

ESTIMATION OF THE AGE OF A TROPICAL REEF FISH USING THE DENSITY OF DAILY GROWTH INCREMENTS

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ABSTRACT

Daily growth increments found in the otoliths of *Pristipomoides filamentosus* (Pisces: Lutjanidae) were used to estimate the ages of the larger and older individuals which are harvested in the commercial deep-sea handline fishery in Hawaii. Previous investigations have been limited to visual counts of all rings present in an otolith, precluding the possibility of aging large fish. A method was devised in which the density of increments within an otolith is related to fish size and age is estimated by regression statistics.

To date 81 otoliths have been analyzed from fish ranging in size from 18 to 70 cm fork length. Sagittae were read from the focus to the antirostral margin of the otolith in frontal sections. Numerous determinations of the density of daily increments were made at measured distances from the focus by microscopic examination and statistical regressions were performed on these data. Integration of the regression equation, bounded by the focus and the otolith margin, provides an estimate of the age in days of the sample specimen. Independent size-frequency data confirm the daily periodicity of the increments.

Overall, approximately 77% of the variation in increment density was explained by the linear model. Error estimates of age were obtained by the Delta Method, a propagation of error technique. In general, the coefficient of variation for age estimates is about 6%, indicating an acceptable level of precision. This new method of obtaining age estimates may prove valuable in the management of tropical fish stocks if assumptions concerning growth interruptions and increment periodicity can be satisfied.

INTRODUCTION

Within the last decade, studies of fish otolith microstructure have revealed the startling fact that these calcified structures often grow by daily accretion of increments. Pannella (1971, 1974) was the first to demonstrate this and others have substantiated his findings for a wide variety of temperate and tropical species in both marine and freshwater environments (Brothers et al. 1976, Le Guen 1976, Struhsaker and Uchiyama 1976, Ralston 1977, Taubert and Coble 1977, Barkman 1978, Methot and Kramer 1979, Wild and Foreman 1980). Brothers (1980), in a recent review of age and growth studies of tropical fishes, discusses the difficulties of conducting otolith microstructure research and cautions against the overzealous application of the method. Nevertheless, he also emphasizes the great potential for analyzing growth patterns by this means.

One problem to date is that all published studies have been limited to counting the total number of increments found in fish of known size and, frequent-

ly, known age. Consequently, studies have been limited primarily to larvae, juveniles, or fast-growing, short-lived species. Age determinations from large, old fish using daily growth increments are currently unavailable, presumably because otolith microstructure becomes more complicated with growth and suitable preparations become more difficult to achieve (Brothers 1980). Furthermore, some investigators have concluded that the explicit one-to-one correspondence between increments and days breaks down among older fish due to interruptions in growth (Pannella 1971, Wild and Foreman 1980).

This paper briefly reports our attempt to age large adult specimens of *Pristipomoides filamentosus* using daily growth increments of otoliths. This is a deepwater, commercial species of snapper (Lutjanidae), found in the Hawaiian Islands and elsewhere in the Pacific. A methodology is developed which estimates the total number of increments in the otolith of a fish in lieu of counting all rings. Additionally, an error estimation technique is applied to the data to assess the reliability of the determinations.

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METHODOLOGY

Specimens of *P. filamentosus* were obtained at various localities around the Hawaiian Islands by hook-and-line fishing. These were kept on ice until returned to the laboratory where both standard length (SL) and fork length (FL) were measured to the nearest millimeter. Fish ranged in size from 18 to 70 cm FL, spanning the full adult size range of the species.

Only the largest otoliths or sagittae were examined in this study and these were removed from the cranium, cleaned, washed, and dried. Samples were then embedded in casting resin and allowed to harden completely. Cast otoliths were sectioned on a Buehler Isomet low speed jeweler's saw. Thin sections were made through the focus along a frontal plane to the most distal portion of the antirostrum (Fig. 1). Sections were then polished with levigated alumina until the surface to be examined was very smooth. Samples were subsequently etched for several minutes in a 1% solution of HCl, washed, and dried. Final preparation of the otoliths consisted of mounting them on glass slides with Euparal and cover slips and allowing about 1 week for them to clear adequately.

