Validation of annual growth increments in the otoliths of the lethrinid *Lethrinus mahsena* and the lutjanid *Aprion virescens* from sites in the tropical Indian Ocean, with notes on the nature of growth increments in *Pristipomoides filamentosus*

Graham M. Pilling

MRAG Ltd.
47 Prince's Gate
London SW7 2QA, United Kingdom
E-mail address: g.pilling@ic.ac.uk

Richard S. Millner
Michael W. Easey

Fisheries Laboratory
CEFAS
Pakefield Road
Lowestoft NR33OHT, United Kingdom

Christopher C. Mees

MRAG Ltd.
47 Prince's Gate
London SW7 2QA, United Kingdom

Shyama Rathacharen

Albion Fisheries Research Centre
Albion, Petite Riviere, Mauritius

Roland Azemia

Seychelles Fishing Authority
P.O. Box 449
Victoria, Mahe, Seychelles

Abstract.—The lutjanids *Pristipomoides filamentosus* and *Aprion virescens* and the lethrinid *Lethrinus mahsena* are commercially important demersal bank and deep slope reef fish from the central Indian Ocean. To obtain von Bertalanffy growth parameter estimates for management purposes, length-based methods are commonly applied by the fisheries institutions of the region. Because the relatively long-lived, slow-growing nature of these species results in a lack of distinct modal progression in length-frequency data, such estimates are unreliable. In an attempt to obtain more reliable growth estimates, the feasibility of age-based methods (where age is determined from annual increments in otoliths) was investigated. Successful validation of annual or daily increments has been reported in two of these species (*P. filamentosus* and *A. virescens*), but not for the target areas of our study: the banks of the Seychelles and Mauritius.

A range of methods was used in an attempt to ensure that the otoliths fulfilled the criteria for use in aging. Two methods are described in this paper: back-calculation and a combination of marginal increment and edge analysis. The results of validation are presented, along with a description of the problems encountered. Marginal increment and edge analysis both indicated that the increments present in the otoliths of *L. mahsena* are annuli. For *A. virescens*, no pattern was present in the marginal increment analysis of older individuals. However, edge analysis offered evidence that the increments present in the otoliths were annuli. The combined marginal increment and edge analysis proved inconclusive for *P. filamentosus*; therefore the increments present in the otoliths of this species could not be validated. Conclusions are drawn regarding the justification of assuming periodicity of increments on the basis of validation achieved in other locations.

Manuscript accepted 4 January 2000.
Fish. Bull. 98:600–611 (2000).

Examination of hard body parts, such as otoliths, frequently reveals the presence of "rings." Laid down incrementally (whether daily, monthly, or annually), these structures provide a means of aging fish. For tropical species, however, the use of otoliths has become relatively common only since the early nineteen eighties (e.g. Loubens, 1978; Morales-Nin and Ralston, 1990; Ferreira and Russ, 1992; Francis et al., 1992; Milton et al., 1995; see also Manooch, 1987, and Fowler, 1995, for reviews). The lack of studies before this time is frequently attributed to the expectation that tropical fish grow consistently in an aseasonal environment. Hence hard parts in tropical fish species were not expected to contain internal structures that relate to fluctuations in growth resulting from the environment.

The physiological basis for the formation of distinct zones in calcified structures of tropical species has not been established conclusively (Ferreira and Russ, 1994). The formation of such zones has been associated with both abiotic and biotic factors (Beckman and Wilson, 1995), such as regular seasonal variances in water temperature (e.g. Reay, 1972; Panella, 1980), photoperiod, feeding, reproduction, and spawning period (e.g. Morales-Nin and Ralston, 1990)

"Indeterminate increments" (increments not related to the annual time scale) are found in all hard parts. These need to be distinguished from increments formed at regular intervals (Fowler and Doherty, 1992). Before increments can realistically be used for aging, they need to be "validated" (Beamish and McFarlane, 1983) to ensure that the structures, such as the otoliths used in our study, can be used as accurate chronometers. They must therefore fulfill a number of criteria (Fowler and Doherty, 1992): 1) the otolith must grow throughout the lifespan of the individual fish; 2) the otolith must show an internal incremental structure; and 3) this structure must correspond to a regular time scale.

Our study concentrated on macro-increments. Aging through micro-(daily)increment counts (e.g. Ralston and Miyamoto, 1983) requires considerable investment in time and equipment and is generally constrained to younger ages, where daily increments remain distinct. These contraints render such techniques unfeasible for stock assessment purposes, particularly in developing countries.

The local fisheries institutions in the study locations of the central Indian Ocean are, to date, limited to the use of length-based methods for aging fish. The results of these methods are felt to be uncertain when applied to long-lived, slow-growing species such as snappers and emperors, owing to modal overlap and a general absence of modal progression (e.g. Langi, 1990). Our study aimed to validate as annual the macro-increments seen in the sagittal otoliths of two species of snapper, *Aprion virescens* (Valenciennes 1830) and *Pristipomoides filamentosus* (Valenciennes 1830), and one species of emperor, *Lethrinus mahsena* (Forsskål, 1775). Validation of the annual nature of increments seen in the otoliths of these species would allow age-based methods of stock assessment to be applied to these commercially important demersal bank and deep-slope reef-fish species from the central Indian Ocean.

Compliance with the three criteria for validation was examined by using two methods: back-calculation (e.g. Manooch, 1987; Van Leewen and Rijnsdorp[1]) and a combination of marginal increment and edge analysis (e.g. Mason and Manooch, 1985; Manooch and Drennon, 1987; Manickchand-Heileman and Philipp, 1996). Back-calculation was used to test the validity of the first two criteria for aging. Marginal increment analysis and edge analysis were then used to validate mark periodicity, fulfilling the third criterion.

Materials and methods

Otoliths of the study species were obtained from commercial fish catches. In waters off both Mauritius and the Seychelles, fishing is frequently limited by rough seas during the SE trade wind period from May to October. Samples of *Lethrinus mahsena* were obtained during excursions of the Mauritian mothership-dory vessels on Nazareth Bank of the Mascarene Ridge (15°S, 61°E). On the Mauritian banks, this species composes about 80–90% of the total catch (Ardil, 1986; Bertrand[2]). In the Seychelles, fishing occurs from smaller vessels, which generally remain on the Mahé Plateau (05°S, 55°E). Otoliths from both *P. filamentosus* and *A. virescens* were obtained from this area.

