

**Fishery Data Series No. 00-41**

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**Age Validation of Burbot Otoliths with  
Oxytetracycline Marks from the Water Supply  
Reservoir at Fort Knox, Alaska**

by

**Lisa Stuby**

December 2000

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Alaska Department of Fish and Game

Division of Sport Fish



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| <b>Weights and measures (metric)</b> |    | <b>General</b>                                    |   | <b>Mathematics, statistics, fisheries</b>                                     |                         |
|--------------------------------------|----|---|---|---|-------------------------|
| centimeter                           | cm | All commonly accepted abbreviations.              | e.g., Mr., Mrs., a.m., p.m., etc.           | alternate hypothesis  | $H_A$                   |
| deciliter                            | dL | All commonly accepted professional titles.        | e.g., Dr., Ph.D., R.N., etc.                | base of natural logarithm   | e                       |
| gram                                 | g  | and   | &   | catch per unit effort   | CPUE                    |
| hectare                              | ha | at  | @   | coefficient of variation  | CV                      |
| kilogram                             | kg | Compass directions:                               |   | common test statistics  | F, t, $\chi^2$ , etc.   |
| kilometer                            | km | east  | E   | confidence interval   | C.I.                    |
| liter                                | L  | north   | N   | correlation coefficient   | R (multiple)            |
| meter                                | m  | south   | S   | correlation coefficient   | r (simple)              |
| metric ton                           | mt | west  | W   | covariance  | cov                     |
| milliliter                           | ml | Copyright   | ©   | degree (angular or temperature)   | °                       |
| millimeter                           | mm | Corporate suffixes:                               |   | degrees of freedom  | df                      |
|                                      |    | Company   | Co.   | divided by  | ÷ or / (in equations)   |
|                                      |    | Corporation                                       | Corp.                                       | equals  | =                       |
|                                      |    | Incorporated                                      | Inc.  | expected value  | E                       |
|                                      |    | Limited   | Ltd.  | fork length   | FL                      |
|                                      |    | et alii (and other people)                        | et al.                                      | greater than  | >                       |
|                                      |    | et cetera (and so forth)                          | etc.  | greater than or equal to  | ≥                       |
|                                      |    | exempli gratia (for example)                      | e.g.,                                       | harvest per unit effort   | HPUE                    |
|                                      |    | id est (that is)                                  | i.e.,                                       | less than   | <                       |
|                                      |    | latitude or longitude                             | lat. or long.                               | less than or equal to   | ≤                       |
|                                      |    | monetary symbols (U.S.)                           | \$, ¢                                       | logarithm (natural)   | ln                      |
|                                      |    | months (tables and figures): first three letters  | Jan.,...,Dec                                | logarithm (base 10)   | log                     |
|                                      |    | number (before a number)                          | # (e.g., #10)                               | logarithm (specify base)  | log <sub>2</sub> , etc. |
|                                      |    | pounds (after a number)                           | # (e.g., 10#)                               | mideye-to-fork  | MEF                     |
|                                      |    | registered trademark                              | ®   | minute (angular)  | '                       |
|                                      |    | trademark   | ™   | multiplied by   | x                       |
|                                      |    | United States (adjective)                         | U.S.  | not significant   | NS                      |
|                                      |    | United States of America (noun)                   | USA   | null hypothesis   | $H_0$                   |
|                                      |    | U.S. state and District of Columbia abbreviations | use two-letter abbreviations (e.g., AK, DC) | percent   | %                       |
|                                      |    |   |   | probability   | P                       |
|                                      |    |   |   | probability of a type I error (rejection of the null hypothesis when true)    | $\alpha$                |
|                                      |    |   |   | probability of a type II error (acceptance of the null hypothesis when false) | $\beta$                 |
|                                      |    |   |   | second (angular)  | "                       |
|                                      |    |   |   | standard deviation  | SD                      |
|                                      |    |   |   | standard error  | SE                      |
|                                      |    |   |   | standard length   | SL                      |
|                                      |    |   |   | total length  | TL                      |
|                                      |    |   |   | variance  | Var                     |

| <b>Weights and measures (English)</b> |                    |
|---------------------------------------|--------------------|
| cubic feet per second                 | ft <sup>3</sup> /s |
| foot                                  | ft                 |
| gallon                                | gal                |
| inch                                  | in                 |
| mile                                  | mi                 |
| ounce                                 | oz                 |
| pound                                 | lb                 |
| quart                                 | qt                 |
| yard                                  | yd                 |
| Spell out acre and ton.               |                    |

