

NOAA Technical Memorandum NMFS

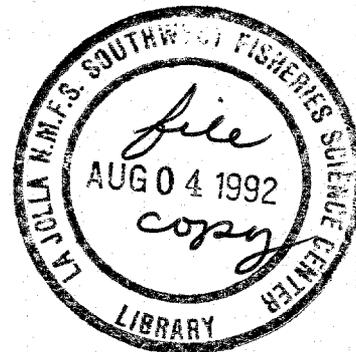


JUNE 1992

**DOCUMENTATION OF THREE COMPUTER PROGRAMS  
USED TO ASSESS DAILY AGE FROM THE HARD  
STRUCTURES OF ANIMALS**

Thomas E. Laidig

Donald E. Pearson



NOAA-TM-NMFS-SWFSC-168

U.S. DEPARTMENT OF COMMERCE  
National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Southwest Fisheries Science Center

## NOAA Technical Memorandum NMFS

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## ABSTRACT

Documentation for the three main daily ageing programs used at the Tiburon Laboratory is discussed. AGE1.EXE is a data collection program, designed to gather data from multiple otoliths and multiple transects from the same otolith. EDIT.EXE is a data edit program for the files created by AGE1.EXE. AGE2.EXE interprets the files from AGE1.EXE and produces daily ages and birthdates for the fish being analyzed. A step-by-step procedure is laid out for each program, and a description of how each program functions is included.

## INTRODUCTION

The ageing of juvenile and larval fish can be expedited through the use of computer programs which utilize links between video cameras, digitizers, and computers. Otoliths from juvenile and larval fish can have visible growth increments<sup>1</sup> (presumed to be daily) when viewed under high magnification with light microscopy (Fig. 1). Computer programs for ageing juvenile fish were originally developed by Rick Methot (Pers. Comm., NMFS, SWFSC, La Jolla). Later, these programs were modified and new ones were created (by Don Pearson) to enumerate and analyze these increments. This document explains the functioning of these programs, both how the programs actually work and how to use each program. Copies of the programs are available from the authors.

Age is typically determined by counting the number of increments along a transect<sup>1</sup> run from the nucleus<sup>1</sup> to the outer edge of the otolith, but this is not always possible. In practice, this task is complicated by the fact that it is often impossible to read daily increments continuously along a single transect from the nucleus to the otolith edge. The transect is often interrupted by irregularities or cracks in the otolith. One way around this problem is to read several transects (perhaps from different otoliths from the same fish) and to piece them

<sup>1</sup> See Appendix A for specific definitions of these terms.

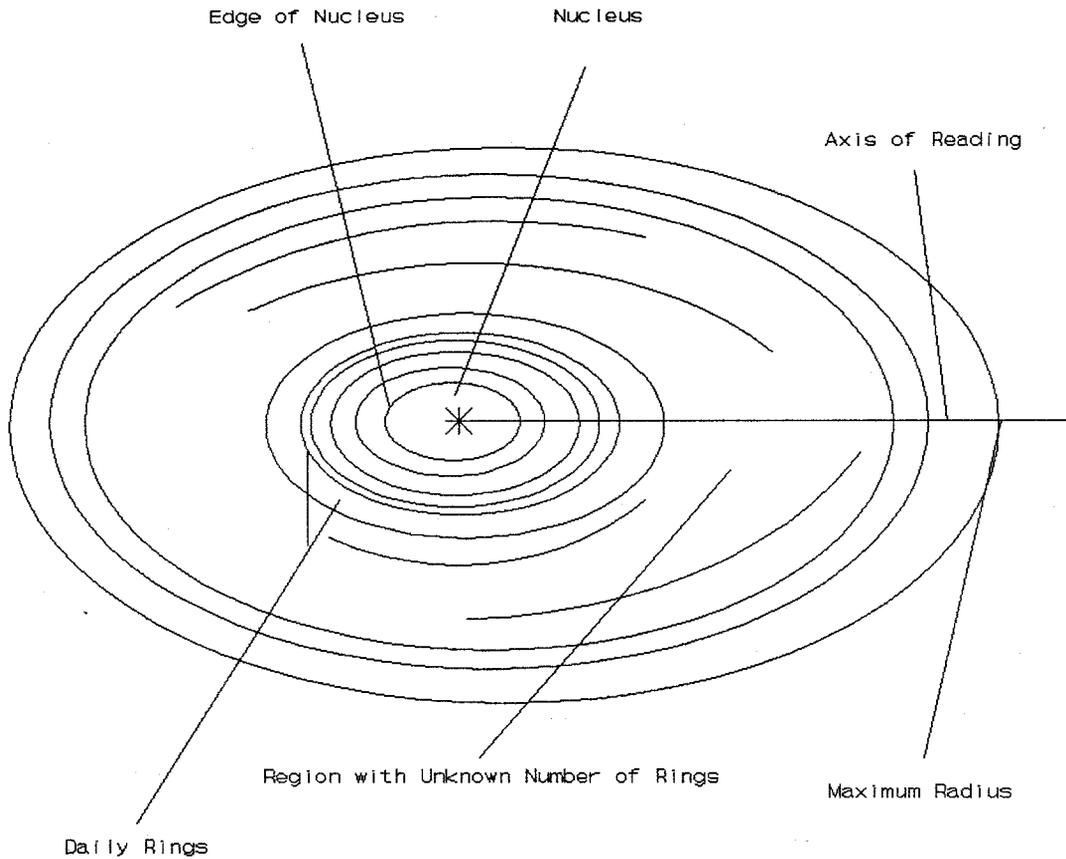


Figure 1. Diagram of a larval fish otolith displaying many of the areas described in the text. Maximum radius refers to the edge of the otolith.

together to form a single complete series for the otolith (called the computed 'best' otolith').

The programs described here (a) automate the data collection process, (b) allow editing of the resulting data, and (c) provide an algorithm for combining a number of transects into a computed

'best' otolith for an individual fish. The programs were written in Microsoft Quickbasic<sup>2</sup> and are compiled. The programs contain many different types of error traps. The program AGE1.EXE inputs measurement data and creates a raw data file for each fish (see summary on page 40). The second program (EDIT.EXE) is used to display and edit the raw data. Since the output data are in an ASCII file, it is possible to edit it with any text editor; however, interpreting the data can be a problem which EDIT.EXE resolves. The third program (AGE2.EXE) inputs a raw data file, generates the computed 'best' otolith using as many of the actual measurements as possible, and outputs the estimated final increment sizes and age of the fish.

These programs were written to simplify ageing analyses and give the user a versatile system with which to study age-related phenomena. The programs may be used to determine ages from fish otoliths, fish fin rays, fish scales, bivalve shells, and any other hard structure that contains growth increments. The measurement capabilities of this program are not limited to measuring the difference between growth increments. Other structures, such as cellular dimensions, sizes of crystalline structures, pigment spacing, etc., may be measured and these distances may be analyzed for different patterns.

<sup>2</sup> Use of trade name does not imply endorsement by NMFS.

## SET-UP

Our system uses the H.E.I. Video Coordinate Digitizer<sup>2</sup> which has an effective dimension of 995 digitizer units. When the user presses a key on the digitizer, the coordinates of the digitizer cursor are sent to the AGE1.EXE program. Although both X and Y coordinates are sent, the program only uses the X coordinates.