Two methods were used to examine whether the otoliths fulfilled the requirements for aging: 1) back-calculation of growth from otolith increments; and 2) marginal increment and edge analysis. A general description of these methods is provided in Blacker (1974) and Williams and Bedford (1974).

Sagittal otoliths were selected for examination because a number of studies have used these structures to age snapper and emperor species successfully elsewhere (e.g. Manooch, 1982; Mason and Manooch, 1985; Morales-Nin, 1989; McPherson and Squire, 1992; Newman et al., 1996), including *Aprion virescens* and *Pristipomoides filamentosus* (Loubens, 1980; Ralston and Miyamoto, 1981, 1983).

One otolith from each fish was embedded in black polyester resin, and a 0.5-mm transverse section taken through the center ("nucleus") of the otolith by using a diamond cutting blade (Bedford, 1983). Sections were then either mounted in clear resin on a microscope slide or stained with acidified neutral red (Richter and McDermot, 1990) and left unmounted. Otoliths were examined with a Zeiss compound microscope equipped with zoom lens and magnification up to 60×. Reflected or transmitted light was used as necessary to identify and count the increments.

In our study, terminology was based on that recommended in Secor et al. (1995). The term "opaque zone" refers to the area that appeared milky under reflected light or dark under transmitted light in unstained samples.

Back-calculation of growth by means of otolith increments

For back-calculation, snapper otoliths were most easily assessed unstained, whereas *L. mahsena* otoliths were most easily assessed stained. Only otoliths that showed clear increment patterns were used for measurements; as a result, seven *A. virescens* otoliths, in which it was difficult to distinguish the first or second increment, were omitted from the samples. Measurements of the total otolith radius, from the nucleus to outside edge, and the distance along the selected growth axis between suspected annual increments were made with the tools described in Millner and Whiting (1996). Unstained *P. filamentosus* and *A. virescens* otoliths were assessed along a growth axis close to the sulcus acousticus (Figs. 1 and 2). This area provides the most consistent and easily interpretable

---

[1] Van Leeuwen, P. I., and A. D. Rijnsdorp. 1986. The analysis of the growth of turbot by back-calculation of otoliths. ICES Council Meeting 1986/G:50

[2] Bertrand, J. 1986. Pour une evaluation des resources en capitaine/dame berri (*Lethrinus mahsena*) des bancs de Saya de Malha. *In* Rapport du Groupe Travaile, French Institute of Research and Exploitation of the Sea (IFREMER)/Albion Fisheries Research Centre (Mauritius). 23–25 Juillet 1985, 39 p. [Available from Albion Fisheries Research Centre, Albion, Petite Riviere, Mauritius.]

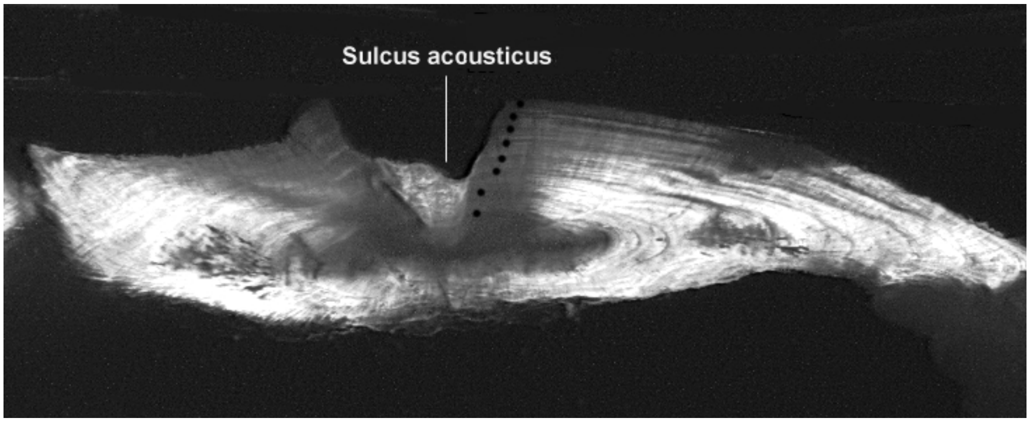

Figure 1

Unstained transverse 0.5-mm section through the sagittal otolith of *P. filamentosus* from Seychelles, viewed with transmitted light. Magnification: 16×. Black dots along sulcus acousticus mark are suspected annual increments.

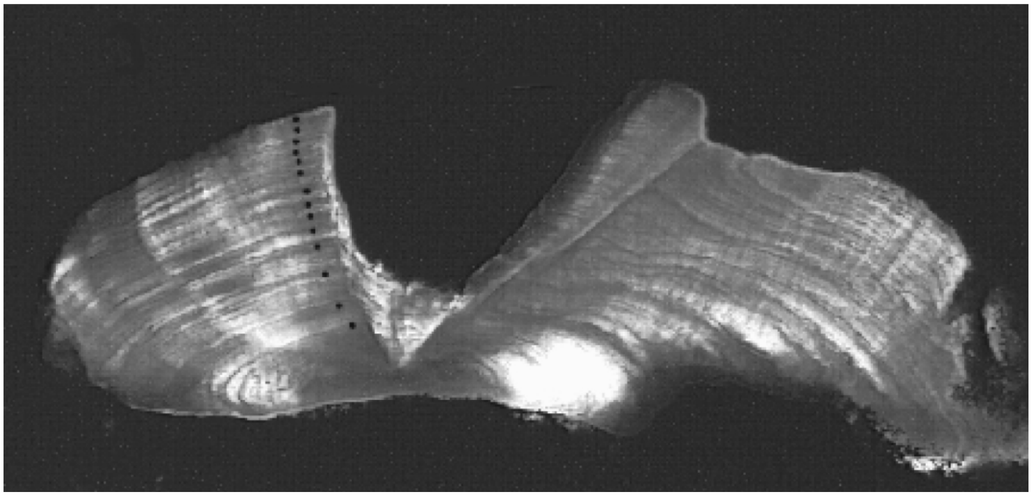

Figure 2

Unstained transverse 0.5-mm section through the sagittal otolith of *A. virescens* from Seychelles, viewed with transmitted light. Magnification: 32×. Black dots along sulcus acousticus mark are suspected annual increments.

incremental pattern. Measurements on stained *L. mahsena* otoliths were performed out to the tip of the dorsal lobe, at approximately 90° to the *sulcus* (Fig. 3). In all species, when the increment structure became too closely spaced to measure individual increments accurately, the thickness of further increments was estimated by interpolation based on the remaining distance from the last measured increment to the outside edge of the otolith, the number of years expected in this zone, and the pattern found in the previous increment widths.

