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ANNUAL RING VALIDATION OF THE SOUTH AFRICAN SARDINE *SARDINOPS SAGAX* USING DAILY GROWTH INCREMENTS

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A total of 21 sardine *Sardinops sagax* otoliths, collected in winter and spring 1994, were examined with a scanning electron microscope to validate growth zones using daily growth increments. Increment numbers and widths for the first, second and third annual growth zones served to back-calculate hatching dates and to validate the assumption that the identified annual rings were deposited in annual intervals. Fish appeared to have hatched throughout 1992. Annual ring deposition periods depended on the individual cases and did not correspond to exactly one year, with some fish estimated to be older than one year at the first annual ring deposition. The second growth zone contained a mean of 266 daily increments, with the second annual ring deposited between November 1993 and June 1994. The third annual ring was visible on six otoliths, and appeared to have been deposited in August and September 1994.

The South African sardine *Sardinops sagax* population partially collapsed in the early 1960s (Berruti and Colclough 1987, Cochrane *et al*. 1991), but stocks are now beginning to recover (Adams *et al*. 1992). This increased importance necessitates better management of the fishery, requiring accurate ageing data. As a result of the environmental variability caused by the complex oceanographic conditions in South African waters (Shannon 1985), sardine deposit a complicated hyaline ring pattern on their otoliths, making accurate age determination extremely difficult. In many otoliths, annual rings, false rings and split annual growth zones appear similar, requiring validation of the annual rings for reliable ageing.

Micro-increments on the otoliths of fish are believed to be deposited daily (Panella 1971, Miller and Storck 1982, Campana and Neilson 1985), and are therefore extremely useful for determining accurate ages of individual fish. Daily growth increments on otoliths of juvenile sardine from Namibian waters have been validated by Thomas (1986), and for the purpose of this study, it was assumed that the increments on the otoliths of the South African sardine are daily.

The object of this study was to validate annual growth zones on sardine otoliths by daily increment counts. Birthdates and times of annual ring deposition were also studied.

MATERIAL AND METHODS

Otoliths were collected from fish landed in commercial catches in July, September and October 1994. Those that showed two or three hyaline (translucent) rings, when viewed under reflected light against a dark background, were selected. The widths of the growth zones, i.e. the area between two consecutive hyaline rings, or between the primordium and the first hyaline ring, were measured (Fig. 1). The otoliths were then photographed by means of a light microscope with a polarizing filter. Otoliths for scanning electron microscopy (SEM) analysis were mounted in resin, ground along the longitudinal plane, polished with 0.05-μm aluminia powder and etched with 0.1M ethylene diamine-tetra-acetic acid (EDTA). They were then mounted on stubs and sputter-coated with gold and examined with a SEM at 600–1000× magnification. All otoliths that showed a clear sequence of daily increments in >90% of the longitudinal plane (from the primordium to the postrostral tip) were photographed. Micrographs were compiled into montages to facilitate enumeration of daily increments and for mapping the positions of the hyaline rings. In some areas of the otoliths, the daily increments were not visible and had to be estimated by interpolation. This was done by taking a mean of the number of increments per centimetre from the sections on either side of the unreadable area. The otoliths were counted by the author three times, at least one week apart. To ensure good-quality results, otoliths were rejected if the primordium was not exposed, more than 10% of the total increments were obtained by interpolation (i.e. more than 10% of the otolith was unreadable), or if the three readings differed by more than 10%.

From a total of 85 otoliths, 21 (25%) were successfully prepared. All otoliths had at least two hyaline rings (i.e. three growth zones) and were assumed to be more than two years old.

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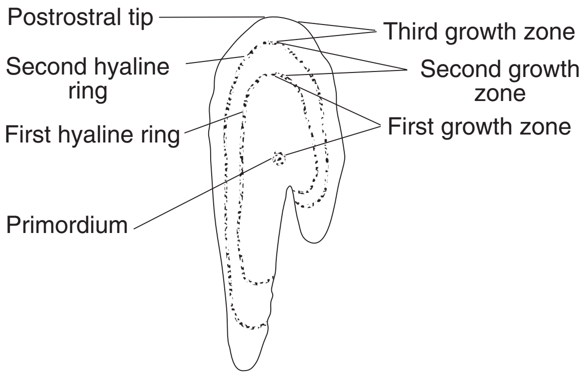

