beta_i\}$ are parameters estimated in the model fitting procedure, and ε is a random error. Standard confirmatory statistics were used to determine the significance of parameter estimates. An exception is the test on temporal variation in which a randomization test was developed to determine probability levels. The residual variation remaining after fitting a linear model was used as input to analyze the data from other perspectives. The residuals, $\hat{\varepsilon}$, were calculated by

$$\hat{\varepsilon} = Y - \hat{Y}$$

where $\hat{Y}$ is the predicted observation from the model.

Adjusting for sex and size selectivity

Size selectivity of sampling gear is known to bias the perception of past growth (Ricker 1969). The fish used in this study were obtained by two different gear types, longline and trawl, which have different selective properties. An average eight-year-old halibut is smaller than the size of full recruitment in the longline fishery, but larger than the average size which is vulnerable to research trawl gear (Myhre 1969). In addition females tend to be larger than males at the same age (Hoag et al. 1979).

The relationship between the zone, sex and gear types, irrespective of time, can be seen graphically by using boxplots (Chambers et al. 1983) (Fig. 2-1). Longline-caught fish grew faster than trawl-caught fish at ages 2 through 4, as indicated by the larger growth zones. Sexual differences in growth became apparent in longline-caught fish by age 2, while sexual differences for trawl-caught halibut did not occur until age 4 (Fig. 2-1). When growth was examined from the years in which fish from both trawl and longlined gear were caught, similar patterns held.

To adjust the growth record for gear selectivity and differences due to sex, I removed variation in growth that was linearly related to the size of the fish at capture and considered the residual variation as the adjusted data set. The correlation coefficients between otolith zone and body size increased with older growth zones (Fig. 2-2). I found that additional variation was explained by including the age of the fish at capture since not all fish were age eight. Because the gear types had different selective properties, separate models were fitted to each zone-gear combination. For each model I used a stepwise approach to determine if both length and age at capture explained significant amounts of variation. In addition, I checked to see if the inclusion of both variables caused co-linearity problems, and tested whether the remaining variation contained differences attributable to sex. In each case I found there were no co-linearity problems and sex was not a factor except when fitting a model to zone 0 growth for each gear. In those cases sexual differences were apparent only after removing the variation attributed to length; sex was not a factor prior to fitting the models. Because the variation explained by sex was slight, I

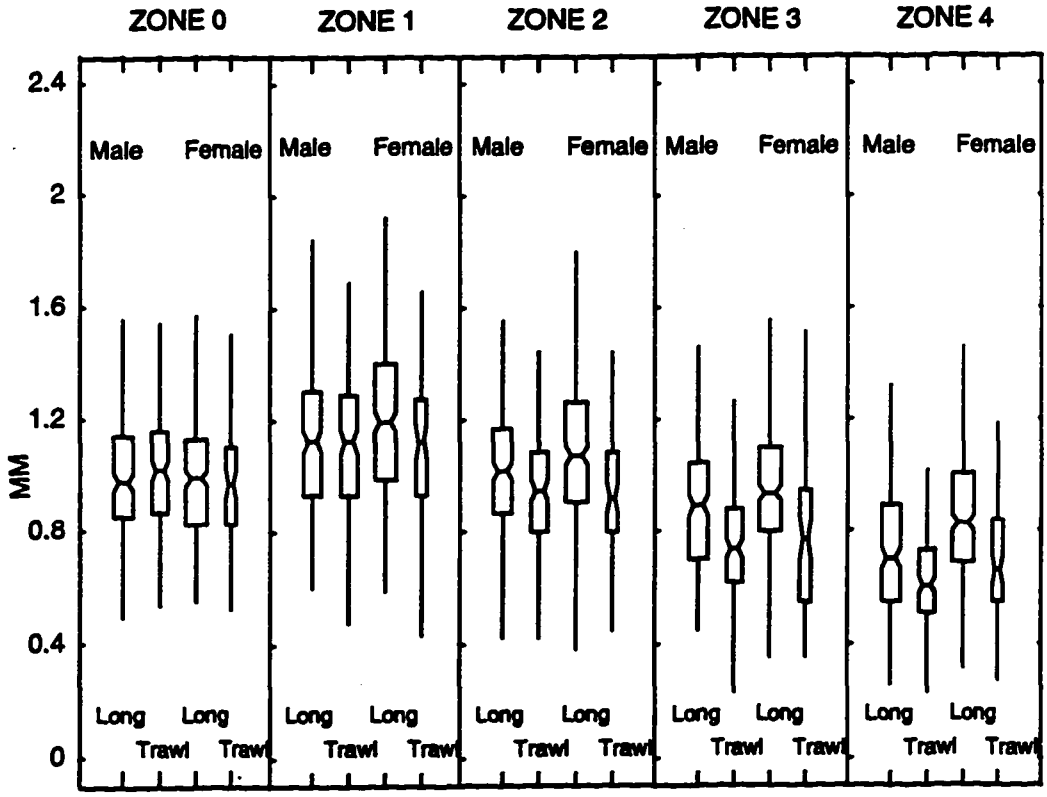

Figure 2-1. Boxplots comparing distribution of the otolith data partitioned by zone, gear type and sex, measured against the ordinate. The boxes contain the middle 50% of the data, the median is represented by the midline, the notches indicate 95% confidence intervals about the median and the lines extending from the boxes cover the range of the data.

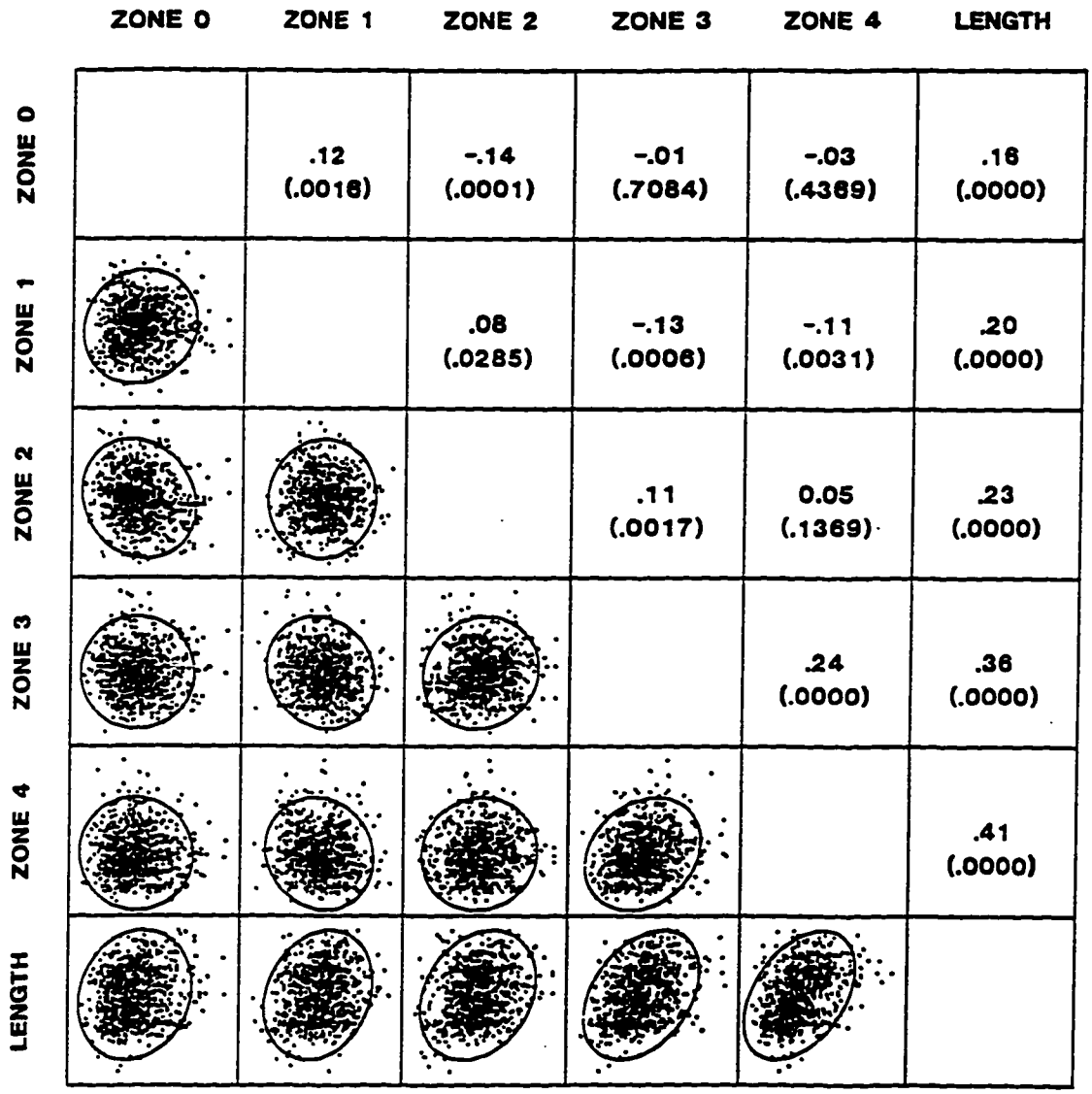

Figure 2-2. Pairwise scatter plots of the individual growth zones (n = 744). Each plot is formed from an intersection of the column and row variables. The ellipses drawn over the scatter plots represent 95% confidence intervals around the mean, with the major axes determined by the unbiased standard deviations of each variable and the orientation determined by the sample covariance. The Pearson correlation coefficients in the upper diagonal refer to the other intersection of variables in the column and rows. The Bonferroni-adjusted significance levels are given in parentheses.

decided significant bias would not be introduced if I maintained consistency with the other growth zone models and subdivided the zone 0 data only by gear type and not by sex.

Table 2-1 shows the final model selected for each category of zone and gear type. For both gear types in zones 3 and 4, length and age as covariates were significant. For zones 1 and 2 with longline-caught halibut, length and age were significant, while for trawl-caught halibut, only length was included. For zone 0 with both gear types, only length was significant. The residuals generated by the separate models were combined, and used in subsequent analysis as the adjusted data set.

Table 2-1. Models used to create data set adjusted for size selectivity and sexual differences in growth by removing variation that is linearly related to body size at capture.

MODEL
$Y_{(0,l)} = 0.161 + 0.186\,L$
$Y_{(0,t)} = -0.227 + 0.295\,L$
$Y_{(1,l)} = -0.058 + 0.346\,L - 0.040\,A$
$Y_{(1,t)} = -0.406 + 0.186\,L$
$Y_{(2,l)} = 0.431 + 0.199\,L - 0.034\,A$
$Y_{(2,t)} = -0.912 + 0.442\,L$
$Y_{(3,l)} = -0.376 + 0.363\,L - 0.042\,A$
$Y_{(3,t)} = -0.645 + 0.438\,L - 0.053\,A$
$Y_{(4,l)} = -1.073 + 0.529\,L - 0.062\,A$
$Y_{(4,t)} = -0.323 + 0.377\,L - 0.078\,A$

For each specimen i, the data Y(i,z,g) was subdivided into growth zone z, (z = 0,...,4) and gear type g, (g = longline(l), trawl(t)) and separate models fitted to each zone - gear combination. I used a stepwise procedure to identify if either log length (L) or age (A) at capture explained significant variation. The residuals from the separate models are combined to form the adjusted data set.

Correlation patterns

Positive correlations between individual growth zones may reveal intrinsic growth (Parma and Deriso 1990), while negative correlations may indicate compensatory growth (Ricker 1975). Figure 2-2 displays the pairwise scatter plots of the individual growth zones and fish length for the original data set. In general there is little indication of a strong relationship, either positive or negative, between zones from one age to the next except for a positive relationship that appears between ages 3 and 4. A table of the correlation coefficients with the Bonferroni adjustment for multiple significance tests (Seber 1984)

shows that some of the relationships, while weak, are still significant due to the large number of samples (Fig. 2-2). Interestingly, slight negative relationships when skipping zones are also significant.

Although individuals from both sexes and gear types during all years were combined in Figure 2-2, the same patterns held when the data were stratified into males, females, longline or trawl caught categories, or divided into pre-1972 and post-1972 year-classes, or included only those individuals eight years of age. In all cases there was the presence of some significant negative correlations when skipping zones and positive correlations between adjacent zones.

The correlation patterns of the adjusted data set confirmed that the positive correlation between otolith zones and fish length had been effectively removed from the raw data. In addition, the positive correlation between adjacent zones was also removed. However the negative correlations when skipping zones were not removed.

Temporal variation

Some of the variation contained in the otolith measurements was explained by temporal factors. By considering each zone separately and using year as a factor, between 8% and 19% of the total sums-of-squares was explained by year using the adjusted data, and between 11% and 18% using the original data. For example, zone 1 growth shows considerable overlap in the distributions by year, but there are some significant pairwise differences in the median growth as indicated by the boxplots (Fig. 2-3). Comparison of the mean growth zones over the year of growth shows that there are some similarities in trends among the first three ages, with a period of low growth in all ages centered in the early 1970's (Fig. 2-4). The time series of mean growth for the adjusted data set was very similar to the original data for age 0 ($r = .96$), and had the largest difference for age 4 ($r = .74$).

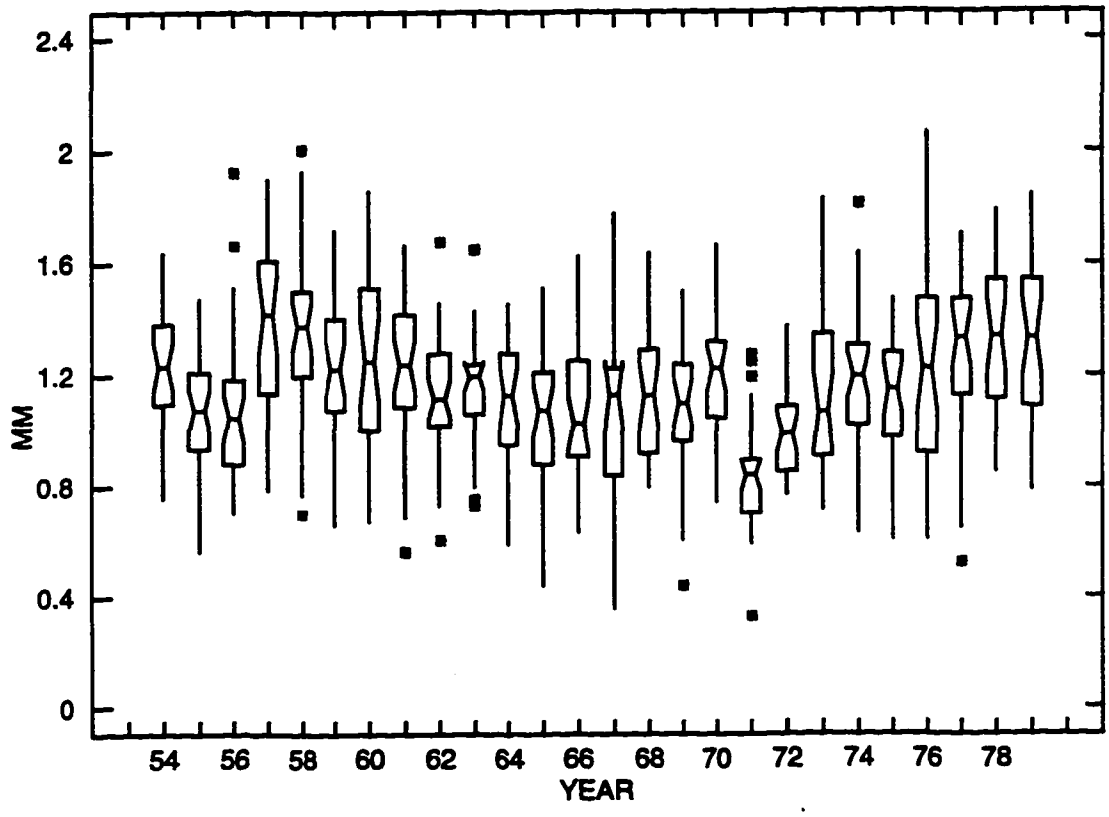

Figure 2-3. Boxplots comparing the distributions of zone 1 growth by year of growth.

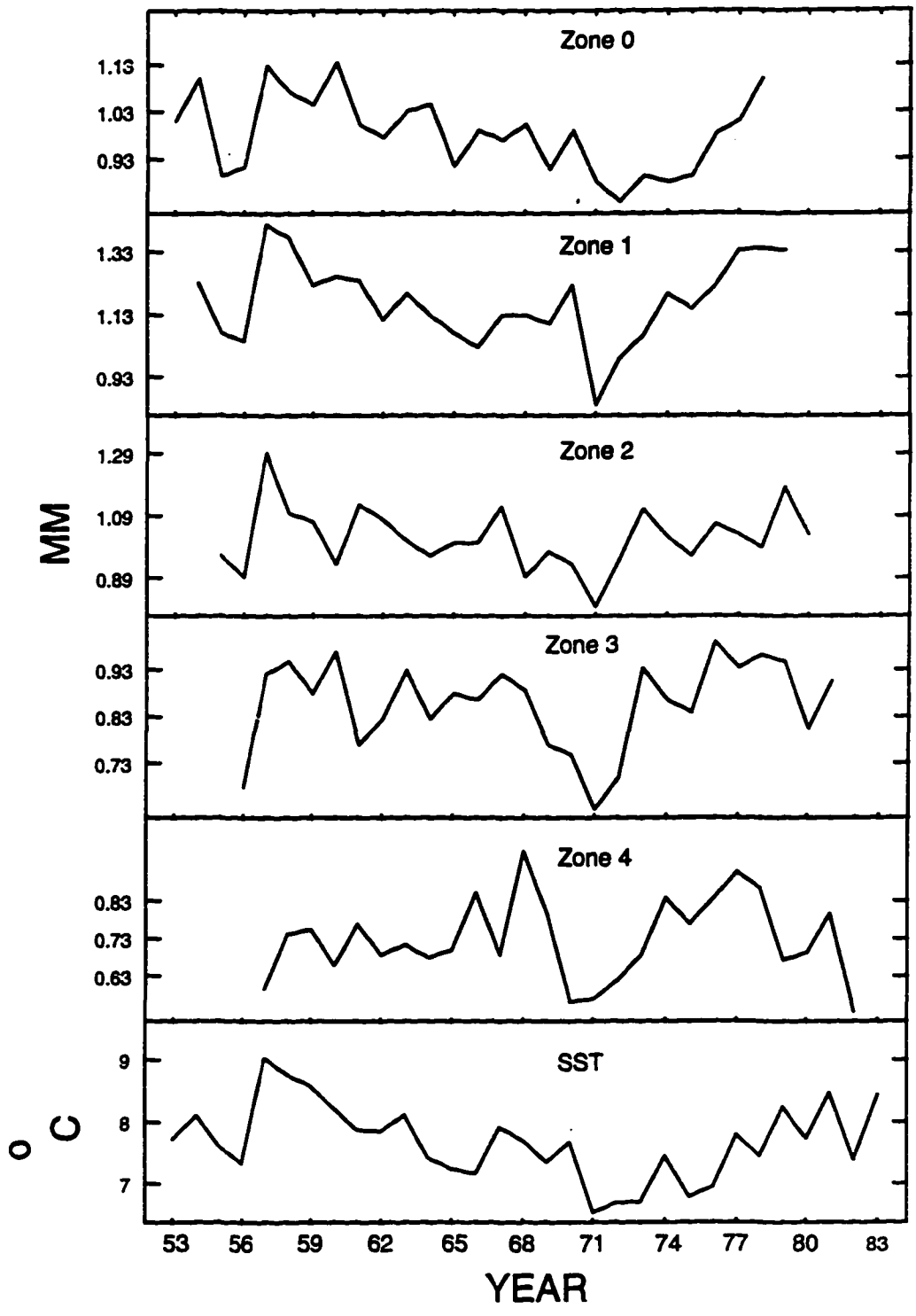

Figure 2-4. Time series of mean growth zones 0-4, by year in which growth occurred. The bottom plot shows the annual average SST from 55° N and 145° and 155° W.

Development of test model

The examination of year and year-class effects was based on the premise that time series of annual growth of two or more ages can be partitioned by either the year in which growth occurs or the year-class to which the fish belongs. Both the original and the adjusted data sets were used as inputs to the models. I denoted each zone measurement as $Y(z,k,j,i)$, where annual growth of fish i from year-class j at age z occurs in year k. The factors are constrained by the relationship $z = k - j$. Schematically, in Figure 2-5, the age of growth lies along the diagonals, and year and year-classes are the rows and columns. The classification design is incomplete: not all year-classes are present in all years. As a result, interactions of year and year-classes could not be estimated and I resorted to examining only main effects by measuring the marginal variation around the row and column means.

I measured year (yr) and year-class (yc) effects by their average sums of squares:

$$SS(yr) = (1 / K)\sum_{k}\left\{\overline{Y}_{[(.)k.]} - \overline{Y}_{(...)}\right\}^2$$

and

$$SS(yc) = (1 / J)\sum_{j}\left\{\overline{Y}_{[(.).j]} - \overline{Y}_{(...)}\right\}^2$$

where $\overline{Y}_{[(z)kj]}$ is the cell mean for measurements from zone $z = 1,...,Z$, year $k = 1,...,K$, and year-class $j = 1,...,J$. A period in a subscript denotes an average over that index. I used the ratio of the average sum of squares (i.e., SS(yr)/SS(yc)) to represent the relative influence of the two effects.

A randomization test was used for measuring the year and year-class effects (Edgington 1980). Randomization tests are procedures for determining statistical significance directly from the data through permutations, and not from statistical tables or through references to an outside population as in

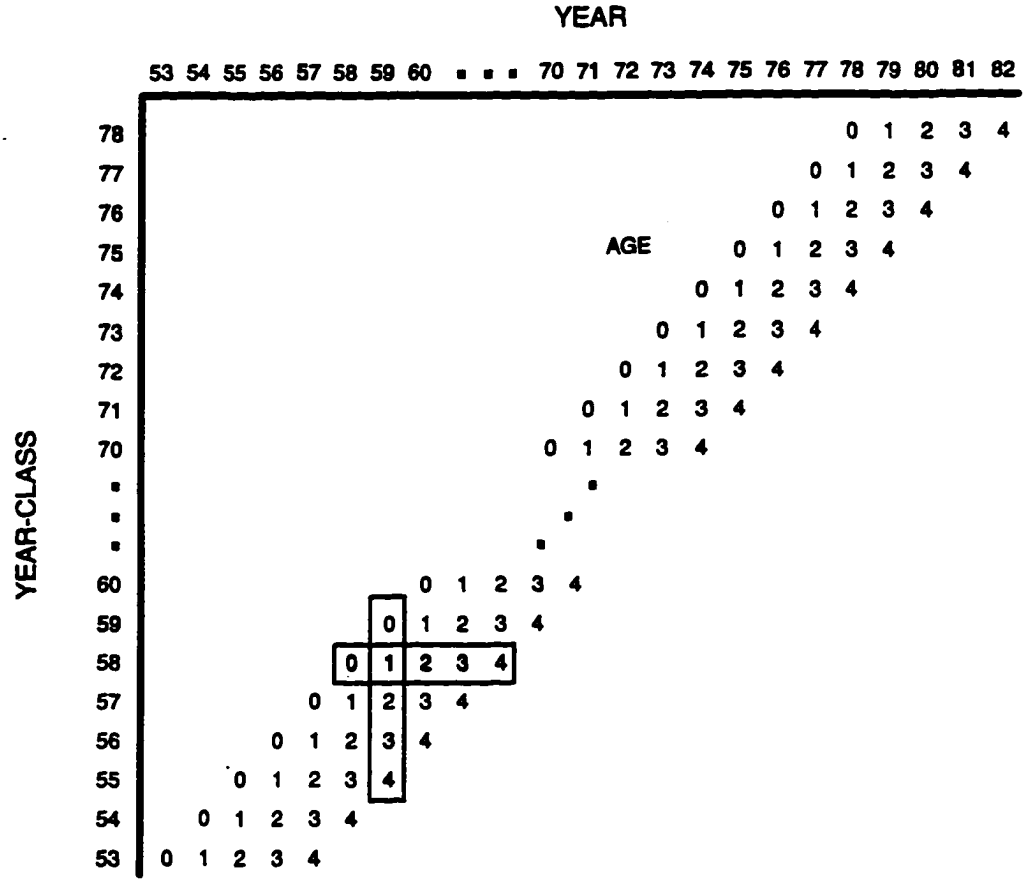

Figure 2-5. Representation of year and year-class effects. Annual growth, z, of each cohort from year-class, k, occurs during year, j, such that k = j + z. Each growth record from age 0 to age 4 occurs along the diagonals.

conventional statistics. The null hypothesis in randomization tests is that all permutations of the dependent variable relative to the explanatory variables are equally likely, or in other words each datum is independent of its assignment to a factor level (Noreen 1989). Randomization tests will produce the same result as the equivalent parametric test in instances where the assumptions of the parametric tests are met (Noreen 1989). They are often computationally advantageous and allow for customized significant testing in situations where there is no parametric equivalent (e.g., Prager and Hoening 1989).

Separate data sets were generated through permutations to determine the significance levels of the two factors independently. The permutations were conducted with respect to the factor tested while at the same time preserving the structure of the data to control for the other factor. Since I was not testing for a zone effect, no shuffling of measurements between the zone types was allowed and the sequence, 0-4, was maintained in the permutations. Since year and year-classes are cross-linked but the design is incomplete (Fig. 2-5), there was a need to be assured that when testing for one factor, the permutated data did not contain, by chance, any combination of zones which contributed to the other factor. In other words, the permutated data should be unbiased and reflect a random background that gives rise to the observed effect.

The method used to assure the separation of the factors can be illustrated with the test of the year effect. The algorithm proceeds by randomly shuffling whole rows (the year-classes in Figure 2-5) along the diagonals defined by the zones. This has the effect of destroying the original sequencing of data in the columns (the observed year effect), while assuring that the new columns contain no combination of zones which appeared in the rows. After such permutation, the SS(yc) from the rows remains unchanged while the SS(yr) from the columns takes on a new value.

For the year-class test I created new data sets by permutating the sequencing of the years. I stipulated that each effect was composed of complete ages and used the largest complete subset that fitted inside the data matrix.

For each test I generated 1000 random data sets and calculated the ratio of the average sums of squares for each data set. For the year test, the ratio SS(yr)/SS(yc) was used, and for the year-class test, the ratio SS(yc)/SS(yr) was used. If the position of the observed ratio was at the extreme right of the distribution of the permutated ratios, then the null hypothesis of no effect was rejected. The probability value p for the test was computed from the relative frequency of permutations to the right of the observed value (Edgington 1980).

Since the denominator in the ratio for each permutated data set remains unchanged, the p-values are equivalent to those generated by comparing the observed SS to the permutated SS. I use the ratio simply to scale the values to provide a quantity with which I can compare the year and year-class effects. For the observed data, a ratio equal to one means both effects are equal, while deviations from one show the relative influence of the effect that is tested. The distribution of ratios calculated from the permutated data will be centered at less than or equal to one.

To determine the standard error and bias of the observed test statistics I used a bootstrap method (Efron 1982). The method is appropriate under the assumption that the individuals whose otoliths were measured represent a random sample drawn from each year-class, irrespective of the time series of years or year-classes. Otolith zone measurements from each year-class were randomly sampled with replacement and the cell means recalculated. The otolith is used as the unit resampled because the zone measurements from each otolith are not independent of one another. The means and standard deviations of the generated statistics were calculated from 1000 replications. The standard deviation is the bootstrap estimate of standard error, and the difference between the bootstrap mean and the observed statistic is the estimate of bias.

Year versus year-class effects

The results from the randomization test of the year effects using all zones is shown in Figure 2-6a. The plot displays the strong influence that the year effect has in explaining variation. Based on the empirical

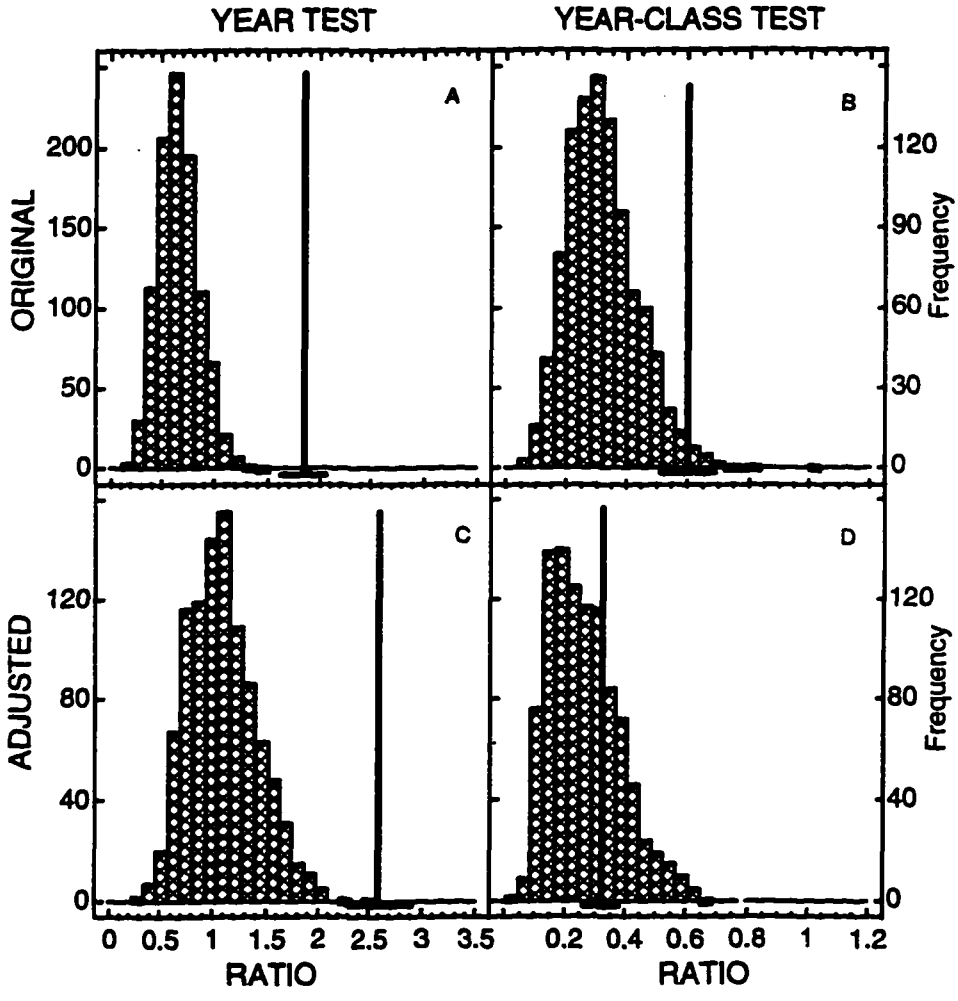

Figure 2-6. Results of the year and year-class tests for the original and adjusted data sets with all zones, 0-4 combined. The vertical bar represents the position of the observed ratio, the horizontal bar represents one bootstrapped estimate of standard error on either side of the observed statistic. The frequency distributions of the 1000 permutation runs are displayed in the histograms. Test of year effect (A) and the year-class effect (B) with the original data. Test of the year effect and the year-class effect (D) with the filtered data.

distribution of the permutations, the observed ratio of 1.85 has a probability of occurrence of 0.001 (Table 2-2). The test of the year-class effect, with an observed ratio of 0.60 (reflecting its weaker influence), may also be significant (Table 2-2). However within a range of two bootstrap standard deviations this probability could extend from 0.062 to 0.005 (Fig. 2-6b). The bias was smaller in the year test than the year-class test, though in both cases bias did not appear large enough to warrant concern over the test results (Table 2-2).

Table 2-2. Summary of year and year-class test results using the original data, the data adjusted for size at capture, and the residuals from a linear regression with SST. The zones refer to the ages that are tested, with the observed ratio measuring the relative influence of the effects. The estimate of standard error and bias of the observed ratio is determined by a bootstrap approach. The bias is given as a percent of the observed ratio and the probability value is determined from the randomization test.

Data and Test		Zones	Observed	S.E.	Bias	% Bias	P
Original:	**Year**	0-4	1.85	0.217	-0.06	3.4	0.001
		0-1	1.39	0.130	-0.04	3.0	0.001
		1-2	1.35	0.128	-0.04	2.8	0.001
		2-3	1.01	0.105	-0.00	0.1	0.002
		3-4	0.85	0.085	0.02	1.8	0.068
	Year-class	0-4	0.60	0.091	0.03	5.7	0.019
		0-1	0.66	0.070	0.03	4.8	0.280
		1-2	0.77	0.080	0.03	3.5	0.114
		2-3	0.92	0.109	0.02	1.7	0.036
		3-4	1.08	0.100	0.00	0.9	0.001
Adjusted:	**Year**	0-4	2.61	0.375	-0.08	3.0	0.001
		0-1	1.32	0.116	-0.03	2.6	0.001
		1-2	1.36	0.130	-0.03	2.3	0.001
		2-3	1.27	0.181	-0.02	1.8	0.001
		3-4	1.31	0.193	-0.08	5.8	0.060
	Year-class	0-4	0.34	0.064	0.03	8.5	0.362
		0-1	0.65	0.068	0.03	5.2	0.284
		1-2	0.74	0.079	0.03	3.6	0.100
		2-3	0.78	0.121	0.04	4.9	0.179
		3-4	0.76	0.125	0.06	8.5	0.133
SST residual:	**Year**	0-1	1.51	-	-	-	0.011
		1-2	1.13	-	-	-	0.006
	Year-class	0-1	0.58	-	-	-	0.792
		1-2	0.93	-	-	-	0.026

The observed year-class effect could be real or an artifact resulting from selectivity effects of the sampling gear. To address this question, I used the data set adjusted for the selectivity factors to test the year and year-class effects using all zones (Fig. 2-6c-d). The probability level for the observed year statistic was the same as the original data: 0.001 (Table 2-2). The year-class test however, showed no significant effect (Fig. 2-6d), suggesting that the year-class effect observed in the original data could be related to the size of the fish at capture and perhaps related to size selectivity of the gear types. Bias, as a percentage of the observed value, was somewhat larger with the adjusted data compared to the other tests.

I extracted adjacent pairs of ages from the data to identify at what ages the temporal effects are significant. Figure 2-7 displays the results of the year and year-class tests using the original data. The probability levels (Table 2-2) are based on the exact values obtained from the permutations. They are not adjusted for the multiple comparisons conducted with the paired zones, though a conservative approach, using Fisher's modified least significant difference method, would be to multiply each p-value by 4 based on the number of paired tests (Edgington 1980). Even without an adjustment, a clear trend with age is evident. The year effect explains all the variation for the youngest ages while no year-class effect is evident (Table 2-2). For ages 2-3, the year effect is still highly significant, however a year-class effect starts to appear important with a probability of 0.036. At the oldest pair of ages, the year effect might be present but it only has a probability of 0.068, while the year-class effect appears to be highly significant at 0.001.

The results of year effect tests for the paired zones in the adjusted data set are similar to those in the original data (Table 2-2). For the year-class test there is no longer a significant effect at the oldest ages, although the probability levels remain in the upper quartile for zones 0 through 4.

Environmental effects

Temperature is an obvious candidate to explain the source of the year effect because its influence on metabolism is well known (Weatherley and Gill 1987). A positive relationship can be expected between

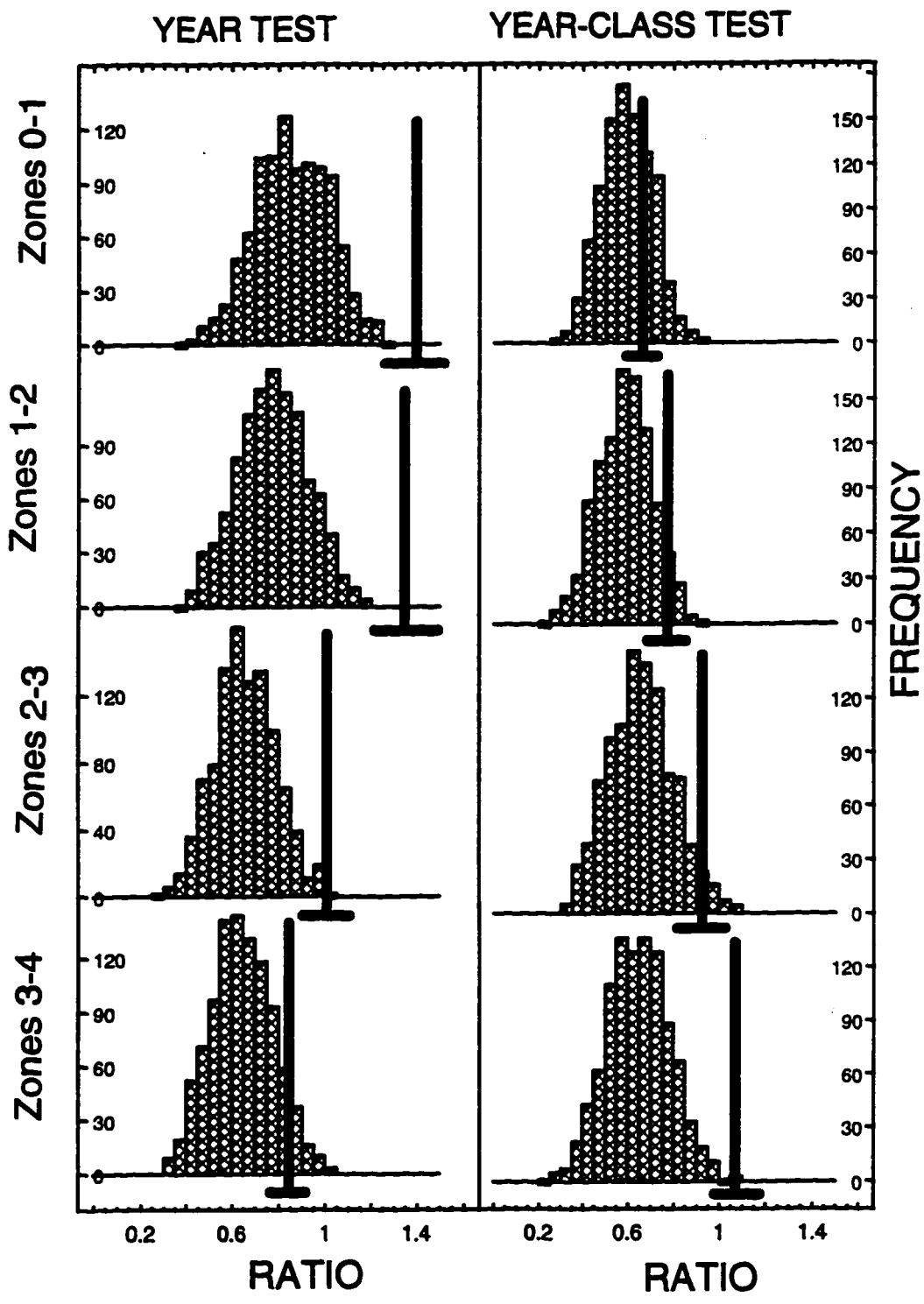

Figure 2-7. Test of year effect and year-class effects for paired zones using the original data.

temperature and growth within physiologically defined limits unless there are constraints on food supply. By using the mean zone measurements from the original data for each year, relatively high positive correlations can be noted for the first two zones with SST (Fig. 2-8). The correlation decreases with the older zones; nonetheless the cold years of 1956 and 1971 still coincide with years of slow growth for zones 4 and 5 (Fig. 2-4). The adjusted data set appeared to be similarly related to SST and was not examined further.