Following the recommendations of Francis (1990) and Ricker (1992), we calculated the geometric mean regression (GMR) of fork length at capture from otolith radius at capture for each species. Back-calculated lengths-at-age for each individual were then derived with the following formula (Ricker, 1992):

$$FL_i = \left(\frac{FL_c - b}{O_c} \times O_i \right) + b,$$

where Fl_i = the estimated fork length at age i;
Fl_c = the fork length at capture;
O_c = the otolith radius at capture;

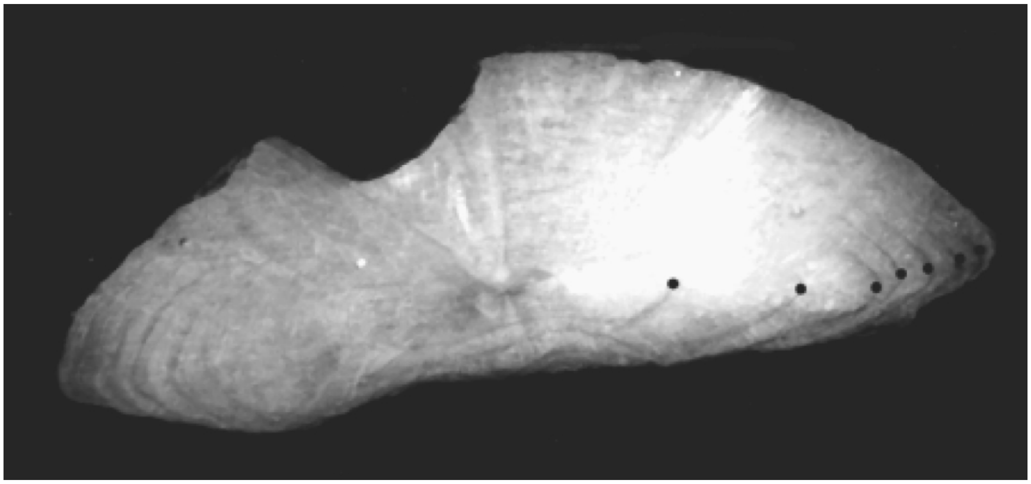

Figure 3

Transverse 0.5-mm section through the sagittal otolith of *L. mahsena* from Mauritius (Nazareth bank), stained with acidified neutral red (see text), and viewed with reflected light. Magnification: 23×. Black dots mark are suspected annual increments.

O_i = the otolith radius at age i; and
b = the intercept of the GMR calculated with the Ricker procedure.

For each species, a von Bertalanffy growth curve was fitted to the back-calculated mean length at age through least squares estimation.

Marginal increment analysis

The thickness of the outer zone in the otoliths was measured by using the image analysis system (see Millner and Whiting, 1996). Measurements were taken from the inside edge (start) of the last opaque band and the edge of the otolith. In stained *L. mahsena* otoliths (indeed in all three study species where otoliths were stained; see "Edge analysis," below), a band was stained on the border between the end of the "translucent zone," and the start of the next opaque zone. Measurements from the last stained band were therefore equivalent to those made for the snapper species. Because zones at the edge of sections taken from the unstained otoliths of both snapper species were not distinct, an average of four measurements was taken in the region of the sulcus (where zones were more easily distinguished). Zones at the edge of the stained *L. mahsena* otolith sections were clearly visible and, therefore, it was possible to obtain a single measurement along the same growth axis as that used for back-calculation.

The pattern expected in the marginal increment resulting from the development of an annual increment would be a minimal marginal increment at the start of the growth period, increasing with time until the measurement fell to a minimum again at the formation of the next period of growth.

The size of the growth zone varies both with time of sampling during the year and the age of the fish. Because younger fish grow faster than older individuals, a larger marginal increment is expected. For this reason, quantitative marginal increment analyses should be standardized for age. Our study was therefore carried out by age class. Owing to the wide range of ages encountered, however, there were insufficient samples to accomplish this standardization fully. It was necessary to combine the ages into two or three groups representing fast, moderate, and slow-growing individuals (Table 1). These classes were based on the growth curves derived from back-calculation, and hence on the assumption that the increments represented annual growth.

For *P. filamentosus*, because sample sizes were either small or because samples could not be obtained in specific months, data were grouped into bimonthly time periods. Sufficient *L. mahsena* and *A. virescens* samples were available to allow the analysis to occur on a monthly basis. For all three species, a mean marginal increment for each time period and growth category was calculated.

Edge analysis

The type of zone at the growing edge of the otolith was identified in each month of the year. Annuli were traceable as a developing single translucent and opaque zone on the edge of the otolith during that growth year.

Problems were encountered in identifying the edge type in unstained *P. filamentosus* and *A. virescens* from Seychelles because of poor resolution of the banding in otoliths, and what appeared to have been the seepage of resin either under or through the edge of the otolith. Staining trials were performed in an attempt to clarify the growth type at the edge of the otolith in these species, and stained otoliths were subsequently examined during edge analysis.

Thin sections of the stained otoliths were assessed for the presence of the band of stain on the growing tip of the dorsal lobe. The percentage of otoliths with a stained band at the growing tip was calculated for each month. Although an extended data set was available for edge analysis, as a result of the seasonal nature of the Seychelles and Mauritius fisheries, sample sizes were either small or not available for certain months.

Results

Based on the assumption that the increments seen in the otoliths were annual, the length and age range of individuals from each species examined in each method is presented in Table 2. The sample size is also shown.

A greater number of samples were examined during edge analysis. This method was less intensive than that required for either back-calculation or marginal increment analysis, allowing more samples to be examined during the period of study and increased the range of lengths and ages examined.

Table 1

Age groups selected for marginal increment analysis, by species.

Species	Growth group	Age class (years)
L. mahsena	Fast	1–5
	Slow	6+
A. virescens	Fast	3–5
	Slow	6+
P. filamentosus	Fast	3–5
	Moderate	6–10
	Slow	11+

Back-calculation

A linear relationship existed between the fork length and otolith radius of fish in all three species, to which the geometric mean regression (GMR) of fork length from otolith radius was fitted (Figs. 4–6).

