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Abstract

The first, second, and third annulus were identified in the dorsal fin rays of lingcod (*Ophiodon elongatus*). These annuli were validated as indicators of age by calculating the total radius of the fin rays of a lingcod that had completed one, two and three years of growth. Fish lengths came from known-age lingcod (from a rearing study and a year-round field sampling program) and modal progression studies. We measured the radii of the first, second, and third hyaline zone (0.36, 0.54, and 0.75 mm, respectively) from commercial and recreational catches of lingcod. The radii were significantly different among the three zones. Further, the radius of each zone corresponded to the total radius of the fin rays of known-age one-, two-, and three-year-old fish, validating them as true annuli.

Introduction

Age determinations are essential to successful fishery management, but often the validity of the age data, based on the analysis of hard parts, has not been established. Incorrect ages could lead to increased uncertainty in harvest guidelines (Beamish and McFarlane, 1983), and could result in overfishing. Age estimates have been validated by various methods including mark and recapture studies, rearing studies (Panfili and Tomás, 2001), identification of strong year-classes, length-frequency analysis (Chilton and Beamish, 1982), and marginal increment analysis (Pearson, 1996).

The dorsal fin rays are the most useful structures for determining the age of lingcod, *Ophiodon elongatus* (Beamish and Chilton, 1977). A complete year of growth was represented by one opaque summer growth zone and one hyaline winter growth zone in a fin ray (Chilton and Beamish, 1982). Ages were typically determined by counting the number of hyaline zones in a fin ray. Cass and Beamish (1983) used oxytetracycline marks in fin rays to validate the annual periodicity of hyaline zones in lingcod between 580 and 770 mm fork length (FL). Beamish and Chilton (1977) concluded that lingcod ages determined from fin rays greater than 490 mm total length (TL) were validated based on the similarity of mean length-at-age estimated using different ageing methods (vertebrae, scales, tagging, and fin rays).

Although the ages of larger individuals have been validated for lingcod, the ages of smaller, younger fish have not. The ages for these younger fish have been assessed by fish length alone, using modal progression and length frequency models (Chatwin, 1954; Beamish et al., 1978). Beamish and Chilton (1977) noted the difficulty of identifying the first two annuli of dorsal fin rays in lingcod. This is perhaps one reason why the fin rays have not been used to ascertain the age of these younger fish. However, the lack of validity of these first few annuli in the fin rays can lead to increased ageing error of older annuli. The correct identification of these first few annuli is pivotal for accurately determining the ages of older fish.

Here we validated the annual periodicity of the first, second, and third hyaline zones in the dorsal fin rays of lingcod. The validation was accomplished by comparing the total radius of the fin ray from known-age fish after one, two, and three years of growth with the measured radius of the first, second, and third hyaline zone. To do this, we first estimated the length of lingcod after one, two, and three years of growth and calculated the total radius of the fin ray corresponding to that fish length. Second, we demonstrated that the growth of fin rays was consistent throughout life by plotting the total radius of the fin ray against fish length. Third, we measured the radius to the first, second, and third hyaline zone. If the radii of the three hyaline zones were significantly different from each other, and they corresponded to the total radius of the fin ray of known-age one-, two-, and three-year-old fish, then these zones could be interpreted as annuli and age estimates based on them could be considered validated. Finally, we examined the pattern of growth in the fin rays to determine if the hyaline zone measurements were stable or if they varied with fish length.

Methods

Fish Collections

A total of 123 benthic juvenile lingcod were collected from San Francisco, Monterey, and Morro Bays, California, in 1993. Juvenile lingcod from San Francisco Bay were collected with a beach seine or a small otter trawl. Juvenile lingcod from Monterey and Morro Bays were collected by divers using spears. Fork lengths were measured to the nearest millimeter on all lingcod collected.

Thirty-seven of the benthic juvenile lingcod collected in San Francisco Bay were transported to Bodega Marine Laboratory, individually tagged, placed in tanks (2,200 l), and held in captivity for up to two years. At approximately six-month intervals, the length of each surviving lingcod was measured. Each fish also was measured upon its death.