Otoliths are processed to make the increments more visible and, thus, possible to count. This processing includes the sanding of the otoliths to create a flat surface for viewing the increments. Next, the otoliths are etched with acid until the increments become visible under 100 X magnification (Laidig, et al. 1991; Woodbury and Ralston 1991). The equipment used consists of a microscope outfitted with a video camera and a monitor equipped with a digitizer. The digitizer interfaces with the video image and allows you to assign specific coordinates to each increment. A computer receives the coordinates from the digitizer and calculates increment distances. The program (AGE1.EXE) calculates the distance between measurements by multiplying the number of digitizer units moved by the size that each digitizer unit represents, in  $\mu\text{m}$ . Since the distance represented by the digitizer unit is different for each level of magnification, different values are used by the program for each level of magnification. The program prompts you when to send information from the digitizer to the computer. The mechanisms by which the computer converts the data from the digitizer into

readable files (i.e., operating systems, memory locations, etc.) are different for different computers, so the specific computer adaptations on our system are not discussed here.

## PROGRAM INSTRUCTIONS

### Conventions Used in This Document

To use the programs in this manuscript, you will be required to input data occasionally. There are three conventions used for specific types of data entry. The first is when a specific key is to be hit, such as the <ENTER> key. In this case, the title of the key will be capitalized and enclosed in <BRACKETS>. If certain letters or words are to be typed exactly as they appear in the text, these will again be capitalized but will be enclosed in double "QUOTATION MARKS". If a term is enclosed in double quotation marks but is in lowercase letters, then you must replace the term(s) used with specific information.

### General Procedure

Otolith increments are recorded from the center of the nucleus outward using AGE1.EXE. Data are stored in a file created by AGE1.EXE. These data can be edited by EDIT.EXE. Once the data are correct, they can be processed by AGE2.EXE to

produce either a hard copy or an output file. The program prompts the reader for information. After data are input, the reader hits the <READY> key<sup>1</sup> on the digitizer (when entering increment coordinates) or the <ENTER> key<sup>1</sup> from the computer keyboard.

#### AGE1.EXE

In general, measurements of increments from a pair of otoliths occur in the following manner:

1. The overall header data<sup>1</sup> for a new fish are manually input.
2. Measurements at selected increments are made along a transect for the first otolith.
3. If required, the otolith is reprocessed (grinding and acid etching) and measurements are made along another transect. This becomes necessary when all increments from nucleus to maximum radius<sup>1</sup> cannot be read in one transect. This is repeated until the separate transects together cover the entire otolith radius.
4. Repeat Steps 2 and 3 for the other otolith. Make sure that the direction of the reading is similar between the two otoliths since the program will combine the readings of both otoliths in the final analysis.

### Starting AGE1.EXE

1. Turn on all equipment and enter the subdirectory in which you wish to work.
2. Type "AGE1", then hit the <ENTER> key to start the program.
3. Hit the <CAPS LOCK> key. The program only accepts inputs in uppercase letters.
4. The program will prompt you to press the <ENTER> key and, shortly thereafter, it will prompt you to hit the <READY> key on the digitizer pad. If the link to the digitizer is good, the Opening Menu will appear. If the link is bad, then the program will abort and you will need to determine the reason for the hardware problem.

### Introductory Menus for AGE1.EXE

1. Select "S", "R", "E", or "X" at the Opening Menu.
2. To start an analysis of a new fish, type "S", then hit <ENTER>. The program will prompt you for the overall header data.
  - A. Input the "date caught" [YY/MM/DD], then hit <ENTER>.
  - B. Input the three-digit "species code", then hit <ENTER>.

- C. Input the single "cruise identification numeral" (or letter), then hit <ENTER>.
  - D. Input "haul number" (up to three-digits), then hit <ENTER>.
  - E. Input the "specimen number", then hit <ENTER>.
  - F. Input the "fish length", then hit <ENTER>.
  - G. If all the data are correct, type "Y", then hit <ENTER>. If not, type "N", then hit <ENTER>. If "N" is typed, the computer monitor screen clears and you input the overall header data again. If "Y" is typed, you return to the Opening Menu.
3. To retrieve a previously saved file, type "R", then hit <ENTER>.
- A. Input the "filename" when prompted. If the filename exists, the program will return you to the Opening Menu. If it does not exist, the program will end. So be careful to type in the correct filename.
4. To input new data, type "E", then hit <ENTER>. You must do Step 2 or 3 before beginning this step. The program will now prompt you for the transect header data.
- A. Input "otolith identification number" (1 or 2), then hit <ENTER>.
  - B. Input "user identification number", then hit <ENTER>.

- C. Input "date of reading" [YY/MM/DD], then hit <ENTER>.
  - D. Input "magnification", then hit <ENTER>.
  - E. If the transect header data<sup>1</sup> are correct, type "Y". If something was input incorrectly, type "N", and answer the questions again.
  - F. Once the transect header data are correctly input, you will be prompted to position the digitizer cursor on the center of the nucleus.
  - G. Once the digitizer cursor is positioned, hit the <READY> key on the digitizer pad to enter the Data Entry Menu.
5. Type "X", then hit <ENTER>, to exit the program.

#### Data Entry Menu for AGE1.EXE (Fig 2)

1. To begin a transect, position the digitizer cursor on either the edge of the nucleus or an increment.
  - A. To measure the nuclear radius, position the digitizer cursor on the rightmost edge of the nucleus. Type "N", hit <ENTER>, and then hit the <READY> key on the digitizer pad.
  - B. To measure an increment first, position the digitizer cursor on the increment. Now, type "U", hit <ENTER>, and then hit the <READY> key.

ENTRY	CODE	COORD	DISTANCE	*	OPTIONS
1	0	134	0.00	*	'N':MEASURE NUCLEUS
2	99	265	57.18	*	'R':MEASURE OTOLITH RADIUS
3	3	342	90.79	*	'#':ENTER # OF INCREMENTS
4	2	417	123.53	*	'B':BACKUP TO PREV. COORD.
5	-1	544	178.97	*	'M':MOVE STAGE
6	4	635	218.69	*	'U':UNKNOWN # OF INCREMENTS
7	3	707	250.11	*	'A':ABORT SESSION (NO SAVE)
				*	'S':SAVE CURRENT TRANSECT
				*	
				*	ENTER CODE?
				*	*****

Figure 2. A typical computer monitor screen display created while using AGE1.EXE. Options on the left refer to options available while entering a transect. Code gives information about how the transect was read. Under code, 0 = starting point at center of nucleus, 99 refers to nuclear radius, -1 refers to a section of unknown number of increments, and any other number refers to the number of increments used in a measurement group. Coord refers to the digitizer coordinates of the corresponding increment. Distance refers to the linear distance ( $\mu\text{m}$ ) from that increment to the center of the nucleus.

2. To measure the number of increments, position the digitizer cursor a few increments to the right of the starting point. Now, input the number of intervening increments counted into the computer and hit the <READY> key (this set of increments is referred to as a measurement group<sup>1</sup>).
3. Repeat Step 2 until the edge of the video image screen, the edge of the otolith, or an area without visible increments is encountered.

4. Once the edge of the video image screen is encountered, the microscope stage will have to be physically moved to continue the transect.
  - A. Carefully identify the increment on which the digitizer cursor is positioned.
  - B. Slowly move the stage so that the noted increment moves to the left side of the video image screen.
  - C. Reposition the digitizer cursor to the same position on the increment as it was previously.
  - D. Type "M", hit <ENTER>, and then hit the <READY> key.
5. When the edge of the otolith is encountered, position the digitizer cursor on the edge, and type "R", hit <ENTER>, and then hit the <READY> key.
6. When an area without visible increments is encountered, the transect is either ended (by typing "S", hitting <ENTER>, and then hitting the <READY> key) or the area is skipped over.
  - A. Position the digitizer cursor on the visible increment to the left of the unreadable area.
    - (1) If this adds additional increments to the transect, then input the number of increments from the last position to this position.

