

CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

THE AGING, VALIDATION, AND BACK-CALCULATION OF THE DATE OF BIRTH AND SETTLEMENT DATE OF YOUNG-OF-THE-YEAR CALIFORNIA HALIBUT (PARALICHTHYS CALIFORNICUS) INTO ALAMITOS BAY, CALIFORNIA USING A NEW TECHNIQUE FOR THE GRINDING OF SAGITTAL OTOLITHS.

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by

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ABSTRACT

THE AGING, VALIDATION, AND BACK-CALCULATION OF THE DATE OF BIRTH AND SETTLEMENT DATE OF YOUNG-OF-THE-YEAR CALIFORNIA HALIBUT (PARALICHTHYS CALIFORNICUS) INTO ALAMITOS BAY, CALIFORNIA USING A NEW TECHNIQUE FOR THE GRINDING OF SAGITTAL OTOLITHS.

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Fifty-six young-of-the-year (YOY) California halibut (Paralichthys californicus), captured from Alamitos Bay, California, were aged using a new method for grinding both faces of sagittal otoliths. The method was developed to produce a microscopic thin section that preserves growth rings at the margins while increasing the resolution of internal growth rings.

The age-length relationship existing between six YOY halibut born in February and December can be described by the linear equation $Y = 1.3x + 26.6$ ($r = 0.96$, $P < .01$) where Y is the age (days) and x is the standard length (mm SL). The standard lengths of YOY halibut ranged from 34 to 81 mm in this data set. The rate of growth calculated from this model was 0.738 mm/day.

The age-length relationship existing between 50 YOY halibut born in March, April, May, and June can be described by the linear equation

$Y = 0.9x + 30.6$ ($r = 0.91$, $P < .01$) where Y is the age (days) and x is the standard length (mm SL). The standard lengths of YOY halibut in this data set ranged from 11 to 142 mm. The rate of growth calculated from this growth model was 1.104 mm/day.

The YOY halibut born in February and December ($n = 6$) were excluded from the following analysis because it was felt that these outliers would have a disproportionate influence on the growth model.

The second degree polynomial equation: $Y = 12.6 + 1.6x - .005x^2$; $r = 0.94$ was calculated for estimated age regressed on the standard length of YOY California halibut ($n = 50$) born in March, April, May, and June. The growth rate calculated from this curvilinear model was 0.86 mm/day for YOY California halibut whose range in standard length was 10 to 80 mm. The model predicted an overall growth rate of 1.25 mm/day for YOY with standard lengths ranging from 10 to 150 mm SL.

The range in standard length and the associated back-calculated age of YOY halibut captured for this study was 11 to 142 mm SL and 38 and 154 days old, respectively. The age at settlement was 25 to 31 days.

Three methods of validation involving the immersion of YOY halibut in various solutions of tetracycline were employed. The daily formation of growth rings was not validated by these methods. Recent work on laboratory reared California halibut has validated the production of growth increments as a daily phenomenon (Lavenberg, pers. comm. 1989).

INTRODUCTION

The California halibut (Paralichthys californicus) is important to both the commercial and the sport fisheries of California (Haaker 1975). It is a benthic spawner that breeds in nearshore waters some distance from the protected and semi-protected environments that young-of-the-year (YOY) halibut choose for settlement (Allen et al In press). The elimination or conversion of these preferred settlement habitats necessitates the understanding of the settlement strategies this species uses if it is to be managed effectively.

The mechanisms by which YOY California halibut move from nearshore breeding sites to protected and semi-protected coastal sites are not well understood. The aging and back-calculation of the date of birth and settlement age is primary to the study of California halibut since estimating the date of birth allows for the prediction of when YOY halibut will be entering the fishery. Moreover, back-calculated settlement ages can be of use in identifying the major biological and environmental determinants correlated to settlement success.

Aging of fish is now a standard tool used to gauge the health of a fishery (Beamish and McFarlane 1987), thus allowing the maintenance of fishery stocks so that a sustained yield can be both predicted and managed. Age studies prior to 1971 were mainly based on annual growth rings found on the scales of fish. Scales are not a reliable structure to use for the aging of fish that are post-maturity (Simkiss 1974). The

scales can undergo reverse mineralization and actually lose calcium under periods of stress. Calcium is the major component of scales and has been found to be resorbed or not deposited on the scales during periods of starvation (Chilton and Beamish 1982; Simkiss 1974). Calcium resorption decreases the number of increments on the scale and results in an underestimation of age.

The method of aging commercially important fish has changed since Pannella (1971) discovered daily growth increments on otoliths of fish. Growth increments found on otoliths and other hard non-calcium resorbing structures has made it possible to accurately age YOY fish (Chilton and Beamish 1982, Geffen 1987). An ever-increasing list of age studies has utilized the technique of reading daily growth rings on otoliths of YOY fish (Brothers et al. 1976; and Jones 1986). Methot (1983) used this technique to age and estimate birth dates for the northern anchovy (Engraulis mordax). Victor (1983), McFarland et.al. (1985), and Boehlert and Yoklavich (1985) employed this technique in estimating settlement times and ages of blue-headed wrasse (Thalassoma bifasciatum), French grunts (Haemulon flavolineatum), and sablefish (Anoplopoma fimbria), respectively.

The otoliths consist of pairs of the following three bones, the sagitta, lapillus, and asteriscus, found in the otic region of the skull (Harder 1975). The bone most commonly used in age studies is the sagitta. Otoliths continue to grow throughout the life of the fish, laying down a new increment each day consisting of an organic layer and a calcium carbonate layer. A problem that was uncovered during the preliminary aging study of California halibut by Allen et al. (1984) was that sagittal otoliths of YOY halibut over 45 mmSL (mm Standard Length)

were too thick to allow for the resolution of the daily growth increments with a light microscope. This was the driving force to develop a new technique to grind down the sagittal otoliths of California halibut ≥ 45 mmSL.

The purposes of the present study were to: 1) develop a technique to grind sagittal otoliths of YOY California halibut to allow for the resolution of growth increments; 2) develop a growth model from which the birth and settlement dates of field caught YOY halibut can be estimated; and 3) obtain an estimate of the rate of growth in YOY California halibut based on this growth model.

This study is part of a larger study of the recruitment and distribution of YOY California halibut (Paralichthys californicus) along the southern California coastline conducted by Allen in conjunction with the Ocean Studies Institute.

MATERIALS AND METHODS

A total of 56 young-of-the-year (YOY) California halibut (Paralichthys californicus) were collected from Alamitos Bay, California (Fig 1). Several net types were used to capture YOY halibut: 1) a standard haul seine measuring 6.1 m x 1.8 m strung with 3.1 mm ace style mesh, 2) a two meter otter trawl, and 3) a two meter beam trawl. The YOY California halibut were measured to the nearest millimeter standard length (mmSL) and then frozen for transport to the laboratory. The sagittal otoliths were removed and ground using the following methods.

OTOLITH REMOVAL PROCEDURE FOR THE CALIFORNIA HALIBUT (PARALICHTHYS CALIFORNICUS).

1. The head was removed with an angled cut behind the opercular bone. This cut was made in two steps: an angled cut starting from the dorsal surface to the pectoral fin, then a cut perpendicular to the main body axis was made from the ventral surface to the pectoral fin. The straight portion of the cut was very close to the anterior base of the hump in the lateral line.

2. The skin and tissues were separated from the skull of the fish, using a dissecting microscope. The opercular connection between the opercule and the skull was then broken and the skull freed of skin and tissue.

3. The skull was pierced in a circular fashion behind the eyes and at the midlevel of the skull. This allowed for the removal of the

uppermost portion of the skull. The bone located between the eyes was then weakened and broken at the point where it meets the brain case. The skull cap was peeled over starting from behind the eyes toward the body. The dorsal sagitta and asteriscus were found adhering to that portion of the skull that was peeled over. The sagitta and asteriscus were enclosed by the same membrane and generally came out together. The lapillus was also located on this inner portion of the skull cap, independent of the sagitta and the asteriscus. The lapillus was contained in a long tubular membrane that attached to the forward portion of the skull. The dorsal lapillus was located in the center of the peeled away skull cap. The lapillus was obscured by a dark inner membrane that lined the skull cap.

4. The posterior section of the skull was removed starting from the region where the vertebrae attach. The brain was teased out by carefully removing the fluid tissues while not changing the positions of the bones remaining in the skull. The ventral sagitta and asteriscus were located in the brain case close to the jaw. The sagitta was in a crevice and was carefully removed because of its brittle and fragile character. The ventral asteriscus generally was removed with the ventral sagitta. The ventral lapillus was located above and central to the position of the ventral sagitta and its chamber.

5. The right and left otoliths were separated and placed in their respective positions on a glass slide. This reduced the amount of error in the identification of the otoliths.

6. The bones were cleaned of all tissues before mounting. This was done by placing the bones in a drop of distilled water on a glass

slide and carefully teasing the membranes away with very fine needles. A dissecting scope was used to magnify the bones and reduce breakage.

7. The sagittal otoliths were then mounted on 3mm x 75mm glass rods using cyanoacrylate (SUPER GLUE) as the mounting medium. The mounting media was chosen for its ability to tightly affix the sagitta to the glass rod. This media reduced micromovements of the mounted sagitta during and after the grinding procedure which prevented fracture of the bone. The glass rod was inserted into the fixture (Fig. 2) which allowed the preferred grinding plane to be adjusted in the x, y, and z axes. The glass rod was used as an accessory light source, by introducing light at the base of the fixture. Light traveled up the glass rod and highlighted the otolith from below. A second light source was used to side-light the otolith. Turning either light on or off allowed different views of otoliths margins.

GRINDING TECHNIQUES

Many common thin sectioning techniques (ie. microtomes) cannot be employed with sagittal otoliths because of the fragile nature of these bones. The resolution of growth rings on sagittal otoliths of YOY California halibut was much improved in individuals measuring ≥ 45 mmSL if the otolith was ground. The grinding of the sagittal otoliths of YOY California halibut ≤ 25 mmSL does not improve the resolution of the growth increments and increases the chance of breakage. The techniques for grinding these small structures have been adapted from many varied sources.

One technique developed by me for the present study was the use of a grinding fixture (Fig. 2). In the past, otoliths have been sectioned

by mounting the bone on a glass slide and then abrading the surface of the bone until a flat plane has been obtained. Because grinding and lapping has been done by hand a truly flat plane has been very difficult to obtain. Without the ability to view and adjust the plane of the otolith while grinding, daily growth rings along the margins of the bones may be lost, leading to under-estimation of age. The use of a grinding fixture assures that a flat plane is produced, thus protecting the daily growth rings found at the margins of the bones.

The fixture used provided a lapping guide for grinding a flat surface. The lapping guide is a circular flat platform that adjusts up and down by turning it clockwise or counterclockwise on a threaded cylinder (40 threads/inch) (Fig. 2). The outer diameter of the lapping guide was divided and scribed with 25 equal increments. Turning the lapping guide one increment would raise or lower the lapping guide in increments of .025 mm insuring that the otolith was not subjected to undo stress and possible breakage.

Initial grinding was done with 1200 grit sand paper (NORTON) attached with double stick tape to a 25.4 X 76.2 mm clear glass slide . The glass slide was inverted so that the abrasive paper was facing down and placed on the lapping guide. With very light pressure the glass slide was moved back and forth allowing the abrasive paper to abrade the sagitta. The initial grind was followed by successive grinds employing 30, 12, and 3 micron (IMPERIAL LAPPING FILM 3M) lapping films attached in the same manner. When a flat, smooth surface had been ground on the sagitta it was removed from the glass rod by immersing it in 15 ml of acetone. The bone was then remounted on a 25.4 X 76.2 mm clear glass

slide with its newly ground flat surface facing the slide. The unground surface of the sagitta was now ready to be ground.

The fixture was then converted to a lapping table by inserting a flat stainless steel disk that had been machined to slip fit into the grinding fixture (Fig 2). The lapping table provided a flat surface to which abrasive papers were attached. The glass slide with the sagitta attached was inverted allowing the sagitta to be ground on the lapping table. The lapping guide was used to obtain flat and parallel sagittal surfaces. The same series of abrasive papers was used to grind and lap the sagittal surface.

The sagitta were then viewed and the increments counted under a light microscope.

PROTOCOL FOR INCREMENT COUNTS

1. The central nucleus was often hard to distinguish, due to the many accessory nuclei (Fig 3) found in California halibut. When in doubt, I considered the nucleus closest to the central axis of the bone to be the central nucleus.

2. Measurements were made along a radius from the central nuclei to the posterior edge of the otolith (Maceina et al. 1987) by using a dial indicator that was adapted to an Olympus CHT compound microscope, with regular bright field illumination. A pointer was used as the stationary point and the distance traveled was recorded on the dial indicator. The first measurement made was that of radius length. This value was then stored in a computer program so that it could later be used as a factor. Segments along the radius that had clear consecutive rings were then measured, with the counted increment number entered

first and the distance units second. These segments were then used to calculate an average number of increments per unit distance. This value was then used as a conversion factor to convert the radius measurement into the total number of increments. This value was then stored and repeated measurements on the same bone were made and stored. Repeated measurements differing more than 5% were not included in the calculation of mean increment number.

BACK-CALCULATION OF DATE OF BIRTH

The mean number of increments calculated from an individual sagitta is equivalent to days of life. A correction factor of four days must be added to this value in as much as YOY California halibut do not produce growth increments during the first four days of life (Lavenberg pers. comm. 1989). The value calculated from the mean number of increments plus the correction factor was then used to back-calculate the date of birth.