Fish lengths, sex, and soft dorsal fin rays from sub-adult and adult lingcod were collected from both the commercial and recreational fisheries. Commercial landings were sampled from Astoria, Oregon to Morro Bay, California from 1992-96. Recreational samples were collected from commercial passenger fishing vessels (CPFVs) between Crescent City and Morro Bay, California from 1993-1996. All length measurements were standardized to fork length using the conversions provided in Laidig et al. (1997).

Fin Ray Preparation and Examination

To prepare fin rays for ageing, they were dehydrated, the skin was removed, and the bare fin rays (number one [anterior] through eight [posterior]) were embedded side by side in polyester resin blocks. The bases of the fin rays were aligned along an axis parallel to, and approximately 5 mm distal to, the bottom of the block. The blocks containing the fin rays were sectioned with a Buehler Isomet[1] low-speed saw, producing a 1-mm thick cross-section of the fin rays. Two cross-sections were taken from each block, the first approximately 5 mm distal to the fin ray base, and the second at approximately 15 mm above the fin ray base. This gave a spatial separation, which allowed more accurate ageing of the fin rays when anomalies, such as irregular growth or inclusions (holes caused by resorption of the center of the fin ray in older lingcod), occurred in a cross-section. Cross-sections were affixed (using clear nail polish) to a microscope slide for viewing.

Fin rays were viewed on a computer monitor linked to a video camera that was mounted on a compound microscope. Fin ray measurements were taken from the video image using the National Institute of Health's Image software[1]. The radius of each of the first three hyaline zones was measured from the center of the fin ray to the distal edge of the hyaline zone along the longest growth axis (Fig. 1A). In addition, the total radius of the fin ray was measured from its center to the marginal edge along the longest axis (Fig. 1B).

Length at Age 1

To determine the length of a lingcod after one year of growth following hatching, we collected and measured young-of-the-year (YOY) lingcod throughout the summer and reared

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[1]Use of tradename does not constitute endorsement by National Marine Fisheries Service.

some of these fish for more than one year. Lingcod were determined to be YOY by means of fish length at time of capture using an age-length curve (Adams et al., 1993). For the determination of when a year of growth had been completed, we selected a hatching date of 28 February because this date was the mean hatching date (ranging from late November to April) as determined from the literature (Miller and Geibel, 1973; Phillips and Barraclough, 1977; Cass et al., 1990; Adams et al., 1993). Also, the hyaline zone should have completely formed in most fish (assuming winter formation) by this date (Beamish and Chilton, 1977; Cass et al., 1990). We plotted lingcod length for these fish against days after hatching and determined that a power curve of the form

$$FL = a \times (age^{b})$$

(where a and b are the model parameters and age = days after hatching) provided the best fit to the data.

Length at Ages 2 and 3

Because we were only able to successfully rear one fish to over two years, we relied on previously published data to determine lingcod lengths after two and three years of growth. To avoid bias from ages determined using hard structures (fin rays, otoliths or scales), we used the results of two modal progression studies. By following the modes of known-age lingcod through time, the length of a one-, two-, and three-year-old lingcod can be determined. Beamish et al. (1978) conducted monthly sampling surveys and found that the first three modes (or ages) in late

February occurred at 284 mm, 362 mm, and 458 mm FL. Karpov et al. (1995) observed that the average length of the modes corresponding to the two- and three-year-old lingcod were 362 mm and 443 mm FL, respectively. To obtain the length of a lingcod at two and three years of growth, we averaged the values from these two independent studies.

Fin Ray Growth

We analyzed the pattern of fin ray growth to determine how the radii of the hyaline zones changed with increasing distance from the base of the fin ray. This information was used to establish the maximum distance distal to the fin ray base that was required for accurate age determination. To analyze this fin ray growth, up to 18 cross-sections were taken slightly over 2 mm apart over the entire length of the fin ray.

Results

Length at Age 1, 2, and 3

The estimated length (from the power curve) of a lingcod after one year of growth was 264 mm FL. The fit of the power curve between FL of lingcod less than two years old and days after hatching was significant ($P<0.05$, $df=110$, $R^2=0.82$, $n=111$; Fig. 2). The average fork length of lingcod in February after two and three years of growth (as determined from two modal progression studies located in the literature) were calculated to be 362 mm and 451 mm, respectively.

