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AGE DETERMINATION OF *ILLEX COINDETII* FROM THE STRAIT OF SICILY BY STATOLITH INCREMENT ANALYSIS

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A total of 649 short-finned squid *Illex coindetti* caught in the Strait of Sicily (Central Mediterranean) was examined to investigate statolith microstructure and estimate the number of growth increments. Two different methods of analysis were employed to count increments, the conventional method by eye and an automatic image-analysis system (IAS). Age estimates (based on the assumption of daily deposition of increments) obtained with the two methods were compared. Maximum ages estimated by eye were 230 and 240 days for males and females respectively. Average IAS estimates were significantly higher (2 days more for males and 5 days more for females) than the conventional ones. Size-at-age relationships were computed from both sets of age estimates using three size indicators: mantle length, body mass and an index calculated as the ratio between the logarithmic values of body mass and mantle length. Size-at-age relationships were similar for both methods of counting increments. Best fits were obtained using the quadratic, power and von Bertalanffy models applied to mantle length, body mass and the new index respectively.

As length frequency distribution (LFD) analyses have shown some limitations when applied to estimating cephalopod growth, recent attention has been aimed at the use of direct methods of age determination, such as size-at-age estimates derived from hard structures, in particular from statoliths (see Jereb *et al.* 1991, Jackson 1994).

The short-finned squid *Illex coindetii* (Verany, 1839) is widespread from the eastern to the western Atlantic and east through the whole Mediterranean Sea (Roper *et al.* 1984). Usually a bycatch of commercial fisheries, it may represent a valuable resource on its own in some areas as a result of its abundance (Strait of Sicily, Central Mediterranean; Jereb and Ragonese 1995a). *Illex coindetii* statoliths were used recently to investigate growth in both the Atlantic and western Mediterranean populations (Sánchez 1995, Arkhipkin 1996, Gonzalez *et al.* 1996). However, only LFD analysis has been used to investigate growth in the population found in the Strait of Sicily (Jereb and Ragonese 1995a).

In the past decade, development of a new computer-based image-analysis system (IAS) has shown great potential for fish otolith research (Secor *et al.* 1995), and its advantages for statolith studies have been emphasized by Jereb *et al.* (1991) and Jackson (1994). To date, however, only one case of "automatic" statolith increment counting by IAS has been reported (Macy 1995).

In this study, specimens of *Illex coindetii* from the Strait of Sicily were examined to investigate statolith microstructure. Growth increments were enumerated both conventionally (eye-piece micrometer) and "automatically" (IAS). Age estimates obtained by the two methods are compared for the first time in squid.

MATERIAL AND METHODS

Short-finned squid were obtained from two experimental bottom trawl surveys carried out in the Strait of Sicily during spring (April 1–29) and autumn (October 9 – November 12) 1995. Hauls of 1 h duration took place during daylight, between the surface and 800 m deep, following the statistical design and methodology reported by Levi (1991). An Italian-type deep-bottom trawl was used (codend 18 mm mesh). The squid were returned to shore frozen. Dorsal mantle length (*ML*) was measured to the nearest mm and body mass (*BM*) to the nearest 0.1 g after thawing. Maturity stages (immature, maturing and mature) were assigned according to the macroscopic scale of Jereb and Ragonese (1995a). Evidence of spawning in females (bundles of spermatophores on the inner side of the mantle, near the gills) was also recorded.

In all, 649 specimens (*ML* 44–202 mm) were analysed. Statoliths were extracted and stored in small plastic vials filled with 96% ethyl alcohol before processing. Terminology and measurement of statoliths was after Lipiński *et al.* (1991). Statoliths were attached to microscopic slides with Pro-tex mounting medium,