Samples were read with a compound microscope using transmitted light at a magnification of 400X. The distance in microns (μ) between the focus and antirostral margin was first measured with a calibrated ocular micrometer. Readings were then made at selected points along the growth axis, wherever increment microstructure was sufficiently clear and interpretable (Fig. 2). At these locations, the density of increments was determined by counting the number of visible increments and measuring the axial length of the segment in which the increments were found. In addition, the axial distance between the midpoint of the sample region and the focus was measured with the ocular micrometer. As many readings as possible were made from each preparation, subject to the restriction that counts only be made in regions where increments were clear and unambiguous.

Data were summarized for each specimen by computing the ratio of segment length in microns to the included number of increments at each specific segment examined in the preparation. This figure measures the average increment width at a given point in the otolith. Plots of average increment width against distance to the otolith focus show an inverse relationship due to declining growth rate with size. Under the assumption that one increment is equal to 1 day it is possible to approximate the growth rate on a size specific basis as follows:

$$\frac{\Delta \ell}{\Delta \text{ increments}} = \frac{\Delta \ell}{\Delta t} \approx \frac{d\ell}{dt} \quad (1)$$

where $\Delta \ell$ is segment length in microns and Δt is time in days. This approximation is reasonable because the localized regions in which the ratios were computed rarely exceeded 2% of the total otolith length. That is to say, the finite approximation to the differential was computed on a sufficiently small scale of time and length.

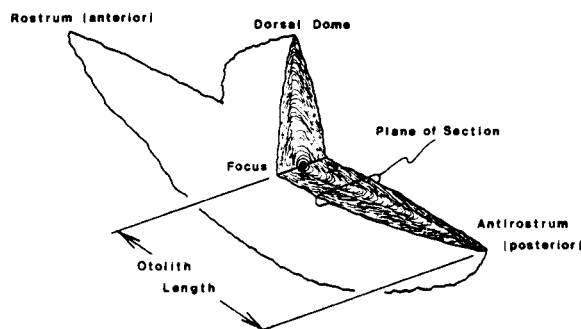


Figure 1. Schematic representation of a sagittal otolith locating the specific points of reference referred to in the text.



Figure 2. Slide preparation of a sagitta from *P. filamentosus* showing increment microstructure.

While plots of $\frac{d\ell}{dt}$ against length show the expected inverse relationship, there is curvilinearity apparent in the data for *P. filamentosus*. A logarithmic transformation of the growth rates removed this trend and the data were analyzed with standard linear regression techniques. The model fitted was:

$$\ln \left(\frac{d\ell}{dt} \right) = \alpha - \beta \ell + \epsilon \quad (2)$$

where ℓ is length in microns, t is time in days (i.e., increments), α and β are model parameters, and ϵ is a normal random variable with mean zero and

finite variance. Separation of variables allows solution of equation (2), which if evaluated as a definite integral, bounded by the focus and margin of the otolith, provides an estimate of the age of the sample specimen in days. More explicitly:

$$\int_{t=0}^T dt = \int_{\ell=0}^L \alpha' e^{\beta \ell} d\ell \quad (3)$$

where T is the age of the fish in days at the time of death, L is the total length of the otolith at death, and α' is the reparameterized version of α from equation (2) (i.e., $\alpha' = e^{-\alpha}$). The general solution of equation (3) is:

$$\tau = \frac{\alpha'}{\beta} (e^{\beta L} - 1) \quad (4)$$

Thus estimates of α , β , and L are all that are necessary in order to estimate the age of the sample specimen.

Confidence limits for age estimations were computed using the Delta Method, a propagation of error technique (Ostle 1963, Seber 1973). Because equation (4) is a nonlinear combination of random variables (α and β) an explicit formulation for VAR{T} is very difficult to obtain. The method used here expands the function around the mean values of the random variables in the first few terms of the Taylor Series. If terms of higher order can be neglected, then an asymptotic estimate of VAR{T} can be obtained. The standard error of the estimate and the coefficient of variation (standard error/estimate \times 100) were computed from this result.

RESULTS

As detailed in the previous section, in order to estimate the age of a specimen, one needs only the total length of the otolith (L), and estimates of the parameters α and β from the regression of otolith growth rate on otolith length (equation 2). Once ages have been estimated for a sufficient number of samples they can be related directly to the lengths of the specimens from which they were obtained, and a growth curve can be generated. This procedure bypasses potential complications due to allometric growth of the otoliths. Nevertheless, it is worth examining the relationship between otolith length and fork length to fully describe the growth process.

