# INTERIM PROGRESS REPORT ON A STUDY OF THE UTILITY OF DATA OBTAINABLE FROM OTOLITHS TO MANAGEMENT OF HUMPBACK CHUB (*GILA CYPHA*) IN THE GRAND CANYON

submitted to:

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## INTRODUCTION

Daily growth increments of otoliths of fishes have been useful in many fishery applications since they have been demonstrated to provide a precise method of ageing individuals and reconstructing individual growth and, possibly, movement or habitat histories. These techniques have not been previously applied to humpback chub, but are believed to have considerable potential for providing knowledge of this difficult to sample and little-understood species. Large temperature and water quality gradients apparently traversed by individuals of this species in the Grand Canyon are of a magnitude likely to produce structural and/or chemical signals in the crystalline calcareous otoliths. If so, since otoliths grow by accretion of daily increments (much like trees develop yearly growth rings), and are stable structures, which unlike scales, are not susceptible to reabsorption except in the most extreme conditions, they retain a structural and chemical chronology of habitats occupied. If the relationships of ambient physical and chemical conditions to otolith structure and composition can be described, a chronology of habitat occupancy and growth for individuals could theoretically be reconstructed with daily precision. Such reconstructions of growth rates, birth dates, movement histories, and possibly, birth place (based on chemistry at otolith formation or during early life), could provide extremely valuable life-history information regarding timing of spawning, cohort recruitment, mortality rates, and data on other population parameters critical for management of this endangered species.

The feasibility of using otoliths and opercles of humpback chub for age estimation of individuals has been preliminarily investigated by examining otoliths and opercles from a total of 47 juvenile (ages 0 through $1^+$) and 43 adult (estimated ages 2 - 23) specimens collected in the Little Colorado River (71 specimens) and mainstream Colorado River (19 specimens) at various places in the Grand Canyon between 1988 and 1992. Studies are continuing, and at this point, due to both sample size and numerous other limitations, and ongoing refinements of techniques, conclusions made here are highly preliminary.

Structures prepared and examined included opercles of 35 specimens, one asteriscus from each of 47 specimens and a lapillus from each of 56 specimens. Seventeen specimens were evaluated using all three calcareous structures (lapillus, asteriscus and opercle). The sagitta was also examined, but found to be unsuitable for ageing purposes due to its long, delicate form and irregular increments after the larval/juvenile stage. Additional lapilli have been removed from other available specimens, and a complete inventory of specimens available for further study of calcified structures is provided.

Studies of micro-spatial variation in chemical composition of selected lapilli is in progress, using the highly accurate proton probe at the Institute of Geological and Nuclear Sciences in Lower Hutt, New Zealand. This method of analysis shows great promise of overcoming what has been indicated in recent literature to be significant inaccuracies of other techniques (Energy Dispersive X-ray diffraction and Wave Length dispersive X-ray diffraction) used in many of the published studies of microspatial elemental analysis of otoliths.

## METHODS

### FIELD COLLECTIONS

Adult and sub-adult humpback chub were collected during late-spring and early summer field seasons in 1989 and 1990. These were euthanized, weighed, measured, and (usually) sexes recorded prior to removal of the majority of muscle tissue and internal organs from the skeleton. Skeletons were then tagged and hung to desiccate in the generally high-temperature, dry air of the field sites. Otoliths and opercles were removed after final preparation as skeletons using dermestid beetles. If otoliths were not found outside the skull after passage through the dermestid colony, they were extracted using forceps with as little damage to the skeleton as possible.

Young-of-the-year collected from 1990 through 1992 were preserved in the field in 95% ethanol. Otoliths were extracted by removal of the dorsal surface of the cranium.

## SPECIMEN PREPARATION AND EXAMINATION

Opercles were cleaned of residual soft tissues and examined under reflected and transmitted light under a binocular stereo microscope. Age estimations reported here were provided by Mr. Gary Scoppetone (United States Fish and Wildlife Service, Reno, Nevada), who has had considerable experience ageing other long-lived regional cypriniform fishes (Scoppettone, 1988; Scoppettone et al. 1986; Scoppettone AND Vinyard, 1991). Subsequent estimates independently done by myself generally agreed well with those of Scoppetone. In the case of disagreement between estimates made by the author and those by Scoppetone (almost always on older individuals), the author carried out a second revision of the specimens, and in all cases, was able to understand how Scoppetone arrived at his estimate. All estimates reported here as derived from opercles are those provided by Scoppetone. When uncertainty existed, both minimum and maximum ages were reported.

Asterisci were removed from skeletonized adults, and then were mounted, sectioned and examined by Mr. Michael McCarthy, who has had experience utilizing otoliths to estimate ages of razorback sucker (McCarthy AND Minckley, 1987), another long-lived, Colorado River endemic cypriniform. The author then examined the same specimens and was unable to estimate ages from them since asterisci in this species appear to grow along temporally variable growth axes. While marks which appear as though they might be annual features are visible, they are discontinuous and it is often impossible to reconstruct chronologies of growth axis shifts. Estimated ages reported here as derived from examination of asterisci are those of by McCarthy, who provided a single age estimate for each specimen.

Dr. Ed Brothers, who has extensive experience in otolith ageing studies involving a wide variety of species, concurred with this conclusion and recommended using the lapillus for ageing in this species. The sagitta was also examined but found to be extremely delicate and increments were clear only during early life. Lapilli were removed from skeletonized specimens or from young-of-the-year as described above and were then mounted, ground and examined by Dr. Ed Brothers. All age estimates based on lapilli are those of Brothers, who provided minimum and maximum estimates whenever uncertainty existed.

All counts of presumed daily increments and annuli were done on specimens by persons unaware of the size or capture dates and locations so as to assure that knowledge of collection conditions did not bias estimates. Whenever uncertainty existed regarding interpretation of one or more marks, estimated minimum and maximum ages were reported. Daily growth increment counts are the average of two counts. Counts were made on a variety of otolith fields (wherever clear increments could be found) and not on a single axis. This was done simply for convenience at this point in the study, but counts and increment measurements could be done along a fixed axis for growth reconstructions.

Length data plotted in all Figures below are actual Standard Lengths, where available, or estimated Standard Lengths derived from actual Total Lengths using regression statistics. This is due to the fact that TL only is the standard measurement taken by field collectors. SL was found to be a much more precise indicator of size with significantly lower variance than TL. The linear correlation coefficient of the SL to TL relationship for all specimens for which both measurements were available (n=126, 13 to 100 mm SL*) was found to be $r^2=.997$. This relationship is described by the following equation (used to compute estimated SL from measured TL):

$$\mathbf{TL = 1.2779979\ (SL) + .0749086}$$

*(note that this relationship was applied, admittedly inappropriately, to compute SL for specimens far larger than the largest specimen used in developing the relationship. This was done only because only TL was available for all larger specimens.)

## RESULTS

Progress on each of the items outlined in the proposal for this study is reported as follows:

(* indicates those items mentioned in proposal as contingent on availability of experimentally spawned ova and cultured larvae/fry and provision of adequate hatchery support facilities and staff.)

1. "A comprehensive bibliography of literature relevant to methods and problems of estimating age and growth of *Gila cypha* and chemical composition of otoliths as related to application of otolith chemistry to reconstruction of the environmental history of individuals." <u>The attached bibliography is provided in fulfillment of this product.</u> New literature relevant to this project is appearing at a very high rate. As evidence of the great interest in this highly specialized field, an international conference on "Fish Otolith Research and Application" held just before this report was finished was attended by 350 persons from 27 countries. The bibliography provided in this report will be updated in the final report. (Note that literature citations in text in this report, reflecting its interim status, are very scarce. The final report will incorporate extensive discussion of the literature).

2. "An inventory of all currently available specimens of *Gila cypha* from the Grand Canyon which are potentially useful for age and growth studies." <u>A complete inventory of all specimens currently housed at the Texas Natural History Collection (TNHC) of the Texas Memorial Museum at The University of Texas at Austin is provided in Appendix 1, as fulfillment of this product.</u> Extensive additional data on each specimen listed in Appendix 1, but not printed there, is available directly from the author. This includes additional collection location and time information, additional tag/recapture data, current locations and shipping dates of various parts (head, body, skeleton, lapilli, asterisci, opercles, gut, etc.) of each specimen, museum (Arizona State University) catalog numbers for some specimens, and notes from field collections and laboratory observations of otolith micro-structure.

3*. "Experimental validation of the periodicity of growth increments in *Gila cypha*." <u>Two approaches have been taken to validate daily increments, but difficulties have been encountered in successful completion of each.</u> In the first experiment, young-of-the-year specimens captured in the Little Colorado River (LCR) during May were caged and subjected to three treatments. One group was moved alternately every three days between the LCR and mainstream, while the other groups were left for the same total period in either the mainstream or LCR. Abrupt temperature changes associated with movements from one river to the other should have produced marks on otoliths which would allow both validation of their periodicity and demonstration of the nature of the mark produced by this movement if it occurred naturally. Unfortunately, the majority of the specimens in all groups did not survive the treatments. Mortality appeared to be related more to effects of being caged than to effects of temperature. Survival was lowest in cages maintained continually in the warm LCR, and highest in those held continually in the cold mainstream. Sample sizes were not intended to be adequate to demonstrate effects of temperature treatments on mortality. Otoliths of these experimental specimens have not yet been examined since such intense stress was felt likely to have invalidated the experiment.

   In the second attempt to validate growth increments (and investigate temperature effects on otolith structure and chemistry - see below), experiments were conducted at Dexter National Fish Hatchery. Since humpback chub were not being cultured, a surrogate species, *Gila elegans*, was used. Eggs were fertilized and incubated until hatching following standard protocol at the hatchery. At hatching, larvae were separated into three groups held at relatively constant temperatures of 15, 21 and 27°C. Specimens were preserved from each treatment at frequent intervals for subsequent analysis of otoliths. Densities and food availability were constant among treatments, but growth rate at all temperatures was very low. Individuals of the same cohort stocked in earthen ponds on the hatchery grew at a much higher rate, but were released to wild habitats (Lake Mohave) without sampling for otoliths. Otoliths of specimens from the temperature treatments have yet to be examined, but recently reported results from several similar studies indicate that resolution of daily increments may be difficult under such low-growth conditions. Specimens from the Dexter temperature treatment studies are at TNHC, but are not included in Appendix 1.

4. "Age estimates (years of age) for 50 selected skeletonized adult specimens of *Gila cypha* collected from the Grand Canyon by Arizona Game and Fish Department in 1989 and 1990." **All age estimates obtained to date from examination of lapilli are presented in Table 1.** Considering only ages estimated from lapilli, totals to date are 18 specimens more than 1 year old and 22 one year olds. Data are summarized graphically in various ways in Figures 12 - 17. Additional estimates utilizing material listed in Appendix 1 (and selected newly provided material), and re-examinations of selected specimens in Table 1, will be completed prior to filing of the final report.

5. "Age estimates (days of age) for 100 selected young-of-the-year *Gila cypha* collected by Arizona Game and Fish Department in 1989 and 1990. Contingent on outcome of 3 (above)." **A total of 18 young-of-the-year (age $0^+$) have been aged to date (Table 1).** Specimens captured subsequent to 1990 and recently provided to the author were also included in this total. Additional material from among that listed in Appendix 1, selected newly provided material, and selected re-examinations of material from Table 1, will be analyzed prior to completion of the final report.

   Presumptive daily growth increments are clearly visible under light microscopy in lapilli of the smallest specimens examined. During earliest portions of otolith growth, these can generally be easily counted, but increment interval becomes increasingly small with increasing age, sometimes resulting in great difficulty resolving increments and counting them without extensive specimen preparation. Some specimens displayed interesting rapid transitions of otolith growth rates (see Plates in Appendix 2).

6. "Analysis of the feasibility of determining annual growth period duration from otoliths of post young-of-the-year individuals of *Gila cypha* for all growth periods throughout age of specimen." **Daily increment counts in year 2 of life for relatively young specimens has proven possible in some specimens without extensive otolith preparation. Data on numbers of increments in years 2 and 3 for specimens where they could be counted are provided in Table 1.** Increasing otolith thickness and narrowing increments as growth slows in later years, make resolution of daily increments in later years of life very difficult. The value of such data at this point, at least for the relatively small sample sizes, was not apparent, and therefore additional effort has not been devoted toward this objective. Though countable increments can be seen under light microscopy in years two and three in some specimens less than 4 years old, counting them becomes very subjective without extensive specimen preparation and (likely) use of SEM.

7*. "Experimental analysis of the effects of temperature changes on otolith structure in *Gila cypha*." **The experiment described under item 3 above (movement of caged individuals between LCR and mainstream Colorado River) was intended also to address this objective.** Apparent failure of that experiment was described above, and otoliths have not yet been examined. Examination of otoliths of selected individuals from this experiment will be completed prior to completion of the final report. Field repetition of this experiment (as has already been done once by AGFD personnel with similar results) would likely again produce the same results. Repetition of this experiment in a hatchery environment with large sample sizes is recommended (see also item 11 below), but selected otoliths from earlier attempts will be analyzed despite perceived "failure" to carry the experiment to completion as designed.