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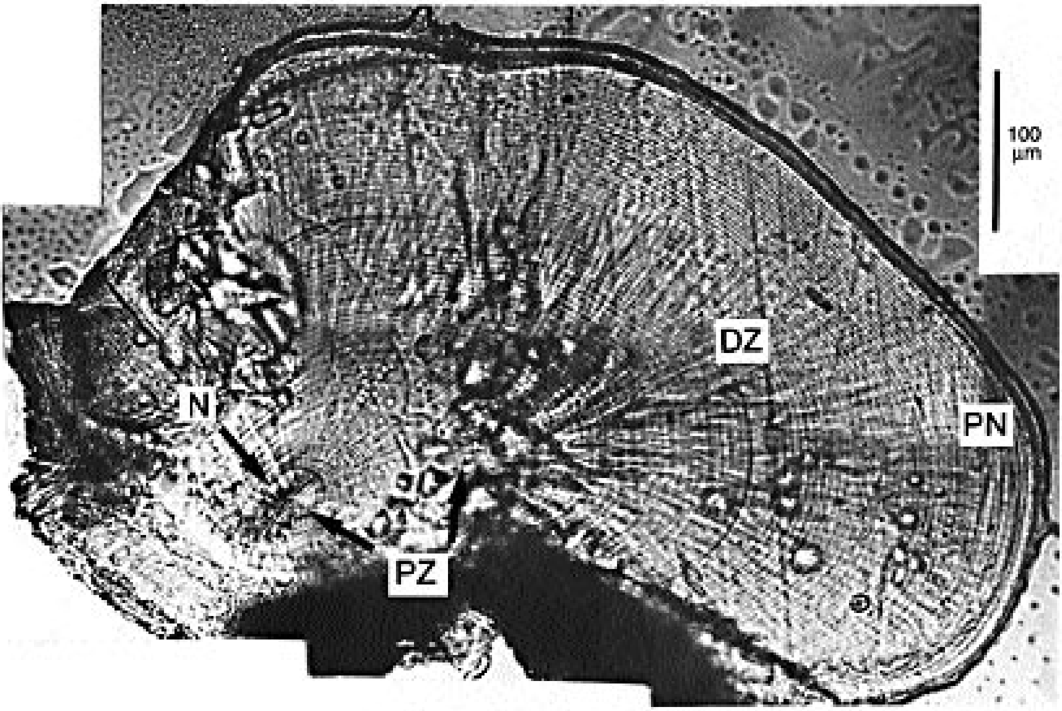

Fig. 1: Light micrograph of the ground statolith from *Illex coindetii* caught in the Strait of Sicily (immature female, 96 mm *ML*, age 109 days). Nucleus (N), postnuclear zone (PN), dark zone (DZ), peripheral zone (PZ)

ground and polished on both sides with wet waterproof sandpaper (600 and 1 000 grit), embedded in Canada balsam and covered with a coverslip. Prepared slides were placed in an oven at 80–90°C for one hour to dry the balsam and to improve the resolution of growth increments. Statolith microstructure was examined under transmitted light using a compound optical microscope (× 400).

Daily growth increments have not yet been validated in *Illex coindetii* statoliths. However, validation and culture studies have shown that statolith increments are produced daily in several other squid species, including the congener *Illex illecebrosus* (see Jackson 1994 for a review). As increments in *Illex coindetii* statoliths closely resemble those of *Illex illecebrosus*, they are for the current purposes assumed to be laid down daily. The total number of growth increments within each statolith was therefore taken to represent squid age in days. Hatching dates were back-calculated.

Growth increments were counted from the nucleus to the edge of the dorsal dome, using two independent methods. One author (AIA) employed the "conventional" reading method using an eye-piece micrometer (Dawe and Natsukari 1991), whereas the other authors employed the image-analysing system (IAS). Results obtained with the first method are herein referred to as "conventional" (C_{res}), and those obtained with the second method as "automatic" or "IAS" (IAS_{res}).