The linear regression lines in Figure 2-8 provide a representation of the strength of the year effect and the importance of SST. The positive relationship between the variables is apparent, particularly for SST and the younger zones. Zone 4 growth does not appear to be strongly linearly related to the other variables except for a somewhat high correlation with zone 3. The regression lines are included in Figure 2-8 to indicate the positive trend in the relationships; they are not intended to imply that linear models are significant or necessarily appropriate for all pairs of data.

Using diagnostic techniques I determined that linear regression models were appropriate when fitted between SST and zones 0 through 2 (Table 2-3). The SST time series is significantly autocorrelated (lag 1: r = 0.47), along with the zone 1 means (lag 1: r = 0.47); none of the other zones were autocorrelated. The residuals from the regressions were well behaved except for zone 1, which had slight, though significant, autocorrelation which suggests that one year old halibut since 1971 have grown at a faster rate than predicted by temperature.

Table 2-3. Regression analysis of sea surface temperatures (SST) and average otolith growth $\overline{Y}_Z$ *for zones 0-2, with the slope coefficient,* b_1*, its associated probability, and the model* R^2*.*

MODEL	slope	p	R^2
$Y_0 = b_0 + b_1SST$	0.100	0.00001	47
$Y_1 = b_0 + b_1SST$	0.153	0.00001	50
$Y_2 = b_0 + b_1SST$	0.094	0.00170	32

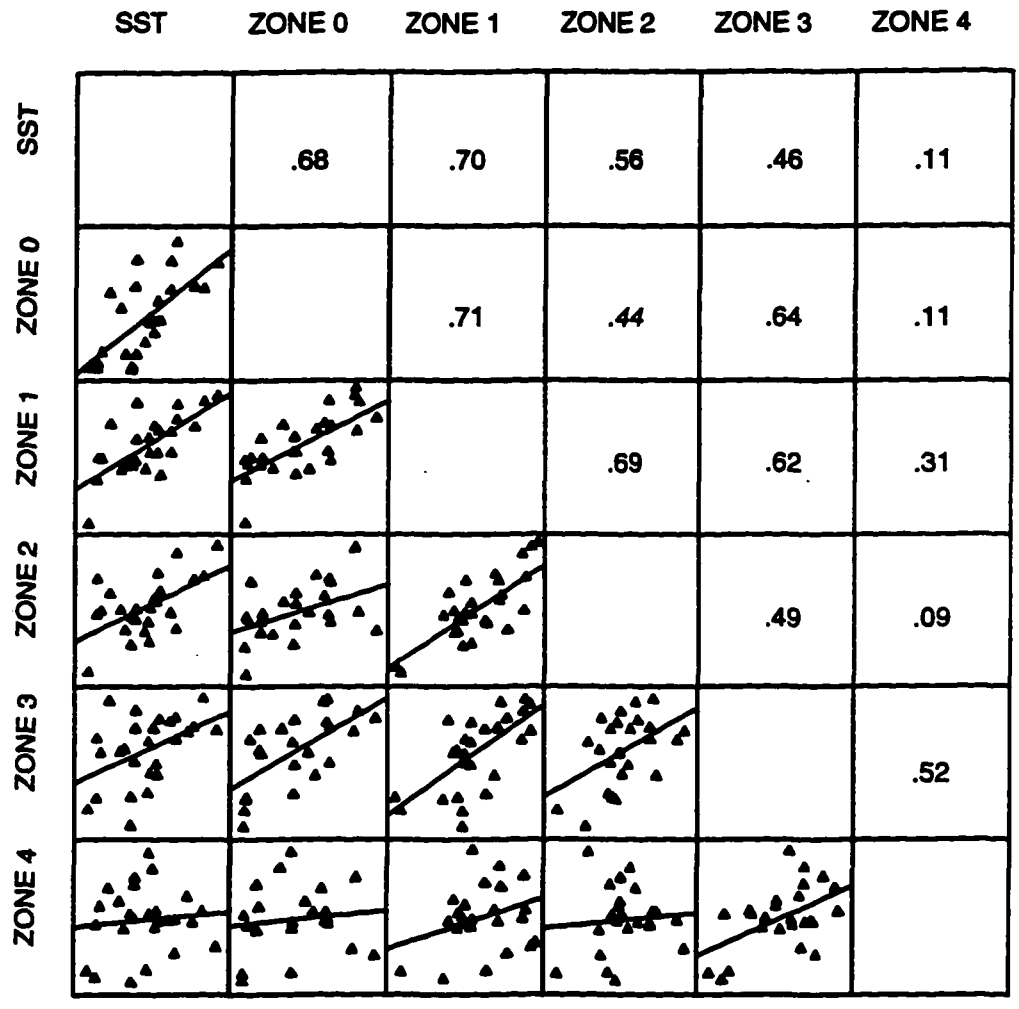

Figure 2-8. Pairwise scatter plots and correlation coefficients of average otolith growth for each zone and SST ordered according to year the growth takes place. Each plot contains the fitted linear regression for each pair of variables. The lines are used for display purposes, they are not intended to imply linear models are significant or necessarily appropriate for all pairs of data.

To determine if these linear relationships with temperature accounted for all the variation attributed to the year effects, I examined the residuals resulting from the models using the randomization procedure for testing year and year-class effects. Since the mean of the growth zones was used, there is only one observation per cell with this design, so bootstrapping the observed statistic was not conducted. The results, using the residuals combined from the paired zones 0-1 and 1-2, are presented in Table 2-2. The observed ratios in the year tests show that year effects remain stronger than year-class effects. The p-values indicate that the effect is significant in both cases, implying that the year effect in the original data was not completely accounted for by the linear relationship with SST. The year-class test on the residuals for zones 1 and 2 suggest that a year-class effect, while smaller than the year effect, is present. This result might be attributable to the autocorrelation of the zone 1 residuals, though it did not appear to result in a significant year-class test for the paired zones 0 and 1.

Year-class strength

The coupling between year-class strength and growth data could take two forms. If year-class strength is determined by predation acting throughout the juvenile period (Sissenwine 1984), and growth is considered a mechanism whereby fish escape predation (Anderson 1988), then a positive relationship between growth and recruitment might be observed. Alternatively, if year-class strength is determined early, such as during the larval stage, as hypothesized by Parker (1989), and growth is regulated by intraspecific competition, then a negative relationship with growth and recruitment might be observed.

Figure 2-9 displays the pairwise scatter plots between the number of recruits and growth zone data with fitted regression lines for the original data set. The data are ordered according to year-class and provide a representation of that effect. It can be seen under the first column that only positive relationships are apparent between recruits and the growth data. However with the possible exception of zones 1 and 2, the relationships are very weak. A linear relationship between zones 3 and 4 in the lower right corner is in accordance with the significant year-class effect I noted with the randomization test. Neither zone appears to be related to year-class strength which may rule out a density-dependent explanation for the

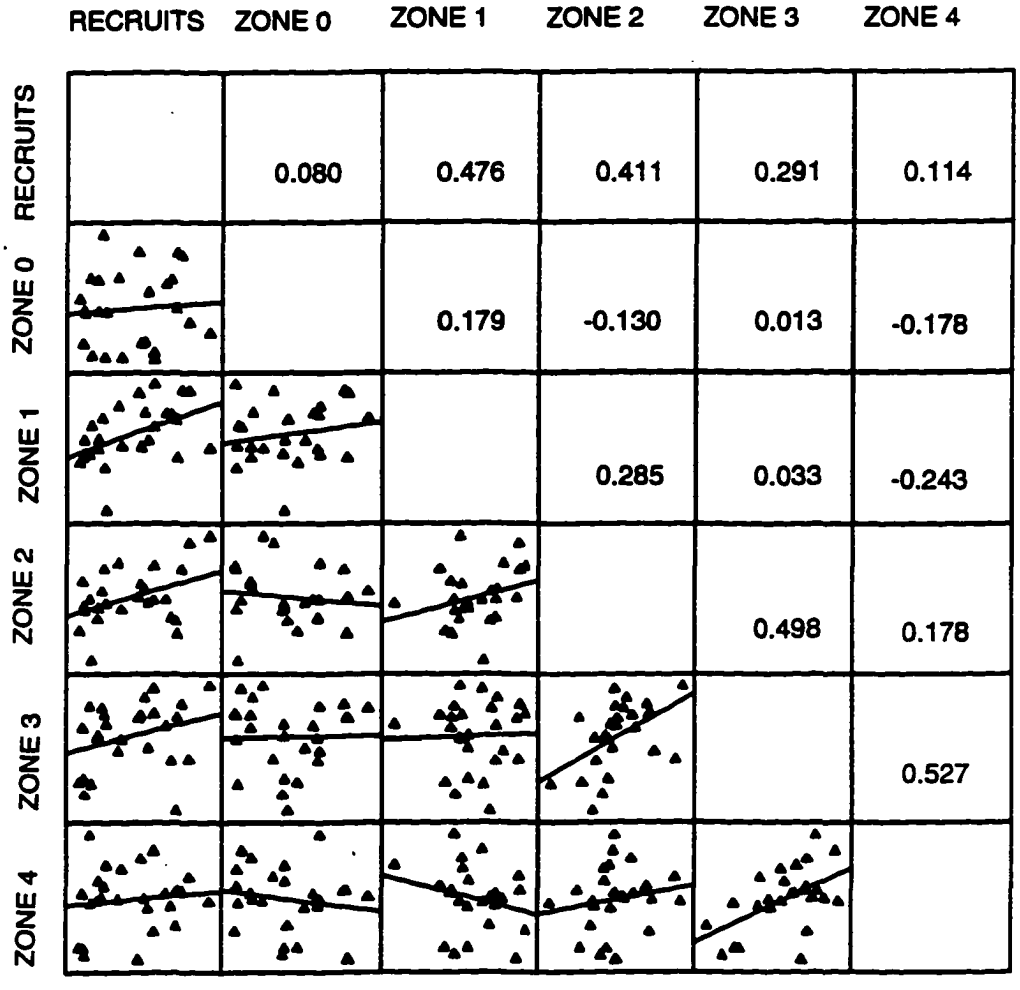

Figure 2-9. Pairwise scatter plots and correlation coefficients of average otolith growth for each zone and recruitment, measured by the number of 8-year-old halibut entering the fishery. The linear regression lines defined by each pair of variables is displayed. The data are ordered by year-class.

year-class effect. The slight positive correlation between adjacent younger zones could be a result of the autocorrelation in the SST noted earlier. Interestingly, the positive relationship between recruitment and zones 1 and 2 may be an indication that growth is a cause and not a consequence of year-class strength. Zone 0 growth, which includes the larval period, however, showed little relationship with recruitment, indicating that factors other than growth may be more important for survival at that age.

Caution should be applied in fitting a model of year-class strength with growth because the time series of year-class strength is strongly autocorrelated (lag 1, r = .74) and is characterized by low frequency trends. A longer time series would be necessary to confirm whether the observed relationships are significant. A model of growth with year-class size could be mis-specified if the relationship between the two variables is not clarified, particularly if year-class size is measured after the period of growth.

The adjusted growth data set showed no improved relationship with year-class strength than was observed with the raw data and was not considered further. In addition, analysis of residuals indicated there was no hidden relationship of growth and year-class strength after accounting for the influence of SST.

Discussion

Year-class effects

Different conclusions can be drawn about the nature of growth depending upon whether year-class or year effects are found important. In this study the year effect was highly significant for the youngest ages while the year-class effect was significant for the oldest pair of ages. Since the unit of growth, the otolith zones, were obtained from the same individuals for each year-class one might expect to see a bias toward year-class effects because measurements for that factor are not entirely independent. The year-class effect was not significant in the data set adjusted for size selectivity of the gear, suggesting that it is a result of sampling bias. The adjusted data set, however, was created by removing variation linearly related to size at capture. Since an individual's actual growth occurs along the year-class, real

expressions of growth may have removed in addition to sampling bias. Only by comparing the growth record of individuals of different ages from the same cohort would it be possible to conclusively separate out the influence of gear selectivity on growth patterns.

If we can assume the year-class effect is real, the next step is to identify the source of the effect. A year-class effect could be caused by intrinsic factors, reflecting an individual's predisposition toward slower or faster growth, or it could be due to extrinsic factors, such as density-dependent growth. To detect intrinsic growth, I examined the correlation patterns of the growth zones from all individuals combined, irrespective of temporal variation (Fig. 2-2). Since the positive correlations between adjacent zones seemed small for the youngest ages, I concluded that intrinsic growth was not a dominant factor. However, I did not rule out the presence of intrinsic growth in the oldest pair of ages. A better way to detect and perhaps measure intrinsic growth would be to consider correlation patterns of individuals within a cohort. The sample size within each cohort (n = 28.6), however, was too small to justify the calculation of those correlations.

Density-dependent growth could generate a year-class effect, provided that growth is constrained by competition within a cohort which operates across more than one age. Density-dependent growth could also appear strictly as a year effect if local densities of the same species or a competitor species change annually, acting much like an environmental factor. One would expect, however, that if any aspect of that density change is related to cohort size, and not just a local phenomenon, then there should be a detectable year-class effect as well. For older ages, where length frequencies overlap with other cohorts, the test of the year-class effect may not be as strong a test as it would be for younger ages. In that instance, both year-class and year effects might appear significant.

Year-class effects in this study appeared to be present in older juveniles, but the relationship of growth to year-class strength was not negative as would be expected under density-dependence. However, a previous study of halibut (Deriso 1985) showed a negative relationship to year-class strength when using weight at age 8, which was determined indirectly from a predictive relationship with otolith size and

weight (Quinn et al. 1985). This might indicate that density-dependent growth begins to occur somewhere between ages 5 and 8. However, the predictive relationship used to estimate size-at-age from otoliths is currently under evaluation because it may not account for interannual variation or possible long term changes in sex ratios of the commercial catch (IPHC 1990). Until this relationship is clarified, discrepancies between this study and previous studies on the relationship of growth to year-class strength and the role of density-dependence must remain unresolved.

It would be interesting to test the relative influence of year and year-class effects on other species for which density-dependence has been recorded. However, separating out the temporal effects using available studies is difficult, because density-dependence may be restricted to a single age (e.g., Peterman and Bradford 1987), or because size-at-age data may be used as the dependent variable instead of growth increments (e.g., Ross and Almeida 1986). A large body of theoretical work (e.g., Ware 1980) is built upon density-dependent growth, yet the empirical evidence for its support does not always seem clear, and frequently alternative explanations, such as enhanced survival of small fish (Bromley 1989), may mimic density-dependent growth. While density-dependent growth is undoubtedly important in certain cases, particularly at high population levels, annual variation induced by environmental factors may be more important in most situations.

A limitation to consider in using otoliths as a proxy for growth, particularly in relying on correlation patterns, is that otoliths may have an entirely different growth dynamic than body growth. In addition, the pattern of otolith growth observed might be dependent upon the particular axis that was measured on the otolith. The measurements taken followed a straight line along what I felt was the axis of greatest otolith growth. If, in some otoliths, this axis of growth was actually curvi-linear and deviated from the straight line prior to the fifth annulus, bias could have been inadvertently introduced in the estimate of growth for the older ages. This could account for some of the negative correlation patterns observed, suggesting that caution in interpreting correlation patterns is warranted in situations where linearity is implicitly assumed.

Year effects

The presence of year effects is easier to interpret than year-class effects and it appears to be robust to sampling bias, as suggested by the significant year effects observed in the adjusted data set. The strong indication that variation in annual otolith growth for juvenile halibut is similar for different ages in the same year can best be explained by a broad scale factor which works across several ages in the same year. Sea surface temperature explained much of the annual variation in otolith growth for the youngest ages. Residual variation unexplained by SST still contained a year effect, suggesting that the actual environment the fish encounter, such as the true bottom temperature, remains unmeasured. The strength of the year effect decreased with the older juveniles who might be subject to different ambient temperatures than the younger halibut which are still restricted to nursery areas. The older juveniles may also be undergoing extensive migrations which could affect growth rates (McCaughran 1987).

I suspect that year effects might be a dominant factor in other growth records. Variation in the growth of English sole (*Parophrys vetulus*) (Kruez et al. 1982) that is explained by density dependence is small in relationship to the environmental factor (Peterman and Bradford 1989). Growth increments, either obtained from hard parts as in this study or by differencing size-at-age data, can be used to test the influence of year and year-class effects. Finding the mechanism to explain the effect may be the most difficult part, reflecting perhaps the inadequacy of environmental time series, or bias due to the collection of the data.

In this study I was fortunate to find a clear explanation for the year effects. Temperatures in the Gulf of Alaska are similar over large areas (Royer 1986). In addition, halibut life history involves broad temporal and spatial scales as evidenced by a long period of larval drift (Parker 1989), an extensive juvenile migration (Skud 1977), and large size and long life for the adults (Hoag et al. 1979). This strategy undoubtedly contributes stability to population levels; Pacific halibut have relatively low annual changes in year-class strength. Long-term cyclic changes in abundance and biomass also suggest the presence of density-dependent regulation (Deriso 1985) with some additional, residual variation due to

fluctuations in larval transport (Parker 1989). Results from this study invite further questions. For instance, if changes in juvenile growth reflect low frequency temperature fluctuations that have been observed in other records (Roden 1989), does this provide a mechanism that helps drive low frequency changes in abundance? The route by which compensatory mechanisms regulate halibut population levels still needs to be delineated and separated from possible confounding effects due to the environment. Extending the otolith growth record back in time, by using the existing otolith collection which goes back to the 1920's, may help resolve these issues. If annual otolith growth is determined by environmental conditions and not density-dependence, as suggested here, a long time series of otolith growth may also prove to be a unique and perhaps useful measure of environmental changes that have occurred in the Gulf of Alaska.

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Chapter Three | Increment width and relative growth rates of Pacific halibut otoliths as a means to investigate larval growth histories.

Abstract

Presumed daily increments in otoliths of larval and juvenile Pacific halibut (*Hippoglossus stenolepis*) were examined to determine if increment widths and relative otolith growth rates (in relationship to otolith size) could be used as indicators of somatic growth rates and serve as a means of identifying halibut from different locations. Otolith growth is largely symmetrical along the sagittal plane during larval development and a concentric check ring is consistently found in the same position which can serve as a reference point. This provides a means for measuring increment widths in relationship to otolith size, and assuming daily increment formation, a means for comparing growth rates and changes in growth rates in otoliths of different individuals. The utility of this approach was confirmed by comparing wild-caught halibut larvae from two areas that exhibited differences in body size and developmental rates at age based on increment count. Comparisons of individual growth and data pooled by area of capture indicate that differences in otolith growth reflect somatic growth, though individual growth trajectories using Lowess smoothing exhibit large within-group variation. Retrospective examinations of otolith growth from juvenile halibut collected from different nursery areas in the Gulf of Alaska and the Bering Sea show a similarity in early larval growth among the Gulf of Alaska stocks with more divergence during later larval growth. The Bering Sea juveniles had much slower otolith growth throughout larval period, indicating some utility for stock separation. The precise relationship between otolith growth and somatic growth remains to be determined. Nonetheless the data on increment widths as a function of otolith size are relatively straightforward to collect, models of growth can be constructed, and relative growth trajectories may identify processes that are important to larval survival and recruitment success.

Introduction

The microstructure of a fish otolith contains information in the form of crystalline deposition patterns. Since Pannella (1971) first showed that otolith increments, observed with transmitted light as alternating opaque and transparent bands, appear to be laid down on a daily basis, a large body of research has focused on the association of increment patterns of otoliths with the growth and development of larval and juvenile fish (reviews by Campana and Neilson 1985, Jones 1986, Stevenson and Campana 1992).

One of the most intriguing uses of otolith information is in retrospective studies that may provide insight on processes that determine year-class size during the larval stages and early juvenile periods. Campana (1984) first suggested that adult otoliths of commercially valuable species could be examined for a record of early growth and that such an examination may reveal correlations with indices of year-class strength. Campana and Neilson (1985) expanded on this idea when discussing future applications of otolith microstructure research. They emphasized its potential use as a predictor of year-class success by examining the otoliths of pre-recruit fish. They also indicated such a study may reveal at what age year-class strength is determined. Despite these suggestions, retrospective studies using older otoliths, with some exceptions (e.g. Rijnsdorp and Leeuwen 1992, Ralston 1995), have generally not been conducted. Difficulties include a recognition that with complex population structure, recruitment is generally measured on a metapopulation level, while the processes that contribute to recruitment operate on a subpopulation level (Bailey 1994). Hence identifying recruitment mechanisms can be difficult without accounting for spatial heterogeneity of the population being studied. In addition backcalculation methods to estimate body size from otolith size requires assumptions on the proportionality of otolith growth and fish growth that a number of studies have questioned (Reznick et al. 1989, Secor and Dean 1989, Hare and Cowen 1995). Another limitation is that for many species a sufficiently extensive time series of otoliths is not available to associate with population data.

Pacific halibut (*Hippoglossus stenolepis*) is an excellent candidate to address the suggestion of Campana and Neilson (1985). It has a long history of fishery management, and catch and fishing effort data have

been collected since the 1930's. Estimates of stock size and year-class strength (determined at the age of recruitment to the fishery) are available through age-structured population models (Deriso et al. 1985), and the population spatial structure is thought to be well-mixed at young ages and across the corresponding size spectrum. Parker (1988) speculated that recruitment success in halibut is related to oceanographic processes which may determine the duration of the larval stages though transport to the nursery areas and may also influence food availability. Because the International Pacific Halibut Commission maintains an extensive collection of adult halibut otoliths extending back to year-classes in the early 1920's, it may be possible to investigate the relative importance of these mechanisms through the record contained in the otoliths.

Otolith growth records of halibut have been examined by Southward (1962), who utilized a backcalculation relationship to examine a time series of annual growth changes and more recently by Hagen and Quinn (1991) [chapter 2] who used annual increment patterns, independent of any backcalculation relationship, to determine temporal factors that influence juvenile otolith growth and by inference body growth. In addition, Hagen (1986) examined microstructure patterns of wild-caught halibut larvae and their association with body size and ontogenetic features.

To utilize increment patterns of otoliths for the investigation of past growth, the primary approach has been to use backcalculation methods. Backcalculation, in its various forms, has a long history in fisheries. The estimates of growth are constructed by collecting measurements of otolith size and fish size, determining an appropriate model that fits that relationship, and then using the model to estimate an individual's previous body size from the radius or diameter that corresponds to the increment location within the otolith. The relationships that are constructed are invariably monotonic and the estimated body sizes are essentially the result of a common transformation which may be scaled to the individual's initial otolith-size to body-size ratio. The difficulties in using backcalculation approaches to examine growth histories include choosing an appropriate model that describes the joint growth of the otolith and the fish (Gutreuter 1987, Campana 1990, Francis 1990). In addition, there is evidence that otolith

growth is not always proportional to somatic growth and can change with the growth rates of individual fish (Reznick et al. 1989, Secor and Dean 1989, Campana and Jones 1992). This latter observation has been suggested as a reason why a predictive relationship of halibut body size from otolith size may vary from year to year (Clark 1992).

Backcalculated relationships from daily increment patterns are particularly problematic when examining young fish (Brown and Bailey 1992, Campana and Jones 1992). Over a short time period otolith growth appears to be influenced more readily by temperature than does body growth (Bradford and Geen 1992, Mosegaard et al. 1988), and otolith growth is known to continue even during starvation (Marshall and Parker 1982). In addition the relationship between otolith and somatic growth can vary with ontogenetic development, indicating the presence of a nonlinear fish-size to otolith-size relationship (Hare and Cowen 1995).

Difficulties also arise in trying to reconstruct larval growth histories from the otoliths of adult fish or older juveniles. In these retrospective studies the temporal information on age or calendar date associated with an increment is frequently difficult or impossible to recover. In addition the initial otolith-size to fish-size relationship at capture provides no real useful information, because the assumptions of proportional or linear growth are unlikely to hold across ontogenetic stages. If the goal is to draw comparisons between different individuals or groups on their relative growth rates, then a backcalculated relationship may be unnecessary. Hare and Cowen (1995) touched upon this in their discussion on the difficulties in reconstructing growth rates in larval fish. In particular they showed that faster otolith growth at a given age will correspond to faster somatic growth when comparing the otolith record of individuals and this can preclude the need to construct backcalculated relationships.

To investigate halibut growth patterns, the approach used here is to consider otolith growth by itself, apart from a presumed relationship with body size, and to use empirical methods to determine if variation in otolith growth can be used as an indication of variation in body size. The width of an increment, if formed daily, can be considered a measure of the growth rate of the otolith at that point.

Along a particular axis, increment widths serve as a longitudinal record of an individual's growth. This longitudinal growth is usually examined as a function of age or calendar date (e.g. Ralston 1995); which was what Hare and Cowen (1995) suggest for comparing growth between individuals. Accurate age estimates however are frequently difficult to determine from older otoliths, or from species in which there is little supporting data on the timing of increment initiation. An alternative which presents several advantages is to examine increment widths as a function of otolith size. Size is much easier to measure than age, and in many animals, metabolic rate is size dependent. In addition, ontogenetic changes, such as the onset of metamorphosis of flatfish, is more related to size than age (Policansky 1983). The specific or relative growth rate of otoliths, which is the amount of new growth with respect to otolith size, can be easily constructed from the data. Kaufmann (1981) argues in favor of this size-based approach for growth analysis and demonstrates that the relationship between relative growth rate and size can be used to fit and analyze a variety of growth models. An empirical determination of whether the otolith growth record corresponds to larger or smaller body size at age can be the basis for applying increment width analysis as a proxy for somatic growth.

The purpose of this study is to develop a consistent methodology for collecting increment width data from halibut otoliths and to evaluate the use of otolith growth rates as a tool for comparing past growth histories. Daily increment formation has not yet been validated for halibut. For that reason otoliths collected from larval halibut of known ages as part of a rearing experiment were examined to determine their temporal periodicity. To determine a suitable plane of measurement, otoliths of wild caught halibut were examined *in situ* and with a scanning electron microscope. Replicate measurements were taken within a subsample of the otoliths to determine the constancy of the measurement patterns. Wild-caught halibut larvae from a previous study (Hagen 1986), that exhibited differences by area in size and development rates as inferred from increment counts, were examined for differences in relative otolith growth rates. Comparisons of individual otolith growth and otolith growth pooled by area were used to evaluate if differences in otoliths correspond to differences in somatic growth. Otoliths of juvenile halibut from widely separate nursery areas in the Gulf of Alaska and the Bering Sea were examined to

determine if the same types of measurements could be collected from older fish. The data from these nursery area samples were pooled and used to investigate spatial variability in growth rate patterns.

Methods

Samples and otolith preparation

In this paper, the otoliths I refer to are the sagittae, the largest of the three otolith pairs. Terminology of otolith microstructure features follows the glossary contained in Secor et al. (1995).

I obtained Pacific halibut larvae of known age from a rearing experiment in Marrowstone WA (Liu 1991). In that experiment the larvae did not survive longer than 20 days post-hatch. Five specimens from 5, 10, and 15 days post hatch were examined for increment patterns. I obtained wild-caught larval halibut from field collections by the International Pacific Halibut Commission during 1985 in the Gulf of Alaska (St-Pierre 1989). From those collections I had previously examined larvae from two areas, Shelikof Strait and Unimak Pass (Figure 3-1), and compared body size (length and depth) and developmental characteristics with otolith diameter and increment count (Hagen 1986). Based on the assumption of daily increment formation, a comparison of the two areas indicated faster growth and development rates for the Shelikof specimens than for the Unimak specimens. Differences were also found in how body length and body depth varied with increment count and developmental stage. In this study, I reexamined a subsample of those otoliths (n = 47) for changes in increment width patterns with size and age. To provide a single index of body size, I approximated body area by taking 1/2 of the product of length and depth (the formula for a rectangle as measured on the diagonals). Body area proved to have a higher correlation with increment count, otolith diameter, and developmental stage than did length (Table 3-1). The otoliths were sufficiently small to allow viewing without additional preparation.

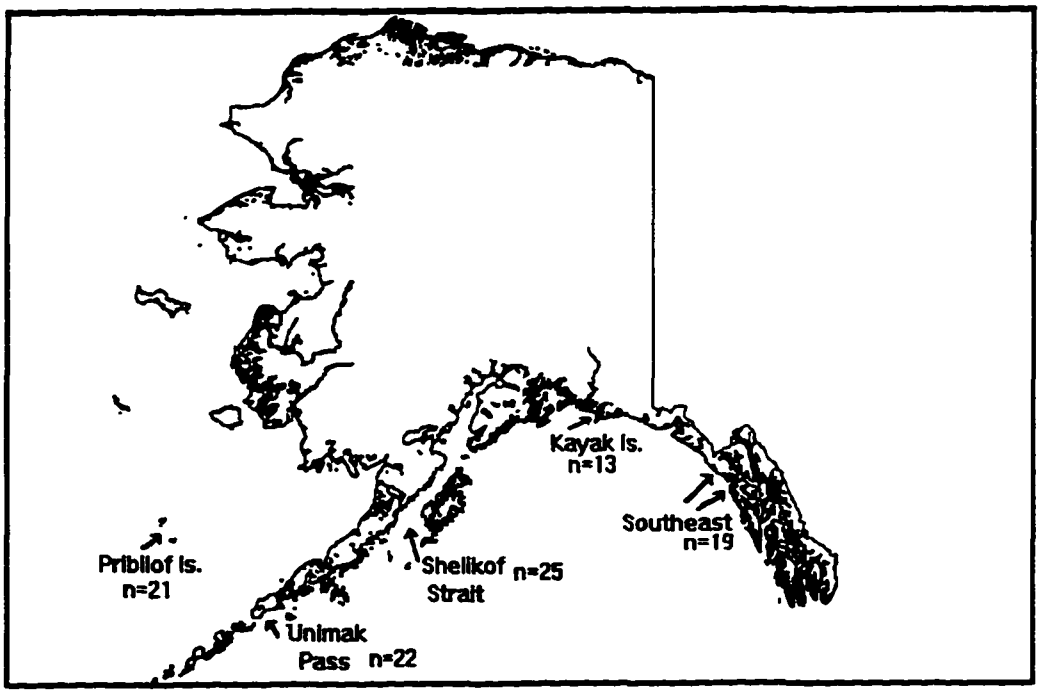

Figure 3-1. Map showing the location of capture and sample sizes of juvenile and larval halibut otoliths examined in this study.

Table 3-1 Spearman rank correlation coefficient of increment count, otolith diameter and developmental stage on body size measurements of larval halibut examined in this study (adapted from Hagen 1986). Developmental stage refers to the 12 stage classification criteria of Thompson and Van Cleve (1936) (stages 5 through 10 present here).

	Increment Count	Otolith Diameter	Developmental Stage
Increment Count		0.862	0.844
Otolith Diameter	0.862		0.933
Body Length	0.728	0.905	0.895
Body Depth	0.828	0.952	0.949
Body Area	0.812	0.953	0.952

The relationship between body area and increment count for the larval specimens used in this study is presented in Figure 3-2. The figure includes two regression lines fitted separately to the two groups to indicate the difference between body size at age for the two areas.

Juvenile halibut (0 and 1 year of age), were collected during summer months at shallow water nursery grounds near the Pribilof Islands in the Bering Sea, Kayak Island in the Gulf of Alaska, and two nearby areas, Palma Bay and Shelikof Bay, on the outside coast of Southeast Alaska. I combined the specimens from Palma Bay and Shelikof Bay because of their proximity and identified them collectively as the Southeast Alaska samples (Figure 3-1). The Pribilof Island samples in the Bering Sea were collected as part of an Outer Continental Shelf Environmental Assessment Project (OCSEAP) by United States Mineral Management Service in 1983. The Gulf of Alaska and Southeast Alaska samples were obtained from surveys by the International Pacific Halibut Commission in 1984. The specimens were generally preserved in 95% ethanol after measuring their lengths in the field. Specimens from the Pribilof Islands however were immediately frozen after capture and later transferred to alcohol. The otoliths dissected from juvenile halibut were mounted in thermoplastic resin on petrographic slides with the sulcus of the otolith exposed. The otoliths were ground down using increasingly fine grit paper to expose the central core. They were then removed from the resin, flipped over, and ground on the opposite surface to produce a thin section that was adequate for viewing with light microscopy.

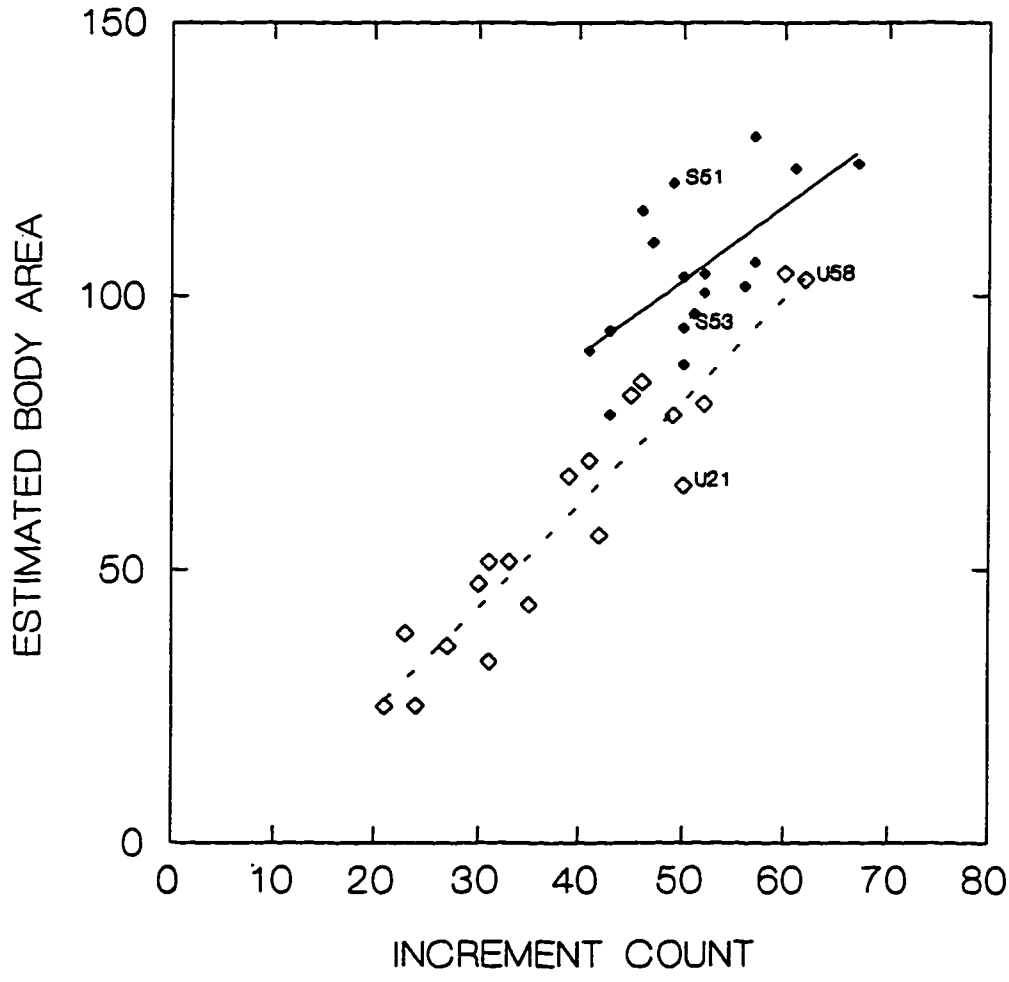

Figure 3-2. Estimated body area (1/2 x length x depth) and increment counts of larval halibut collected from Shelikof Straits (♦) and Unimak Pass (◇) and used in this study for comparing increment width measurements. Linear regression lines fitted separately to each area. Labeled points indicate specimens discussed in the text and in figures 5 and 6.