The von Bertalanffy growth parameters derived from mean back-calculated length-at-age data from *A. virescens*, *P. filamentosus* and *L. mahsena* are shown in Table 3. Initial L_∞ estimates for *L. mahsena* were low compared with the maximum lengths seen in length-frequency distributions from the region (52 cm). The asymptotic length, L_∞, was therefore constrained to levels in keeping with the maximum length found in the distributions, and the remaining von Bertalanffy growth parameters were re-estimated.

For the snapper species, both L_∞ and K estimates derived from the back-calculated length-at-age data were lower than those historical estimates derived with length-based methods. For *L. mahsena*, constraining L_∞ to a level in keeping with the maximum length found in catches resulted in estimates of K comparable to those estimated by Bautil and Samboo (1988).

Marginal increment and edge analysis

The number of individuals examined in marginal increment analysis are presented in Table 4 by species and time period.

Because the region between translucent and opaque zones took up the stain, the presence of a stained area at the otolith edge during edge analysis indicated the start of opaque band formation.

Table 2

The length range, age range, and sample size of individuals examined by method and species. Ages are based on the assumption that the increments identified in the otoliths are annuli.

	Method		
Species	Back-calculation	Marginal increment analysis	Edge analysis
L. mahensa			
Lenth range (cm)	22–42	22–42	20–50
Age range (yr)	3–14	3–14	3–16
n	222	220	568
A. virescens			
Lenth range (cm)	37–87	37–87	30–99
Age range (yr)	3–19	3–19	3–27
n	81	141	1259
P. filamentosus			
Lenth range (cm)	22–73	22–73	—
Age range (yr)	3–30	3–30	—
n	85	242	—

L. mahsena Marginal increment analysis indicated an overall increase in the marginal increment between January and May, falling in August (Fig. 7). This pattern was clearest in the younger, faster-growing growth group. A unimodal distribution was found in the plot of the proportion of stained edges found in each month, as well as a high proportion of individuals showing opaque growth commencing between May and August (Fig. 8).

A. virescens With relatively few samples at certain key times during the year, patterns in the marginal increment could not be identified clearly (Table 4). The width of the outer zone increased until June in younger, faster-growing individuals. In subsequent months, a lack of samples prevented the identification of an indicative pattern (Fig. 9). No pattern was found in the marginal increment of older individuals. Staining improved the clarity of the type of growth present on the edge of the otolith. Edge analysis of these specimens showed a unimodal distribution of the percentage of stained edges by month. A high proportion of individuals exhibited opaque zone formation in the period between October and March (Fig. 10).

P. filamentosus No overall pattern of increase in the marginal increment was found (Fig. 11). This result was partly due to the lack of samples in the months of May and June. The legibility of the increments in the otoliths of this species was poor, and staining failed to improve the clarity of the otolith zones. Edge analysis was confused by indeterminate increments formed in the translucent growth period. Their presence on the edge of the stained otolith at the time of capture led to the misidentification of opaque zone formation and thus confused the identification of an overall pattern. As a result, validation could not be achieved with this method.

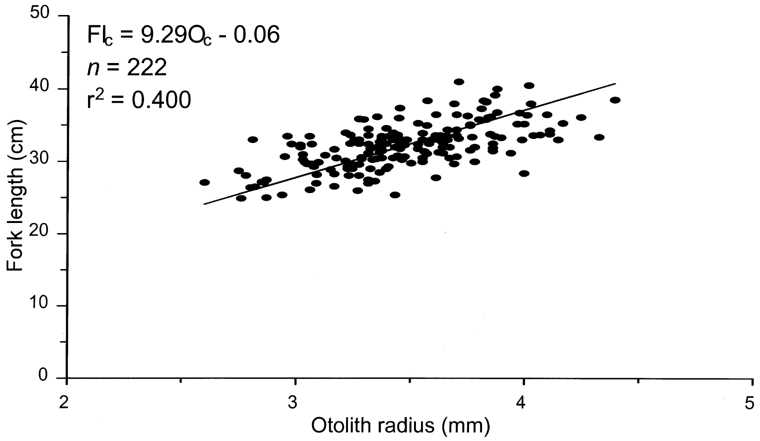

Figure 4

Geometric mean regression of fork length (cm) from otolith radius (mm) for *L. mahsena.*

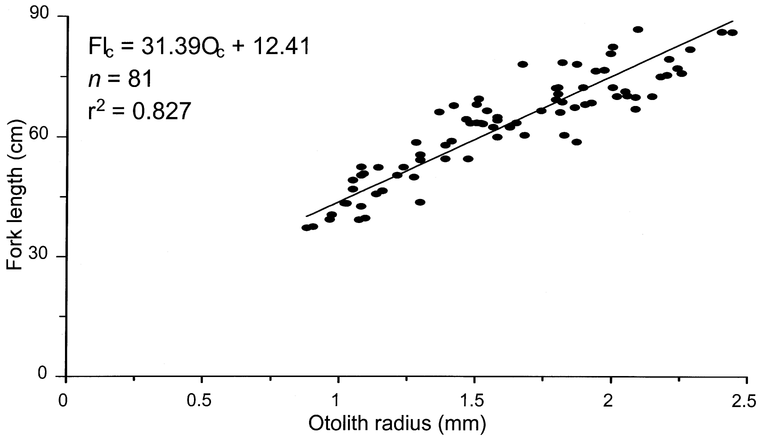

Figure 5

Geometric mean regression of fork length (cm) from otolith radius (mm) for *A. virescens.*

Discussion

Back-calculation indicated that there was a direct linear relationship between fork length and otolith radius. The otoliths of each species showed an internal structure, with increments widely spaced near the nucleus and narrower and more evenly spaced toward the edge. Growth curves derived from back-calculated length-at-age data indicated an exponential decrease in increment width with assumed increasing age. The otoliths of the three species therefore fulfilled the first two criteria for their use in aging, at least over the age range seen in the samples, in that the otolith grew throughout the life of the fish and showed an internal structure of increments. The pattern in *P. filamentosus* was, however, confused by the presence of indeterminate increments.