VALIDATION

Validation in this and any aging study is important to insure that the growth increments are truly daily growth increments (Geffen 1987). To do this a relationship must be demonstrated between a known period of time and the number of daily growth increments produced in that period of time. To be valid, this relationship must have a correlation coefficient very close to one.

One aspect of validation involves the placement of a mark on a growth increment of an otolith that fixes a specific point in time. Marking strategies have included both the denial and augmentation of

food resources in order to produce a difference in the growth increments (Victor 1982). Other techniques involve the use of chemicals, such as tetracycline, that leave a fluorescent mark on the otolith.

The most common validation technique used for marking otoliths is the intraperitoneal injection or whole fish immersion in a tetracycline solution, thus producing a fluorescent mark on the otolith (Hettler 1984).

Three different immersion methods have been attempted, by me, to produce a fluorescent mark on the otoliths of YOY California halibut. Immersion in tetracycline was favored over injection of tetracycline as a technique that would not traumatize the fish and expose it to infection. Immersion can be readily employed under field conditions.

One method used was modeled after Hettler (1984). The technique involved the immersion of YOY halibut in a solution of 250 mg tetracycline per liter of 33 ppt NaCl for two hours. The sodium chloride solution is used instead of sea water because it lacks the components that bind with tetracycline making it unavailable to mark the bone. The YOY halibut were maintained in the laboratory for seven days after immersion. The otoliths were then removed and examined for the presence of the fluorescent mark. The sample size for this experiment was 6.

A second method was modeled after Schmitt (1984). YOY halibut were immersed in a solution of 250 mg tetracycline per liter of 33 ppt seawater for 12 hours. The sample size was 27. The fish were caged in the field for seven days so that the environmental cues that promote the formation of growth increments would be present.

A third validation experiment involved immersing of juvenile fishes in a tetracycline solution using the method described by Campana and Nellson (1982). Field caught YOY California halibut were immersed in a 0.02% oxytetracycline/saline solution for 24 hours after which they were caged in the field for ten days. They were then sacrificed and kept frozen until the otoliths could be removed and examined under a fluorescent microscope. The sample size was 25.

Caging of the animals in the field

A cage resembling a frustum pyramid (Rasis 1984) (Fig 4) with one m^2 of bottom surface was built from steel reinforcing rods and fitted with 3.1 mm ace style-netting. The physical characteristics of the net are as follows: 1) total volume, $.29 m^3$; 2) total available surface area, $3 m^2$; and 3) total bottom area available to juvenile halibut for perching, $1 m^2$. The opening of the cage was an $.254 m$ nylon zipper sewn into the netting on the top of the cage.

Statistical Analysis

Means, variances, and standard deviations were calculated where appropriate. The Tukey-Kramer method for multiple (unplanned) comparisons among pairs of means with unequal sample sizes was used to determine if the means calculated for increment number and standard length versus birth months were significantly different at the $p < 0.05$ level.

Linear regression and correlation analysis was used to test the associations of birth month with standard length and daily age. The

linear growth models developed allowed for comparison by birth month and the calculation of growth rates.

Curvilinear regression was used to develop a growth model for YOY California halibut. Correlation analysis was utilized to test the significance of association between the two variables.

RESULTS

AGING

A total of 56 young-of-the-year (YOY) California halibut (Paralichthys californicus) were aged. The YOY halibut ranged in standard length from 11 mm to 142 mm. The distribution of standard lengths of the YOY halibut, in 10 mm size classes has been plotted in figure 5.

Mean counts of daily growth increments for each sagittal otolith allowed for the calculation of birthmonth. When sorted by birthmonth, the YOY halibuts standard length in millimeters (mm SL) was plotted versus its age (days) (Fig 6).

The mean standard lengths and ages for YOY halibut were calculated for each birthmonth (Fig 7). The number of YOY halibut with birthdates calculated to be in the following birthmonths are as follows: February n = 5, March n = 12, April n = 13, May n = 21, June n = 4, and December n = 1.

The age-length relationship existing between the 56 YOY halibut can be described by the linear equation $Y = 31.26 + 0.94x$ ($r = 0.89$, $P < 0.01$) where Y is age in days and x = millimeters standard length (Fig. 8).

The results of the unplanned comparisons (Tukey-Kramer test) of the mean daily growth increments of YOY halibut when grouped by birthmonth are reported in Table 1. A significant difference was found between the mean daily increment counts for the birthmonths of March and June ($p <$

0.05, df = 50). There was no significant difference demonstrated between the means of the daily increment counts for the birthmonth comparisons of February and March, February and April, February and May, February and June, March and April, March and May, April and May, April and June, and May and June.

Table 2 shows the results of the unplanned comparisons (Tukey-Kramer test) of the mean standard lengths of the YOY halibut when grouped by birthmonth. A significant difference was found between the mean standard lengths of YOY halibut with the birthmonths of March and June, April and June, and May and June ($P < 0.05$, $df = 50$). There was no significant difference between the mean standard lengths of YOY halibut with the birthmonths of February and March, February and April, February and May, February and June, March and April, March and May, and April and May.

The age-length relationship existing between the six YOY halibut born in the months of February and December (Fig. 9) can be described by the linear equation $Y = 1.3x + 26.6$ ($r = 0.96$, $P < .01$) where Y is the age (days) and x is the standard length (mm SL). The standard lengths of the YOY halibut ranged from 34 to 81 mm in this data set.

The age-length relationship existing between the 50 YOY halibut born in the months of March, April, May, and June (Fig. 10) can be described by the linear equation $Y = 0.9x + 30.6$ ($r = 0.91$, $P < .01$) where Y is the age (days) and x is the standard length (mm SL). The standard lengths of YOY halibut in this data set ranged from 11 to 142 mm.

The rate of growth predicted by the linear growth model, for YOY halibut with the birthmonths of February and December, $Y = 1.3x + 26.6$ is 0.76 mm/day. The rate of growth predicted by the linear growth

model, for YOY halibut with the birthmonths of March - June, $Y = 0.9x + 30.6$ is 1.11 mm/day.

The YOY halibut with the birthmonths of February and December (n = 6) were excluded from the following analysis because it was felt that these outliers would have a disproportionate influence on the growth model. The following results provided the basis for exclusion: 1) the significant differences in the means of the ages (days) of these fish; 2) the significant differences in the regression coefficients between both linear growth models; and 3) substantial differences in the growth rates predicted by each model.

CURVILINEAR GROWTH MODEL

The growth model produced from the application of curvilinear regression to YOY halibut with the birthmonths of March - June (n = 50) is $Y = 12.6 + 1.6x - .005x^2$ ($F_{(49,3)} = 107.8$ ($P \ll .001$), $R^2 = 87.5$) (Figures 11 and 12).

The growth rate calculated from the model is 0.86 mm/day for YOY halibut whose range in standard length is 10 to 80 mm. The overall growth rate predicted from the model for YOY halibut whose standard lengths ranged from 10 to 150 mm SL is 1.25 mm/day.

The smallest and youngest halibut in this study measured 11 mm SL and its age was estimated to be 21 days. The regression equation above would predict that a YOY halibut measuring 11 mm SL would be 38 days old. The oldest fish in this study measured 132 mm SL and its age was estimated at 158 days. The curvilinear growth model estimates that a YOY halibut measuring 132 mm SL would be 146 days old. The largest YOY halibut (142 mm SL) was estimated to be 112 days old. The curvilinear

growth model estimates that a YOY halibut 142 mm SL would be 154 days old.

AGE AT SETTLEMENT

The length at which YOY California halibut settle is between 8 and 12 mm SL (Allen 1988, Kramer and Hunter 1987). The curvilinear growth model (Fig. 11) estimates the ages of settling YOY halibut to be in the range of 25 to 31 days. The YOY halibut with the shortest standard length in this data set (11 mm) was judged to be approximately 30 days old.

VALIDATION

The results of the three validation experiments are as follows:

Validation after Hettler 1984

In this experiment, the solution killed 33% of the YOY California halibut during the two hour treatment period. The smallest of the fish in the sample died while the larger fish did not. Examination of the sagittal otoliths from YOY halibut revealed that the tetracycline did not produce the expected fluorescent mark. Hence this technique failed to produce a reliable mark that could validate the growth increments as being daily in nature.

Validation after Schmitt 1984

All of the YOY California halibut (n=27) survived the immersion in the solution for 12 hours. The fish also survived the subsequent seven

days of caging in the field. When the otoliths of these fish were examined for the presence a fluorescent mark, none was found. Hence this treatment also failed to reveal any reliable marks that could validate the daily production of the growth increments.

Validation after Campana and Neilson 1982

There was 100% survival of 25 YOY California halibut used in this treatment. They survived immersion in a 0.02% oxytetracycline/saline solution for a period of 24 hours. The juvenile fish also survived the subsequent ten day caging period. When the otoliths were examined no fluorescent band was found. This method did not validate the hypothesis that growth increments correspond in a 1:1 ratio with daily age.

While these experiments have not yielded any evidence that the increments used for the estimations of daily age are in fact laid down in a daily fashion. However, since it is widely agreed that these increments are correlated with daily cycles (Beamish and McFarlane 1987), I have assumed that these increments have to be laid down in a daily fashion. Recent research on hatchery reared California halibut has validated the growth increments as daily growth increments (Lavenberg, pers. comm. 1989)

CAGING

The cage (Fig. 3) performed quite well in all of the validation attempts. A total of 27 YOY halibut were caged for seven days with 100% survival and 25 YOY halibut for ten days with a 100% survival. Since there was no augmentation of food supplies, it is assumed that the YOY

halibut were feeding on small crustaceans that were attracted to the net structure.

DISCUSSION

The ability to age California halibut (Paralichthys californicus) is important to the management of this species. The use of hard, non-calcium resorbing structures that record daily growth increments, such as otoliths, has become common practice in the aging of fish (Chilton et al. 1982). However, viewing the growth increments on the otoliths of larger fish is difficult because of bony overgrowth (Ralston 1974). The otoliths of young-of-the-year (YOY) California halibut with standard lengths ≥ 25 mm have otoliths that are too thick to allow good resolution of growth increments (Allen 1988). The development of a grinding technique to increase the resolution of growth increments was necessary.

The technique of grinding both surfaces of the sagittal otoliths improves the resolution of growth increments. The development of the grinding fixture (Fig. 2) greatly enhances the ability to produce planar sections that preserve the increments found at the margins of the otolith (Brothers 1987). One advantage of this technique is that expensive machines, ie. bone saws and lapping machines, are not necessary to prepare a sagittal otolith for increment counting. Another advantage is that the combined preparation time of a single sagittal otolith is about one hour and all grinding can be done in a single day.

The three tetracycline immersion techniques (Hettler 1984; Schmitt 1984; Campana et al. 1982) did not produce the expected fluorescent mark on otoliths of YOY California halibut. Because the otoliths would not take a mark, the growth increments found were not validated as daily

growth increments. The daily production of growth increments on the otoliths of YOY halibut is an assumption based on the daily production of growth increments on otoliths of many diverse species (Brothers et al. 1976; Campana et al. 1982; Campana 1983; Jones 1986; Victor 1982; Warlen 1987; and Young et al. 1988). Immersion in tetracycline was not a useful technique for the production of fluorescent marks on otoliths of YOY California halibut. Intraperitoneal injections of the tetracycline solution should be employed for future daily increment validations.

The formation of the first growth increment in laboratory reared California halibut has been estimated to occur four days after fertilization of the egg (Lavenberg pers. comm. 1989). This delay in the first growth increment production is not unusual (Brothers et al. 1976; Warlen 1987). Brothers et al. (1976) found that northern anchovy (Engraulis mordax) produce the first growth increment six days after the egg is fertilized. Warlen (1987) found that five days pass between hatching and the production of the first growth increment on the otoliths of Gulf menhaden (Brevoortia patronus). Campana (1984) found that 4 to 6 days pass after hatching before the first growth increment is produced on the otoliths of the starry flounder (Platichthys stellatus).

Two studies (Haaker 1975; Allen 1988) have reported that the main settlement of YOY California halibut into the bay and estuarine environment is during the spring months of April, May, and June. The standard length of YOY halibut newly settled into Alamitos Bay has been found to be between 8 and 12 mm standard length (mm SL) (Allen 1988).

The range of standard lengths of the YOY halibut captured from Alamitos Bay (Fig. 1) for this study was between 11 and 142 mm (Fig. 5).

The partitioning of YOY California halibut by birth month (Fig. 6) has yielded some interesting results (Fig. 7). The YOY halibut with the birth months of December and February are older than expected for their standard lengths. The YOY halibut have a mean standard length that is nearest the mean standard length of YOY halibut with the birth month of May. The mean age of YOY halibut with the birth months of December and February is nearest the mean age of YOY halibut born in June (Fig. 7).

A significant difference ($p < 0.05$, $df = 50$) existed between the mean daily ages of YOY halibut born in March and June (Table 1), as was expected. Spawning is from February to July (Allen 1988) and follows a normal distribution, so fish born in March are more likely to have been born later in March while fish born in June are more likely to have been born earlier in June. One would expect that fish born in February would have a younger mean daily age than those born in later months; however, the mean daily age of the fish born in February is much greater than that of the fish born in March, and is similar to the mean daily age of fish born in June (Fig. 7). The observed difference between February and March cannot be demonstrated statistically, but this difference is thought to be a real difference, and not an artifact of sampling.