8. "Analysis of micro-spatial (=chronological) variation in elemental composition in otoliths of 20 selected individual *Gila cypha* specimens from the Grand Canyon with evaluation of the utility of such techniques for reconstruction of movement history of individuals." **X-ray diffraction analysis**

**for elemental composition, as employed in early studies of microdistribution of elements in otoliths, and as proposed initially for this study, is now indicated to be of limited utility in this application due to low precision and relatively high detection levels.** Newer techniques capable not only of detecting much lower concentrations, but which are also much more precise, are now beginning to be used in this application. Consequently, it was decided to further investigate alternative analytical techniques. A few samples were sent in December 1992 to Dr. Graeme Coote for analyses on the proton probe at the Institute of Geological and Nuclear Sciences in Lower Hutt, New Zealand. Preliminary results from that work are anticipated within a month or two of the date of this report.

9*. "Comparisons of total elemental composition among otoliths of 5 selected individual specimens of young-of-the-year *Gila cypha* captured in the Little Colorado River, otoliths of 5 hatchery-reared young-of-the-year *Gila cypha*, and otoliths of 5 selected *Gila cypha* suspected or known to have moved between the Little Colorado River and mainstem Colorado River in the Grand Canyon. Among sample comparisons would be used to investigate the hypothesis that elemental composition of otoliths reflects ambient water composition. Emphasis would be placed on a search for elemental markers which might be applied to determine origin (birthplace) of specimens." This set of analyses has also been delayed temporarily awaiting additional exploration of alternative analytical techniques. It is also proposed to incorporate in this analysis some other specimens from individuals of known histories in other waters. These would include bonytail reared as part of the temperature effects experiment described above.

10*. "Analysis of the isotopic composition of a subsample of the same (or comparable) specimens described in 9 (above). Among sample comparisons will be used to investigate the hypothesis that isotopic composition of otoliths reflects ambient water composition and or temperature. Emphasis would be placed on a search for isotopic markers which might be applied to determine origin (birthplace) of specimens." Currently, radioisotope analyses generally require sample sizes that approach or exceed whole lapilli of adult chubs. Since age and otolith structural data will be required for interpretation of results from these analyses, radioisotope studies will be performed on selected samples after completion of ageing. Alternate techniques which might be capable of revealing micro-spatial distribution of radioisotopes in sectioned otoliths is being investigated as a preliminary step toward attainment of this objective.

11*. "Experimental analysis of the effects of ambient temperature on otolith elemental and isotopic composition of individuals reared in constant water quality conditions." See item 9 above.

12. "Assessment of the utility of age, growth and correlative environmental history data obtainable from otoliths for humpback chub population monitoring and management in the Grand Canyon, and recommendations for future studies." See Conclusions and Discussion.

## CONCLUSIONS AND DISCUSSION

Presumptive daily increments are clearly visible in humpback chub otoliths (Figure 1). Counts of all such increments have been relatively easily and reliably done on any specimens captured in the LCR prior to their first winter or prior to movement to the mainstream Colorado. Increments are generally clearly visible to the margin (Figure 2), thus providing reliable ageing of these specimens with daily precision. Relatively little preparation is generally required with such very small specimens. Though daily periodicity of increment formation under a diversity of conditions has not been validated, the limited and highly preliminary data available so far, and a voluminous literature on other species, indicate that increments formed in the LCR are almost certainly daily. Back-calculated hatch dates for young-of-the-year captured in the LCR (Table 1) agree well chronologically with anecdotal field observations of likely reproductive activity. Periodicity of increment formation should be validated, as should time of first increment formation. The literature indicates that the first increments form almost always within a few days before or after, or exactly upon, hatching. Preserved specimens of bonytail chub from experiments carried out at Dexter should be useful for determination of time of first increment formation in that closely related species, but the same should be done with humpback chub if hatchery stocks are obtained and artificially spawned.

Most specimens taken from the mainstream Colorado River have proven much more difficult to read than are specimens of similar sizes from the LCR. Daily age estimates are impeded by narrow, poorly defined increments and odd patterns. Poorly defined increments have been reported in the literature from other species when held in cold, constant temperatures, so it is not surprising to find such structures in this river. Though age estimates were not obtainable for all specimens of young-of-the-year from the mainstream Colorado, most could be aged. Figure 3 illustrates poorly defined increments in a specimen from the mainstream Colorado which could be counted, but only with some difficulty. Some specimens taken from the mainstream Colorado display unusual patterns of very abrupt transitions between periods of presumptive rapid growth, probably in habitats with thermal fluctuations on a diel cycle (as indicated by well-defined increments), to periods of very narrow increments such as might be typical of much lower temperatures (Figure 4). Some specimens displayed interesting repeated rapid transitions among brief (several days) periods of each rapid and slow growth (Figure 5). These marks are very similar to otolith structural patterns purposefully produced in hatcheries to mark batches of fish for stocking (Brothers, 1990) so that hatchery and batch origin can be determined upon recapture. Such marks are produced in hatcheries through temperature manipulations.

It appears to be possible to age adult specimens on the basis of presumptive annular marks (Figure 6 and Figure 7), but, once again, periodicity of such marks has not been validated. Due to the larger size of otoliths from adults, this process requires additional specimen preparation (grinding). Distances between annual marks could be easily measured for reconstruction of individual growth histories. Rigorous validation of annual periodicity of such marks will likely require study of otoliths from mark-recapture studies. Use of chemical marking (Tetracycline or Alizarin), in conjunction with PIT tags, would be preferable, but much progress could be made utilizing non-chemically-marked specimens with histories well-known from the standard, ongoing mark-recapture program.

Presumptive daily increments can also be resolved in otoliths of adults, and in some cases, are clear enough so that those between annular growth interruptions (Figure 8) may be counted. Such counts likely reflect the length of the growing season experienced in each year by individuals. Increments, however, generally become less clear with increasing age (Figure 9 as compared to Figure 1), but may still be countable in later years (e.g. year 6, Figure 8). It does not appear practical to expect to determine birth dates of specimens that have entered or passed through their first winter.

Remarkably abrupt transitions in growth rates are also apparent in adults (Figure 10). Such abrupt marks are likely related to abrupt temperature changes, such as might be encountered by specimens moving between the LCR and mainstream during summer months. Evidence of frequent movements back and forth over short periods between cold and warm waters might be reflected in otolith structural patterns such as illustrated in Figure 11.

## Table 1

Tag number, capture locality, lengths, weights, and estimated ages of humpback chub from the Grand Canyon. Specimens are separated by river of collection, and within rivers sorted by estimated ages based on lapilli. Minimum and maximum ages are provided whenever uncertainty existed, and independent estimates are listed for each different calcareous structure examined. Unless otherwise stated, all ages or estimated dates are based on age estimates from lapilli. Date of first increment formation is an approximation of hatching date. Numbers of daily increments in the first and second years of life approximate lengths of growing seasons in those years of life.

TABLE 1  OTOLITHS EXAMINED

| TAG NO. | TAG TYPE | TAG COL. | RIVER | CAPTURE LOCALITY | DATE CAPTURED | N | SL (mm) | TL (mm) | WT (gm) | SEX | MIN. AGE (DAYS) (LAP) | MAX. AGE (DAYS) (LAP) | DATE 1st INCREMNT FORMED (LAP) | MIN. AGE (YRS) (LAP) | MAX. AGE (YRS) (LAP) | INCRE-MENTS YR. 1 | INCRE-MENTS YR. 2 | EARLY YEAR CLASS | LATE YEAR CLASS | MIN. AGE (YRS) (ASTER) | MAX AGE (YRS) (ASTER) | MIN. AGE (YRS) (OPERC) | MAX. AGE (YRS) (OPERC) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *LITTLE COLORADO RIVER* | | | | | | | | | | | | | | | | | | | |
| 588 | CAR | YE | LCR | LCR; ABOUT 250 M ABOVE MOUTH | 5/28/89 | 1 | | 461.00 | | | | | | 22 | 23 | | | 1966 | 1967 | 18 | 18 | 21 | 21 |
| 461 | CAR | YE | LCR | LCR; ABOUT 250 M ABOVE MOUTH | 5/15/89 | 1 | | 382.00 | | F | | | | 16 | 16 | | | 1973 | 1973 | 14 | 14 | 15 | 16 |
| 340 | FL | BL | LCR | LCR; SALT CANYON | 5/2/89 | 1 | | 350.00 | 316 | M | | | | 13 | 16 | | | 1973 | 1976 | 14 | 14 | 16 | 16 |
| 548 | CAR | YE | LCR | LCR; AT MOUTH | 5/23/89 | 1 | | 436.00 | 655 | M | | | | 13 | 15 | | | 1974 | 1976 | 22 | 22 | 14 | 17 |
| 550 | CAR | YE | LCR | LCR; ABOUT 250 M ABOVE MOUTH | 5/26/89 | 1 | | 461.00 | | | | | | 13 | 14 | | | 1975 | 1976 | 13 | 13 | 15 | 15 |
| 591 | CAR | YE | LCR | LCR; AT MOUTH | 5/25/89 | 1 | | 425.00 | 770 | F | | | | 12 | 13 | | | 1976 | 1977 | 22 | 22 | 14 | 14 |
| 350 | FL | BL | LCR | LCR; SALT CANYON | 5/6/89 | 1 | | 340.00 | 303 | M | | | | 10 | 18 | | | 1971 | 1979 | 13 | 13 | 11 | 11 |
| 951 | CAR | OR | LCR | 180/ NS D HOOP | 5/7/90 | 1 | | 195.00 | 87 | F | | | | 10 | 11 | | | 1979 | 1980 | | | | |
| 496 | CAR | YE | LCR | LCR; FOUR MILES ABOVE MOUTH | 5/17/89 | 1 | | 284.00 | 107 | M | | | | 9 | 10 | | | 1979 | 1980 | 15 | 15 | 5 | 5 |
| 439 | CAR | YE | LCR | LCR; ABOUT 250 M ABOVE MOUTH | 5/8/89 | 1 | | 370.00 | 393 | F | | | | 8 | 10 | | | 1979 | 1981 | 14 | 14 | 12 | 12 |
| 947 | CAR | OR | LCR | 192/ SS D HOOP | 5/7/90 | 1 | | 342.00 | 343 | M | | | | 8 | 9 | | | 1981 | 1982 | | | | |
| 338 | FL | BL | LCR | LCR; SALT CANYON | 5/2/89 | 1 | | 357.00 | 316 | F | | | | 6 | 8 | | | 1981 | 1983 | 12 | 12 | 6 | 6 |
| 474 | CAR | YE | LCR | LCR 1/4 MI ABOVE COLORADO MAIN | 10/18/90 | 1 | | 158.00 | | | | | | 6 | 7 | | | 1983 | 1984 | | | | |
| 497 | CAR | YE | LCR | LCR; 4 MILES ABOVE MOUTH | 5/17/89 | 1 | | 295.00 | | F | | | | 6 | 6 | | | 1983 | 1983 | 12 | 12 | 5 | 5 |
| 498 | CAR | YE | LCR | LCR; 4 MILES ABOVE MOUTH | 5/17/89 | 1 | | 230.00 | 61 | F | | | | 5 | 5 | | | 1984 | 1984 | 13 | 13 | 3 | 3 |
| 343 | FL | BL | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 108.00 | | | | | | 2 | 2 | | | 1987 | 1987 | 2 | 2 | 1 | 1 |
| 495 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/17/89 | 1 | | 223.00 | 87 | M | | | | 2 | 2 | 190 | 183 | 1987 | 1987 | 5 | 5 | 3 | 4 |
| 341 | FL | BL | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 126.00 | | | | | | 1 | 1 | 244 | 164 | 1988 | 1988 | 3 | 3 | 1 | 2 |
| 346 | FL | BL | LCR | LCR; NEAR SALT CANYON | 5/4/89 | 1 | | 121.00 | 14 | | | | | 1 | 1 | 212 | 65 | 1988 | 1988 | 2 | 2 | 1 | 1 |
| 460 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 75.99 | 100.36 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 465 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 71.67 | 96.36 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 470 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 86.23 | 111.12 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 491 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 59.20 | 79.47 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 502 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/24/89 | 1 | | | | | | | | 1 | 1 | 180 | 78 | 1988 | 1988 | 2 | 2 | 1 | 1 |
| 510 | CAR | YE | LCR | O/EXPERIMENTAL | 4/26/90 | 1 | 74.65 | 94.81 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 557 | CAR | YE | LCR | O/LCR WARM CONTROL | 4/26/90 | 1 | 67.99 | 86.67 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 570 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 83.29 | 104.52 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 588 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 98.16 | 127.88 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 482 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 28.38 | 37.33 | | | 63 | 63 | 3/13/90 | 0 | 0 | 63 | | 1990 | 1990 | | | | |
| 494 | CAR | YE | LCR | LCR - 1/4 MILE UP | 10/18/90 | 1 | | 55.00 | | | 200 | 200 | 4/1/90 | 0 | 0 | 200 | | 1990 | 1990 | | | | |
| 512 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 26.92 | 35.58 | | | 64 | 64 | 3/12/90 | 0 | 0 | 64 | | 1990 | 1990 | | | | |
| 514 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 14.24 | 18.44 | | | 34 | 34 | 4/11/90 | 0 | 0 | 34 | | 1990 | 1990 | | | | |
| 529 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 26.79 | 35.85 | | | 63 | 63 | 3/13/90 | 0 | 0 | 63 | | 1990 | 1990 | | | | |
| 543 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.92 | 22.09 | | | 50 | 50 | 3/26/90 | 0 | 0 | 50 | | 1990 | 1990 | | | | |
| 567 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 12.96 | 16.87 | | | 36 | 36 | 4/9/90 | 0 | 0 | 36 | | 1990 | 1990 | | | | |
| 580 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 23.86 | 31.05 | | | 58 | 58 | 3/18/90 | 0 | 0 | 58 | | 1990 | 1990 | | | | |
| 581 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 20.12 | 26.12 | | | 53 | 53 | 3/23/90 | 0 | 0 | 53 | | 1990 | 1990 | | | | |
| 1 | O | O | LCR | LCR; 975 M ABOVE CONFLUENCE | 5/23/88 | 1 | | 355.00 | 400 | F | | | | | | | | | | 14 | 14 | | |
| 3 | O | O | LCR | LCR; 975 M ABOVE CONFLUENCE | 5/23/88 | 1 | | 361.00 | 353 | M | | | | | | | | | | 16 | 16 | | |
| 339 | FL | BL | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 278.00 | | M | | | | | | | | | | 9 | 9 | 3 | 4 |
| 344 | FL | BL | LCR | LCR; 100 M ABOVE BIG CANYON | 5/4/89 | 1 | | 235.00 | 122 | | | | | | | | | | | 10 | 10 | 6 | 6 |
| 348 | FL | BL | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 118.00 | | | | | | | | | | | | 2 | 2 | | |
| 349 | FL | BL | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 105.00 | | | | | | | | | | | | 2 | 2 | | |
| 4 | O | O | LCR | LCR; 975 M ABOVE CONFLUENCE | 5/23/88 | 1 | | 342.00 | 322 | M | | | | | | | | | | 14 | 14 | | |
| 440 | CAR | YE | LCR | LCR; 200 M ABOVE MOUTH | 5/9/89 | 1 | | 390.00 | 465 | F | | | | | | | | | | 13 | 13 | 16 | 16 |
| 441 | CAR | YE | LCR | LCR; AT MOUTH | 5/9/89 | 1 | | 330.00 | 283 | M | | | | | | | | | | 11 | 11 | 15 | 16 |
| 442 | CAR | YE | LCR | LCR; AT MOUTH | 5/9/89 | 1 | | 369.00 | 404 | F | | | | | | | | | | 16 | 16 | 17 | 18 |
| 444 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/8/89 | 1 | | 274.00 | 137 | F | | | | | | | | | | | | 7 | 7 |
| 490 | CAR | YE | LCR | LCR | | 1 | | | | | | | | | | | | | | | | 14 | 14 |

