Automated Enumeration by Computer Digitization of Age-0 Weakfish *Cynoscion regalis* Scale Circuli*

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There has been extensive use of daily otolith growth increments in age and growth studies of age-0 fishes (Campana and Neilson 1985). Recently, the daily ageing method has been extended to scales (Szedlmayer et al. In press). However, visually counting increments is tedious, time consuming, and subject to human error (Rice 1987). In an effort to automate the counting of increments or daily circuli in scales of age-0 juvenile weakfish *Cynoscion regalis*, a microcomputer-based system was used to digitize the video image of a scale, store the light intensities from a radial transect, and count circuli. Circuli were also counted visually to verify the accuracy of the software. Others have used microcomputer-based systems to aid in increment counting (Tzeng and Yu 1988, Thorrold and Williams 1989, Karakiri et al. 1989), but to our knowledge the present algorithm is the first method that completely automates increment counting with a high degree of accuracy.

Materials and methods

Age-0 juvenile weakfish were collected from the York River, Virginia (for further collection methods, see Szedlmayer et al. 1990). Fish, 50–140 mm standard length (n = 45), were anesthetized with tricane methanol sulfate (50 mg MS-222/L seawater), and scales removed from just below the midbody lateral line curve. The scales were placed on a glass slide in water and cleaned with a paint brush, then permanently mounted with a methacrylate copolymer, and covered with a glass cover slip (Flo-Texx liquid cover slip, Lerner Lab.).

For the visual method, scale circuli were counted twice by the same reader, along a central radius from the focus to the edge, on the anterior side, at 125× magnification on an Olympus BH-2 microscope. If counts were not the same, they were counted a third time. Only 1 out of 45 required a third count, and for that scale the counts that were the same were used for comparison with automated computer counts.

For automated counting, scales were digitized using the same magnification and radius as visual counts. Scale images were detected by a Ikigami ITC-510 video camera (625-line resolution) mounted on the microscope, digitized by a Matrox PIP-512B image analyzer, and stored in computer memory in a matrix of 512 × 512 picture elements (pixels) with 256 gray levels for each pixel. A PC-AT 286 computer with a 10-meg Hz processor, 1-meg RAM, and math coprocessor controlled the image analyzer. A 40-megabyte hard disk and a floppy disk drive were used for image and data storage.

Once the image was digitized, it was displayed on a Panasonic PM 205A video monitor (1000-line resolution) and a mouse was used to control the movement of a cursor mark projected on the image. The cursor was then positioned to select two points defining a transect from the focus to the edge of the scale perpendicular to the circuli (Fig. 1). Light intensities (gray levels) of three transects, each one pixel apart and one pixel wide, were simultaneously stored to the hard disk. Each transect was then analyzed using a Fortran program to identify and count scale circuli. The algorithm applied a moving average (7, 8, 9, and 10 pixel averages were tried) to smooth each transect and then searched for local minima (e.g., 10 pixels on either side of the inflection point corresponds to a local minimum within a search width of 20 pixels; search widths of 18, 20, 22, and 24 pixels were tried). The Fortran counting algorithm compared adjacent pixels and determined if light intensity increased or decreased. Subsequently, an increment was counted only after the following two criteria were satisfied: (1) an inflection point was detected, i.e., a change in light intensity from decreasing to increasing, and (2) the inflection point was the minimum light intensity within the specified search width. Depending on the scale size, one to five images were needed to complete a scale count (i.e., with smaller scales the complete scale was included in the digitized image, but with larger scales several images were needed at the same magnification to include all circuli from the focus to the edge). The computer counts (aver-

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Figure 1
Photograph of a weakfish scale with 29 circuli (29 circuli + edge = count of 30). Arrow depicts counting transect from focus to perimeter along the anterior growing side (transect line = 0.5mm).

ages of the three counts from individual scales) were then regressed against the visual counts.