A significant difference ($p < 0.05$, $df = 50$) existed between the mean standard length comparisons of YOY halibut with the birth months of March, April, and May when compared to June (Table 2). This again is an expected result. What was surprising is that there was no significant difference demonstrated between YOY halibut born in February compared to

those born in June. The YOY halibut born in February were comparable in mean standard length to those born in May (Fig. 7).

Three linear growth models comparing age-length data were produced for the YOY halibut: 1) for all YOY ($n = 56$, $r = 0.89$) (Fig. 8); 2) YOY halibut born in December and February ($n = 6$, $r = 0.96$) (Fig. 9); and 3) YOY halibut born March - June ($n = 50$, $r = 0.91$) (Fig. 10). The linear growth models show that when the influence of YOY born in December and February was removed from the data set, the correlation coefficient became more positive for YOY halibut with the birth months March - June over that calculated for all YOY. Young-of-the-year halibut born in December - February were found to have a very strong association between age and standard length.

Growth rates calculated from the linear growth models for YOY halibut with the birth months of December and February (0.7mm/day) and March - June (1.1mm/day) yielded different growth rates. The YOY halibut born in March - June grow 1.5 times as fast as those born in December and February. Young-of-the-year halibut born in December or February may maintain a slower rate of growth until there is an increase in the favorable biotic/abiotic growth factors found in protected and semi-protected environments. This is similar to what Warlen (1987) found for early versus late recruiting Gulf menhaden (Brevoortia patronus). A YOY halibut with a birth month that is correlated to colder water temperatures and lower rates of production would have a slower rate of growth. Campana (1984) found that metamorphosis and settlement of starry flounder (Platichthys stellatus) into the bays is determined by size, not age. This suggests that fish delay their growth in standard length during time periods with poor growth conditions.

A curvilinear relationship between otolith increments (age in days) and fish length (mm SL) appears to be a characteristic of flatfish with pelagic larvae (Campana 1984, Brothers 1987). The California halibut is a flatfish with a pelagic larval phase. A curvilinear relationship was explored for the YOY halibut with the birth months of March - June (n = 50) (Fig. 11). The second degree polynomial $Y = 12.6 + 1.6X - .005X^2$, ($F_{(49,3)} = 107.8$, $P < .001$) (Y = age in days, X = mm SL) calculated from the age-length data predicts that the smallest YOY halibut (11 mm SL) in this sample would be 30 days old. This is close to Allen's (1988) estimate that the settlement of YOY California halibut in Alamitos Bay occurs when they are between 20 and 29 days old. Allen (1988) did not correct settlement ages for the time delay between birth and first increment formation. In light of this, the corrected settlement ages would be, using the estimated four day delay (Lavenberg, pers. comm. 1989), to be between 24 and 33 days old which is consistent with these results.

Young-of-the-year halibut were estimated to grow to 80 mm SL in their first year (Allen 1988). In this study, the calculated growth rate for YOY halibut whose range in standard length was 10 to 80 mm was 0.86 mm/day. This estimate falls in the range of growth rate estimates of YOY California halibut (10 - 80 mm SL) calculated from Allen (1988); for the years 1983, 1984, and 1985, the growth rates were 0.35, 0.71, and 0.98 mm SL/day, respectively (Allen 1988). The age estimates from this study indicate that in their first year of life halibut may attain a standard length greater than 150 mm. The growth rate predicted from the model for YOY halibut whose standard lengths ranged from 10 to 150 mm is 1.25 mm/day (Table 3).

Prior studies (Allen 1988; Kramer et al. 1987) have assumed that YOY California halibut belonged in a size class ≤ 80 mm SL. The results of this study suggest halibut grow to a much larger size in their first year of life. The YOY with the greatest mean age in days (158) had a standard length of 132 mm. This individual fish was just over 5 months old. The YOY halibut with the largest length (142 mm SL) was not the fish with the greatest number of growth increments.

One aspect of sustained yield management is the estimation and prediction of settlement of YOY into a fishery. Settlement success is very labile in many fish populations (Sissenwine et al 1988). The curvilinear growth model produced for YOY California halibut (Fig. 12) born in March - June allows for the prediction of a YOY halibut's age based on its standard length. This is a useful tool that can be used to age and release field collected YOY halibut while gathering information about major environmental factors that may have an influence on the settling strategies of these fish.

Several sources of error can be associated with reading sagittal otoliths in YOY California halibut. The sagittal otoliths of YOY halibut have a central nucleus surrounded by several accessory nuclei (Fig. 4) which have growth increments arising from them. The increments associated with accessory nuclei converge with increments of the central nucleus. This creates convergence zones where the increments are not uniform in width. Convergence zones can produce growth increments that are greater or smaller in width than surrounding areas devoid of accessory nuclei (Campana 1984).

The method for calculating the number of growth increments per measured radii involves taking an average of the number of increments

per unit distance. The calculated average number of increments per unit distance can be directly influenced by the non-uniform growth increments of convergence zones. The method of measurement could not separate out areas in which these accessory nuclei were present. This phenomenon is a common problem associated with the aging of flatfish; a similar problem was encountered by Campana et al. (1984) when looking at the growth increments on the otoliths of starry flounder (Platichthys stellatus).

An additional source of error arises in the measurement of the radii. Each radius was measured from the center of the central nucleus out to the edge of the otolith. The length of the radius from the center of the central nucleus to the first growth increment includes an area where no growth increments exist. The inclusion of this length of radius results in the estimation of a greater number of growth increments. The difficulty in the interpretation of the first growth increment was recognized in the preliminary aging study (Boehlert et al. 1984). The variability in the distance measured from the center of the central nucleus to the first visible growth increment provided the basis for the decision to include this distance in the measurements of radii. The measurements would be a less variable and more accurate, although biased, estimate of the number of increments.

The presence of finely spaced increments adds another source of error. The resolution of daily increments is difficult when the increments are close together. A miscount of the number of increments, due to their close proximity, can result in an under or over-estimation of age. Finely spaced daily growth increments may result from several sources. YOY halibut may have differing growth rates depending on birth

month; halibut born in the months of March, April, May, and June have faster rates of growth. Halibut born outside of those months will have a slower rate of growth, which would produce finely spaced growth increments. Another source of finely spaced daily growth increments may be the result of a delay in the metamorphosis of the larval halibut in order to locate suitable nursery grounds. The possibility exists that early settling YOY halibut encountering unfavorable growth conditions would produce finely spaced growth increments.

The cage (Fig. 3) used in this study worked well in all of the validation procedures. A 100% survival rate was achieved with 27 YOY halibut caged for seven days and 25 YOY halibut caged for ten days. There was no augmentation of food supplies. It was assumed that the YOY halibut were feeding on small crustaceans that were attracted to the net structure. Non-augmentation of food resources was not seen as a factor that would cease increment deposition as Ralston (1976) found that butterflyfish do continue to deposit increments during periods of starvation.

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APPENDIX A

FIGURES

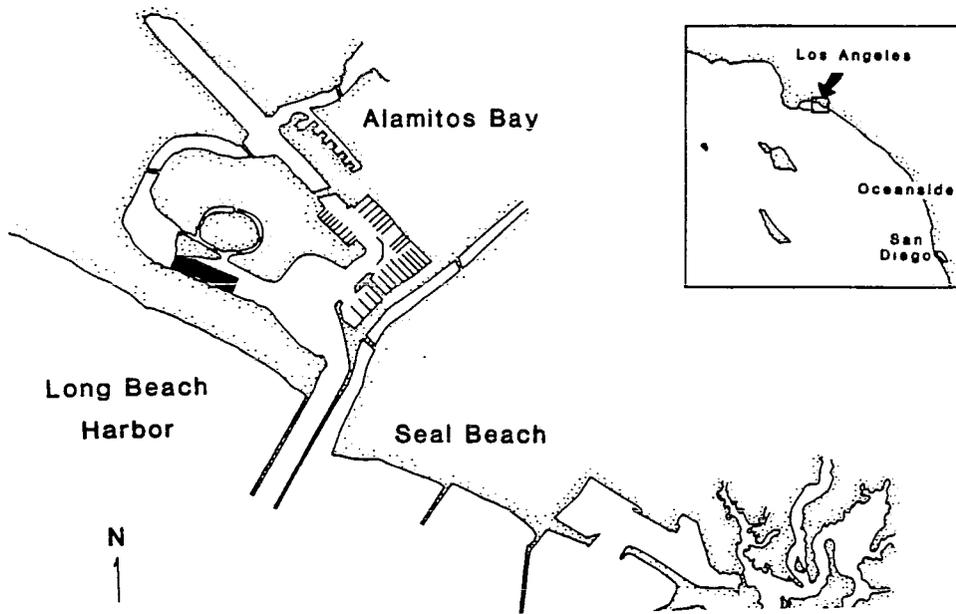


Figure 1. Location of sampling site (blocked out area) in Alamos Bay, California.

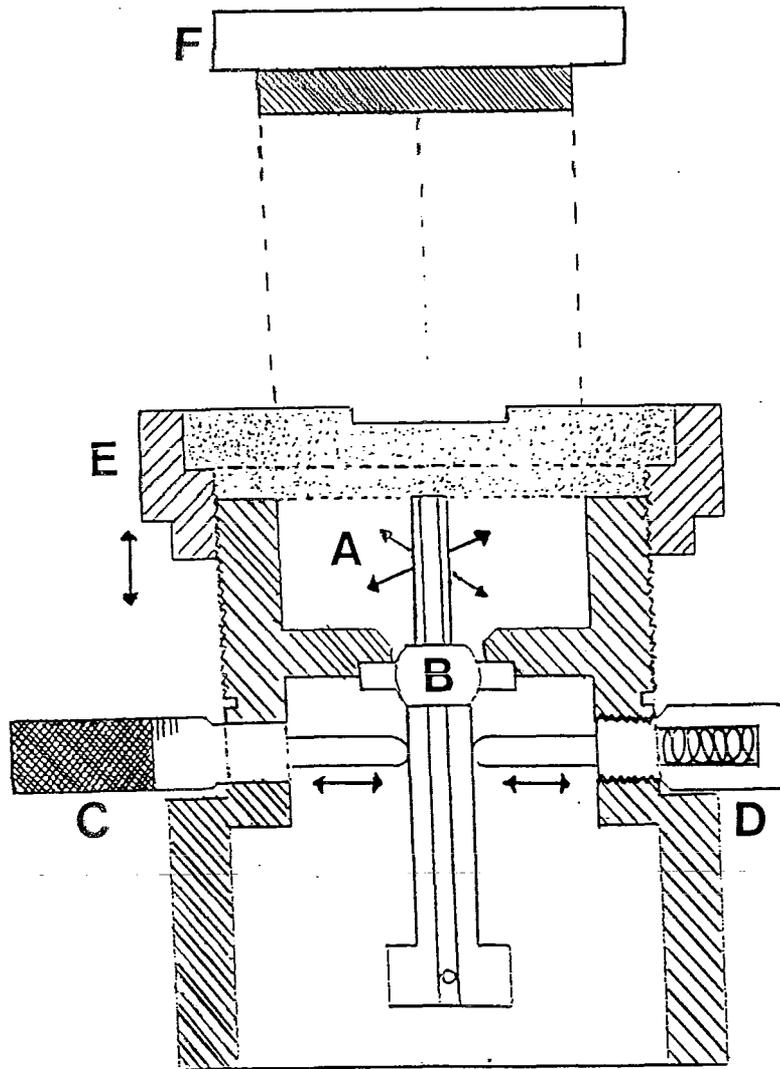


Figure 2. The grinding fixture in cross-section showing the most important features along with the relative motion of moving parts. These features are: A) glass rod holder, B) ball bushing, C) micrometer adjustment head (two at 90 degrees to each other), D) return spring (two at 90 degrees to each other), E) lapping platform, F) lapping table.

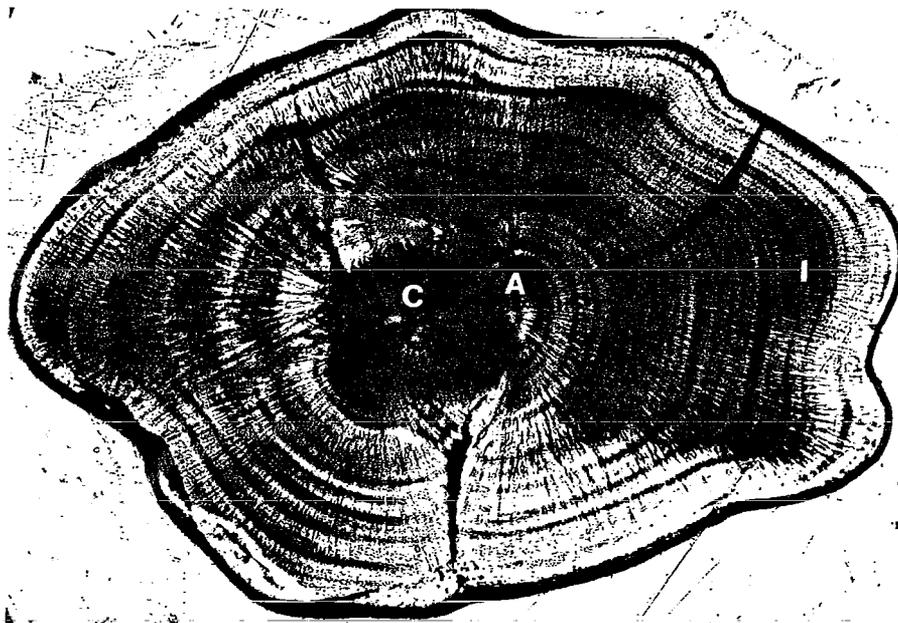


Figure 3. Sagittal otolith of a YOY California halibut (10X), both surface have been ground using the grinding fixture. Features of the otolith are as follows: C) central nucleus, A) accessory nucleus, I) growth increment

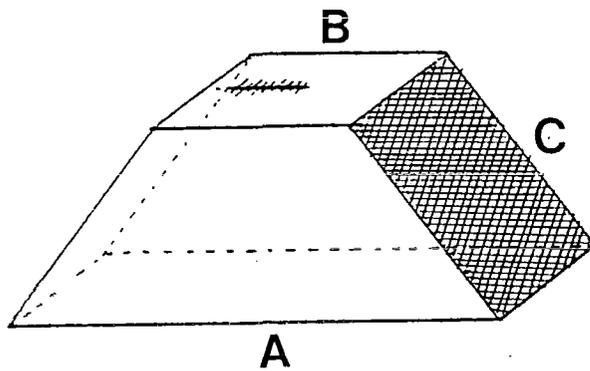


Figure 4. The enclosure used to cage YOY California halibut in the field for purposes of validation. The enclosure features one square meter of bottom area. The sides of the enclosure measure: A) 1 meter, B) 0.5 meter, C) 0.61 meter. The netting is 3.2 mm ace-style mesh.