| <b>Time and temperature</b>        |     |
|------------------------------------|-----|
| day                                | d   |
| degrees Celsius                    | °C  |
| degrees Fahrenheit                 | °F  |
| hour (spell out for 24-hour clock) | h   |
| minute                             | min |
| second                             | s   |
| Spell out year, month, and week.   |     |

| <b>Physics and chemistry</b> |        |
|------------------------------|--------|
| all atomic symbols           |        |
| alternating current          | AC     |
| ampere                       | A      |
| calorie                      | cal    |
| direct current               | DC     |
| hertz                        | Hz     |
| horsepower                   | hp     |
| hydrogen ion activity        | pH     |
| parts per million            | ppm    |
| parts per thousand           | ppt, ‰ |
| volts                        | V      |
| watts                        | W      |

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RESERVOIR AT FORT KNOX, ALASKA**

by

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## ABSTRACT

Two hundred thirty one burbot from the water supply reservoir of the Fort Knox gold mining complex were injected with 25 mg/kg oxytetracycline (OTC) in May 1995. Forty-seven sagittal otolith pairs were collected from these burbot over a period of five years in order to validate ages past the OTC mark. The mark was made visible using a mercury lamp attached to a compound microscope. One otolith from each pair was thin-sectioned through the nucleus and the other left whole. Total and age past the OTC mark were determined for each otolith and compared. One reader conducted all aging. The mark was readily visible for 89% percent of the thin-sectioned otoliths and 85% of the whole otoliths.

The proportion correctly aged past the OTC mark was 1.0 for the thin-sectioned otoliths and 0.67 (SE = 0.01) for whole otoliths. On average, the reader found the presence and intensity of the OTC mark to have been better for the thin-sectioned versus the whole otoliths. The null hypothesis that total ages from whole otoliths were similar to thin-sectioned otoliths was rejected. The mean total age was 4.57 years for whole otoliths (SE = 1.05) and 5.11 years (SE = 1.16) for thin-sectioned otoliths.

Visibility and readability of the annuli past the OTC mark using a compound microscope with ultraviolet light were excellent for the thin-sectioned otoliths and good for the whole otoliths. This technique has potential applications to future age validation studies.