Hyaline Zone Widths

There was a significant linear relationship between the total radius of the fin rays and FL ($P<0.05$, df=342, $R^2=0.94$, n=344; Fig. 3). By applying the lengths of lingcod after one, two, and three years of growth to this relationship, we estimated the length of the total radius of the fin rays after one, two, and three years of growth to be 0.37, 0.58, and 0.78 mm, respectively.

There were significant differences among the radii of the first, second, and third hyaline zone (ANOVA, $P<0.05$, df=254; Fig. 4). Some variation in the radius of the first three hyaline zones was observed, but there was little overlap. The mean radius of the first hyaline zone was 0.36 ± 0.05 mm; the second hyaline zone, 0.54 ± 0.05 mm; and the third hyaline zone, 0.75 ± 0.05 mm. These values were similar to the sizes of the total radius of the fin rays after each year of growth calculated above.

Fin Ray Growth

The average radii of the first, second, and third hyaline zone on fin ray number four decreased for cross-sections taken at increasing distance from the fin ray base (Fig. 5). Hyaline zone one became increasingly smaller in size until 20 mm from the base when the size stabilized at approximately 0.23 mm. It remained this way until it became indistinguishable from the center of the fin ray at approximately 30 mm from the fin ray base. Hyaline zones two and three also reduced in size with increasing distance from the base and they became indistinguishable from the center of the fin ray by 37 and 45 mm above the fin ray base, respectively. The radius of each hyaline zone remained fairly constant for the first 13 mm distal to the fin ray base (Fig. 5). Although the radius of the hyaline zones decreased, there was no overlap between the radius of a hyaline zone and the next smaller hyaline zone until approximately 25 mm above the fin ray base. At this size, the width of hyaline zone two was smaller than the largest width of hyaline zone one. Similarly, the width of hyaline zone three at 27 mm from the fin ray base became smaller than largest width of hyaline zone two.

Discussion

This study validated the first, second, and third hyaline zone in dorsal fin rays of lingcod as first, second, and third annulus, respectively. To validate these hyaline zones, we showed that (1) the hyaline zones were related to annular events and were produced only once in a year, (2) the fin rays widened proportionally to fish growth, and (3) the hyaline zones in the fin rays were permanent and unchanging. From our collection and rearing studies, lingcod were found to be approximately 264 mm FL after their first year of growth (Fig. 2). In February, Chatwin (1954), using modal progression, observed the mean length of the first mode (age one fish) of captured lingcod to be 274 mm FL. Similarly, Beamish et al. (1978) found the mean length of the first mode of collected lingcod was 284 mm FL. These values are similar but slightly larger than our length after one year of growth. In another modal progression study, Miller and Geibel (1973) found a slightly smaller length after one year of growth. They collected small lingcod off central and northern California from February and March and calculated the average length of the first mode (age one fish) to be 243 mm FL. These investigations corroborate the results from our research, which showed the length of a lingcod after one year of growth to be approximately 264 mm FL. The calculated lingcod length after one year of growth of 264 mm FL equated to a total radius for the fin ray of 0.37 mm, which is very similar to that observed for the first hyaline zone at 0.36 mm. Because the first hyaline zone equated to an annual event (the completion of the first year of growth), we can say that the first hyaline zone is validated as the first annulus.

To ascertain the validity of the second and third annuli, we used fish lengths calculated by modal progressions. The use of lengths from modal progression studies allowed for an age-structure independent measure of length-at-age. Because, during modal progressions or length-frequency analyses, collections were made throughout a time period, these lengths would be related to annual events (like date of sample). Here, we selected two modal progression studies and averaged the fish length after two and three years of growth. This produced average lengths of 362 mm and 451 mm FL, respectively. These lengths are similar to fish lengths in February calculated from other studies that assessed age using hard structures. Using data from Miller and Geibel (1973), we determined the length of lingcod in February after two and three years of growth by linear interpolation to be 404 mm and 501 mm FL, respectively. Similarly, the estimated length of a two- and three-year-old lingcod from Puget Sound from a growth curve (Stick and Mandapat, 1981) was 372 mm and 441 mm FL. In Cass et al. (1990), the estimated length after two and three years of growth was 382 mm and 490 mm FL, respectively. Although variable, the lengths among all studies are similar with a lingcod after two years of growth averaging 382 mm and after three years averaging 477 mm FL. These values are slightly higher than our values of 362 mm and 451 mm. This may be due to geographic differences (since the latter two studies were from Canadian waters), annual variation in lingcod growth, or natural variation in this species. The closeness of the results between these two different ageing methods lends credence to the length values we obtained in this study.