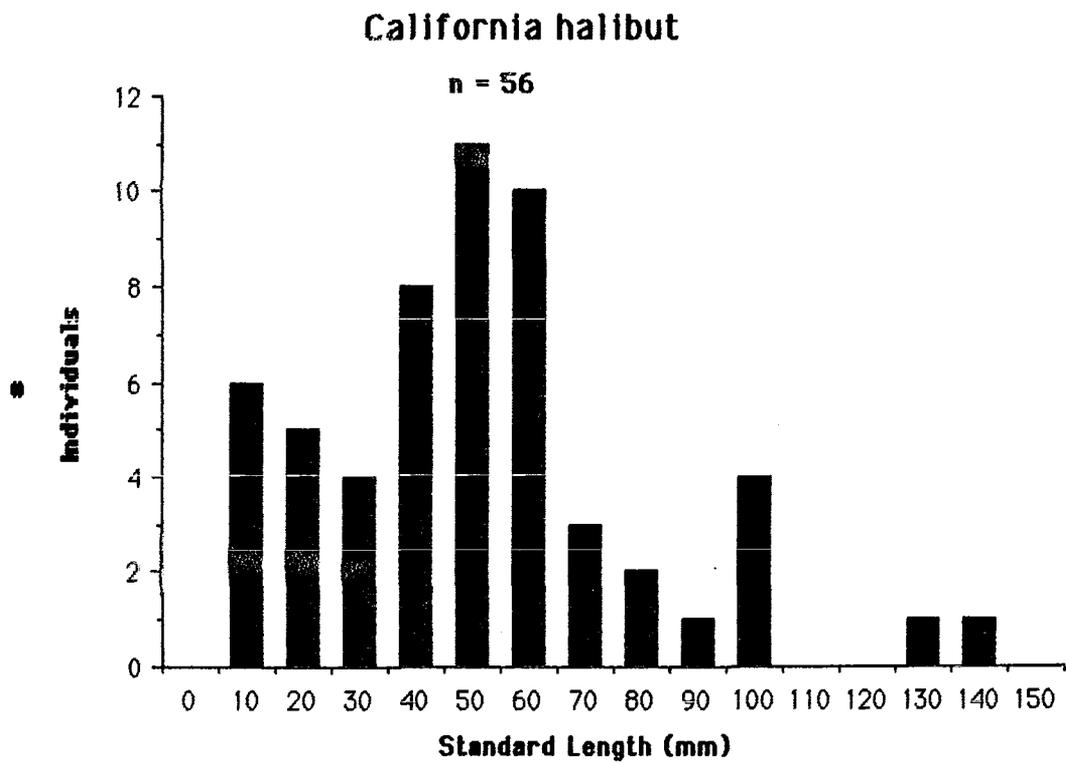


Figure 5. Distribution of standard lengths of YOY California halibut in 10 mm size classes collected in the years 1984, 1986, 1987, and 1988.

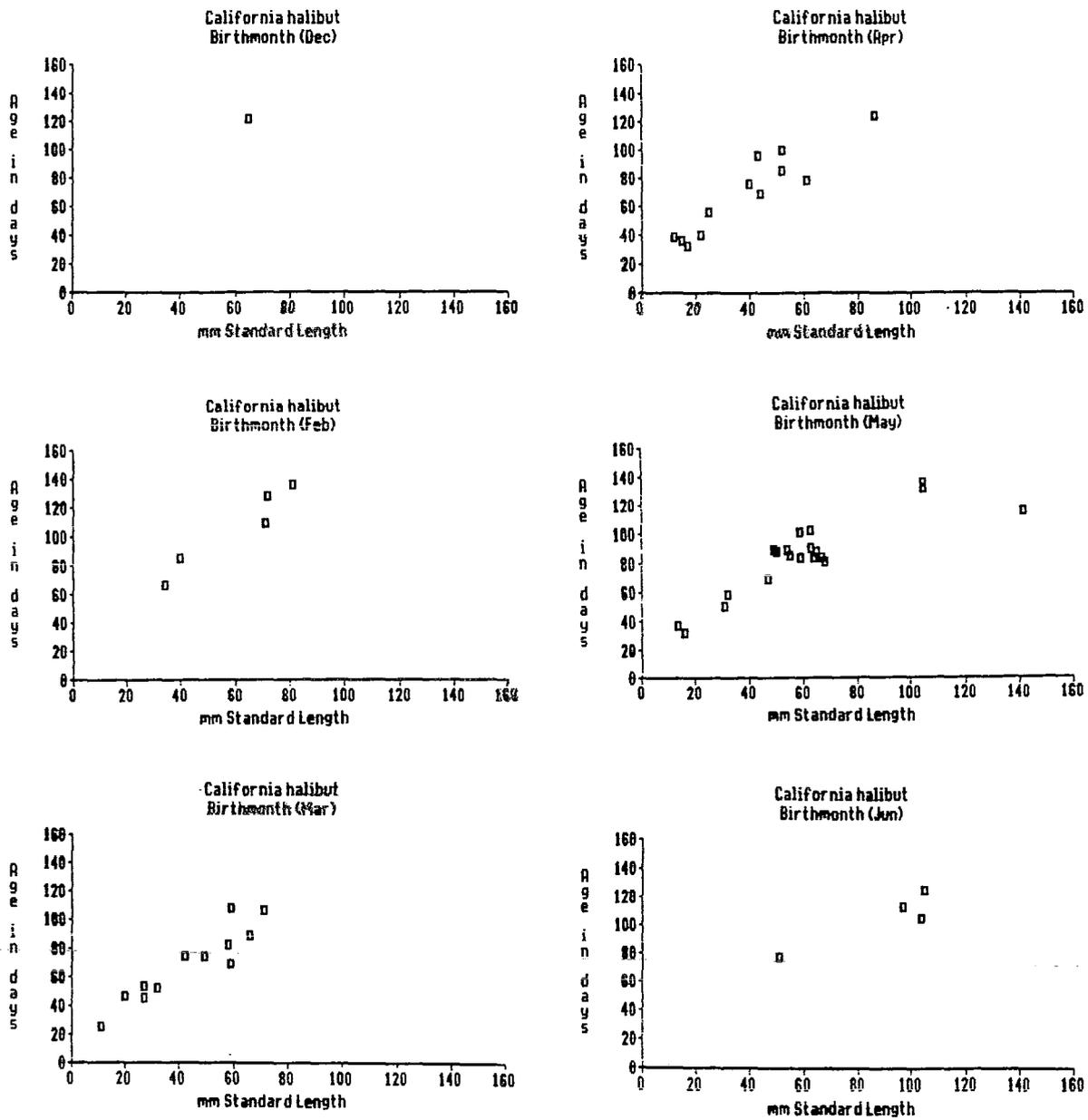


Figure 6. Relationship between daily age and standard length (mm) when separated by birth months (n = 56).

California halibut

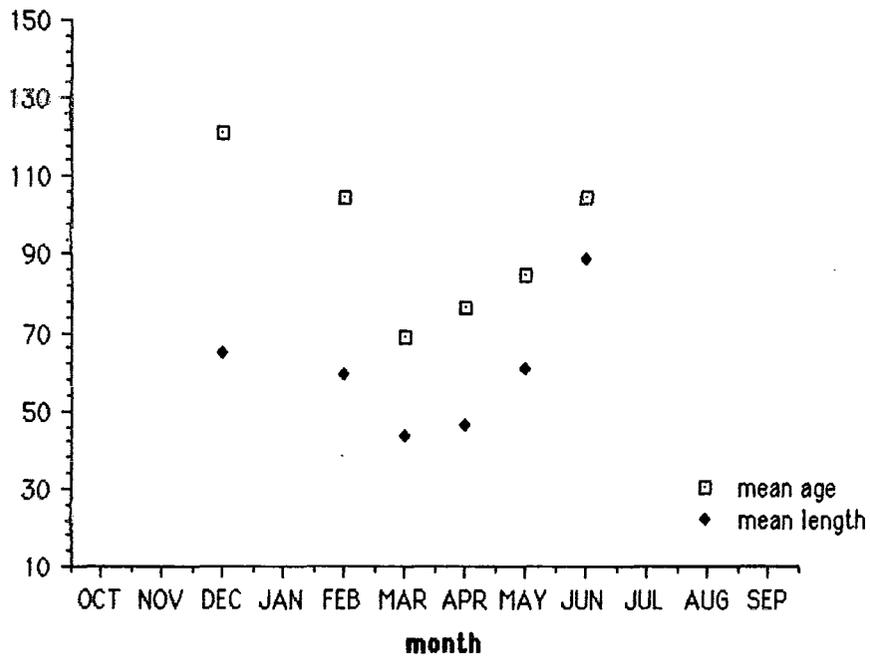


Figure 7. Means for both standard length (mm) and age (days) of YOY California halibut (n = 56) plotted by birth month for the years 1984, 1986, 1987, and 1988.

California halibut

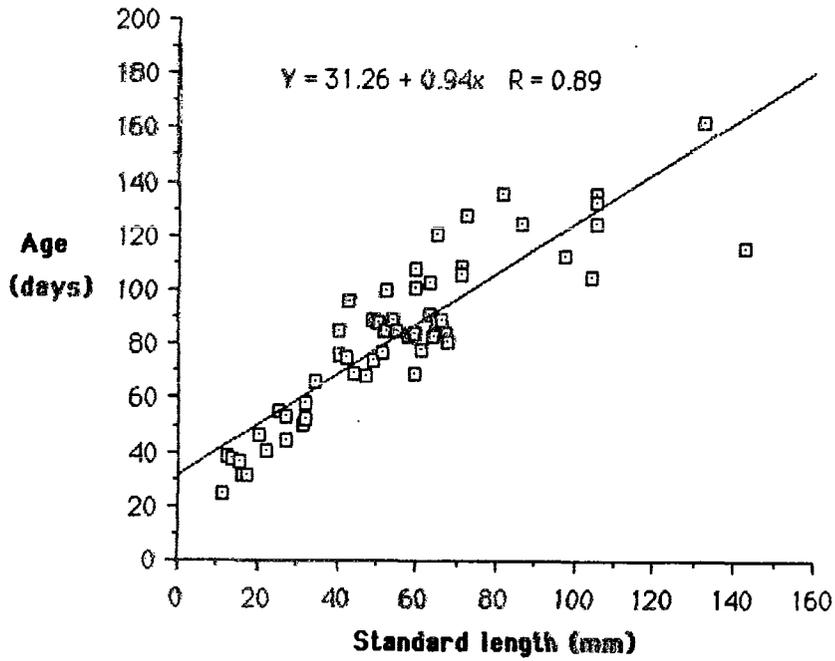


Figure 8. Linear relationship between standard length (mm) and age (days) for YOY California halibut (n = 56) born December - June.

California halibut

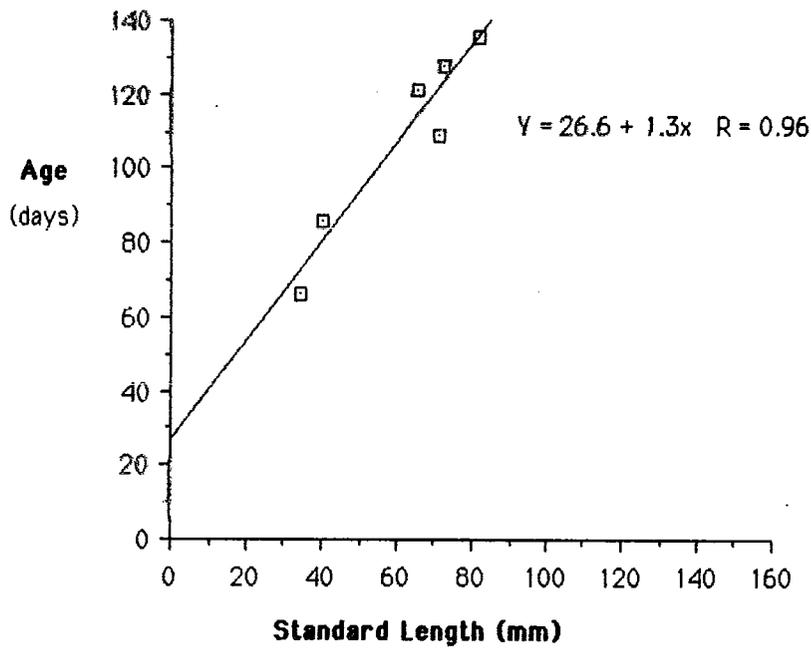


Figure 9. Linear relationship between standard length (mm) and age (days) for YOY California halibut (n = 6) born in December and February.