Fig. 1: Diagram of an otolith showing the hyaline rings and growth zones measured using a light microscope

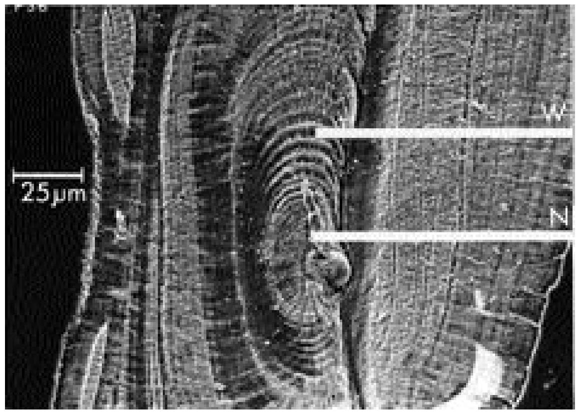

Fig 2: Scanning electron photomicrograph of the primordium of a typical sardine otolith, showing the different increment widths, where *N* denotes narrow rings in the first 10–15 days after hatching and *W* denotes wider rings in the following 10–20 days

RESULTS

The results illustrated the variability in hyaline ring deposition; some fish deposited the first hyaline ring after only about 200 days, whereas others deposited a hyaline ring after 400–500 days. The size of the first growth zone ranged from 1.3 to 0.48 mm, with between 517 and 207 daily increments respectively (Table I). The second growth zones varied in size from 0.68 to 0.27 mm, containing between 390 and 143 daily increments respectively. Third growth

Table I: Size of growth zones, number of daily increments (*DI*) and the mean increment widths (*MDIW*) in the growth zone for the first, second and third growth zones on sardine otoliths

First growth zone				Second growth zone				Third growth zone				
Otolith	Size (mm)	*DI*	*MDIW* (μm)	Otolith	Size (mm)	*DI*	*MDIW* (μm)	Otolith	Size (mm)	*DI*	*MDIW* (μm)	Total age (days)
PV39	1.3	342	4.2	PV56	0.68	390	2.3	PV56	0.34	358	1.2	1 011
SP36	1.28	411	3.7	SP10	0.68	339	2.2	SP27	0.026	155	2.2	751
SP25	1.2	397	2.1	PV44	0.06	349	1.3	PV46	0.26	224	1	747
PV16	1.18	351	3.6	PV33	0.6	307	1.5	SP26	0.24	213	1.1	937
PV45	1.14	361	3.2	PV46	0.58	310	1.1	PV41	0.24	162	1.2	773
SP5	1.14	356	3.8	SP20	0.53	372	1.2	PV39	0.23	216	1.3	741
SP8	1.06	333	3.7	SP4	0.52	306	2.5	SP36	0.22	123	1.7	721
SP28	1.06	310	3.9	SP37	0.48	317	1.3	SP10	0.22	262	1.2	953
SP37	0.98	279	3.9	PV41	0.48	315	1.3	PV45	0.21	156	1.8	758
SP26	0.96	517	2.2	SP8	0.44	323	2.1	SP25	0.19	102	1.9	681
PV21	0.95	345	3.8	PV16	0.42	202	2.3	SP37	0.19	98	1.3	694
SP30	0.94	326	3.1	PV21	0.42	288	2	SP30	0.18	250	0.8	844
PV41	0.94	207	2.8	SP27	0.36	236	2.5	PV33	0.18	202	1.3	868
SP27	0.92	360	4.5	SP30	0.36	220	2.5	PV16	0.16	115	1.7	668
SP20	0.91	363	2.3	SP5	0.34	253	1.2	PV21	0.16	139	1.5	785
SP4	0.9	320	2.1	SP26	0.34	207	1.6	PV44	0.16	182	1.2	853
PV33	0.9	359	2.2	SP36	0.34	187	2.3	SP28	0.16	111	1.7	709
PV44	0.88	264	3.2	SP28	0.33	288	2.3	SP4	0.14	91	1.7	717
PV46	0.83	213	3	PV45	0.33	241	2.1	SP5	0.12	122	0.5	731
PV56	0.66	228	3	SP25	0.32	182	2.8	SP8	0.12	96	1.5	752
SP10	0.48	300	2.2	PV39	0.27	143	1.4	SP20	0.12	64	1.1	799
Mean	0.95	326	3.08		0.42	266	1.9		0.18	154	1.3	