The IAS consisted of a Pentium processor PC with two monitors, a monochrome video camera, and a monochromatic frame-grabber board upgraded to a dual monitor configuration. Specific software (OPTIMAS 1992) was used for enhancement of the statolith image and data collection. This software allows the operator to perform the basic applications given within the package and/or to build up specific applications to fulfil specific requirements. The latter approach was followed in this study, and the program IASAS-Increment Reading (Bonanno *et al.* 1996) was developed. The program identifies growth increments as a continuous series of peaks and valleys along a luminance (or "grey" values) scale. One of the most signi-

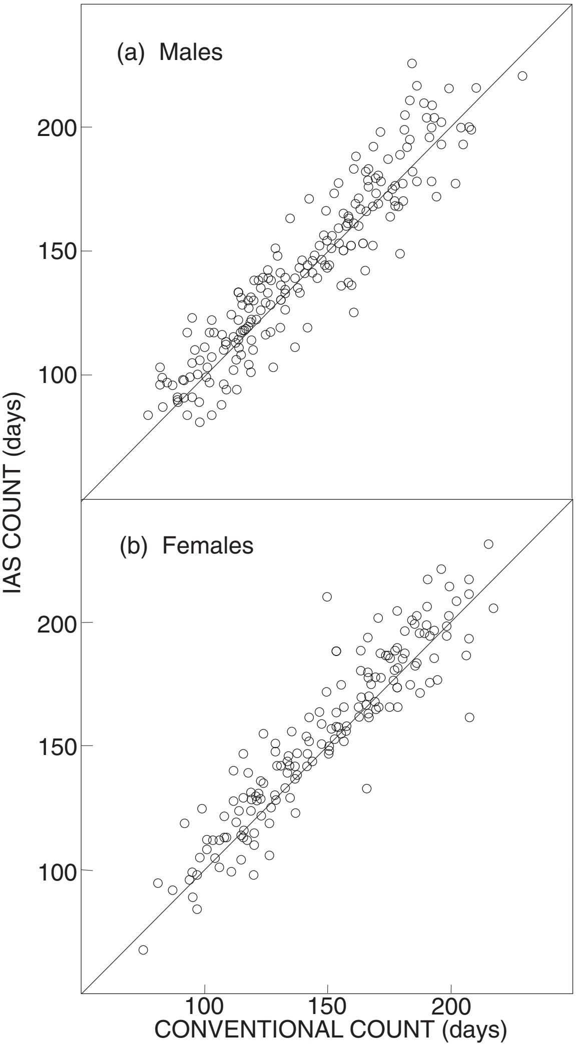

Fig. 2: Relationships between conventional and IAS counts for (a) male and (b) female *Illex coindetii*

Table I: Descriptive statistics for *Illex coindetii* used to compare conventional (C_{res}) and automatic (IAS_{res}) increment counts; *DIFF* denotes the difference between C_{res} and IAS_{res} (equivalent to age). *CV* is the coefficient of variation of the IAS_{res} and *SE* the standard error of the mean values

Statistic	*ML* (mm)	*BM* (g)	C_{res}	IAS_{res}	*CV* (%)	*DIFF*
Males (n = 201, Paired t-test: H_0 DIFF = 0; df = 200; t = 2.44; p = 0.016)*						
Minimum	46	4	77	81	0.1	−41
Maximum	171	191	230	226	18.8	36
Mean	109.8	58.9	141.6	143.8	6.6	−2.2
SE	1.93	2.8	2.42	2.47	0.26	0.91
*Females (n = 165, Paired t-test: H_0 DIFF = 0; df = 164; t = 5.145; p = 0.000**)*						
Minimum	41	3	75	68	0.7	−61
Maximum	202	246	240	256	20.2	46
Mean	131.5	78.7	150.4	155.5	6.5	−5.1
SE	3.12	4.64	2.63	2.73	0.28	1.00

* Significant ($p < 0.05$)
** Highly significant ($p < 0.01$)

ficant utilities implemented was the facility to count increments along three different axes, having the same origin and ending on the same increment, so gaining an idea of the precision associated with each age estimate. As an image of an entire statolith is rarely acquired at once, the procedure must generally be repeated on subsequent areas until the whole reading is complete, so it is actually "semi-automatic". Increments were counted on images under a 600 × magnification objective, using transmitted light.