Results and discussion

Application of a nine-pixel smoothing interval on the transect data combined with a 20-pixel local-minima search width, produced the highest coefficient of determination (r^2 0.99) between the automated and visual counts (Fig. 2). Other combinations of smoothing interval and search width resulted in lower coefficients. The present program allows adjustment of smoothing interval and search width to optimize its use with other fish species. Computer counting was approximately 3.3 times faster than visual counting. The visual method showed a slightly higher precision compared with computer counting, but savings in time more than compensates for this small increase in error (Table 1). In addition, the concentration needed and subsequent fatigue in visual counting compared with computer counting are difficult to measure, yet computer counting was considered much easier by all scale readers.

Microcomputer systems that can digitize increments from scales, otoliths, and other bony structures have been previously reported and demonstrated potential for automated counting (McGowan et al. 1987). The present system makes an advance over other systems by using the local-minimum method of increment identification. Previous methods usually use threshold-light intensity levels to identify increments that may produce discrepancies between computer

Table 1
Comparison of counts and counting times of scale circuli from weakfish *Cynoscion regalis* between computer and visual methods from a random subsample of those counted. Each scale was from a different fish.

	Visual counts				Computer counts				
Scale	1st	2d	3d	Time (s)	1st	2d	3d	$\bar{x}$	Time (s)
1	36	36		46	38	38	38	38.0	13
2	32	32		42	31	31	32	31.3	16
3	32	32		41	31	32	33	32.0	13
4	37	37		46	39	38	38	38.3	12
5	32	32		42	31	31	32	31.3	16
6	30	30		39	30	30	30	30.0	12
7	50	51	50	106	51	52	50	51.0	13
8	60	60		82	64	62	61	62.3	35
9	46	46		51	48	47	49	48.0	19
10	43	43		45	44	44	44	44.0	15
11	58	58		94	60	60	60	60.0	26
12	64	64		74	66	67	65	66.0	25
13	60	60		80	61	58	61	60.0	31
14	49	49		56	50	50	50	50.0	15
15	58	58		69	60	58	58	58.7	14
				$\bar{x}$ 60.9					$\bar{x}$ 18.3
				SD 21.7					SD 7.4

Paired t-statistic = 8.85, t critical value (level = 0.05) = 2.145, therefore there is a significant difference between counting time.

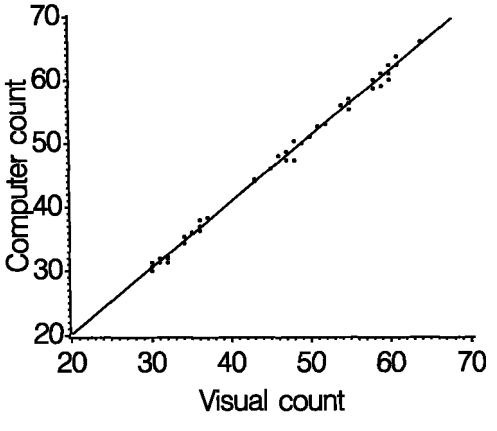

Figure 2
Linear regression of computer automated count on visual count of scale circuli from weakfish, *Cynoscion regalis* (N 45 fish, r^2 0.99, $y = 1.03x - 0.3$).

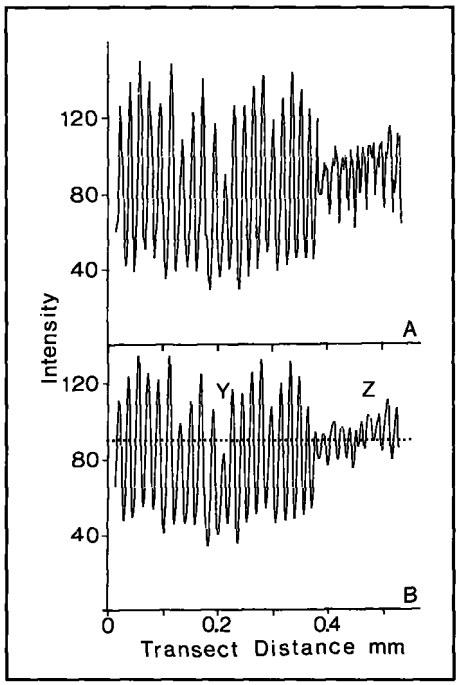

Figure 3
Light intensities along a transect taken from a single scale by distance in mm. (A) Light intensities of a single transect. (B) Smoothed data using a nine-point moving average. Dashed line represents a threshold light intensity of 90. Lowering the threshold to include peak Y would eliminate peaks under Z.