Our measurements for length after two and three years of growth corresponded to fin ray radii at the time of their birthdays of 0.58 mm and 0.78 mm, respectively. These predicted radii are similar to the widths of the second and third hyaline zone measured in our study (0.54 mm and 0.75 mm). These measurement studies and the fact that the hyaline zones were found to be significantly different from each other substantiate that these hyaline zones were true annuli.

As the lingcod grew, the radii of the fin rays increased. Although this relationship had some scatter (Fig. 3), continuous growth was seen in the fin rays. This scatter may reflect differences in growth rates. Genetic deformities, biologic events (such as attempts at predation), and environmental causes (such as physical eroding of the fin rays on the substrate) can all lead to misshapen fin rays. Although this does not appear to change the age estimate for the fish, the radial measurements for each annulus may vary slightly from the expected values.

Although annulus width was shown to remain constant as the lingcod grew in length, other challenges exist to determining lingcod age, including resorption of the fin ray and the presence of check marks (hyaline zones other than annuli). As lingcod grow, the center of the fin rays may become resorbed. This space is then occupied by connective tissue, blood vessels, or nerve bundles (Beamish and Chilton, 1977). When this inclusion occurs, it can obscure or eliminate the first few annuli. In this case, the use of the radius measurement for the third annulus can help to establish the placement of succeeding annuli during age determination (Chilton and Beamish, 1982).

Another potential problem for age validation is the confusion of check marks with annuli. Chilton and Beamish (1982) suggest that a check mark is generally much narrower than an annulus and it is rarely continuous around the entire fin ray. Many check marks have been observed in lingcod fin rays, from a variety of causes (Chilton and Beamish, 1982). Temperature changes, stress, and photoperiod have been shown to change otolith daily and annual increment formation (Taubert and Coble, 1977; Campana and Neilson, 1985; and Schramm, 1989). Chalanchuk (1984) suggested that temporary bad weather or food shortages may cause "false" annuli. Some annuli split in fast-growing fish and may be counted as two annuli (Cass and Beamish, 1983). "Annuli" have even been shown to be formed twice a year in *Thunnus thynnus* (Compeán-Jimenez and Bard, 1983). Besides the confusion of determining an annulus from a check mark, Chilton and Beamish (1982) state that the first two annuli may become obscured by check marks as the fish grow. In this case, measuring the third annulus may be the only method of determining the accurate age of lingcod.

The manner in which the fin ray grows can potentially cause problems in determining the age of lingcod. If the fin rays grew from the bottom and were pushed upward, the information contained in them would vary depending on the height of the cross-section of the fin ray above the fin ray base. However, if the new tissue was deposited on the outside of the fin ray by the skin covering the fin ray, the information enclosed in the cross-section would hold the entire history of the fish. This would be true only for heights shorter than the length of the fin ray at the time of the first annulus formation. If data were taken from the outer region of the fin rays, then the first year's information would not be included. From our data, it appears that the fin rays grow by laying tissue down on the outside of the fin ray. The annular radii do not vary greatly with height above the fin ray base and are visible for much of the length of the fin ray. The first annulus becomes obscured and vanishes around 30 mm up from the fin ray base. This is expected because the length of a fin ray for a one-year-old fish is approximately 30 mm. Furthermore, the radius of the second annulus decreases and becomes similar in size to that of the first annulus at approximately 25 mm above the base. Therefore, when making cross-sections, the analysis should be conducted within 25 mm of the fin ray base so that all of the pertinent information will be retained in the fin ray cross-section.

Recent stock assessments indicate that lingcod biomass on the west coast has fallen below fifteen percent of original biomass estimates (Jagielo et al., 2000; Adams et al. 1999). Because stock assessments are based on age-structured models, protection of lingcod will only be enhanced by the use of validated ages. With these more accurate ages, better stock assessment models can be created. With these data, managers can implement strategies for rebuilding lingcod populations.