Statoliths from the spring sample (n = 407) were used to compare IAS and conventional results. Of these, 41 (18 males and 23 females) were considered "not readable" by the IAS. This was attributed to poor preparation of some statoliths (debris in the embedding resin), but a χ^2 test was also used to test the possible presence of a sex effect on readibility. For the remaining 366 statoliths, the difference between the conventional and the automatic results ($DIFF = C_{res} - IAS_{res}$) was tested separately for males and females after an analysis of variance was performed to test for any significant sex effect. An approximation of the coefficient of variation was calculated for each IAS estimate as follows:

$$CVIAS = (IAS_{max} - IAS_{min})/IAS \times 100 ,$$

where IAS_{max} and IAS_{min} are maximum and minimum values of the IAS.

Table II: Analysis of variance table. The columns give degrees of freedom, the mean square for each source of variation, the *F* statistic and the *p*-value corresponding to each *F* statistic

Source of variation	*df*	Mean square	*F*	*p*
Sex	1	776.66	4.67	0.031*
Hatching period of males	4	356.77	2.18	0.072
Hatching period of females	4	56.92	0.339	0.852
Maturity stage of males	2	305.87	1.84	0.161
Maturity stage of females	2	8.06	0.048	0.953

* Significant ($p < 0.05$)

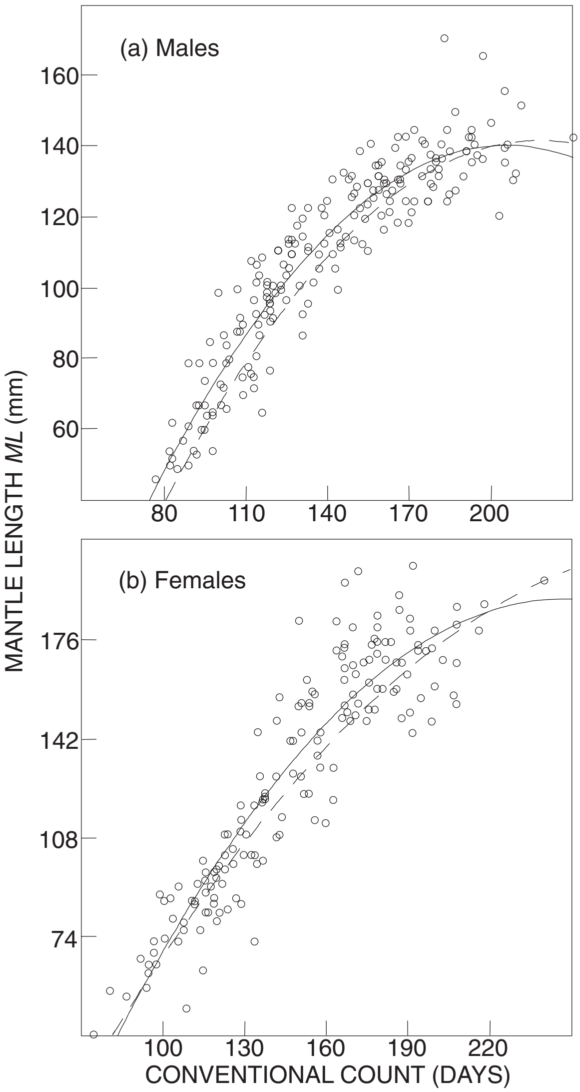

Fig. 3: Relationship between mantle length and the number of increments (age, days) for (a) male and (b) female *I. coindetii*, according to the models and coefficients listed in Table IV. The dashed lines represent the age curve determined using the IAS, the solid lines the conventional count