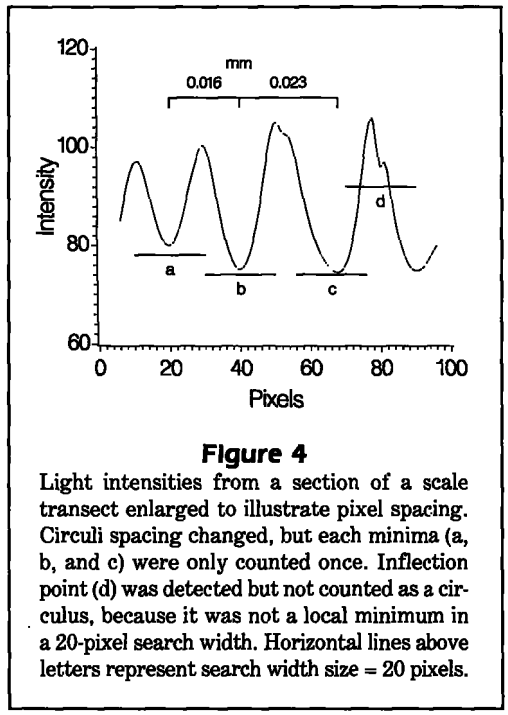

Figure 4
Light intensities from a section of a scale transect enlarged to illustrate pixel spacing. Circuli spacing changed, but each minima (a, b, and c) were only counted once. Inflection point (d) was detected but not counted as a circulus, because it was not a local minimum in a 20-pixel search width. Horizontal lines above letters represent search width size = 20 pixels.

and visual counts, because background intensity levels change as the transect moves across the otolith or scale. Thus, some areas may be counted incorrectly when they fall below the selected threshold level as shown for a transect across a weakfish scale (Fig. 3). The local-minimum method solves this problem, since identification of increments is only dependent on adjacent pixel-light intensity levels. The local-minimum method also responds to changes in increment spacing: as increment spacing increases (measurements from one typical scale ranged from 10.3 to 22.9 m^{-6}) the algorithm moves greater distances (more pixels) along the transect, but does not count more increments until another minimum is detected. For example, in Figure 4, increments a, b, and c were each counted as one circulus despite changes in circuli spacing, but inflection point d was not counted because it was not a local minimum within a 20-pixel search width.

Increments narrower than the selected search width would cause errors (e.g., microincrement spacing width = 10 pixels, but search width = 20 pixels). However, this problem can be corrected in three ways: (1) reduce the search width; (2) increase the magnification of the scale or otolith, e.g., from 125 to 400; or (3) increase the number of pixels between increments, i.e., increase the resolution of your system as discussed below. In addition, the true limit of counting narrow increments is not the algorithm, but the resolution limit of the light microscope. After projection of the image onto the video monitor, one pixel corresponds to an actual distance of about 0.2 m^{-6} (with the light microscope at 1000×). This 0.2 m^{-6} size is the maximum theoretical resolution of any light microscope (Eastman Kodak Co. 1980). In addition, "...the functional limits are invariably higher than those derived theoretically" (Campana et al. 1987), therefore several pixels may be present even between the smallest detectable increments. Other advantages of the present system, as well as other systems, include elimination of data entry and associated transcriptional errors, and establishment of repeatable criteria for ageing of fishes.

A disadvantage of the present system and other similar systems is that clearly defined increments are needed. This was not a problem with weakfish scales because circuli are distinct (Fig. 1), but application to otolith increments may need further refinement. Another disadvantage is that most systems need multiple images to complete a transect reading of a single scale or otolith, which may increase processing time and errors. The multiple-image-per-transect problem results from a limiting number of pixels (512 × 512 pixels) in our digitizer, such that lower magnifications (25 or 40) that would encompass the entire transect do not contain enough pixels for accurate identification of increments. Systems with greater pixel resolution (e.g., 1024 × 1024) would alleviate this problem, and are now commercially available.

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