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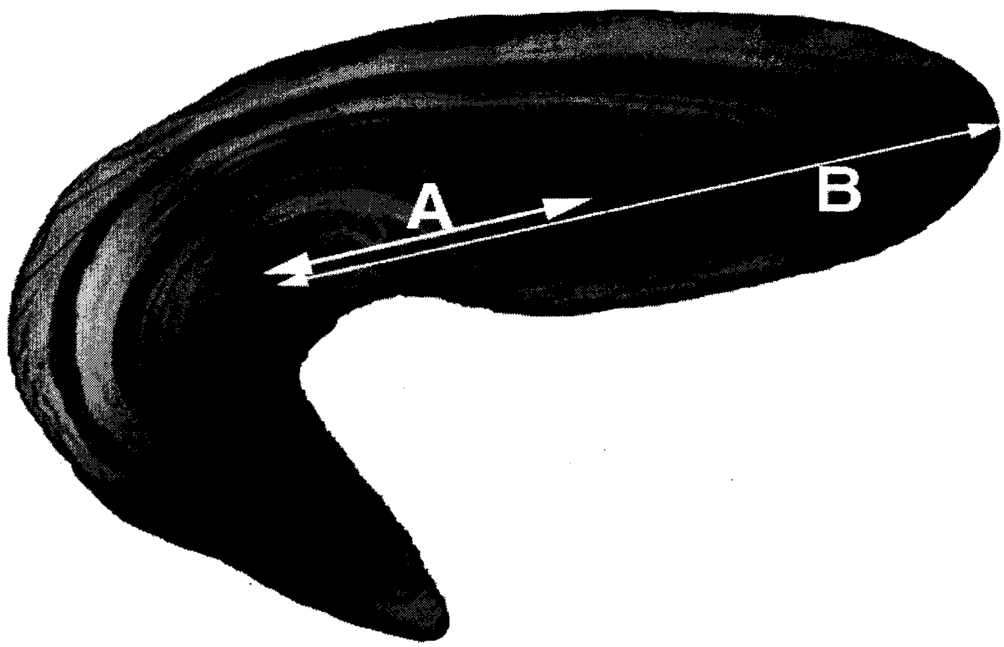

Figure 1. A cross-sectional view of a lingcod (*Ophiodon elongatus*) fin ray showing three hyaline zones. A) The radius of the first hyaline zone as measured from the center of the fin ray to the edge of the first hyaline zone along the longest axis. B) The radius of the fin ray as measured from the center of the fin ray to the marginal edge along the longest axis.