- (2) If the right end of the unclear area is off the video image screen, then move (by following Step 4) the digitizer cursor position more to the left.
    - (3) If this still does not allow the right side of the unclear area to be seen, then continue to Step 6.D.
  - B. Now, move the digitizer cursor to the first visible increment after the unreadable area.
  - C. Type "U", hit <ENTER>, and then hit the <READY> key.
  - D. If the right edge of the unclear area cannot be seen, then position the digitizer cursor to some identifiable structure (a crack, a dark area, a partial increment, etc.) on the right side of the video image screen.
    - (1) Now, using this structure as a marker, perform a move (Step 4).
    - (2) Repeat Step 6.D.(1) until the right edge of the unclear area is visible on the video image screen.
    - (3) Repeat Step 6.B. and Step 6.C.
7. If you notice that an increment was missed or that some data were input incorrectly, use the backup ("B") command to correct it.

- A. First, reposition the digitizer cursor on the correct increment to which you want to go back.
  - B. Type "B", then hit <ENTER>.
  - C. Input the "entry number" of the increment to which you wish to return, and hit <ENTER>.
8. To end a transect without saving, type "A", then hit <ENTER>.
    - A. You will now be asked if this is correct.
      - (1) If yes, type "Y", then hit <ENTER>, and the program will end without writing to the disk.
      - (2) If no, type "N", then hit <ENTER>, and the program will continue as before.
  9. To end and save a transect, type "S", then hit <ENTER>.

This will return you to the Opening Menu.
  10. Repeat Steps 1-9 until the entire otolith is read.

#### EDIT.EXE

This program is used to edit and display the data files created by AGE1.EXE. Before using this program, we suggest that you print a hard copy of the data file, and that you put a copy of the file to be edited in a different subdirectory, since you may scramble your data.

Starting EDIT.EXE

1. Turn on all equipment and enter the subdirectory in which you wish to work.
2. Type "EDIT", then hit <ENTER> to start the program.
3. Hit the <CAPS LOCK> key. The program only accepts inputs in uppercase letters.

Menus for EDIT.EXE

1. Examine Figure 3 to select a choice.
2. For instructions, type "I", then hit <ENTER>.
3. To retrieve a file for editing, type "R", then hit <ENTER>.
  - A. Input the "name of the file" you would like to retrieve, then hit <ENTER>.
4. To save a file, type "S", then hit <ENTER>.

'I' - The instruction screen  
'R' - Retrieve a file  
'S' - Save a file  
'D' - Display a file either to the printer or the screen  
'X' - Exit the program  
'T' - Switch a transect between otoliths  
'C' - Change a value within a transect  
'E' - Erase an entire transect  
'M' - Change the maximum radius value in one otolith  
'A' - Add an entire transect manually

Figure 3. The options available while using EDIT.EXE.

5. To display a file, type "D", then hit <ENTER>.
  - A. Type "P" to print the file.
  - B. Type "S" to display the file on the screen.
6. To exit the program, type "X", then hit <ENTER>.
7. To switch a transect from one otolith to another, type "T", then hit <ENTER>.
  - A. Input the "transect number" to be changed, and hit <ENTER> or, to return to the Opening Menu, type "99", then hit <ENTER>.
  - B. You will be prompted to input a yes ("Y") or a no ("N") to the question of whether the transect contains the nuclear radius.
8. To erase an entire transect, type "E", then hit <ENTER>.
  - A. You will be asked if you really want to do this. If yes, type "Y", then hit <ENTER>. If no, type "N", then hit <ENTER>.
  - B. If yes, input the "transect number" and hit <ENTER>.
9. To change a value within a transect, type "C", then hit <ENTER>.
  - A. You will be prompted for any changes to the overall header data.
    - (1) If you want to change the overall header data, type "Y", then hit <ENTER>.

- (a) Input new "sample date", and hit <ENTER>, or type the number "0" to keep the same date.
  - (b) Input new "species code", and hit <ENTER>, or type the number "0" to keep the same code.
  - (c) Input new "haul number", and hit <ENTER>, or type the number "0" to keep the same haul number.
  - (d) Input new "specimen number", and hit <ENTER>, or type the number "0" to keep the same specimen number.
  - (e) Input new "fish length", and hit <ENTER>, or type the number "0" to keep the same fish length.
- (2) If no, type "N", then hit <ENTER>. You will return to the Opening Menu.
- B. Once the transect header data are correct, input the "measurement number" you wish to change, and hit <ENTER>.
  - C. To complete the changes, type "99", then hit <ENTER>.
  - D. You will return to the Opening Menu.
10. To change a maximum radius, type "M", then hit <ENTER>.
- A. Input the "otolith number", and hit <ENTER>.
  - B. Input the new "otolith radius", and hit <ENTER>.

11. To add a new transect, type "A", then hit <ENTER>.
  - A. You will be asked if the new transect will contain the nuclear radius.
    - (1) If yes, type "Y", then hit <ENTER>.
    - (2) If no, type "N", then hit <ENTER>.
  - B. You will be asked if the new transect will contain the maximum radius.
    - (1) If yes, type "Y", then hit <ENTER>.
    - (2) If no, type "N", then hit <ENTER>.
  - C. Input the "date of reading" for the new transect, then hit <ENTER>.
  - D. Input the "reader identification number" for the new transect, then hit <ENTER>.
  - E. Input the "magnification" used for the new transect, then hit <ENTER>.
  - F. Now, input the "new data", one measurement at a time, and hit <ENTER>.
  - G. To end, type "-1", then hit <ENTER>. This will return you to the Opening Menu.

AGE2.EXE

1. Turn on the computer and printer and enter the subdirectory which contains the files created by AGE1.EXE.

2. Type "AGE2", then hit <ENTER> to start the program.
  - A. Input the "filename" when prompted.
  - B. Input whether you want an output file or not.
    - (1) Type "Y", then hit <ENTER> for yes.
    - (2) Type "N", then hit <ENTER> for no.

## PROGRAM DESCRIPTIONS, HINTS, AND CAUTIONS

AGE1.EXE

This program obtains raw measurement data from the digitizer and produces an output file for each fish examined. It performs no analysis and is strictly for data collection. This program is used to gather data from one or both sagittal otoliths from a single fish. It can be used for fish that are younger than 300 days. A related program, AGE1000.EXE, can handle data from fish up to 1000 days old, but only data from one otolith may be entered. Since the format for the two programs is similar, we will only refer to AGE1.EXE.

Use of AGE1.EXE

A copy of the file MAG.DAT should be located in the subdirectory in which you plan to work. MAG.DAT is a small data file containing the magnification calibrations of the microscope (Fig. 4). This file also contains the theoretical first increment size<sup>1</sup> for the species being studied. If the theoretical first increment size for the species in question is not known, then you should examine numerous specimens and try to determine the value, or consult the literature. If it is not possible to get this information, the result is slower operation

```
=====
4 10 25 40 100
3.18 1.268 0.4365 0.3172 0.1227
0.4
=====
```

Figure 4. The structure of the MAG.DAT file. The top row represents the magnification of the five different lenses on our microscope. The second row represents the magnification factors that correspond to each lens (the number of  $\mu\text{m}$  corresponding to one digitizer unit). These factors will vary greatly between different systems. The bottom row represents the theoretical smallest size of the first increment.

and decreased accuracy if interpolation is required near the nucleus.

The program begins by testing, and often retesting, the link between the computer and digitizer. If the link is good, the Opening Menu will appear. If the link is bad, then the program will abort and you will need to determine the reason for the problem. Check the cabling first. A loose cable is often the reason for the interruption in the signal. Restarting the program will also often clear the problem.