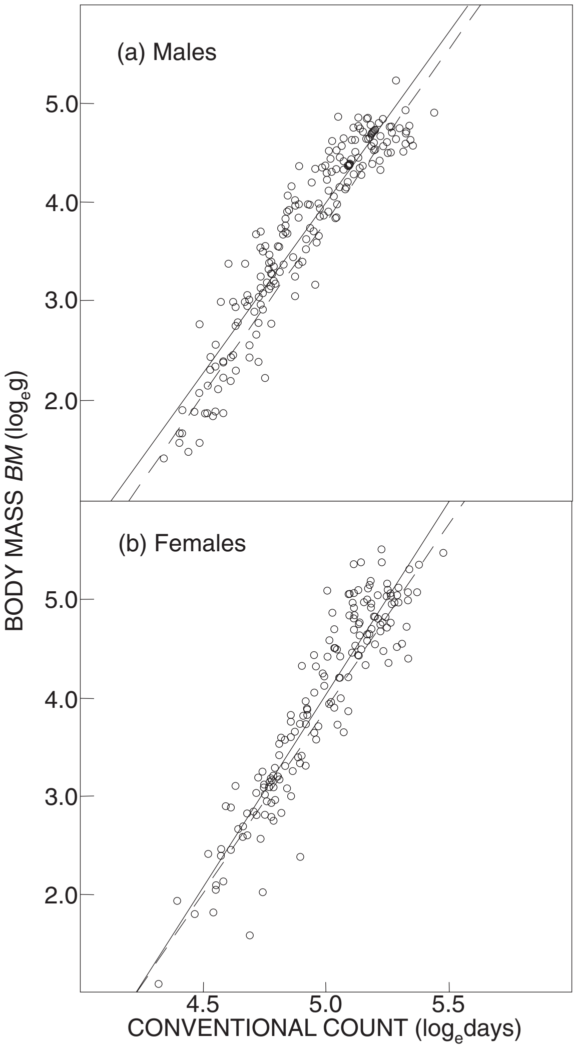

Fig. 4: Relationship between body mass and the number of increments (age, days) for (a) male and (b) female *I. coindetii*, according to the models and coefficients listed in Table IV. The dashed lines represent the age curve determined using the IAS, the solid lines the conventional count

Five hatching periods obtained by back-calculation (using conventional reading results; Period 1 = August-September 1994; Period 2 = October 1994; Period 3 = November 1994; Period 4 = December 1994; Period 5 = January 1995) were also examined to test any related increment-counting differences.

Absolute (daily) growth rates (*AGR* = *SIZE*/*AGE*) were computed to assess possible differences between different hatching and maturation periods. *ML* and *BM* were both considered as size indicators, and *AGE* was taken as the result of the conventional reading technique.

Coefficients of the length-mass relationships were computed for each sex on the base of the classic allo-

Table III: Mean absolute daily growth rate (*AGR*) in mantle length (*ML*, mm) and body mass (*BM*, g) for male and female *Illex coindetii* hatched in different periods. %*M* and *n* denote the percentage of mature squid and the total number of squid. *SE* of mean in parenthesis

Hatching period	Males				Females			
	n	%Mat	AGR_{ML}	AGR_{BM}	*n*	%Mat	AGR_{ML}	AGR_{BM}
1. September 1994	17	100	0.689 (0.011)	0.550 (0.015)	19	89.5	0.856 (0.018)	0.775 (0.054)
2. October 1994	50	98.0	0.780 (0.008)	0.593 (0.016)	47	80.8	0.941 (0.013)	0.700 (0.030)
3. November 1994	44	59.1	0.807 (0.009)	0.415 (0.020)	41	24.4	0.894 (0.020)	0.457 (0.039)
4. December 1994	65	9.2	0.809 (0.012)	0.238 (0.013)	45	0	0.803 (0.016)	0.220 (0.017)
5. January 1995	25	0	0.686 (0.018)	0.102 (0.009)	13	0	0.670 (0.030)	0.100 (0.013)

metric (power) formula after $\log_e$ transformation of the data. The variable *LRSIZE* = $\ln BM/\ln ML$ was computed and tested as a single condition index.