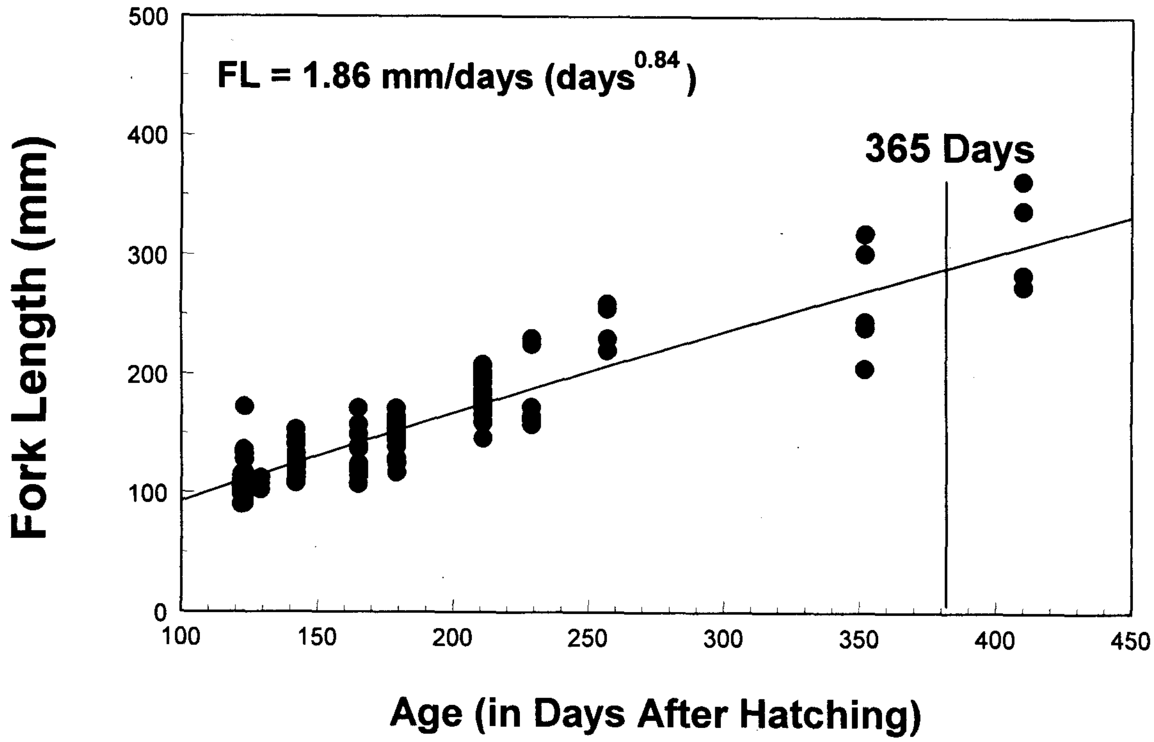

Figure 2. Relationship of fork length versus age (days after hatching) for lingcod (*Ophiodon elongatus*) under two years old (n=111). The curved line represents the predicted values determined from a power curve. The solid vertical line represents one year after hatching.