Confirmation of the third criterion (that increments seen in the otoliths corresponded to a regular time scale) was not demonstrated so readily. Validation could not occur through methods such as tetracycline injection owing to practical limitations imposed by the fisheries and to the depths inhabited by certain species. *Pristipomoides filamentosus*, for example, is caught at over 75 m depth. The majority of specimens would be dead or heavily stressed through barotrauma on reaching the surface (DeMartini et al., 1996) and useless for tagging programs. Marginal increment and edge analysis was therefore used.

Some evidence for the annual nature of the increments seen in the otoliths of *L. mahsena* was found in the pattern of the marginal increment through the year (Fig. 7). This evidence was confirmed by the unimodal distribution resulting from edge analysis (Fig. 8), indicating that opaque band formation was initiated once a year, from May to August.

For *A. virescens*, identification of the pattern in marginal increments (Fig. 9) was confused by the poor resolution of the unstained sections and the relatively small samples sizes, rendering the results inconclusive. However, the unimodal plot resulting from edge analysis on stained specimens from this species indicated that the transition between the translucent and opaque bands occurred once a year (Fig. 10), between October and March.

From this evidence, we considered the increments seen in the otoliths of both *L. mahsena* from Mauritius and *A. virescens* from Seychelles validated as annual deposits.

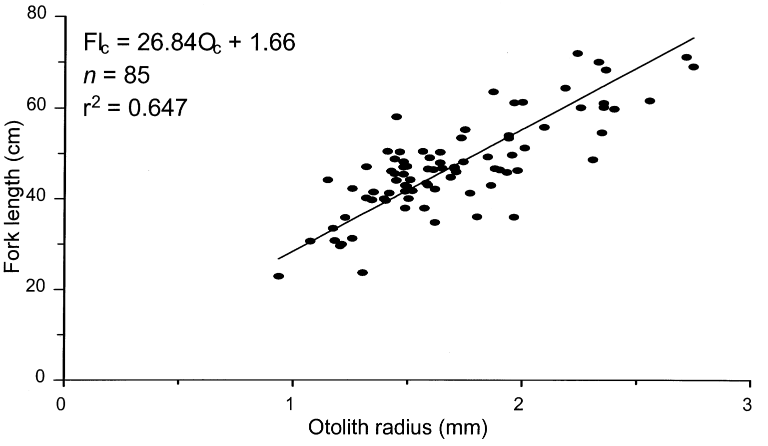

Figure 6

Geometric mean regression of fork length (cm) from otolith radius (mm) for *P. filamentosus*.

Opaque zone deposition is thought to occur during periods of increased growth, whereas the corresponding "translucent zone" is formed during periods of low metabolic activity (Beckman and Wilson, 1995). The period of opaque zone initiation in *L. mahsena* from the Mauritian bank, from May to August, corresponded to the season of the southeast trade winds, a period of rough weather. The period of opaque zone deposition in *A. virescens* from Seychelles, from October to March, coincided with the northwest monsoon season from mid-November to mid-March.

In reviews of otolith studies in tropical latitudes, Beckman and Wilson (1995) and Fowler (1995) found that, for the majority of tropical species studied, the period of opaque growth coincided with spring and summer months, as seen in *L. mahsena* from the Mauritian bank. *Aprion virescens* from Seychelles appears to contradict this general pattern its opaque zone formation begins during winter months. The authors also performed edge analysis studies for 1090 *L. mahsena* individuals from Seychelles (not presented). These studies indicated that in this area, *L. mahsena* also exhibits opaque zone initiation during winter. Of the twenty-nine tropical species reviewed by Fowler (1995), only four showed opaque zone formation in the winter period. Timing of opaque zone formation in these two species may be driven by local phenomena. If opaque zone formation is linked with increased food availability, the monsoon periods experienced in Seychelles at this time, combined with local upwelling, may improve local productivity and feeding conditions. Although little empirical data exist to support this theory, algal blooms have been identified around Seychelles at this time of year.[3]

Although edge analysis indicated differences in the timing of opaque zone deposition at Nazareth bank (Mauritius), and Seychelles (Figs. 8 and 10), patterns in the marginal increment analysis for these locations were similar (Figs. 7 and 9). This similarity may result from the relatively small data sets available for marginal increment analysis, requiring ages to be grouped into age classes, and the difficulty experienced in measuring the marginal increment in *A. virescens* otoliths from Seychelles. Similarities may therefore result from inadequacies in the data that obscure the true pattern.

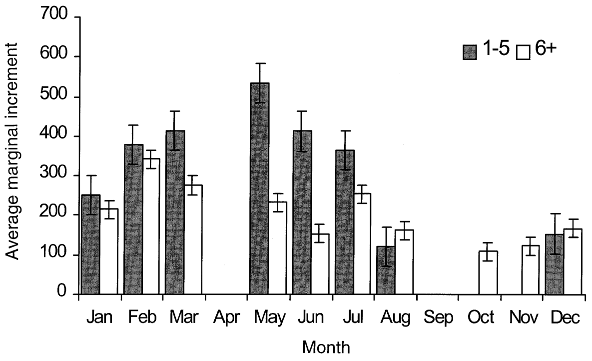

Figure 7

Marginal increment plot by month for *L. mahsena*. Marginal increment measured in VID units (pixels). Data segregated as in Table 1. Error bars denote one standard error.

The results of marginal increment analysis for *P. filamentosus* were inconclusive because problems were experienced in identifying and measuring the outer zone in the otolith. Staining did not improve the clarity of the zones owing to the frequent appearance of "indeterminate increments." Validation was therefore considered to be unsuccessful for *P. filamentosus* in Seychelles and contrasts with that achieved in Hawaii where microincrements were used (Ralston and Miyamoto, 1981, 1983). During our study, ini-

---

[3] Tarbit, J. 1980. Demersal trawling in Seychelles waters. Fisheries Bulletin 5, Fisheries Division, Seychelles. Seychelles Fishing Authority, P.O. Box 449, Victoria, Mahé, Seychelles.

Table 3

Von Bertalanffy growth parameters estimated from back-calculated length data and published sources. Method: L = parameters derived through length-based methods, O = parameters derived from otoliths.