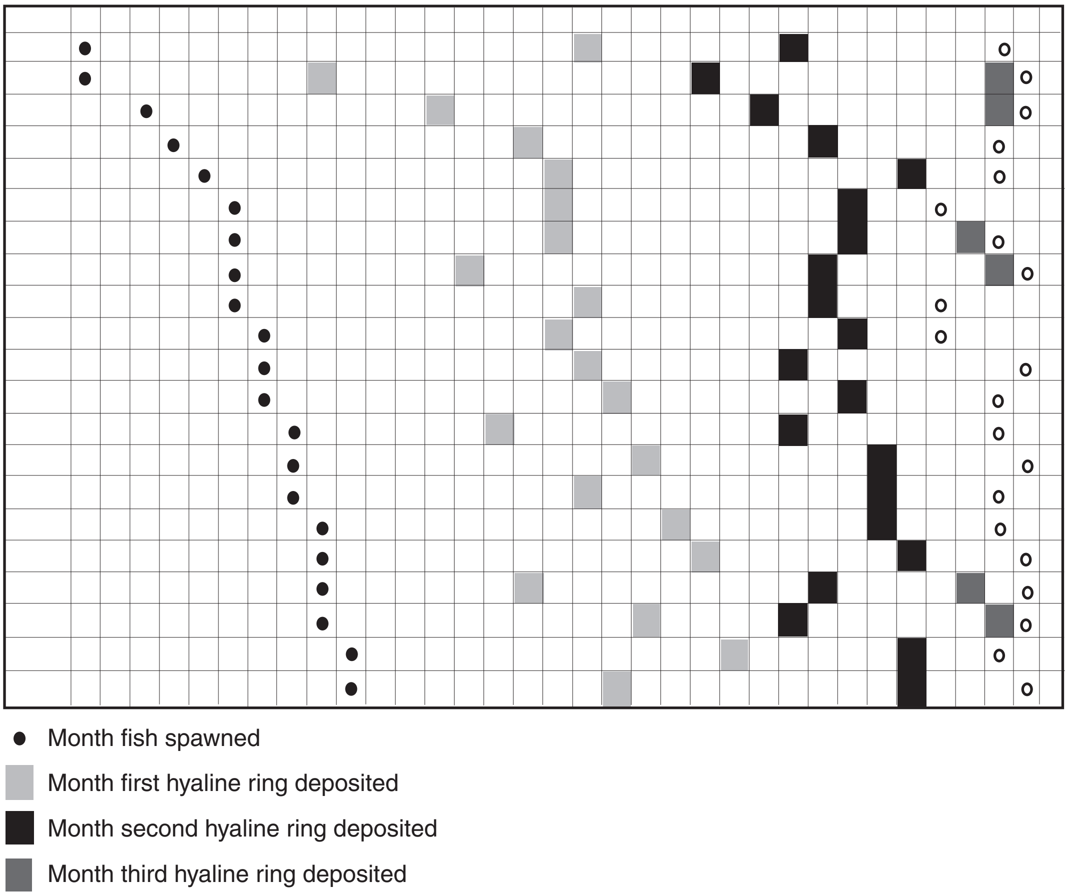

Fig 3: Diagram showing the approximate spawning date, hyaline ring deposition and catch date of the sardine otoliths under study. The results are displayed in order of fish spawned the earliest

zones, which represented the marginal increment after the second hyaline ring, consisted of between 64 and 224 daily increments. A fourth growth zone was found in six otoliths, in which the third growth zones should have represented a growth of one year. Those otoliths had between 162 and 358 daily increments in the third growth zone, with zone size ranging between 0.16 and 0.34 mm respectively (Table I).

Size of the first growth zone varied widely. Daily increments were usually narrow (c. 0.5 µm) within the first 10–15 days of hatching, widening within the next 2–5 days (Fig. 2). Increments remained 4–8 µm wide for the subsequent 10–20 days. After about 50 days, the rings varied in width, with no obvious pattern, except that they all appeared to have a maximum ring width of between 9 and 12 µm, equivalent to between 80 and 100 days respectively.