Figure 3 shows that, over the range of sizes examined, otolith length is allometrically related to the fork length of *P. filamentosus*. The data were fitted to the linearized form of the power function and the estimated exponent (0.631) forces the re-transformed regression through the origin. This is a

minor constraint because otolith formation occurs very early in life, usually during the larval stage (Brothers et al. 1976).

A total of 81 sagittae were analyzed in this study and overall these provided 2,957 separate estimates of $\frac{d\ell}{dt}$ along the antirostral growth axis. The data were pooled initially and the natural logarithm of growth rate computed. The results are plotted against distance to the otolith focus in Figure 4.

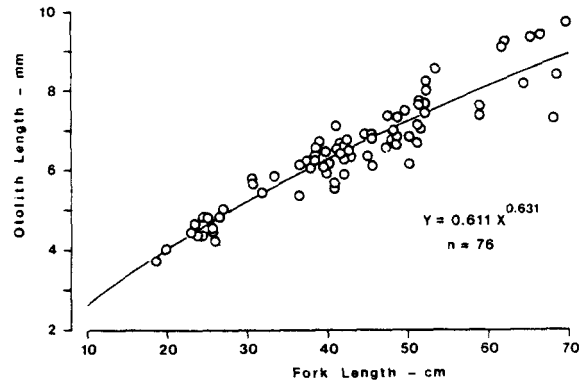


Figure 3. The relationship between otolith length (focus to most distal antirostral margin) and fork length in *P. filamentosus*.

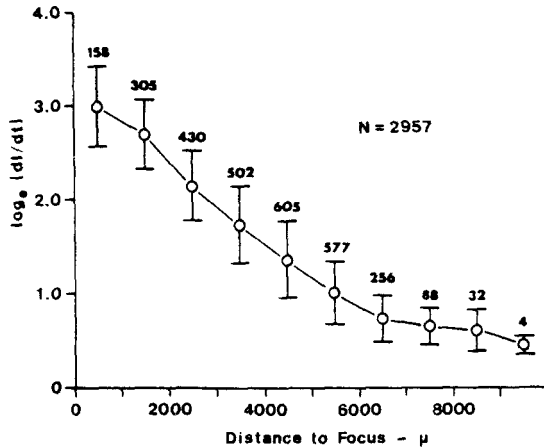


Figure 4. Pooled growth rate data for all 81 sample specimens. Circles are means bracketed by standard deviations. Numbers above are sample sizes for each length class.

It is apparent that after the otolith reaches 6,000 microns in length, an alteration occurs in the rate at which growth rate declines, maintaining a higher rate than would otherwise be expected. *Pristipomoides filamentosus* reaches reproductive maturity at about 38 cm FL (unpublished data), showing a close correspondence to this particular otolith length (see Fig. 3). These two observations are

counterintuitive to one another and together they suggest that growth interruptions may commence in this species at sexual maturity. If true, an incomplete otolith time record would result, tending to lead to an overestimation of growth rates. This hypothesis may explain the higher than expected growth rates of large fish as illustrated in the figure. Consequently, all data gathered at otolith lengths in excess of 6,000 microns were treated as equivocal and excluded from all remaining analyses. This reduced the total number of density observations from 2,957 to 2,585. Separate model regressions were then performed for each of the 81 sample specimens using the restricted date set. Age estimates were computed according to equation 4.

One means of evaluating the reliability of the age estimates obtained is to examine the frequency distribution of the coefficients of variation. Of the 81 samples analyzed, 14 had coefficients of variation greater than 10%. Figure 5 shows that the median value of this distribution is 6%. Thus, a typical preparation provides an age estimate for which the 95% confidence interval of age is $\pm 12\%$ of the estimate. It follows that for individuals up to 4.2 years of age it is possible, on the average, to assign to them an age in years with 95% confidence. Estimates from older specimens become less precise. The certainty with which age can be assigned to the nearest year drops off to 75% for individuals between 7 and 8 years old.