Species	*K*	L_∞ (FL)	*n*	Method	Reference
P. filamentosus	0.11	62.3	85	O	Our study
	0.29	81.7	—	L	Mees (1993)
	0.24	75.8	—	L	Mees and Rousseau (1997)
	0.146	78	—	O	Ralston and Miyamoto (1983)
	0.33–0.36	78-86	—	O	Hardman-Mountford et al. (1997)
A. virescens	0.13	79.0	81	O	Our study
	0.32	95-104	—	L	Mees (1993)
	0.31	65.6	—	O	Loubens (1980)
L. mahsena	0.38	34.1	222	O	Our study
	0.09	52.0	222	O	Our study, L_∞ = 52 cm
	0.1	61.7	—	L	Bautil and Samboo, (1988)
	0.32	58.9	—	L	Dalzell et al. (1992)

Table 4

Numbers of individuals examined for marginal increment analysis by monthly (*L. mahsena* and *A. virescens*) or bimonthly (*P. filamentosus*) interval.

	Age class						
	L. mahsena		*A. virescens*		*P. filamentosus*		
Month	1–5 yr	6+ yr	3–5 yr	6+ yr	3–5 yr	6–10 yr	11+ yr
Jan	14	8	—	4	95	29	20
Feb	13	5	—	—			
Mar	19	5	4	8	—	16	18
Apr	—	—	3	15			
May	15	9	10	17	—	—	—
Jun	4	11	6	11			
Jul	15	8	10	—	8	—	4
Aug	21	4	5	6			
Sep	—	—	—	8	11	19	5
Oct	—	17	—	4			
Nov	—	19	6	16	—	7	10
Dec	13	20	—	8			

tial examination of *P. filamentosus* otoliths was performed by using scanning electron microscopy, in an attempt to validate the “suspected” annuli through micro-increment counts (Brothers and Mathews, 1987). Unlike the results reported from studies in Hawaii, no consistent pattern of micro-increments was found, although it should be noted that our study was very limited in scope (*n*=4). Hardman-Mountford et al. (1997), also using specimens from the Seychelles, related assumed “monthly increments” to assumed annuli, finding approximately 12.4 monthly increments within each annual increment. Consistent banding of this type could not be found during our study for specimens collected from the same region, even though we had a larger sample size.

The lack of consistent annual increments in *P. filamentosus* may be due to the biology of the species. Spawning occurs throughout the year, with two peaks (Mees, 1993), and may not provide a sufficient stimulus for zone formation. *Pristipomoides filamentosus* is found at greater depths (between 75 and 150 m) than the other study species (around 30 m) in conditions that may be buffered against environmental change. Hardman-Mountford et. al. (1997), however, indicated that the 20–24.5°C temperature range at this depth (Mees, 1993) is sufficient to leave

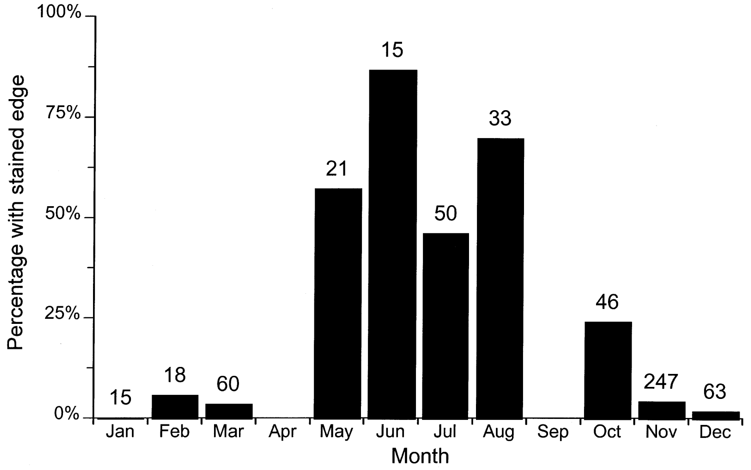

Figure 8

Proportion of *L. mahsena* otoliths with stained edges on the growing tip of the dorsal lobe, by month. Numbers denote sample size.

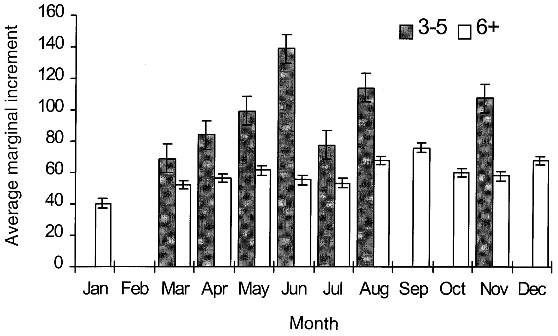

Figure 9

Marginal increment plot by month for *A. virescens*. Marginal increment measured in VID units (pixels). Data segregated as in Table 1. Error bars denote one standard error.

increments on scales in some species (Chey, 1933, in Panella, 1980). If this species is sensitive to relatively small changes in temperature, currents, food supply or food quality, relatively short periods of fluctuation in these factors may result in frequent "indeterminate increments." Even if validation had been achieved, *P. filamentosus* otoliths appear unsuitable for routine aging procedures owing to the difficulties in differentiating periodic increments from other increments not related to a regular time scale.

These difficulties highlight the dangers in assuming the periodicity of increments in otoliths on the basis of validation of that species or a similar species in another location (e.g. Smith and Kostlan, 1991) and also identify the need for thorough validation studies. For example, our study indicated that the assumption of annual, and possibly daily periodicity for increments in the otoliths for *P. filamentosus* in Seychelles, based on published data, would have been incorrect.

Published work (e.g. Sparre and Venema, 1998) suggests that consistent periodic increments do not form in otoliths of tropical species; this is particularly so in the case of low-latitude species. The validation results obtained by our study contrast sharply with this view; indeed, our results add to the expanding number of tropical species for which the validation of annual increments has been successful.

The results of models used in fisheries management, e.g. analytical yield per recruit models (Beverton and Holt, 1957), are sensitive to uncertainty in the estimates of input parameters such as the von Bertalanffy growth parameters (Mees and Rousseau, 1997). Current estimates of growth and mortality in fish in Seychelles and Mauritius

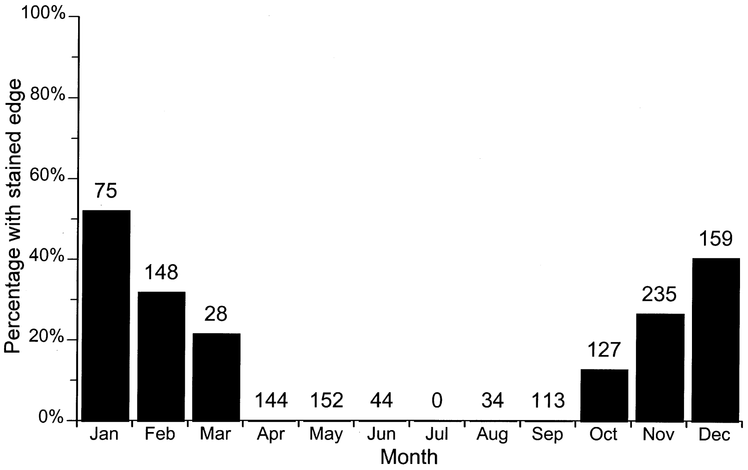

Figure 10

Proportion of *A. virescens* otoliths with stained edge on the growing tip of the dorsal lobe, by month. Numbers denote sample size.