TABLE 1. OTOLITHS EXAMINED

| TAG NO. | TAG TYPE | TAG COL. | RIVER | CAPTURE LOCALITY | DATE CAPTURED | N | SL (mm) | TL (mm) | WT (gm) | SEX | MIN. AGE (DAYS) (LAP) | MAX. AGE (DAYS) (LAP) | DATE 1st INCREMNT FORMED (LAP) | MIN. AGE (YRS) (LAP) | MAX. AGE (YRS) (LAP) | INCRE-MENTS YR. 1 | INCRE-MENTS YR. 2 | EARLY YEAR CLASS | LATE YEAR CLASS | MIN. AGE (YRS) (ASTER) | MAX AGE (YRS) (ASTER) | MIN. AGE (YRS) (OPERC) | MAX. AGE (YRS) (OPERC) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 0 | 0 | LCR | LCR; 975 M ABOVE CONFLUENCE | 5/23/88 | 1 | | 348.00 | 351 | | | | | | | | | | | 15 | 15 | | |
| 500 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/24/89 | 1 | | 182.00 | 62 | F | | | | | | | | | | 4 | 4 | 3 | 4 |
| 501 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/24/89 | 1 | | | | | | | | | | | | | | 3 | 3 | 2 | 2 |
| 503 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/24/89 | 1 | | | | | | | | | | | | | | 1 | 1 | 1 | 1 |
| 504 | CAR | YE | LCR | LCR; 1225 M ABOVE MOUTH | 5/24/89 | 1 | | | | | | | | | | | | | | 1 | 1 | 1 | 1 |
| 519 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 522 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 533 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 534 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 2 | 2 | | |
| 536 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 538 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 546 | CAR | YE | LCR | LCR; AT MOUTH | 5/26/89 | 1 | | 372.00 | | | | | | | | | | | | 16 | 16 | 15 | 15 |
| 549 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 8 | 8 | 4 | 4 |
| 552 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 1 | 1 | | |
| 553 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | | | | | | | | | | | 2 | 2 | | |
| 590 | CAR | YE | LCR | LCR; AT MOUTH | 5/15/89 | 1 | | 373.00 | | | | | | | | | | | | 13 | 13 | 12 | 12 |
| 595 | CAR | YE | LCR | LCR; AT MOUTH | 5/7/89 | 1 | | 386.00 | 456 | F | | | | | | | | | | | | 14 | 14 |
| 598 | CAR | YE | LCR | LCR; 200 M ABOVE CONFLUENCE | 5/7/89 | 1 | | 104.00 | 8 | | | | | | | | | | | | | 1 | 1 |
| 600 | FL | RE | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 309.00 | | M | | | | | | | | | | 13 | 13 | | |
| 605 | FL | RE | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 121.00 | | | | | | | | | | | | 3 | 3 | 1 | 1 |
| 612 | FL | RE | LCR | LCR; ATOMIZER FALLS | 5/6/89 | 1 | | 108.00 | | | | | | | | | | | | 2 | 2 | | |
| 902 | CAR | YE | LCR | LCR; AT SALT CANYON | 5/13/89 | 1 | | 350.00 | 331 | F | | | | | | | | | | 13 | 13 | 2 | 13 |
| | | | | **COLORADO RIVER MAINSTREAM** | | | | | | | | | | | | | | | | | | | |
| 469 | CAR | YE | COL | COLORADO RIVER MAINSTEM RM 63.9L | 10/22/90 | 1 | | 202.00 | | | | | | 9 | 10 | | | 1980 | 1981 | | | | |
| 266 | CAR | OR | COL | COLORADO RIVER AT RM 64.5 | 6/24/92 | 1 | 25.35 | 33.25 | | | | | | 1 | 1 | | | 1991 | 1991 | | | | |
| 274 | CAR | OR | COL | COLORADO RIVER AT RM 122.5 | 4/6/91 | 1 | 28.10 | 35.34 | | | | | | 1 | 1 | 100 | | 1990 | 1990 | | | | |
| 322 | CAR | OR | COL | COLORADO RIVER AT RM 122.5 | 4/6/91 | 1 | 29.00 | 37.49 | | | | | | 1 | 1 | 80 | | 1990 | 1990 | | | | |
| 331 | CAR | OR | COL | COLORADO RIVER AT RM 122.5 | 4/6/91 | 1 | 29.92 | | | | | | | 1 | 1 | 110 | | 1990 | 1990 | | | | |
| 365 | CAR | OR | COL | COLORADO RIVER AT RM 64.5 | 4/15/92 | 1 | 30.40 | 37.82 | | | | | | 1 | 1 | 80 | | 1991 | 1991 | | | | |
| 287 | CAR | OR | COL | COLORADO RIVER AT RM 70.3 | 5/12/91 | 1 | 33.50 | 42.10 | | | | | | 1 | 1 | 90 | | 1990 | 1990 | | | | |
| 371 | CAR | OR | COL | COLORADO RIVER AT RM 193.9 | 5/23/91 | 1 | 48.70 | 60.00 | | | | | | 1 | 1 | 130 | | 1990 | 1990 | | | | |
| 381 | CAR | OR | COL | COLORADO RIVER AT RM 64.5 | 6/24/92 | 1 | 26.97 | 35.45 | | | | | | 1 | 1 | | | 1991 | 1991 | | | | |
| 398 | CAR | OR | COL | COLORADO RIVER AT RM 122.0 | 4/18/92 | 1 | 39.05 | 46.73 | | | | | | 1 | 1 | | | 1991 | 1991 | | | | |
| 455 | CAR | YE | COL | O/COLO. COLD CONTROL | 4/28/90 | 1 | 75.84 | 94.84 | 44 | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 511 | CAR | YE | COL | COLORADO; 0.2 MI ABOVE CARDENAS | 10/22/90 | 1 | | 69.00 | | | | | | 1 | 1 | | | 1989 | 1989 | | | | |
| 461 | CAR | YE | COL | COLORADO MAINSTEM; RM 65.3 L | 10/20/90 | 1 | | 58.00 | | | | | | 0 | 0 | | | | | | | | |
| 539 | CAR | YE | COL | COLORADO RIVER MAINSTEM; RM70.9 | 10/22/90 | 1 | 29.40 | 37.40 | | | | | | 0 | 0 | | | | | | | | |
| 276 | CAR | OR | COL | COLORADO RIVER AT RM 68.1 | 9/13/91 | 1 | 25.10 | 31.65 | | | 110 | 110 | 5/28/91 | 0 | 0 | 110 | | 1991 | 1991 | | | | |
| 320 | CAR | OR | COL | COLORADO RIVER AT RM 68.1 | 6/24/92 | 1 | 19.53 | | | | 45 | 45 | 5/10/92 | 0 | 0 | 45 | | 1992 | 1992 | | | | |
| 328 | CAR | OR | COL | COLORADO RIVER AT RM 192.3 | 9/24/92 | 1 | 19.55 | 25.10 | | | 98 | 98 | 6/18/92 | 0 | 0 | 98 | | 1992 | 1992 | | | | |
| 295 | CAR | OR | COL | COLORADO RIVER AT RM 122.1 | 6/26/92 | 1 | 14.17 | 17.50 | | | 43 | 43 | 5/14/92 | 0 | 0 | 43 | | 1992 | 1992 | | | | |
| 378 | CAR | OR | COL | COLORADO RIVER AT RM 68.1 | 6/24/92 | 1 | 14.30 | | | | 48 | 48 | 5/7/92 | 0 | 0 | 48 | | 1992 | 1992 | | | | |
| 387 | CAR | OR | COL | COLORADO RIVER AT RM 68.1 | 6/24/92 | 1 | 13.70 | | | | 41 | 41 | 5/14/92 | 0 | 0 | 41 | | 1992 | 1992 | | | | |
| 488 | CAR | YE | COL | MAINSTEM COLORADO; RM 65.3 L | 10/20/90 | 1 | | 33.00 | | | 143 | 143 | 5/30/90 | 0 | 0 | 143 | | 1990 | 1990 | | | | |

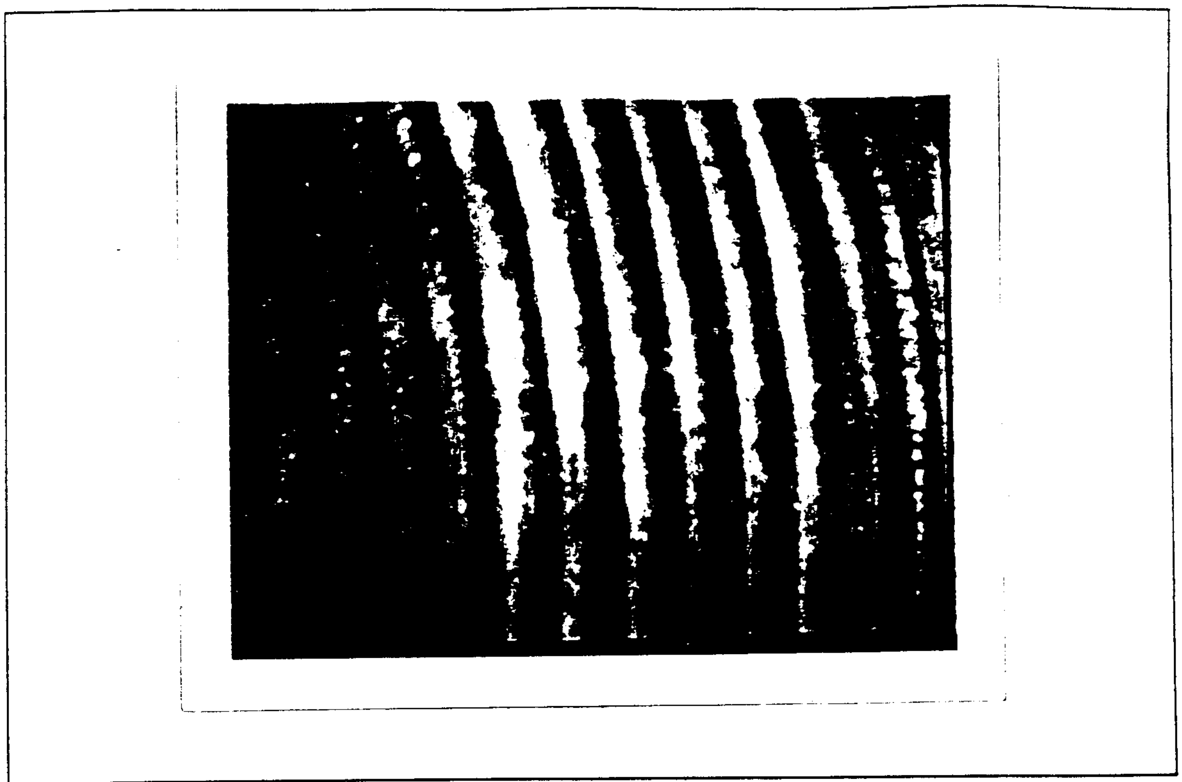

Figure 1. Clear presumptive daily increments in the first year of growth of a $1^+$ year-old fish (tagno 502 YC) captured in the LCR about 1 km above its mouth.