The approximate month of hatching of each fish was estimated by back-calculation (Fig. 3). The deposition of hyaline rings in relation to the catch date indicated that the sardine under study were hatched between February and November 1992, with the first hyaline rings deposited between October 1992 (Otolith PV56) and December 1993 (Otolith SP25). The first hyaline ring deposition occurred most frequently between March and September 1993 (autumn/spring). The majority of the second hyaline rings were deposited in autumn, between March and May 1994. One otolith showed that a second hyaline ring was deposited as early as November 1993. In

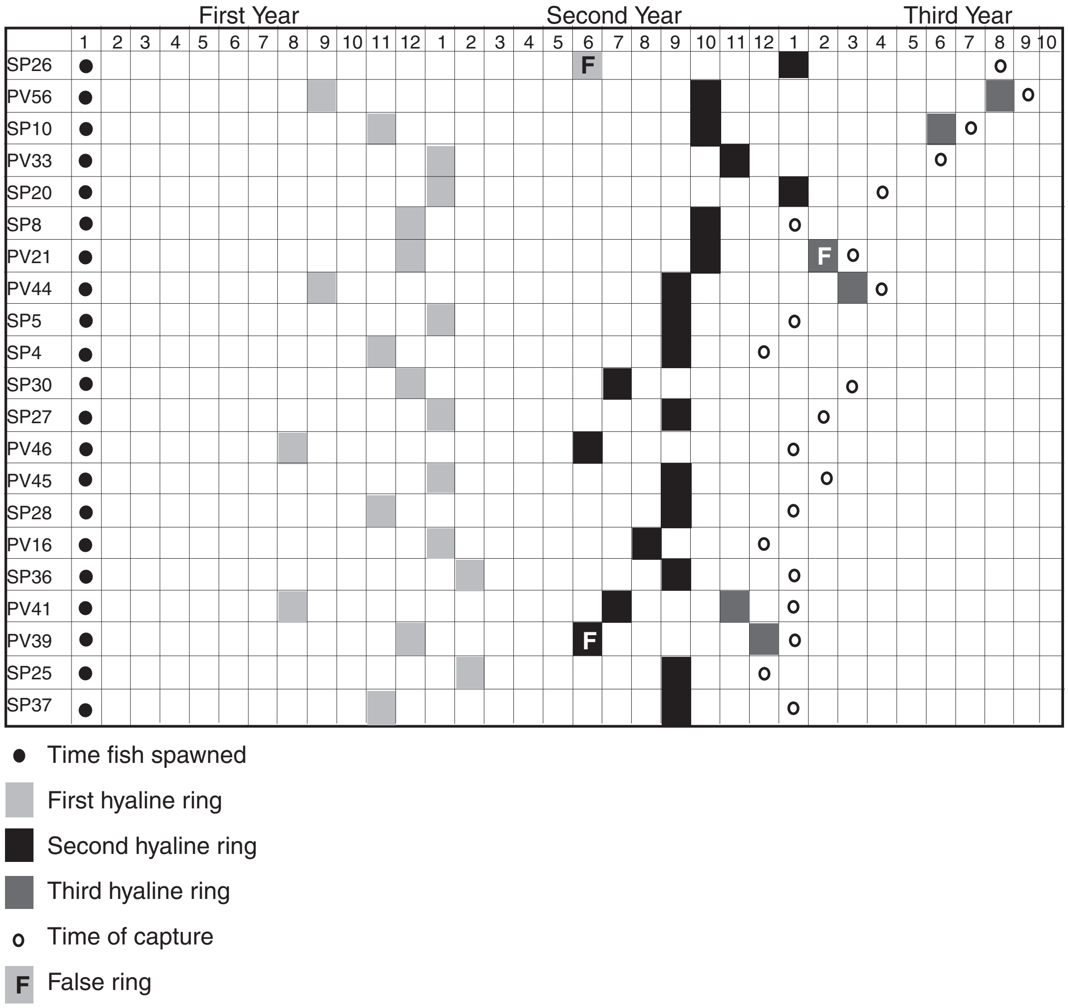

Fig 4: Diagram showing the actual time between first, second and third hyaline rings. Data are arranged as if all the fish were spawned in the same month

other otoliths, second hyaline rings were deposited as late as July 1994, indicating that the second hyaline ring can be completed any time between midsummer and midwinter. Although there were only six otoliths with third hyaline rings, all six were laid down in early spring (August–September).

Some of the hyaline rings were found to be false, i.e. having the same appearance as annual rings, but not validated as such. It is possible that the first hyaline rings on Otoliths PV41, PV46 and SP26 were false, because they were deposited after 207, 213 and 517 days respectively. The second hyaline ring on Otolith PV39 was also probably false, because it appeared to be deposited after only 143 days. Because the third hyaline ring on Otolith PV21 was apparently deposited after only four months, that ring was not considered to be an annual ring.