A subsample of both larval and juvenile halibut otoliths was examined with a JOEL 50 Scanning Electron Microscope (SEM) to confirm the presence of the increments that were observed with the light microscope. These otoliths were given a final polish using 0.3 μm alumina polish slurry to remove scratches and etched with dilute tri-sodium ethylenediaminetetraacetate (EDTA) to provide the surface topology necessary for SEM photography.

Analysis

Commercially available image processing packages were used to help enhance the appearance of the microstructure patterns when viewing the otoliths with transmitted light microscopy and to take measurements of increment widths. The otoliths were viewed at 1000x magnification and the images initially digitized with a software package at a resolution of 512 x 512 pixels. A sharpening filter was used to enhance the contrast of the increment edges but this did not affect spatial resolution. Under these conditions the minimum sampling unit for measurements was 0.21 μm, which approaches the minimum size for distinguishing objects using light microscopy. Because these increments were quite small, the sampling units for measuring increment widths were frequently in single digits. Additional samples of otoliths were later measured using a higher digitizing resolution (640 X 480). A comparison of data from otoliths measured at both resolutions (n = 7) showed no difference using the methods identified below.

The increment width data were collected along transect lines (Figure 3-3). Because the otolith nucleus was frequently obscured by cracks or fractures, I used as a reference point for the start of the transect lines a check ring that I had noted as appearing consistently at 57 ± 4 μm (n = 61) in diameter (Hagen 1986). This same check ring could be observed in the otoliths from juveniles at essentially the same diameter (54 ± 9 μm, n = 8) and was visible when the nucleus frequently was not (unpublished data). To use the check ring as a starting point presumes that it is a result of a common physiological event and that otolith growth is unrestricted along a plane that measurements are taken. This first assumption was

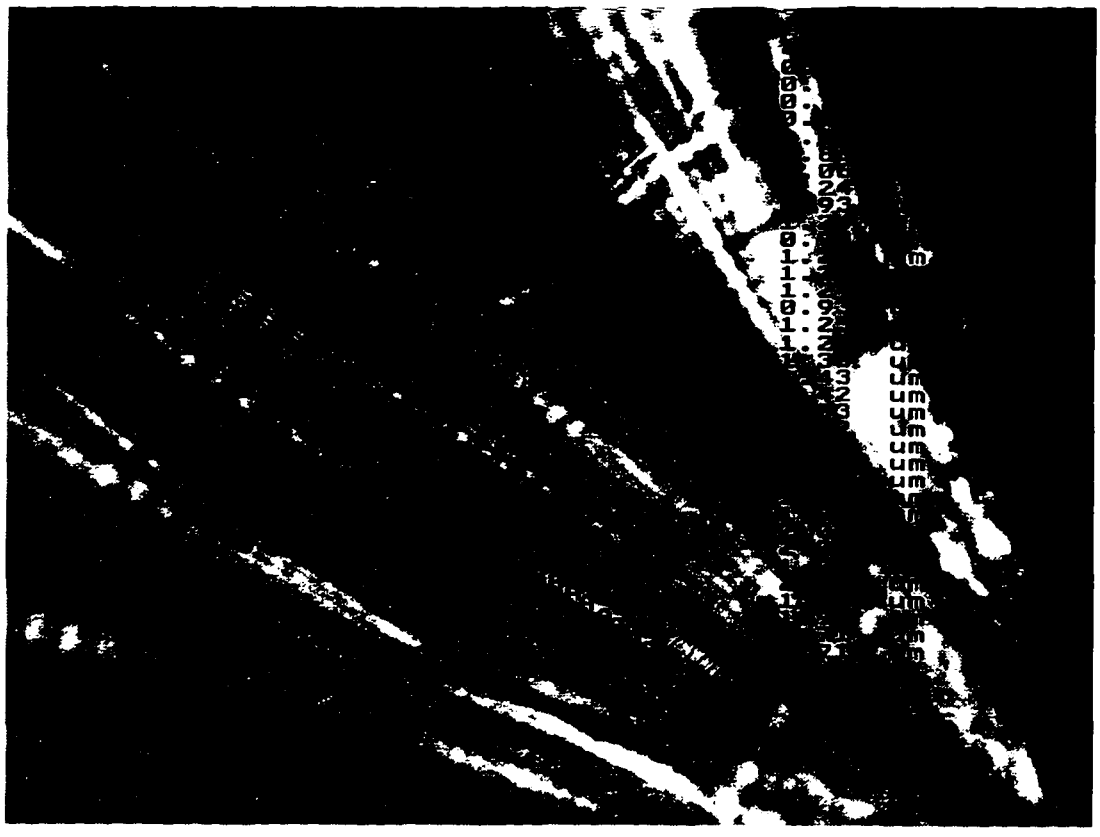

Figure 3-3. Light microscopy image of an otolith showing the line transect used in collecting increment widths with the measurements displayed on the photograph. No filtering was applied to this image.

not validated, but the constancy in the appearance and location of the check rings suggests that it might be associated with first feeding and the end of yolk sac absorption (Hagen 1986, chapter 4). To check the assumption of unrestricted growth, I examined otoliths *in situ* with light microscopes and took SEM photographs of otoliths at different cross-sections. After choosing a standard plane I took replicate transects in a subsample of the juvenile otoliths (n = 26) to compare within-otolith differences in increment widths that might be due to the choice of axes.

For data analysis, I used increment widths, defined as the distance between the adjoining points of L-zone to D-zone transitions (light to dark changes), as the dependent variable. Along a given transect, increment width, G_t, can be considered a measure of daily growth rate in units μm* d $^{-1}$. For the independent variable, I measured the distance from the check ring to the start of the corresponding increment width measurement and then added 28.5 μm to each measurement based on the average radius of the larval check. I refer to this distance as the otolith radius, R_t, in units μm. I also examined changes in relative growth rate, defined as $Z_t = G_t / R_t$, as a function of otolith radius. Relative growth rate has units d $^{-1}$. It is typically examined as a function of age, but in this instance it will be considered as a function of size.

Structuring the data in this manner, where increment width is considered a measure of otolith growth rate and where the rate is examined as a function of otolith radius, provides some practical advantages. Because true age does not need to be known and the only temporal assumption required is that increments are formed daily, the full growth record does not need to be measured. Differences between otoliths on where the first increment is visible can be easily accommodated and gaps in the increment record along the transects is allowed. Comparisons between individuals however should be made only where both data sets overlap.

I explored the shape of the growth trajectories using a Lowess smoothing method (Cleveland 1979) on the bivariate plots of growth rates and otolith size. Lowess is a locally weighted least squares method that minimizes the median of the absolute value of the residuals. It is useful for displaying trends in data

where the error distributions are unknown and to reveal patterns in data that may otherwise be difficult to detect. For each Lowess line on data sets greater than 20 observations, I used a window of 2/3 of the data for smoothing at each point. For data sets below 20 observations I used a smoothing window of 1.0. In each case I used three iterations to compute the estimates.

To compare differences in the relationship of increment width to otolith radius, I used analysis of covariance (ANCOVA) methods (Sokal and Rohlf 1981). The otolith radius R_{ij} associated with the ith measurement from the jth transect or group is used as the concomitant variable in a linear relationship with increment width G_{ij}. The ANCOVA model is written,

$$G_{ij} = \mu + \alpha_j + \beta_j R_{ij} + \varepsilon_{ij}$$

where μ is the grand mean, ε is the error, and β is the slope. The slopes β_j are examined first to determine if a common slope can be applied prior to testing for significance of the adjusted group means α_j.

I restricted the comparison to portions of the otolith that appeared to consist of a linear increase in increment widths. Transformations were applied if necessary to linearize the data. I used this method for comparing individual transects of longitudinal growth as well as cross-sectional growth; which is the data pooled from individuals from the same nursery areas.

Results

Validation and otolith structure

The attempt to validate the periodicity of the increment patterns from the halibut of known age was largely unsuccessful. While increment patterns were present in some specimens, they were faint and the counts variable and not reflective of the known ages. Validation attempts from rearing experiments are frequently unsuccessful because the conditions are generally much different from those found in the wild

(Jones 1986). The increment patterns in wild caught larvae were similar in appearance to what Campana (1992) describes as typical of daily increment formation as opposed to subdaily increments or periodic check rings. In addition, Hagen (1986) argued that the assumption of daily increment formation was consistent with other information about the timing of halibut larval development and life history changes. Nonetheless, to the extent that this analysis relies on daily increment formation, the results must be considered tentative.

Figure 3-4 shows the location of the otolith within a 24 mm larva. This view of the otolith is along the distal surface: the side opposite of the macula and where the sulcus groove forms during later otolith growth. The sagitta appears to be suspended in the chamber and not constrained by the sides. Along this viewing plane the otolith appears roughly symmetrical, though a slight deformity can be seen.

An SEM photograph of a juvenile otolith that was cross-sectioned along the transverse plane shows the larval check ring surrounding the otolith center as well as a series of deep check rings parallel to the proximal surface (Figure 3-5a). The check rings likely indicate interrupted growth which contributes to the formation of the sulcus groove. The other SEM photograph (Figure 3-5b) is from a juvenile halibut otolith which was ground down from the proximal surface revealing the sagittal plane of the otolith microstructure. The sagittal plane is the one from which increment measurements were collected in both the larval and juvenile specimens. Two concentric rings are apparent. The inner ring is the one which I had noted as appearing consistently as a dark ring in larval otoliths at 23 μm diameter (Hagen 1986). In the SEM photograph it appears as a groove after etching, indicating that it was likely composed of EDTA - soluble protein. Optically dense protein deposits are noted in the nucleus of other species and have been associated with the fusing of separate primordia. The outer check ring is the one that appears at about the same radius in all halibut otoliths and serves as the point of origin for the measurements of increment width.

From the dozen wild-caught larval and juvenile halibut otoliths examined with SEM, no increment formation was apparent inside the outer check ring. This was consistent with observations made with

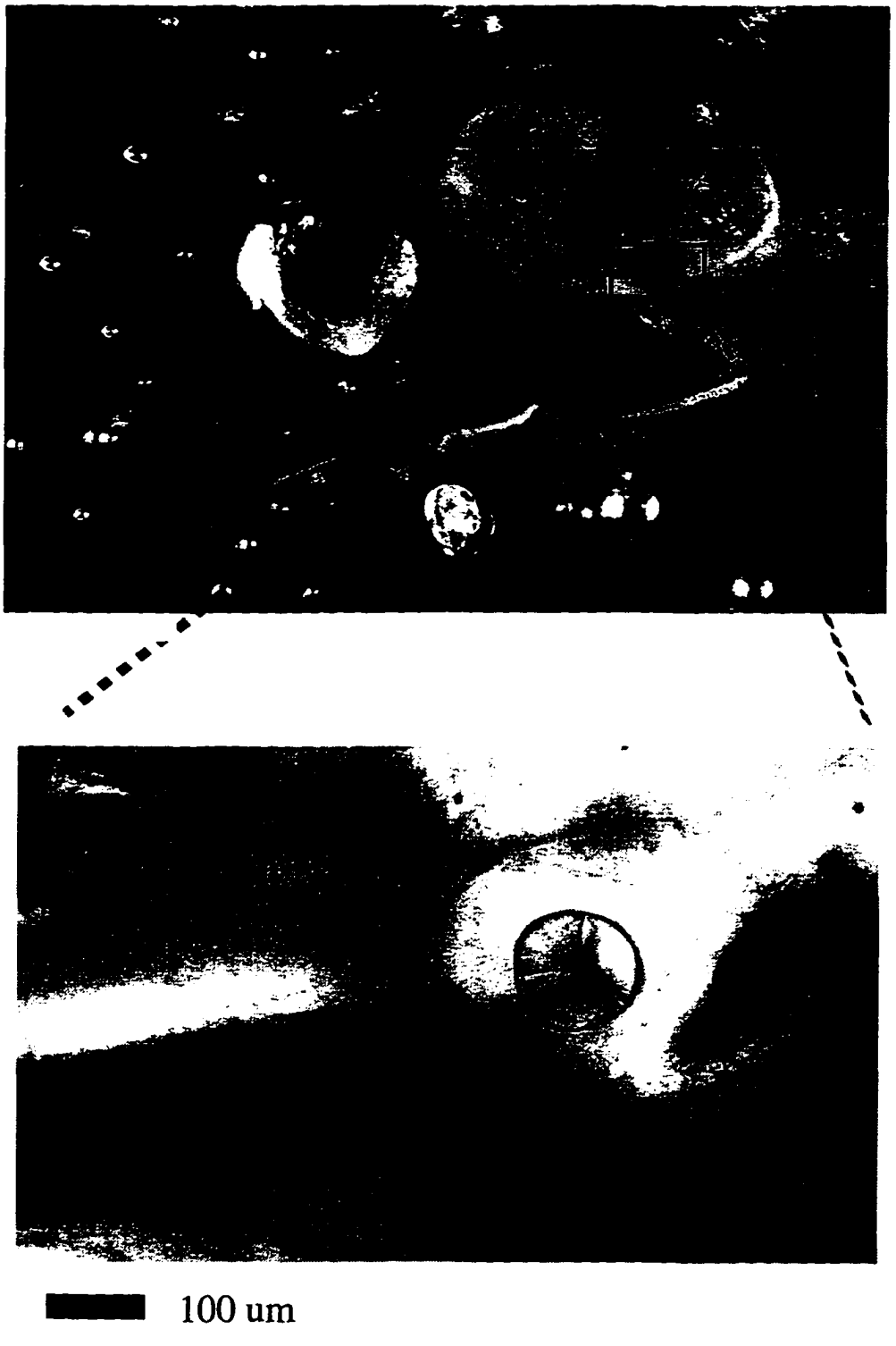

Figure 3-4. Photograph of a lateral view of a larval halibut embedded in epoxy resin and sectioned to reveal the location and orientation of a sagitta otolith within the sacular vestibule.

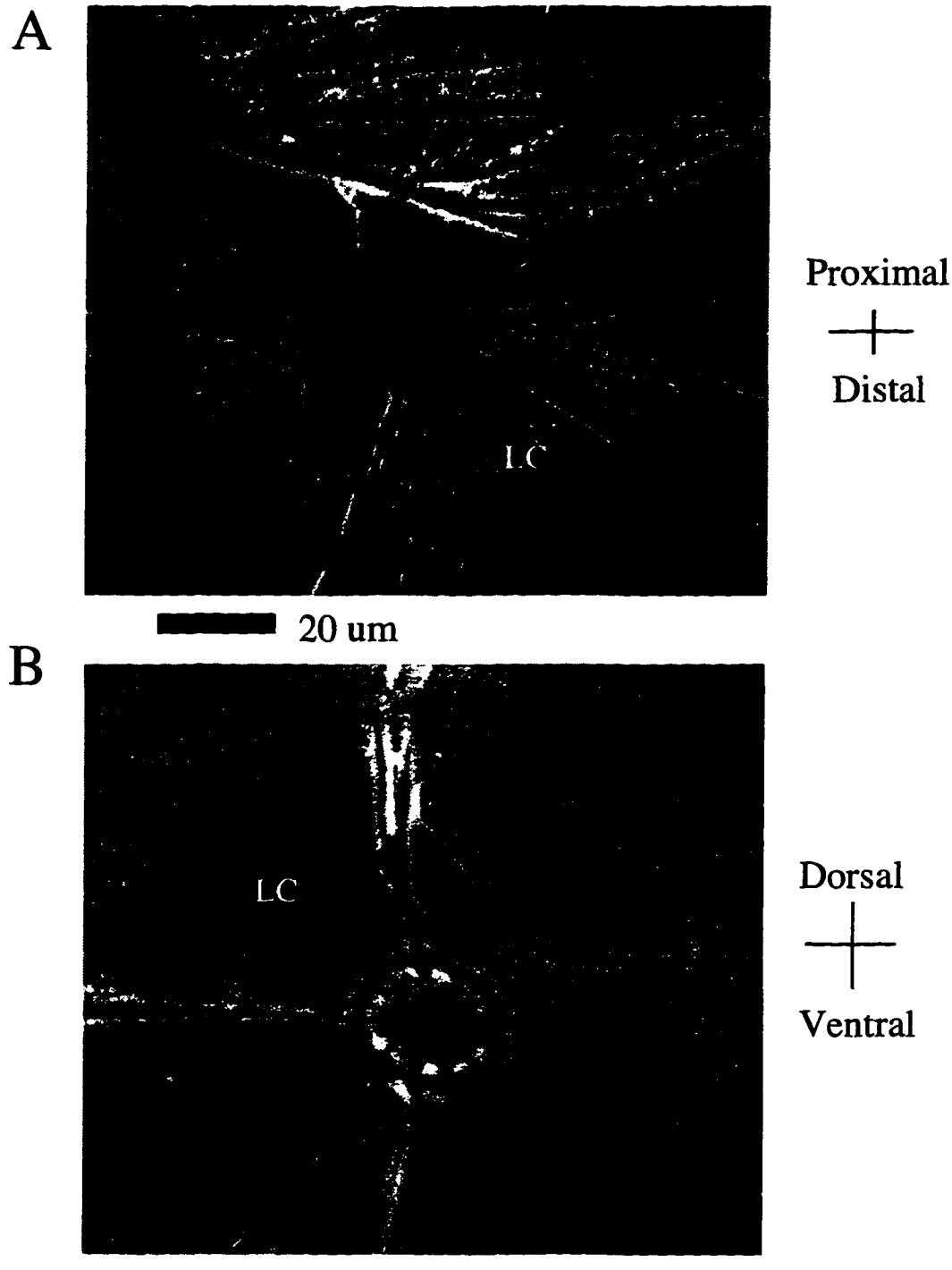

Figure 3-5. Scanning electron photograph of juvenile halibut otoliths: A) cross sectioned along the transverse plane showing the larval check ring (LC) and a series of deep check rings parallel to the sulcus groove and B) ground down along the sagittal plane which is the orientation of the otolith when collecting measurements on increment widths. The larval check is used as a reference point in collecting measurements.

light microscopy. The hatchery-reared larvae examined for validation, however, did appear to have some increment formation outside the inner check ring unlike the wild fish. This discrepancy could be due to differences in rearing conditions. Alternatively the increments were perhaps present in wild larvae but might be at a slightly different viewing plane or require other etching techniques to view them with SEM (Neilson 1992).

Otolith growth

To evaluate within-otolith variation as a possible source of error, two transects were made at different axes on a sample of 26 juvenile otoliths where clear increments were visible. Separate analysis of covariance tests were made on each pair of transects. The results showed that for 18 of the otoliths a common slope and intercept could be fitted to the increment width and otolith radius data on both transects ($p > 0.05$, average $df = 75$). In five of the eight remaining otoliths one transect covered a longer distance than the other. When the tests were restricted to the area of overlap, the two transects shared a common slope and intercept ($p > 0.05$, average $df = 45$). In the three remaining otoliths nonlinearity appeared to be introduced in one of the transects as a result of inadvertently measuring subdaily increment widths within the larger increments. On the basis of this examination, where there appeared to be overall consistency of measurements between replicates, I concluded that within-otolith variability was not a significant factor, chose the largest data set from the pair and collected only one data set per fish in the remaining samples.

The examination of the larval samples provided an opportunity to see how well individual otolith growth rate trajectories track differences in somatic growth. The relationship between the estimate of body area and increment count for the two sampling locations is shown in Figure 3-2. The display includes two linear regression lines fitted for each area. An ANCOVA test indicates that where the increment count overlaps (between 40 and 63), the slopes are not significantly different from each other ($p = 0.196$) and there is a significant difference in adjusted mean length ($p < 0.001$). This indicates faster body growth for the Shelikof Strait specimens than the Unimak Pass specimens under the assumption of daily

increment formation. Figures 3-6 and 3-7 display increment width and relative growth rate as a function of otolith radius for four of the individuals labeled in Figure 3-2.

In Figure 3-6 the two larvae, U21 and S51, had the same increment counts indicating that they are presumably of the same age, but the Shelikof Strait specimen, S51, was much larger and more advanced developmentally using the criteria of Thompson and Van Cleve (1936) (stage 9 verses stage 7). This difference in somatic growth is reflected in the increment width patterns. At any given otolith radius the increment widths were smaller for the Unimak Pass specimen, indicating the velocity of growth was slower. This is also shown in the plot of relative growth as a function of otolith radius where it shows as a difference in mean relative growth. The display also shows with the smoothed line that the Unimak Pass specimen had a change in growth velocity at about 44 μm.

Figure 3-7 contrasts otolith growth between individuals, U58 and S53, which had essentially the same otolith radius at 106 μm. However the Unimak pass specimen, U58, had greater increment count (62 versus 50), was slightly larger, and was more advanced developmentally (Thompson and Van Cleve (1936) stage 9 versus stage 8: primary distinction based on eye position). The data in Figure 3-7 covered only about 80% of the growth record. However, the otolith growth record shows that the Unimak Pass specimen grew much slower at any given size than the Shelikof Strait specimen. The similarity in otolith diameter most likely has to do with the age difference, which provided the additional increase in body size.

The variability in growth patterns can be illustrated by the superimposition of Lowess line plots of individual growth rates. Figure 3-8 displays these trajectories from 47 of the larval otoliths from both areas. Overall, there is a trend of increasing increment width with otolith radius. The shorter data sets in this display appear to have greater curvature than the longer data sets. This curvature may be real and reflect a relative growth spurt at small radii, but it could also be a function of sample size and a difference in the number of points used to smooth each data set in the Lowess smoothing. The plot of

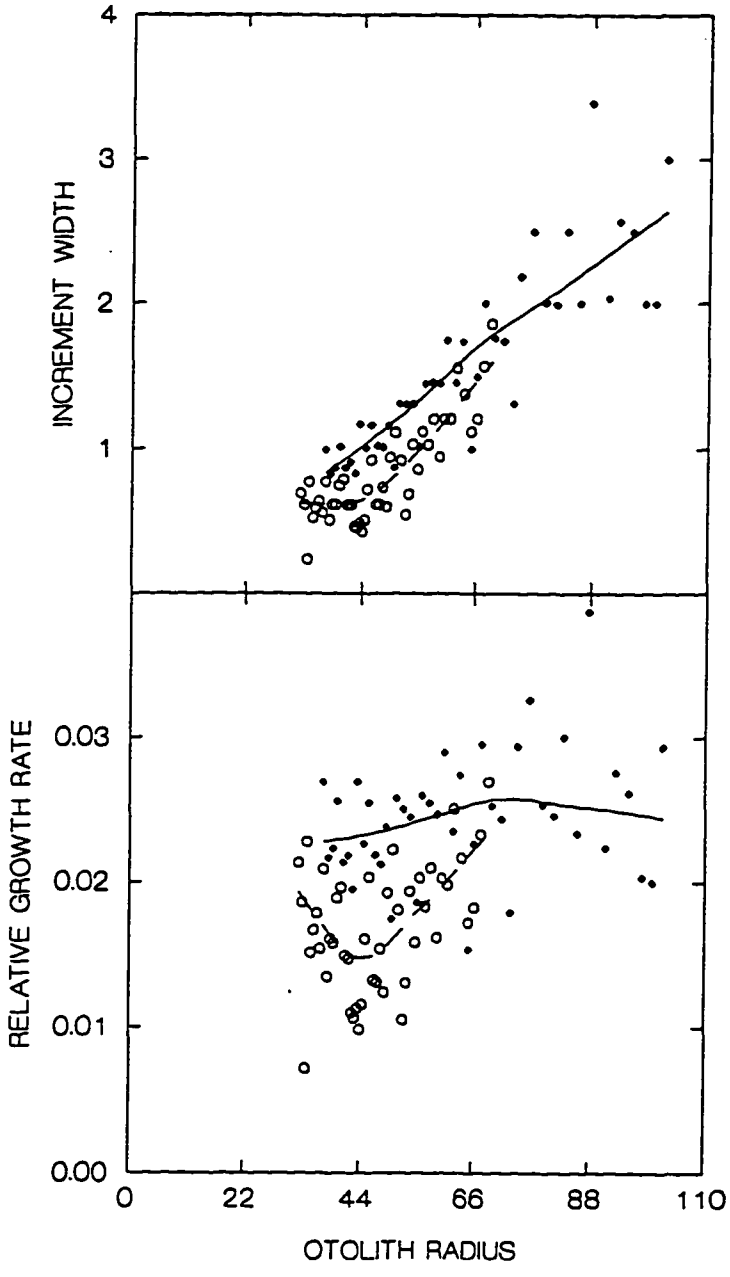

Figure 3-6. Increment width and relative growth rates of otoliths as a function of otolith size for specimens, U21 (dashed line) and S51 (solid line) identified in figure 3-2. Lowess line indicates trends in the data using a 2/3 smoothing window.

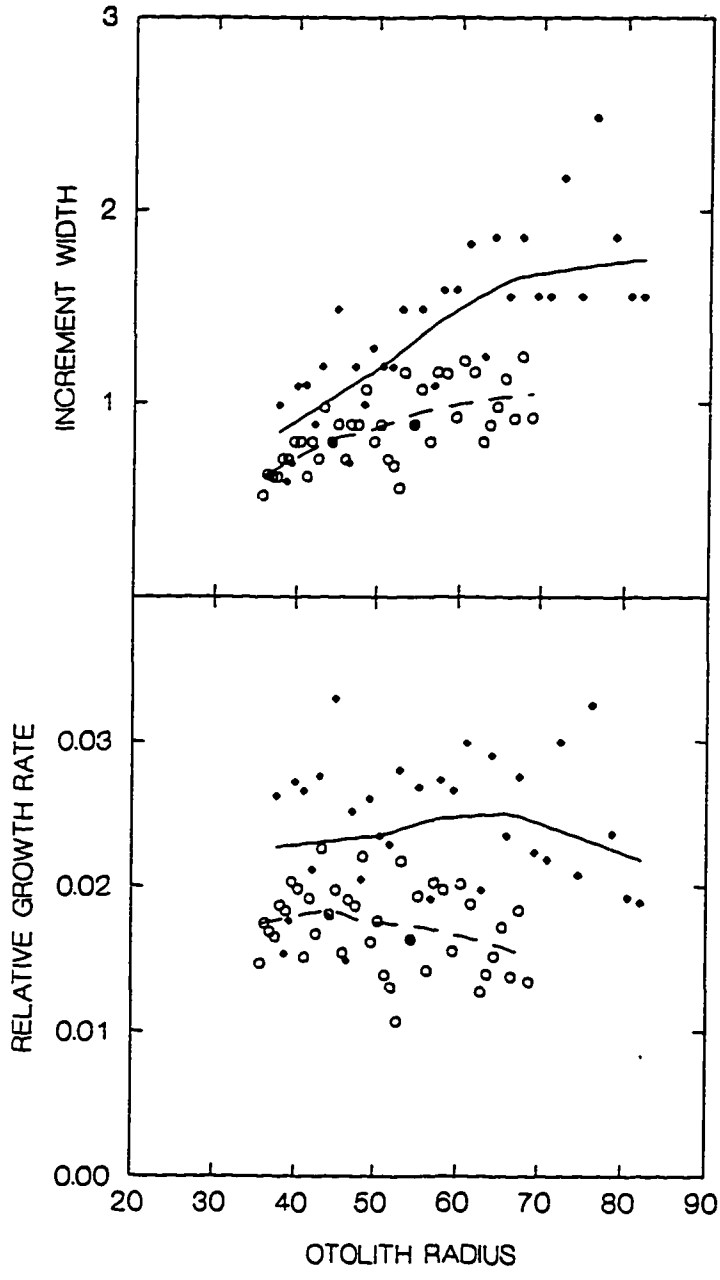

Figure 3-7. Increment width and relative growth rates of otoliths as a function of otolith size for specimens, U58 (dashed line) and S53 (solid line) identified in figure 3-2. Lowess line indicates trends in the data using a 2/3 smoothing window.

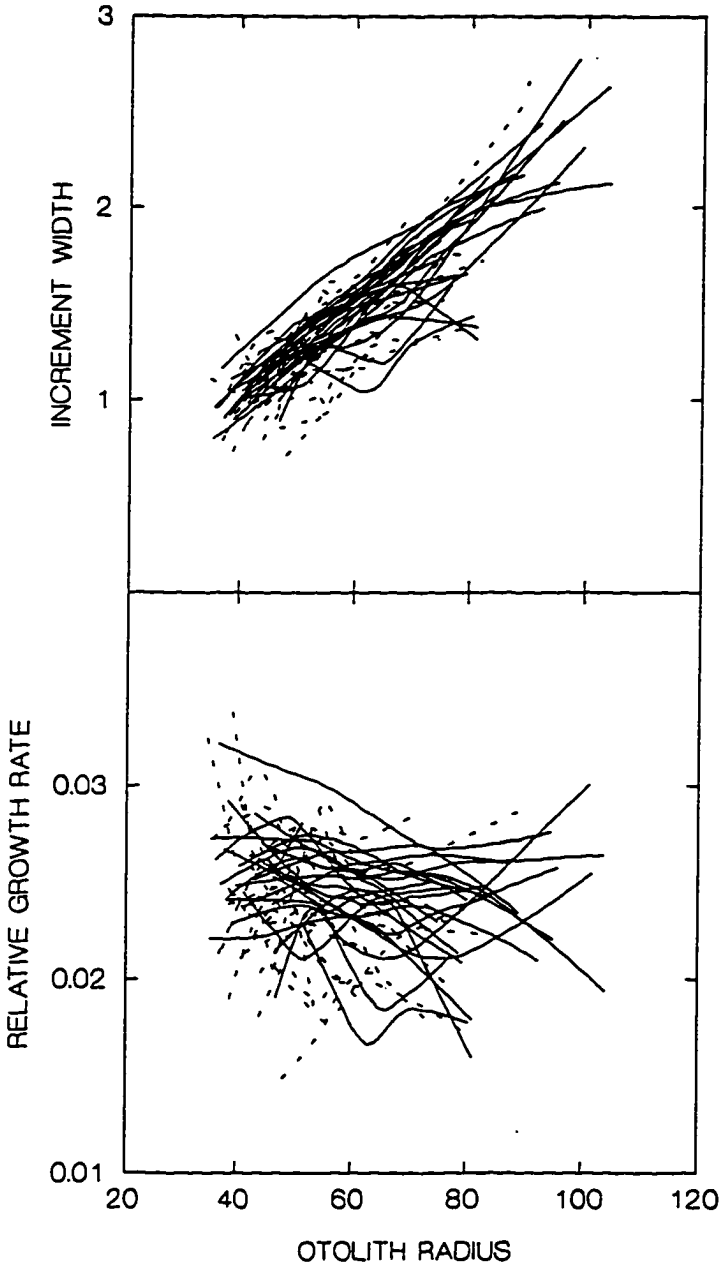

Figure 3-8. Lowess smoothed trajectory plots of increment width and relative otolith growth rates as a function of radius for each Unimak Pass (dashed line) and Shelikof Strait (solid line) specimen. Lowess line smoothed with 2/3 data window for individuals with greater than 20 data points and 1.0 window for data under 20 points.

relative growth shows similar variability, with some individuals exhibiting an increase in relative growth at different otolith sizes, while others show steady or declining changes in relative growth.

The test for group differences was made by pooling the data from each of the two areas. Separate Lowess smoothing indicates that the Unimak samples have a slower relative growth than the Shelikof Strait samples with a slight change in growth at about 60 μm (Figure 3-9). The increase in increment width with otolith radius is roughly linear in both areas. The slight difference between the two areas can be confirmed with ANCOVA, where the initial test for difference in slopes shows no significance (p = 0.277). As shown in Table 3-2, otolith radius is a significant covariate and there is a significant difference in adjusted mean increment width between the two areas. However, the mean-square error for location is not large and the significance may be partially a result of large sample sizes.

Table 3-2. Analysis of covariance on increment width as a function of otolith radius and area of capture for the pooled data from the Unimak Pass (n=22) and Shelikof Strait (n=25) larvae.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
Area	1.528	1	1.528	17.929	0.000
Otolith Radius	104.613	1	104.613	1227.544	0.000
Error	104.311	1224	0.085		

The juveniles collected from Pribilof Islands (n= 21), Kayak Island (n=13) and Southeast Alaska (n=19) show a similar amount of individual variability during larval growth as was observed with the larval samples (Figure 3-10). For some individuals, growth rates appear to accelerate while for others it appears nearly linear. A graph of Lowess lines from the combined data from each area including the two larval samples is shown in Figure 3-11. It is apparent that the Pribilof Island sample shows the slowest otolith growth rates while the Gulf of Alaska and Southeast samples are similar in shape. The Kayak Island specimens however have growth rates higher than the other areas during the later larval period, but are otherwise similar during early growth. The relative growth plots indicate the increase in their growth takes place at an otolith radius of about 70 μm.

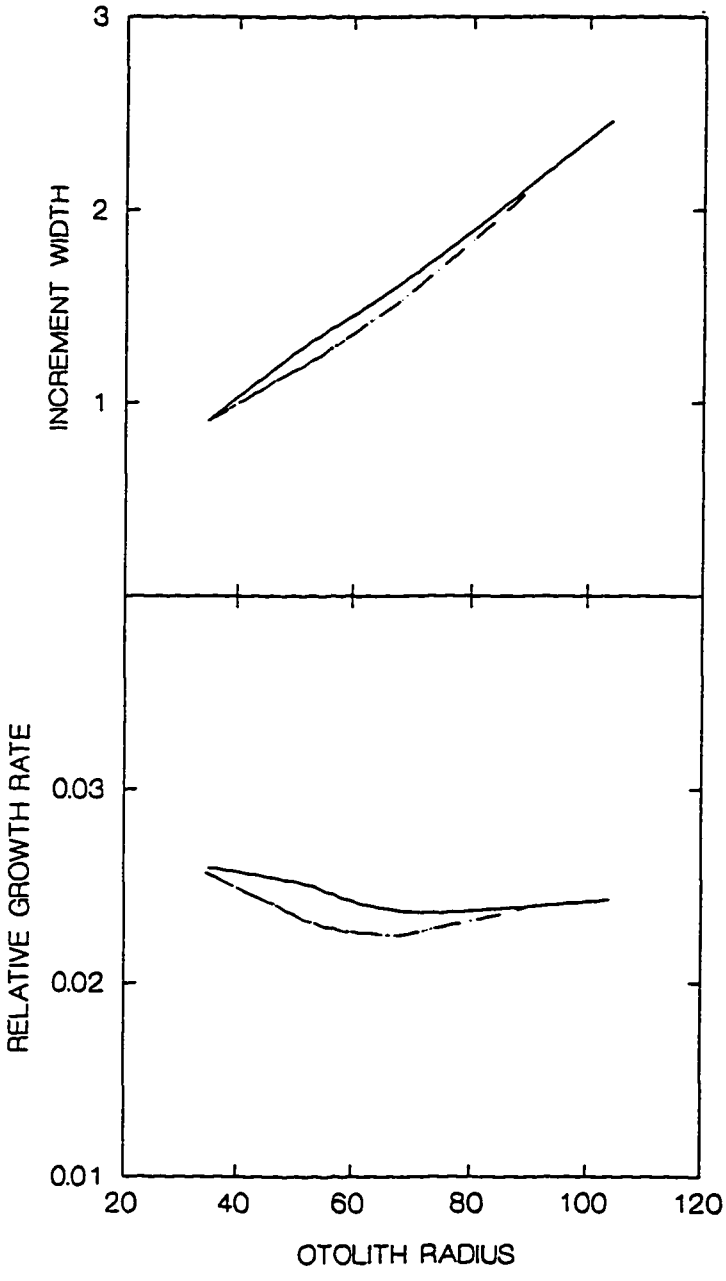

Figure 3-9. Lowess smoothed plots of pooled data from all individuals within each area; Unimak Pass (dashed line) and Shelikof Strait (solid line).