California halibut

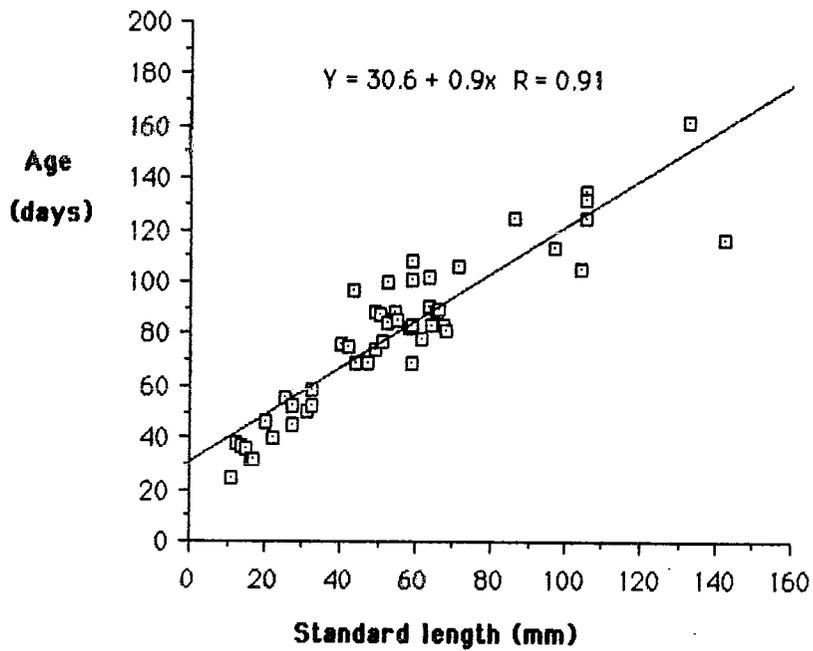


Figure 10. Linear relationship between standard length (mm) and age (days) for YOY California halibut (n = 50) born March - June.

California Halibut

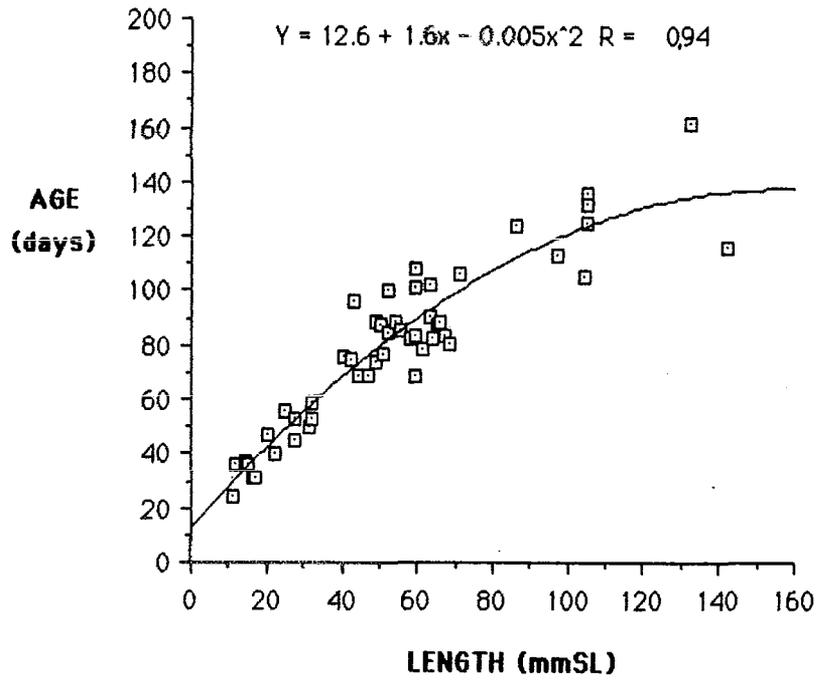


Figure 11. Curvilinear relationship between standard length (mm) and age (days) for YOY California halibut (n = 50) born March - June.

MODEL PREDICTING AGE *P. californicus* (YOY)
 $Y = 12.6 + 1.6X - 0.005X^2$ (with 95% C.I.)

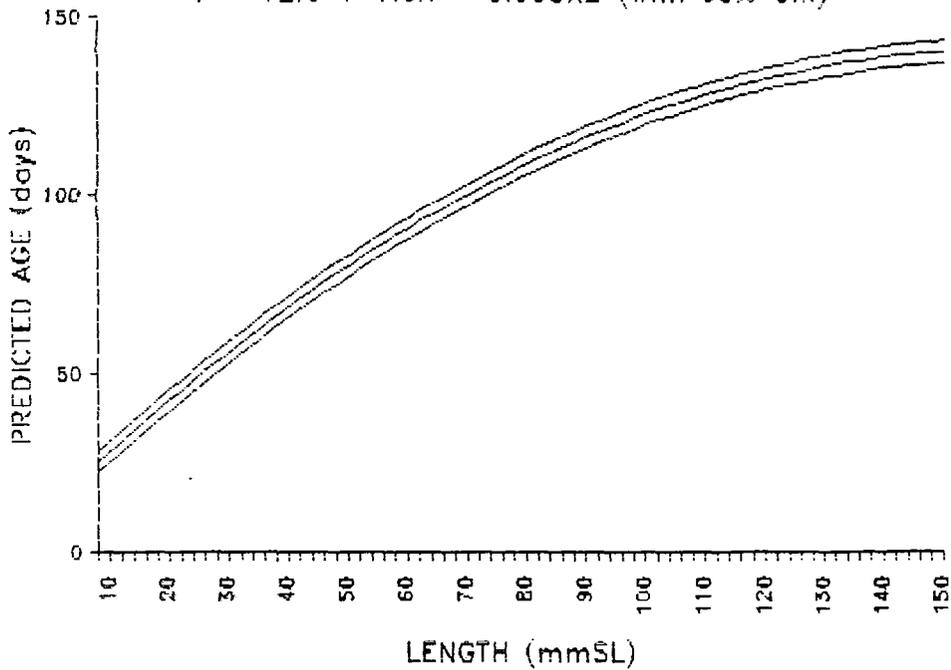


Figure 12. Curvilinear growth model for YOY California halibut born March - June (n = 50) showing the 95% confidence interval.

APPENDIX B

TABLES

Table 1. Results of Tukey-Kramer test for multiple comparisons among paired means with unequal sample sizes when applied to the means of increment number versus birth month ($p = 0.05$, $df = 50$), (*) indicates a significant difference.

Birth months	FEB	JUN	MAY	APR	MAR
FEB	----	41.1	39.3	41.5	42.0
JUN	.2	----	27.2	30.4	31.0
MAY	20.2	20.0	----	27.9	28.6
APR	28.7	28.5	8.5	----	31.6
MAR	36.3	36.1*	16.1	7.6	----

Table 2. Results of Tukey-Kramer test for multiple comparisons among paired means with unequal sample sizes when applied to the means of standard length (mm) versus birth month ($p < 0.05$, $df = 50$), (*) indicates a significant difference.

Birth months	JUN	MAY	FEB	APR	MAR
JUN	----	26.0	39.2	29.0	29.6
MAY	28.7*	----	37.5	26.6	27.3
FEB	29.7	1.0	----	39.7	40.1
APR	43.1*	14.4	13.4	----	30.2
MAR	45.9*	17.2	16.2	2.8	----

Table 3. The interpolated growth rates of flatfish from previous studies for comparison with the calculated growth rates of this study.

Study	Growth Rate (mm/day)
Allen et al. 1984	
<u>Paralichthys californicus</u>	.53
Allen et al. 1988	
<u>Paralichthys californicus</u> 1983	.35
1984	.71
1985	.98
Alhossaini et al. 1988	
<u>Pleuronectes platessa</u>	.16-.88
Campana 1984	
<u>Platichthys stellatus</u>	>.5
This study	
<u>Paralichthys californicus</u> (mm SL < 80)	.86
(mm SL < 150)	1.25

APPENDIX C

Annotated Bibliography of Age-growth Studies in Fish Emphasizing Flatfish, Otolith Validation, and Grinding Techniques.

Alhossaini, M. and T.J. Pitcher. 1988. The relation between daily rings, body growth and environmental factors in plaice, Pleuronectes platessa L., juvenile otoliths. J. Fish Biol. 33:409-418.

ABSTRACT: The deposition rate of sagittal otolith rings in post-metamorphosed plaice, Pleuronectes platessa, was considered in relation to body growth under three feeding levels and two photoperiod regimes. Feeding levels and photoperiods significantly influenced the number of rings; more rings were deposited at higher levels of feeding and in longer photoperiod regimes. There was also a linear relationship between fish growth and the number of rings. The results suggest that sagittal rings could be used for ageing wild juvenile plaice during the period of the growing season when growth rate is greater than 0.1 mm/day. NOTES: First

validated the increments then used them for ageing. Experimented with the effects of temperature, photoperiod, and feeding levels to see if the ring deposition was affected by these factors. Cites that there is no evidence that temperature is an environmental modifier of otolith increments (Brothers 1981, Campana 1984).

To validate they injected .025ml/gram body weight oxytetracycline hydrochloride (OTC). Ground plane section of the sagitta, by hand, with sandpaper with grits of 800 and 1200 initially and finished with carborundum aluminum oxide in distilled water. They counted

increments found on the long axis with a hand counter and repeated the counts three times. They used mean number of rings when variation was less than 10%.

Found OTC bound to the otolith in 12 hrs. Produced marks in 99% of the fish. The mortality rate was 90%. No significant difference was found between tanks (ANOVA). Feeding levels produced no significant differences on ring number or sagittal growth (ANOVA). Two photoperiods did show a significant difference in mean number of rings produced and sagittal growth. Twenty-four hours of light produced more increments than 12 hours dark:12 hours light regimes.

Beamish, R.J. and G.A. McFarlane. 1987. Current trends in age determination methodology. In The Age and Growth of Fish, Edited by R.C. Summerfelt and G.E. Hall, 1987. The Iowa State University Press, Ames, Iowa 50010.

ABSTRACT: The most important advancement in age-determination studies from 1970 until the present has been the discovery of daily growth rings. To date, this discovery has been limited in its application to very young or short lived fish. Recent studies have demonstrated that scale ages for some species can result in serious underestimates of age. These underestimates occur for freshwater and marine species but appear to be more serious for marine fishes. The awareness that scale ages were not always reliable, renewed the interest in developing accurate methods of age determination and evaluating the implications of ageing error in stock assessment. The realization that fish live for long periods, sometimes with minimal growth, has stimulated interest in the importance of longevity.

NOTES: Aggregates of needle-like crystals of calcium carbonate formed on the outer margin of the otolith and produced an incremental zone. A second zone, called the discontinuous zone, formed between the incremental zones. The incremental zones were predominantly calcium and the discontinuous zone predominantly organic matrix. Both zones formed in one day.

There are conflicting reports in the literature regarding the effects of various environmental factors on the daily growth rings. There is agreement on an endogenous rhythm being responsible for the initiation of the increments. Various studies concluded that feeding frequency, photoperiod, and temperature did or did not have an effect on number and width of increments. Was clearly shown that rings do form during periods of starvation (see Taubert and Coble 1977; Marshall and Parker 1982; Campana 1983).

Found that scale ageing has underestimated the ages in many fishes. Otoliths, unlike scales, continue to grow through out the fish's life. Scales do not grow when there has not been an elongation of the fish's length, hence scale growth increments are not laid down. The method of aging marine fish using scale is not reliable.

There were problems with separating growth checks from annuli. Growth increments form on otoliths during periods of starvation. Fish can live for years with little or no growth (50 - 60yrs). The scales method is inappropriate for aging older fish. Other, more reliable, bony structures are better for aging older fish ie. fin rays, whole vertebrae, cleithra, or opercular. Otoliths do not

grow at a constant rate, they slow down. Increments become crowded around the margins of the otolith in older fish.

Boehlert, G.W.;Yoklavich,M.M. 1984. Variability in age estimates in Sebastes as a function of methodology, different readers, and different laboratories. Calif. Fish and Game., Vol 70 NO.4:210-224
NOTES: The sagitta was used for this study. The reader variability in age increased with fish length. The first growth bands, on the otolith were difficult to interpret.

Brothers, E.B., C.P. Mathews, and R. Lasker 1976. Daily growth increments in otoliths from larval and adult fishes. Fishery Bulletin 74(1):1-8.

ABSTRACT: Daily growth increments have been found in the otoliths of fish larvae. The daily nature of these layers was verified by examining larval fish of known age reared in the laboratory. A simple technique for observing these marks is described and can be used on otoliths from larvae and adults. This provides a convenient method for determining early growth in fishes and is particularly useful for fishes which do not lay down seasonal rings.

NOTES: Purpose of paper was to show that daily rings do exist both in adults and larvae of several species of fish. They used two methods to age otoliths these are; 1) SEM; and 2) a simple technique is described that does not call for expensive equipment. Ageing is important in that it can be used to assess the stocks of commercial fishes.