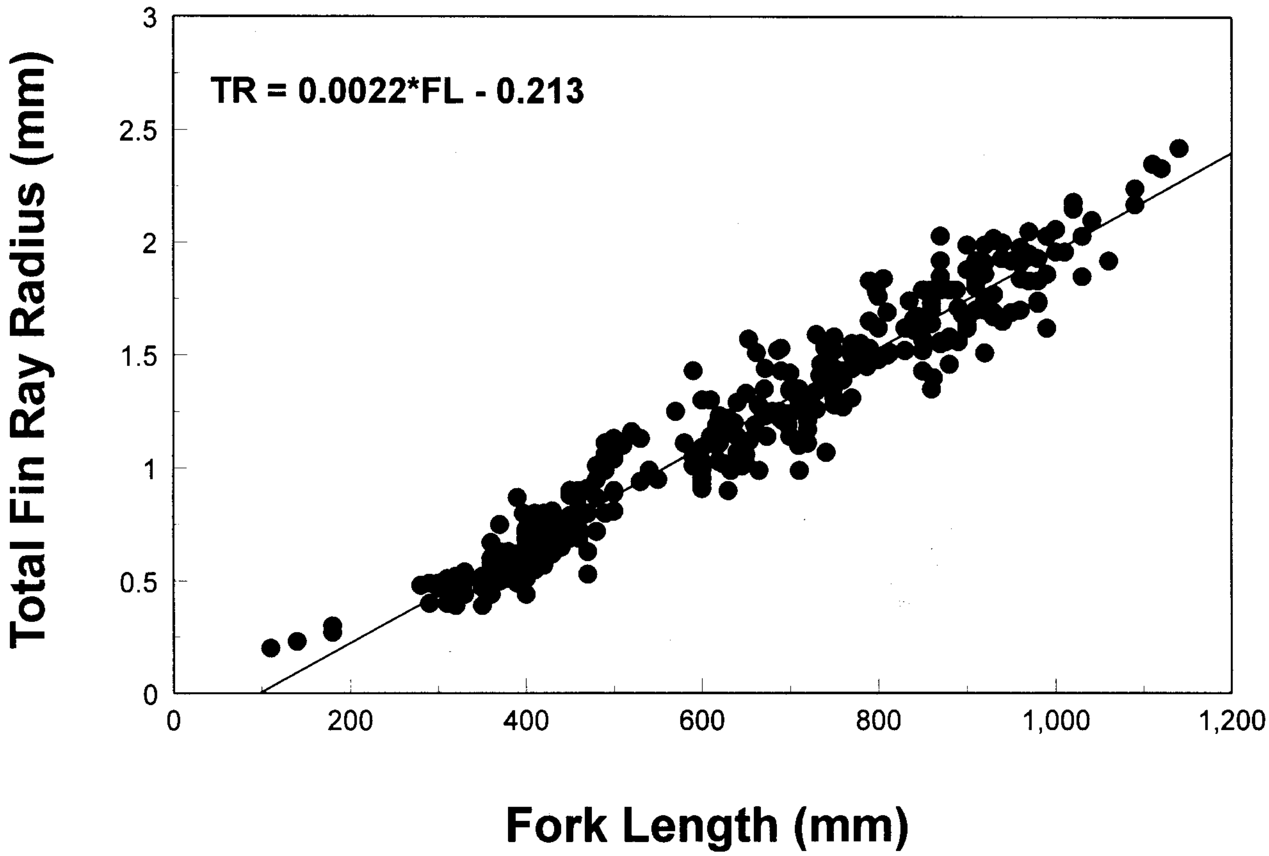

Figure 3. Relationship of the total radius of the fin ray (for fin ray number four) versus fork length for lingcod (*Ophiodon elongatus*). Solid line represents predicted values from the linear model (n=344).

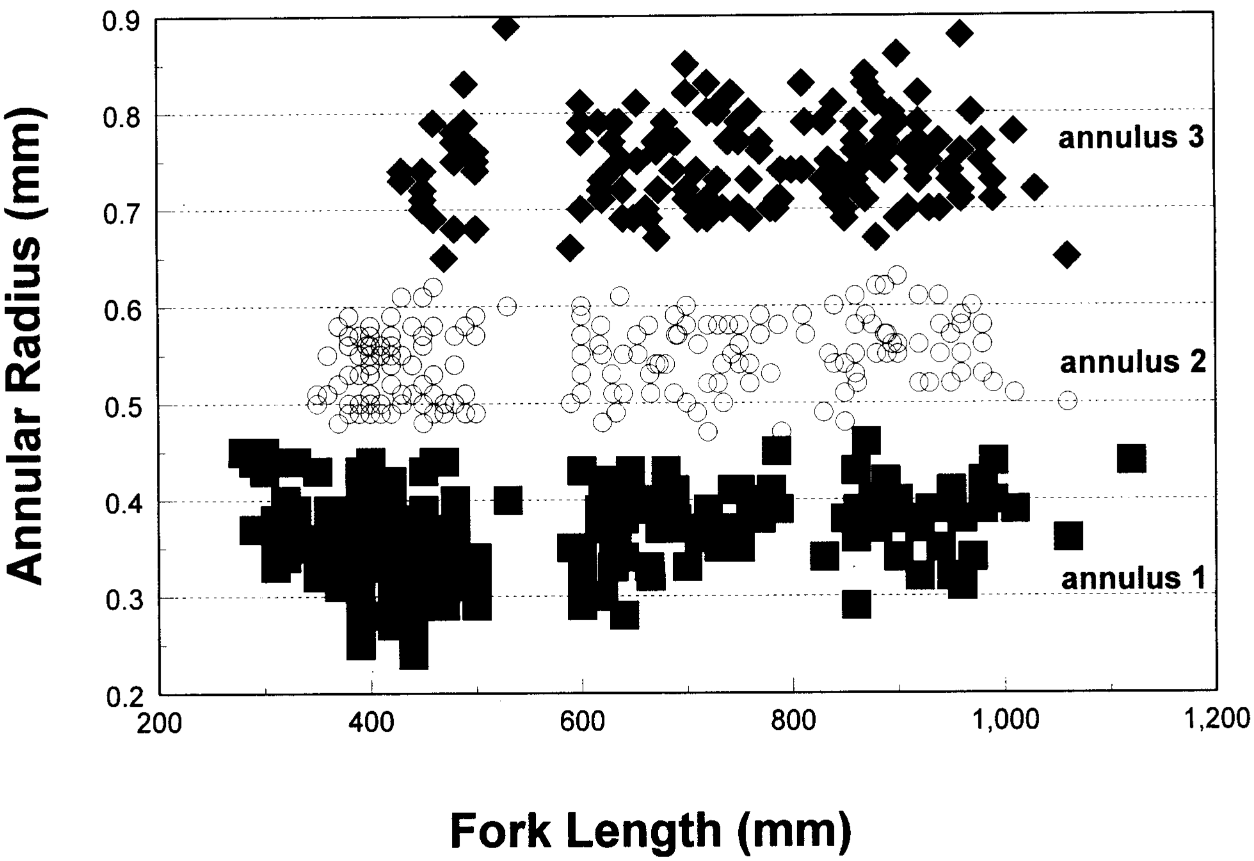

Figure 4. A comparison of the radii of the first three hyaline zones in lingcod (*Ophiodon elongatus*) fin rays over change in fish length (n=256).