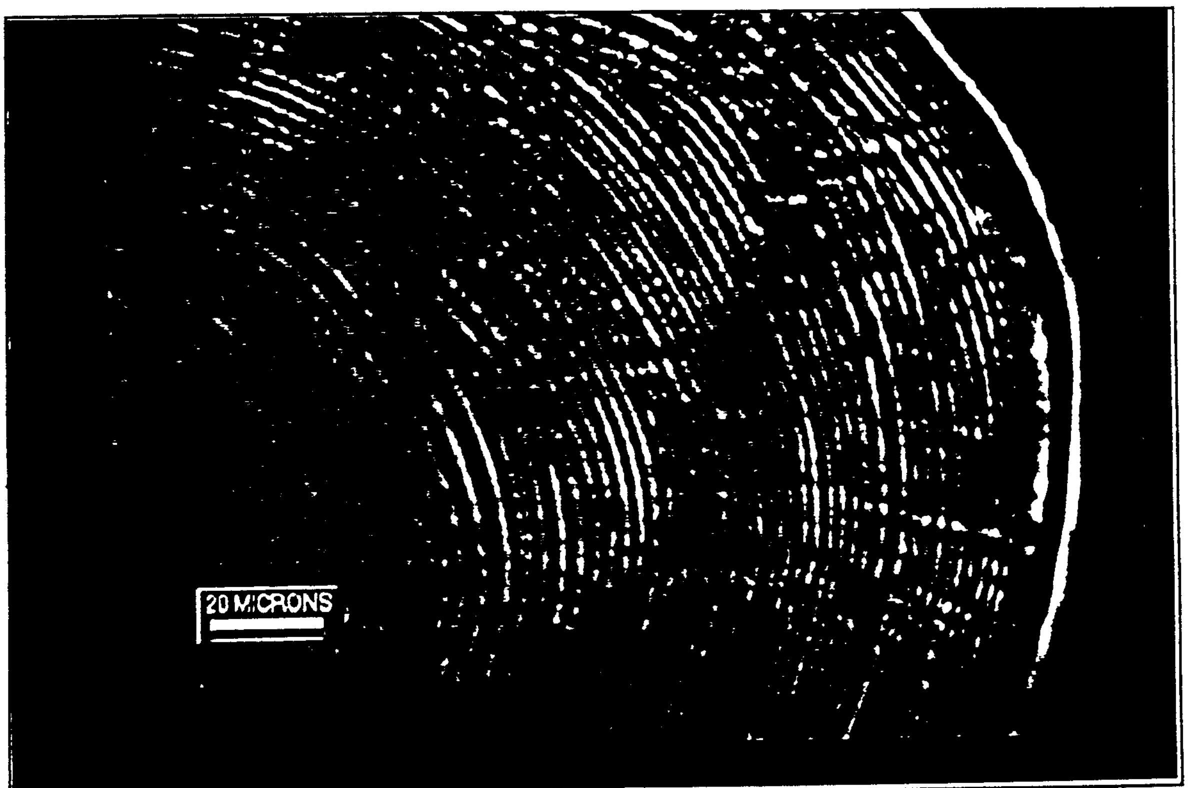


Figure 2. Daily increments to edge in lapillus of an age $0^+$ specimen from River Mile 68.1 collected September 13, 1991. Note clear increments extending to edge. Specimen estimated to be 110 days old (thus, estimated hatch date = 5/26/91).

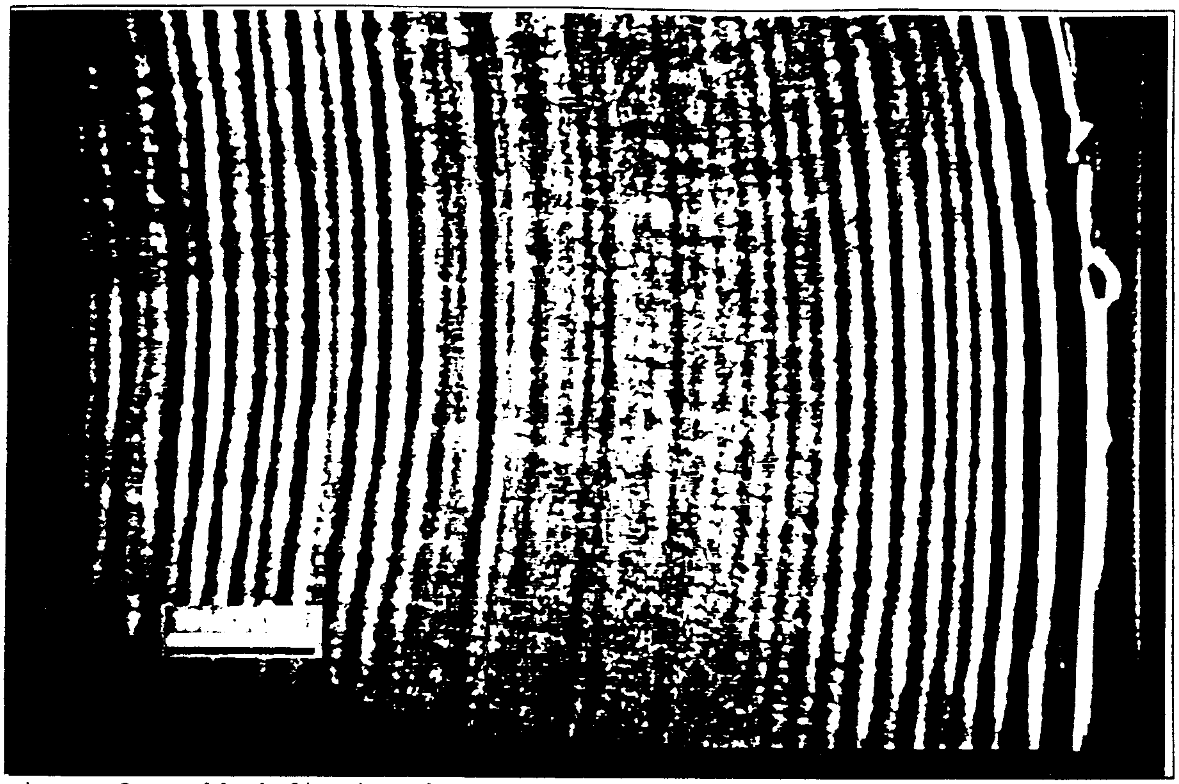

Figure 3. Well defined and poorly defined increments in lapillus from a young-of-the-year specimen (tag number 328) from the mainstream Colorado at River Mile 192.3.

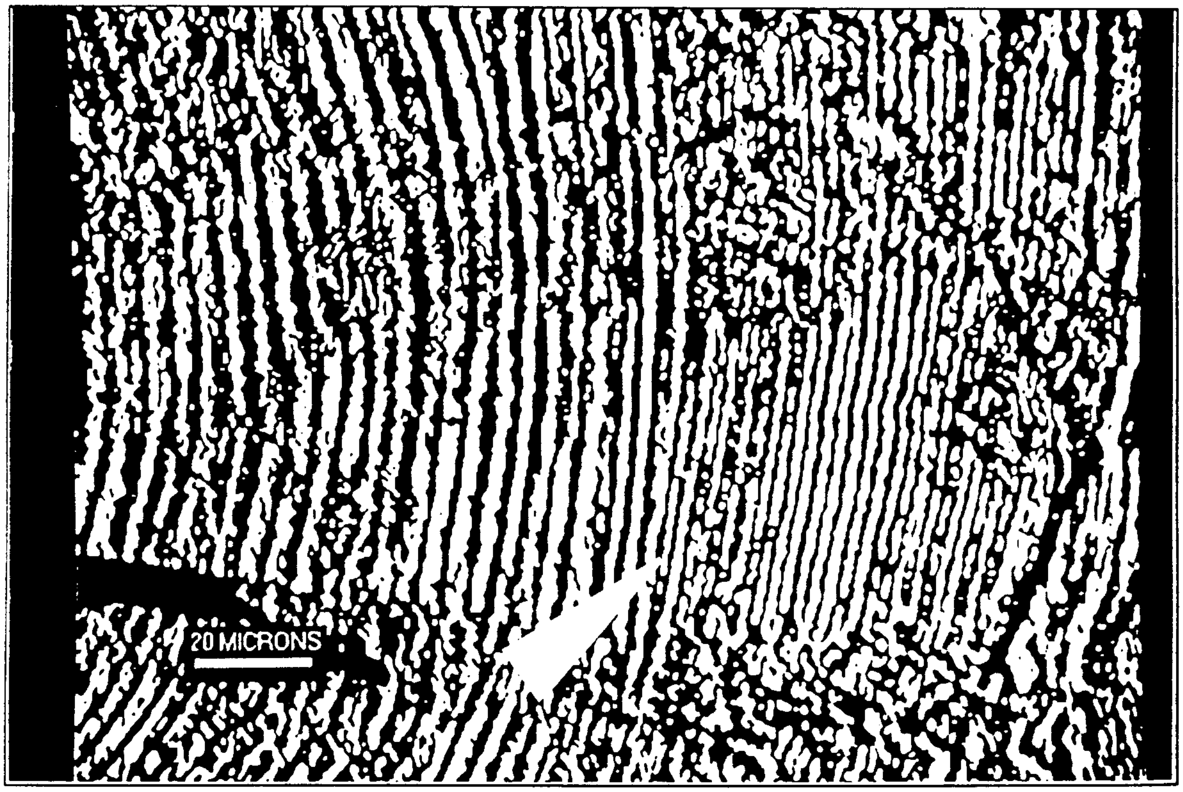


Figure 4. Rapid transition from wide to narrow growth increments in a specimen taken in the mainstream Colorado at River Mile 65.3 on October 20, 1990. Specimen estimated to be 143 days old.

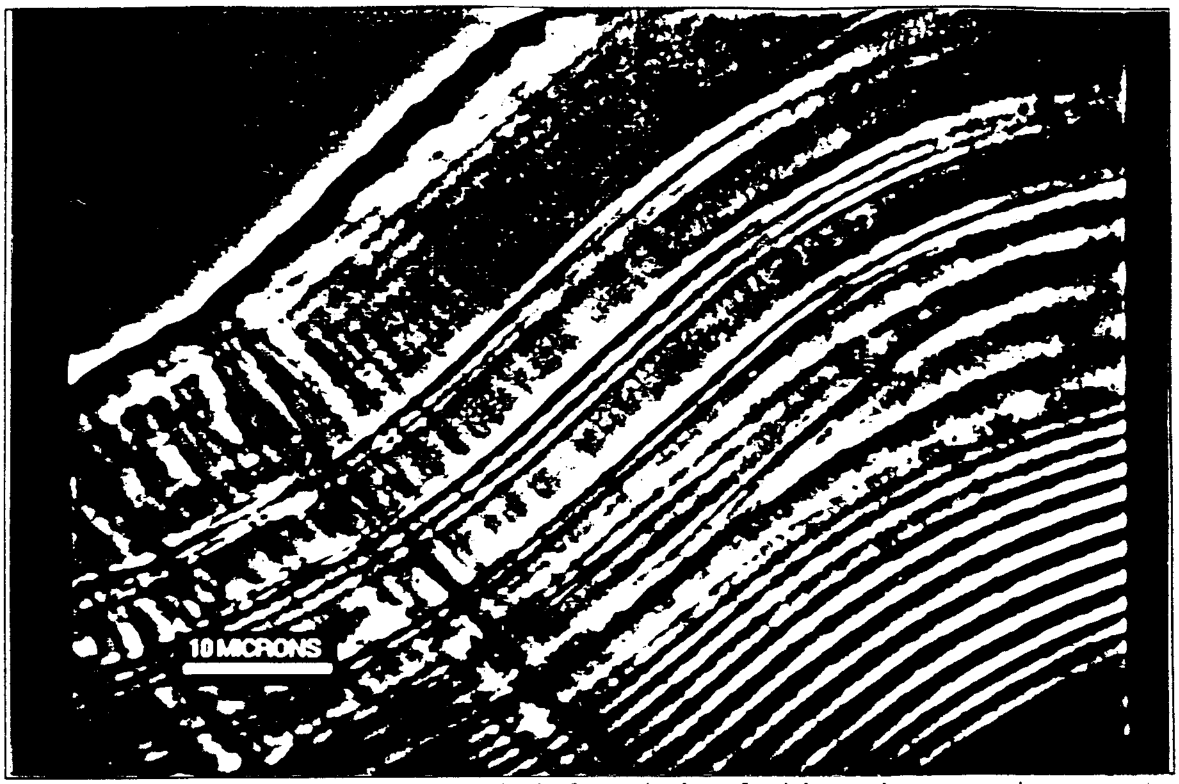


**Figure 5.** Fluctuations between brief periods of wide and narrow increments near edge of lapillus from specimen (tagno 539) taken in mainstream Colorado River at River mile 70.9 on October 22, 1990.

