

NOTES

Frequency of Increment Formation on Sagittae of North Pacific Albacore (*Thunnus alalunga*)

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An examination of sagittae from 116 albacore (*Thunnus alalunga*) caught in the North Pacific, injected with tetracycline, tagged, released, and subsequently recaptured in sport and commercial fisheries showed that detectable increments are formed on these otoliths at an average rate of 0.954 per day. We take this as a confirmation of daily increment formation in North Pacific albacore sagittae. The slight departure of observed mean increment counts from the expected rate of one per day may be due to an occasional interruption of otolith growth, or to a systematic bias in detecting daily increments or interpreting otolith microstructure. The estimated rate of detectable increment formation applies explicitly to albacore of fork lengths between about 50 and 100 cm. If the same rate holds for fish smaller than 50 cm, as is likely, most albacore taken in sport or commercial catches can be aged accurately by applying our methods and expanding the increment count by 5%.

Un examen des sagittae recueillies chez 116 germons (*Thunnus alalunga*) capturés dans le Pacifique nord — qui ont reçu une injection de tétracycline, puis ont été étiquetés, relâchés et recapturés au cours de pêches sportives et commerciales — a révélé que des zones de croissance discernables se forment sur ces otolithes à un taux moyen quotidien de 0,954. Les auteurs considèrent ce taux comme une confirmation de la formation d'une zone de croissance par jour dans les sagittae du germon du Pacifique nord. Le faible écart entre les dénombrements moyens observés de zones de croissance et le taux escompté, soit une zone par jour, peut être dû à un arrêt occasionnel de la croissance des otolithes ou à un biais systématique dans le dénombrement des zones de croissance quotidienne ou l'interprétation de la microstructure otolithique. Le taux estimatif de formation de zones de croissance discernables ne s'adresse qu'aux germons dont la longueur à la fourche varie d'environ 50 à 100 cm. Si le même taux s'applique, comme il est probable, aux poissons de taille inférieure à 50 cm, on pourra déterminer l'âge précis de la plupart des germons capturés par les pêcheurs sportifs et commerciaux à l'aide des méthodes présentées en ajoutant 5 % aux dénombrements des zones de croissance.

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In the decade since Pannella's pioneering studies of otolith microstructure (Pannella 1971, 1974), the validation and counting of daily growth increments on otoliths have enabled precise age determination in several species of teleosts, and more accurate modeling of life histories and the biological phenomena shaping them. In species with well-defined increments, it is now possible to study growth and other processes in considerable temporal detail. Under ideal circumstances one can estimate age-specific vital rates, and associate changes in these with coincident, small-scale perturbations of environmental factors or other influential variables. However, use of the otolith techniques must be preceded by rigorous testing of basic assumptions about increment identification and

interpretation. In particular, as emphasized recently by Beamish and McFarlane (1983), the true frequency of increment formation must be established.

The otolith daily increment approach has shown particular promise for aging of tunas. Other methods based on scales, spines, or vertebrae have often produced inconclusive results (Shomura 1966). Daily formation of increments on tuna sagittae was first postulated for two tropical species, yellowfin tuna (*Thunnus albacares*) by Wild and Foreman (1980) and skipjack tuna (*Katsuwonus pelamis*) by Uchiyama and Struhsaker (1981). In experiments to estimate rates of increment deposition, Wild and Foreman confirmed the daily formation of increments in yellowfin tuna between 40 and 110 cm fork length

(FL), but rejected the hypothesis for skipjack tuna of 42–64 cm FL. Radtke (1983) demonstrated daily formation of increments on sagittae of skipjack larvae hatched and maintained for 5 d in captivity. He concluded that the first daily increment in skipjack is formed 1 d after hatching. Brothers et al. (1983) studied otolith microstructure in larvae of the Atlantic bluefin tuna (*T. thynnus*). They hypothesized that increments are deposited daily on bluefin sagittae, but were unable to pinpoint the age that the first increment appears. They found two or three diffuse increments which were presumably deposited prior to the onset of exogenous feeding, suggesting that the first increment is laid down at hatching or even earlier.