Otolith prepared by wet lapping with silicon carbide grit (400-600). Final polishing compound was a 1 micron grit diamond.

Bone was examined in immersion oil which will cause a loss of resolution if left in oil. Magnifications of 1800X-600X were used to examine the bone. Larval otoliths were examined without grinding by placing the bone on a glass slide covered with immersion oil and a coverslip.

Rings begin to form only after the yolk sac is absorbed, about 6 days in the northern anchovy. Suggests that with pacific hake the daily increments should be used and not the annular rings. This is because of the great propensity for error when using the annuli. Daily marks also found in the squid Loligo opalescens. This technique is suited for ageing larvae, juveniles, and fast growing species. Daily marks may be used on some fish that are up to 6 years old, and also used on some tropical fish. Many of the fish used were from California.

Campana, S.E. 1984. Lunar cycles of otolith growth in the juvenile starry flounder, Platichthys stellatus. Marine Biology 80:239-246.

ABSTRACT: The otolith microstructure of juvenile starry flounders, (Platichthys stellatus) sampled from a monitored environment was examined for evidence of lunar periodicity. Three types of biweekly cycles were observed in all of the flounder otoliths; two of the cycles were correlated with a tidal modulation of the environment. Through a multiple regression model, much of the day to day variability in increment width could be related to daily variations in temperature, salinity, and tidal mixing. My results suggest that a 15-d increment width cycle was entrained by the interaction of 15-d tidal cycle with temperature and salinity. The same tidal cycle/temperature interaction probably produced a

biweekly pattern of increment contrast. However, the presence of otolith checks formed at weekly and/or biweekly intervals could not be so explained, although checks were consistently formed on the new and full moons.

NOTES: This paper may explain the near weekly growth checks that are found on otoliths of California halibut. Thin sections, of the otoliths, were produced by grinding both surfaces (n=31).

Campana, S.E. 1984. Microstructural growth patterns in the otoliths of larval and juvenile starry flounder, Platichthys stellatus. Can. J. Zool. 62:1507-1512

ABSTRACT: Microstructural growth patterns were examined and interpreted in the otoliths of both wild and lab. reared starry flounders. Growth increments were not formed with a daily periodicity in laboratory reared larvae. However, increment counts increased with the degree of sample preparation, suggesting that increments near the resolving limit of light microscopy were not being observed. Increments in wild flounder sagittae were more clearly defined under both light and electron microscopy; in addition, larval and juvenile growth patterns could be easily differentiated. A transition zone between the growth regions corresponded to the size and age at metamorphosis. An increase of increment width with larval age resulted from a curvilinear relationship between otolith diameter and fish size. Larval growth rates of wild fish remained relatively constant at 0.25 mm/day until metamorphosis; juvenile growth rates were substantially higher. Metamorphosis was characterized by a sudden but temporary decline in growth rate.

NOTES: Compared; 1) otolith increment counts vs time; 2) otolith diameter vs total length; and 3) increment width vs increment number. No significant difference was found between the right or left sagittae (paired t-test). A magnification of 400X was used and poorly prepared specimens were discarded. The bones were mounted using instant glue. Electron microscopy was compared to light microscopy. The growth increments were not well resolved at the margins because the grinding plane was above the margins. This lead to an underestimation of the number of increments at the edges of the bones. A transition zone in increment formation was found in wild juveniles. The transition zone separated two distinct incremental sequences (inner and outer) which differed in both increment width and appearance. Increment growth at first is very circular surrounding the central nucleus (CN). During the transitional phase (metamorphosis), the circular increments are deformed by the increments which form around accessory nuclei (AN). The width of the increments surrounding the ANs is greater than those of the CN. Metamorphosis occurs at about 45 days in the lab reared specimens. Daily increment formation has been validated in the starry flounder (Campana and Neilson 1982). This study was not validated but the increment clarity, width regularity, and absence of gaps in the growth record suggests that these increments are daily. Starry flounders metamorphose at a certain size not at a certain age. Otolith diameters, at metamorphosis, are therefore very similar. Estimated dates of metamorphosis correspond closely to the date of first appearance in estuarine nursery area. Metamorphosis is felt to be insufficient to produce a radical

change in the microstructure of the otolith, but the change of the habitat and behavior of the fish may ie. higher temperature, food availability, and bottom settling.

Campana, S.E. 1983. Feeding periodicity and the production of daily growth increments in the otoliths of steelhead trout (Salmo gairdneri) and starry flounder (Platichthys stellatus). Can. J. Zool. 61:1591-1597.

ABSTRACT: Juvenile steelhead trout and starry flounders were reared 64-76 days under various experimental feeding regimes to determine if feeding periodicity influenced the production of daily growth increments on the otoliths. Both species produced daily increments when fed thrice daily, daily, or once every three days, as well as through 26-32 days of starvation. Daily growth increments were also deposited in vateritic ("crystalline") otoliths, which constituted 27% of the trout sagittae sampled. Feeding frequency affected increment appearance and the incidence of subdaily increments in trout, but not in flounders. The difference in effect was probably due to the higher metabolic rate of trout. Increment deposition in all flounders was far more variable than in trout, and some flounders apparently ceased formation all together.

NOTES: Daily increments were used to age larval fish. Daily growth rates were calculated and used to assess life history changes in individual fish. Photoperiod was rejected as a factor in ring deposition. Studies have supported daily temperature fluctuations as a factor in increment periodicity. Purpose of the study was to examine the role of feeding periodicity and starvation in the production of daily growth increments in otoliths.

Campana, S.E., and J.D. Neilson 1982. Daily growth increments in otoliths of Starry Flounder (Platichthys stellatus) and the influence of some environmental variables in their production. Canadian Journal of Fisheries and Aquatic Science 39:937-942

NOTES: Intraperitoneal injections of oxytetracycline hydrochloride (OTC) (100 mg OTC/kg fish) resulted in deposition of a fluorescent band on the sagittae of flounders. The injected volume was 0.025 ml., similar results were obtained by immersing the fish in a 0.02% OTC/saline solution for one day. The uptake rate of OTC was studied. Tetracycline incorporation into the otolith was evident in 97% of the flounders. The band was formed in less than one day (50% after 10 hours, 100% after 24 hours). Temperatures were controlled to mimic those found in the field. Differing photoperiods of 24 to 36 hours were used. All otoliths were photographed at 500X-1250X under both white and ultraviolet light using a fluorescence microscope with a 35mm camera attachment. They used excitation filter bands from 450 to 490 nm and barrier filters at 510 and 515 nm. To determine position of the fluorescence band they used paired photographs one under white light, and one under ultraviolet light. The otoliths were stored in the dark for up to four months without any decrease in the intensity of the band. A glue/otolith area of fluorescence may be produced, but it was easy to distinguish from that of a true band. All counts were replicated three times in a random sequence by two readers.

Chambers, R.C., Leggett, W.C., and J.A. Brown 1988. Variation in and among early life history traits of laboratory-reared winter flounder Pseudopleuronectes americanus. Mar. Ecol. Prog. Ser. 47:1-15

ABSTRACT: We analyzed variation in and among early life history traits of laboratory-reared winter flounder Pseudopleuronectes americanus. We measured lengths and ages at hatching and metamorphosis, lengths at 2 wk intervals through the larval period and into the first 3 wk of the juvenile period, and after 23 wk of juvenile life. Variation in traits was quantified and compared by the coefficient of variation ($CV = SD/mean$). CVs for lengths-at-ages increased from hatching (0.03) to an asymptotic value of $\sim 10\%$ by 2 to 4 wk after the first feeding. There were 2 peaks in variation in lengths-at-age during the early life history; one at Week 4 (the approximate midpoint of the larval period) and another Week 10 (during the latter portion of the metamorphic window and early juvenile life). These may reflect commensurate changes observed in growth rates. Variation in length at hatching and metamorphosis was relatively low (3.0 and 5.3%, respectively), and less than variation in age. Length-at-transitions were also less variable than length at fixed ages during the larval and juvenile periods. These findings imply a target size for life period transitions. Length and age at metamorphosis were positively correlated, as were sequential average lengths calculated from fortnightly measures. Length and age at metamorphosis, and growth and developmental rates through the entire larval period, were positively correlated when calculated from the bivariate measure of individuals. The pattern of

variation in and correlations among early life history traits established in the larval period appears to propagate through metamorphosis and into early juvenile life. The population consequences of these associations, and their potential use in analyzing the results of early life history studies, are discussed.

NOTES: Growth rate of juveniles estimated to be 0.145 mm/day. The age at metamorphosis was 59.5 days. Metamorphosis is defined as the point at which the iris of the migrating eye was visible from the right side of the body upon inspection at 6X magnification.

Chapman, C.J. and O. Sand 1974. Field studies of hearing in two species of flatfish Pleuronectes platessa (L.) and Limanda limanda (L.) (Family Pleuronectidae). *Comp. Biochem. Physiol.* 47A:371-385

NOTES: Two main sensory systems have been suggested as the acoustic receptors in flatfish. These are; 1) the paired otolith organs in the labyrinth; 2) and the lateral-line organs. Each otolith organ consists of groups of sensory haircells, maculae, loaded by the otoliths. The otoliths are dense crystalline bodies whose movements in a sound wave are delayed relative to the maculae, thus creating a shearing movement of the haircells. The otolith organs are therefore particle motion detectors.

Chilton, D.E., and R.J. Beamish 1982. Age determination methods for fishes studied by the Groundfish program at the Pacific Biological Station. *Can. Spec. Publ. Fish. Aquat. Sci.* 60: 102 p.

ABSTRACT: This manual describes methods and equipment used to estimate the ages of groundfish species studied by the Pacific Biological Station. Procedures for routine identification of annuli on scales, otoliths, otolith sections, fin-ray sections, and spines

are described and illustrated. Although the techniques are applied to species found off Canada's west coast, they should be applicable to other marine and freshwater fishes from temperate and arctic waters. Because some of the procedures are experimental and many have not been validated, it is expected that this manual will be revised several times.

NOTES: "Ability to age fish accurately is essential to the total understanding of the dynamics of fish populations." Advocate using length frequencies to age younger fish. Validation is very important and the OTC injection method is suggested. The marking, release, and recapture of fish is suggested.

Structures for ageing:; 1) Scales - cycloid in soft rayed fishes and ctenoid in spiny rayed fishes. "Scales form a protective outer covering for fish and increase in size as the fish grows. The major increase in scale size occur prior to maturity. After maturity, the growth of most fish, and especially males, is reduced. This reduced body growth rate can affect scale growth and change the pattern of circuli formation. This means that the appearance of the annulus for younger fish may not be the same for older fish. If fish growth is very slow or nonexistent then scale growth may be minimal or nonexistent. Annuli can become very crowded on the edge of the scale and often cannot be identified. In addition, during the latter years, calcium may be reabsorbed from the scales resulting in some reworking or breaking of the circuli." Scales may clear with time, and should only be used to estimate age in pre-maturity fishes; 2) Otoliths - 3 pairs of bones, otolith and sagittae are used interchangeably. The formation of the otolith is not isometric

throughout life. "As the fish ages, more material is deposited on the internal surface than on the external surface and it is possible that no material is deposited on the external surface in any particular year." Bone resorption is not known to occur from the otoliths. They are the best permanent pattern of fish growth.; and 3) Fin rays - much more difficult to handle and interpret.

Collins, R.A. and J.D. Spratt 1969. Age determination of northern anchovies, Engraulis mordax, from otoliths. (Calif. Dept. of Fish and Game) Fish Bulletin 147:39-55

NOTES: Whole sagittal otoliths were used to age fish. Compared the scale age with the otolith age. The otoliths were not ground but were submersed in water and annular bands read. An appendix that suggests a standard terminology for otolith readers is provided. The counts were controlled for variability by producing consistent counts before the data was collected. Contrasted age vs length.

Cowan, J.H. Jr. 1988. Age and growth of Atlantic croaker, Micropogonias undulatus, larvae collected in the coastal waters of the northern Gulf of Mexico as determined by increments in saccular otoliths. Bulletin of Marine Science 42(3):349-357.

ABSTRACT: The age and growth rate of larval Atlantic croaker, Micropogonias undulatus, collected in the coastal waters of the northern Gulf of Mexico were estimated from growth increments in saccular otoliths. Examination of the data suggests that the growth increments were daily and they were used to generate the linear growth equation, which described the growth of Atlantic croaker larvae from an estimated 40 to 80 days old. The reported spawning season of Atlantic croaker in northern Gulf waters

(September to March) was not delineated in this study. However, data interpreted indicated that little spawning occurred after January in the winter of 1981-1982. The relationship between maximum otolith diameter and larva total length was also examined.

NOTES: The otoliths were ground and then etched before viewing with polarized light. Age was regressed on length. The daily nature of increment formation was not validated in this study.

Echeverria, Tina. W. 1987. Relationship of otolith length to total length in Rockfishes from Northern and Central California. Fishery Bulletin 85(2):383-387

NOTES: Compared total length to otolith length for 30 species of rockfish. The otolith was measured through its longest axis with an ocular micrometer to the nearest 0.1 mm. Linear regression was used to analyze the data. Outliers were discarded from the data set before calculations. The outliers were attributed to errors in measuring, collecting, or storing.

Geffen A.J. 1987. Methods of validating daily increment deposition in otoliths of larval fish. In: The Age and Growth of Fish, Edited by R.C. Summerfelt and G.E. Hall. 1987. The Iowa State University Press, Ames, Iowa 50010.