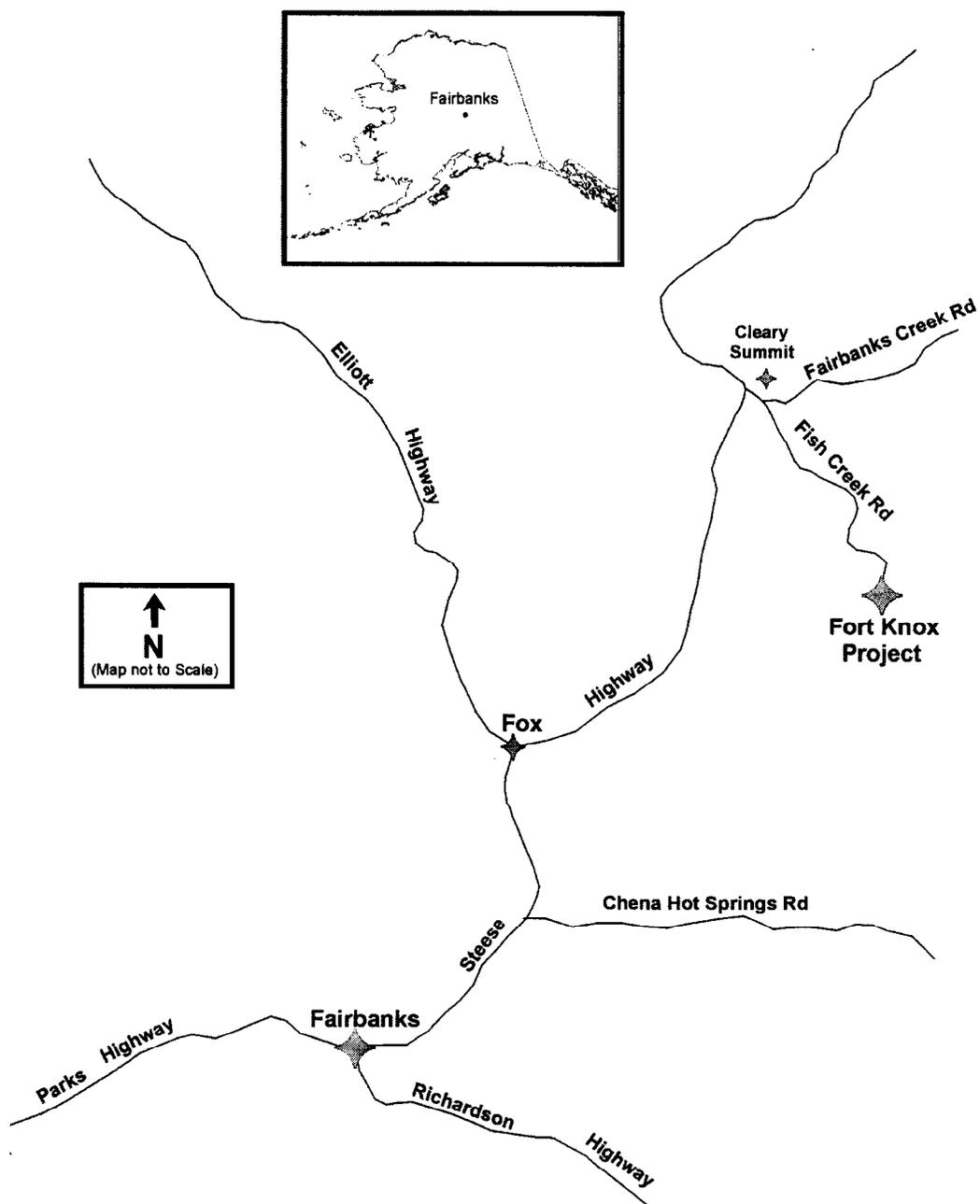
Key words: age validation, burbot, *Lota lota*, sagittal otoliths, oxytetracycline, thin-sectioned otoliths, whole otoliths, ultraviolet light, compound microscope, dissecting microscope.

## INTRODUCTION

Burbot *Lota lota* are a popular sportfish, which are sought year-round by anglers in interior Alaska. The Fort Knox hard-rock gold mining complex is located in the Fish Creek drainage near Fairbanks, Alaska (Figure 1). The project includes an open-pit mine, mill, tailing impoundment, water supply reservoir (WSR), and related facilities (Ott and Morris 2000). The WSR is approximately 165 acres and contains approximately 3,500 acre-feet of water. This reservoir was created via a freshwater impoundment dam in Fish Creek. Impoundment of water began November 1995. Sampling conducted by the Alaska Department of Fish and Game, Habitat Division during 1994 showed that young burbot, which ranged in length from 110-210 mm and were thus assumed to range in age from 0-4 years, inhabited the Fish Creek drainage (Ott et al. 1995). Because the system is small, access and fishing are restricted, and a large proportion of the population can be captured, it has been an ideal location to conduct an age validation study (Evenson 1996).

The study area at Fort Knox in 1995 consisted of two small settling ponds (0.9-1.2 ha) connected by a creek stretch. During a mark-recapture analysis of each pond, thirty-three burbot were sacrificed for age analysis. Ages based on readings of whole otoliths ranged from 2-5 years. Age 0 and age 1 fish were absent from the sample, as were fish older than age 5 (Evenson 1996). In fall 1996 and 1997, young-of-the-year burbot were abundant in fyke-net catches (Ott and Townsend 1997, Ott and Morris 1999).

Otoliths are the preferred structure for aging burbot. The scale is the most popular bony structure for estimating the age of fish (Everhart and Youngs 1981). However, burbot scales are minute, imbedded and often lack discernable annuli. Other structures such as fin rays and vertebrae have been used (McCrimmon and Devitt 1954, Guinn and Hallberg 1990). Of these, vertebrae yield reliable age estimates, however, require more time to process and read than otoliths. Guinn and Hallberg (1990) found that vertebrae and otoliths yielded similar age estimates and differed little



**Figure 1.-Map showing the location of the Fort Knox hard-rock gold mining complex. Map taken from Ott and Morris (2000).**

in regard to precision of those estimates. However, due to ease of preparation, otoliths have remained the primary structure used in age determination of burbot. Otoliths, like vertebrae, require the sacrifice of the fish, which can limit the number of samples that can be collected from a population.