Based on the necessary assumption (one discrete sample was used for comparison) of infra-cohort invariance within the whole growth pattern, possible differences between size-at-age curves computed with conventional reading results and IAS results were tested, using *ML*, *BM* and *LRSIZE* as size indicators. After a preliminary exploratory analysis on scatterplots, interpolated by a smoothing technique (Lowess, in SYSTAT 1992), five models were tested: the quadratic and linear models to investigate *ML*, the log-linearized power model to investigate *BM*, the von Bertalanffy model to investigate *LRSIZE* and the Gompertz model for all three parameters. A weighted regression was employed for IAS age estimates, where the weighting factor *WF* corresponded to the reciprocal of the IAS result variation coefficient (i.e. *WF* = 1/*CVIAS*). The mean square error was used as an index to compare the goodness of fit of the different models and ageing methods.

Statoliths from the autumn sample ($n = 242$) were examined conventionally to count growth increments within different growth zones of the statolith.

RESULTS

Growth increments within statolith microstructure can be grouped into three growth zones, namely the inner postnuclear zone, the median dark zone and the outer peripheral zone (Fig. 1). They start depositing from the rounded nucleus (maximum diameter 20–24 µm). The postnuclear zone is translucent under transmitted light, and growth increments are narrow (1.8–2 µm). In 95% of the squid examined, there was a prominent check within the inner postnuclear zone (Fig. 1). The number of increments between the nucleus and this check ranged from 7 to 20 (mean 11.4, *SD* = 2.44). The total number of growth increments within the inner postnuclear zone (PNZ) was analysed separately for squid captured in spring and autumn. Within each sample, the frequency distribution was unimodal and close to a normal distribution. Mean values were similar for males and females (Spring: male PNZ = 32.7, *SD* = 3.74; female PNZ = 33.4, *SD* = 3.92. Autumn: male PNZ = 38.7, *SD* = 4.02; female PNZ = 38.5, *SD* = 4.06), but different when comparing the two seasons. At the transition between the inner postnuclear and the dark zones, there was no check. Growth increments decreased in width in the periphery of the PNZ, then broadened rather sharply in the inner part of the dark zone. The dark zone was opaque and bore the widest growth increments (4–5 µm), but then the increments narrowed gradually (to 2–2.5 µm) in the outer part of the peripheral zone. The transition between dark and peripheral zones could not be distinguished by increment width (Fig. 1).

Conventional age-at-length data analysed in relation to different maturity stage gave slightly different results for the spring and autumn samples, most evident when considering females. Modal sizes of largest squid (females of 160–170 mm *ML*, males of 130–140 mm *ML*; Jereb and Ragonese 1995a) was at ages of about 180 and 200 days respectively. First maturity in females was at an age of 120 days in the autumn sample and at 150 days in the spring sample. Age of the spawning females (autumn sample) ranged between 150 and 178 days. Males start maturing a little earlier than females. The youngest mature males in the samples analysed were about 130 and 120 days old in the spring and autumn samples respectively.

The hypothesis that sex had a significant effect on statolith readability by the IAS was rejected ($\chi^2 = 1.60$, $p > 0.2$), indicating that problems encountered by the system were probably related to statolith preparation.

A graphic comparison of the results of conventional and IAS increment counting is presented in Figure 2. In both cases, there was an almost continuous linear correlation and no particular increase of variance. Statistics relevant to the analysis are given in Table I. Although the average values of the counts were very close for the two methods, the difference variable value (*DIFF*) is higher in females than males, whereas the approximate mean coefficient of variation appeared to be the same in both sexes. The different size range of males and females could be considered one possible source of the discrepancy between the results for the two sexes, but there was no significant trend in the relationship between *DIFF* and size (*ML*, *BM* and *LRSIZE*) using a linear (Pearson) correlation ($0.066 < p < 0.895$). Results indicated that IAS counts tend to exceed conventional counts. A paired *t*-test showed that the IAS counts were statistically higher ($p < 0.05$) than conventional ones (Table I), with a mean value of +2 and +5 increments for males and females respectively. Results of the ANOVA carried out to test whether the differences related to hatching time and maturity stage are provided in Table II. For both sexes, the differences appeared to be independent of maturity stage and hatching period.