ABSTRACT: Early literature on larval fish otoliths has documented the presence of otolith increments, but only a few attempts have successfully validated a daily rhythm of ring deposition. Utilization of otolith data is limited by the need to validate rates of increment formation. Full-scale validation studies are time consuming, labor intensive, and often technically difficult. Some basic assumptions for daily increment validations are

outlined, and several validation techniques are summarized in a framework of alternative methods for rapid identification of daily or non-daily deposition rhythms. New approaches to validation may provide faster and easier, but less reliable, alternatives. The ultimate choice of a validation method will always be a compromise between desired reliability and available resources.

4 assumptions; 1) ring deposition is at a constant rate throughout the larval period; 2) initiation of ring deposition is constant between individuals; 3) ring deposition rate is independent of larval growth rate, age, or season; and 4) deviations can be predicted and modeled.

NOTES: Advantages of marking wild fish; 1) fish caught in wild represent surviving larvae avoiding one of the bias involved with using reared populations; 2) do not have to rear the fish, which has its own problems; and 3) marking and release assures that growth conditions during the experimental period are natural.

Haaker, P.L. 1975. The biology of the California halibut, Paralichthys californicus (Ayres) in Anaheim bay, pp. 137-151. In E.D. Lane and C.W. Hill (editors) The marine resources of Anaheim bay, California Department of Fish and Game, Bulletin 165.

NOTES: A length-weight relationship for preserved halibut was produced. The curvilinear formula is $\text{Log } W = 5.03 - 10 + 3.088 \text{ Log } SL$, where W = weight in g after preservation and SL = standard length after preservation. The preservative used was seawater and formaldehyde solution. A difference in the growth rates of males and females was found with females growing faster than males. Age and growth data came from annular rings on otoliths backed up by

scale ages. Used oil of anise to clear the thick bones. Scales were removed from the eyed side just posterior to the lateral line arch. All scales were read three times on separate occasions. A table of food habits is presented which may be useful to determine what the caged fish may be eating.

Harder, W. 1975. Anatomy of Fishes Part 1. E. Schweizerbart'sche Verlagsbuchhandlung (Nägele U. Obermiller) Stuttgart 612 p.

NOTES: (pages 327-330.) Otoliths slide across the cilia of the sensory padding and stimulate the sensory cells. They are an organ used for detecting gravitational attractions and thereby relating the position of the fish (aka statoliths). The sagittae was named for its arrow shape. Growth rate of the otolith differs with season. The hyaline zone is translucent, hazy or cloudy feature of the bone. The medial side of the bone is concave while the lateral side is convex. The sulcus is a furrow that is located on the medial side of the bone. The otoliths from both sides of the body are not always perfectly symmetrical.

Hettler, William. F. 1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Transactions of the American Fisheries Society. 113:370-373.

ABSTRACT: Otoliths of the larvae of spot Leiostomus xanthurus and pinfish Lagodon rhomboides were marked with a band that fluoresced in ultraviolet (UV) light after live larvae had been immersed for 1-2 hours in tris-buffered, 100-500 micrograms/liter solution of tetracycline in a 1% NaCl solution. Dimethyl sulfoxide did not significantly increase tetracycline uptake. After otoliths were marked, their radii increased approximately 18% in 8 days for spot

and 15% for pinfish, but distinct daily growth increments could not be determined even though diel periodicities in feeding and photoperiod were provided in the laboratory.

Hourigan, T.F. and R.L. Radtke. 1989. Reproduction of the Antarctic fish Nototheniops nudifrons. Marine Biology 100:277-283.

ABSTRACT - The reproductive biology of the Antarctic fish Nototheniops nudifrons (Lonnberg, 1905) was analyzed by the examination of the gonads of fish collected in March and April 1985 in trawls near Low Island, Antarctic Peninsula, and compared to direct observations of reproduction and early larval development in the laboratory from March to October of the same year. Males and females reached sexual maturity at an age of 4 to 5 yrs. Mature males differed in coloration from immature males. Ovaries of sexually mature females contained two distinct size modes of vitellogenic oocytes, representing two separate clutches of developing eggs. Females spawned 100 to ~3,500 demersal eggs, which were laid in a nest in crevices or under rocks, and guarded by the male for about 4 mo. Females did not assist in nest defense or egg care. Most spawning in the field and in the laboratory occurred in late fall and early winter (May to June). A second clutch may be spawned in spring (November and December). Eggs hatched after 124 d, and larvae were raised for 38 d. Otoliths of larvae contain internal microincrements, which are deposited in a daily fashion, and are visible by light microscopy. The otoliths of 32 adult fish were examined by scanning electron microscope, and counts of microincrements in these otoliths allowed the back-calculation of

hatching dates. Estimated hatching dates were between September and May.

NOTES: The ages of fish were estimated from the standard length. Growth curves were determined from a sample of 32 fish which were aged by counting the number of microincrements in their sagittal otoliths viewed with scanning electron microscopy. These microincrements were deposited on a daily basis, and fish length was related to the number of microincrements in the sagitta in an almost linear fashion, best described by the equation:

$$\text{mmSL} = 0.0407 (\text{days}) + 23.56 \quad (R^2 = 0.095, p < 0.0001)$$

Jones, Cynthia and Edward B. Brothers 1987. Validation of the otolith increment aging technique for Striped Bass, Morone saxatilis, larvae reared under suboptimal feeding conditions. Fishery Bulletin 85(2):171-178

NOTES: One problem identified with ageing methods based on otolith counts was that inadequate resolution of narrow bands with the light microscope could lead systematic under-estimations of increment count. Different feeding strategies produced the following correlation coefficients; 1) well fed laboratory reared larvae, $r = 0.96$ ($n=63$); 2) starved laboratory reared larvae, $r = 0.77$ ($n=43$); 3) larvae that were starved then fed had a regression coefficient of 0.90 ($n=12$); and 4) larvae that were starved and fed intermittently had a regression coefficient of 0.96 ($n=12$).

Age estimation using the light microscope gave accurate estimates of age when the larvae were well fed and did not experience any type of deprivation. When the larvae were fed intermittently or starved the accuracy of this method went down. The scanning

electron microscope (SEM) improves the accuracy of age estimation, but it is far more costly both in terms of money and time. The use of SEM is suggested as a method to assess the accuracy of the light microscope counts or age estimates.

Kelley, D.F. 1988. Age determination in bass and assessment of growth and year-class strength. *J. mar. biol. Ass. U.K.* 68:179-214.

ABSTRACT: Scale readings from 6269 bass caught in 1946-86 on various parts of the U.K. coast, with a few from the Channel Islands and France, have been analyzed to determine U.K. growth patterns and assess the relative abundance of successive year-classes. In the U.K. growth was fastest on the south-east coast, slowest on the west coast. In the Channel Islands growth was initially slower than on the south-east coast but faster after maturity; though less fast than on the Biscay coast of France. Year-classes of exceptional abundance tended to grow more slowly than classes of normal abundance. There was evidence in each region of a slight long-term fall in growth rate. Best growth occurred in warm summers. The growth period commenced, and annulus formed, at dates ranging from early May for the youngest juveniles to mid July for adults; terminating in each case in October. Males grew rather more slowly than females and appeared less long-lived and less hardy, except on the south-east coast, where, exceptionally, samples were caught well offshore and included large, generally faster growing fish; this suggests a behavioral difference between the sexes rather than consistently slower growth in males. Explanations are offered for occasional fast growing fish, which occur mainly on the south-east coast and mainly with

particular year-classes, notably 1966 and 1969. Problems of interpretation are considered and solutions offered.

Lai, H.L., D.R. Gunderson, and L.L. Low 1987. Age determination of Pacific cod, Gadus macrocephalus, using five ageing methods. Fishery Bulletin 85(4):713-723.

ABSTRACT: A comparative study of age determination methods for Pacific cod, Gadus macrocephalus, was carried out using dorsal and pectoral fin rays, scales, otoliths, and coracoids. A preliminary validation using the modal length of a strong year class confirmed that sections of dorsal fin rays were the most reliable ageing method. A Monte Carlo method was developed for converting scale ages to dorsal fin-ray ages. An analysis by log-linear model was developed for testing the effects of ageing method and age class on repeatability of age reading.

NOTES: There may be false checks in the first year of life that are present on the scales of YOY cod. Scales provided very low precision. Validation method a comparison of mean lengths at age estimated from the five ageing methods to modal lengths of a strong year class.

Lancraft, T.M., Hopkins, T.L., Torres, J.J. 1988. Aspects of the ecology of the mesopelagic fish Gonostoma elongatum (Gonostomatidae, Stomiiformes) in the eastern Gulf of Mexico. Mar. Ecol. Prog. Ser. 49(1&2):27-40

NOTES: Otolith increments were used to age the fish and calculate growth rates. Otoliths were removed from both fresh and frozen fish, cleaned and stored dry. Image enhancement and magnifications of 400X - 1000X were used for increment counts. The otoliths were

counted by one observer 2X on separate days. If counts varied by 5% were counted over. Daily rings were distinct measuring 1-3 microns in width. Regression of growth increments on fish length produced this model; $Y (SL) = 12.97 + 0.34X$, $X = \text{increment}$, $r^2 = 0.96$. The sample size was 84 with a range of 23 - 187 mm SL.. Linear growth has been reported in stomiliformes and deep living myctophids. Cites that Pannella (1980) found that fish exhibiting pronounced diel activities (e.g. vertical migration and distinct feeding periods) tend to have more distinct daily increments than non-migratory and non-cyclical feeding fish. This fish displays both. The author uses this as his validation for the daily nature of the growth increments. A metamorphic growth check was found in these fish at 23 mmSL or at the 60 increment range.

Layzer, James. W. and Michael D. Clady 1987. Phenotypic variation of young-of-year Bluegills (Lepomis macrochirus) among microhabitats. *Copeia* 1987(3):702-707

Fourteen percent of the otoliths used in this study were discarded for following two reasons; 1) poor nucleus resolution; and 2) not all rings were present. Two counts of all distinct rings were made if the counts differed by two or less the average was used. If the difference was greater than two the rings were counted again and the average of all three counts was used to estimate age.

McCaughran, D.A. 1987. Growth in length of Pacific halibut. In: The Age and Growth of Fish, Edited by R.C. Summerfelt and G.E. Hall. The Iowa State University Press, Ames, Iowa 50010.

ABSTRACT: Mark-recapture data was used to estimate growth in length of Pacific halibut (Hippoglossus stenolepis) by sex, region, and year classes (1935-1965). Analysis showed that males and

females form separate families of growth functions; females grow faster and attain a larger size than males. There was little difference in growth of both sexes from 1935 to 1965 and no relationship to stock biomass was found. Migrating halibut were shown to grow slightly faster than non-migratory individuals. Growth was greater in the central part of the range and diminished toward the ends.

NOTES: This paper deals with the age and growth estimations of the Pacific halibut. It does not deal with daily age. Does report that the female halibut grow faster and attain a larger size than males. A Comparison of migratory and non-migratory Pacific halibut was made. Migratory halibut were found to be faster growing than non-migrants. The energy used for migration is, reportedly, not derived from the energy needed for reproduction because migration takes place in these fish before the onset of sexual maturity.

Mugiya, Y., N. Watabe, J. Yamada, J. M. Dean, D.G. Dunkelberger, and M. Shimizu 1981. Diurnal rhythm in otolith formation in the goldfish, Carassius auratus. J. Biochem. Physiol. 68A:659-662.

ABSTRACT: 1. The presence of diurnal rhythm in otolith formation was examined physiologically in the goldfish Carassius auratus. 2. Calcium 45 uptake by otoliths indicated that otolith formation slowed down or stopped at sunrise and resumed in three hours. 3. Plasma calcium level increased toward sunrise and fell significantly in the next three hours. 4. The increasing phase of plasma calcium corresponded to the period of rapid decrease in calcium 45 in the culture medium.

NOTES: The otoliths, blood and the aquaria were sampled for calcium 45 for 24 hours. There was a cessation in calcium deposition at

sunrise. The graph of the calcium 45 uptake in the otolith shows a constant increase over the 24 hour period.

"Although we have no data to explain how changes in plasma calcium (diffusible) concentration positively correlate with otolith formation, it should be noted that otolith formation slowed down during which the elevated level of plasma calcium fell. Calcium ions are well known to be essential for activating secretory cells to release their products (Thorn 1976). As the otolith is a secretory product by the macular cells (Dunkelberger et al 1980), a decrease in trigger calcium (possibly descending phase in plasma calcium level) might cause a stop in otolith formation through the inactive secretion of otolith precursors."

Pannella, G. 1971. Fish otoliths: Daily growth layers and periodical patterns. *Science* 173:1124-1127.

NOTES: Otoliths were used in the past for seasonal ageing purposes. The otoliths are made of calcium carbonate in the form of aragonitic prisms and an organic matrix. The otolith grows in an environment that is completely isolated from the external environment and immersed in the labyrinth endolymph.

Sections of the otolith tangential to the medial longitudinal plane of maximal growth show the increments best. A section is produced by holding down the inner side of the sagitta with one finger during the grinding process, which should be done with very fine grit (Carborundum size 2600). Grinding and acid etching (1% aqueous HCL Exposure time 45-180 seconds) were used in combination to produce ridges on the bone. An acetate sheet was then placed over the otolith and melted into the depressions of the bone. This

acetate sheet could then be used to count the rings and observe the structure. Preliminary observation of the growth patterns in the sagittae of other species, living at various depths and different climates, supports daily growth as a universal feature of fish otoliths.