### Starting a New Fish Menu for AGE1.EXE

The program uses this information to assign an output filename (See Appendix B for an explanation of the file naming conventions). If a file with the same filename exists in the current subdirectory, the program aborts and returns to DOS to prevent overwriting an existing file. Every fish that is analyzed needs a distinctive filename for the program to work properly.

### Data Entry Menu for AGE1.EXE

From this menu, you are able to measure the distance from the center of the otolith to the otolith increments, while the computer records the data. All transect measurements begin from the center of the nucleus. The program logs positions measured only on the horizontal axis, and only accepts digitizer cursor positions to the right of the previous position. For this reason, rotate the image until the desired transect is horizontal and to the right of the initial digitizer cursor position. The image can be rotated by moving either the video camera, the microscope slide, or remounting the otolith on the slide.

Every transect must begin with the "N" command (nuclear radius) or the "U" command (unknown number of increments). If the transect contains the nuclear radius, this must be the first measurement, and the nuclear radius (the first complete dark ring from the center) can only be measured once. For the program to

work properly, the edge of the nucleus must be closer to the center than any other subsequent increment measurement.

If fish age (total increment counts) is the only datum of interest, then use larger measurement groups (3-5 increments) up to a maximum of 20 increments. If more than 20 increments are entered, then the computer will beep and you will need to redo the count with at least two smaller intervals. If data on increment widths are of interest, then use measurement groups that are as small as possible (1-2 increments). If the individual increment widths in a measurement group are too small (less than 0.5  $\mu\text{m}$ ), then the computer will beep and that measurement group will not be recorded. The program can be modified to accept smaller increment sizes if desired. The program averages the increment size and stores the linear distance of each increment from the center of the nucleus.

When the maximum otolith radius is input, no more measurements are accepted and you are returned to the Opening Menu.

If the backup command ("B") is used, all the data after the entry to which you backed up will be erased. To exemplify the use of the backup command, examine Figure 2. If you wanted to return to entry 4, you would position the digitizer cursor on the increment that occurred at digitizer coordinate 417. Now, type "B", "4", and then hit <ENTER>. The computer monitor screen would update, and entries 5, 6, and 7 would be erased.

When a transect is ended with the "A" command, the program is exited without saving the file and no data or partial files are written to disk.

#### Description of File Created by AGE1.EXE

The file that is produced by AGE1.EXE contains all of the header data (overall and transect), array pointers, and individual increment measurement data which are used by AGE2.EXE. In addition, it contains information about how the otolith data were entered into the computer, such as how many increments were measured in each measurement group, where gaps occurred during the ageing process, etc.

#### EDIT.EXE

This program is used to edit and display the data files created by AGE1.EXE. This program is useful for correcting data entry errors (such as incorrect header or increment data) or viewing the data without creating a hard copy. Before using this program, we suggest that you print a hard copy of the data file, and that you put a copy of the file to be edited in a different subdirectory, since you can scramble the data easily.

To determine the transect and measurement number, look at the computer monitor screen display or the hard copy. An example

of the hard copy output is located in Figure 5. Space is allocated in each file for seven transects per otolith, with transect numbers 0-6 reserved for otolith 1 and transects 7-13 reserved for otolith 2. Transects 0 and 7 contain the nuclear radius (one nuclear radius for each otolith), and the maximum otolith radius could be contained in any of the transects. The switch transect option was included because experience has shown that a transect is sometimes inadvertently assigned to the wrong otolith by the reader.

No changes are saved until the save command is issued. In this way, you can analyze the effects of the changes to the data without overwriting the file on disk. However, if you retrieve a new file without saving the old file, your changes will be lost. No measurement in an otolith should be larger than maximum radius, and no measurement should be smaller than the nuclear radius, or problems will result when using AGE2.EXE (see below).

#### AGE2.EXE

This program uses the data recorded by AGE1.EXE to estimate final increment sizes and fish age. Only complete files will be accepted by AGE2.EXE. A complete file must have at least one transect from one otolith, and must contain the nuclear and otolith radius for that otolith.

SAM DATE	CRUISE	SP CD	HAUL	SPEC#	LENGTH
870618	A	604	10	1	8.1

MAXIMUM RADIUS OF OTOLITH 1= 56.95714  
 MAXIMUM RADIUS OF OTOLITH 2= 0

OTOLITH # 1

TRANSECT #(ARRAY POSITION) 0

READER: 3

DATE READ: 871208

MAGNIFICATION: 100

MEASUREMENT# 0	16.4418
MEASUREMENT# 1	17.5461
MEASUREMENT# 2	18.6504
MEASUREMENT# 3	19.7547
MEASUREMENT# 4	21.1453
MEASUREMENT# 5	22.5359
MEASUREMENT# 6	23.9265
MEASUREMENT# 7	25.9306
MEASUREMENT# 8	27.9347

TRANSECT #(ARRAY POSITION) 1

READER: 3

DATE READ: 871208

MAGNIFICATION: 100

MEASUREMENT# 0	-25.6783
MEASUREMENT# 1	27.5463
MEASUREMENT# 2	29.8497
MEASUREMENT# 3	32.3321
MEASUREMENT# 4	34.8549
MEASUREMENT# 5	-43.6740
MEASUREMENT# 6	47.8971
MEASUREMENT# 7	52.6230
MEASUREMENT# 8	56.95714

Figure 5. A typical output from EDIT.EXE. This example is for a fish with the filename 87A60401.010 (which is the same file as used in Appendix B and Figure 8). In TRANSECT #1, the minus sign in front of MEASUREMENT # 0 and 5 is representative of an area of unknown number of increments. SAM DATE refers to date caught, SP CD refers to species code, and SPEC# refers to specimen number. The example shows two transects from a single otolith. The program AGE1.EXE can accommodate up to seven transects for each of two otoliths. See the text for further details.

### Linking the Transects

The program goes through a number of steps to combine the input data and produce a complete reconstructed otolith<sup>1</sup> (sequence of increment measurements from the center of the nucleus to the otolith edge) (Fig. 6, 7). First, the program reads the data from the file and links all the transects from one otolith (a maximum of seven transects) into a single transect. It repeats this for the other otolith (if it was analyzed). To do this, the program starts with the transect that contains the nuclear radius (Fig. 6). Then it uses continuous measurements until the end of the transect, a gap in the measurements (determined by a special coding convention within the file), or until the maximum otolith radius is reached. If a gap is found, or if the transect ends at a value less than the maximum otolith radius, the remaining transects are searched for a measurement that overlaps the first transect at this measurement. If multiple transects have measurements that overlap this measurement, then the transect that covers the greatest linear distance is used (Fig. 6). After a new transect has been selected, its overlapping section is removed and an additive scaling factor (to compensate for minute differences in measurements) is used to align the new transect with the first transect.