In order to illustrate the time between hatching and hyaline ring formation, the data were arranged as if all fish were hatched in the same month (Fig. 4). Excluding Otolith SP26, the first hyaline ring was deposited between 7 and 13 months after hatching.

The second and third hyaline rings were less dependent on the hatching date, and ring deposition appeared to be regulated individually, over an increasing number of months.

DISCUSSION

South African sardine spawn throughout the year, but tend to have spawning peaks between August and February (Crawford 1980, Akkers *et al*. 1996). The present results substantiate the view that sardine have a protracted spawning period, at least for the year under study. Unlike anchovy *Engraulis capensis,* which appear to deposit the first hyaline ring in response to specific seasons (Waldron 1994), hyaline rings in sardine are deposited throughout the year, likely in response to physiological or environmental cues.

Thomas (1983) found that the majority of hyaline rings in adult sardine from Namibian waters were laid down during late winter, which agrees with the deposition period for the third hyaline ring in the sardine under study. The author found that the majority of young fish deposited hyaline rings between March and September (autumn and winter). In the present study, 76% of first hyaline rings were formed during that period, although the deposition period varied considerably more than in Thomas's study. This could be attributable to the larger size range of fish sampled in the current study, because only specific length groups were examined by Thomas (1983). Kerstan (1995) found that 50% of juvenile South African sardine deposited their first hyaline ring in September or October, and in some fish as early as April. On average, the first hyaline ring is deposited a year after hatching, indicative of an annual ring.

The number of daily increments between the hyaline rings appears to decrease as the fish age. The daily increment counts between the second and the third hyaline rings were less than 365 days (representing one year of growth). The mean count was 218 daily increments, with only one otolith (PV56) having a count of more than 300 increments. That otolith had a relatively large third growth zone of 0.34 mm (average = 0.18 mm), although the daily increments were of average width for the third zone. The fact that the number of daily increments was less than 365 between consecutive annual rings could be attributable to some rings being too small to be identified (Campana and Neilson 1985, Parrish *et al.* 1994), or that they were not deposited daily as the fish grow older (Miller and Storck 1982, Morales-Nin 1987). Also, the time between hyaline ring deposition could be less than one year (Radtke *et al*. 1985).

There are a few inaccuracies inherent in the current methods. If the otoliths are not ground correctly to expose the postrostral tip, daily counts could be underestimated by as much as 30 days, because increments can be very narrow (0.5 μm). Such an omission would not affect the counts of the number of days between each hyaline ring, but rather shift the ring deposition and hatching date in relation to season. The number of counts, and consequently the time between each hyaline ring, would be affected by the counting and ring interpretation errors. In some areas of the otolith it was difficult to identify true daily increments from sub-daily increments.

The shortest time between hatching and first hyaline ring deposition appears to be seven months. Because annual ring completion tends to commence first in the smaller individuals and is coincidental with minimal somatic growth (Kerstan 1995), it is possible that early deposition of annual rings is more a response to poor growth conditions than to hatching. Daily increment deposition patterns indicate that growth usually peaks 80–100 days after hatching. However, if environmental conditions were poor during that period, this increase in growth would not occur. This would result in a smaller first growth zone on the otolith and perhaps early annual ring deposition. Most otoliths that showed small first growth zones appeared to compensate by having large second growth zones. This concurs with the findings of Kerstan (1996), who found that small sardine appear to "catch up" by compensatory growth in following years. In otoliths where the second growth zone was too large to be the first annulus and daily increment numbers indicated more than one year's growth (e.g. Otoliths PV26 and PV36), it is likely that the first annulus was suppressed.

The third hyaline ring of Otolith PV39 was probably the second annual ring, because it was deposited 12 months after the first annual ring. This implies that the second hyaline ring was a false ring and that the fish had just completed its second year of life. Such problematic otoliths would result in erroneous age-group assignments. A method to overcome errors caused by false rings is to measure otolith sizes at each age. Exceptionally narrow second growth zones are unlikely to be annual.

Unfortunately, there appears to be no objective or independent means of judging an annual growth zone in South African sardine using SEM, because the hyaline rings on the otolith do not always coincide with obvious changes in daily increment widths. The deposition of hyaline rings in the sardine probably does not reflect a sudden change in the growth rate of the fish.