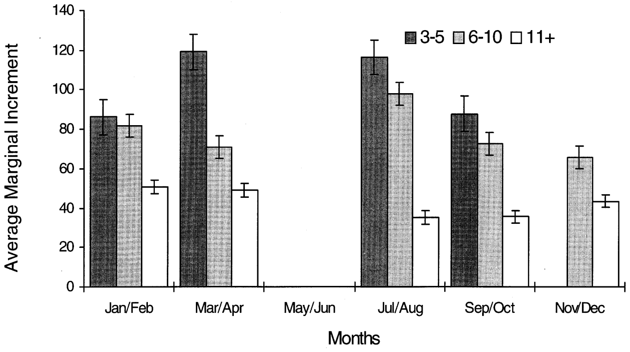

Figure 11

Marginal increment plot by month for *P. filamentosus.* Marginal increment measured in VID units (pixels). Data segregated as in Table 1. Error bars denote one standard error.

are derived through length-based methods, which for long-lived, slow growing species are considered uncertain. The preliminary back-calculated growth parameters from our study are the first otolith-based estimates of growth for the study species at these particular locations. Although these estimates are not definitive, they do suggest that current length-based growth estimates for *A. virescens* may have overestimated mean growth rate and asymptotic length. For *L. mahsena*, growth rate may have been underestimated, and asymptotic length overestimated. However, by constraining the value of L_∞ to levels in keeping with the maximum size found in length distributions from the region (52 cm), we obtained a similar growth rate to that identified by Bautil and Samboo (1988). If snappers do indeed have a longer life span than that indicated by length-based assessments, they may be more vulnerable to overfishing than previously thought. Future work will involve the estimation of more definitive growth parameters based on length-at-age data derived from otoliths.

Acknowledgments

The authors wish to thank the Directors and staff of CEFAS, Albion Fisheries Research Centre and the Seychelles Fishing Authority who collaborated on this project and provided the data necessary for this analysis. Three anonymous reviewers are thanked for their helpful comments on the manuscript. The project to which this paper relates was funded by the Department for International Development of the UK through the Fisheries Management Science Programme.

Literature cited

Ardil, J. D.
1986. Current status of the fishery in Mauritius. Food and Agriculture Organisation of the United Nations (FAO) Southwest Indian Ocean Project (SWIOP) document RAF/79/065/WP/37/87/E, 30 p.

Bautil, B., and C. R. Samboo.
1988. Preliminary stock assessment for the Mahsena emperor (*Lethrinus mahsena*) on the Nazareth Bank of Mauritius. *In* Proceedings of the workshop on the assessment of the fishery resources in the southwest Indian Ocean (M. J. Sanders, P. Sparre, and S. C. Venema, eds.), p. 260–267.

Beamish, R. J., and G. McFarlane.
1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112:735–743.

Beckman, D. W., and C. A. Wilson.
1995. Seasonal timing of opaque zone formation in fish otoliths. *In* Recent developments in fish otolith research, no. 19 (D. H. Secor, J. M. Dean, and S. E. Campana, eds.), p. 27–43. The Bella W Baruch Library in Marine Science.

Bedford, B. C.
1983. A method for preparing sections of large numbers of otoliths embedded in black polyester resin. J. Cons. Int. Explor. Mer 41:4–12.

Beverton, R. J. H., and S. J. Holt.
1957. On the dynamics of exploited fish populations. Fish. Invest. Minist. Agric. Fish. Food GB (2 Sea Fish.) 19, 533 p.

Blacker, R. W.
1974. Recent advances in otolith studies. *In* Sea fisheries research (R. Harden-Jones, ed.), p 67–90. Elek Science, London.

Brothers, E. B., and C. P. Mathews.
1987. Application of otolith microstructural studies to age determination of some commercially valuable fish of the Arabian Gulf. Kuwait Bull. Mar. Sci. 9:127–157.

Dalzell, P., S. Sharma, and G. Naith.
1992. Estimation of exploitation rates in a multispecies emperor (Pisces: Lethrinidae) fishery in Fiji, based on length frequency data. *In* Papers on fisheries science from the Pacific Islands, vol. 1: Inshore fisheries research project, technical document 1, p. 43–50. South Pacific Commission, Noumea, New Caledonia.

DeMartini, E. E., F. A. Parrish, and D. M. Ellis.
1996. Barotrauma-associated regurgitation of food: implications for diet studies of Hawaiian pink snapper, *Pristipomoides filamentosus* (Family: Lutjanidae). Fish. Bull. 94:250–256.

Ferreira, B. P., and G. R. Russ.
1992. Age, growth and mortality of the inshore coral trout *Plectropomus maculatus* (Pisces: Serranidae) from the central Great Barrier Reef, Australia. Aust. J. Mar. Freshwater Res. 43:1301–1312.
1994. Age validation and estimation of growth rate of the coral trout *Plectropomus leopardus* from Lizard Island, Northern Great Barrier Reef. Fish. Bull. 92:46–57

Fowler, A. J.
1995. Annulus formation in otoliths of coral reef fish—a review. *In* Recent developments in fish otolith research, vol. 19 (D. H. Secor, J. M. Dean, and S. E. Campana, eds), p. 46–63. The Bella W Baruch Library in Marine Sciences.

Fowler, A. J., and P. J. Doherty.
1992. Validation of annual growth increments in the otoliths of two species of damselfish from the southern Great Barrier Reef. Aust. J. Mar. Freshwater Res. 43:1057–1068.

Francis, R. I. C. C.
1990. Back-calculation of fish length—a critical review. J. Fish Biol.:883–902.