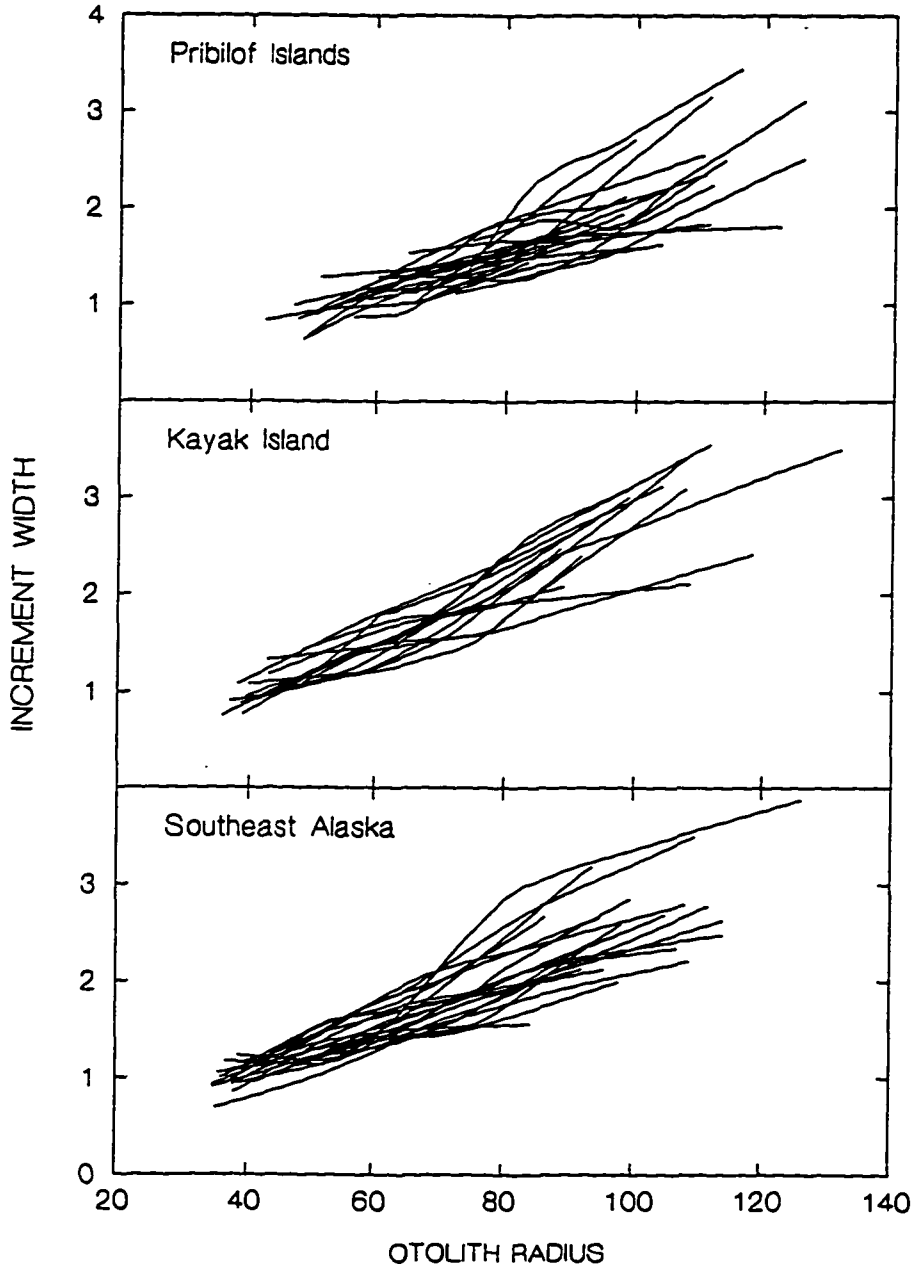

Figure 3-10. Lowess smoothed trajectory plots of individual larval growth from otoliths of juvenile halibut obtained from Pribilof Islands, Kayak Islands and Southeast Alaska.

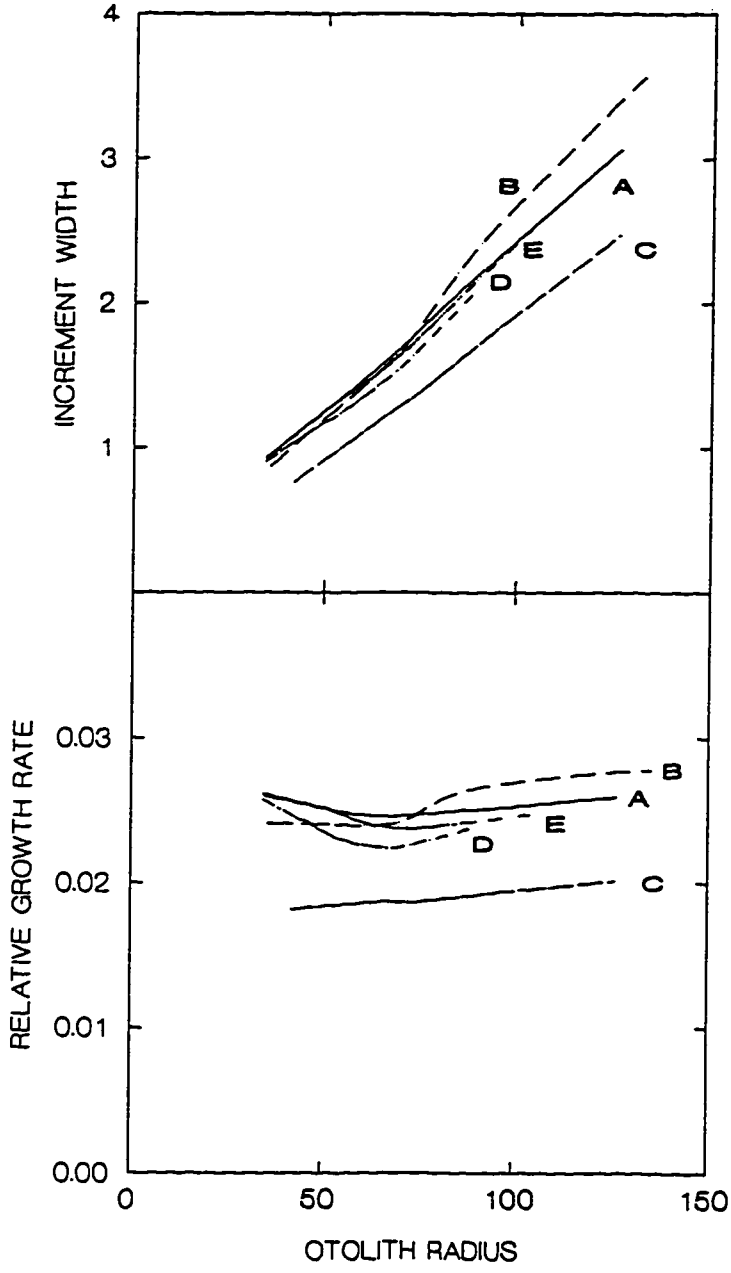

Figure 3-11. Lowess smoothed plots of pooled data from each sampling location for juvenile and larval halibut. A). Southeast Alaska, B) Kayak Island, C) Pribilof Islands, D) Unimak Pass, E) Shelikof Strait.

Discussion

The results from this study confirm that differences in increment width formation during larval development reflect differences in somatic growth rate in larval fish. This is detectable both with the individual otolith growth history (longitudinal data), as well as the pooled data where the growth history is combined from groups of individuals. If this relationship is valid for halibut from the different nursery areas then it appears that larval growth is much slower in the halibut that settle out around the Pribilof Islands than those in Gulf of Alaska or Southeast Alaska. This information could have utility for stock separation. From tagging studies, the Bering Sea is known as a nursery area for young halibut that eventually recruit to the commercial fisheries in the Gulf of Alaska and Southeast Alaska and there is also a resident population of halibut that remains in the Bering Sea (Best 1979). The Unimak Pass larvae are progeny of stocks that spawn in the Gulf and are likely to settle out in nursery areas in the southern end of Bristol Bay. The larval patterns from the Pribilof samples were distinct enough to suggest that they are progeny of spawning populations within the Bering Sea and do not come from the Gulf of Alaska. The distinction between the patterns may have some utility for distinguishing populations that likely intermix within the Bering Sea as juveniles (Dunlap et al. 1964).

The difference between increment patterns may also have utility for recruitment investigations. From the Gulf of Alaska samples, the pooled data from the juvenile and larval otoliths appear to have a good deal of similarity, though as shown with the Unimak Pass and Shelikof Strait otoliths, statistically significant differences could be detected. If significant interannual variation can be shown by this method, it might be possible to examine adult otoliths from known year-classes and recover the pattern of their early otolith growth. The correspondence, if any, with year-class size may prove informative in a couple of ways. Growth rate is thought to be a contributing factor in determining survival rate primarily as a means of outgrowing predators (Anderson 1988). But some studies on predation also indicate that faster growth may be of negative consequence when the predators are size selective (Bertram 1996).

Differences in the growth record, in conjunction with information on recruitment success, may provide useful indication on whether growth plays an important role, either positive or negative, in survival.

Another attribute of the otolith growth record which might have informative properties is the change in the velocity of growth as indicated in the smoothed plots of relative growth rates. This change appeared to be pronounced in some individuals and was detectable in some of the areas when the data were pooled. The biological interpretation of a change in the velocity of otolith growth is unknown. The variation between individuals indicates it is not physiologically predetermined. Instead it is likely a result of a change in feeding success or the encounter of a water masses of different temperatures. One possibility is that an increase in otolith growth rates is an indication of the larval movement from deeper waters to warmer surface waters. In comparing the Shelikof Strait and Unimak Pass larvae, Hagen (1986) attributed a difference in growth rates between the larvae to a 2° C difference in sea surface temperatures between the areas of capture. In retrospective studies it may not be feasible to determine the precise location nor the calendar date in which the growth changes took place. Nonetheless, tracking such variation in relationship to year-class size might prove interesting. Parker (1988) hypothesized that variability in larval transport resulting from low frequency oceanographic changes is the primary determinant of halibut year-class strength. The presumption is that survival is directly related to how quickly the young are transported to nearshore nursery areas. While any retrospective examination is necessarily filtered by the record of the survivors, a correlation of accelerated growth with year-class size could prove to be an interesting means to examine that hypothesis. .

By modeling relative growth rates as a function of size, it is still possible to estimate the relative age at a given otolith size even though the data are not explicitly collected for that purpose. This can be illustrated by considering the differences in growth rates between the Pribilof Island samples and those in the Gulf of Alaska. The question can be asked how many days (post yolk-sac absorption) does it take for a larva to reach an otolith radius of 100 μm. For purposes of illustration here the data will be modeled with the linear equation, $Z = \alpha + \beta R$, where Z is relative growth rate and α and β are the

estimated parameters by least squares. The parameter estimates for separate regressions are $\alpha = 0.0231$, 0.0168 and $\beta = 0.0000324$, 0.0000308 for the Gulf of Alaska and the Pribilof Island specimens, respectively. Because $Z = G/R$, the equation $G = dR/dt$ for the daily change in growth at radius R can be rewritten as $dt = dR/(\alpha R + \beta R^2)$. This is the differential equation form of the logistic model and it can be integrated and solved for time t, through the method of partial fractions. The solution with respect to initial condition $R = R_0$ is

$$t - t_o = \frac{1}{\alpha}\left[\ln\left(\frac{R}{R_o} * \frac{(\alpha + \beta R_o)}{(\alpha + \beta R)}\right)\right] .$$

R_o and t_o can be chosen as the initial larval check radius and day of formation, respectively. Solving this equation for each of the areas using $R = 100$, $R_o = 28.5$ and $t_o = 0$, indicates that the mean time from the larval check formation to when the otolith reaches a size of 100 μm corresponds to 50 days for the Gulf of Alaska stocks and 68 days for the Pribilof Island samples. These calculations illustrate the substantially slower growth of Pribilof Island samples in contrast to the Gulf of Alaska stocks, and show how age information can be extracted from the increment data. These estimates are based on the assumption that increment widths on portions of the otolith that were not measured are similar to those that were.

In this study I primarily used a data analytic approach to draw comparisons of growth rates and relative growth rates of otoliths among individuals and among areas in a manner that included both longitudinal and cross-sectional perspectives. Longitudinal data are generally treated in a manner distinct from most size and age analyses because of the interdependency of the measurements (Chambers and Miller 1995). Nonparametric methods, such as Lowess smoothing, have been suggested as being particularly beneficial for investigating longitudinal growth because it can reveal patterns not easily observed with traditional parametric models (Gasser et al. 1984). For cross-sectional studies of growth, which are comprised of one data point per individual, parametric models are generally applied, such as the

Gompertz, von Bertalanffy or the logistic, as shown above. Kaufmann (1981) illustrates in his size-based study how relative growth rates collected from cross-sectional data can be used in model selection. Schnute's (1981) generalized growth equation can also be adapted for this size-based approach. In particular Schnute's (1981) model makes explicit use of the property of acceleration to help guide model selection. Cross-sectional studies are useful when drawing comparisons between populations from different areas or time periods or as inputs into population dynamic models. However several authors have pointed out the problem of individual variability in the estimates of growth parameters applied to a population (e.g. Sainsbury 1980). This individual variation was apparent here in the longitudinal plots of otolith growth, where different individuals may show spurts of growth or growth retardation.

My pooled data approach which combined increment growth from various individual growth histories presents a blending of the cross-sectional and the longitudinal approaches. The pooling can either be by age or by size; the latter was done here. I argue that a size-based approach in this case makes much more intuitive sense than does an age-based approach which is commonly used in both longitudinal and cross-sectional studies of growth. This is because size is likely the better measure of physiological condition than is age (Kaufman 1981, Kirkpatrick 1984), and in the absence of complete longitudinal records, which is likely to be the case in retrospective studies, the age information would be difficult to collect. The disadvantage of pooling by size is the inability to determine calendar date, and thus observe specific environmental effects. However if the data are partitioned, for instance by year-class, then environmental effects could still be examined as an aggregate effect.

The results of this study are dependent on the assumption of daily increment formation as well as a general correspondence between otolith growth and fish growth. A number of studies have pointed out that otolith growth and fish growth can at times be disjointed depending on the scale of observation (Bradford and Geen 1987), the ontogenetic stage of development (Hare and Cowen 1995), and rearing conditions (Mosegaard et al. 1988). There is general agreement that on short time scales, otolith growth

is conservative in relationship to fish growth, but otolith growth will also show more direct response to temperature than somatic growth. Mosegaard et al. (1988) suggest that otolith growth rate reflects the fish's metabolic rate, but metabolic rate and somatic growth rate may track each other under adequate food and temperature. Without direct experimentation, inferring a particular larval size at age based on increment patterns does not seem prudent. However the approach used here, that of examining otolith growth rates with respect to otolith size, is likely to be an improvement on methods that attempt to compare a particular body size at age as the means of comparison. As seen in Table 3-1, otolith size is more highly correlated with larval size and development stage than it is with age or its increment count. It would seem that for purposes of comparison, the size of the fish and not its age would be a better predictor of future growth.

I limited this study to the middle and late periods of larval otolith growth. Early growth was not available for study because the increments were not observable. Transitional larval and early juvenile growth was not examined because the otolith growth at those stages is no longer symmetrical due to the presence of accessory primordia. This would result in significant within-otolith variability on the velocity of growth along any particular axis. The inferences on halibut growth that I was able to draw here, though based on a single line, are likely to be reflective of otolith growth as a whole, as measured by an increase in volume, because of this symmetry. How to extract consistent growth increment data corresponding to transitional and juvenile stages remains a challenge.

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Chapter Four | Elemental Composition of Pacific Halibut Otoliths and its use for Stock Separation.

Abstract

The elements Sr, Na, K, S, and Ca within otoliths of Pacific halibut (*Hippoglossus stenolepis*) were evaluated as natural tags to identify halibut to their nursery area origin using a wave-length dispersive X-ray microprobe. The collection included otoliths from age zero and one halibut in three widely separated near-shore areas in Alaska in 1987 and 1988, otoliths obtained from one of the areas 29 years earlier, and adult otoliths in 1986. The distributions within otoliths were found to vary in relationship to ontogenetic changes and structural features. Elevated levels of Sr/Ca ratios were associated with the yolk-sac period of larval development, and variation in both Ca and Sr/Ca coincided with annuli in older halibut otoliths. Sulfur was above detectable limits only during early larval growth. Levels of Na and K show some concentric deposition patterns during larval development, but were also associated with non-concentric structural features. Na was concentrated in a boundary line that delineates larval crystal growth from growth originating from accessory primordia. The potential for stock separation was evaluated using ANOVA models for both larval and juvenile periods of growth. The Sr/Ca ratio does not appear to be useful for stock separation, given its high within-otolith variation and non-significant variation attributed to the area of capture. Na and K both show more promise with significant variation attributed to area and low between-individual variation. K had the lowest within-otolith variation; however there was some significant variation between the left and right otoliths within individuals. Halibut from the same area collected 29 years apart appear to have significant differences in elemental concentrations of both larval and juvenile growth.

Introduction

Pacific halibut (*Hippoglossus stenolepis*) is a large, long-lived flatfish with a wide distribution in the North Pacific Ocean that extends along the continental shelf from Santa Barbara, California to Hokkaido, Japan (IPHC 1978). Halibut life history involves extensive movement of individuals at all developmental stages. Adult halibut move from shallow feeding grounds in the summer to deep water spawning grounds on the edge of the continental shelf during the winter (St-Pierre 1984). The eggs are pelagic, hatching after about two weeks depending on temperature, and the larvae drift with prevailing currents for several months until they are brought to various nearshore nursery areas (McFarlane et al. 1991, Parker 1988, St-Pierre 1989). Settlement and transformation to juvenile body shape take place in shallow nursery areas where the young may reside for two years (IPHC 1985). As juveniles get older they move into deeper water and may eventually undertake extensive migrations (Skud 1977, Hilborn et al. 1995). Juvenile migration is considered to be a mechanism to allow the population to maintain its geographic distribution by compensating for larval drift (Skud 1977). After sexual maturity, it is thought that movement patterns are limited to the summer feeding and winter spawning grounds (Skud 1977).

Current management of halibut is based on a catch-quota system that identifies broad regions as separate management units. However an important consideration in the management of halibut is the extent of the juvenile migration (Quinn et al. 1990). Estimates of migration or movement are necessary to evaluate the impact that the interception or by-catch of juveniles will have on the adult population (Sullivan et al. 1994).

Otoliths are promising structures as natural tags because of their ability to record information (Campana and Neilson 1985). In addition, otoliths have been collected by the International Pacific Halibut Commission (IPHC) for over 50 years to provide age estimates. If information about migration patterns could be found within otoliths, this historical collection may provide a unique time series to help answer current questions about population dynamics.

Trace elements in otoliths have shown promise for identifying stock composition for various species

(Mulligan et al.1987, Edmonds et al. 1989, 1991, Thresher et al. 1994, Severin et al. 1995). Widespread application, however, has not materialized due to difficulties in applying the methodology (Gunn et al. 1992) and recognition that there is no direct or simple relationship between otolith chemistry and exogenous sources of the more common trace elements (Kalish 1991, Sadovy and Severin 1994). Nevertheless, the potential payoff is high if otoliths could be used to identify some components of the halibut population.

Addressing this potential, though, requires careful consideration of the limits of the technology used to detect the elements and the use of studies designed to uncover sources of variation. The distributions of elements within otoliths may reflect changes in the fish's ontogeny as it switches between different food sources or moves to new areas, they may correspond to structural features in the otolith that are unrelated to external events, such as the boundary zones that separate different fields of crystalline growth (e.g. Hagen 1995, [Chapter 5]), or they may be a result of unknown causes, but still provide a geographic signature indicating the location of the fish during that period of otolith growth. Understanding the nature of the variability of elements within otoliths is necessary in utilizing that information to identify stocks.

The most practical location to delineate halibut stocks is in nursery areas. Many halibut nursery areas - defined by the presence of age 0 halibut - appear to be persistent locations of settlement and have been surveyed occasionally by IPHC (Best and Hardman 1982, IPHC 1985, St-Pierre 1989). Halibut in these areas may come from different spawning grounds, and their otoliths may contain a record of elements from the period of larval drift as well as elements laid down during the period of nursery area residence. Because changes in temperature or water chemistry over time may also be a source of variability, specimens from the surveys taken in the same area at different years can be used to determine whether differences in elemental composition reflect geographic variation as opposed to temporal variation.

This study was undertaken to determine if elemental concentrations might be useful for identifying halibut to their nursery areas. The distribution patterns of trace elements within larval and nursery area

portions of juvenile otoliths are examined and that information is used as a guide to quantitatively examine elements in the otoliths of halibut from known nursery areas. An examination of variation explained by the different factor levels, pertaining to otoliths, individuals, and areas, is used to provide recommendations for the direction of future studies. Samples obtained from the same area but in different years are included to see if temporal variation might be an important factor.

Methods

Otoliths used in this project were from zero and one year-old juveniles collected by small mesh trawl from three nursery areas in 1987 and 1988. The areas were the southern end of Bristol Bay, Patton Bay just outside of Prince William Sound, and Shelikof Bay in Southeast Alaska (Figure 4-1). The locations chosen represent widely separate areas of settlement. In addition, a second set of otoliths collected from Shelikof Bay in 1958 was also examined, along with otoliths from adult halibut collected from Sitka, Alaska in 1986.

The 1958 Shelikof Bay otoliths were obtained from the IPHC archives where they had been stored in glycerin, while the other otoliths were stored dry. Microscopic examination indicated that glycerin infiltration was limited to the crystalline layers immediately below the outer surface, but the possibility that the glycerin contamination could affect the results cannot be ruled out.

Each otolith was mounted sulcus side up on a petrographic slide using a thermal plastic resin. Otoliths were ground along the distal face using a succession of fine grit silicon carbide paper followed by polishing using 0.05 μm aluminum oxide paste. The glass slide was then heated to melt the resin allowing the otolith to be flipped and the other side similarly ground and polished. Processing both sides of otoliths was necessary to locate and expose the otolith nucleus. Each otolith was treated identically with the order of preparation from the different groups randomly intermixed to avoid differentially contaminating all otoliths from one area. After preparation the otoliths were rinsed in distilled water using an ultrasonic cleaner.

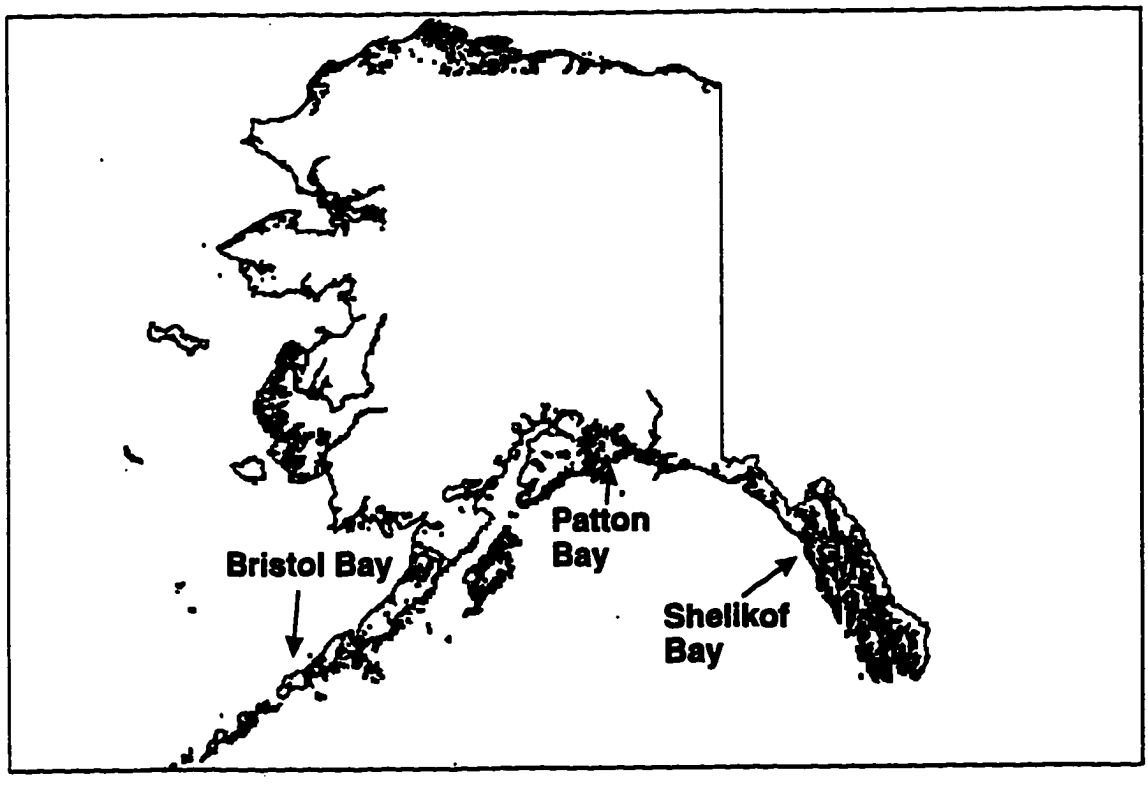

Figure 4-1. Locations of samples of halibut collected for this study.

Elemental concentrations were measured with a Cameca SX-50 electron microprobe housed at the Department of Geology and Geophysics at the University of Alaska Fairbanks. It was equipped with one energy dispersive and four wavelength X-ray spectrometers. Initially the energy dispersive spectrometer (EDS) was used for rapid identification of elements within selected scanned areas. However, in most instances, promising elements initially observed with EDS were found not to be present when using the more accurate wave-length dispersive spectrometer (WDS). Though EDS allows the simultaneous detection of a large number of possible elements, the potential for detecting false positives is also large. For that reason after an initial exploratory examination of different otoliths in different regions, quantitative examination was conducted using WDS tuned to detect specific elements confidently found to be present. These elements include Na, Sr, S, K, and Ca.

For each analysis targeting on a single locus, the beam diameter was 20 μm, counting time for each element was 20 seconds, accelerating voltage was 15kV, and current was 10 nA. Samples were randomized before analysis and standards were reanalyzed periodically to monitor instrumental drift. The calibration using standards and calculation of detection limits follow Severin et al. (1995). The element counts were converted to percent contribution for data analysis. Table 4-1 contains detection limits and typical precision estimates for each element (K. Severin pers. com.).

Table 4-1. Summary of standards used in microprobe analysis to determine detection limits and typical measurements and standard error encountered in the otoliths.

		Detection Limits		*Typical Measurements*	
Element	*Standards*	*Wt %*	*Counting Error*	*Wt %*	*Error*
Ca	Calcite	0.077	0.026	38.403	0.261
K	Osumolite	0.036	0.012	0.103	0.014
S	CaSO4	0.045	0.015	0.033	0.016
Na	NaCl	0.026	0.009	0.367	0.025
Sr	Strontianite	0.045	0.015	0.173	0.019

Following Severin et al. (1995), a locus was considered acceptable if the Ca concentration at that location was above 35%. Locations with low Ca were found to be associated with cracks in the otolith. Excluding those loci resulted in an unequal number of data points.

Strontium forms a carbonate which can substitute for calcium carbonate in the inorganic portion of the otolith. For that reason, following other studies (e.g. Thresher et al. 1994, Severin et al. 1995), I used the ratio Sr/Ca in my analyses. Some studies (e.g. Kalish 1990, 1991) have also used calcium ratios in analyses of other elements to account for differences in the amount of material removed at each locus by the electron beam. However, Gunn et al. (1992) found that there was no difference in the relationship between the original element concentrations and the calcium ratios under most beam conditions. For that reason and because I was interested in the variation of elements independent of calcium content, I did not use calcium ratios in the analysis.

Distribution within otoliths

Two types of methods were used to explore how elements are distributed within otoliths: line transects and X-ray mapping. A sequence of line transects was made within an otolith of a juvenile halibut from Patton Bay. The line transects were made along two orthogonal axes crossing the nucleus. The first line transect was along the dorsal - ventral axis and the second was the posterior - anterior axis. Individual loci along these transects were 22 μm apart. After completion, the otolith was etched in 5% tri-sodium ethylenediaminetetraacetate (EDTA) for 3 minutes and an SEM photograph taken (Figure 4-2). Transects were also made in an adult otolith. The otolith was sectioned along the proximal - distal plane and a transect taken from the nucleus to anterior axis. The beam size was 10 μm , and the loci spaced 20 μm apart.

The X-ray maps provide a pictorial means for examining the relative distribution of elements. Maps were made of otoliths from a Patton Bay juvenile, two Shelikof Bay juveniles and an adult otolith. The technique involves rastering the electron beam over a given area on an otolith and converting the X-ray counts of elements into digital images (Chandler 1987). The brightness of each pixel reflects the concentration of the element being mapped at that location. Figure 4-3 is a transmitted light photograph of the Patton Bay juvenile otolith after the map was completed. The otolith was scanned with a 1 μm

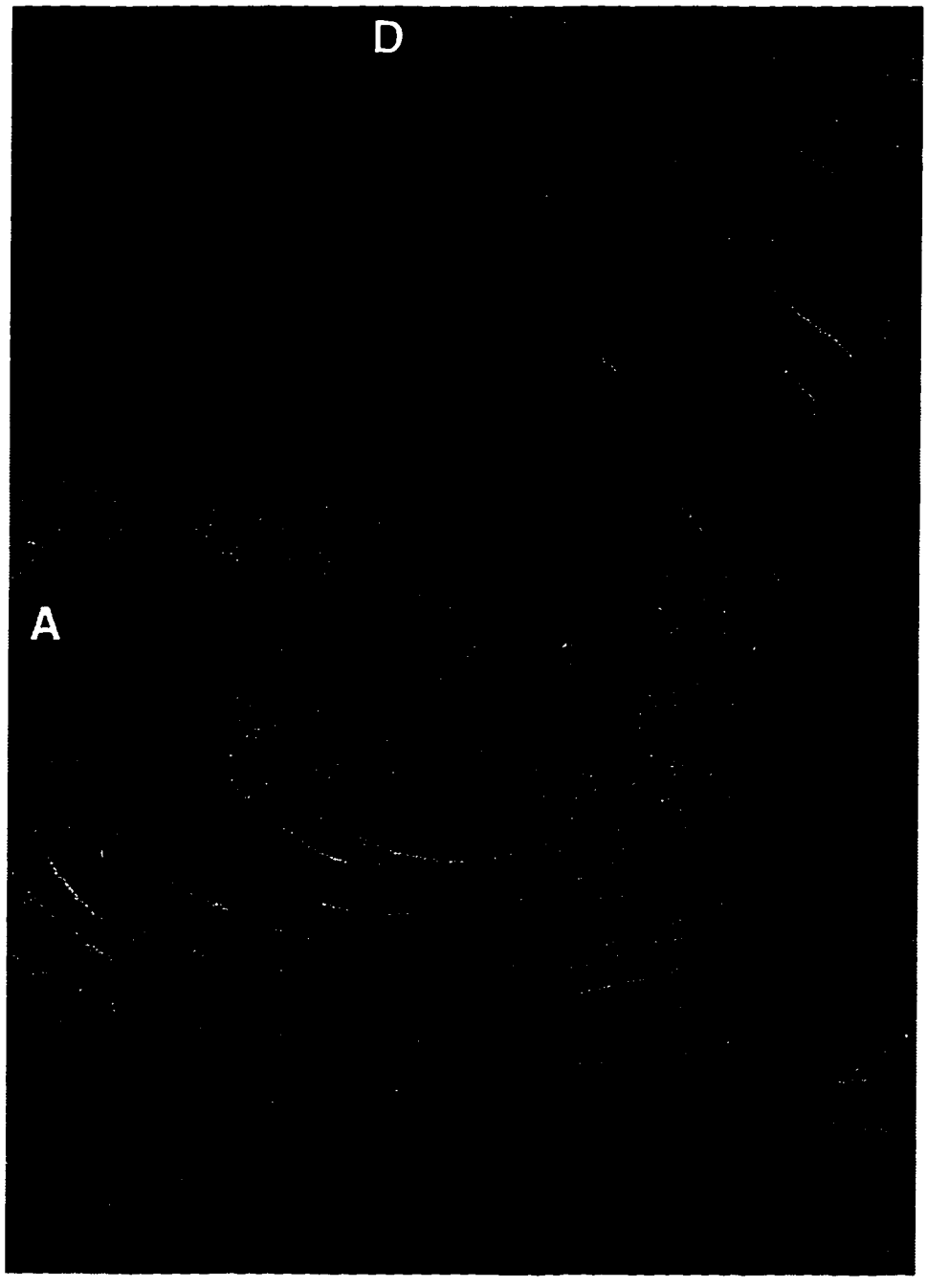

Figure 4-2. SEM photograph of an otolith of a Patton Bay juvenile halibut showing the location of probe footprints along transects across the sagittal plane in relationship to the anterior (A), and dorsal (D) axes.

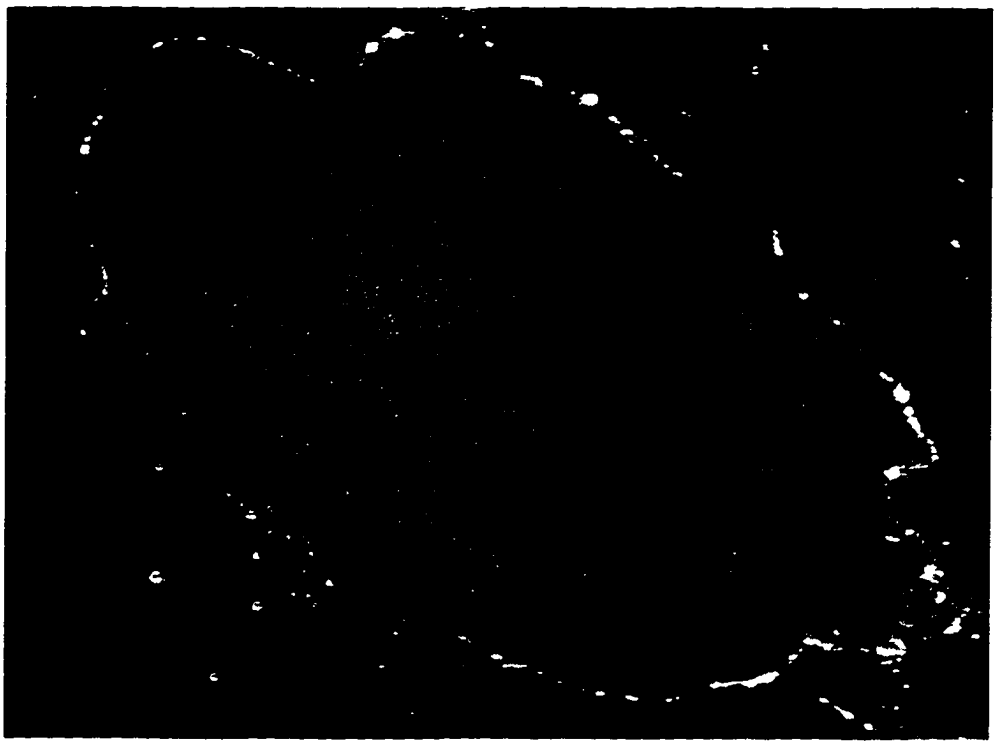

Figure 4-3. Reflected light micrograph showing the location of the X-ray intensity map of a Patton Bay halibut otolith as indicated by the slight charring of the otolith surface (arrow).

beam, pausing for 0.2 seconds at each location over a 256 μm by 256 μm area. Slight charring of the otolith by the probe indicates its location. Maps from both Shelikof Bay otoliths were made with a 2 μm beam rastered over a 512 by 512 μm area. The adult otolith was scanned with a 10 μm beam over a 5.12 mm by 5.12 mm area. The maps for the different elements were converted into a digital gray scale image and viewed and measured with image processing software (Optimas Corp, Seattle Wa).

The distribution of elements from both the line transect and X-ray map methods were compared with structural features of the otolith examined through light microscopy. In addition, the data were compared with information on halibut ontogeny using the relationship of otolith size to halibut developmental stage following Hagen (1986).

Stock Separation

To evaluate the utility of using these element concentrations for stock separation, I collected data from randomly selected loci within the otoliths, treating the juvenile portion of the otolith separately from the larval portion. For purposes of this paper I define the larval portion as that area between 60 and 200 μm from the otolith's center. That location corresponds to the distance from the larval check ring (see chapter 3) to the approximate otolith size that Hagen (1986) observed for late stage halibut larvae captured with plankton nets. The juvenile portion of the otolith was considered as the area outside the boundary line that delineates the larval crystal (see chapter 5) but within 700 μm from the center. This portion should correspond to early summer residence within the nursery area. Because discontinuities or cracks along radial lines converge in the otolith center, there were fewer clear areas within the larval portion of the otolith than the juvenile portion. For that reason the larval model was restricted to only three loci.

The analysis was conducted by treating each element as the dependent variable in separate models and using a three factor mixed model analysis of variance design (Model III ANOVA; Winer 1971). The factor levels were: area of capture - using 4 areas, treating the two samples from Shelikof Bay as distinct

areas; individuals within area - using 3 individuals from each area; otolith side - considering a difference between the left and right otoliths; and within otolith - using 3 randomly chosen loci for the larval period and 5 randomly selected loci for the juvenile period. Because of damage to some of the otoliths during preparation, the design is incomplete in that not all otolith pairs were available for analysis for each individual. Prior to running the models, the data were examined for normality using Kolmogorov-Smirnov tests. No transformations were found necessary.

For each element Y, I estimated a full model containing all the factors and their interactions, which has the following form

$$Y = \text{constant} + Area + Side + Area*Side + Indiv[Area] + Side*Indiv[Area] + \text{error}$$

where *Area* of capture is treated as fixed effect, otolith *Side* (left or right) is a fixed effect which has an interaction term with *Area*, and *Individual* is random effect that is nested within *Area* as denoted by the brackets. The remaining term, the interaction of otolith *Side* with *Individual* within *Area*, is also a random effect. The error term contains the within otolith variation from either the juvenile or larval portions of the otoliths. Under a mixture model, the denominator used in the F-ratios for tests of significance depends upon whether the factor tested is considered a random or a fixed effect. Table 4-2 shows the F-ratios and the mean squared error terms used for testing each term in the model for significance.

Table 4-2 The F-ratio used for testing the significance of the corresponding factor under a mixed model.

Source	F-Ratio for testing	Source	F-Ratio for testing
Area	MS(Area) MS(Indiv[Area])	Indiv[Area]	MS(Indiv[Area]) MS(model error)
Side	MS(Side) MS(Side*Indiv[Area])	Side*Indiv[Area]	MS(Side*Indiv[Area]) MS(model error)
Area*Side	MS(Area*Side) MS(Side*Indiv[Area])		

One method for evaluating the different elements for stock separation is to compare how the variation is partitioned among the different sources. However because of missing cases in the design, the full models were not completely factorial. Under this situation the use of least squares estimators will allow tests of significance for each effect, but the predictors in the models may be correlated which can preclude simultaneous estimation of the mean squares of each effect (Wilkinson et al.1996). This presents a problem in trying to determine the partitioning of variation as well as in choosing a reduced model because the variation that remains after removing an effect will not necessarily be pooled in the error term.