Absolute growth rates (*AGR*) for the various hatching periods are listed in Table III. *AGR* in terms of mantle length was lower in juveniles and old specimens of both sexes, whereas *AGR* in terms of body mass increased continuously with age, consistently in females and with a trend in males. The onset of maturity appeared to have no particular effect on *AGR*, with the exception of a slight decrease in the oldest specimens. As expected, males matured earlier than females, and *AGR* diverged after this critical period. Mature females grew faster than males.

The estimated length-mass relationships were similar to those computed previously by Ragonese and Jereb (1992), and yielded the results below.

Sex	Intercept	*SE* intercept	Slope	*SE* slope	r^2
Males (n = 201)	–11.170	0.205	3.198	0.044	0.964
Females (n = 165)	–9.674	0.141	2.830	0.029	0.983

Descriptive statistics computed for the *LRSIZE* variable show how the index tends to reduce the differences in size and to allow an easier comparison between sexes.

Statistic	Males	Females
Number	201	165
Minimum	0.375	0.296
Maximum	1.028	1.048
Mean	0.793	0.814
Standard error *SE*	0.011	0.012

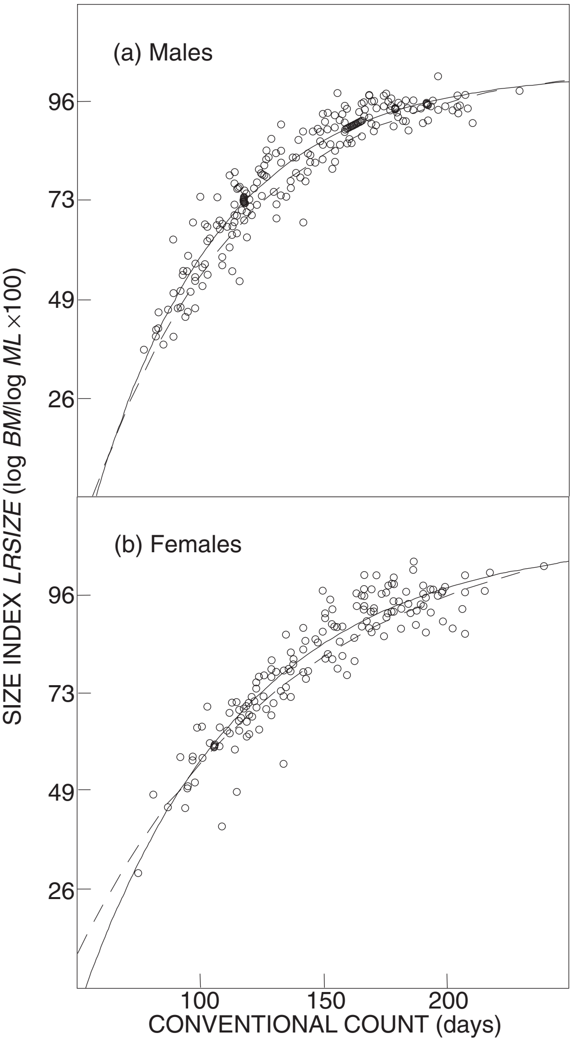

Fig. 5: Relationship between *LRSIZE* index and the number of increments (age, days) for (a) male and (b) female *I. coindetii*, according to the models and coefficients listed in Table IV. The dashed lines represent the age curve determined using the IAS, the solid lines the conventional count

Size-at-age curves computed using the different models are shown in Figures 3–5, and coefficients of the estimates are provided in Table IV. The best fit was observed for the von Bertalanffy model with *LRSIZE* as size indicator for both IAS and conventional age-determination methods. The Gompertz model fitted the data poorly and no reasonable esti-

Table IV: Size-at-age of *Illex coindetii* males and females which gave the minimum mean square error (*MSE*) of the models tested. The standard error of the estimated coefficient is given in parenthesis. VBF = von Bertalanffy form