Burbot otoliths are often read whole and/or sectioned under a dissecting microscope. Sectioned otoliths may be read burned or unburned. Several past investigators have reported similar precision in ages between the two methods (Evenson and Merritt 1995, Evenson 2000, Arndt and Hutchinson 2000). Beamish and McFarlane (1983) stressed the importance of proving an aging structure and/or technique is valid. Assignment of true ages using a particular structure and aging technique can only be proven by a validation method.

When a biologist is limited to structures such as otoliths, which require the sacrifice of the fish, validation can only be achieved by marking the structure (McFarlane and Beamish 1987). Oxytetracycline (OTC) is commonly used for this purpose because it binds with proteins in the blood and is incorporated in newly forming and mineralizing bone and cartilage (Frost et al. 1961). The OTC is fluorescent under ultraviolet light and can accurately mark the date of application (Campana and Neilson 1985).

During May 1995, burbot in the Fort Knox WSR were captured and tagged as part of a mark-recapture experiment. Some of these fish were also injected with OTC for later otolith collection. The specific objective of this study was to:

1. estimate the proportion of correctly aged annuli past the OTC marks of recaptured burbot otoliths such that the proportion is within 15 percentage points 95% of the time.

In addition, a project task was to compare the proportion of correctly aged annuli past the OTC marks of paired sectioned versus whole otoliths.

## **METHODS**

### **SAMPLING**

Two hundred thirty-one burbot were injected with OTC on 10 and 18 May 1995. Sampling was conducted in two settling ponds, which were later flooded to form the WSR. The burbot were captured using a combination of fyke nets, minnow traps and hoop traps. Lengths of captured fish ranged from 152 to 331 mm.

The 231 burbot were weighed to the nearest 0.1 g and given an interperitoneal injection of 25 mg/kg OTC (McFarlane and Beamish 1987) to induce a permanent fluorescent mark on bony structures. Five of the OTC-labeled fish were captured and otoliths extracted in November 1995. In addition, 15 fish were sampled during May in 1997, 15 in 1998, 10 in 1999, and 2 in 2000. Both sagittal otoliths were removed from the burbot skulls, cleaned with water and placed in coin envelopes to dry. From time of extraction until time of reading, care was taken to minimize exposure to light, which can cause fading of the photolytic OTC mark. Choate (1964) and Trojnar (1973) stated that direct light could negatively affect the fluorescent properties of OTC.

## **AGING**

### **Whole Otoliths**

Whole otoliths were first soaked in water for approximately one to two days. All otoliths were then ground before annuli could be deciphered and enumerated. The otoliths were ground on the convex, distal side by rubbing first the anterior half and then the posterior half on a whetstone. Readings were performed on the sulcus surface using a dissecting microscope set at 0.8X to 1.6X magnification. The otoliths were placed in water and read using reflected light against a black background.

The whole otolith was examined and aged in several directions, particularly out to the anterior edge (Figure 2). Because burbot otoliths grow much more slowly on the posterior edge, annuli on this side tend to be crowded and not as clear (Chen 1969), particularly as the fish get older. Burbot otoliths are comprised of alternating hyaline and opaque bands. Chen (1969) noted that the otoliths of Tanana River burbot began formation of the opaque ring from June to July. He assumed the hyaline ring to form in April to May, similar to what had been noted for Lake Erie burbot (Clemens 1950). All of the Fort Knox burbot sampled in May showed a thin, dark hyaline band along the margin while viewed under transmitted light. The opaque nucleus, the hyaline ring, and the first opaque ring were considered as the first year's growth (Mackay et al. 1990). Every successive pair of hyaline and opaque rings was thus taken to be an additional year's growth (Chen 1969, Bailey 1972). Annuli for this study were defined as the hyaline zones, which were typically (but not always) the zones of slower growth and appeared dark under reflected light (Figure 3a).