Our aim was to estimate the rate of increment deposition on sagittae of North Pacific albacore (*T. alalunga*), a temperate-water migratory tuna, with the ultimate objective of developing an accurate aging method. We based our study on a large sample of experimental fish growing in the wild over an extended period of time. These tuna may range over 40° of latitude between subtropical waters and those of the subarctic and undertake migrations as long as 6000 km in the span of a few months. In other species, similar investigations have usually been with a relatively small number of captive fish under controlled laboratory conditions (e.g. Campana and Neilson 1982; Neilson and Geen 1982; Volk et al. 1984). Only a few studies have been carried out in natural settings (e.g. the mark-and-recapture experiments by Wild and Foreman (1980) and Victor (1982)).

Experimental Methods

The increment deposition rate was estimated, in the manner of Wild and Foreman (1980), by counting increments on sagittae of albacore which had been caught at sea, injected with tetracycline, tagged, released, and later recaptured by fishermen, and then comparing the counts with the known periods of liberty.

In 1977 and 1978 albacore were caught in the eastern North Pacific, primarily by pole-and-line fishing, aboard the commercial fishing vessel *Linda*, chartered by the American Fishermen's Research Foundation.¹ Of those fish landed in good condition, 2544 were measured, injected intramuscularly with a 1.5-mL titre of 100 mg/mL oxytetracycline hydrochloride solution, tagged with a Floy² FT-1 "spaghetti" dart, and returned to the sea. The tetracycline, a calciphilic fluorochrome compound, was incorporated rapidly into the growing periphery of each sagitta, creating a mark on the developing otolith increment which could later be detected by its fluorescence under ultraviolet light (Milch et al. 1957; Weber and Ridgway 1962, 1967). By the end of 1982, 182 of the tagged albacore had been recaptured, and 125 returned with intact otoliths. The sagittae were dissected from these specimens, cleaned in household bleach, rinsed in distilled water, dried, and stored in darkness.

Without further preparation, the otoliths were mounted distal-side up in Plasticene, placed on a culture microslide, and viewed through a compound microscope at magnifications of 480 and 1250× under a 200-W mercury burner ultraviolet lamp. Immersion oil ($n_d 23^\circ\text{C} = 1.515$) was used to improve transparency and resolution. The excitation wavelength was restricted by

¹The American Fishermen's Research Foundation administers revenues derived from a landing assessment paid by the U.S. albacore industry on U.S.-caught albacore.

²Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

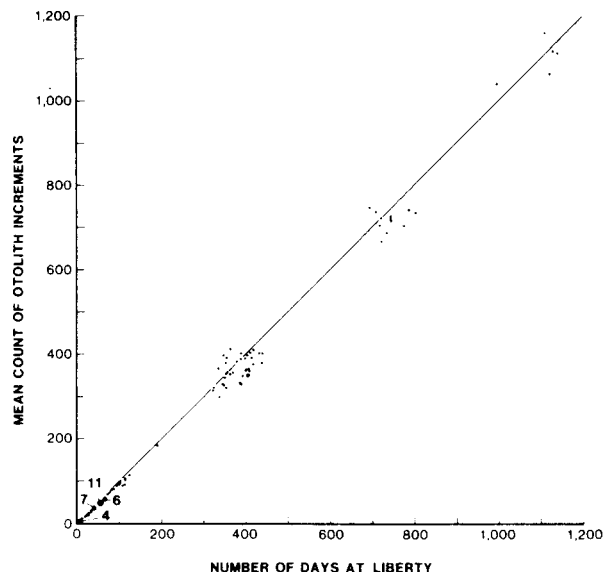


FIG. 1. Observed relationship between mean increment count on sagittae of albacore and days at liberty (points) after injection with tetracycline, and expected relationship under hypothesis of daily increment formation (line). Numerals indicate multiple observations at certain points.

BG-12, B(50-nm), and U(400-nm) filters. A 530-nm barrier filter reduced autofluorescence.

Counts of increments were made by R.N. on a single otolith selected randomly from each pair of sagittae. When the selected otolith was damaged or otherwise unusable, the remaining one was read. Earlier experiments showed there was no difference between average increment counts on left and right sagittae. Increments were counted along the major axis of the post-rostrum, where definition of increments was best, from the proximal edge of the fluorescent mark to the margin of the otolith. The counting path was usually not linear. Rather, it varied with otolith topography and was selected to maximize the clarity of apparent growth structures. On thick otoliths, or on otoliths in which the growing surface had obscured (or had been obscured by) increments deposited earlier, frequent refocusing was necessary.