Platt, C. 1973. Central control of postural orientation in flatfish I. Postural change dependence on central neural changes. *J. Exp. Biol.* 59:491-521

NOTES: Flatfish metamorphose from a larval form which swims upright to an adult that lies on one side. The otolith organs do not change their orientation and have essentially shifted their position by 90 degrees. This permanent change in body orientation requires a permanent change in the mechanism that determine posture. Three bilaterally symmetrical pairs of otolith organs exist in regular teleosts. The utriculus contains an otolith that is horizontally positioned. The sacculus and lagena each contain an otolith that is vertically positioned. In flatfish it is the sacculus that is responsible for the behavioral response to tilt rather than the utriculus. Shear force on the sensory epithelium exerted by the weight of the otolith is known to be adequate for the tilt responses. The eyes will migrate in these fish but the otoliths will not shift their position. In the flatfish the saccular otolith (sagitta) lies at roughly 45 degrees to the dorso-ventral axis, the two otoliths thus at 90 degrees to each other. The utricular otolith (lapillus) on each side is perpendicular, and the lagenar otolith (astericus) is parallel, to the dorso-ventral axis. Since the dorso-ventral axis of the adult flatfish is normally horizontal to gravity, the lapillus is vertical and the astericus is

horizontal. Adult flatfish have no swimbladder their posture is primarily determined by the otolith organs.

Platt, C. and A.N. Popper 1981. Fine structure and function of the ear. pp 3-38 In: Hearing and sound communication in fishes. W.N. Tavolga, A.N. Popper, and R.R. Fay, Editors. Springer-Verlag New York Inc. pp. 608

NOTES: The transference from mechanical to electrochemical energy by the hair cells in fish ears results from the bending of the apical bundle of stereocilia by lateral shearing forces. The shapes of the otoliths in teleost ears are species specific. The shape of the otolith may affect the frictional forces acting on the otolith and set up drag.

Ralston, S.V. 1976. Age determination of a tropical reef butterfly fish utilizing daily growth rings of otoliths. Fishery Bulletin 74:990-994

NOTES: Saggital otoliths were used exclusively. Otolith preparation was as follows; 1) extracted from fish; 2) covering membranes were removed; 3) placed in a 2% aqueous HCL solution for several minutes, rinsed, and dried; 4) mounted on depression slides in Euparal, covered with a coverslip allowed to sit two weeks.

The larger the fish the more difficult it was to read the otolith increments. Peripheral ring increments become smaller with growth.

Rice. J.A. 1987. Reliability of growth-rate estimates derived from otolith analysis. In : The Age and Growth of Fish , Edited by Robert C. Summerfelt and Gordon E. Hall. 1987. The Iowa State University Press, Ames, Iowa, 50010.

ABSTRACT: Otoliths are assumed to be daily when the slope of the ring count regressed on the known age is not significantly

different from one. This acceptance of the null model greatly increases the risk of making a type II error or failing to reject a false null hypothesis. All ring count-age relationships exhibit variability and in some cases ring counts clearly do not correspond to age in days, either because deposition does not occur daily, or because it does not produce detectable rings. Hence, validation that the rings we observe are, in fact, daily is a prerequisite for using otolith analysis in age-growth rate determination.

NOTES: When regression is used to back-calculate the size of a fish, there may be a greater chance of making a type II error. The regression slope of ring count on age will not be significantly different from 1.0 when the relationship is actually daily. However, the same result may also occur when the true slope is very different from 1.0 but the data are too variable to distinguish it from a slope of 1.0. The power calculation for regressions is shown in equation form.

Scarfe, H. 1975. Techniques of gem cutting. Watson-Guptill Publications. One Astor Plaza, New York, New York. pp.172.

Grit size refers to the particle size that can fit through a grit mesh of that particular size i.e. 60 grit means that the particles can fit through a mesh screen that has 60 openings to the linear inch.

Table 1.

Micron size	Grit mesh size
1	14000
3	8000
6	3000

15	1200
30	600
45	325

Other chart from Contempo Lapidary 1987 catalog.

1/2	48000
1	24000
3	8000
6	3000
15	1200
30	600
45	320

Simkiss, K. 1974. Calcium metabolism of fish in relation to ageing. pp. 1-12. In: The Ageing of Fish T.B. Baernal (ed.).

NOTES: Calcium relations are very important in the ageing of fish because all of the structures used in ageing are all calcium dependent structures ie. scales, otoliths, and fin rays.

Plasma calcium

Some data:	m.moles/liter
seawater	10.0+/-0.1
teleosts	2.5 +/- 0.1 - 3.2 +/- 0
freshwater	0.9 +/- 0.1

All fish regulate their plasma calcium levels within narrow limits. Kidneys, intestine, gills and skin all function in calcium regulation. In marine teleosts, seawater is swallowed, as the fluid passes along the intestine there is a fall in the concentration of monovalent ions and an increase in the divalent ions especially

magnesium and sulfate. Marine teleosts drink seawater at 0.2 - 2.0% B.W./hr. Fluids from the intestines of the summer flounder Paralichthys lethostigma have been analyzed in depth the results are as follows:

(Calcium Conc. in m.moles/liter)

seawater	10
rectal fluid	12.1
urine	18.6

It is well documented that marine teleosts drink seawater. An analysis of the calcium uptake from seawater has also been done, the results are as follows:

(Calcium conc. in micro moles/hour)

seawater swallowed	42.6
absorbed	29.2 (68%)
rectal excretion	13.4 (32%)
renal excretion	3.3 (11%)

The intestines have been found to absorb 5 times the amount of calcium than Magnesium or sulfate ions. The kidney in marine teleosts functions to excrete Magnesium and Sulfate ions. The general body surface does pick up some of the ions. The scales have been found to under go reversed mineralization "Crichton Effect". The scales of salmon and sea going trout undergo calcium resorption when fish stops feeding in the deeper ocean waters and migrates into fresh water. If a fish detained at the river mouth it will undergo more severe resorption. If it goes up the river and returns quickly it will not have had a great deal of resorption. Otoliths are found in the labyrinths of fish ears and are very variable structures. They are deposited from the endolymphatic

fluid of the labyrinth. There is generally a very low level of bone recycling in the fish. Annual rings have been found in inanimate submerged objects such as glass from bottles.

Sissenwine, M.P., Fogarty, M.J., and Overholtz, W.J. 1988. Some fisheries management implications of recruitment variability. In: Fish Population Dynamics second edition. J.A. Gulland Editor. John Wiley and Sons. pp 422

EXCERPT: "Recruitment is highly variable in many fish populations. Explaining the causes of recruitment variability is a central problem in fisheries research. Numerous biotic and abiotic factors can affect recruitment levels. Recruitment variability is a direct result of random variation on mortality rates during the early life stages. Most fishes are highly fecund and a relatively small change in mortality rates during the egg and larval stages will have a large effect on the number surviving to recruitment.

The importance of identifying the biological and physical determinants of recruitment variability has long been recognized. Hjort (1914) hypothesized that fluctuations in recruitment were caused by; 1. variability in the prey available to the larvae. 2. advective loss during drift from spawning to nursery grounds. Cushing (1973) extended Hjort's first hypothesis to formulate the "match-mismatch" hypothesis relating recruitment variability to the timing of the production cycles of larvae and their prey. More recently, Hunter (1982) and Sissenwine et al (1984b) proposed that greater emphasis be placed on the effects of predation, particularly on the larvae. Sissenwine (1984) suggested that the mortality rate of post-larval and juvenile fish may play an

important role in determining recruitment. Rothschild (1986) provided an important synthesis of recruitment processes and proposed a "bio-dynamic" theory which relates the mortality of early life stages to the contact between predators and prey under the influence of temperature, light, and water motion."

Victor, B.C. 1982. Daily otolith increments and recruitment in two coral-reef wrasses, Thalassoma bifasciatum and Halichoeres bivittatus. Mar. Biol. 71(2):203-208

NOTES: Tropical fish are hard to age because of the lack of cycles (temperature, photoperiod, etc.). Otoliths are stone like calcium carbonate accretions situated within the semicircular canals of bony fishes which assist in balance and sound perception. Denied or augmented food resources to different groups of fish in order to develop a mark on the sagitta or lapilli. The fish studied with food augmentation was the slippery dick (Halichoeres bivittatus). Augmentation involved bringing 20 sea urchins to a particular location on a reef for 5 consecutive days.

The bluehead wrasse (Thalassoma bifasciatum) was denied food resources for 4 days after capture in order to produce a otolith mark.

Results: Banding was found on the otoliths of treated fish.

Victor, B.C. 1986. Delayed metamorphosis with reduced larval growth in a coral reef fish (Thalassoma bifasciatum). Can. J. Fish. Aquat. Sci. 43:1208-1213.

ABSTRACT - Analyses of daily otolith increment sequences in the bluehead wrasse, Thalassoma bifasciatum, from San Blas Islands of Panama indicated that (1) the duration of the planktonic larval

period of this species ranges from 38 to 78 d, (2) those larvae with extended larval durations reach settlement size at the same age as average individuals but then remain planktonic for several more weeks, and (3) the estimated larval growth rate during this period of delayed metamorphosis ($0.08 \text{ mm} * 1/\text{d}$) is distinctly lower than both the estimated growth rate before attaining settlement size ($0.26 \text{ mm} * 1/\text{d}$) as well as the growth rate of juveniles after settlement ($0.31 \text{ mm} * 1/\text{d}$). The ability to delay metamorphosis, the first documented for a vertebrate, may be an adaptation for maximizing the return of planktonic larvae to coastal waters.

Warlen, S.M. 1987. Age and growth of larval Gulf menhaden, Brevoortia patronus, in the northern Gulf of Mexico. Fishery Bulletin 86(1):77-90.

ABSTRACT: Experiments on laboratory spawned and reared larval gulf menhaden, Brevoortia patronus, showed that they formed one otolith growth increment per day and that the increments could be used to estimate their age. Wild larvae from collections in the northern Gulf of Mexico along three transects (Cape San Blas, Florida; Southwest Pass, Louisiana; and Galveston, Texas) were aged. Gompertz growth equations were used to describe the relationship between age and standard length for larvae collected at various locations, and in different seasons and years. MANOVA tests and subsequent pairwise tests were used to test for differences among these growth curves. For the most extensive data set (Southwest Pass, Louisiana), there were significant differences in growth between early season (December) and late season (February) larvae. Early season larvae grew faster than late season larvae. Growth of

larvae also differed among December collections and among February collections. The growth model for the pooled data for all wild larvae predicted that they grew from 2.4 mmSL at hatching to 20.4 mmSL at 62 days.

NOTES: Standard length was measured to the nearest 0.1 mm. The otoliths of this species was easily visible without grinding. The sagittae was the only structure used. The correlation coefficient was; $r = .928$. This study was validated by using laboratory reared fish. The first increment formed five days after the first exogenous feeding.

Wilson, K.H., and P.A. Larkin. 1980. Daily growth rings in the otoliths of juvenile sockeye salmon (Oncorhynchus nerka). Can. J. Fish. Aquat. Sci. 37:1495-1498

NOTES: Caught and dyed pinhead salmon upon emergence from their eggs in the gravel beds. The fry were dyed with neutral red which marked the otolith. One sagitta from each fish was used for the study. Crystalline and other unreadable otoliths were discarded. Otoliths were affixed to a glass slide with the sulcus (ie. proximal surface) upward. The otoliths were ground lightly on a sintered glass plate with aluminum oxide and clarified with glycerine before photographs were taken. Ring counts were made on the projected images of these photographs. The rings on the otoliths were formed daily. The average date of the first discernible ring was used as the date of emergence. Excessive grinding will remove rings. Rings will also be obscured by calcium overburden where grinding is insufficient.

Yoklavich, M.M. and G.W. Boehlert 1987. Daily growth increments in otoliths of juvenile black rockfish, Sebastes melanops: an evaluation of autoradiography as a new method of validation. Fishery Bulletin 85(4):826-832.

NOTES: Conflicts in daily growth increments versus studies that have found that the increments do not represent the true age of the fish. Nondaily increment formation explained by 1) the inclusion of subdaily increments and 2) the methodological errors in preparing and viewing the otoliths.

Several techniques for validation studies have been used; 1) laboratory reared fish; 2) chemical marks OTC, strontium, Calcium 45; and 3) stress marks cold shock, removal from field.

Results: Calcium-45 marks were not as clearly discernable as the OTC method. Both marking methods produced the same results. OTC proved easier to use and handle, although not all otoliths showed clear increment formations after the injection of OTC. Sixty-seven percent of these fish did not show prominent daily increments. They suggest use either an injection of OTC or use stress to mark the otoliths.

Young, J.W., C.M. Bulman, S.J.M. Blaber, S.E. Wayte. 1988. Age and growth of the lanternfish Lampanyctodes hectoris (Myctophidae) from eastern Tasmania, Australia. Marine Biology 99:569-576.

ABSTRACT: The age, growth and mortality of the lanternfish Lampanyctodes hectoris (Gunther) were investigated by examining the otoliths, length - frequency data, and seasonal abundance of fish collected from the continental slope waters of the eastern Tasmania between April 1984 and June 1985. Although L. hectoris can live to

be three years old, it has a high annual mortality (79%) and few live past their first year. Growth, described here by a variation of the Von Bertalanffy model, takes place mainly in the first six months of life and only during late spring and summer. The hypothesis of daily deposition of primary growth increments in myctophids was supported by comparing the back-calculated birth dates of daily aged individuals of L. hectoris with the spawning season of this species.