To provide a means for comparing variation explained by the different elements along different sampling levels or units, I formulated an alternative model to analyze data, using as a guide the results obtained from significance testing in the full model. The justification of this approach is given in the results section. The alternative model treats the data as if they came from a fully nested design where each level is a random effect (Model II ANOVA; Winer 1971, Sokal and Rohlf 1981). In this approach, area is considered a random sampling from all possible nursery areas, and as before the selection of individuals is considered a random sample from within the nursery area. The selection of an otolith within a fish is also considered a random sample under this model, in which the left or right otolith is interchangeable. The selection serves as a measure of variation within fish from conditions that can influence elemental deposition. This variation between sides is distinct from within-otolith variation from repeated measurements which is contained in the error term. The fully nested model takes the form

$$Y = Area + Indiv[Area] + Side[Indiv] + \text{error}$$

where otolith side nested within-individuals replaces the interaction term of otolith side and individuals-within-areas under the full ANOVA model. The advantage of using this form is that the magnitude or variance of all the model effects could be estimated simultaneously. I used a variance component approach to estimate the variation explained by each factor level and converted the variance into

percentages (Winer 1977, Sokal and Rohlf 1981). Due to the missing data however, confidence bounds around the variance could not be made (SAS System Inc).

Following development of the ANOVA models, boxplots (Chambers et al. 1983) were used to compare distributions of the data within factor levels and to examine, in particular, differences between the Shelikof Bay samples from 1958 and 1987.

Results

Distributions of elements within otoliths

Both the line transect data and the X-ray intensity maps from the juvenile otoliths reveal evidence of concentric distributions of the elements Na, K, S, Ca and the ratio Sr/Ca around the otolith center. The pattern of distribution, however, varies among those elements.

Calcium concentrations, as shown in both the dorsal - ventral and anterior - posterior line transects, is symmetrical (Figure 4-4). The concentrations are low at the otolith center and also in a band at about 100 μm which is most apparent in the dorsal-ventral transect (Figure 4-4). Calcium concentrations along both transects were significantly autocorrelated (Lag 1: $R > 0.49$). The X-ray intensity map of calcium (Figure 4-5a), shows two distinct bands of elevated concentrations at approximately 70 and 160 μm in diameter. Calcium is in low concentration at the otolith center (about 9 μm in diameter) which corresponds to a diffuse dark ring observed in larval otoliths (Hagen 1986). The absence of calcium likely indicates a higher protein content at that location. Protein rich zones can also be observed by the effects of charring the otolith with the SEM when creating the map (Figure 4-3a). The larger scale X-ray maps from the Shelikof juveniles show some delineation of the accessory primordia, but no concentric patterns evident during juvenile growth (Figure 4-6a).

The ratio Sr/Ca is symmetrical in both the dorsal - ventral and the anterior - posterior line transects (Figure 4-4). The ratios are elevated in a span approximately 60 to 80 μm in diameter around the otolith

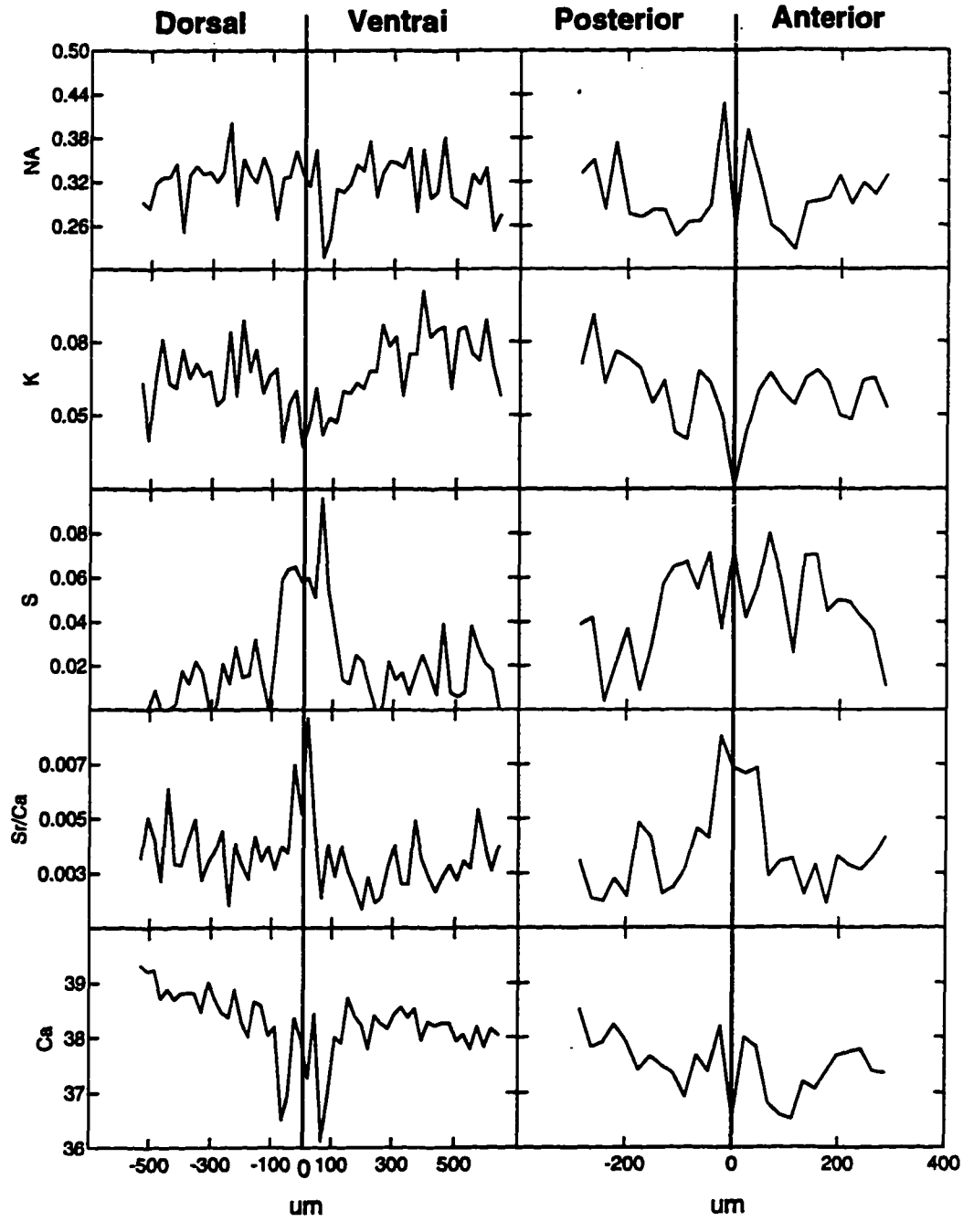

Figure 4-4. Percentage by weight of Na, K, S, Ca and the ratio Sr/Ca along dorsal-ventral and posterior-anterior transects in the otolith of the juvenile halibut shown in figure 4-2. The central primordia is at the origin. Units in µm

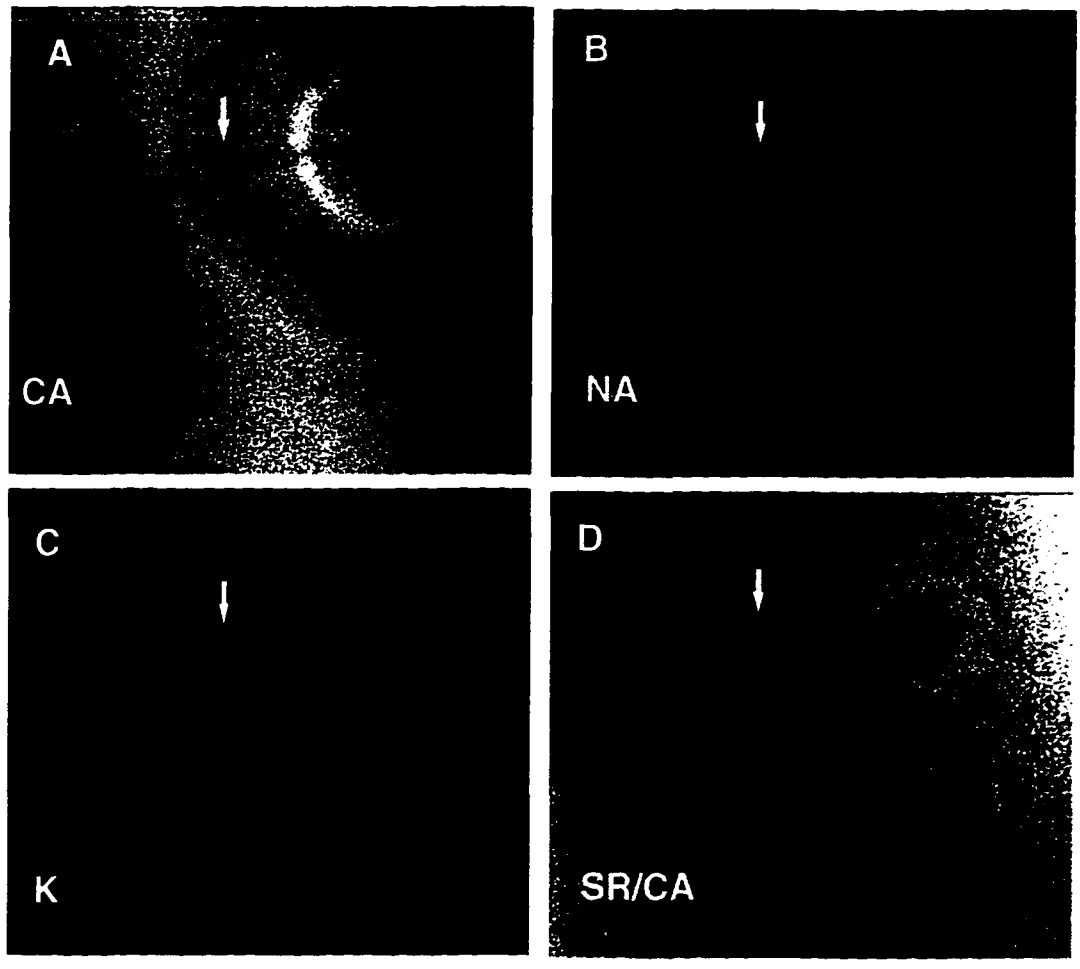

Figure 4-5. X-ray intensity maps showing the relative concentration of elements in juvenile halibut otolith from Patton Bay. Each map covers a 256 μm by 256 μm region with the central primordia in the upper right corner and the image gray scale was stretched to increase contrast and a 3 x 3 averaging filter applied to reduce noise. a) Ca. b) Na. c) K. d) the ratio Sr/Ca. The arrow identifies a radial line that emanates from the otolith center.

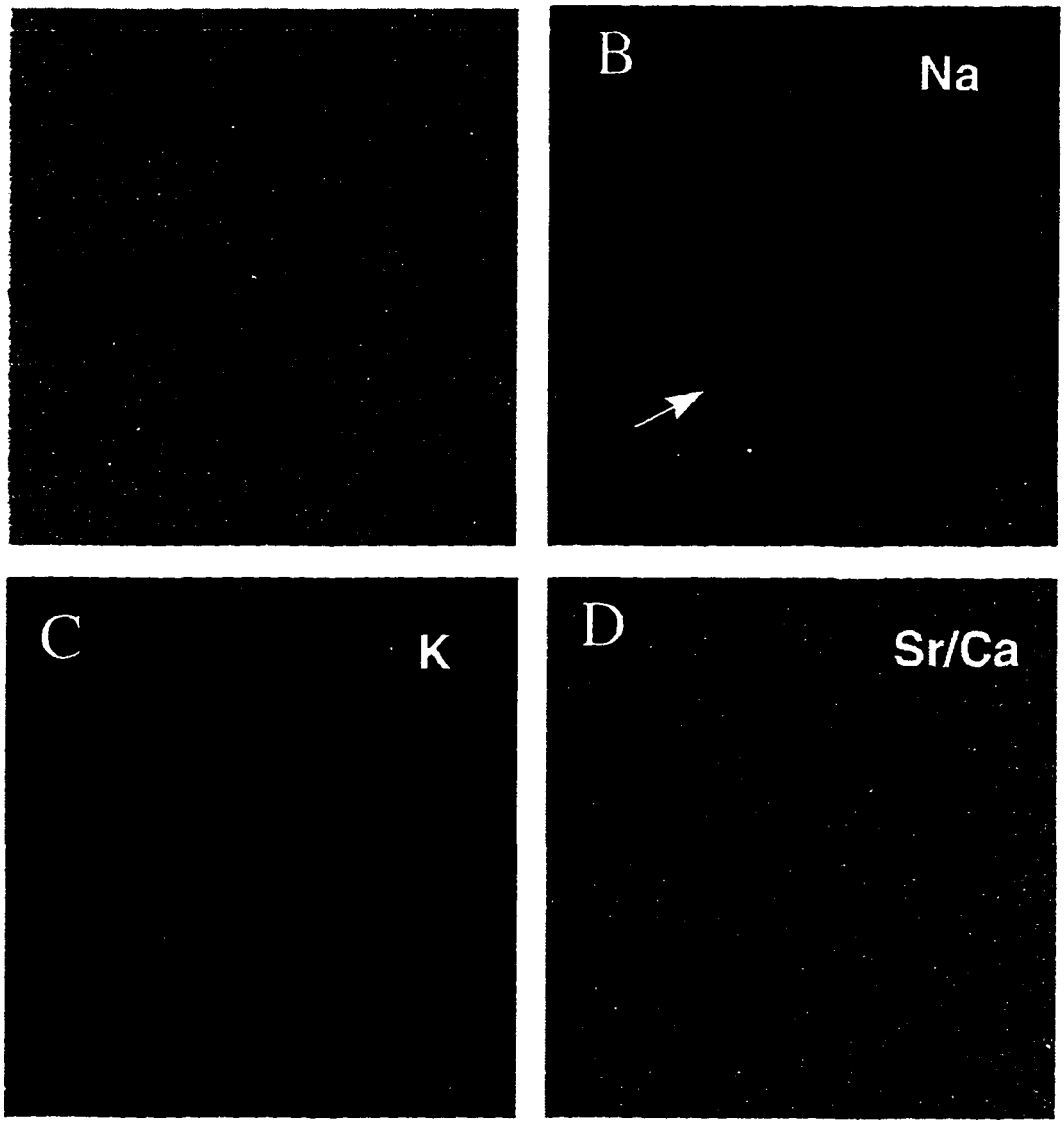

Figure 4-6. X-ray intensity maps showing the relative concentration of elements in juvenile halibut otolith from Shelikof Bay. Each map covers a 512 μm by 512 μm region. The image gray scale was stretched to increase contrast. a) Ca. b) Na. c) K. d) the ratio Sr/Ca. The arrow points out boundary line separating crystal growth fields that becomes most apparent with a change in Na levels.

center. Outside the otolith center the Sr/Ca ratios were not significantly autocorrelated. The X-ray intensity map of Sr/Ca ratios (Figure 4-5d) clearly showed two concentric patterns. The ratio is highest in a span approximately 60 μm in diameter with a further drop at 160 μm. In larval otoliths, Hagen (1986) observed a check ring in the microstructure consistently at 57 μm and hypothesized that it corresponds to end of yolk-sac absorption and first feeding. This ring was also constantly observed in juvenile otoliths prepared for this study. Outside this larval area, no concentric patterns of Sr/Ca are evident in the X-ray maps of the Shelikof Bay juveniles (Figure 4-6c).

Sulfur concentrations, as seen in the dorsal - ventral line transect (Figure 4-4), are also elevated in a 160 μm span in the otolith center, which corresponds to an otolith diameter of a late larval stage halibut approximately 20 mm S.L. (Hagen 1986). Outside this span the concentrations of S dropped below the level for accurate detection (Table 4-1). In the anterior - posterior axis, levels of S were elevated in a broader area, but again dropped to low levels. It appears that S is distributed asymmetrically in the otolith. No X-ray intensity maps were made of S, however, due to limitations on the number of detectors and time constraints.

From the transect data, levels of the element K were significantly autocorrelated (Lag 1: $R > 0.40$), but exhibited only slight symmetry around the otolith center (Figure 4-4). The X-ray maps, however, from both the Patton Bay (Figure 4-5c) and the Shelikof Bay juveniles (Figure 4-6c) show some concentric banding during the late larval stage.

Unlike K, the levels of Na were not significantly autocorrelated along either transect. However there were two peaks next to the otolith center in the anterior - posterior axis but not along the dorsal-ventral transect (Figure 4-4). In contrast to the line transect data, the elemental map of Na from the Patton Bay juvenile (Figure 4-5b) indicates subtle concentric banding at a similar location as Ca. Slightly elevated concentrations of Na also occur near the central primordia and both Na and K show elevated concentrations associated with a radial line emanating from the primordia (Figure 4-5b,c indicated by arrow). This line is not an artifact due to preparation, as is the dark line that transverses the Ca map

(Figure 4-5a). Rather it appears to be associated with the boundary lines that separate crystalline facets that grow out of the nucleus. X-ray maps from the Shelikof Bay juveniles also show concentrations of Na associated with the boundary line that delineates the larval crystal growth from that which stems from accessory primordia (see chapter 5) (Figure 4-6b).

The distributions of elements in the adult otoliths provide a larger scale view of elemental variation. Elemental concentrations along the transect of a cross-sectioned otolith from a six year old are similar to the juvenile otolith, in that K is significantly autocorrelated (Lag 1: R = 0.361), while Na is not. For both elements, the concentrations appear generally stationary from the nucleus to the edge. The ratio Sr/Ca, however, is significantly autocorrelated (Lag 1: R = 0.63), and it has a significant autocorrelation up to lag 10. The variation in the amount of Sr/Ca along the transect line can be seen in Figure 4-7. The patterns however do not appear to be associated with translucent or opaque zones seen with the luminescence profile using a transmitted light microscope.

The X-ray map of the second adult halibut otolith was taken along the proximal face of the otolith (Figure 4-8a-c). The map covered the first five annuli (the translucent zones) in the otolith as seen with the transmitted light microscope (Figure 4-8a). The nucleus of the otolith was not exposed, because doing so at this viewing plane would have ground through most of the latter annuli. Similar to the transect data, K and Na showed little variation in intensity, however levels of both of Ca and Sr/Ca appeared to vary in association with the annuli. Calcium was in low concentrations in narrow bands that seem to be associated with check rings and was in higher concentrations corresponding to the opaque zones (Figure 4-8b). The ratio Sr/Ca also showed banding associated with the number of annuli; however the concentrations appeared highest just to the inside of the check rings (Figure 4-8c). The banding was weakest at the first annulus, perhaps due to some overlaying material on the otolith center, but it appeared more prominent by the third annulus. The white spots in the Sr/Ca map are a result of the dividing the Sr count at that spot by a Ca count of zero; it does not indicate a high count of Sr.

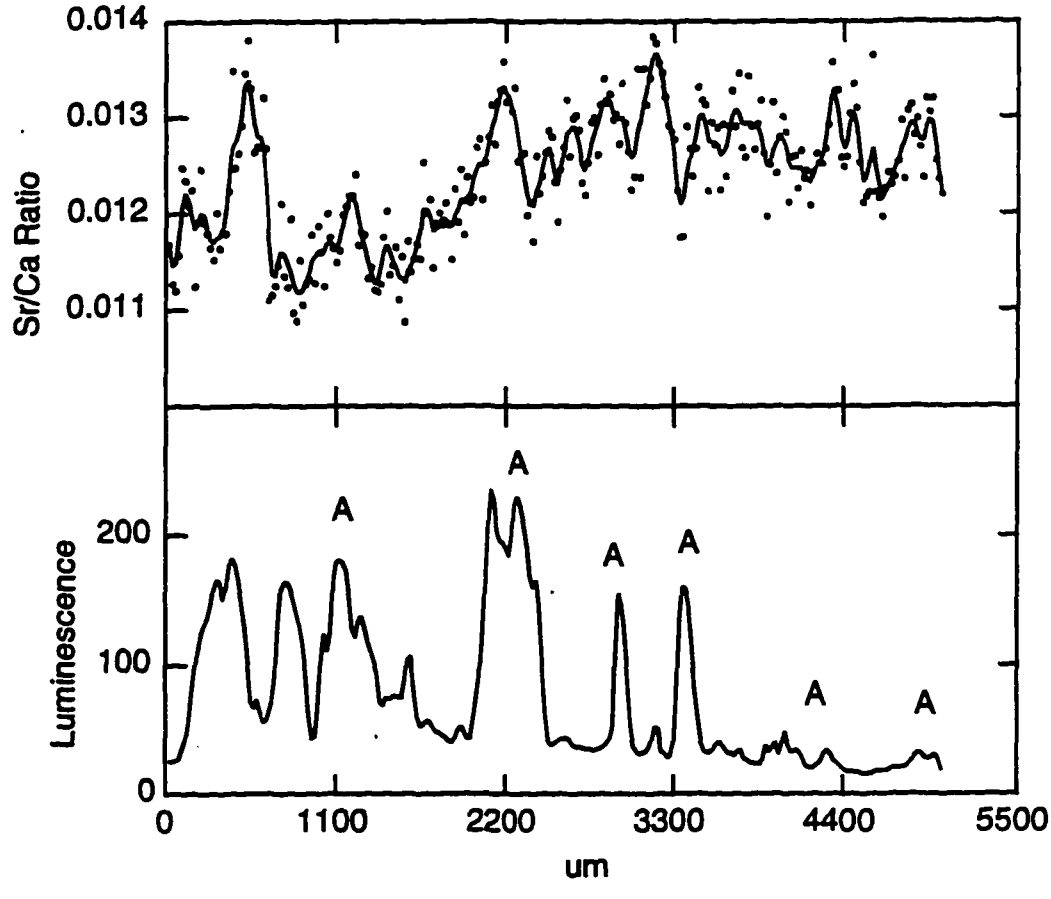

Figure 4-7. Sr/Ca ratios along a line transect from the nucleus to the anterior edge in the cross section of a six old halibut with a lag three moving average. Luminescence profile along the same axis using an 8 bit gray scale. Photo taken with transmitted light source. Approximate location of apparent annuli indicated by letter A.

Figure 4-8. Adult halibut otolith used in X-ray scanning, with the polished proximal surface exposed. (a) Transmitted light photograph, the five points are adjacent to the five translucent zones (light bands), that in halibut are interpreted as annuli. (b) X-ray intensity map of Ca concentrations. The image gray scale was stretched to increase contrast and a 3 x 3 averaging filter applied to reduce noise. (c) X-ray intensity map of Sr/Ca ratios. The image gray scale was stretched to increase contrast and a 3 x 3 averaging filter applied to reduce noise.

Stock Separation

To evaluate the use of these trace elements for stock separation, I first applied the full model ANOVA to all data sets for the purpose of determining what factors are likely to be important sources of variation. Because not all otolith pairs within each individual were available for examination, there were missing cases in the design. To compensate yet allow for statistical testing, the degrees of freedom used in calculating mean square estimates were reduced (Wilkinson et al. 1996). This has the consequence of reducing the power of the tests for detecting statistical significance.

The results from the full model tests of the larval portion of the otoliths indicate that the area of capture did not explain significant amounts of variation for K and Na (Table 4-3). However, p-values were only slightly above 0.05, so larger sample sizes might have given the test more power to detect differences. For Sr/Ca and S models the p-value was quite high indicating that little to no variation can be attributed to the capture area. The only other factor which showed significance was individuals-within-area in the S model.

Table 4-3. The result from the larval portion of the otolith showing the F-ratio, degrees of freedom, and the p-value for each element and source of variation from the full model ANOVA. The degree of freedoms varied with the different models due to missing cases and an adjustment based on least squares estimation.

	Na		K		Sr/Ca		S	
Source	F-ratio (df)	p	F-ratio (df)	p	F-ratio (df)	p	F-ratio (df)	p
Area	5.831 (2, 4)	0.065	8.652 (3, 3)	0.054	0.042 (1, 5)	0.844	6.555 (1, 5)	0.922
Side	0.000 (1, 7)	0.987	0.040 (1, 7)	0.848	0.159 (1, 7)	0.701	0.150 (1, 7)	0.709
Area*Side	0.276 (2, 7)	0.766	0.072 (2, 7)	0.931	0.066 (2, 7)	0.936	0.020 (3, 7)	0.995
Indiv[Area]	0.766 (4, 31)	0.555	1.368 (3, 31)	0.271	0.740 (5, 31)	0.599	3.826 (5, 31)	0.008
Side*Indiv[Area]	1.103 (7, 31)	0.386	1.555 (7, 31)	0.187	0.678 (7, 31)	0.689	1.329 (7, 31)	0.271

The results from the full model for the juvenile portion (Table 4-4) indicated that area of capture was a significant factor for explaining K and Na variation, but not Sr/Ca. The variation associated with individual-within-area was significant for all three elements. The only other effect that had significant variation was the interaction of otolith side and individuals-within-area for K. Due to counts below the detection limits, S was not analyzed in the juvenile portion of the otolith.

Table 4-4. The result from the juvenile portion of the otolith showing the F-ratio, degrees of freedom, and the p-value for each element and source of variation from the full model ANOVA's. The degree of freedoms varied with each model as a result of least squares estimation due to missing cases.

	Na		K		Sr/Ca	
Source	F-ratio (df)	p	F-ratio (df)	p	F-ratio (df)	p
Area	4.354 (3, 7)	0.050	21.860 (3, 7)	0.0006	0.889 (3, 6)	0.499
Side	0.136 (1, 6)	0.725	0.0765 (1, 7)	0.780	0.024 (1, 8)	0.882
Area*Side	0.019 (3, 6)	0.996	0.0204 (2, 7)	0.980	1.087 (2, 8)	0.382
Indiv[Area]	2.457 (7, 83)	0.024	2.442 (7, 83)	0.0251	2.579 (6, 83)	0.024
Side*Indiv[Area]	0.595 (6, 83)	0.733	3.002 (7, 83)	0.0073	0.193 (8, 83)	0.991

Neither otolith side nor the interaction of otolith side and area was a significant factor in any of the models examined and the p-values associated with those terms were quite high. The only term in which otolith side did play a role was in the interaction with individuals-within-area in the juvenile model for K. In that model the significance of the term indicates that a measurable amount of variation within-individuals is explained by grouping the data as to either the left or right otolith. But since there is no systematic difference between the left and right otolith outside the individual, otolith side here provides a measure of within-individual variation with the remaining error term containing the variation within otoliths.

The lack of a systematic difference between right and left otoliths, but the presence of a significant interaction term in one of the models, provides justification for examining the data in terms of a nested design. This is reasonable from a biological perspective. The process by which elements are incorporated into the otolith crystal involve pathways of ion transport through the blood and endolymphatic system to deposition on the otolith crystal. The pair of otoliths taken together provide a measure of variability in this pathway within the same fish. In the absence of any systematic difference between fish, the left or right otolith is essentially interchangeable with respect to the other in that neither one represents a fixed level within the fish. This idea is reflected in how the terms are used in the mixture model. Otolith side by itself is considered a fixed effect, but since individuals collected within areas were randomly selected, the interaction of the terms is also a random effect.

The results of fitting the data to a nested model and calculating the percentage of variation explained by each factor level indicated a similarity between the larval and juvenile regions of the otoliths for the elements Na, K and the ratio Sr/Ca, even though they represent broadly different physiological stages and different geographic locations (Table 4-5). For the ratio Sr/Ca and the element S, the majority of variation is contained within the otoliths and essentially no variation is attributable to the nursery area of capture. The ratio Sr/Ca and the element S have the highest variation attributed to the individual-within-area. For the element K, the majority of variation is explained by the nursery area, but some variation occurs between and within individuals. For Na the variation explained by the within-area and within-individual factor levels are different in the larval and juvenile models, but the other factor levels are essentially similar.

Table 4-5. Percentage of variance components explained by each factor level for Na, Sr/Ca, K and S in the larval and juvenile models. Sulfur concentrations were below detectable limits in the juvenile portion of the otolith so no model was estimated.

	Na		K		Sr/Ca		S
SOURCE	Larval	Juvenile	Larval	Juvenile	Larval	Juvenile	Larval
Area	35.3%	35.9%	66.4%	69.9%	0.0%	3.3%	0.0%
Indiv[Area] (within Area)	0.0%	13.7%	9.1%	2.3%	26.9%	17.2%	19.9%
Side[Indiv] (within Individual)	14.0%	0.0%	4.6%	7.0%	0.0%	0.0%	15.4%
Error (within Otoliths)	50.7%	50.4%	19.9%	20.9%	73.1%	79.5%	64.6%

The reason for the high variation explained by area with Na and K, and the marginal variation by area with Sr/Ca can be seen graphically with boxplots showing the distributions of the measurements grouped by area (Figure 4-9) (Chambers et al. 1983). For Na and K, both the larval and juvenile data show that the Bristol Bay sample does not differ from the 1958 Shelikof Bay sample but does differ from the Prince William Sound and Shelikof Bay 1987 samples. The two Shelikof Bay collections do not show much overlap with respect to Na and K, while the Sr/Ca ratios in all the areas show a good deal of overlap.

Discussion

Distribution of elements within otoliths

Though only a few juvenile otoliths were examined for patterns of element depositions, both the line transect data and the X-ray intensity maps clearly show that the distributions of most elements are not uniform during larval and juvenile growth. Of the two approaches, the X-ray maps provide a superior means of detecting patchy distributions of elements and for pulling out subtle concentric patterns that might otherwise be difficult to detect with a single transect line. X-ray maps require more resources and time to complete; however they can also save effort in trying to interpret how elements are distributed. With the exception of Sr mapping (e.g. Sadovy and Severin 1994), results from elemental mapping have not been reported in the otolith literature.

The association of trace ions with structural features, such as Na with the boundary line between the larval and juvenile fields of crystal growth, has not been reported in other studies. Because these structural features form as a result of otolith growth over a period of time, the concentration of ions at these locations is unlikely to be related to environmental conditions or to the individual's particular geographic location. The mechanisms of otolith growth that might allow the deposition of trace elements are not well understood. The inorganic portion of otoliths is composed of polycrystalline aggregates of

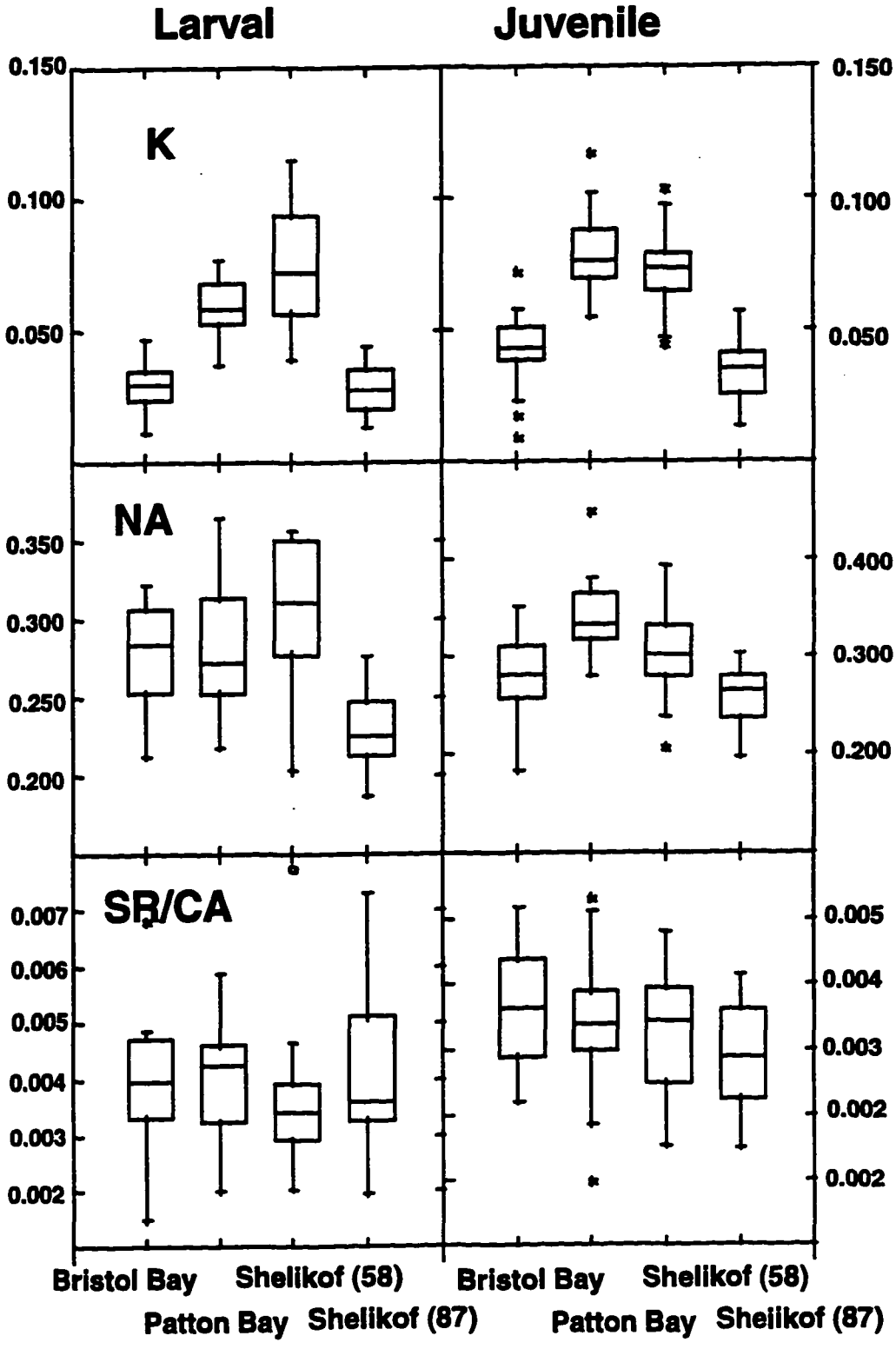

Figure 4-9. Boxplots showing the distribution of Na, K and Sr/Ca by relative amounts grouped by area for both the larval and juvenile portions of the otoliths.

calcium carbonate in aragonite form (Lowenstam and Weiner 1989). The precipitation of Na and K ions may occur in the interstitial space between separate prisms or cones of aragonite. These ions may also be associated with one of the constituent proteins that make up 0.3 to 10% of the otolith by weight (Degens et al. 1969). Many organisms use proteins to promote or inhibit mineralization along specific axes as a means to control the overall shape of the structure (Lowenstam and Weiner 1989). In conjunction with specific proteins, these trace ions, which have an ionic radius smaller than that for calcium, could play a role in determining the shape of the otolith. However, without further investigation of otolith protein composition (e.g. Gauldie et al. 1990, Asano and Mugiya 1993) and the crystallography of otoliths (Radtke and Shafer 1992), information about such roles must remain speculative. As an aside, sulfur, which was observed at only small levels in the larval period of otolith growth, is also a constituent of one of the amino acid side chains that have been identified in some otoliths (Asano and Mugiya 1993).

The concentric patterns of the elements Ca, Na, and K during larval growth are similar to patterns observed by Thresher et al. (1994). Toole et al. (1993) also noticed elevated Sr/Ca ratios near the otolith center using line transect data on Dover sole otoliths, suggesting that these patterns are not species specific. In addition, the otolith primordium was high in protein and low in calcium, which has also been noted by others (Thresher et al. 1994).

The correspondence of elevated levels of Sr at a diameter similar to where a check ring occurs might be an indication of yolk-sac utilization. Kalish (1990) showed that elevated Sr of a parent can be supplied through vitellogenesis to yolk of the progeny in salmonids. A similar mechanism may explain the distribution of Sr in halibut otoliths: where the drop in Sr levels is an indication that all the yolk has been utilized, and Sr from other sources is not in as high a concentration as that available from the maternal source.

No strong elemental pattern appeared to be associated with the location on the otolith that might indicate a metamorphosis or settlement mark. Otoliths from transitional-state halibut larvae or newly settled

juveniles were not available to determine a mean size of the otolith at settlement. The initiation of accessory primordia, which has been observed in late stage halibut larvae (Hagen 1986), appears too variable to be a reliable indictor of settlement (Chapter 5). In a similar search for settlement marks in Dover sole, Toole et al. (1993) did not observe any specific change in Sr/Ca ratios that would indicate a change of habitat.

In the adult halibut otoliths, the X-ray maps of Ca and Sr/Ca bands coincided in number and placement to the annuli seen with light microscopy. The line transect data, however, did not produce a good match with what appeared to be annuli zones. The association of Sr/Ca ratios with annuli marks has been seen in other species (e.g. Sadovy and Severin 1994, Lecomte-Finiger 1992, Secor 1992, Toole et al. 1993), but in some individuals these associations are noted as not always being consistent (Sadovy and Severin 1994). Difficulties both in interpreting annuli marks and the limited information obtained from a single transect line could explain the discrepancies.