If no transect overlaps the first, then the program searches the remaining transects and locates the transect with the

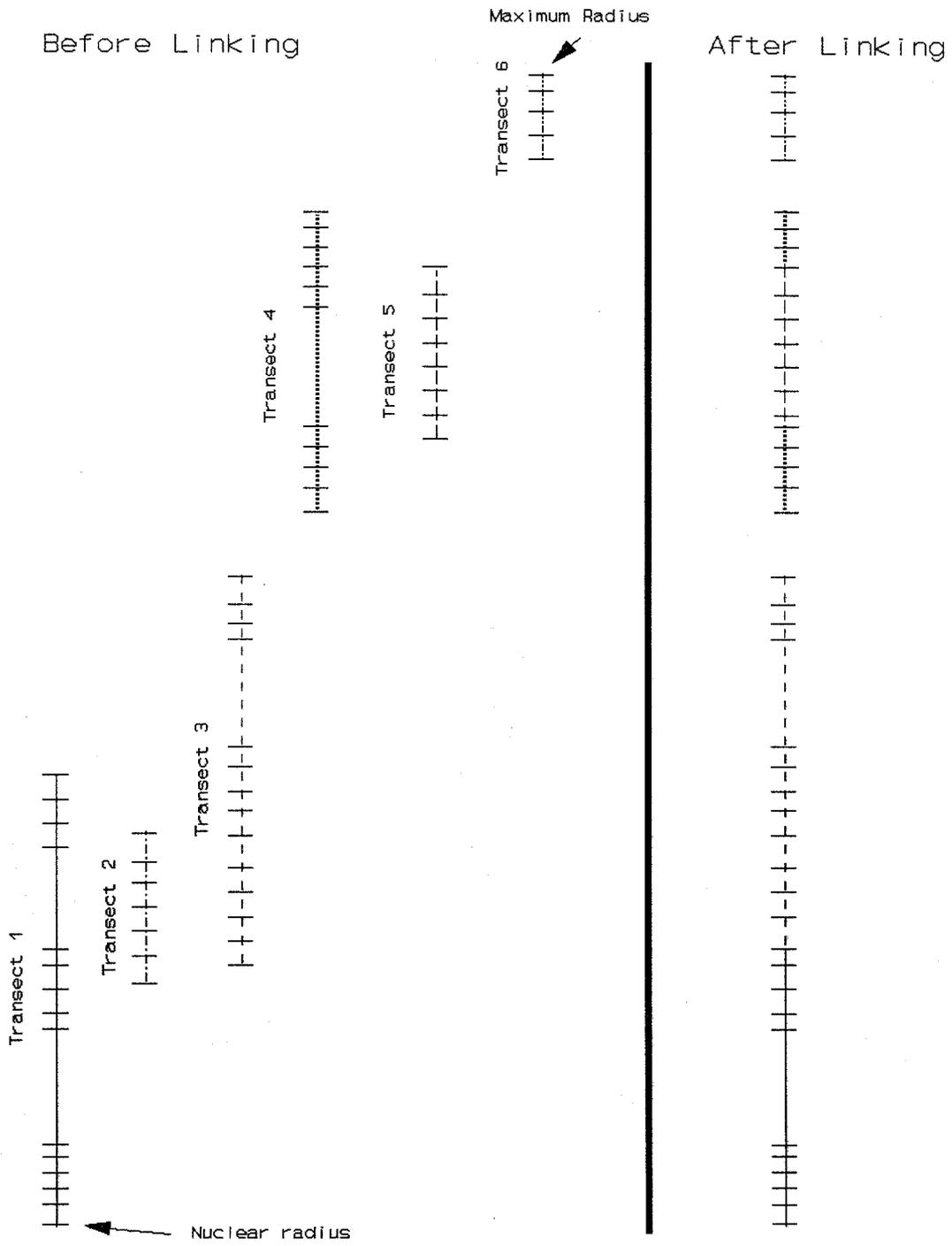


Figure 6. Diagrammatic presentation of the method involved in linking transects to create a single reconstructed transect by AGE2.EXE.

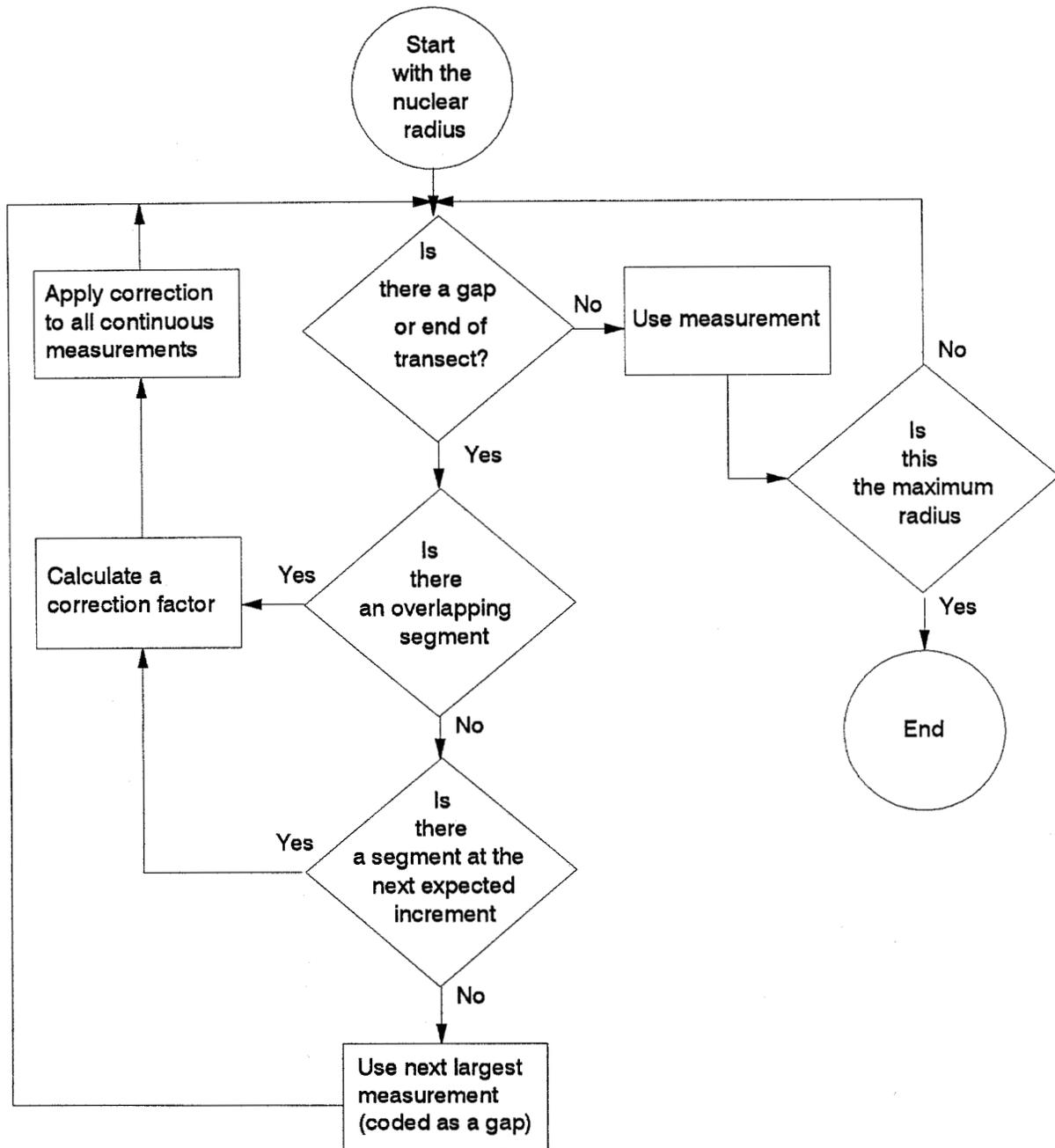


Figure 7. Flow chart of the steps used in AGE2.EXE for linking segments to create a reconstructed transect.

smallest linear distance measurement occurring after the last measurement in the first transect. The average increment size of the last three increment measurements of the first transect is calculated, and this value is temporarily added to the last measurement. The program then searches the remaining transects for a value matching (or nearly matching) this value. If it finds one, then it links the new sequence using the same search and counting procedures described above.

If no value is found that fits the next expected size, then the program searches all transects for a transect with the next largest measurement. The program searches all transects in single increment size steps and uses the same method of counting measurements as above. When a segment<sup>1</sup> is finally located, the first measurement is coded as an unknown number of increments.