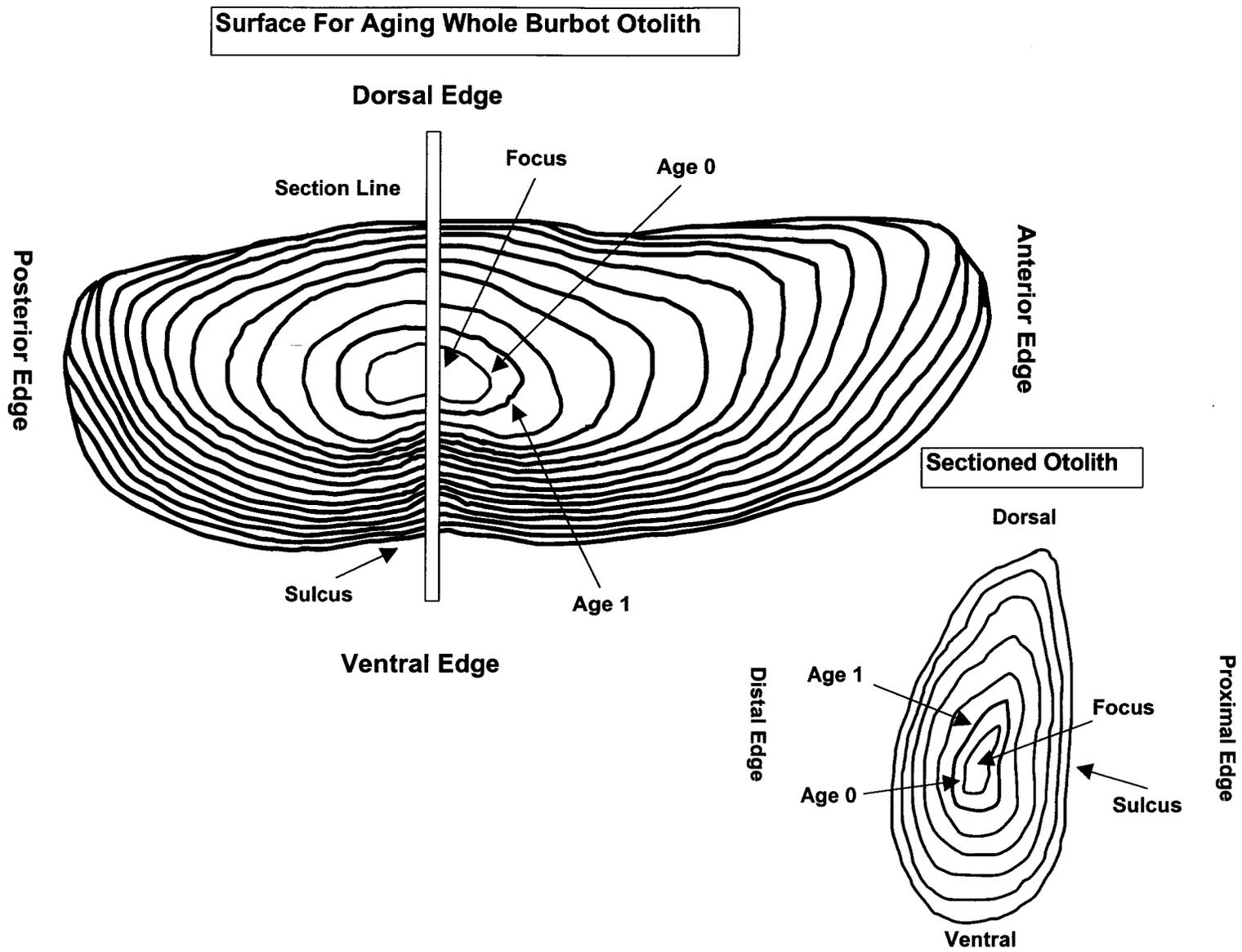
The whole otoliths were viewed under reflected ultraviolet light using a dissecting microscope. The OTC mark was viewed using an Olympus BX 40 compound microscope equipped with a mercury lamp. Magnification varied between 2X and 10X depending on the size of the otolith and reader preference. The ultraviolet light caused the OTC mark to fluoresce, followed by the opaque area and dark hyaline zones (Figure 3b). Annuli definition was better under transmitted light, although the OTC mark faded (Figure 3c). With transmitted light, the hyaline rings appear light and the opaque growth zone becomes dark. For reflected light, the annulus is dark and the growth zone is light (Chilton and Beamish 1982). When both types of light sources are used, the reader must be aware of the difference. For this study, a single age assignment was made using all three methods for viewing whole otoliths.