Variation of Sr/Ca ratios in otoliths has been associated with the onset of spawning (Kalish 1991). Since first spawning halibut are thought to be older than age 5 (Schmitt and Skud 1978), the banding seen here is likely associated with seasonal temperature changes and / or changes in growth rates (Sadovy and Severin 1994). The number and seasonal timing of opaque and translucent or hyaline zones in otoliths are not well defined for many species (Beckman and Wilson 1995). While the mechanisms that give rise to Sr and Ca variation in otoliths are not well understood (Radtke and Shafer 1992), matching this variation with variation in annuli patterns may help in interpreting both sets of patterns. In addition, Sr/Ca ratios could help in investigating alternative methods of identifying the onset of sexual maturity through the otoliths (Rijnsdorp and Storbeck 1995).

Stock Separation

Though sample sizes were small, significant differences by area were found for the elements Na and K. Thus measuring concentrations of Na and K in either larval or nursery area regions of the otolith has

potential to identify different components of the adult halibut population. Potassium in particular appears to have the strongest potential based on the relatively small within-otolith variation and the high variation among areas. The presence of some small variation between the left and right otoliths within individuals raises some questions as to the mechanism that gives rise to the variation among areas.

It is possible that the differences in the elemental concentrations between the fish from the different nursery areas could arise as a result of differences in ocean chemistry. However the elements examined here are common constituents of seawater, and variation in salinity is generally minimal in oceanic waters: an observation that has been made by others (e.g. Edmonds et al. 1995). This does not preclude the utility of examining trace elements in either the larval or the juvenile portion of the otolith for stock separation, but the mechanism that give rise to the observed differences are not likely to be directly related to water chemistry.

In a similar study also using the WDS microprobe, Thresher et al. (1994) found differences between nursery area fish, but also saw correlation within otoliths on the level of elements found early in life. The authors suggest that there is a 'base level' of elements within individuals which may be modified by ontogenetic changes but persists throughout the first few years of the fish's life. They propose that these levels in the otoliths may be genetically determined or stem from some type of entrainment resulting from the fish's initial exposure to the concentration level of elements.

The results of this study are consistent with the observations of Thresher et al. (1994). For both Na and K, there is a similarity in the variance components for larval and juvenile portions of the otoliths. Either location could serve to identify halibut to nursery areas, despite the fact that larval halibut have a long period of larval drift and presumably encounter diverse conditions. Ontogenetic changes in element concentrations appear to be common during the early larval stages. Similar trends as those shown in the quantitative transects and mapping were also found during non-quantitative exploratory examinations of other otoliths. In addition, there was no apparent shift in element concentrations that would indicate a transition to larval settlement has taken place, and counts of Sr/Ca and K were also autocorrelated along

the transect lines. These observations support the conclusion that there could be a connection between the level of element concentrations in the larval and nursery areas that is unrelated to changes in habitat.

To distinguish between the two explanations for elements appearing at a 'base level' in otoliths, Thresher et al. (1994) suggested looking for year-class differences within nursery areas. Individuals from separate year-classes in the same area presumably have a similar genetic makeup, but are likely to experience different environmental conditions early in life. In this study, the 1958 and 1987 Shelikof Bay samples did not have similar amounts of Na or K. This would support the entrainment hypothesis over the genetic hypothesis to explain 'base level'. Because the 1958 samples had been stored in glycerin for close to 30 years, the possibility of storage contamination or leaching of trace ions cannot be ruled out as an alternative explanation. I found no indication through EDS or WDS examination that there was infiltration of trace elements into the otolith, but any leaching of the ions, if associated with proteins soluble in a glycerin and water solution, would not be detected.

In the relatively short history of microchemistry analysis of otoliths, initial promise has been tempered by indications that the complexity of methodology and the nature of otolith development can confound any simple interpretation (Jones 1995). This cyclic process whereby a method may eventually be accepted or discarded depends upon the utility of the method for extracting meaningful information. The microprobe is now known to be limited to detecting the most common trace elements (Gunn et al.1992), and concentrations of these elements are known to be mediated by physiological factors (Kalish 1991). However, stock separation is feasible in some instances (Thresher et al. 1994, Severin et al. 1995).

The approach I used here, where I examined the percentage of the variance explained by each level, is particularly useful for evaluating the reliability of new techniques (Dunn 1989). It is common practice in many pilot studies to use discriminant analysis methods for stock separation, relying on a single set of variables to characterize each specimen. Such approaches can be useful where there is a large number of samples from each area and all the areas of interest have been sampled. However those methods don't necessarily provide insights into the mechanisms that give rise to differences or indicate potential

difficulties of within sample variability. A comparison of the variance components between the elements studied here suggests that each likely has a different function or reason for its presence within the otoliths. When trying to use this information as indicative of geographic location, such differences need to be addressed.

The elements, Na and K, have potential to identify halibut that use the Bristol Bay nursery areas from those that reside in the Gulf of Alaska. The data do not have to be collected from a particular location on the otolith, though the period of nursery area residence is recommended. To reduce variation, beam size could also be increased to integrate the counts over a larger area. These elements may not prove sufficient if follow-up work finds that there is some significant within-area variability. New methods are now becoming available for detecting trace elements at specific locations on otoliths at much lower concentrations than possible with the microprobe (e.g. Fowler et al. 1995). Additional elements could provide a more robust means for separating stocks; however similar cautions must be taken in regard to interpreting their presence in otoliths. Variance component analysis, along with consideration of otolith crystallography, can provide a useful means for selecting trace elements as candidates for stock discrimination.

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Chapter Five [1] Evaluating crystalline shapes in juvenile Pacific halibut otoliths as a stock separation tool.

Abstract

The otolith microstructures of young Pacific halibut (*Hippoglossus stenolepis*) contain distinctive polygonal patterns associated with transition from late larval to juvenile stages. These patterns are derived from the placement of secondary sites of nucleation and mark the boundary between different fields of crystalline growth. Variations in shape and orientation of the patterns were examined to determine if they could be used for stock separation. Sagittal otolith pairs were taken from six newly settled halibut from three widely dispersed nursery areas in Alaska. Fifteen measurements were collected from each otolith. A partial nested analysis of variance model contrasted the pattern variation between otolith pairs with that between individuals and areas. The results indicate that the mechanisms which cause the pattern formations are not under tight biological control and there is little utility in measuring the patterns to identify the halibut population according to their nursery areas. It is observed that sites of secondary nucleation are frequently associated with the junction of separate crystal fields that arise from the otolith's center; suggesting that the diversity of patterns may arise from sensitivity to initial conditions.

Introduction

Pacific halibut (*Hippoglossus stenolepis*) is a large, long-lived flatfish with a wide distribution in the North Pacific Ocean that extends along the continental shelf from Santa Barbara, California to Hokkaido, Japan (IPHC 1978). Larval halibut are pelagic for several months (Thompson and Van Cleve

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1936, St-Pierre 1989) and during that time their transport to nursery areas is dependent on wind- and buoyancy-driven circulation (Parker 1988). From tagging studies of juveniles it is known that a significant proportion of the population may undertake extensive migrations (Hilborn et al. 1995). This juvenile migration is thought to be a mechanism by which halibut compensate for larval drift and maintain dispersed spawning populations (Skud 1977).

Management of halibut is based on a catch-quota system that identifies broad regions as separate management units. However, uncertainty about migration rates of halibut between these regions can cause significant adjustments to the population models which are used to estimate yields (Quinn et al. 1990). In addition, by-catch of juvenile halibut by the trawl fishery is of increasing concern because of its potential effects on recruitment to the longline fishery (Sullivan et al. 1994). Estimating this loss to the different management units requires knowledge of migration rates of the juveniles. Currently, the only estimates of migration of halibut stem from tagging studies (Quinn et al. 1990, Hilborn et al. 1995). However, differential tagging mortality, tag-induced behavioral effects, and incomplete reporting of tag recoveries add uncertainty to those estimates. In addition, tagged juveniles are likely to be in the process of migration, making it difficult to identify site-specific patterns to migration.

An ideal tag would be a natural marker that could identify the fish to its nursery area of origin, or the spawning grounds from which it originated. This study was conducted to determine if a microstructure pattern formed in otoliths during the first year of life could be used as a tag to identify halibut to area of origin. Otoliths are an ideal candidate for examination, because unlike other sites of calcium deposition, calcium carbonate in otoliths is generally not reabsorbed (Campana and Neilson 1985). The deposition patterns which form early in life can be recovered in older fish by removing the overlaying material.

In halibut, similar to other marine fish, the otolith starts to grow from a central nucleus, and in late larval stages, forms secondary sites of nucleation (Hagen 1986). Campana and Neilson (1985) referred to nucleation sites as primordia; the secondary nucleation sites are called accessory primordia and the central nucleus may be composed of one or more primordia. In other flatfish, accessory primordia have

been shown to correspond to the times of metamorphosis from larval to juvenile stages (Toole et al. 1993, Sogard and Able 1992). Eventually growth from the accessory primordia encases the growth that stems from the central primordia, and a boundary line demarcating the two fields of growth appears as a complex polygon (Figure 5-1). This line is quite prominent when viewed along the sagittal plane of the otolith and readily recovered from older fish. Variation in this shape and its association with nursery area are examined in this paper. If shape can be used to identify halibut to nursery area, it may be possible to recover that information from adult halibut otoliths which are collected for age-structured population analysis (Quinn et al. 1990).

For the purposes of this paper, the growth stemming from the central primordia, which is encased by the boundary line, will be referred to as the larval crystals. The growth stemming from the accessory primordia will be referred to as the accessory crystals. This terminology, though not common to otolith discussions, reflects a classification that is similar to the descriptions of other biomineralized structures (Lowenstam and Weiner 1989).

The approach taken here is to extract measurements of halibut larval crystals collected from different nursery areas and to include both the left and right otoliths in the analysis. An analysis of variance model is then used to contrast the pattern variation between otolith pairs with that between individuals and areas. By using otolith pairs it is possible to determine the extent that pattern formation is under biological or environmental control or whether it is unrelated to extraneous sources.

Methods

Juvenile halibut (age zero) from three nearshore areas in Alaska were collected for this study. The sites were nursery areas in Bristol Bay, Prince William Sound, and Southeast Alaska. Left and right sagittae otoliths from six individuals from each area were removed and mounted sulcus side up on glass slides using thermoplastic resin. The otoliths were ground using series of silicon carbide paper (500, 1200 grit), followed by 9 micron lapping paper with a 0.3 micron alumina polish to expose the otolith

Figure 5-1. Larval crystal of a halibut otolith observed using Nomarski interference contrast illumination.

crystal. The surfaces of the otoliths were examined using reflected bright field and interference contrast (Nomarski) illumination. Under these light sources the boundary lines demarcating the larval crystal were easily observed. Interference contrast, in particular, provided a perspective of topographic relief to what is essentially a smooth plane. When it is gradually exposed during the grinding sequence, the larval crystal achieves its largest size when the exposed plane includes the otolith nucleus. To determine an estimate of measurement error that was likely to be encountered with small differences in grinding depth, measurements were made on a subsample of otoliths at various points in the grinding sequence. Based on these observations, there appeared to be less than 2% difference in linear measurements between slightly different grinding planes that included the otolith nucleus. Given this consistency of the measures, a single measurement plan through the nucleus sufficed and for the purpose of this study no attempt was made to quantify the crystal shapes in three dimensions.

Measurements of larval crystal shape were collected with the aid of an image processing program (Optimas Corporation, 190 West Dayton Street, Edmonds WA., USA 98020). A line drawn from the nucleus to the anterior axis of the otoliths, as indicated by the farthest point along the rostrum, was given an angle of zero. Fifteen measurements were taken on each otolith. Three of the measurements included angles in relationship to the reference line, five were linear measurements of distance, two were indices of shape, one was the measurement of area, and four were the moments of a distribution of linear measurements (Table 5-1). The data were collected for each otolith by tracing the shape of the larval crystal and identifying the location of the central nucleus. From the outline of the larval crystal, the area contained by the outline was obtained, along with the length of the major axis, its breadth, and the distance and orientation of the nucleus to the area's center of mass (Figure 5-2). These measures provide a degree of asymmetry in the growth of the crystal. Also obtained from the larval crystal shape were two indices, circularity and rectangularity which measure similarities of the shape to a circle and rectangle (Table 5-1).

Table 5-1. Suite of measurements taken for each larval crystal shape.

1.	**Area:** size of the larval crystal (μm^2)	9.	**MinRadiAngle:** angle of MinRadi from reference line (degrees)
2.	**Circularity:** the ratio of the area perimeter squared divided by the area (unitless)	10.	**MaxRadi:** longest radial distance from the nucleus to the crystal boundary (μm)
3	**Rectangularity:** the ratio of the crystal area to the area of a containing box oriented along the longest axis (unitless)	11.	**MaxRadiAngle:** angle of MaxRadi from reference line (degrees)
4	**MajorAxislength:** the length of longest axis through the area (μm)	12.	**MeanRadi:** mean of all radial distances (n=360) from nucleus to the crystal boundary (μm)
5.	**Breadth:** the width of the otolith measured perpendicular to the major axis (μm)	13.	**SDRadi:** standard deviation of distribution of radial measurements
6.	**COMdistance:** distance from the area center of mass to the nucleus (μm)	14.	**SkewRadi:** skewness of distribution of radial measurements
7.	**COMangle:** angle of COMdistance to reference line (degrees)	15.	**KurtRadi:** kurtosis of distribution of radial measurements
8.	**MinRadi:** shortest radial distance from the nucleus to the crystal boundary (μm)		

A second series of measurements was obtained by collecting 360 radial measurements in one degree increments from the nucleus to the outline of the larval crystal (Figure 5-3). The mean, variance, skewness and kurtosis of these measurements were calculated. In addition, the minimum and maximum radial measurements were recorded along with their angles with respect to the reference line.

To analyze the data I used a combination of exploratory data analysis, methods for analyzing directional data, and analysis of variance (ANOVA) models. I used both otoliths in the analysis, even though in halibut, similar to other flatfish, the otoliths are asymmetric with the right otolith being thicker than the left otolith. This asymmetry can complicate the model design. The advantage of using both otoliths,

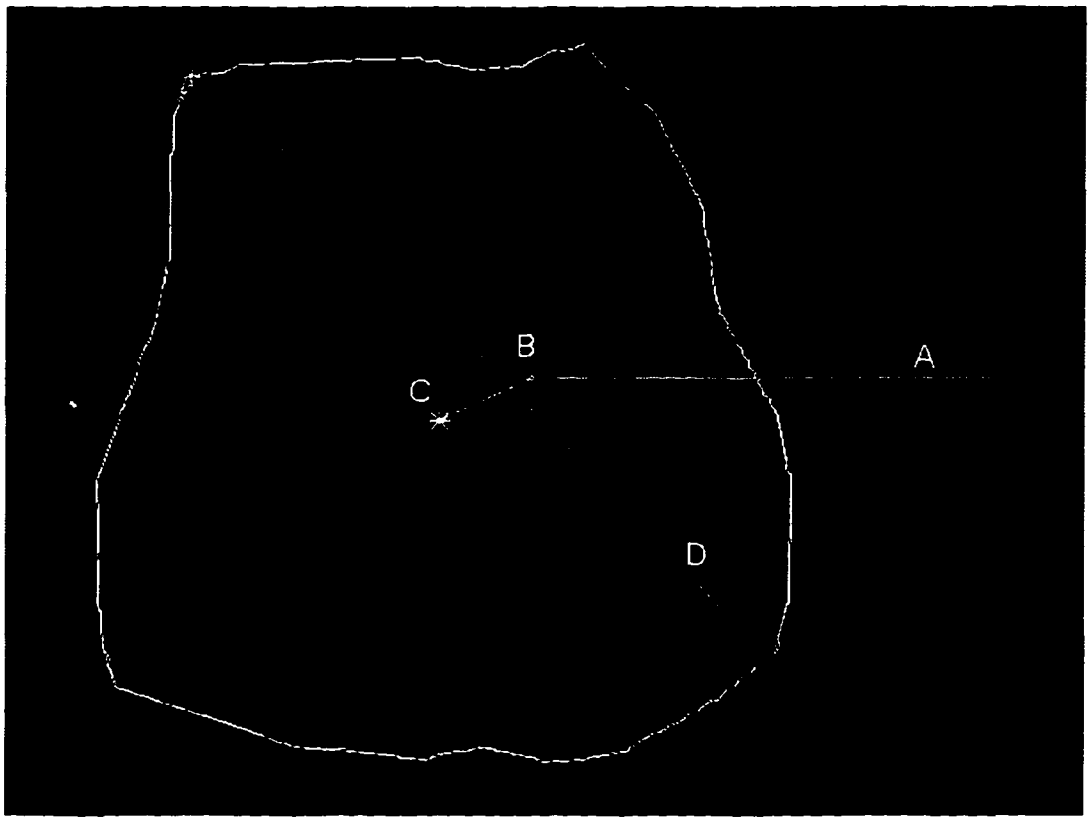

Figure 5-2. Digitized image of a larval crystal of a halibut otolith using Nomarski interference contrast illumination. Outline of larval crystal shape is indicated by the white line. Reference line A indicates direction towards anterior axis. Point B shows location of central nucleus. Point C indicates the center of mass of the area of the larval crystal. Measurement COMdistance (#6 in Table 1) is the distance C to B. COMangle (#7) is the angle, counterclockwise, of ABC. Line D is the MajorAxisLength (#4), as determined by larval crystal shape. Breadth (#5), not shown, is width of the area at 90 degrees to the MajorAxisLength.

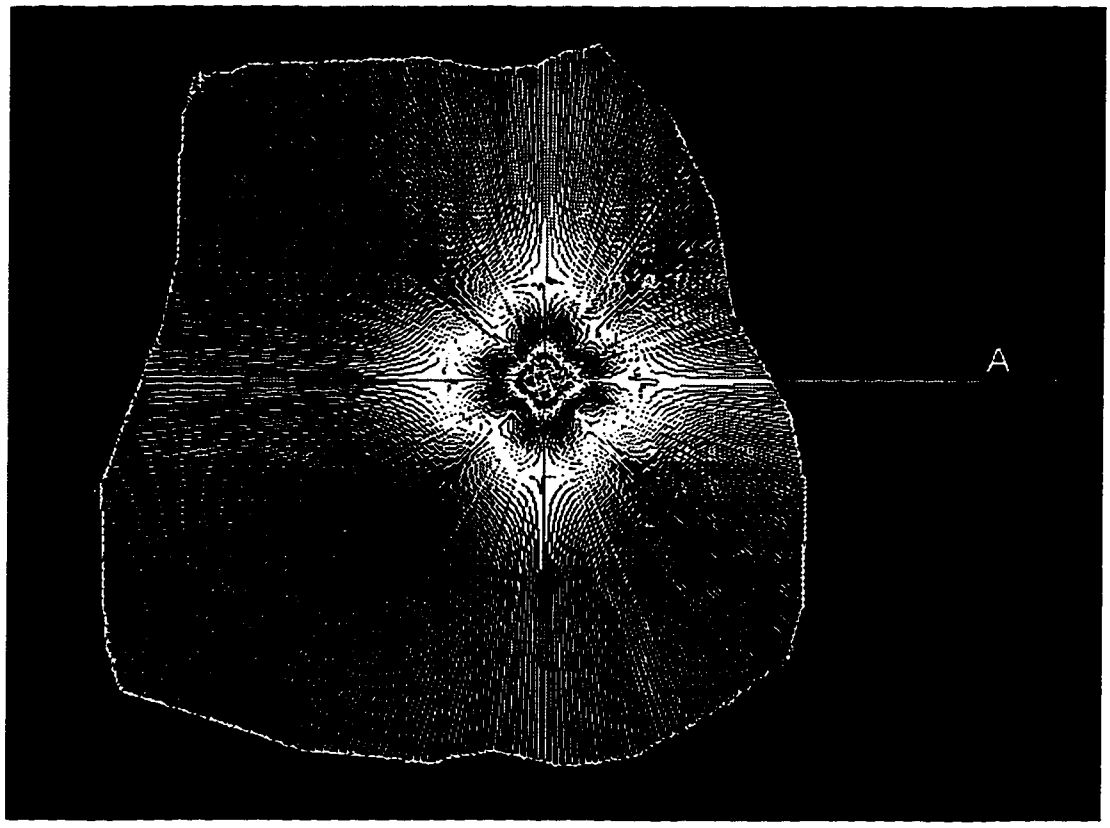

Figure 5-3. Digitized image of a larval crystal of a halibut otolith using Nomarski interference contrast illumination. The 360 radial lines emanate from the central nucleus and extend to the boundary of the larval crystal. The minimum and maximum length radial lines were extracted including their angle with respect to the reference line A.

however, is that it provides a measure of variation found within an individual and this can assist in understanding the processes which may ultimately control the variation in pattern.

The three angle measurements were examined for mean angle, dispersion, and Rayleigh's test of significance (Batschelet 1981), for the different factors of otolith side and capture location.

For the other twelve measurements, I examined the extent to which variation in the shape can be explained by the effects of location, individual, and otolith side. I first checked for normality of each variable Y and applied a partial hierarchical nested analysis of variance model (Winer 1971). In this design, location L is treated as a fixed effect, individual I within location is treated as a random effect, nested by location, and the otolith side S is treated as a fixed effect which can have interactions with location and with individual within location The model can be written as

$$Y = C + L + I\,[L] + S + S{*}L + S{*}I\,[L]$$

where C is a constant and the brackets ' [ ] ' indicate the associated factor is nested, and ' * ' indicates an interaction effect between factors. Since there is no within-cell variation (i.e. only one otolith pair per fish), the model is complete and, as written, there is no error term. To test for the effects of location the F statistic $F_L = MS(L)/MS(I\,[L])$ is used, while to test for the other effects x, $F_x = MS(x)/MS(S{*}I\,[L])$ where MS is the mean squared error.

Because separate testing was done but the measurements were not independent of each other, I applied an adjustment to p-values based on a sequential modified Bonferroni procedure (Wright 1992) . This adjustment provides for a conservative test which avoids erroneous conclusions. An alternative to using separate tests is to apply a MANOVA approach, which can combine all measurements simultaneously and account for covariance of the dependent variables. Unfortunately the partial hierarchical nested design used here proved to be problematic and I was unable to determine if a MANOVA equivalent to this particular design has been developed. But given the broad suite of measurements examined, I did not feel a MANOVA test would reveal strong patterns that were otherwise undetectable using separate tests.

Results

Nomarski interference contrast illumination provided a perspective on the otolith morphology distinct from that commonly observed with transmitted light or even with scanning electron microscopes. While the method did not give an indication of daily increment deposition, it did show the boundary of the larval crystal, and in addition brought out other patterns that were unexpected. For example the cone that seems to surround the nucleus in Figure 5-1 was apparent in all specimens examined. The three dimensional appearance of the structure is somewhat an artifact of this type of illumination but it does indicate a difference in the refraction of light. This feature did not appear to coincide with an event such as metamorphosis or first feeding that might be expected from an examination of larval increments (Hagen 1986). One possible explanation is that it shows the location of the sulcus during the early larval stages, and the pattern is visible with Nomarski lighting because there is a shift or folding in the orientation of the crystalline axes at that point.

Since the purpose of the study was to see, in a practical sense, if there was some simple measure of the larval crystal that could be used to delineate stocks, a variety of measurements were taken from each otolith to ensure that all attributes of shapes were considered. Many of the measurements were positively correlated with one another. For example, area was significantly correlated, using a Bonferroni adjustment, with seven of the other variables. Other measures, such as breadth, showed fewer significant positive correlations. The indices circularity and rectangularity were not significantly correlated with any other measurement.

The three angle measurements were considered separately in the analysis due to the difficulties in trying to linearize circular scales (Batschelet 1981). In Table 5-2, I pooled the left and right otoliths from all nursery areas to see if there was a consistent direction for growth away from the nucleus (COMangle), and for the orientation of the minimum (MinRadAngle) and maximum (MaxRadiAngle) radial lengths.

There did appear to be a consistent direction associated with maximum radial length (Table 5-2), but no indication that the other measurements were different from a random distribution as indicated by Rayleigh's test (Batschelet 1981). When the data were broken down by otolith pair and nursery area (Table 5-3), Rayleigh's test was not significant for any of the groups, except that for the right otoliths from Prince William Sound, there was a tendency for the larval crystal to grow more toward the posterior axis of the otolith. In general though, the lack of consistency within any of the angle measures suggests that the orientation of the shapes is quite variable.

Table 5-2. Mean Angle a, dispersion s, the Rayleigh test statistic R, and adjusted probability level p, for tests of directedness, for each of the three angle measurements (see table 5-1).

	COMAngle	MaxRadiAngle	MinRadiAngle
Mean Angle (a)	154.02	172.00	47.22
Dispersion (s)	84.78	81.83	98.76
Test statistic (R)	12.05	12.98	8.15
Probability level	.08 > p > .04	.04 > p > .02	p > .40

From the ANOVA models of the other measurements no significant location effect was observed (Table 5-4). Knowledge of a fish's nursery area of origin does not explain any significant variation in the patterns, and thus the use of any of these patterns as a natural tag does not appear feasible.

Table 5-3. Mean angle with respect to anterior end of the otolith for three measurements of larval crystal shape, by location of capture and otolith side. No measurements were found significant ($p < 0.05$) after applying a Bonferroni adjustment for multiple testing.

	COMAngle		MaxRadiAngle		MinRadiAngle	
	Right	Left	Right	Left	Right	Left
B. Bay	152.6	89.3	146.3	164.7	15.9	125.7
PWS	64.2	169.3	63.8	161.8	75.4	22.2
SE AK	165.2	132.5	162.0	132.7	52.5	24.2

As with the location effect, there was no significant variation in the patterns that could be attributed to the fact that an otolith pair comes from the same individual (Table 5-4). In addition there was insignificant variation explained by the otolith side factor or its interaction with location. The only measure that appeared to have any significance using the Bonferroni adjustment was the index of rectangularity, in which the left otolith is, on the whole, closer in shape to square than is the right otolith. If that effect is indeed real, it could be a reflection, and an early indication, of the asymmetric differences in otolith thickness that is apparent in juvenile and adult halibut otoliths.

Table 5-4. Unadjusted and (modified Bonferroni adjusted) p-values for model $Y = C + L + I[L] + S + S*L + S*I[L]$, where L is nursery area location, I is individual and S is otolith side '' indicates significant at $\alpha = 0.05$*

Variable	L	I[L]	S	S*L
Area	.67 (1.00)	.91 (1.00)	.94 (1.00)	.70 (1.00)
MajorAxisLength	.64 (1.00)	.78 (1.00)	.78 (1.00)	.75 (1.00)
Breadth	.50 (1.00)	.82 (1.00)	.05 (0.55)	.01 (0.12)
Circularity	.14 (1.00)	.26 (1.00)	.41 (1.00)	.05 (0.50)
Rectangular	.76 (1.00)	.15 (1.00)	.003 (0.04)*	.44 (1.00)
COMdistance	.10 (1.00)	.99 (1.00)	.85 (1.00)	.74 (1.00)
Min Radi	.53 (1.00)	.07 (0.70)	.37 (1.00)	.01 (0.12)
Max Radi	.25 (1.00)	.80 (1.00)	.79 (1.00)	.75 (1.00)
Mean Radi	.85 (1.00)	.88 (1.00)	.78 (1.00)	.58 (1.00)
SD Radi	.09 (1.00)	.98 (1.00)	.66 (1.00)	.62 (1.00)
Skew Radi	.34 (1.00)	.05 (0.55)	.94 (1.00)	.80 (1.00)
Kurtosis Radi	.52 (1.00)	.03 (0.36)	.86 (1.00)	.14 (1.00)

The next lowest p-values were breadth and minimum radius length in the interaction effect of side and location (Table 5-4). In both cases the effect is due to differences in the shape of Prince William Sound otoliths (Figure 5-4). For breadth, the left otolith is larger than for the other two areas and for the minimum radius length, the right otolith is shorter than for the other areas. While these effects are not significant, they do suggest that some characteristics may distinguish Prince William Sound samples from the other samples. The other two areas, however, show no distinguishing variables which separate them.

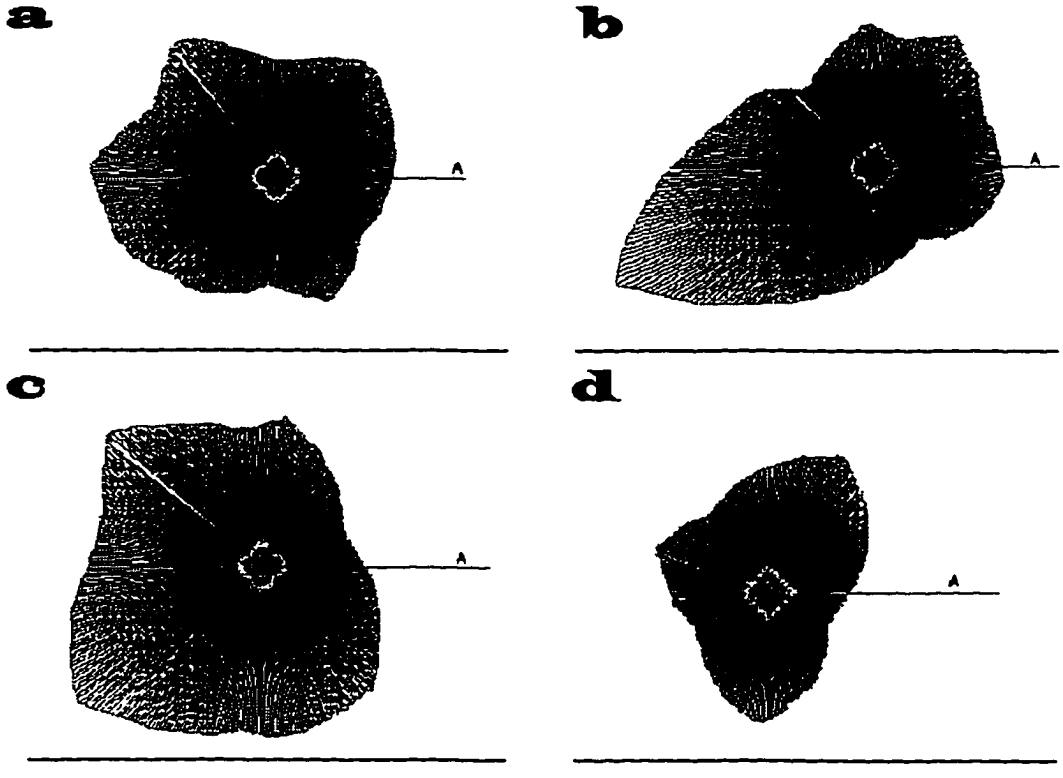

Figure 5-4. The radial lines extracted from the outline of larval crystal shapes from two pairs of otoliths collected in Prince William Sound illustrating the variability in shape of the crystalline patterns, with the left otolith (a) and the right otolith (b) of one halibut and the left otolith (c) and right otolith (d) of the other halibut. Line A is the reference line indicating the anterior axes of each crystal shape.

Discussion

Lowenstam and Weiner (1989), in their review of biomineralization, note that organisms from a wide variety of taxa may have large differences in the degree of control exerted during the mineralization of various structures. This control is exerted in the number and sites of nucleation, the differential growth of particular crystal facets, and the resulting crystalline morphology. The authors go on to note that "even straightforward measurements of the dimensions of the individual crystals can provide important clues about the type of control exerted over crystal formation." (p. 105).

From the results of this study, the larval crystal shape was not a useful indication of a halibut's nursery area of origin. Furthermore, the general lack of systematic difference between left and right otoliths and high within-individual variation suggest that the processes that influence the formation of the larval crystal shape are not under tight biological control.

From the measurements in this study and from examining cross-sectioned otoliths, the larval crystals are spatially constrained by the accessory crystals in three dimensions. The shape of the crystals is determined by the relative differences in growth rates between the larval and accessory crystals, and the number, timing, and relative placement of the accessory primordia. Faster growth in the accessory primordia is inferred from increments that pass through both crystal fields but have wider spacing in the accessory primoridia field than in the larval crystal field (P.T. Hagen, unpublished data). This is known to be a common phenomenon with other species (Toole et al. 1993, Campana and Neilson 1985). Faster growth is an indication that the accessory crystals are energetically more favorable sites of ion deposition and it may indicate that a shift has taken place in the direction of ion transport to crystals (Mann et al. 1989). Gauldie and Nelson (1990) propose a pH gradient as the mechanism which directs crystalline growth of otoliths.

The sites of the accessory nucleation were not explicitly measured in this analysis of shape, primarily because it would require a three dimensional perspective. However from Figure 5-4 it is reasonable to

infer that primordia are likely located close to where the outline of the crystal shapes are concave. The relative location of these sites help determine the final crystal shape.

Control over sites of nucleation is one of the primary means organisms use to form shape-specific biomineralized structures. Control is apparently maintained through the organic components. So-called macromolecules can at times inhibit or promote nucleation along particular crystallographic axes (Lowenstam and Weiner 1989). The rapid crystalline growth radiating from the accessory primordia, however, would tend to suggest that the molecular form of control, if it does occur, is inhibitory for the most part. Otherwise, we would expect to see more sites of nucleation than just the three or four which can be inferred from the outline of the larval crystal shape.

One observation from examining larval halibut otoliths (Hagen 1986) is that sites of accessory primordia frequently correspond to radial lines extending from the central nucleus. The central nucleus of halibut otoliths is likely composed of multiple primordia in close proximity which implies that the larval crystal is in fact composed of multiple prisms. The junctures of these different prisms might be energetically more favorable sites for new nucleation if there is a discontinuity in the crystalline surface. Under this scenario, the location of accessory primordia may in some part be related to the spatial orientation of primordia at the very start of otolith formation. Given the 3-dimensional nature of otolith formation it is difficult to determine if this observation is true in all cases of accessory primordia location, but to the extent that it is more than a chance association, it would indicate that final larval crystal shape is sensitive to conditions at the otolith's initial formation.

For halibut it appears that there is likely little functional need to tightly control the sites of secondary precipitation. Indeed given the function of sagittae otoliths for use in sound reception (Popper and Combs 1980), it is the outer shape of the otolith which likely provides utility to the fish. Perhaps not as critical are the details of how the otolith achieves that shape.

Extending these results of this analysis to other species may not necessarily be straightforward. Toole et al. (1993) noted a difference in the occurrence of accessory primordia between the left and right otoliths

of Dover sole. Dover sole apparently have a long and protracted transition from larval to juvenile life stages. Under these conditions, the shape of the otolith during the transitional period may be more critical, and in these instances, the organisms may exhibit greater biological control over the sites of secondary nucleation. What this study does show, however, is the importance of systematically incorporating both the left and right otoliths when extracting information from otoliths.

Despite the results of this study, it may be premature to conclude that there is no utility in examining other otolith patterns of halibut to identify stocks. Hagen and Quinn (1991) found that annuli patterns of young halibut follow broad long-term changes in temperature. To the degree that nursery areas are subject to different environmental conditions, it may be possible to use patterns such as the size of age zero or age one annuli to identify widely separated nursery grounds. In addition, though it may require more detailed preparation, daily increment widths from the first year's growth may also provide a means of distinguishing halibut nursery grounds. In either of these approaches, the results here suggest that within-individual pattern variation also needs to be identified. Incorporating this variation can help determine the extent to which a pattern reflects extrinsic influences versus being a function of happenstance.

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Chapter Six | Summary and an ontogenetic review

Each chapter in this thesis shares a common theme: the examination of variability in Pacific halibut otolith patterns for the purpose of determining if these patterns can provide insight into the biology and population structure of halibut. From the fish's perspective, the purpose for having otoliths - for balance and sound reception - is much different than the purpose we biologists have for examining them. Otoliths are essentially calcium carbonate crystals containing small amounts of protein and their growth is mediated biologically. The variation of patterns observed in otoliths may have importance in terms of the otolith's function, may be an indirect record of factors that affect the biological mediation, and/or may be the result of processes that are solely characteristic of crystal formation. To help separate these different processes and determine the utility of the patterns as sources of information, the general approach I took in this thesis was to examine variation in terms of various factor levels and how these patterns may be explained by temporal and spatial effects.

In the introductory chapter, I provided the rationale for quantifying otolith patterns and reviewed previous work on otoliths and halibut. In chapter 2, I investigated how annuli patterns of juvenile halibut varied temporally over a 26 year period, and by partitioning age-specific variation into year and year-class effects, investigated the relative importance of environmental and intrinsic sources of growth. In chapter 3, I examined patterns of larval increment widths, and by showing a correspondence of daily increment width and somatic growth, investigated patterns of growth as a means for examining mechanisms of year-class success. In chapter 4, I examined variation in some trace elements within and between otoliths and focused on how the pattern of elements may reflect ontogenetic changes and potentially be used as a natural tag. In chapter 5, I examined variability in the larval core shape of otoliths, investigated how these patterns arise, and evaluated whether they are useful for discriminating fish from different nursery areas.

Each of the otolith patterns I examined varied in a different manner with respect to factor levels and temporal or spatial effects. The larval core shape appears to contain most variation within fish, with no systematic difference between the left and right otoliths and no variation explained by location. The sample sizes were not large and thus the power of the tests for detecting significant effects was not great. Nonetheless for any stock separation purpose, it appears that the shape of the larval core does not convey any information about nursery area location.

The examination of elemental composition was limited to the more common trace elements; however the distribution of patterns indicates some complex relationships. Strontium levels at times reflect ontogenetic changes such as during yolk-sac feeding, as well as seasonal growth through annuli patterns. However strontium does not appear to be useful for stock identification. Sodium shows some variation attributed to location, but it also shows concentrations in portions of the otoliths that delineate the larval core boundary and thus is not likely to be useful for stock identification. Potassium, on the other hand, appears to show good variation by nursery area of capture, but similar to Na, it does not appear to be free of interannual variation. Because the mechanism of incorporation of these trace elements into otoliths is not well understood, the ability to draw useful information from trace elements in otoliths may be limited.