If the program encounters an area with an unknown number of increments within a transect, it goes to the preceding measurement and repeats all of the steps described above for linking transects, except no alignment is performed. If a segment is found in another transect to fill the unknown area, as much of it as possible will be used, even if it exceeds the original gap that it was filling; hence, there is no need for multiple realignments at both ends.

When the maximum otolith radius is encountered, the linking process stops. At this point, the otolith radius can be (or has been) adjusted to compensate for the slight alterations needed to

align the segments (using a scaling factor). This entire process is repeated for the second otolith.

Figure 6 illustrates the linking procedure. Transect 1 contains the nuclear radius (even if the reader didn't read it first, it will always be the first transect). This transect is used until a gap is encountered (in Figure 6, a gap is signified by a lack of horizontal lines). The program searches the other transects for measurements which overlap. Both transects 2 and 3 have measurements which overlap; however, transect 3 has more continuous measurements so it is used. A small scaling factor is calculated to align the measurements in transect 3 with the overlap on transect 1. When a gap in transect 3 is encountered, a transect with overlapping measurements or a transect with a measurement at the place of the next expected increment cannot be found. The program therefore uses the next largest linear measurement (found in transect 3). A gap is now left between transect 3 and transect 4. Transect 4 is used until a gap is found; transect 5 is then used completely, even though it more than fills the gap in transect 4. The program finds additional measurements in transect 4 that it can use and it adds these to the developing series. Transect 6 is then added to complete the linking.

The program then searches each otolith, looking for cases in which multiple unknown regions are within two measurements of each other. When this situation is encountered, the measurements bounded by the unknown areas are eliminated and the entire region

is assumed to contain an unknown number of increments. The logic for this is that a large gap may be more accurately filled using data from the other otolith rather than relying on incomplete data.

#### Selecting the Computed 'Best' Otolith

Once the transects have been linked, the computed 'best' otolith is selected. The reconstructed otolith (number 1 or 2) with the most increments is considered the most accurate and is labelled as the 'best' (Fig. 8, 9). If the number of increments is similar between the two otoliths, then the reconstructed otolith with the longest sequential series of measurements from the center of the nucleus is considered the 'best' otolith.

#### Filling the Gaps in the Computed 'Best' Otolith

Once the computed 'best' otolith is selected, the program fills the unknown areas. Each gap in the computed 'best' otolith is examined for size, and, if the size is less than or equal to 5% of the total distance from the edge of the nucleus to the maximum otolith radius, then the gap is filled by linear interpolation (Fig. 8). The mean increment size for the gap is calculated by averaging the three increments prior to and following the gap. The number of expected increments within the gap is then calculated by the following method. If the gap

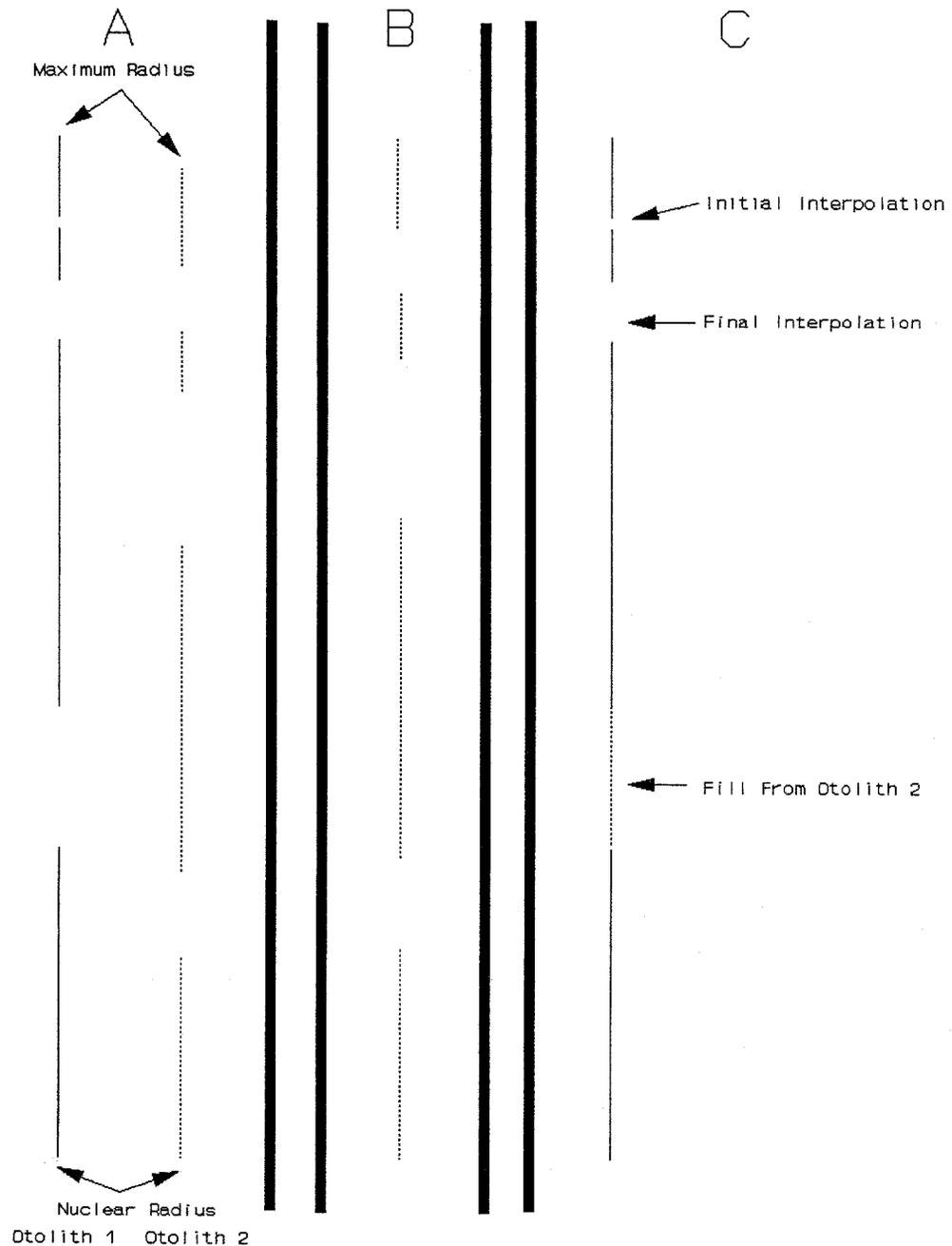


Figure 8. Diagrammatic presentation of the method used to select the computed 'best' otolith and fill in unknown areas by AGE2.EXE. A. Otolith 1 is selected since it has the most measurements; B. otolith 2 is scaled to otolith 1; C. gaps in 'best' otolith are filled from otolith 2 and linear interpolation.