The presence and quality of OTC rings on otoliths viewed under ultraviolet illumination were scored in one of four categories based on the criteria established by Weber and Ridgeway 1967:

- (1) no mark
- (2) poor (present, but neither clear or intense)
- (3) fair (clear, but not intense)
- (4) good (clear and intense)

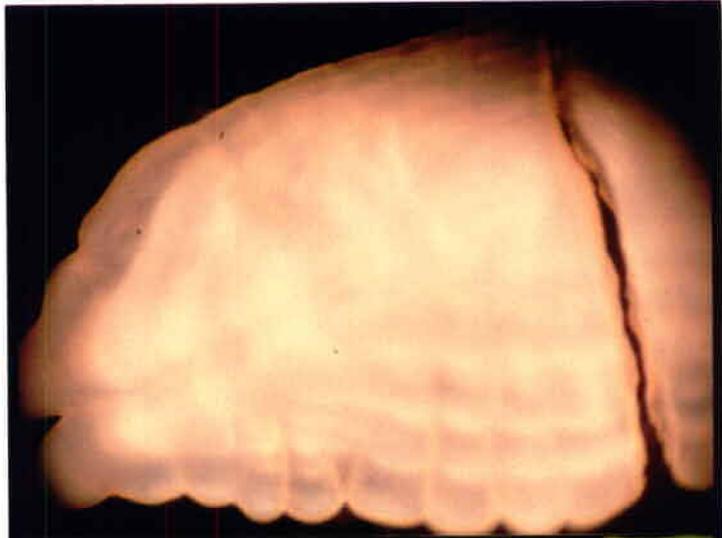
### **Thin-Sectioned Otoliths**

The burbot otolith was sectioned through the nucleus, encapsulating the distal to proximal edges (Figure 2). The otolith was first ground to the focus using a diamond-grinding wheel, mounted on a glass slide with Crystalbond Thermoplastic, and further ground to a thin section,

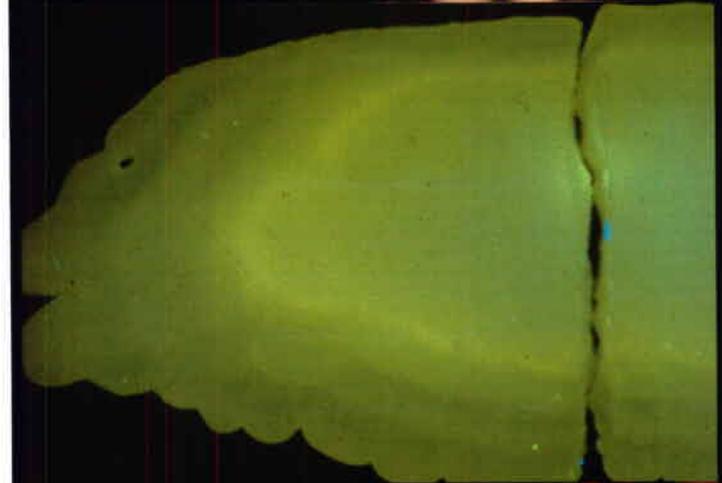


**Figure 2.-Drawing of burbot otolith showing areas used for counting and location of sectioning.**

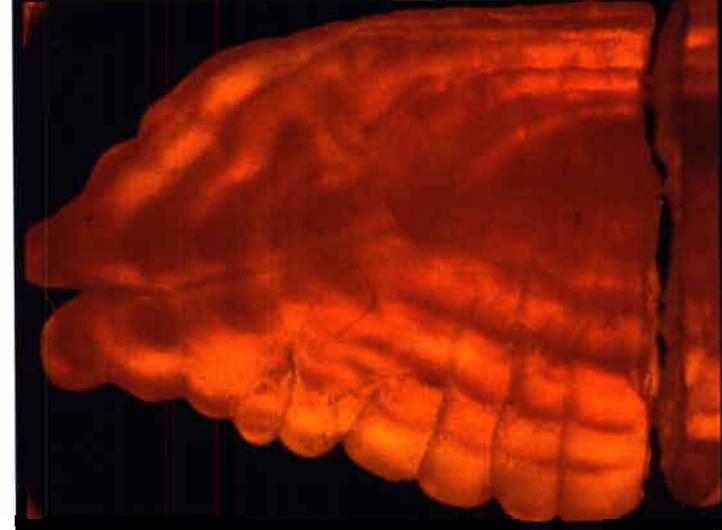
a)



b)



c)