In contrast, larval otolith growth history, as contained in increment patterns, appears to be a good indicator of somatic growth rates. Using increment width as a function of otolith size provides a means for pooling data and comparing growth differences between areas. Features such as the point of accelerated otolith growth may have meaningful interpretation in regards to the investigation of recruitment success and for identifying Bering Sea halibut from those in the Gulf of Alaska.

Finally, annuli patterns, representing juvenile growth up to age five, indicate that environmental factors are more responsible for annual growth than density dependence or other factors associated with year-class size. Thus, increases in juvenile growth rates may increase the size of the year-class that is recruited to the commercial fisheries.

In order to put the results of these investigations into perspective, I'll describe in this chapter how the otolith patterns may arise in relationship to the ontogeny of halibut. I'll consider the developmental stages of halibut from egg to sexual maturity, covering both microstructure patterns and macrostructure features in the otolith. I will also touch upon factors recorded in otolith patterns which may determine year-class strength and take the liberty to include some additional observations on halibut otoliths that were not addressed specifically in the other chapters. In addition I will include information on the development of larval halibut that stem from Thompson and Van Cleve's (1936) original field studies, my own master's thesis (Hagen 1986), and McFarlane et al.'s (1991) report on rearing larvae through yolk-sac development. I will also utilize information from rearing studies of Atlantic halibut (*Hippoglossus hippoglossus*). In describing otolith formation I will use a perspective of the otolith as a crystal that grows through acellular processes, and is therefore under different regulatory control than somatic growth. This approach is more commonly used in studies of biomineralization than in fisheries work. Reviews on the field of biomineralization can be found in Simkiss and Wilbur (1989), Lowenstam and Weiner (1989), and Mann et al. (1989). From the fisheries literature, the most recent comprehensive review on otolith microstructures can be found in Stevenson and Campana (1992).

Figure 6-1 is presented to set the stage. It shows the relationship using a log-log transformation between otolith size and fish size for both larval and juvenile halibut up to age two that were examined in this thesis. The gaps in the data include egg, yolk-sac larvae, transitional stage postlarvae and newly settled juveniles. I will use the otolith patterns from the older halibut to draw inferences about growth and development during these periods. The close association of otolith-size to fish-size in Figure 6-1 illustrates the promise that otolith patterns may have for reconstructing the growth of halibut. The apparent shift in relationship between the larval and juvenile stages can serve as a reminder that the processes that regulate otolith and somatic growth are not under the same control. I will conclude this chapter with some recommendations and ideas for further investigations.

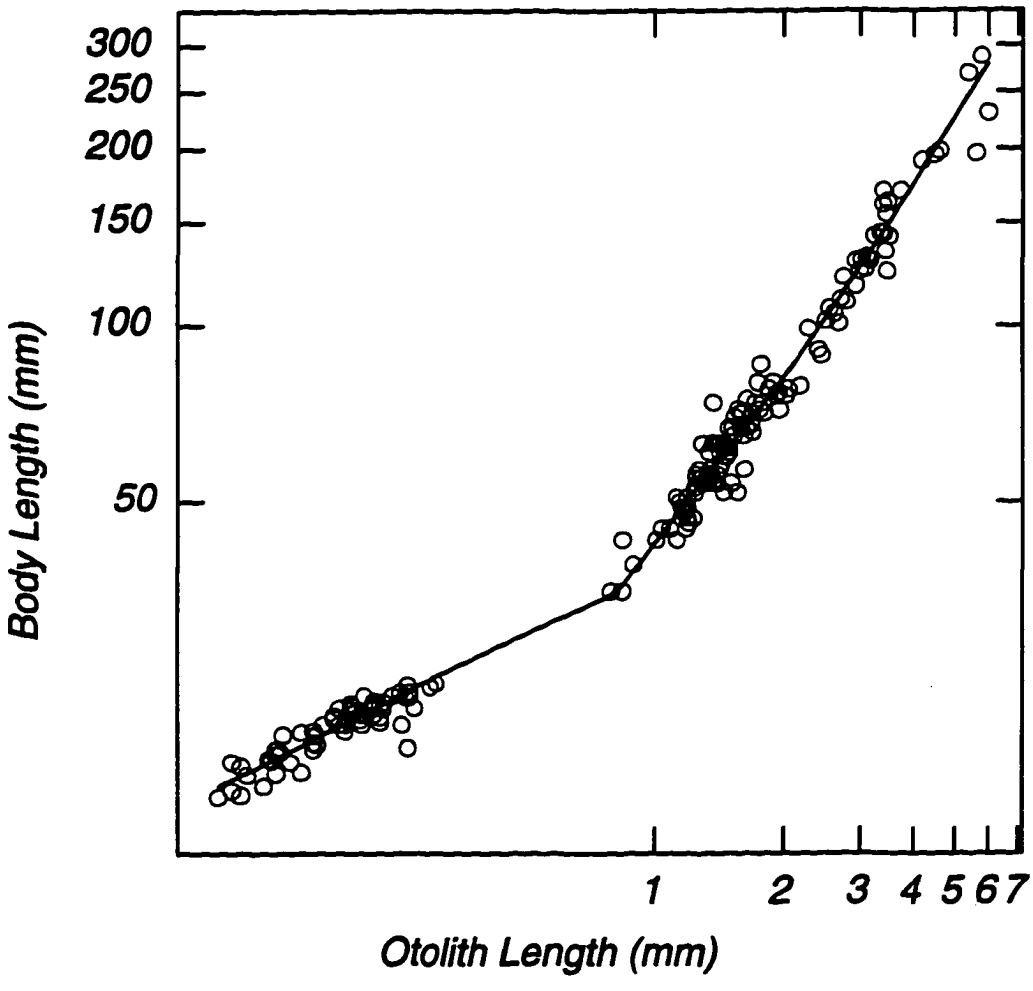

Figure 6-1. Relationship of body length and otolith length (log transformed scale), for larval and juvenile halibut examined in this thesis (n=251). Fitted Lowess smoothed line indicates linear trends.

Embryonic Development

Pacific halibut spawns in deep water off the edge of the continental shelf at depths of 200 to 600 meters with the period of peak spawning occurring between late December to late January (St-Pierre 1984). If its behavior is similar to Atlantic halibut that spawn in fjords along the Norwegian coast, a male halibut will position itself above a female as she releases her eggs in batches while they are swimming along the contours of a deep basin. This behavior was inferred from the capture of halibut with sunken gillnets when commercial fishing took place during the winter spawning period (V. Oiestad, Bergen Norway, pers. comm.). Adult Pacific halibut are traditionally captured with baited longline gear. They apparently do not feed durring the act of spawning and may even move to shallower water between releases of egg batches (St-Pierre 1984), so making inferences regarding spawning behavior from longline catch and survey records is problematic.

McFarlane et al. (1991) successfully reared halibut larvae through yolk-sac development and combined their observations with field data on oceanographic conditions. Their results confirmed the observations of Thompson and Van Cleve (1936), Van Cleve and Seymour (1953), and St-Pierre (1989). Newly fertilized eggs are positively buoyant and will rise in the water column. The time to hatching is largely dependent on temperature but it may range from 12 to 30 days depending on location and depth of spawning.

The otoliths will begin to form prior to hatching at specific sites or chambers in the developing labyrinth system of the embryo. There are actually three pairs of otoliths that form: the sagittae, lapilli, and asterisci, each in their own area. The sagittae is what I refer to when using the generic term otoliths. They are the primary subject of this thesis and have been collected by IPHC for age estimation. The lapilli are similar in size and shape as the sagittae during larval growth, but their growth is slower in juvenile and adult halibut, and while they contains similar types of patterns as the sagittae, they are rarely collected. The third otolith pair, the asterisci, are the smallest and appear to be of a different crystalline morphology than the other otoliths and thus are not useful as recorders of growth. Both the

sagittae and lapilli contain calcium carbonate in the aragonite crystal morphology, which is commonly associated with patterns and growth rings in biomineralized structures of other organisms such as mollusks.

The initial site of nucleation of calcium carbonate for the otolith is referred to in the fisheries literature as the primordium. Nucleation results from a condition of supersaturation of charged ions in solution with the availability of substrate, perhaps containing charged protein molecules, that can attract and hold ions. Once the initial crystal seed is laid down, subsequent precipitation will take place on the exposed facets of the growing crystal, provided a supply of ions continues. In my observations of otoliths of newly hatched larvae (chapter 3), it appears that there are actually multiple primordia that serve as the seeds of crystal deposition in halibut (Figure 6-2). In salmonids, multiple primordia are easily observed in the otolith center and appear to be quite variable in number and in relative position. However studies trying to extract useful information from the patterns of primordia distribution have proven unsuccessful (Neilson et al. 1985). This is not surprising. The location of a nucleation site is generally considered a difficult process to control by organisms that grow mineralized structures outside the cellular walls. If there is no functional purpose in precisely controlling the sites of nucleation, then there is little reason to expect that the organism would expend the energy in mediating the process. In halibut, these primordia are much smaller than in salmonids and appear to form more adjacent to each other. As a result, the shape of the otolith appears to be concentric during early larval development on most axes and, like other fish otoliths, the primordia become buried within the otolith as growth takes place on all sides.

The small size of the halibut otolith at first nucleation makes it difficult to observe the multiple primordia when examining the otoliths of older halibut. Evidence of their presence however can be observed in the radial lines that emerge from the otolith center. These lines are present in the otoliths of almost every species; however they are seldom acknowledged. The role they play is one of demarcating separate fields or lattices of crystal growth. During crystal growth, calcium ions and carbonate ions

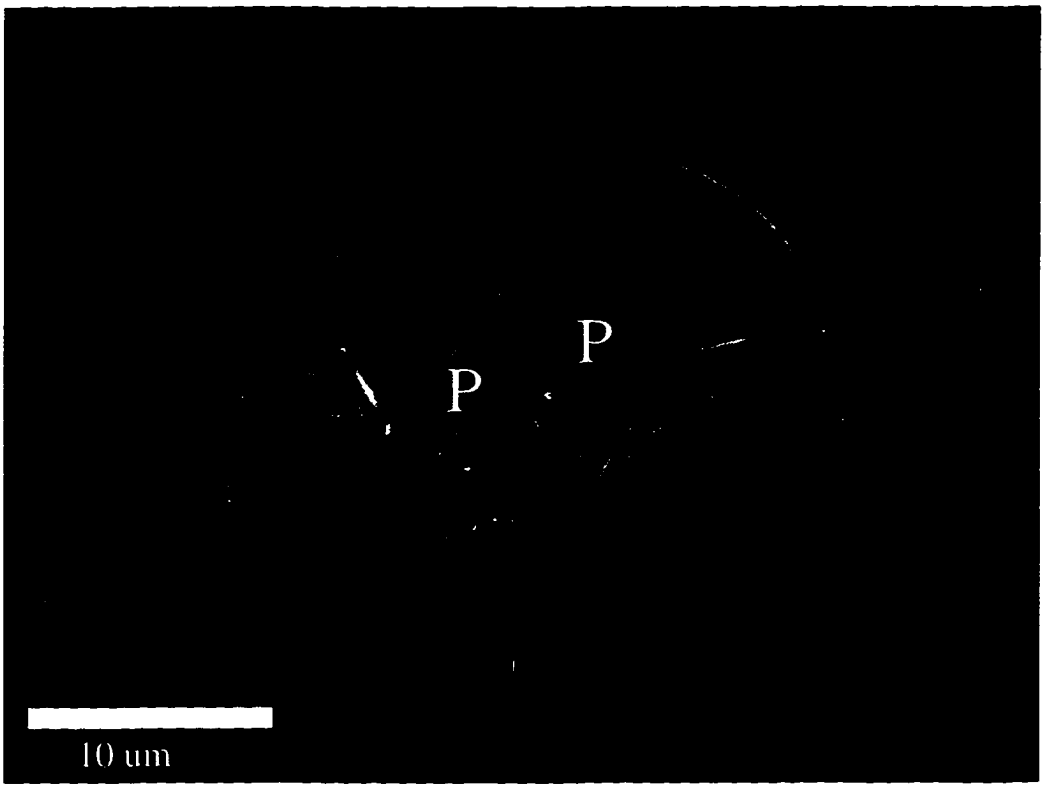

Figure 6-2. Otolith from a fifteen day post-hatched halibut larva using transmitted light microscopy (1000x). P denotes separate primordium. Scale length is 10 μm.

alternately fit into the crystal lattice to produce repeated translations of the unit cell. The unit cell is what defines a particular crystal morphology such as aragonite. Given this type of growth, it is not possible for a unit cell to be formed from separate crystal lattices that arise from separate primordia. Consequently, the radial lines that emerge from the otolith center will continue as long as ions are provided to the adjacent crystal lattices. In chapter 4, I pointed out through elemental mapping how Na seemed to be concentrated along the radial line. Other patterns I examined and will mention later also appear to be related to this feature.

A related phenomenon worth mentioning here is that aragonite in its pure state is noted for a characteristic of growth called twinning. What this refers to is that at sites of nucleation, a crystal can bifurcate at a specific angle due to the particular arrangement of the ions. Thus from a single primordium, two separate crystal fields may be formed, provided the supply of ions is available to both fields. At points later on, the crystal may bifurcate again, developing potentially quite complex patterns and variability in shape. It is for the occurrence of multiple primordia and bifurcation of crystal growth that the otolith is more properly called a polycrystal.

Returning to the otolith patterns that form prior to hatch, the multiple primordia appear to be surrounded by a dense protein layer that appears as a dark ring with transmitted light microscope and was soluble in EDTA as shown in the SEM photo (figure 6-3). I observed this ring in the newly hatched larvae, but I did not have any prehatch otoliths available to determine if the timing of the ring was coincident with a hatching event. Similar protein dense rings have been observed in other species which are not associated with hatching but rather appear to indicate the 'fusion' of separate primordia. In salmonids, a sign that such fusion is completed is pigmentation of the eye (personal observation). In halibut, eye pigmentation appears to occur approximately six days after hatching (McFarlane et al. 1991).

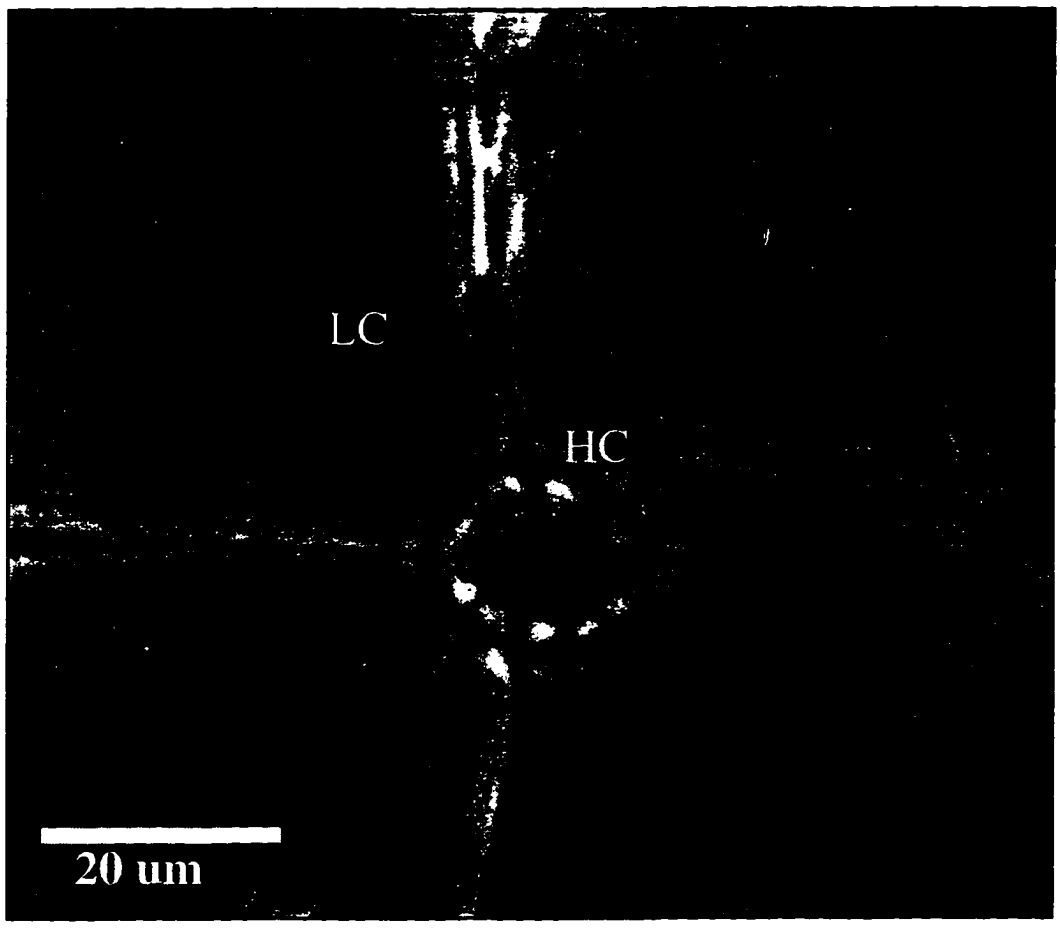

Figure 6-3. SEM photo of an otolith of a halibut juvenile showing inner hatch ring (HC) and larval check (LC). Photo taken from the sagittal plane. Scale at 20 um.

Yolk Sac Larval Development

Halibut larvae hatch at a primitive state at about 7.5 mm in length and will remain at depth for several days until the eye pigmentation is completed, after which they become more active and are able to move around freely (McFarlane et al. 1991). Observations with Atlantic halibut indicate that the hatching mechanism of halibut is unique compared to other marine fish (Helvik and Walther 1993). This is likely a result of their unusually primitive state of development at hatching. In addition, experiments show that high light intensity inhibits hatching, oxygen levels have no effect, and high turbulence will delay hatching slightly (Helvik and Walther 1993). This appears similar to studies on hatching success in Pacific halibut (Liu et al. 1994).

The developmental rate of the larvae is closely determined by temperature. At 6°C it will take 55 days from hatching to complete utilization of the yolk reserves at a length of 13.5 mm (McFarlane et al. 1991). During that time the mouth and internal digestive track and organs will develop. McFarlane et al. (1991) did not see any indication that halibut larvae were attempting to feed prior to yolk-sac absorption. In Atlantic halibut Haug (1990) suggests that the larvae can and do feed prior to yolk-sac absorption. However Tyler and Blaxter (1989) show that yolk-sac larvae of Atlantic halibut are drinking water to osmoregulate, so the intake of food could be a consequence of this need.

My observations of the otolith during the period of yolk sac utilization are based on the record contained in otoliths of older field-caught halibut which were past that developmental stage. The exception was a few artificially reared halibut collected soon after hatching. In the field-caught halibut, no increment patterns were observed at the area corresponding to yolk-sac absorption when examined with light or scanning electron microscopes. However with the artificially reared halibut, some irregularly appearing increment patterns were observed using high resolution light microscopy (Figure 6-2).

The respective presence and absence of increments in the artificially reared and field caught halibut is likely a result of differences in rearing conditions. The cause of increment formation in otoliths has been

the subject of considerable research, largely because counts of increments (which appear as alternating light and dark bands when viewed with light microscope) can provide a means to age the fish in terms of days. In many species, daily increment formation does not arise unless the fish is exposed to daily fluctuations in temperature or sunlight or is undertaking some circadian behavior. However cold temperatures can also inhibit increment formation even if other conditions are met. Otolith increments are thought to arise from a temporary cessation of calcium carbonate precipitation, along with the inclusion of a protein band or matrix. This process appears to occur in the growing surface of the entire otolith and is unaffected by the boundary lines that demarcate the different fields of crystalline growth. Details on the protein structure of otoliths are not well known. But it is likely that multiple proteins are involved, each with their own stereochemical properties that influence the arrangement of the calcium carbonate ions in the lattice in different ways. For the wild-caught halibut larvae it is probable that the cold dark waters where they reside do not provide the conditions necessary for increment formation. However as discussed in Chapter 3, even with scanning electron microscopes, very faint and narrow rings can be difficult to observe. Consequently the presence of increments before yolk-sac absorption cannot be discounted.

The first prominent structure that appears outside the otolith center is a thin dark concentric ring at approximately 57 microns in diameter when the otolith is viewed along the sagittal plane (Figure 6-3). I refer to this structure in chapter 3 as the larval check. It has not been possible to determine conclusively whether the larval check is associated with the end of yolk-sac absorption, since no specimens at that stage were available for examination. However it appears to be ubiquitous in all halibut otoliths at the same approximate diameter, suggesting that it is associated with a physiological change and not environmentally induced. In other species, hatching and first-feeding has been known to induce check ring formation (Campana and Neilson 1985).

An interesting feature in a few larval otoliths (3 out of 65) was double check rings at the location of the larval check. The two check rings were similar in appearance and ranged from 2 to 8 μm apart. No other

check rings were observed elsewhere during larval growth. One explanation for the second check is that it might indicate a significant time delay between successive feedings. If this interpretation is correct and the first check ring marks the completion of yolk-sac reserves, then the percentage of individuals that have a second check ring might prove interesting as an indirect measure of the possibility of food limitation early in life, where the record of 'near misses' is preserved in the otoliths of the survivors. This could provide a rather unique means to evaluate, through the examination of specimens from different year-classes, whether the critical period concept (Hjort 1914) plays a role in determining year-class strength of halibut. The critical period concept and its variants (e.g. match mis-match hypothesis of Cushing (1975)), is based on the supposition that the end of the yolk-sac period is the most critical point in the survival of larval fish and that the rates of successful first-feeding can vary annually. The precise event or change which is associated with the larval check and the occasional second check ring should, however, be determined experimentally.

One feature in the otolith associated with the position of the larval check is a drop in strontium levels as indicated with X-ray mapping in chapter 4 (Figure 4-5d). The elevated levels of strontium in the otolith during the period of yolk-sac absorption would suggest it is derived from yolk, and the rapid drop in strontium would indicate the exhaustion of yolk reserves. In this way the otolith can be said to carry a record of the parent when it underwent vitellogenesis. Whether or not variation in the level of strontium, or other trace elements, can be used to provide information about the location or feeding success of the parent is still unknown.

Postlarval Development

Postlarvae is the term that refers to the developmental stages between yolk-sac absorption and metamorphosis to the juvenile stage. In Thompson and Van Cleve's (1936) study they delineated 9 stages of development which cover the postlarval period through the completion of metamorphosis. They based the stages not on size, but on the appearance of various physiological traits. I had previously reviewed the criteria used by Thompson and Van Cleve (Hagen 1986), and found the assigned stages in

wild caught larvae generally followed changes in otolith size and increment counts. I had also examined unpublished data from Thompson and Van Cleve's study and concluded that changes in body shape are not isometric but takes place in stanzas, where there is an initial increase in body depth with little change in length, followed by an increase in both length and depth. Because a single measurement such as body length can be somewhat misleading when establishing growth relationships, I used a transformation of length and depth to provide an index of body area to compare with increment patterns for the analysis in chapter 3.

As the postlarval halibut develop, their active movement and change in buoyancy will bring them up higher in the water column. The duration of the postlarval period may vary, but it is likely to last several months. As discussed by Parker (1988), various wind and tidal driven currents are likely responsible for transporting halibut larvae and postlarvae toward nearshore nursery areas. An interesting point to consider is how behavioral patterns of halibut may also help direct their movement. From an icthythoplankton survey by the National Marine Fishery Service in 1984, ten out of eleven halibut postlarvae captured were taken at night. The survey gear was a neuston net which covers the upper 15 cm of the water column. This suggests that halibut post-larvae undertake diurnal vertical migration. This behavior is common with other larvae, where the nighttime movement is thought to be a means to avoid predation and/or seek out preferred food types. It also may be important for enhancing growth, because the warmer surface waters allow a vertically migrating fish to assimilate food more efficiently than if they had remained in the colder waters at depth (Wurtsbaugh and Neverman 1988). In addition, movement through the water column may influence the direction of transport as the postlarvae move towards near-shore waters.

As the postlarval halibut grows, the otolith increments, which first appear just outside the larval check, gradually become wider when viewed along the sagittal plane (Figure 3-3). The relationship between increment width and otolith size is approximately linear, but there also appears to be a good deal of variability both within individuals as they get older and between individuals from different locations.

When the data are smoothed and examined in terms of relative otolith growth rates some subtleties in the patterns appear that may have some utility for comparing differences between individuals, locations, and possibly year-classes. As was shown in chapter 3, differences in somatic growth between individuals are reflected in the differences in increment widths. From the empirical observations it appears that at any particular developmental stage or size, the faster growing individuals will have the larger increments. By itself increment widths may not be a good indication of growth rates over short time periods. However when averaged over a long time period, the measurements should reflect, on a comparative basis, actual differences in somatic growth, provided the growing conditions are not extreme. The pooled data, by area or time period, can provide a tool for comparing relative growth rates temporally or geographically.

Increment widths can be interpreted as measures of the velocity of otolith growth along the axis of measurement. For many individuals there appears to be an acceleration in otolith growth at à particular otolith size. It is possible that this point of change may be an indication that the postlarvae have moved further up in the water column and are starting to undertake the diurnal movement to the surface waters at night. Such speculation may be difficult to validate with field caught postlarvae, but it would be consistent with the notion that changes in metabolic activity and behavior can result in changes in the increment patterns in the otoliths (Campana and Neilson 1985). Examining this type of variation contained in the increment patterns of adults from different year-classes may be one means to evaluate the effect of broad-scale oceanographic changes on recruitment success as proposed by Parker (1988) and Parker et al. (1995).

When the otolith is viewed on a cross-sectional plane, a different perspective of otolith growth can be obtained. As shown in figure 6-4, the larval check ring is compressed on the proximal and distal surfaces; this is an indication the direction of growth has changed. One result is that daily increments are widest at mid-depth in the otolith and are narrower towards both surfaces as shown in the cross-sectioned otolith in Figure 1-1. On the proximal surface of the otolith (the 'interior' side), deep check

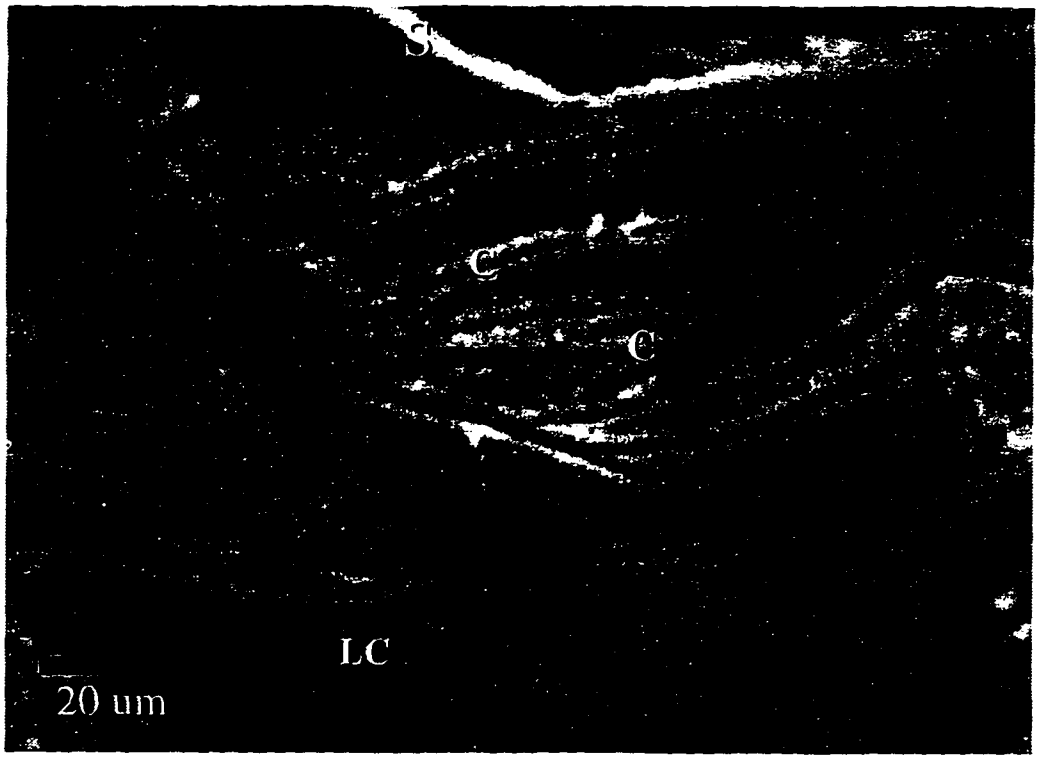

Figure 6-4. SEM photo of cross-section of otolith of juvenile halibut, revealing larval check (LC) and check rings (C) associated with the formation of the sulcus groove (S). Scale at 20 μm.

rings begin to appear (Figure 6-4). These check rings are not continuous like the larval check ring but are wider and more pronounced. It is along the proximal face that the sulcus groove forms. The sulcus is an indentation in the otolith which houses the sensory epithelia of the macula, the sense organ that detects otolith movement. The sulcus initially appears in a larval otolith as a concentric depression and later develops into an elongated groove seen in juvenile and adult otoliths. Traces of the larval sulcus depression can be seen in the Nomarski reflected light images of ground and polished otoliths of juvenile halibut in Figures 5-1 and 5-2. Normarski illumination accentuates subtleties in reflected light, so while the physical depression is not necessarily present, its location can still be viewed by the angle of the aragonite crystals that were exposed after grinding.

The formation of check rings along the proximal face is likely associated with the process that helps sculpture the shape of the sulcus groove. The pattern of the checks suggests that this process involves episodic periods of otolith growth (Figure 6-4). An intriguing speculation is whether this could also serve as an indirect indication of anabolic and catabolic processes in growth. Gauldie and Radtke (1990) proposed that such a process is responsible for the periodicity of check formation during seasonal changes. An analysis of check ring patterns might provide some indication as to the mechanisms that give rise to the pattern; however care must be given to any measurement system given the three dimensional nature of otolith patterns.

Another prominent pattern in halibut otoliths, the accessory primordia, starts to appear in the late stage halibut postlarvae. Accessory primordia are new sites of nucleation that form on the growing surface of the otolith (Figure 6-5). When viewed under high magnification, accessory primordia are characterized by numerous long and thin crystals which emanate from a common point. Elongated crystals of aragonite are generally considered a sign of rapid growth in biomineralized structures, while slow growth is denoted by short and wide crystals. During the initial period of rapid crystal growth from the accessory primordia, increments are still being laid down on the rest of the otolith but they appear to be

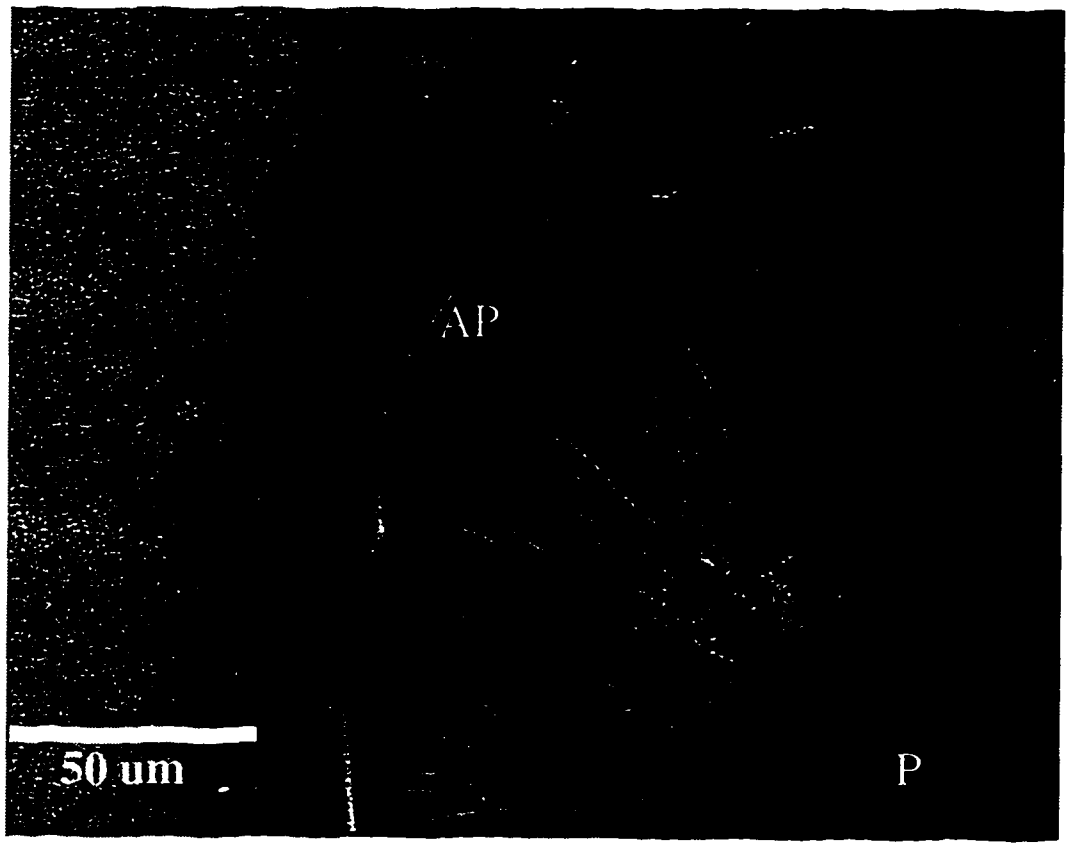

Figure 6-5. Otolith of a larval halibut showing newly formed accessory primordium (AC) in relationship to the central primoridia (P). Scale at 50 μm.

much narrower than the increments which are simultaneously laid down upon the accessory primordia (Figure 6-6). This suggests that in terms of 'competition' for calcium carbonate ions, the accessory primordia crystals are more favorable as sites of deposition.

To my knowledge, the question of what gives rise to the accessory primordia has not been investigated in the fishery literature. Accessory primordia are a common feature in flatfish sagittae, but are also seen in a number of gadiform species as well. As discussed in chapter 5, I frequently observed the placement of accessory primordia in association with one of the radial lines that mark the boundary of the separate crystal bundles that emanate from the otolith center. One possible explanation for the presence of accessory primordia can be made through consideration of the otolith as a crystal and the role protein may play on the orientation of the crystalline lattices. As discussed in the biomineralization literature, one of the determinants of crystalline shape is the direction from which ions are available. All else being equal, a crystal will grow towards that gradient. However proteins, which are incorporated onto the otolith either as a sheath surrounding the crystal bundles or interstitially as part of the protein matrix, may also help direct the precipitation of calcium carbonate onto particular crystal facets or inhibit crystal growth on other facets by way of their stereochemical properties (Addadi and Weiner 1985). During late stages of larval development, a shift in the direction of ions available to a growing otolith starts to take place. Precipitation will still occur along the orientation established early in life, but in localized areas a condition of supersaturation may develop. A feature, such as a surface discontinuity associated with the boundary area between crystals, may be all that is necessary for a new site of nucleation to form. The subsequent rapid growth is an indication that these are energetically more favorable as sites of precipitation, and it is also likely an indication that crystal growth at these points is not initially under tight biological control.

The appearance of the first accessory primoridium might be associated with a particular developmental stage, but as discussed in chapter 5, the orientation of the accessory primordia may arise from happenstance and does not reflect processes controlled by external events or biological functions.