**Figure 6.** Annuli in the lapillus of the oldest specimen examined (tagno 586), estimated to be 22 or 23 years old.

Figure 7. Thirteen to 15 annuli visible in lapillus of specimen 548, as seen in reflected light.

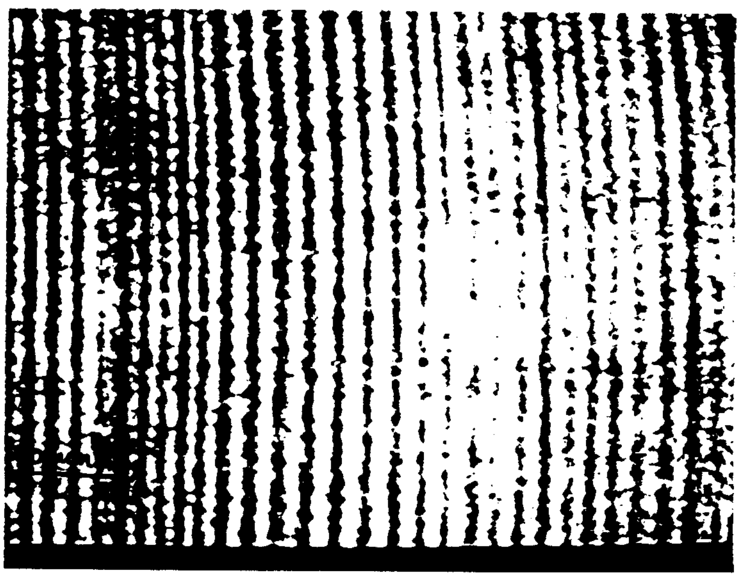

Figure 8. Daily increments visible in that portion of the lapillus corresponding to year 6 of life of a 13 to 15-year old specimen (tag number 548).

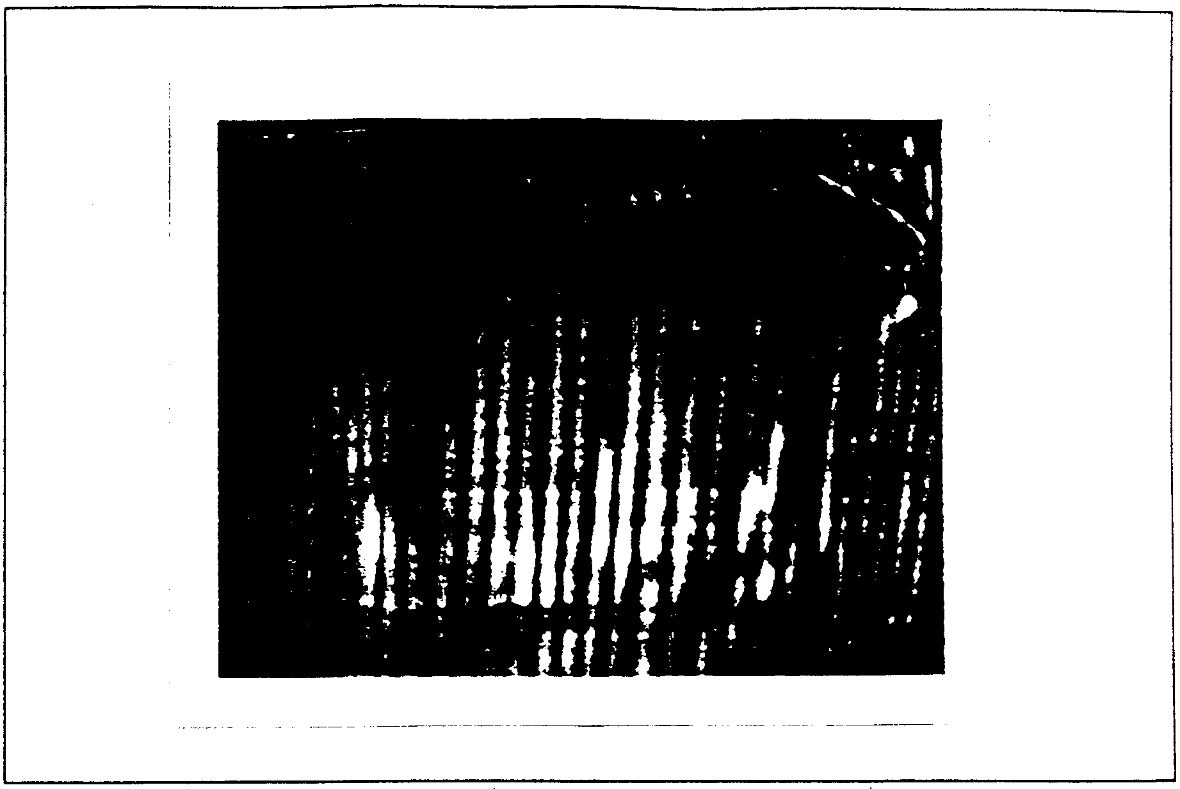

Figure 9. Increments in second year of growth of a $1^+$ age specimen (tag number 502) captured in the Little Colorado River.

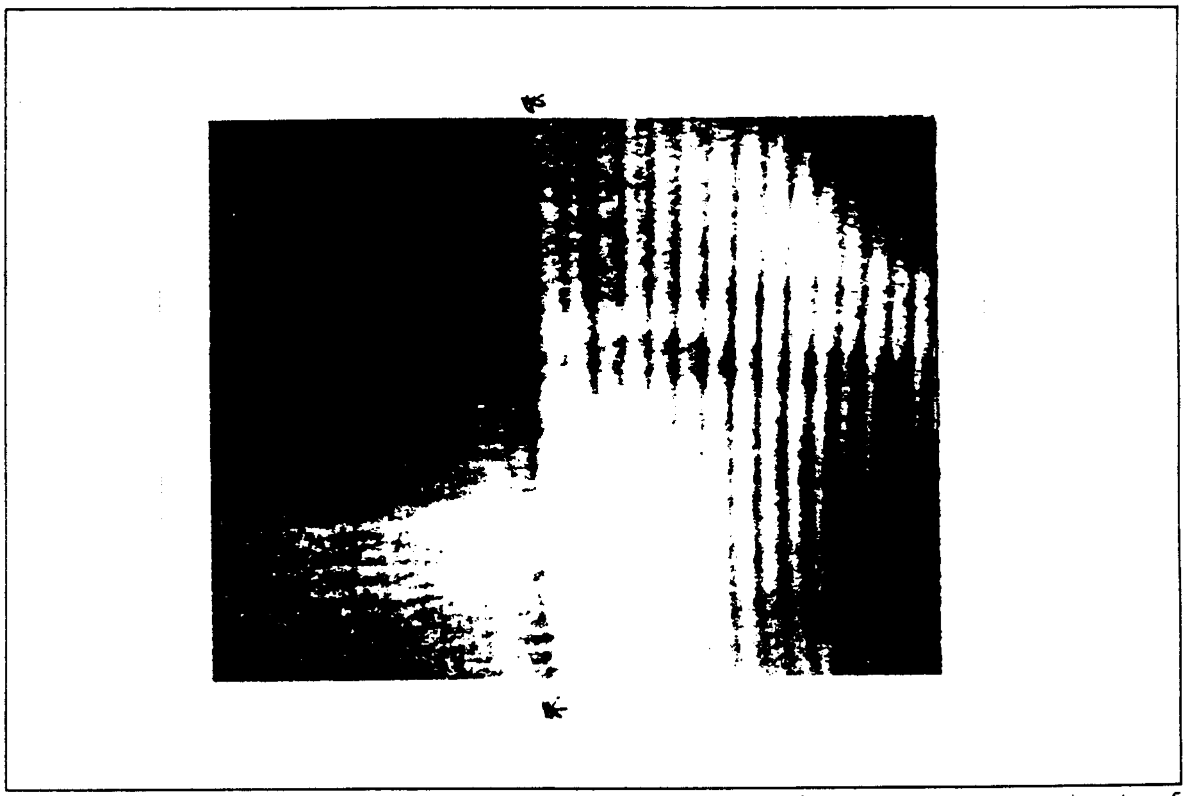

Figure 10. Abrupt transition from wide to narrow increments near start of 3rd growing season in specimen 495.

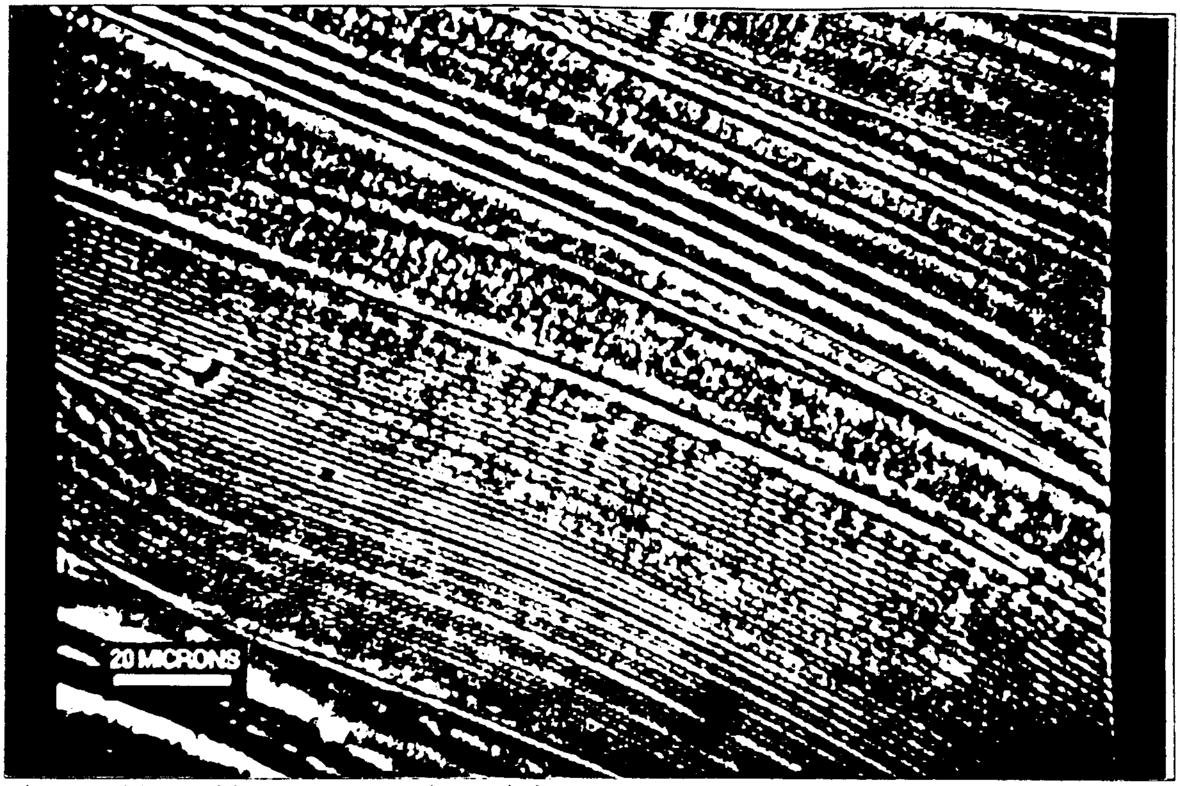


Figure 11. Odd patterns in adult specimen (tag number 495) possibly produced by repeated movements across temperature differentials.