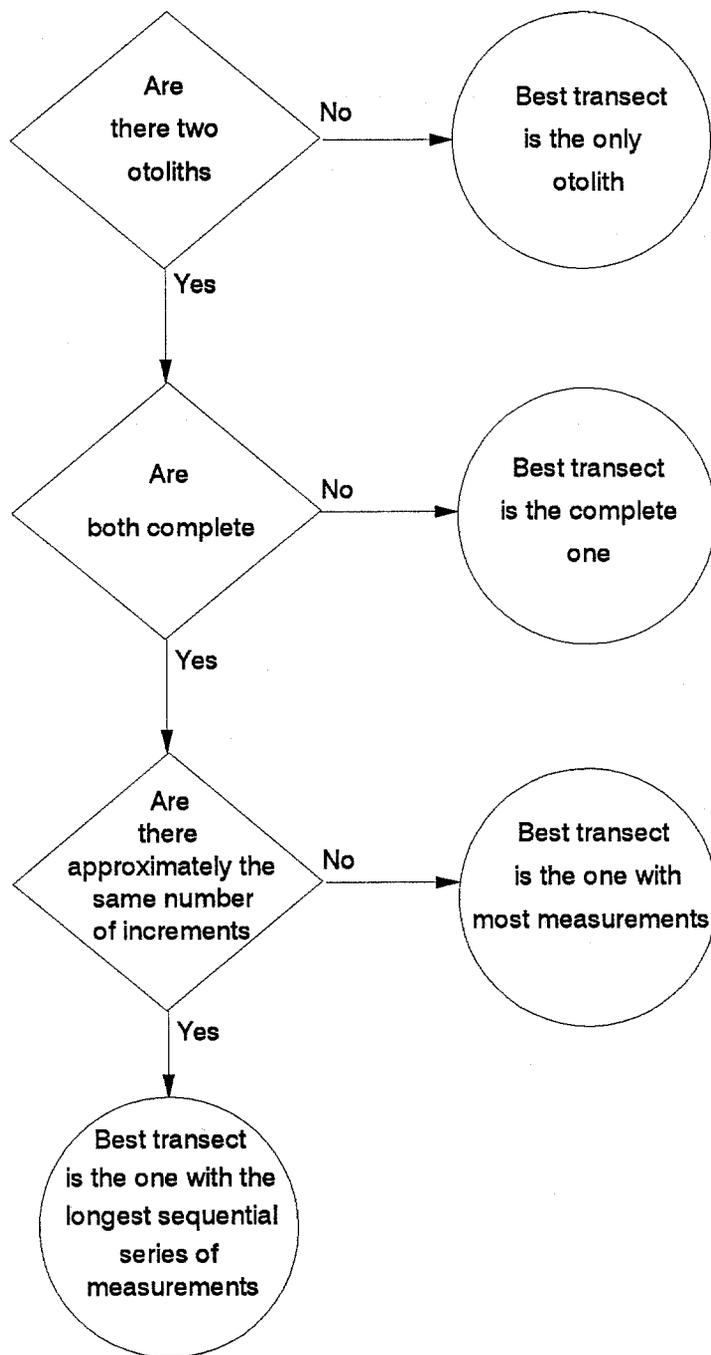


Figure 9. Flow chart showing how AGE2.EXE selects the best reconstructed transect.

contains less than one increment, it is said to contain no increments and the unknown number of increments code is removed. If the gap contains a fractional number of increments, then the gap is apportioned into the nearest whole number of increments.

The rest of the unknown areas are filled by using measurements from the other reconstructed otolith or by interpolation (Fig. 8). In filling the gaps using data from the other otolith, the program first scales the measurements of the otolith to match the size of the computed 'best' otolith. To scale the otoliths, each measurement in the other otolith is multiplied by the ratio of the difference between the maximum radius of each otolith, divided by the difference in the nuclear radii. The formula for this is

$$(\text{Maxrad}_{\text{best}} - \text{Nucrad}_{\text{best}}) / (\text{Maxrad}_{\text{other}} - \text{Nucrad}_{\text{other}}),$$

where  $\text{Maxrad}_{\text{best}}$  = the maximum radius of the 'best' otolith

$\text{Nucrad}_{\text{best}}$  = the nuclear radius of the 'best' otolith

$\text{Maxrad}_{\text{other}}$  = the maximum radius of the other radius

$\text{Nucrad}_{\text{other}}$  = the nuclear radius of the other radius.

Once this is accomplished, the program uses a similar system as used with the linking of the transects above to find a segment in the other reconstructed otolith that matches the unknown area of the computed 'best' otolith. Finally, any remaining gaps are interpolated using a procedure identical to that explained above.

An example of how the program selects and fills the gaps in the computed 'best' otolith is located in Figure 8. The two reconstructed otoliths from a fish are shown after the linking process (see Panel A). Since otolith 1 has more measurements, it is selected as the computed 'best' otolith. Otolith 2 is scaled to otolith 1 (Panel B). In Panel C, the computed 'best' otolith is completed by first interpolating to fill the small gaps, then attempting to fill gaps using sections from otolith 2, and finally by interpolating the remaining gap.

#### Determining Age, Birthdate, and Output of Results

Once the computed 'best' otolith has been determined and the gaps have been filled, the program determines the age of the fish. This is done by counting all measurements (Fig. 10). The birthdate is then calculated by subtracting the age of the fish from the date of capture.

#### Interpreting the Output from AGE2.EXE

The main body of the output lists the measurements of each increment on an otolith (Fig. 11). The left column contains the increment number. If the increments are presumed (or verified)

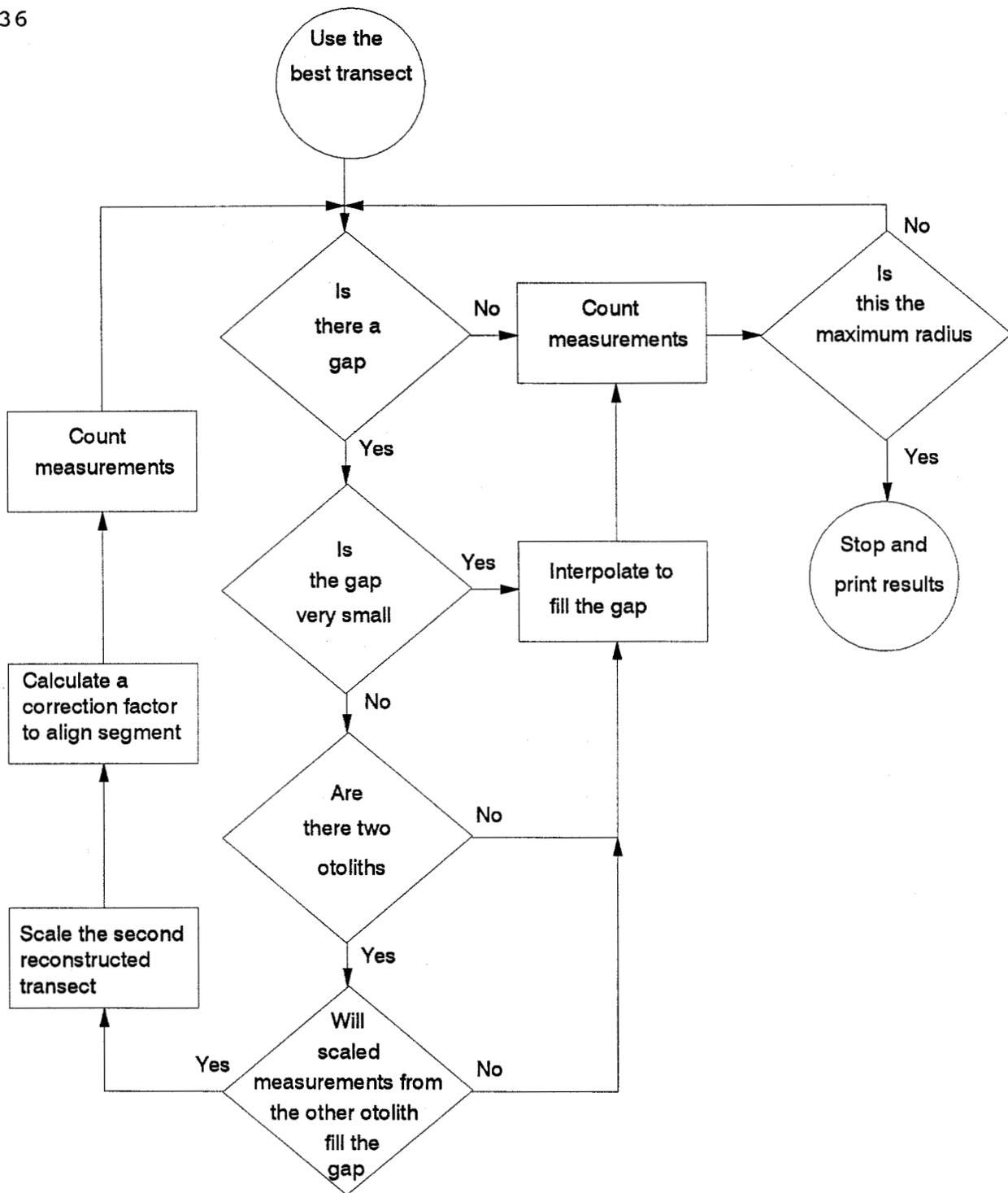


Figure 10. Flow chart showing the steps used by AGE2.EXE to determine the age of the computed 'best' otolith.

```
=====
RESULTS
```

```
SPECIES:      604          HAUL #:    10
SPECIMEN:     01          LENGTH:   8.1
SAMPLE DATE:  870618     CRUISE:   A
```