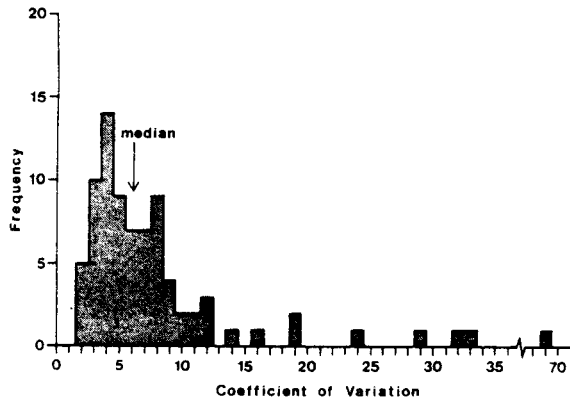


Figure 5. Frequency distribution of coefficients of variation (standard error of age estimate/age estimate $\times 100$) from the regression model ($n = 81$).

The ability of the regression model presented in equation 2 to explain variation in otolith growth rate ($\ln \frac{d\ell}{dt}$) is summarized in Figure 6. The median

value of the frequency distribution of coefficients of determination (r^2) obtained in this study is

about 77%. Hence, the regression approach utilized here appears reasonably effective in explaining variation in growth rate and ultimately in predicting growth patterns.

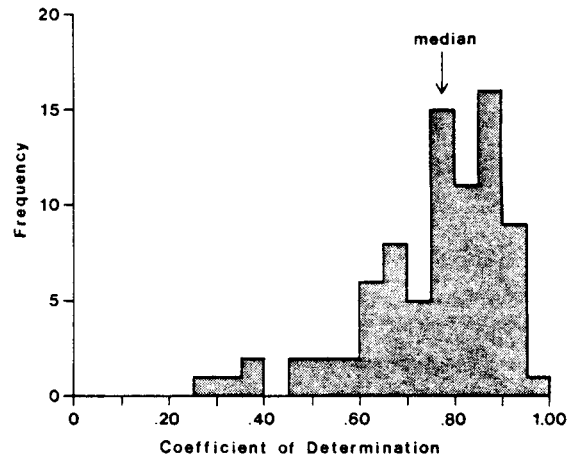


Figure 6. Frequency distribution of coefficients of determination (r^2) from the regression model ($n = 81$).

The results presented in Table 1 summarize the estimates of the parameters α and β , as well as the sample sizes of the 81 regressions. Variation in the two regression parameters is attributable in large part to variation in growth among individuals of *P. filamentosus* within the population. While some of the variability is due to the estimation procedure itself (approximately 19% for α and 22% for β), most is caused by natural variation in growth among individuals. The average sample size of the 81 regressions was 31.9. In other words, approximately 32 separate density estimations were used in each otolith examined after the equivocal data were deleted.

When the fork lengths of the sample specimens are plotted against their estimated ages (Fig. 7) the general pattern of growth in *P. filamentosus* emerges. Only 57 points are presented in the figure because estimations whose coefficients of variation exceeded 10% were omitted. Furthermore, several of the points are based on the average of two determinations taken from the left and right sagittae of the same fish. Thus, the points presented in this figure represent all of the fish (as opposed to otoliths) for which accurate estimations were obtained.

DISCUSSION

Several assumptions, both explicit and implicit, are made when the model (equation 3) is applied to

Table 1. Summary of regression parameter estimates and sample sizes from the increment density model.

	Mean	Standard Deviation	n
α parameter	3.16	0.465	81
β parameter	0.00040	0.00011	81
Sample size of regressions	31.9	12.9	81

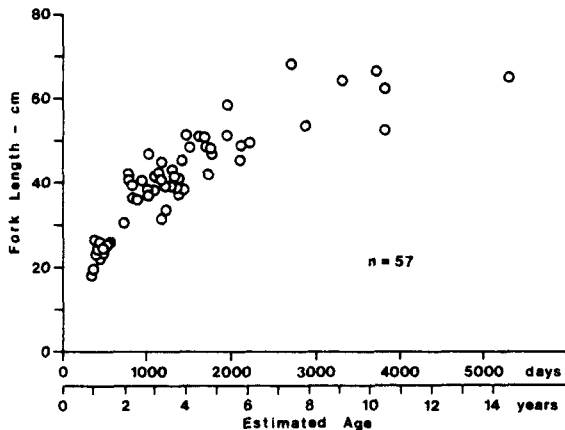


Figure 7. The relationship between estimated age and fork length in *P. filamentosus*.

increment density data taken from otoliths. One is that it is appropriate to use the interpolative power of the regression equation to predict growth rates in intermediate regions where microstructure is visually ambiguous. This assumption implies that similar processes are occurring in regions of the otolith that appear different. It is not an unreasonable assumption, however, because one can enhance the quality of a sample simply by increasing the amount of time and care devoted to a preparation, bringing out distinct increments in regions which otherwise would remain uninterpretable. Presumably, the visual quality of a preparation is largely limited by the ability of the investigator to unveil its contents.