Sex	Ageing method	Model	*MSE*
		Variables: y = mantle length (mm); x = age (days)	
Males	Conventional	Quadratic: $y = -0.0058\,x^2 + 2.392x - 106.2$ *SE*s – (0.0006) (0.1610) (11.27)	89.4
	IAS	Quadratic: $y = -0.0054\,x^2 + 2.355x - 114.9$ *SE*s – (0.0007) (0.2244) (16.32)	45.8
Females	Conventional	Quadratic: $y = -0.0056\,x^2 + 2.768x - 151.9$ *SE*s – (0.0010) (0.2971) (21.88)	240.8
	IAS	Quadratic: $y = -0.0035\,x^2 + 2.113x - 109.0$ *SE*s – (0.0010) (0.3130) (23.71)	72.4
		Variables: y = body mass ($\log_e$ g); x = age ($\log_e$ days)	
Males	Conventional	Power-log: $y = -13.454 + 3.493x$ *SE*s – (0.453) (0.092)	0.105
	IAS	Power-log: $y = -13.802 + 3.524x$ *SE*s – (0.638) (0.130)	0.054
Females	Conventional	Power-log: $y = -15.466 + 3.899x$ *SE*s – (0.618) (0.124)	0.137
	IAS	Power-log: $y = -14.799 + 3.737x$ *SE*s – (0.736) (0.147)	0.043
		Variables: y = LRSIZE (($\log_e$ g)/($\log_e$ mm)); x = age (days)	
Males	Conventional	VBF: $y = 1.037*(1-\exp-0.0194*(x-56.4))$ *SE*s (0.0148) (0.0011) (1.92)	0.0024
	IAS	VBF: $y = 1.064*(1-\exp-0.0161*(x-54.9))$ *SE*s (0.0248) (0.0013) (3.12)	0.0015
Females	Conventional	VBF: $y = 1.106*(1-\exp-0.0147*(x-51.7))$ *SE*s (0.0214) (0.0010) (2.68)	0.0031
	IAS	VBF: $y = 1.162*(1-\exp-0.0111*(x-41.5))$ *SE*s (0.0281) (0.0007) (3.53)	0.0011

mates for any of the size indicators could be obtained with that model.

DISCUSSION

The postnuclear zone of the statolith is considered to represent the larval stage of *Illex* (Balch *et al*. 1988, Arkhipkin 1990). In this study, the mean number of growth increments of the autumn sample (mainly summer-hatched squid) indicated a larval stage of about 39 days, similar to that of African populations of *I. coindetii* (Arkhipkin 1996). The paralarval period of the spring sample (mainly autumn and winter-hatched squid) was 33 days. This may be related to more favourable conditions during paralarval development.

Results obtained with the IAS and *ad hoc* developed program were very satisfactory in terms of time saving, considering the time taken for both increment reading and automatic and direct storage/analysis of data. Moreover, facility to obtain a range of measures within a "single" reading event permits weighting of the estimates, which is not possible with the conventional method. Differences between the estimates obtained by the two methods were statistically significant, but small. As for the IAS estimate ranges, which in some cases were high, there tends to be considerable variation in the readability of statoliths among different species and within the same species (Jackson 1994). This problem (poor readability) is likely to be one of the main limitations of an IAS. The necessity to change focus repeatedly or to "extrapolate" in difficult cases is a more difficult problem to overcome with IAS than with the conventional method. Readability of *I. coindetii* statoliths was acceptable, but problems caused by debris within the embedding resin and by overground zones could not be discounted as sources of variability during increment reading. In view of the unmistakable advantages of the IAS as an analytical method, improvement of preparation techniques is an important goal to pursue in future research.

The new condition index (*LRSIZE*) used in the size-at-age models minimized sex- and ageing-method-related differences in the plots, improving growth pattern comparisons between sexes and age determination methods. This index consequently yielded better results than either *ML* or *BM*, in both absolute and relative terms.

Different models appear better suited to define growth during various phases of the life history of most squid species studied (see Jackson 1994 for a review), including *I. coindetii* (Arkhipkin 1996, Gonzalez *et al*. 1996). The need to develop a more comprehensive model for squid growth is an important goal of future research (Ragonese *et al*. 1991, Jackson 1994). The present study contributes valuable information towards realizing this goal.

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