AGE	RADIUS	INC SIZE	DELT SIZE	STATUS
1	16.44	0.00	0.00	0
2	17.55	1.11	1.11	0
3	18.65	1.10	-0.01	0
4	19.75	1.10	0.00	0
5	21.15	1.40	0.30	0
6	22.54	1.39	-0.01	0
7	23.93	1.39	0.00	0
8	25.93	2.00	0.61	0
9	27.93	2.00	0.00	0
10	29.85	1.92	-0.08	1
11	32.33	2.48	0.56	1
12	34.85	2.52	0.04	1
13	37.24	2.39	-0.13	INTERP
14	40.16	2.88	0.49	INTERP
15	43.67	3.51	0.63	1
16	47.90	4.23	0.72	1
17	52.62	4.72	0.49	1
18	56.96	4.34	-0.38	RADIUS

```
AGE = 17   BIRTHDATE:  151
AGE DETERMINED FROM 1 OTOLITH(S)
BEST OTOLITH TRANSECT WAS  1
TOTAL TRANSECTS = 2
=====
```

Figure 11. A typical output from AGE2.EXE. Details explained in text. This example is for a fish with the filename 87A60401.010 (which is the same file used in Appendix B and Figure 5).

to be formed daily, then the increment number represents the age in days. The second column contains the distance from the increment to the center of the nucleus. The third column contains the increment width. The fourth column contains the difference in increment width from the previous increment width. The last column contains the source of each measurement. Numbers in this column refer to the original transect number from AGE1.EXE. For example, a '1' means the measurement came from transect 1 and a '0' means the measurement came from transect 0. These measurements may not directly match the measurements that were actually measured from the video image, as scaling factors may have been applied in the linking process. INTERP refers to linear interpolation of gaps less than 5% of the otolith radius. FILL indicates measurements taken from the other otolith. F. INTERP indicates linear interpolation of any remaining gaps which it could not fill. RADIUS represents the maximum otolith radius which may or may not be the same as actually measured since, again, some correction factors to align transects and segments may have taken place.

The status column is useful in determining how valid an age might be. Otoliths requiring a great deal of interpolation or filling are expected to be less accurate than those requiring only a little. Additionally, if there is a lot of switching between transects, accuracy can be expected to decrease. The program makes every effort to reduce the amount of switching and interpolation.

The calculated age and birthdate (days since the beginning of the calendar year) are located at the bottom of the main section. Below this is a listing of how many otoliths were analyzed, how many transects were completed, and which reconstructed otolith was considered the 'best' otolith.

Two related programs (FORCEOTO.EXE and AGE2000.EXE) to the programs explained above were developed. The former allows the user to designate which reconstructed otolith is the computed 'best' otolith. This option allows the user to compare the ages produced by both otoliths. The latter is used in conjunction with AGE1000.EXE and performs analysis on fish that are older than 300 days.

SUMMARY OF STEPS FOR  
DETERMINING THE DAILY AGE

1. Mount otoliths.
  2. Etch and grind otoliths.
  3. Take increment measurements on a transect for otolith 1.
  - \* 4. Repeat Steps 2 and 3 until entire otolith is completed.
  - \* 5. Repeat Steps 2 through 4 for otolith 2.
  
  - \* 6. Edit files
  
  - + 7. Link all transects and create reconstructed otoliths for each otolith.
  - + 8. Determine computed 'best' otolith.
  - + 9. Interpolate small gaps in computed 'best' otolith.
  - + 10. Fill large gaps in computed 'best' otolith from the other reconstructed otolith.
  - + 11. Interpolate remaining gaps.
  - + 12. Calculate age and print results.
  
  13. Interpret results.
- \* - Optional step  
+ - Steps under computer control

For Steps 3-5 use AGE1.EXE.  
For Step 6 use EDIT.EXE.  
For Steps 7-12 use AGE2.EXE.

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1991 Interannual variation in growth rates and back-calculated birthdate distributions of pelagic juvenile rockfishes (Sebastes spp.) off the central California coast. Fish. Bull., U. S. 89:523-533.

APPENDIX A  
GLOSSARY OF TERMS

'Best' otolith: A linked (reconstructed) series of transects which the program has determined to be the most accurate representation of the entire otolith increment structure in that fish.

<ENTER> key: The key on the computer keyboard that signals the end of a data input step. Also called the <RETURN> key.

Increment: A space representing a single day's growth as delineated by both a light and dark band.

Maximum radius: The measurement from the center of the nucleus to the extreme edge of the otolith along the axis selected for reading.

Measurement group: A group of increments measured by a single access to the digitizer. Usually 1-2 increments, but up to 20.

Nucleus: The central section of the otolith representing the size of the otolith on the first day of life.

Overall header data: Descriptive information about the fish that contains the cruise number (or letter), date caught, a three-digit species code, up to a three-digit haul number, up to a two-digit specimen number, and the standard length.

## APPENDIX A

(Cont.)

<READY> key: The key on the digitizer pad that signals the end of a digitizer coordinate input to the computer.

Reconstructed otolith: Sequence of increment measurements from the center of the nucleus to the otolith edge, created from linking all transects of that otolith.

Segment: A series of continuous measurements on a transect. It may include one or more measurement groups and may include the entire otolith.

Theoretical first increment size: Smallest measured size of a first increment after the nuclear radius for a given species. This value is determined by the user, and manually entered into the MAG.DAT file. This size is used for interpolation of increment sizes when the actual first increment size cannot be measured.

Transect: A series of one or more measurement groups arranged in one or more segments which may include the entire otolith or only a portion.

Transect header data: Descriptive information about each transect that contains the otolith number, the user's identification number, the date of reading, and the magnification used during that reading.

APPENDIX B  
FILENAME STRUCTURE

Output files for AGE1.EXE are named according to the following convention:

Y C S S S N N . H H H

where

YY = Year of collection  
C = Cruise designation (either a numeral or letter)  
SSS = Species code  
NN = Specimen number  
HHH = Haul number of collection

An example of this structure would be a file with the name

87A60401.010

where

Year = 1987  
Species code = 604  
Haul number = 10  
Specimen number = 1  
Cruise letter = A

Output files from AGE2.EXE are named according to the following convention:

S S S Y C N N . H H H

where

YY = Year of collection  
C = Cruise designation (either a numeral or letter)  
SSS = Species code  
NN = Specimen number  
HHH = Haul number of collection

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- 159 Report of a marine mammal survey of the eastern tropical Pacific aboard the research vessel *McArthur*,  
P.S. HILL, A. JACKSON and T. GERRODETTE  
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