Ages of adults as estimated from opercles are in fairly good agreement with those estimated independently from lapilli, however, the single independent estimates from asterisci are generally higher (Figure 12). Lapilli are clearly the preferred structure for ageing. Both daily increments and annuli in asterisci are comparatively much more difficult to interpret. Opercles appear to provide a reasonable means of obtaining yearly ages of adults and have the advantage of requiring less preparation than do otoliths. Opercles, however, have the disadvantage, like all bone, of being susceptible to periodic mobilization of calcium and other elements which could also alter structure. Otoliths are well know to be much more stable than bone. Additionally, opercles do not provide the same detailed daily history within years that can be obtained from otoliths. This

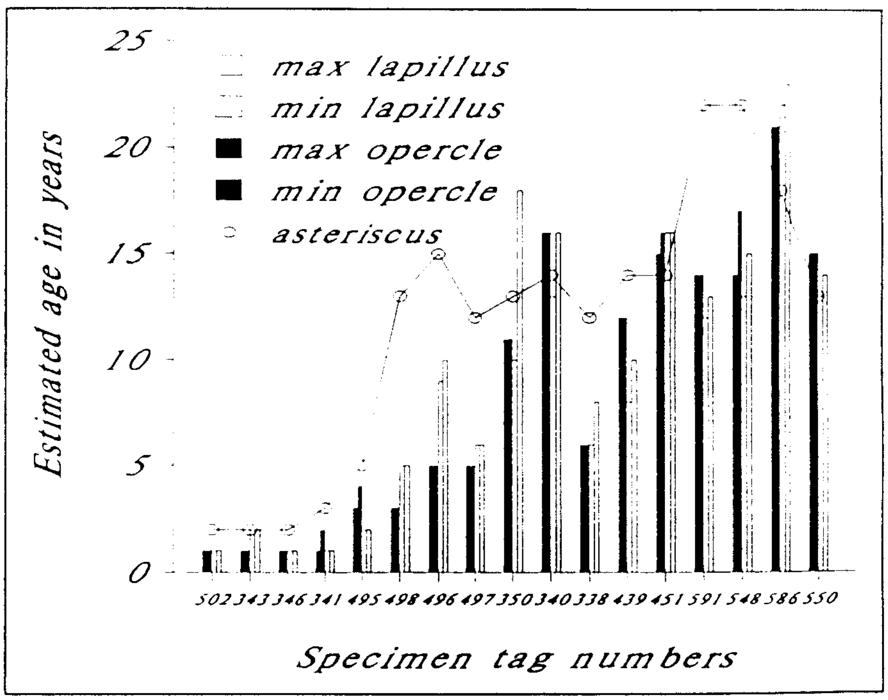


Figure 12. Minimum and maximum age estimates using lapilli and opercles (bars) and asterisci (circles) for fish > 1 yr. old.

might be significant once larger sample sizes have been analyzed. Year-specific natural marks in daily increments have been utilized as useful cohort markers in other species, and could be useful in validating ages. It is probable that events such as unusual summer floods in the LCR might produce characteristic patterns that would unambiguously allow assignment of those zones to that event in specimens captured many years subsequently. Utilization of either lapilli or opercles for yearly ageing or for annual growth estimation will require validation of the periodicity of presumptive annuli. It seems probable that stress associated with such marked and rapid transitions as that experienced when moving between the LCR and mainstream would form marks on either otoliths or opercles which could be mistaken as an annual mark.

On the basis of data compiled to date from lapilli and opercles it appears that growth rates of humpback chub in the Grand Canyon are highly heterogeneous. Size is not a good predictor of age (Figure 13 and Figure 14). Variations in growth rate may be a function of inherent individual variation and/or temporally and spatially expressed habitat effects. On the basis of the small sample examined to date, variation in growth rate appears to be expressed early in life, and to be markedly affected by habitat. Nearly all young-of-the-year from the LCR were larger than others of similar age taken from the cold mainstream Colorado River (Figure 15). Similarly, one-year old fish taken from the LCR in April and May of 1990 averaged more than double the size of one-year olds taken from the mainstream almost exactly one year later. This is despite the indication from estimated ages that the 1991 yearlings from the mainstream had been growing for 30 to 50 days more in the year of capture than had the 1990 yearlings from the LCR (Table 1). Though comparisons of first year growth between these 1989 and 1990 year classes have not been done (but would be possible with back-calculation techniques using increment widths), the effect of lower mainstream temperatures on growth appears large, and is probably significant in terms of consequences for mortality rates. In most well-studied fisheries, lower growth rates are associated with higher mortality. Measurements of daily growth increments could easily be used to quantify the effect of life in the mainstream on growth rates.

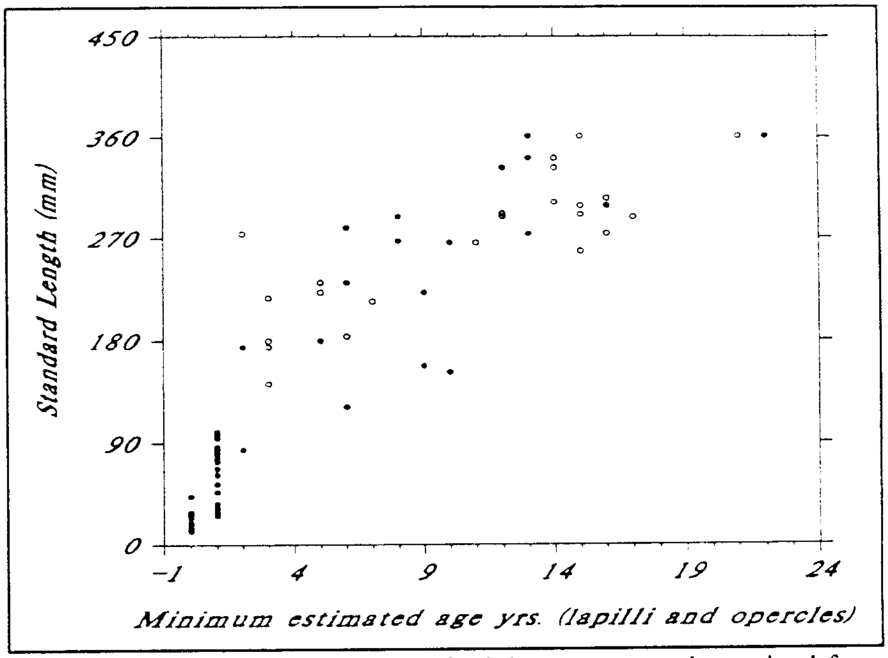


**Figure 13**. Scatter of relationship of minimum ages as determined from lapilli (solid circles) and opercles (open circles) and Standard Length of humpback chub.

Part of the extensive variation in the relationship of estimated age and Standard Length may be due to sexual dimorphism, but not nearly enough data are available in the small data set developed to date to allow attempts to factor out this source of variation (Figure 16 and Figure 17).

Marked structural changes in daily increments found thus far are promising in that it is likely that environmental changes of the magnitude required to produce such dramatic structural changes in the otoliths are likely to have produced by changes in otolith chemical composition which might be found to correlate with ambient water quality or temperature. The unique physical and chemical properties of water

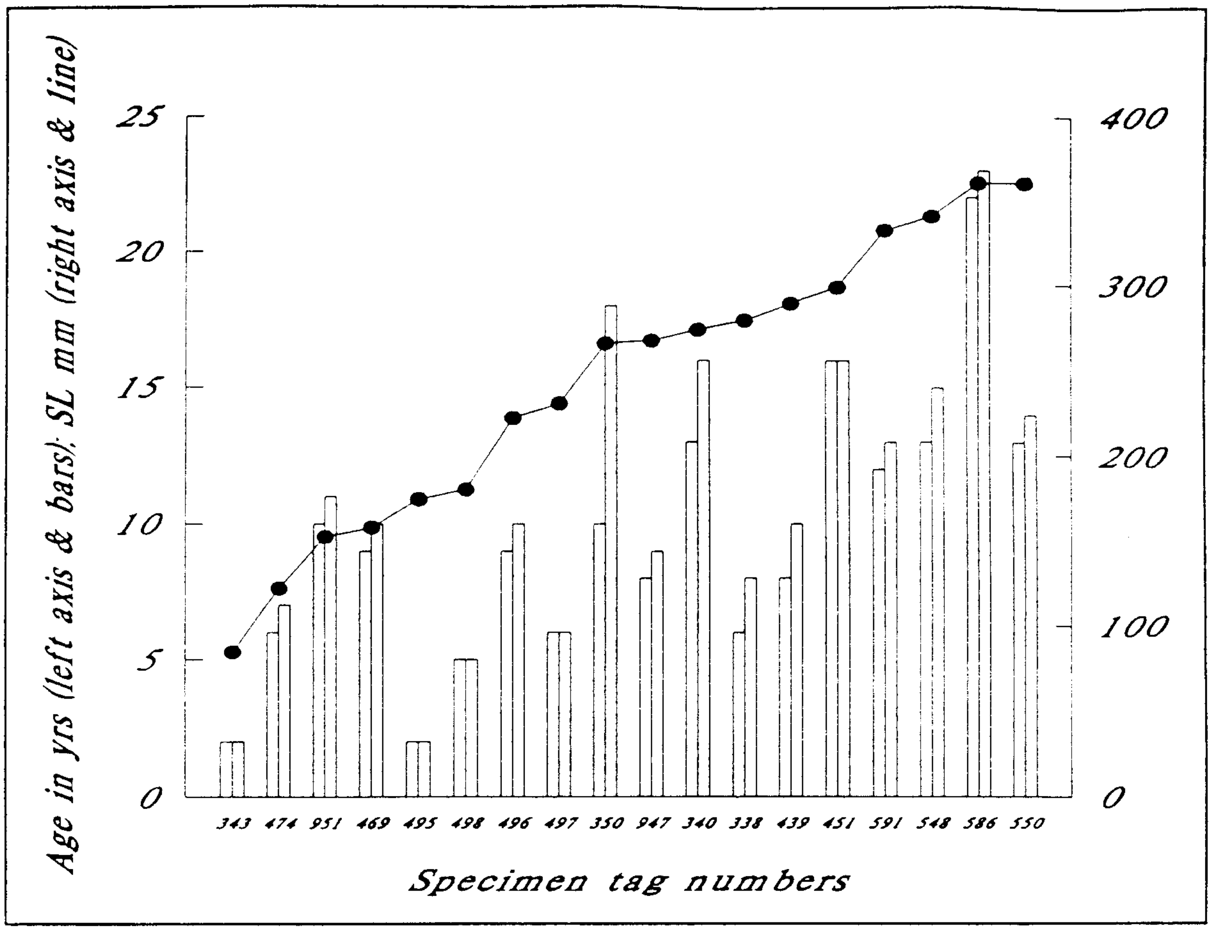


**Figure 14.** Minimum and maximum age in years estimated from lapilli and Standard Length for all specimens estimated to be more than one year old.

quality in the LCR that clearly distinguish it from the mainstream Colorado River seem to provide a very appropriate system in which to test rapidly developing hypotheses of the relationship of otolith chemistry to environmental factors.

## REQUESTS FOR ADDITIONAL SPECIMENS, DATA AND SUGGESTIONS

### ACCURACY OF THE OTOLITH DATA BASE

The entire inventory of specimens currently available to the author for otolith studies is provided in Appendix 1 and (with much more detail) in a file on disk (hbinvtry.xls). Some questions remain regarding exact collection localities for some specimens, as well as habitat conditions. It is hoped that these data can be provided by the field crews who collected them and that they will generally proof once again the entire data base. Additionally, sex is unknown for many specimens from which it might still be obtainable from preserved materials not currently available to the author. Sex determinations are needed since current scatter in the distribution of length at age (Figure 13) greatly compromises precision of attempts at back-calculation of lengths at various ages as will be required to reconstruct growth histories of individuals. Removal of the effect of sexual dimorphism in size from the length-age relationship would almost certainly allow more precise reconstructions of growth histories.

### ADDITIONAL SPECIMENS

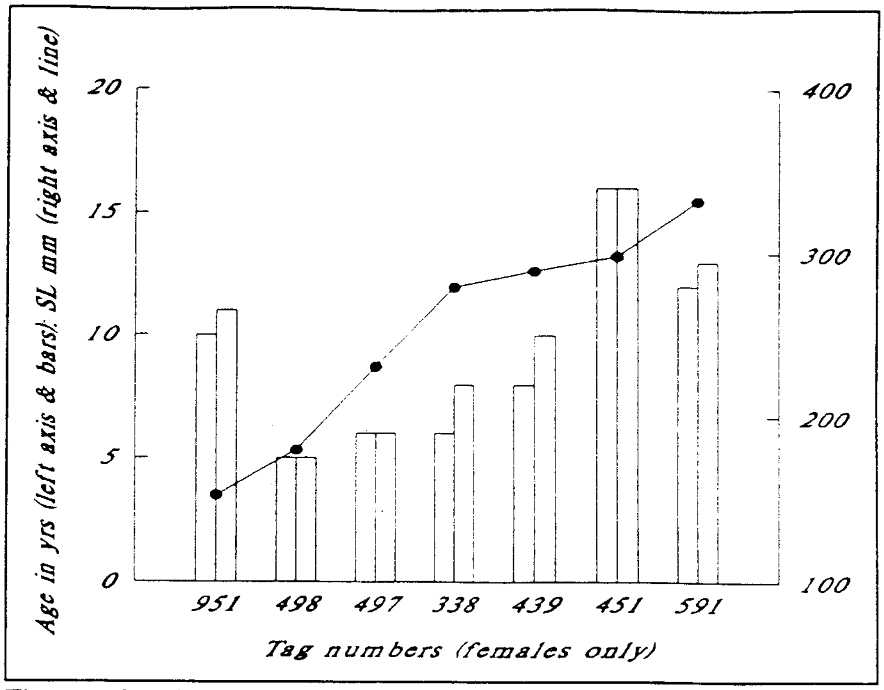


Figure 16. Minimum and maximum age estimates (bars) and Standard Length (dots) as determined from lapilli for all specimens verified to be females.