The fluorescing tetracycline marks were unmistakable in 116 of the 125 sagittae examined, and were nearly always confined to a single increment. Absence of a fluorescent mark could be attributed to procedural errors. In one batch of fish, the tetracycline solution used was outdated and improperly handled. In another, incorrect injection technique led to extrusion of the titre.

On sagittae from most smaller albacore or those recaptured within approximately a year of release, eight independent counts were made; otherwise four replicate counts were taken. Replicate increment counts and the exact time at liberty were known for 116 albacore. The number of days at liberty within the sample ranged from 7 to 1142. The smallest fish was 51 cm FL at release, the largest 97 cm FL at recapture.

Analysis and Discussion

Our analysis of increment counts showed that increment formation on sagittae of North Pacific albacore is almost

certainly a daily event; an average of about 0.95 increments were counted for every day at liberty. The slight departure from a 1:1 relationship could be due to systematic undercounting of indistinct increments, or to periodic interruption of increment deposition.

Assuming a constant average rate of increment formation, we expected to find a linear relationship between mean increment count and days at liberty. Further, we supposed that both additive and multiplicative systematic counting errors could occur, as well as random errors proportional to the expected count. Preliminary statistical tests showed that the additive bias component was insignificant. Apparently there was no significant lag between injection and appearance of the tetracycline mark, and no other significant additive error (e.g. consistently misjudging the position of the tetracycline mark, or undercounting peripheral increments at a rate independent of the total number of increments present). An appropriate statistical model of the observed mean increment count for the i th fish was therefore established as

$$y_i = \alpha x_i \{1 + \beta + \epsilon_i\}$$

where

y_i = mean increment count

x_i = days at liberty

α = true increment deposition rate, assumed constant

β = proportional counting bias, assumed constant

ϵ_i = proportional counting error, with mean 0 and variance σ^2/m_i

m_i = number of replicate counts on the i th fish.

When constants are combined, the model reduces to a zero-intercept linear regression

$$y_i = \theta x_i + \epsilon'_i$$

where

$$\theta = \alpha(1 + \beta) \quad \text{and} \quad \epsilon'_i = \alpha x_i \epsilon_i.$$

Note that α and β are confounded; systematic undercounting will therefore introduce a negative bias into estimates of the deposition rate. The theoretical variance of the mean count is

$$V(y_i) = (\alpha x_i)^2 \sigma^2 / m_i,$$

consistent with empirical estimates of $V(y_i)$ computed from the replicates. Assuming a normal distribution of the ϵ_i , we computed a maximum likelihood estimate of θ using an iteratively reweighted Gauss-Newton algorithm, minimizing

$$\sum_{i=1}^n w_i \{\epsilon'_i\}^2,$$

where w_i is a statistical weight proportional to $V^{-1}(y_i)$ and n is the number of fish in the sample (116).

For the entire sample the estimate of θ was $\hat{\theta} = 0.954$, with a computed standard error of $\hat{\sigma}_{\hat{\theta}} = 0.006$ (Fig. 1). The high precision in $\hat{\theta}$ reflects the low counting variability; the coefficient of variation among replicate counts was about 3%. Similar estimates of $\hat{\theta}$ were obtained when the data were partitioned by days at liberty; for albacore recaptured within 200 d of release, $\hat{\theta} = 0.941$ ($n = 51$), and for fish at large over 200 d, $\hat{\theta} = 0.969$ ($n = 55$). In each case the hypothesis that $\theta = 1$ was rejected (t -test, $P < 0.01$).

The significant departure from a 1:1 relationship could arise in at least two ways. First, increment deposition could occur with a daily rhythm, but be subject to occasional interruptions, so that the average rate of increment formation is less than 1 per day. Second, increments could be added daily, but be systematically undercounted by about 5% (i.e. assume $\beta = -0.05$). A reasonable case could be made for either undercounting or growth interruption, and indeed both factors could be involved.