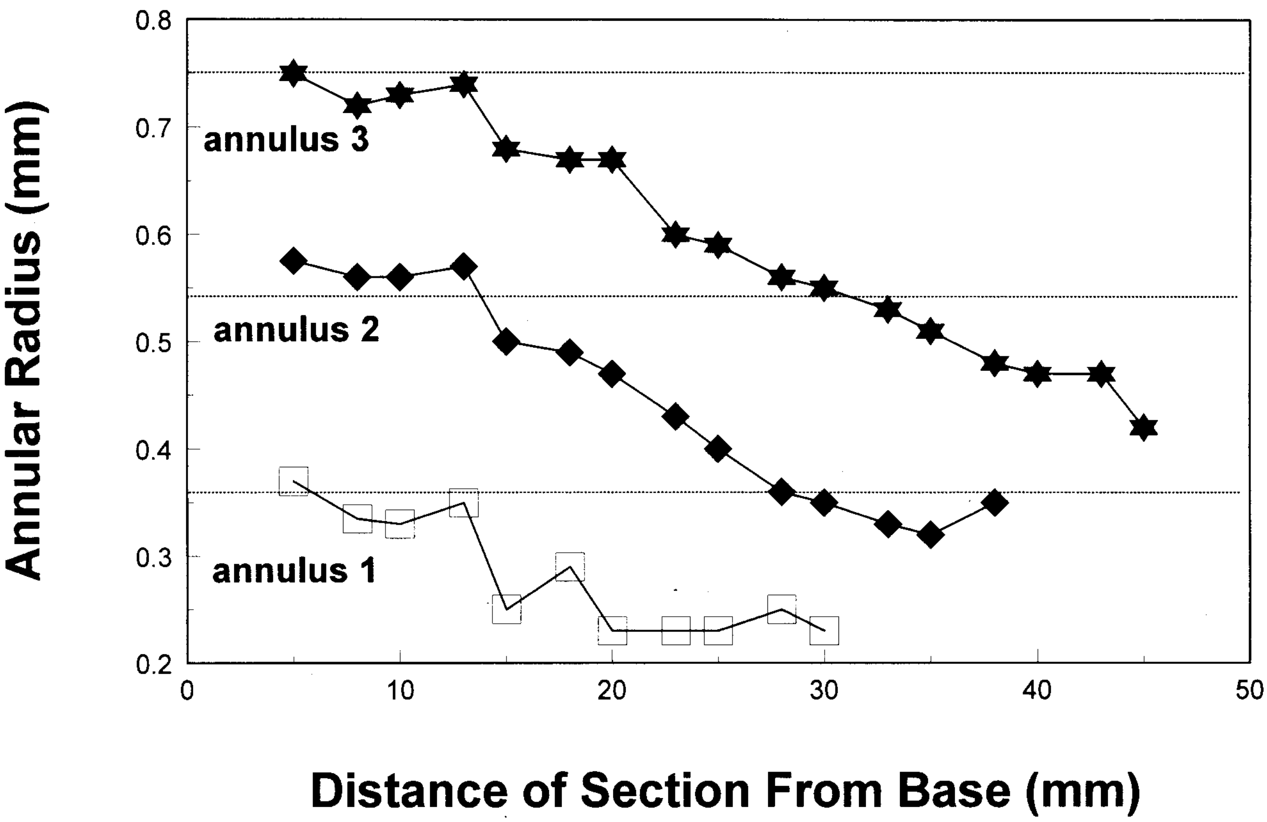

Figure 5. Radii of the first three hyaline zones (annuli) dependent on distance from the fin ray base (fin ray number four) for lingcod (*Ophiodon elongatus*). Dashed lines indicate the average radius of each hyaline zone as determined in this study.

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