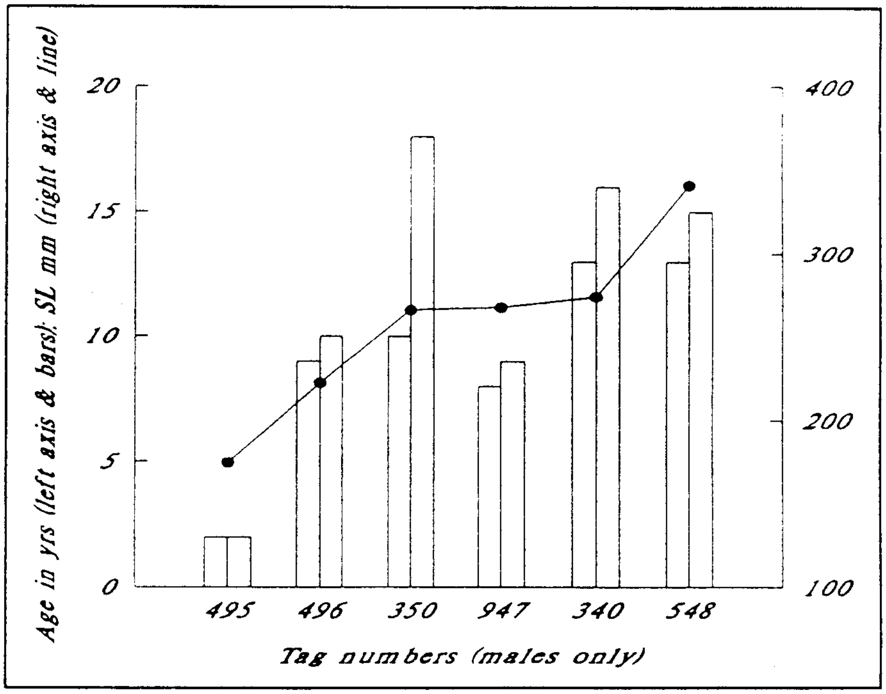


Figure 17. Minimum and maximum age estimates (bars) and Standard Length (dots) as determined from lapilli for all specimens verified to be males.

information would allow blind comparisons of otolith and tag-derived histories. Particularly valuable would be multiply-recaptured young adult fish with clearly demonstrable growth between original capture and recaptures. Also useful might be radiotelemetered individuals known to have passed through major environmental gradients such as the LCR-Mainstream interface during the growing season in which sacrificed. Especially informative for future work would be intentional chemical marking of otoliths of selected individuals in the field (utilizing Tetracycline or Alizarin). If accompanied by PIT-tagging, otolith-marked individuals recaptured in the future could be used to validate periodicity of both daily and annual marks.

<u>3. Specimens captured far from the Little Colorado River.</u> Humpback chub encountered in the lower Grand Canyon may or may not be part of the LCR gene pool. In particular, it seems unlikely that small specimens taken in lower canyon reaches originated in the LCR. To date relatively few specimens from locations far below the LCR have been made available for otolith research and it is recommended that otoliths of specimens taken in the future from lower canyon areas be taken for ageing, examination of daily increment patterns in the first year of life, and analyses of chemical composition. Some specimens already examined from the lower Canyon which are of ages that would seem to make it quite unlikely that they were born in the LCR. For example, specimen 328, a 98-day old fish, was taken at River Mile 192.3, or about 130 miles below the LCR. Unfortunately, no 1991 year class young-of-the-year from the LCR have yet been

examined to determine dates of spawning in that river. Presumably specimens not yet processed, but listed in Appendix 1, collected in May, June and July of 1991, include at least some young-of-the-year, and if so, spawning dates in 1991 will be estimated in the next report. Similarly, since specimen 328 was analyzed blind shortly before preparation of this document, without knowledge of its capture location, no particular attention was paid to searching for increment patterns early in life which might support the hypothesis that it hatched and grew for some time in warm tributary waters before moving to the mainstream. Patterns presumed to depict this movement have been found in other specimens (e.g. Figure 4 or Figure 10). This hypothesis will now be investigated.

Hatching date estimates obtained to date, while based on relatively few specimens, indicate May and June hatches in 1992 (5 specimens - all from Colorado mainstream) and hatches in March and April in 1990 (9 specimens - all from the LCR). Interestingly, a single specimen taken about 5 miles below the LCR in the mainstream Colorado in October, 1990, was estimated to have hatched about May 30 of that year, nearly two months later than the latest estimated hatch date from the same year in the LCR. This may simply be an artifact of small sample size and/or sampling bias since young-of-the-year specimens, if such were present and collected in the LCR in late May or later in the summer of 1990, were not available to the author (Appendix 1).

## ENVIRONMENTAL DATA

**Though the magnitude of physical and chemical differences between the mainstream and LCR are obvious to even untrained observers, quantitative descriptors of chemical composition and physical attributes of these waters will be required for analysis of hypotheses that otolith composition reflects ambient water quality.** Verbal requests for such data have been made several times to representatives of AGFD, and though advised they exist in data bases maintained by other groups (e.g. USGS), and that they would be provided, the author has not yet received them. Specifically, data on temporal and spatial variability in temperature in each river will be required, as will comprehensive water quality data. Since the otolith chemical analyses will be exploratory, data on as many water quality parameters as possible would be useful. This would include data on rare elements, heavy metals, isotopes, etc.. Any elements or isotopes which might uniquely characterize either river would be of particular interest. Precise identification of isotopes released in the Zuni River basin by an accidental spill a number of years ago would be of great interest, as would studies of its subsequent distribution downstream. Additionally, in order to analyze effects of discharge on growth rates and year-class strength, detailed discharge data will be required for each river. Ideally, chemistry and discharge data covering the past three decades might be provided. This period has been chosen to cover the entire estimated lifetime of the oldest specimens analyzed to date.

Temperature and discharge data covering the periods from which young-of-the-year specimens are available might prove especially valuable. It is likely that unusual spring or summer meteorologic events such as unusual cold spells during normally warm months, or dramatic floods, will produce event-specific, unique natural otolith banding patterns. These patterns could then be used as markers which would allow subsequent validation of both daily and annual ageing techniques, and future back-calculations of birth dates of adults in which such event-specific marks can be located. A few cases in which such unique, natural marks have proven valuable in management of commercial marine fisheries have recently been reported (1993 Otolith Research and Applications Conference).

## GENERAL SUGGESTIONS

As discussed above, otoliths are clearly indicated by preliminary work to have considerable potential for humpback chub management applications in the Grand Canyon. It is hoped that this preliminary report will provoke comments from the management community which will assist the author in determining what future research pursuits are likely to provide the most useful contributions to those trying to make informed management decisions.

## SUMMARY

Preliminary data obtained from otoliths of humpback chub from the Grand Canyon are provided. Age estimates with near daily precision appear to be easily obtainable from young-of-the-year specimens while in the Little Colorado River, yet the daily deposition of increment formation has yet to be rigorously validated in the lab or in field experiments. The conclusion that they are daily, however, is supported by evidence that hatch dates estimated from otoliths generally agree with field evidence of timing of spawning activity. Reliable resolution and counting of daily increments from periods spent in the cold, near constant-temperature waters of the mainstream Colorado River appears not to always be possible with standard light microscopy techniques, but might be attainable with Scanning Electron Microscopy.

Otoliths of humpback chubs appear to provide reasonable estimates of yearly ages of adult specimens. Up to about three years of age, length of the growing season can be estimated from daily increments between annuli.

Highly preliminary data from small samples analyzed to date provide interesting insights into the biology of humpback chub in the Grand Canyon. Growth rates in the mainstream Colorado River are strongly indicated to be much lower than those attained in the LCR. Some specimens from the mainstream appear to have spent several brief periods in waters much warmer than the mainstream, perhaps tributary mouths. Indications of very abrupt changes in growth rates have been found in many specimens, and is presumed likely to correspond to inter-habitat movements, such as passage from the LCR to mainstream Colorado and returns to the LCR. Back-calculated hatching dates indicate considerable variation in timing of reproduction among years, and relatively young ages of specimens taken far downstream of the LCR.

Very recent literature on temperature and salinity effects on elemental composition of otoliths indicate that it is very likely that at least some elements can be found that would provide a unique mark for time periods spent in the LCR or at least, non-mainstream Colorado River habitats. Though there is almost no literature on concentrations of elements expected in freshwater fish otoliths, they can clearly be expected to be near the detection capabilities of analytical equipment now commonly in use on the many studies being published on marine species. New techniques are quickly becoming available and analyses of micro-spatial distribution of elements across humpback chub otolith transects will be completed prior to the completion of the final report from this study.

## ACKNOWLEDGMENTS

Dr. Ed Brothers provided training and general advice regarding otolith preparation and reading, as well as readings of all lapilli. Mr. Gary Scoppetone graciously provided his estimates of ages from opercles, and Mr. Michael McCarthy mounted, ground and made age estimates from the asterisci.

## APPENDIX 1

Tag numbers, capture locality and date, length, weigth and sex of specimens collected and available for examination of otoliths but not yet analyzed.

APPENDIX 1 - SPECIMENS AVAILABLE FROM WHICH OTOLITHS HAVE NOT YET BEEN EXAMINED

| TAG NO. | TAG TYPE | TAG COL. | RIVER | CAPTURE LOCALITY | DATE CAPTURED | N | SL (mm) | TL (mm) | WT (gm) | SEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **SPECIMENS NOT YET ANALYZED** | | | | | | | |
| | | | **COLORADO RIVER MAINSTREAM** | | | | | | | |
| 373 | CAR | OR | COL | COLORADO RIVER AT RM 64.5 | 4/2/91 | 1 | 42.64 | 55.20 | | |
| 461 | CAR | YE | COL | COLORADO MAINSTEM; RM 65.3 L | 10/20/90 | 1 | | 58.00 | | |
| 484 | CAR | YE | COL | 0/COLO. COLD CONTROL | 4/26/90 | 1 | 86.99 | 109.04 | 44 | |
| 536 | CAR | YE | COL | 0/COLO. COLD CONTROL | 4/26/90 | 1 | 86.13 | 113.14 | 44 | |
| 572 | CAR | YE | COL | 0/COLO. COLD CONTROL | 4/26/90 | 1 | 66.34 | | 44 | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAIN | 7/14/91 | 1 | | | | |
| | | | COL | AGFD; REACH = MAINSTREAM | 7/11/91 | 1 | | | | |
| | | | **LITTLE COLORADO RIVER** | | | | | | | |
| 2 | O | O | LCR | LCR; 875 M ABOVE CONFLUENCE | 5/23/88 | 1 | | 330.00 | 289 | F |
| 594 | CAR | YE | LCR | LCR | 5/5/89 | 1 | | 131.00 | 22 | |
| 450 | CAR | YE | LCR | LCR; 598 M ABOVE MOUTH | 5/6/89 | 1 | | 364.00 | 413 | M |
| 521 | CAR | YE | LCR | LCR | 5/15/89 | 1 | | | | |
| 580 | CAR | OR | LCR | LCR; FOUR MILES ABOVE MOUTH | 5/14/89 | 1 | | | | |
| 0314 | FL | YE | LCR | LCRMO; PARA TRAM | | 1 | | 352.00 | 377 | M |
| 201 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 297.00 | | F |
| 201 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 243.00 | | M |
| 215 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 163.00 | | M |
| 223 | | OR | LCR | 1200/ HOOP | 4/20/90 | 1 | 48.84 | 64.00 | | |
| 227 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 20.90 | 26.86 | | |
| 229 | | OR | LCR | 2400/ RUN RIFFLE | 5/6/90 | 1 | 19.83 | 24.98 | | |
| 231 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 77.62 | 97.35 | | |
| 236 | | OR | LCR | BELOW FALLS | 6/6/90 | 1 | 19.21 | 24.88 | | |
| 237 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 68.08 | 86.69 | | |
| 239 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 22.07 | 28.74 | | |
| 241 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 87.63 | 107.87 | | |
| 243 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 243.00 | | U |
| 244 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 74.53 | 94.51 | | |
| 245 | | OR | LCR | 2400/ RUN RIFFLE | 5/6/90 | 1 | 18.09 | 22.70 | | |
| 246 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 21.42 | 26.70 | | |
| 247 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 22.88 | 29.31 | | |
| 249 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 208.00 | | M |
| 249 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 76.56 | 96.27 | | |
| 255 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 366.00 | | M |
| 270 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 246.00 | | M |
| 271 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 22.82 | 29.14 | | |
| 276 | | OR | LCR | 2400/ RUN RIFFLE | 5/6/90 | 1 | 26.46 | 34.02 | | |
| 282 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 69.15 | 88.92 | | |
| 283 | | OR | LCR | 200/ HOOP | 4/20/90 | 1 | 86.51 | 113.00 | | |
| 290 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 70.76 | 87.61 | | |
| 291 | CAR | BL | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | | 361.00 | | M |
| 297 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 24.19 | 31.44 | | |
| 298 | CAR | RE | LCR | 180/ NS D HOOP | 6/1/90 | 1 | | 250.00 | 118 | F |
| 316 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 84.62 | 105.29 | | |
| 320 | | OR | LCR | BELO FALLS | 5/6/90 | 1 | 18.69 | 23.32 | | |
| 346 | | OR | LCR | 110/SIDE CHANNEL AT MOUTH | 5/7/90 | 1 | 21.31 | 26.58 | | |
| 360 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 75.36 | 93.86 | | |
| 364 | | OR | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 74.65 | 95.09 | | |
| 365 | CAR | OR | LCR | LCRMO @ CONFLUENCE | 4/28/90 | 1 | | 119.00 | | M |
| 391 | CAR | RE | LCR | 1225/ HOOP | 6/1/90 | 1 | | 258.00 | 142 | M |
| 452 | CAR | YE | LCR | 0/EXPERIMENTAL | 4/26/90 | 1 | 62.92 | | | |
| 456 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 83.37 | 109.61 | | |
| 458 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 18.96 | 23.53 | | U |
| 462 | CAR | YE | LCR | 0/EXPERIMENTAL | 4/26/90 | 1 | 83.62 | | | |
| 464 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 60.90 | 72.51 | | |
| 467 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 93.04 | 118.99 | | |
| 471 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 20.87 | 26.98 | | |
| 472 | CAR | YE | LCR | 0/COLO. COLD CONTROL | 4/26/90 | 1 | 81.39 | | 44 | |
| 475 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 14.07 | 18.35 | | |
| 476 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 94.65 | 119.02 | | |
| 477 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 54.55 | 69.73 | | |
| 481 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 80.86 | 101.58 | | |
| 483 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 19.95 | 25.81 | | |
| 506 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 66.33 | 92.25 | | |
| 507 | CAR | YE | LCR | 0/EXPERIMENTAL | 4/26/90 | 1 | 74.89 | | | |
| 508 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 26.80 | 35.62 | | |
| 515 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 50.55 | 65.41 | | |
| 516 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 58.07 | 74.75 | | |