We have no evidence to dismiss the possibility of systematic undercounting. However, if such a bias exists it apparently arises from difficulties in increment detection related to characteristics of the otolith or otolith preparation technique, rather than from systematic reader error. In analyses of the variation in mean increment counts recorded by different readers examining the same otoliths, between-reader differences were not significant when considered over a large number of otolith specimens. Because of the difficulties inherent in increment identification and interpretation, indistinct or atypical daily increments could have escaped detection, particularly with sagittae of the larger albacore, on which increments near the margin are usually thinner than those closer to the otolith core and detectable in whole mounts only by careful focusing. In other studies of tuna otoliths, further treatments, e.g. acid etching and acetate replication, have sometimes been applied to sagittae from larger fish in an effort to improve increment definition. However, the etching techniques carry the risk of removing increments from the otolith margin. This would have been particularly troublesome in our case, since counts were made from a mark sometimes quite near the edge.

Growth interruption might also have contributed to the slight departure of the average increment count from the expected count under the daily deposition hypothesis, but without further experiments this can only be surmised. Wild and Foreman (1980) reviewed the literature on frequency of increment formation, and noted that whereas daily formation of increments may be the rule for many species, in some fish subdaily structures are formed under certain conditions, and in some the deposition of increments is apparently interrupted due to starvation, maturation, or other factors.

The physiological basis of increment formation in sagittae is only sketchily known for a few species (Pannella 1980; Mugiya et al. 1981), and has not been studied in albacore. Experiments with a variety of species exposed to different light-dark cycles support the view that this process occurs with a daily periodicity tied to an internal clock whose pulse is entrained by diurnal cycles in various environmental factors (Taubert and Coble 1977; Tanaka et al. 1981). Some subdaily patterns of otolith growth have also been observed and irregular and periodic interruptions may occur (Pannella 1980). Studies involving diet manipulation in captive fish suggest that the regular deposition of identifiable increments may be checked by starvation, temporary chemical imbalances, or by competing nutritional or energetic needs. In some experiments, feeding schedules have been shown to affect the number of increments deposited per day (e.g. Brothers 1978; Methot and Kramer 1979; Neilson and Geen 1982). Since albacore are highly active, migratory fish (Graham and Laurs 1982), it is conceivable that in the natural environment, patchiness of food supply may produce fluctuations in growth rate, and disturb the regular deposition of increments. This view is supported by the striking patterns of variability in observed increment width, which could mirror, to some degree, changing growth conditions. In laboratory studies with other species it has been shown that food ration and prey

type affect average increment width and rate of fish growth (e.g. Taubert and Coble 1977; Volk et al. 1984).

In their experiments with tagged skipjack tuna, Wild and Foreman (1980) found that otolith growth had been interrupted, to the extent that increment counts on sagittae underestimated days at large by about 25%. However, they considered it unlikely that in skipjack tuna growth retardation of such magnitude could be due to starvation, or to energy demands of migration. Instead, judging their fish to be sexually mature, they considered maturation and reproductive activity to be likely causes of the interruption in increment deposition. We found no evidence that this was so in albacore. Although all 116 fish in our analysis were considered immature (<85 cm FL) when tagged, 11 of these were presumed mature when recaptured, the largest being 97 cm FL. For these large fish the average ratio of mean increment count to days out was 0.954, identical to our estimate of θ for the whole sample.

The calibration of increment counts on sagittae of North Pacific albacore has been accomplished. Provided otolith increments accumulate at the same rate on albacore <51 cm FL as they do on larger ones, our results may be used to convert increment counts on albacore sagittae to estimates of absolute age (in days). The conversion factor, corrected for statistical bias in θ , is

$$C = \hat{\theta}^{-1} \{1 - (\hat{\sigma}_{\hat{\theta}}/\hat{\theta})^2\} \\ = 1.05.$$

The assumption of daily increment deposition in very small fish will be difficult to test because North Pacific albacore have not been spawned in captivity and wild juveniles are rarely caught. However, the studies of otolith development in larvae of skipjack tuna by Radtke (1983), and in Atlantic bluefin tuna by Brothers et al. (1983), suggest it is reasonable to assume daily increment formation on albacore sagittae, at least from the time of yolk-sac absorption, i.e. within the first few days posthatching. Therefore, there is little doubt that counts of increments on sagittae of harvested albacore, adjusted upward by 5%, provide accurate estimates of absolute age.

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