Francis, R. I. C. C., L. J. Paul, and K. P. Mulligan.
1992. Ageing of adult snapper (*Pagrus auratus*) from otolith annual ring counts: validation by tagging and oxytetracycline injection. Aust. J. Mar. Freshwater Res. 43:1069–1089.

Hardman-Mountford, N. J., N. V. C. Polunin, and D. Boullé.
1997. Can the age of tropical species be determined by otolith measurement? A study using *Pristipomoides filamentosus* (Pisces: Lutjanidae) from the Mahé Plateau, Seychelles. Naga 20:27–31.

Langi, S.
1990. The applicability of ELEFAN for use in analysing three species of deep-sea snappers (*Etelis corruscans*, *Pristipomoides flavipinnis* and *P. filamentosus*, Family Lutjanidae). Fishbyte 8(1):21–25.

Loubens, G.
1978. Biologie de quelque especes de poisson du lagon Neo-Caledonien. I. Determination de l'age (otolithometrie). Cahiers ORSTOM, Series Oceanographie 16:263–283.
1980. Biologie de quelques especes de poissons du lagon Neo-Caledonien III. Croissance. Cal. Indo-Pac. 2:101–153.

Manickchand-Heileman, S. C., and D. A. T. Philipp.
1996. Reproduction, age and growth of the Caribbean red snapper (*Lutjanus purpureus*) in waters off Trinidad and Tobago. *In* Biology, fisheries and culture of tropical groupers and snappers (F. Arreguín-Sánchez, J. L. Munro, M. C. Balgos, D. Pauly, eds.), p. 137–149. ICLARM, Manila, Philippines.

Manooch, C. S.
1982. Ageing reef fishes in the Southeast fisheries centre. *In* The biological basis for reef fishery management: proceedings of a workshop held October 7–10, 1980 at St. Thomas, Virgin Islands of the Unites States (G. R. Huntsman, W. R. Nicholson, and W. W. Fox, eds.), p. 24–43. U.S. Dep. Commer., NOAA, Beaufort, North Carolina 28516.
1987. Age and growth of snappers and groupers. *In* Tropical snappers and groupers: biology and fisheries management (J. J. Polovina and S. Ralston, eds.), p. 329–363. Westview Press Inc., Boulder, CO.

Manooch, C. S., and C. L. Drennon.
1987. Age and growth of yellowtail snapper and queen triggerfish collected from the US Virgin Islands and Puerto Rico. Fish. Res. 6:53–68.

Mason, D. L., and C. S. Manooch.
1985. Age and growth of the mutton snapper along the east coast of Florida. Fish. Res. 3: 92–104.

McPherson, G. R., and L. Squire.
1992. Age and growth of three dominant *Lutjanus* species of the Great Barrier Reef inter-reef fishery. Asian Fish Sci. 5 (1):25–36.

Mees, C. C.
1993. Population biology and stock assessment of *Pristipomoides filamentosus* on the Mahé Plateau, Seychelles. J. Fish Biol. 43:695–708.

Mees, C. C., and J. A. Rousseau.
1997. The potential yield of the lutjanid fish *Pristipomoides filamentosus* from the Mahé Plateau, Seychelles: Managing with uncertainty. Fish. Res. 33:73–87.

Millner, R. S., and C. L. Whiting.
1996. Long-term changes in growth and population abundance of sole in the North Sea from 1940 to the present. ICES J. Mar. Sci. 53:1185–1195.

Milton, D. A., S. A. Short, M. F. O'Niell, and S. J. M. Blaber.
1995. Ageing of three species of tropical snapper (Lutjanidae) from the Gulf of Carpentaria, Australia, using radiometry and otolith ring counts. Fish. Bull. 93(1):103–115.

Morales-Nin, B.
1989. Growth determination of tropical marine fishes by means of otolith interpretation and length frequency analysis. Aquatic Living Resources Nantes 2:241–253.

Morales-Nin, B., and S. Ralston.
1990. Age and growth of *Lutjanus kasmira* (Forsskål) in Hawaiian waters. J. Fish Biol. 36(2):191–203.

Newman, S. J., D. M. Williams, and G. R. Russ.
1996. Age validation, growth and mortality estimates of the tropical snappers *Lutjanus adetii* and *L. quinquelineatus* from the Central Great Barrier Reef, Australia. Mar. Freshwater Res. 47:575–584.

Panella, G.
1980. Growth patterns in fish sagittae. *In* Skeletal growth of aquatic organisms (D. C. Rhoads and R. A. Lutz, eds.), p. 519–560. Plenam Press, New York, NY.

Ralston, S., and G. T. Miyamoto.
1981. Estimation of the age of a tropical reef fish using the density of daily growth increments. *In* The reef and man (E. D. Gomez, C. E. Birkeland, R. W. Buddemeier, R. E. Johannes, J. A. Marsh, and R. T. Tsuda, eds.), p. 83–88. Proceedings of the fourth international coral reef symposium, Manila, 1981, vol. 1.
1983. Analyzing the width of daily otolith increments to age the Hawaiian snapper, *Pristipomoides filamentosus*. Fish. Bull. 81(3):523–535.

Reay, P. J.
1972. The seasonal pattern of otolith growth and its application to back-calculation studies in *Ammodytes tobianus*. J. Cons. P. Int. Explor. Mer 34:485–504.

Richter, H., and J. G. McDermot.
1990. The staining of fish otoliths for age determination. J. Fish Biol. 36:773–779.

Ricker, W. E.
1992. Back-calculation of fish lengths based on proportionality between scale and length measurements. Can. J. Fish. Aquat. Sci. 49:1018–1026.

Secor, D. H., J. M. Dean, and S. E. Campana.
1995. Recent developments in fish otolith research. Belle W. Baruch library in marine science no. 19, South Carolina Press, Columbia, SC, 735 p.

Smith, M. K., and E. Kostlan.
1991. Estimates of age and growth of Ehu *Etelis carbunculus* in four regions of the Pacific from density of daily increments in otoliths. Fish. Bull. 89:461–472.

Sparre, P., and S. C. Venema.
1998. Introduction to tropical fish stock assessment. Part 1, Manual, FAO Fisheries Technical Paper 306.1, rev 2. FAO, Rome, 407 p.

Williams, T., and B. C. Bedford.
1974. The use of otoliths for age determination. *In* Ageing of fish (T. B. Bagenal, ed.), p. 114–123. Unwin, Surrey, UK.