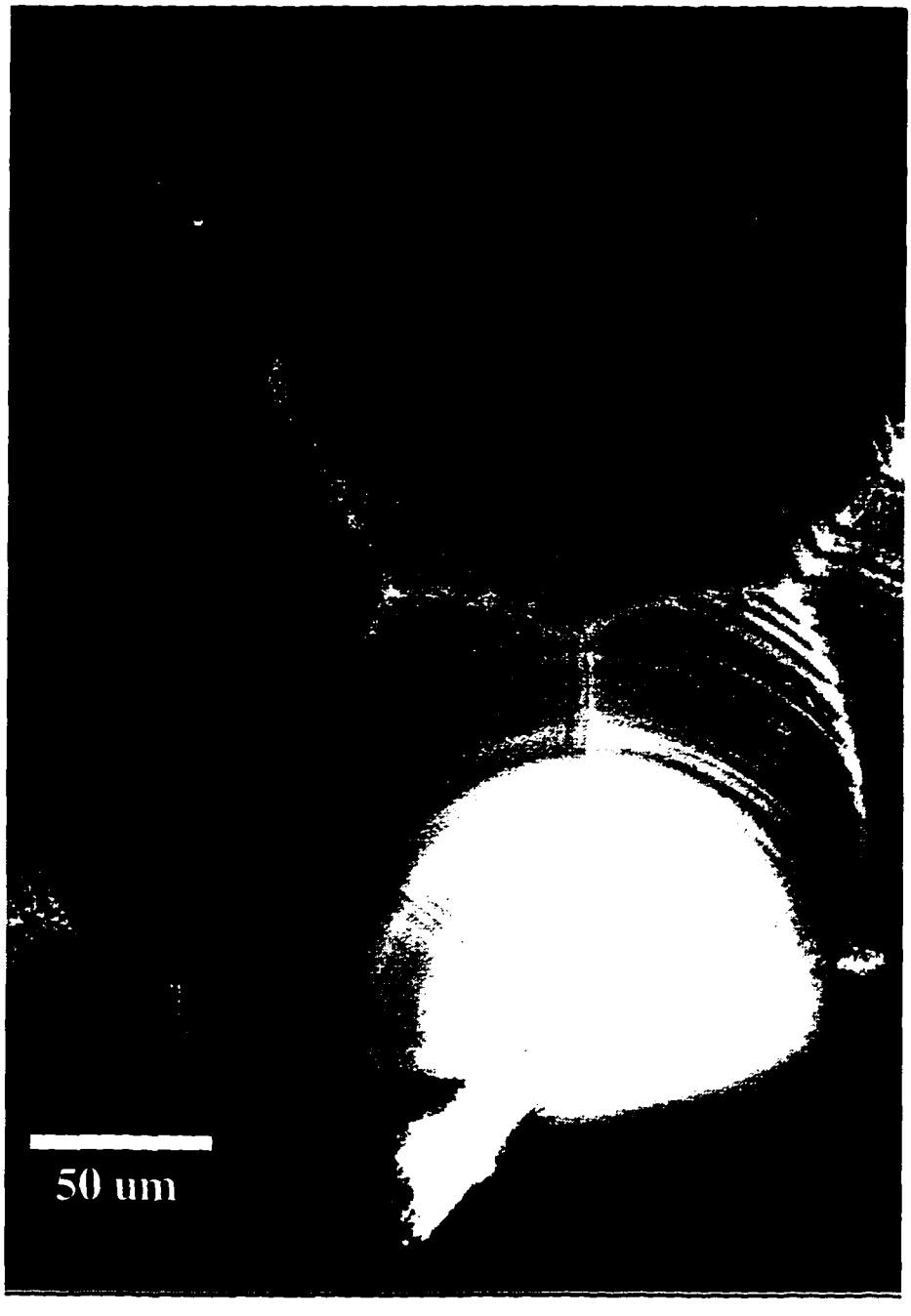

Figure 6-6. Otolith of a juvenile halibut showing the variation in increment widths that cross different crystal fields as a result of the accessory primordia formation. Photo taken along the sagittal plane with transmitted light. Larval increments appear washed out as a result of fracture through the otolith center and the refraction of light. Scale at 50 μm.

The postlarval specimens in which I found accessory primordia were at an advanced stage of development (stage 10, according to the criteria of Thompson and Van Cleve 1936 and discussed by Hagen 1986) and they were collected with pelagic trawl gear, using double oblique tows down to midwater depths. However in 1985 in Bristol Bay, I collected a halibut larva at a similar developmental stage with bottom trawl gear in a near-shore area. This indicates that there could be quite a bit of variability in habitat associated with the appearance of the accessory primordia.

Metamorphosis

Metamorphosis in flatfish generally refers to the physiological changes that accompany the transition from a pelagic to a benthic orientation. In halibut, the length of this transition period is likely to be variable. The diurnal vertical movement of postlarvae noted earlier may facilitate the ability for halibut to select appropriate nursery areas for settlement. By moving back up in the water column the larvae can continue to drift if the bottom type does not appear suitable. This process of up and down movement has been noted with Dover sole larvae during settlement (Toole et al. 1993), and it seems to be a behavior documented with Japanese flounder (Tanaka et al. 1989). With some of the early rearing studies on Atlantic halibut, it was noted that halibut larvae would remain pelagic in conical shaped containers with only a small area for settlement, but upon transferring to containers with flat bottoms settlement would readily take place, indicting some ability to delay metamorphosis.

The otoliths I've observed from Pacific halibut juveniles do not appear to contain a feature in the microstructure that would point to a time when settlement is completed. However because the smallest otoliths I've observed were from 37 mm S.L. juveniles and settlement likely occurs around 30 mm in length, it is difficult to evaluate whether a particular pattern might indicate settlement. In all juvenile otoliths I've observed, the larval crystal (as defined in chapter 5) is completely encased by growth which stems from the accessory primordia crystals (e.g. Figure 6-7). Others working with flatfish (i.e. Sogard

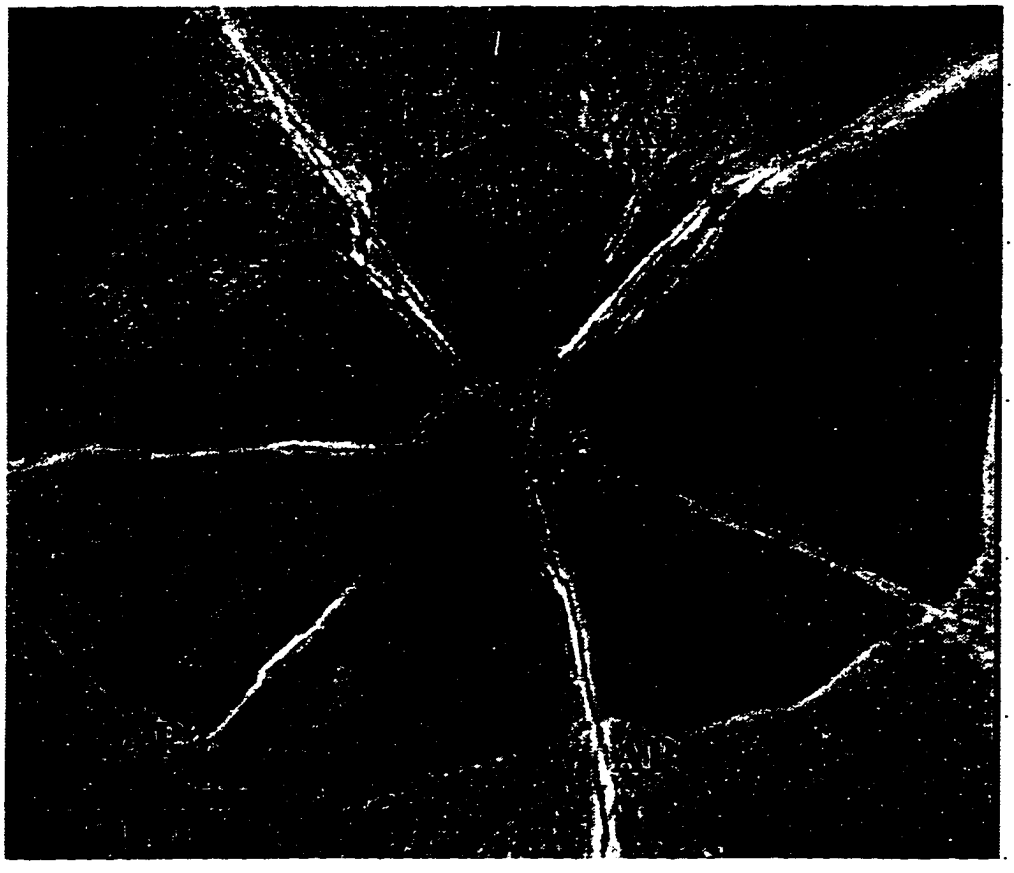

Figure 6-7. SEM photo of an otolith of a juvenile halibut showing on the sagittal plane the location of the central primordia (P) and larval crystal shape formed by the relative position of the accessory primordia (AP). Scale is 100 μm.

and Able 1992) have used the increment location associated with the accessory primordia as a reference point for examining age and growth in nursery areas.

Toole et al. (1993), in an extensive study of otolith growth in Dover sole (which has an even longer pelagic period that halibut), note that the first accessory primordia can also be laid down prior to settlement. The authors propose however that the completion of settlement may correspond to the point at which the larval crystal is completely contained, and that the increments associated with that point might serve as a reference location. It is tempting to suggest that halibut otoliths might have similar characteristics of growth, however as I argue in chapter 5, the mechanisms that likely give rise to these accessory primordia do not indicate to me any reason why the shape of the larval crystal should be dependent on timing of settlement.The width of the increments laid down during the time of accessory primordia formation may vary depending on where they are measured on the otolith: the larval crystal area or the primordium itself (Figure 6-6). This makes it difficult to draw inferences about body growth directly from the increment widths during this time period. Using an average width spanning the different growth fields could also be problematic because not all accessory primordia will have their origin at the same plane of measurement. A three dimensional perspective in measuring increment widths would clearly help in tracking growth differences during metamorphosis, but it would also be technically difficult to obtain.

It should be noted that the presence of accessory primordia in halibut otoliths is unique to the sagittae. The lapilli otoliths I've examined from adult halibut do not contain such structures. Instead they have a largely concentric pattern of increment growth that appears to continue through metamorphosis after which the increments develop an elliptical shape (Figure 6-8). The lapilli of adult halibut exhibit cone or pyramid-like shapes with the apex containing the earliest growth increments. This characteristic makes it a very easy structure with which to extract early life history information. Unfortunately, the lapilli are also quite small in adults (< 3 mm) and are difficult to locate in the brain capsule. I am not aware of any

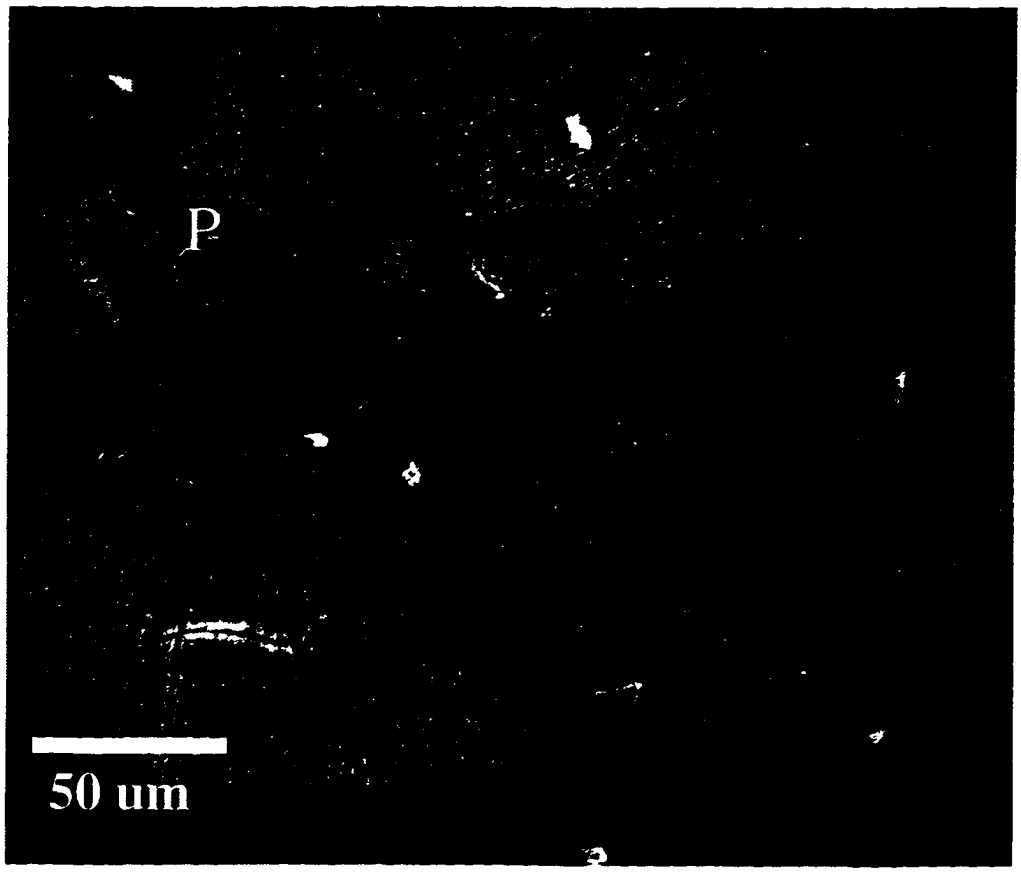

Figure 6-8. Microstructure of a lapillus otolith from a six year old halibut showing the location of the central primordia (P) and larval and juvenile growth fields . Accessory primordia are absent. SEM photo. Scale is 50 μm.

systematic collection of halibut lapilli. However for purposes of investigating transitional and early juvenile residence in nursery areas, the lapilli might be useful structures to collect in future field studies.

Another opportunity to look for a settlement mark in otoliths is through their elemental composition. In chapter 4, my examination of the trace elements Sr, Na, K, and S did not reveal, however, any strong signal that would indicate a change from pelagic to benthic life. Toole et al.(1993) also reported that no trace elemental signature was present when looking at the transitional area on the otolith of Dover sole. However there appeared to be some differences in the relative concentrations of protein and calcium during that period. The trace elements that are commonly examined with X-ray microprobes - the technique used here - probably play a physiologically mediated role in the otolith and do not directly reflect ambient concentrations in the environment. Strontium concentrations may be inversely related to otolith growth, with the exception of the elevated concentrations at the otolith center which may have a maternal origin. Similarly, I suspect that Na and K are also associated with a difference in growth rates and more specifically associated with specific proteins that may partially block precipitation of calcium ions.

New techniques are now becoming available that can detect other elements in otoliths that are at concentrations approaching parts per billion (Fowler et al. 1995). The methods however require even more sophisticated equipment and it remains to be seen whether elements at such trace concentration levels reflect ambient environmental conditions, or are also biologically regulated. Because the otoliths reside in the fish's brain, the necessity for filtering chemicals from entering the brain must be considered and caution should be applied in presuming that trace element concentrations will provide a definitive geographic signature.

Juvenile Development

For many flatfish, the nursery area residency is thought to be a period where compensatory processes take place that may regulate and dampen fluctuations in recruitment. Predation on newly settled

individuals in particular is thought to play an important role (Bailey 1994). Behavior such as burying beneath the sand is a common phenomenon among flatfish, and it likely evolved in response to predation pressure. Food limitation may also provide competitive pressure that regulates population size. In cases of both predation and food limitation, growth rates of an individual may play an important role in survival. Because the off-shore spawning of halibut results in the larvae distributed over wide areas, newly settled halibut are generally not the numerically dominant flatfish in any nursery area. Potential predators of flatfish, such as crangon shrimp or other fish (van der Veer et al. 1990), are not likely to specialize or target on halibut over other competing prey types of similar size. As a prey item, one means to reduce susceptibility to predation is to grow quickly through a window of vulnerability. Rapid growth might also be a mechanism to reduce competition for food with other flatfish. Diet studies show there is potential for a large overlap in prey preference for juvenile flatfish which can be found inhabiting the same depth and bottom type as halibut, with some ability for changing food type in response to competition (Holladay and Norcross 1995). Halibut may also minimize that type of competition by 'out-growing' other flatfish through more active feeding or greater assimilation efficiency, allowing them to target on larger size prey types than other similar aged flatfish.

As they get older, juvenile halibut move into deep water, and a component of the population may undergo extensive migrations as shown from tagging studies. McCaughran (1987) noted that the individuals that do migrate tend to have a higher growth rate than the non-migrating component, but it is not known if that is a result of genetic or environmental causes. The need to identify the different components of the population, as an aid to understanding the population structure, was the primary motivation behind the examination of elemental concentrations in chapters 4 and the larval crystal pattern in chapter 5. While both approaches provided insight into otolith growth, neither was completely successful in serving as a natural tag.

Otolith increments formed during juvenile growth are fairly easy to observe in halibut otoliths and could be used to investigate growth in relationship to survival. Finding a consistent location or reference point

on the otolith that indicates the start of nursery area residence would simplify such an investigation (e.g. May and Jenkins 1992). As previously indicated, a reference point similar to the larval check ring may not be easily determined in halibut otoliths. However even lacking such a reference point, it still may be possible to estimate otolith growth differences within and between individuals, nursery areas, and year-classes using a random sampling approach to measure increment widths at various locations on the otolith during the first summer's growth.

Near the end of the first summer's residence in a nursery area, the halibut may have reached a size of 50 to 70 mm, depending on the location of the nursery area. In addition to clear and readily observed increment patterns as shown in the introductory chapter (Fig 1-1), the summer growth is periodically interrupted by check ring formation (Figure 6-9). These checks are discontinuous and may appear at various locations throughout the otolith, but are more concentrated on the posterior and ventral portions. Figure 6-10 shows a portion of an otolith during late summer growth which was slightly fractured during grinding to reveal the layering nature of the otolith that accompanies the check ring formation. Check rings in other species have been associated with tidal cycles, but this association has not been found to be true in all cases, even with the same species in different nursery areas. Checks have also been shown to be induced by stress, associated with sculpturing of the otolith shape as mentioned earlier, and may arise from physical constraints due to space limitations in the otic capsule. To the extent that check ring formations reflect stanzas of growth, they remain one of the more intriguing structural features in otoliths.

In addition to patterns seen in the microstructure, annuli marks, frequently referred to as translucent zones, are larger scale structures which form during the late winter. When viewed with reflected light, the annuli appear as dark bands in contrast to the white opaque zones which form during summer growth. These optical bands in otolith have been used to age fish since the turn of the century, and halibut since 1914. The reason for their optical properties is generally thought to be associated with differences in the ratios of protein and calcium, with winter growth having a higher concentration of

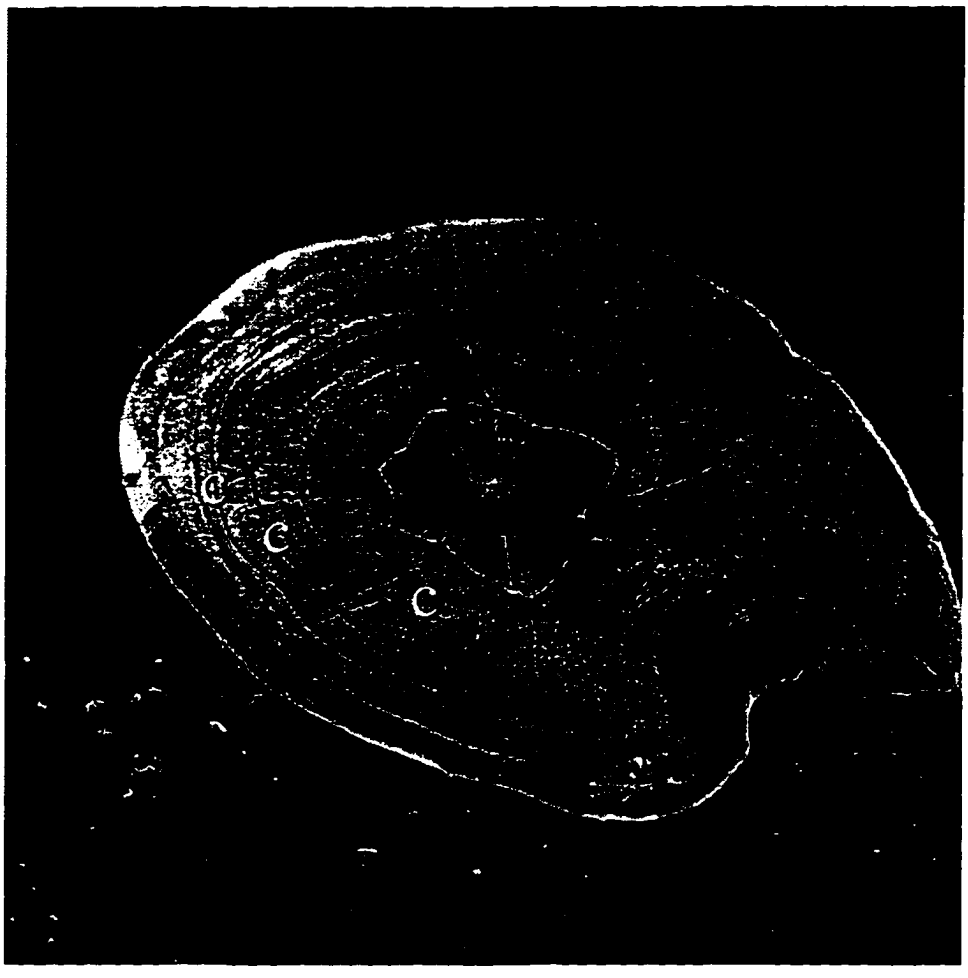

Figure 6-9. SEM photo of an otolith of a juvenile halibut showing first summer's growth and location of check rings, c, on the ventral side. Specimen tilted during photo, length at longest axis is 1.4 mm..

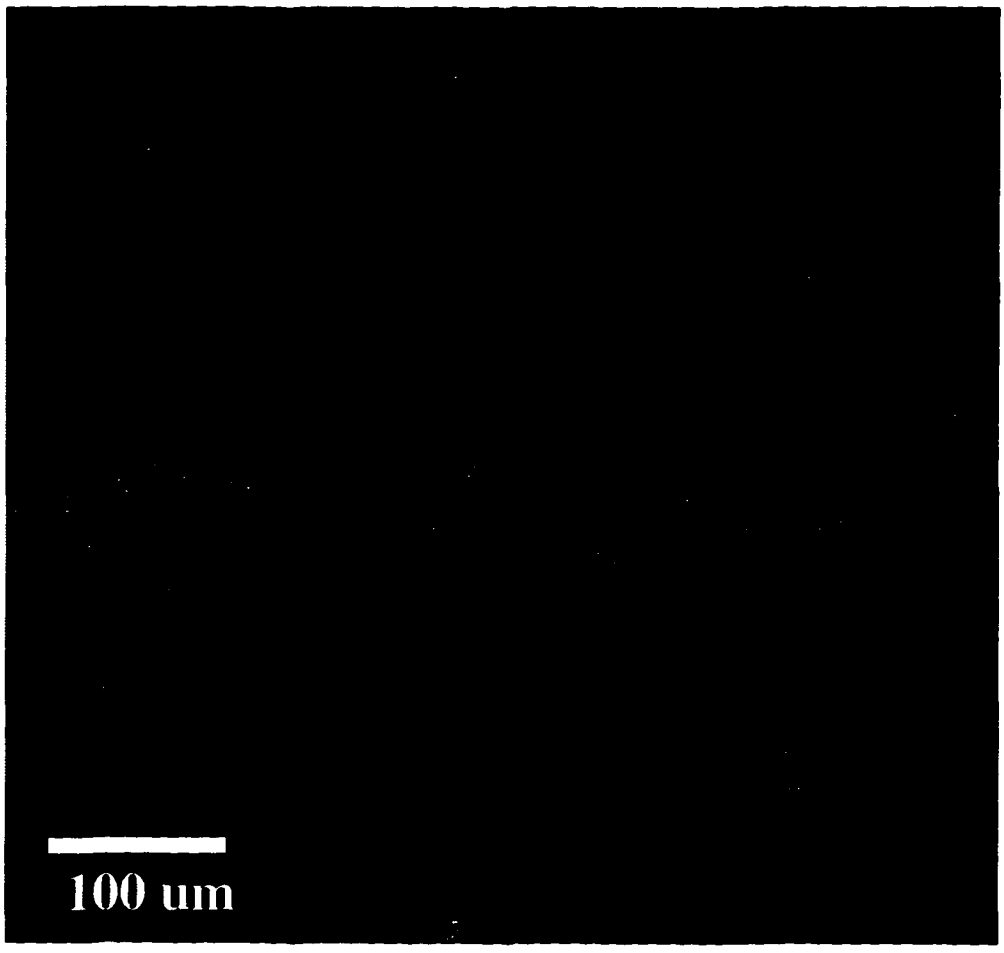

Figure 6-10. SEM photo of juvenile halibut otolith showing the layering characteristics of check rings on the ventral portion of otolith formed during nursery area residence. Scale is 100 μm.

protein. The banding of calcium and its association with annuli could be seen in the elemental map in chapter 4 along with bands showing the Sr/Ca ratio (Figure 4-8). Combined with the increase in frequency of check ring formation during winter, these features provide the means for aging the fish in terms of years.

Measurements of the annuli patterns provide another means to investigate growth histories, albeit not on as fine a scale as the microstructure increments. Annuli measurements are much easier to obtain and, as shown in Chapter 2, when the otolith growth history is examined in terms of the widths of the annual growth zones, it can be used investigate temporal influences of growth and possibly population regulation.

The primary results from chapter 2 showed that variation in average annual growth of ages 0 through 3 over a 26 year period varied significantly by the year of growth and not the year-class. Further, the temporal variation by year was significant and positively correlated with a temperature record. Growth in these early years was positively correlated with the size of the year-class as determined by the number of eight-year-olds entering the commercial fishery. This correlation was not large, but it was significant. This suggests that growth in nursery areas is perhaps a cause and not a consequence of recruitment variation. Density dependent growth, which is commonly presumed to be a mechanism that dampens recruitment variation in nursery areas, was not evident during early growth as shown from the otolith growth zone data.

The conclusion that variation in growth is determined by the year of growth and not the year-class may not necessarily apply to just the 26 year time period I examined in chapter 2. Southward (1967) also analyzed growth patterns from IPHC's historical otolith collection. In his study, which used traditional back-calculation methods, he compiled a growth history of halibut extending, in some areas, back to 1900. Southward (1967) concluded that long term growth changes were due to both environmental causes and as a response to density dependence, but he was not able separate out either effect. In investigating the methods for measuring year and year-class effects on growth, I reanalyzed some of

Southward's data on size at age of halibut taken from Portlock Grounds (Southward 1967: Table 6). I used the mean lengths for ages 1 through 3 and converted the data to annual growth by each cohort spanning the years 1911 through 1958. I then applied the permutation tests of year and year-class effects. The results indicate that significant variation in annual growth is explained by the year of growth ($p = 0.003$) and not the year-class ($p = 0.998$). This result is similar to that I observed directly from the otolith measurements for the time period 1953 to 1982. Southward (1967) noted from his data that there was an increase in size at age over time. The method I applied does not contradict those findings, but rather it is a means to determine 'how' that size is achieved; either from annual events which affect all ages similarly or annual events which affect only the cohort of fish. The similarity in results from the two data sets would seem to suggest that environmental factors, such as temperature, influences growth of young halibut, while the year-class the fish belongs to does not account for variation in annual growth. The year-class effect might be expected to be significant if, for instance, the growth of young halibut was regulated by the numbers in the cohort under a density dependence scenario.

Parker et al.'s (1995) investigation of halibut recruitment found a correspondence between low frequency changes in halibut recruitment and an 18.6-year tidal modulated cycle of oceanographic changes, referred to as the lunar nodal cycle. Recruitment was measured as the biomass of the eight-year-olds entering the commercial fishery. In this case biomass, which is the combined weight of all individuals, was smoothed with a 3-year running average and lagged back to the year-class date. While a variety of mechanisms were postulated that may explain this association, the primary assumption was that biomass changes were linked to tidal events during the larval stages which directly determined recruitment in numbers. However the three cycles that constituted the data series had their highest correlation with the lunar nodal cycle with a phase shift of 2.5 years. While not precluding the importance of the larval and postlarval periods on recruitment in terms of numbers, the use of a smoothed index based on growth that takes place at different ages leaves open the suggestion that the association of recruitment biomass with the lunar nodal cycle may not be limited to the 6 month larval period as hypothesized by Parker et al. (1995).

Royer (1993) showed an association of the same lunar nodal cycle with temperature anomalies in the North Pacific. A sea surface temperature index used in chapter 2 showed a strong correlation with otolith growth at ages 1 through 3. All these growth zone measurements were positively correlated with the length of halibut at age eight, which suggests that early growth contributes to the weight of the eight-year-olds and hence the total biomass of recruits entering the commercial fisheries. Predation within nursery areas, as Bailey (1994) discusses, can be a stabilizing force to dampen recruitment variation, and faster growth, as I mentioned earlier in this section, can be a means for a potential prey to increase their chances for survival. Thus growth can also influence recruitment in terms of abundance. A lunar nodal cycle indeed might be a driving force behind long-term changes in halibut year-class strength, but I would suggest the mechanism may operate even more directly through the effects of temperature on the growth rates of young halibut at ages 0 through 3.

It is likely that there are a number of critical periods in the early life of young halibut, and environmental conditions which promote or inhibit survival may act in concert during these periods. The low frequency changes in halibut abundance and the similar low frequency changes in the physical environment, such as that driven by the lunar nodal cycle, suggests that causal mechanisms are involved. However because so few cycles have been observed it might be difficult to distinguish between competing theories or to prove causation without additional evidence. Examination of the historical otolith collection may provide some indications. Time however may also provide new information. A grand experiment is currently taking place by way of man's activities in putting greenhouse gases into the atmosphere. Starting in the early 1970's, there was upswing in ocean temperatures which appears to be consistent with to the lunar nodal cycle. Air temperatures have also tracked that cycle making it difficult to determine if global warming was indeed taking place. The recent indications are that air temperatures have remained high while the nodal cycle has declined, however the ultimate effect on ocean temperatures remains to be determined. Amongst the other ramifications of global warming, the possible uncoupling of temperature fluctuations from a lunar driven mechanism may provide an

opportunity to learn about what critical points in the early life of halibut are responsible for changes in abundance. Perhaps we will know by the next cycle.

The results from chapter 2 were predicated on a general correspondence between otolith size and fish size, such that a faster growing fish will have larger annular otolith increments. Recently, studies with other species have shown that the otolith-size and fish-size ratios are generally not constant with respect to somatic growth rates. This was suggested by Clark (1992) as possible explanation for why otolith size has not been a consistent predictor of the body size of adult halibut over the several decades that it has been measured. Campana (1990) examined the consequences of this phenomenon on traditional back-calculation methods. He showed that slower growth rates will result in an underestimation of previous size at age (Lee's phenomenon) with the greatest discrepancies on the size estimates of youngest ages. This may explain why Southward's (1967) examination of growth using back-calculation did not follow a periodic trend at young ages which might have been expected if temperature, driven by a lunar nodal cycle, was playing a role. A reexamination of that data series should be undertaken in light of this possibility.

It was concern over the possible 'uncoupling' between otolith size and fish size, and the pitfalls associated with back-calculation, that the analysis in chapter 2 was based on width of the otolith zone measurements directly. However it is still probable that the width of the otolith zone measurement does not directly correspond to a proportional change on body growth. I would argue however that the relationship should be close and is at least monotonic, which on a relative scale would allow a ranking of growth and would not adversely influence the method for testing year and year-class effects. Hare and Cowen (1995) make a similar argument in regards to using increment widths as a comparative means for examining growth in larval fish. They present a schematic showing why a relative growth approach avoids the problems associated with back-calculation methods when there are individual differences in somatic growth rates.

If there is a desire to make use of back-calculation to determine previous size-at-age in young halibut, then the relationship between otolith-size and fish-size needs to be established precisely. However as shown in chapter 2, gear selectivity and sexual dimorphism can likely play a large role in the perception of the past growth and on the otolith-size to fish-size relationship. This suggests that field collections at all ages and sizes should be made carefully to avoid selectively biased samples.

One explanation for the potential uncoupling between otolith growth and fish growth was given by Mosegaard et al. (1988) who suggest that otolith growth is more influenced by the fish's metabolic rate than its somatic growth. They showed experimentally that otolith growth and somatic growth respond differently to temperature changes, and that there is a difference in the temperature range that supports optimal growth. In addition, a number of studies have shown that the otolith continues to grow even during periods in which starvation is taking place and for very old fish, somatic growth may cease, while the otolith will continue to lay down increments.

A metabolic model of otolith formation may explain the residual variability in otolith-size and fish-size relationships between individuals that are reared under the same conditions and temperatures. Cui and Liu (1990) in an examination of individual differences in energy allocation within and between species note that there is a trade-off between energy devoted to metabolism and that devoted to growth. This individual variation along with the variation in feeding rates largely gives rise to differences in size at age. Thus it seems likely the variation in otolith-size and fish-size within a cohort may be a reflection of differences in metabolic strategies for allocating food. These differences play a role in the ability of individuals to adapt to changing environmental conditions and it serves as the basis for natural selection. So while otolith growth may not directly reflect somatic growth, it is likely a measure of factors that are nonetheless important for individual survival and population growth.

As an aside, I've notice that some of the older fishery literature, including work conducted by IPHC (Thompson 1936), makes reference to finding differences in the head size of adult halibut from different locations. Their comments indicate that a large head size is commonly considered a characteristic of

slower growth, which has been confirmed through age studies. The recent fisheries literature seldom mentions such observations. However, if there is a difference in relative head size between faster and slower growth halibut, it would be consistent with the findings that slow growing halibut have larger otoliths than fast growing halibut. In this case, otolith growth would be tracking head growth, and both have an allometric relationship with body size.

Adult halibut

Adult halibut can be defined by their sexual maturity, which is typically more a function of size than age, and will vary between males and females. The adults will apparently exhibit a high degree of site fidelity (home ranges) as indicated from the sonic-tracking studies in Glacier Bay (Phillip Hooge, U.S.G.S. Biological Resource Division, pers. comm.). Younger halibut however may also exhibit similar behavior as indicated from the same study, but they are also thought to exhibit shoaling behavior at times and extensive migrations are known to take place through tagging studies. This might an indication that population structure of halibut is heterogeneous, with sub-populations that may exhibit different behavioral patterns. The existence of sub-populations of halibut was one of the primary observations that came from the earliest work on halibut (Thompson 1936). This was shown through measurements of shape, early tagging studies, and the observations of long term local depletion due to fishing effort. Migration of halibut was initially considered not common enough to be important in the maintenance of the local populations. It was much later that the intermixing of halibut sub-populations came to be emphasized, primarily through the movement at the early life stages (Skud 1977). This emphasis on the intermingling of stocks across broad areas coincided with the decisions associated the United States instituting a 200 mile fishing limit and the elimination of Canadian halibut fishing in U.S. waters.

Adult halibut were not explicitly examined in this study, though their otoliths were used in chapter 2 to reconstruct juvenile otolith growth histories and adult otoliths were examined for the elemental

composition of juvenile growth in chapter 4. Inferences however may still be drawn based on some of the patterns that were observed.

As shown in chapter 2, there was an increase in the importance of year-class effects in explaining temporal variation with age in the older juveniles. In other words, annual growth appears increasingly autocorrelated with age and this trend likely extends to adult halibut. The result is that growth of adults is likely characterized by low frequency patterns, where past growth predicts future growth and environmentally induced changes are no longer the sole influence on annual growth. As I noted earlier, I did not find evidence of density dependence in the growth record of young fish. However it may be reasonable to suggest that such competition plays a role at older ages, where halibut are no longer competing for resources with other species, but rather as top predators compete with each other and across multiple age classes. Territoriality and competition for preferred feeding areas perhaps plays a part. There has apparently been a long term trend of increasing growth rates of adult halibut over time as noted from IPHC survey data. Separating out environmental factors from a density dependent response due to commercial fishing, bycatch removals or other factors has yet to be determined.

Extending the type of information to extract from otoliths, it would be interesting to consider whether the onset of sexual maturation can be detected. Such information could contribute new ways of assessing the population by providing a maturation age for use in age-structured population models. Attempts at sexing adult halibut through otoliths, however, has been unsuccessful and previous studies using annuli growth zones to detect sexual maturation in other species have been not been validated. A recent study of plaice suggests that there might be some possibility to detect first maturation in females through their otolith zone spacing but more work is still needed (Rijnsdorp and Storbeck 1995). Another method to consider is the elemental composition approach. The strontium/calcium balance in particular may be affected by the onset of vitellogenesis. I am not aware of any studies that have looked at the question specifically but based on work by Kalish (1991), it might be worth an examination. I would also suggest

that the method of elemental mapping, as shown in chapter 4, might prove useful for identifying subtle changes in strontium patterns.

To close this chapter, a final consideration is given to a puzzle which brings this back to the beginning. W.F. Thompson, who, along with R. Van Cleve, made the first and most complete investigation of halibut life history in the 1920's and 1930's, also spent considerable effort at identifying the population structure of halibut at different locations. How the population structure is maintained given the long larval drift period and wide distribution of progeny was a mystery then and it remains so today. The male and female halibut swimming side by side during spawning must have had a means of finding each other as well as means of selecting a spawning location. If an area is favorable for launching progeny into the currents of the North Pacific, and those progeny have a high survival rates, then it seems reasonable to expect that some mechanism is in place to maintain that area as a spawning site over time and over generations. The migration of young halibut is clearly the mechanism that counters the direction of the larval drift, but do the progeny actually return to the feeding and spawning areas of their parents and if so how? W.F. Thompson, who worked with Pacific salmon and studied their well known homing ability, also felt the same model applied to halibut (O.A. Mathisen, JCFOS pers. comm.). If site-specific homing does take place with halibut, is the mechanism through imprinting similar to salmon? But for halibut, the young are released as eggs and their developmental rates are slow; they could have drifted several hundreds of miles from a spawning location before any sensory organs become functional. A genetic explanation likely plays a part, but in other species it only provides the individual the ability to read a compass, it does not provide the map. Sinclair (1988), in an essay on stock composition, would probably argue that it is the area the larvae reside in that determines the population structure, and specific spawning sites that contribute to a common larval retention area are largely irrelevant in terms of population regulation. Cury (1994) however suggests that any spawning population persists as a result of some type of imprinting process, even though the mechanisms may not be known. So the question remains as to how local spawning and feeding sites for halibut get

established across broad geographic areas, what brings the halibut to them, and if they are persistent across generations, how is that maintained.

A final thought attempts to address this latter point. Strontium in the center of the otolith is apparently derived from the parent through the yolk, and yolk-sac absorption takes place over a period of months while the larva develops. Can other elements arrive by the same pathway into the newly forming brain of the larva, perhaps influencing some neurological development? Is there an elemental signature specific to the feeding areas of the adults through which the elements can be passed to the yolk in the developing eggs? If the answer to both is yes, then it presents a potential mechanism that can provide an imprinting signal. In this case the signal is delivered slowly as the larva drifts and develops miles away from the parents. The successful progeny then returns from a distant nursery area with a 'compass' that seeks out that same signal. An elemental signal as hypothesized here can potentially be uncovered through the examination of the otoliths using new technology.

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