A second and more important assumption is that it is reasonable to extrapolate the growth of reproductive individuals based on the pattern of growth preceding gonad maturation. Because all data gathered at otolith lengths in excess of 6,000 microns were deleted, age estimates obtained from the model (equation 3) are bound by this constraint. Based on the information provided by Figure 4 it is probable that otolith increments become useless as chronometers past maturity due to the existence of growth interruptions. Both Pannella (1971) and Wild and Foreman (1980) reached similar conclu-

sions in their studies of *Urophycis chuss* and *Katsuwonus pelamis*, respectively. Clearly, if the additional energy burden incurred at maturity is substantial, extrapolation beyond 6,000 microns may be an unrealistic assumption and the growth rates of large fish may be overestimated. In this regard, it is worth noting, however, that the proportion of ovarian tissue to total body weight in *P. filamentosus* is not great, ranging from 1 to 4% among Stage III spawning females (unpublished data). Similarly, most of the accepted models of fish growth (von Bertalanffy, Gompertz, etc.) do not treat maturity as a growth singularity, i.e., a time at which the pattern of growth changes abruptly. Nevertheless, this is a critical assumption and future research in this area is planned.

A final assumption is that one increment takes 1 day to form. Other evidence, to be reported on elsewhere, has been gathered which strongly supports the conclusion that increment periodicity is daily in *P. filamentosus*, at least for individuals less than 40 cm FL (see above). Marking of otoliths in vivo by tetracycline injection, shifts in field sampled size-frequency distributions, and comparisons with the growth rates of other snapper species using an "auximetric grid" (Pauly 1980), all indicate that this assumption is realistic.

Given the above assumptions, the model appears to be effective in describing patterns of growth. The frequency distribution of coefficients of determination (Fig. 5) shows that a large percentage of the variation in growth rate within individuals (77%) is accounted for by this simple approach. Furthermore, estimates of age appear sufficiently precise (coefficient of variation $\approx 6\%$) to assign ages to relatively old individuals. It is important to note that in the expansion of equation 4 about the expected values of the parameters α and β , the higher order terms, do tend to zero as sample size increases. Thus, in this case, the Delta Method does provide a formulation for the asymptotic variance of the age estimates.

The actual model of growth fitted to the data (equation 2) deviates somewhat from the standard von Bertalanffy growth curve (Fabens 1965, Ricker

1975). The latter model assumes $\frac{dL}{dt}$ to be a decreasing, linear function of length, rather than $\propto n \frac{dL}{dt}$ as presented here. The outcome of the pre-

sent method of curve fitting is to postulate that no upper bound on size exists. Given an infinite amount of time to grow, based on the empirical evidence, *P. filamentosus* should approach infinite length and not an asymptotic L_{∞} . Other authors have reached similar conclusions with other species

of fish (see for example Parker and Larkin 1959, Paloheimo and Dickie 1965).

Acquisition of age and growth data for tropical species of fish is of great practical importance. Pauly (1980) has shown that it is possible to estimate the rate of natural mortality given estimates of the basic growth parameters. In addition, an age-length key can be generated using the results presented in Figure 7 (manuscript in prep.). Once mortality rates have been calculated it is possible to undertake an analysis of growth overfishing (Beverton and Holt 1957) and rational decisions concerning the management of exploited stocks of tropical fishes can be made. At present this type of approach is uncommonly applied to tropical stocks due to the difficulty in attaining age and growth estimates.

Many tropical species have never been aged because of uniform growth and related difficulties. The method presented here may help overcome some of these problems, if the previously discussed assumptions can be satisfied. Most important is to verify the periodicity of the increments the investigator is studying and to assess the likelihood of growth interruptions. While this field of research is becoming reasonably well established, it is premature for researchers to rely on the literature to validate their own interpretations. Much more experimental work is needed first.

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