**Figure 3.- Photographs of whole otoliths viewed under a) reflected light against a black background, b) ultraviolet reflected light, and c) transmitted light.**

encapsulating the focus in the transverse plane (Secor et al. 1991). An Olympus BX40 compound microscope equipped with a mercury lamp was used to illuminate the OTC mark. The otoliths were viewed from 2X to 10X powers in a darkened room. The ultraviolet light was reflected and caused the OTC mark to fluoresce (Figure 4a). After the mark was ascertained, a small amount of transmitted light was allowed through which highlighted the annuli (Figure 4b). The annuli were aged similarly to those for whole otoliths, except the thin-sections were not viewed under reflected light using a dissecting microscope. To better highlight the annuli, a polarizing filter was used which darkened the background and enabled better viewing contrast of the annuli. However, the polarizing filter usually masked the OTC mark (Figure 4c). Similar to the whole otoliths, mark intensity was noted for the thin-sections using the above criteria.

## DATA ANALYSIS

The procedures outlined by Freund (1984) were used to determine that approximately 43 fish marked with OTC were necessary to estimate proportions within the objective criterion of  $\pm 15$  percentage points 95% of the time. Forty-seven fish were recaptured and OTC marks were visible on 40 whole and 42 thin-sectioned otoliths.

Two readings by one experienced reader were performed on each pair of thin-sectioned and whole otoliths. The reader was aware of the range of capture dates but did not have knowledge of these dates for individual samples. The two ages for each of the paired samples were compared and a third reading was conducted if they differed. The estimated ages past the OTC mark were then compared with the true age. To assess the accuracy of ages obtained from the paired structures, the proportion and variance of burbot otoliths whose estimated age reflected the true age was calculated for thin-sectioned and whole otoliths as:

$$\hat{p} = \frac{a}{n} \quad (1)$$

$$V[\hat{p}] = \frac{\hat{p}(1 - \hat{p})}{n - 1} \quad (2)$$

where:

a = number of fish whose assigned ages agreed with the true age; and,

n = total number of known-age structures in the sample.

Contingency table analysis was used to determine if thin-sectioned and whole otoliths had equal error rates past the OTC mark by testing the hypothesis:

$H_0$ : proportion of correct readings independent of technique ( $P_{\text{whole}} = P_{\text{sectioned}}$ )

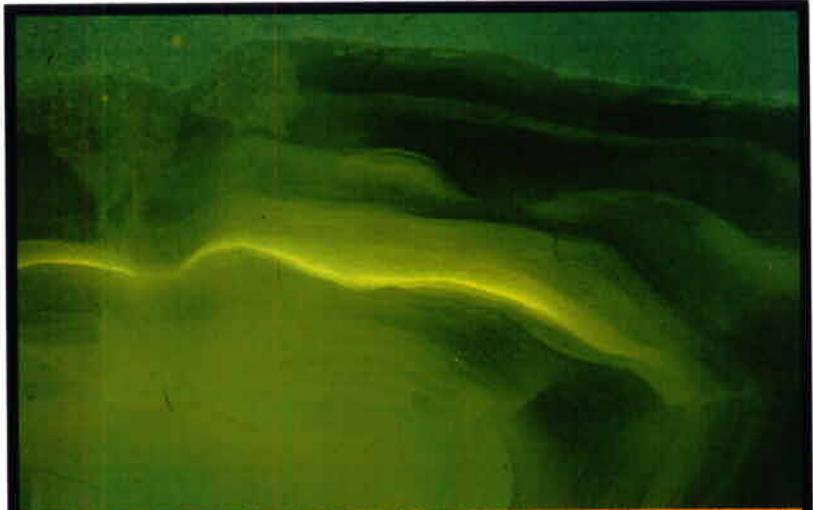
$H_a$ : proportion of correct readings is dependent on the technique ( $P_{\text{whole}} \neq P_{\text{sectioned}}$ ).