APPENDIX 1 - SPECIMENS AVAILABLE FROM WHICH OTOLITHS HAVE NOT YET BEEN EXAMINED

| TAG NO. | TAG TYPE | TAG COL. | RIVER | CAPTURE LOCALITY | DATE CAPTURED | N | SL (mm) | TL (mm) | WT (gm) | SEX |
|---|---|---|---|---|---|---|---|---|---|---|
| 520 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 77.43 | 97.00 | | |
| 523 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 78.29 | 98.99 | | |
| 525 | CAR | YE | LCR | 5432/ SIPAPU | 5/5/90 | 1 | 90.94 | 119.14 | | |
| 526 | CAR | YE | LCR | 0/EXPERIMENTAL | 4/26/90 | 1 | 82.74 | | | |
| 527 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 90.21 | 118.03 | | |
| 528 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 27.52 | 35.54 | | |
| 530 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 72.04 | 91.41 | | |
| 537 | CAR | YE | LCR | 0/LCR WARM CONTROL | 4/26/90 | 1 | 73.08 | | | |
| 539 | CAR | YE | LCR | | 10/22/90 | 1 | 29.40 | 37.40 | | |
| 540 | CAR | YE | LCR | 0/LCR WARM CONTROL | 4/26/90 | 1 | 63.80 | | | |
| 541 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 22.05 | 29.29 | | |
| 542 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 17.85 | 23.68 | | |
| 544 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 58.25 | 75.26 | | |
| 546 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 60.42 | 75.07 | | |
| 551 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 78.43 | 96.72 | | |
| 555 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 19.65 | 26.35 | | |
| 556 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 67.86 | 82.81 | | |
| 558 | CAR | YE | LCR | 0/LCR WARM CONTROL | 4/26/90 | 1 | 82.44 | | | |
| 560 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 82.85 | 102.52 | | |
| 562 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 84.41 | 106.84 | | |
| 565 | CAR | YE | LCR | 1/4 MILE UP LCR | 10/18/90 | 1 | | 105.00 | | |
| 568 | CAR | YE | LCR | LCR - 1/4 MI. UP | 10/18/90 | 1 | | 64.00 | | |
| 569 | | YE | LCR | 5432/ SIPAPU | 4/26/90 | 1 | 78.70 | 99.70 | | |
| 571 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 83.92 | 110.18 | | |
| 577 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 82.46 | 103.30 | | |
| 579 | CAR | YE | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 26.38 | 34.34 | | |
| 582 | CAR | YE | LCR | 0/LCR WARM CONTROL | 4/26/90 | 1 | 91.12 | | | |
| 583 | CAR | YE | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 81.86 | 105.49 | | |
| 587 | | YE | LCR | 5432/ SIPAPU | 5/2/90 | 1 | 74.79 | 93.91 | | |
| 589 | CAR | YE | LCR | LCR; AT MOUTH | 5/5/89 | 1 | | 370.00 | 366 | M |
| 62 | CAR | BL | LCR | 90/ ANGLING | 5/10/90 | 1 | | 390.00 | 480 | U |
| 749 | CAR | YE | LCR | LCR; AT MOUTH | 5/9/89 | 1 | | 175.00 | 36 | |
| 864 | CAR | OR | LCR | LCR; SALT CANYON | 5/11/89 | 1 | | 319.00 | 275 | F |
| 943 | CAR | OR | LCR | 100/ HOOP | 5/8/90 | 1 | | 246.00 | 141 | U |
| 962 | CAR | OR | LCR | 180/ NS D HOOP | 5/9/90 | 1 | | 287.00 | 174 | M |
| 965 | CAR | OR | LCR | 180/ NS D HOOP | 5/9/90 | 1 | | 302.00 | 226 | M |
| 976 | CAR | OR | LCR | 0/ PARA TRAM | 4/20/90 | 1 | | 385.00 | 476 | F |
| 984 | CAR | OR | LCR | 0/ PARA TRAM | 5/1/90 | 1 | | 415.00 | 644 | M |
| 986 | CAR | OR | LCR | 100/ HOOP | 5/8/90 | 1 | | 223.00 | 104 | F |
| 988 | CAR | OR | LCR | 192/ SS D HOOP | 5/8/90 | 1 | | 227.00 | | F |
| 996 | CAR | OR | LCR | 5432/ SIPAPU | 5/8/90 | 1 | | 283.00 | 85 | F |
| | NON | | LCR | 200/ HOOP | 4/20/90 | 1 | | 113.00 | 10 | U |
| | NON | | LCR | 1200/ HOOP | 4/20/90 | 1 | | 106.00 | 11 | U |
| | NON | | LCR | 1200/ HOOP | 4/20/90 | 1 | | 75.00 | 3 | U |
| | NON | | LCR | 192/ SS D HOOP | 4/21/90 | 1 | | 123.00 | 18 | U |
| | NON | | LCR | 180/ NS D HOOP | 4/26/90 | 7 | | | | |
| | NON | | LCR | 5432/ SIPAPU | 5/11/90 | 1 | | 72.00 | | U |
| | NON | | LCR | 180/ NS D HOOP | 5/12/90 | 1 | | 52.00 | | U |
| | NON | | LCR | 13854/ HOOP | 5/2/90 | 1 | | 142.00 | 20 | U |
| | LP2 | | LCR | 9904/ HOOP | 5/9/90 | 1 | | 145.00 | | M |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 23.93 | 30.61 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 25.70 | 34.65 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 19.71 | 25.29 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 25.23 | 34.17 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 15.91 | 19.83 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.26 | 21.22 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 14.96 | 19.35 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.23 | 19.54 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 19.06 | 24.46 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 15.45 | 20.21 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 20.37 | 25.98 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 18.38 | 23.15 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 20.39 | 25.81 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 17.25 | 22.40 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 19.30 | 24.14 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 21.01 | 27.87 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.92 | 20.95 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.62 | 20.19 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 20.57 | 26.84 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 21.01 | 26.54 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 15.04 | 17.86 | | |
| | NON | | LCR | 9650/ SALT TRAIL CAMP | 5/15/90 | 1 | 16.96 | 20.23 | | |
| | NON | | LCR | 380/ L SIDE MT | 5/12/90 | 1 | | 34.00 | | U |
| | NON | | LCR | 380/ L SIDE MT | 5/12/90 | 1 | | 37.00 | | U |
| | NON | | LCR | 550/ R SIDE MT | 5/12/90 | 1 | | 32.00 | | U |
| | NON | | LCR | 550/ R SIDE MT | 5/12/90 | 1 | | 34.00 | | U |
| | NON | | LCR | 550/ R SIDE MT | 5/12/90 | 6 | | | | |
| | NON | | LCR | 692/ L SIDE MT | 5/12/90 | 1 | | 31.00 | | U |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 85.19 | 111.41 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 81.32 | 103.96 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 74.36 | 97.70 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 93.57 | 116.51 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 75.73 | 100.66 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 71.46 | 95.11 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 73.58 | 98.41 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 78.42 | 106.38 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 84.09 | 109.47 | | |

APPENDIX 1 - SPECIMENS AVAILABLE FROM WHICH OTOLITHS HAVE NOT YET BEEN EXAMINED

| TAG NO. | TAG TYPE | TAG COL. | RIVER | CAPTURE LOCALITY | DATE CAPTURED | N | SL (mm) | TL (mm) | WT (gm) | SEX |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | 68.86 | 90.72 | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | | | | |
| | NON | | LCR | 5432/ SIPAPU | 5/8/90 | 1 | | | | |
| | NON | | LCR | 1200/ HOOP | 5/11/90 | 1 | | 113.00 | 16 | U |
| | NON | | LCR | 821/ HOOP | 5/14/90 | 1 | | 86.00 | | U |
| | NO | | LCR | 0/ PARA TRAM | 4/20/90 | 1 | | 352.00 | 377 | M |
| | NO | | LCR | 180/ NS D HOOP | 5/12/90 | 1 | | | | U |
| | NO | | LCR | 100/ HOOP | 4/23/90 | 1 | | 353.00 | 405 | F |
| | NO | | LCR | 8904/ HOOP | 5/5/90 | 1 | | 97.00 | 6 | U |
| | UP2 | | LCR | 180/ NS D HOOP | 5/3/90 | 1 | | 150.00 | 27 | U |
| | | | LCR | AGFD; MILE 1800; LCR; RIGHT | 3/30/91 | 1 | | 72.00 | | |
| | | | LCR | AGFD; MILE 1800; LCR RIGHT | 3/30/91 | 1 | | 73.00 | | |
| | | | LCR | AGFD; REACH 22; MILE 1800 | 3/30/91 | 1 | | 130.00 | | |
| | | | LCR | AGFD; MILE 1800; LCR; RIGHT | 3/30/91 | 1 | | 69.00 | | |
| | | | LCR | AGFD; REACH 22; MILE 1800 | 5/2/91 | 1 | | 72.00 | | |
| | | | LCR | AGFD; REACH 22; MILE 1800 | 5/2/91 | 1 | | 126.00 | 15 | |
| | | | LCR | AGFD; REACH 22; MILE 1920 | 5/3/91 | 1 | | 135.00 | 20 | |
| | | | LCR | AGFD; REACH 22; MILE 1920 | 5/5/91 | 1 | | 118.00 | 63 | |
| | | | LCR | AGFD; REACH 22; MILE 1900M | 5/13/91 | 1 | | 76.00 | 36 | |
| | | | LCR | AGFD; REACH 22; MILE 2200 | 6/20/91 | 1 | | 30.00 | | |
| | | | LCR | AGFD; REACH 22; MILE 0900 | 6/21/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1830 | 6/27/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1854 | 6/27/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1920 | 6/28/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 10760 | 7/1/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 2500 | 7/6/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9970 | 6/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 7.0 K | 6/16/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1850 | 6/20/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9400 | 6/23/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1830 | 6/27/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1790 | 6/30/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9970 | 6/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9970 | 6/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9970 | 6/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 7.0 K | 6/16/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1850 | 6/20/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9400 | 6/23/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9400 | 6/23/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9400 | 6/23/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 9400 | 6/23/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1830 | 6/27/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1790 | 6/30/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1790 | 6/30/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1790 | 6/30/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1790 | 6/30/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; MILE 22; REACH 1750 | 7/7/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 1855 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/11/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/14/91 | 1 | | | | |
| | | | LCR | AGFD; REACH 22; MILE 0010 | 7/14/91 | 1 | | | | |