In conclusion, it is possible to identify true annual rings and to determine the age of the South African sardine correctly, providing that the specific growth patterns of the otoliths are considered and that otolith age determinations are accompanied by growth zone measurements.

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LITERATURE CITED

ADAMS, N. J., SEDDON, P. J. and Y. M. VAN HEEZIK 1992 — Monitoring of seabirds in the Benguela upwelling system: can seabirds be used as indicators and predictors of change in the marine environment? In *Benguela Trophic Functioning*. Payne, A. I. L., Brink, K. H., Mann, K. H. and R. Hilborn (Eds). *S. Afr. J. mar. Sci.* **12**: 959–974.

AKKERS, T. R., MELO, Y. C. and W. VEITH 1996 — Gonad development and spawning frequency of the South African pilchard *Sardinops sagax* during the 1993–1994 spawning season. *S. Afr. J. mar. Sci.* **17**: 183–193.

BERRUTI, A. and J. COLCLOUGH 1987 — Comparison of the abundance of pilchard in Cape gannet diet and commercial catches off the Western Cape, South Africa. In *The Benguela and Comparable Ecosystems*. Payne, A. I. L., Gulland, J. A. and K. H. Brink (Eds). *S. Afr. J. mar. Sci*. **5**: 863–869.

CAMPANA, S. E. and J. D. NEILSON 1985 — Microstructure of fish otoliths. *Can. J. Fish. aquat. Sci.* **42**(5): 1014–1032.

COCHRANE, K. L., HAMPTON, I. and B. A. ROEL 1991 — Current status of South African anchovy and pilchard resources. *S. Afr. Shipp. News Fishg Ind. Rev*. **46**(1): 22–23.

CRAWFORD, R. J. M. 1980 — Seasonal patterns in South Africa's Western Cape purse-seine fishery. *J. Fish Biol*. **16**(6): 649–664.

KERSTAN, M. 1995 — Seasonal uncoupling of otolith and somatic growth in South African pilchard (*Sardinops sagax*) and its applications. *ICES Doc*. **C.M. 1995/P:6**: 17 pp. (mimeo).

KERSTAN, M. 1996 — Quantification of growth compensation and depensation in the South African sardine (*Sardinops sagax*) population. *ICES Doc*. **C.M. 1996/H:10**: 15 pp. (mimeo).

MILLER, S. J. and T. STORCK 1982 — Daily growth rings in otoliths of young-of-the-year largemouth bass. *Trans. Am. Fish. Soc*. **111**: 527–530.

MORALES-NIN, B. Y. O. 1987 — The influence of enviromental factors on microstructure of otoliths of three demersal fish species caught off Namibia. In *The Benguela and Comparable Ecosystems*. Payne, A. I. L., Gulland, J. A. and K. H. Brink (Eds). *S. Afr. J. mar. Sci*. **5**: 255–262.

PANELLA, G. 1971 — Fish otolith: daily growth layers and periodical patterns. *Science* **173**: 1124–1127.

PARRISH, D. L., VONDRACEK, B. and W. J. ECKMAYER 1994 — Accuracy and precision of daily age estimates for walleyes from Ohio hatcheries and Lake Erie. *Trans. Am. Fish. Soc*. **123**: 601–605.

RADTKE, R. L., FINE, M. L. and J. BELL 1985 — Somatic and otolith growth in the oyster toadfish (*Opsanus tau* L.). *J. expl mar. Biol. Ecol*. **90**: 259–275.

SHANNON, L. V. 1985 — The Benguela ecosystem. 1. Evolution of the Benguela, physical features and processes. In *Oceanography and Marine Biology. An Annual Review* **23**. Barnes, M. (Ed.). Aberdeen; University Press: 105–182.

THOMAS, R. M. 1983 — Back-calculation and time of hyaline ring formation in the otoliths of the pilchard off South West Africa. *S. Afr. J. mar. Sci*. **1**: 3–18.

THOMAS, R. M. 1986 — Growth of larval pelagic fish in the South-East Atlantic from daily otolith rings in 1982/83 and 1983/84. *S. Afr. J. mar. Sci*. **4**: 61–77.

WALDRON, M. E. 1994 — Validation of annuli of the South African anchovy, *Engraulis capensis*, using daily growth increments. *ICES J. mar. Sci.* **51**: 233–234.