Contingency table analysis was also used to determine if the relative intensity of the OTC mark is similar for thin-sectioned and whole otoliths by testing the hypothesis:

$H_0$ : relative intensity of OTC mark is independent of technique

$H_a$ : relative intensity of OTC mark is dependent of technique.

a)



b)



c)



**Figure 4.-Photographs of thin-sectioned otoliths viewed under a) ultraviolet reflected light, b) ultraviolet reflected and transmitted light, and c) transmitted light with a polarizing filter.**

A t-test for paired comparisons was performed to test the hypothesis that mean total age from whole otoliths was similar to thin-sectioned otoliths. This statistical method tested whether the mean of sample differences between pairs of readings was significantly different from a hypothetical mean, zero under the null hypothesis. The standard error over which this mean was tested was the standard error of the mean difference (Sokal and Rohlf 1995).

## RESULTS

A total of 47 otolith pairs were collected and examined. One pair of otoliths that lacked the OTC mark were both sectioned to see if the mark occurred in only one otolith. The OTC mark was not visible for five of the thin-sectioned otoliths and seven of the whole otoliths (Table 1). Thirty-eight pairs of otoliths showed visible OTC marks on both otoliths.

The proportion which reflected the true age past the OTC mark was 0.67 (SE = 0.01) for whole otoliths and 1.0 for thin-sectioned otoliths (Figure 5). Likewise, the null hypothesis that correct ages past the OTC mark was independent of technique was rejected ( $\chi^2 = 14.25$ ,  $df = 1$ ,  $P \leq 0.01$ ). These results show that the accuracy in age assignment is greater for thin-sectioned otoliths than for whole otoliths (Table 2).

On average, the reader rated the OTC mark presence and intensity for whole otoliths a 2.61 and 3.02 for thin-sectioned otoliths. The null hypothesis that relative intensity of an OTC mark is independent of technique was rejected ( $\chi^2 = 9.27$ ,  $df = 3$ ,  $P = 0.03$ ). A greater proportion of whole otoliths were given a score of two relative to the thin-sectioned otoliths and a greater proportion of thin-sectioned otoliths were given a score of three relative to the whole otoliths (Table 3). The reader observed that thin-sectioned otoliths were easier to age than whole otoliths and the OTC mark was easier to decipher.

The hypothesis that total ages from whole otoliths are similar to thin-sectioned was rejected ( $t = 3.84$ ,  $df = 45$ ,  $P \leq 0.01$ ). The overall mean for total ages was 4.57 years for whole otoliths (SE = 1.05) and 5.11 years (SE = 1.16) for thin-sectioned otoliths.

Data for these analyses are archived as described in Appendix A.

## DISCUSSION

Age estimates are important for interpreting life history parameters of fish populations and in developing management strategies for these populations. Past work on interior Alaska burbot has encompassed assessment of recruitment age, which is important for assigning length restrictions in a sport fishery. Ages have been used to ascertain natural mortality for catch at age analyses, which lead to yearly estimates of abundance (Evenson 1994, 1996-1998, Stuby and Evenson 1999). Thus, validating structures and methods for acquiring accurate ages is an important part of good management of a fish stock and/or population. Estimating the age of a fish requires that a pattern of growth be identified on some structure from the fish, be related to the growth history of the structure, and be shown to form once a year throughout the life of the individual fish.

**Table 1.-Correct ages for whole and thin-sectioned otoliths for ages past a visible OTC mark. Otoliths collected from burbot from the WSR at Fort Knox in 1995, 1997-2000.**

| Otoliths       | Age             | Age Past OTC Mark |    |    |   |   | Total <sup>a</sup> |
|----------------|-----------------|-------------------|----|----|---|---|--------------------|
|                | Assignment      | 0                 | 2  | 3  | 4 | 5 |                    |
| Whole          | Correct         | 3                 | 11 | 7  | 5 | 0 | 26                 |
|                | Incorrect       | 1                 | 3  | 6  | 3 | 2 | 13                 |
|                | OTC not Visible |                   |    |    |   |   | 7                  |
|                | Total           | 4                 | 14 | 13 | 8 | 2 | 46                 |
| Thin-Sectioned | Correct         | 5                 | 13 | 13 | 9 | 2 | 42                 |
|                | Incorrect       | 0                 | 0  | 0  | 0 | 0 | 0                  |
|                | OTC not Visible |                   |    |    |   |   | 5                  |
|                | Total           | 5                 | 13 | 13 | 9 | 2 | 47                 |

<sup>a</sup> Total correct/incorrect ages reflect those otoliths for which the OTC mark was visible or assumed to be visible.

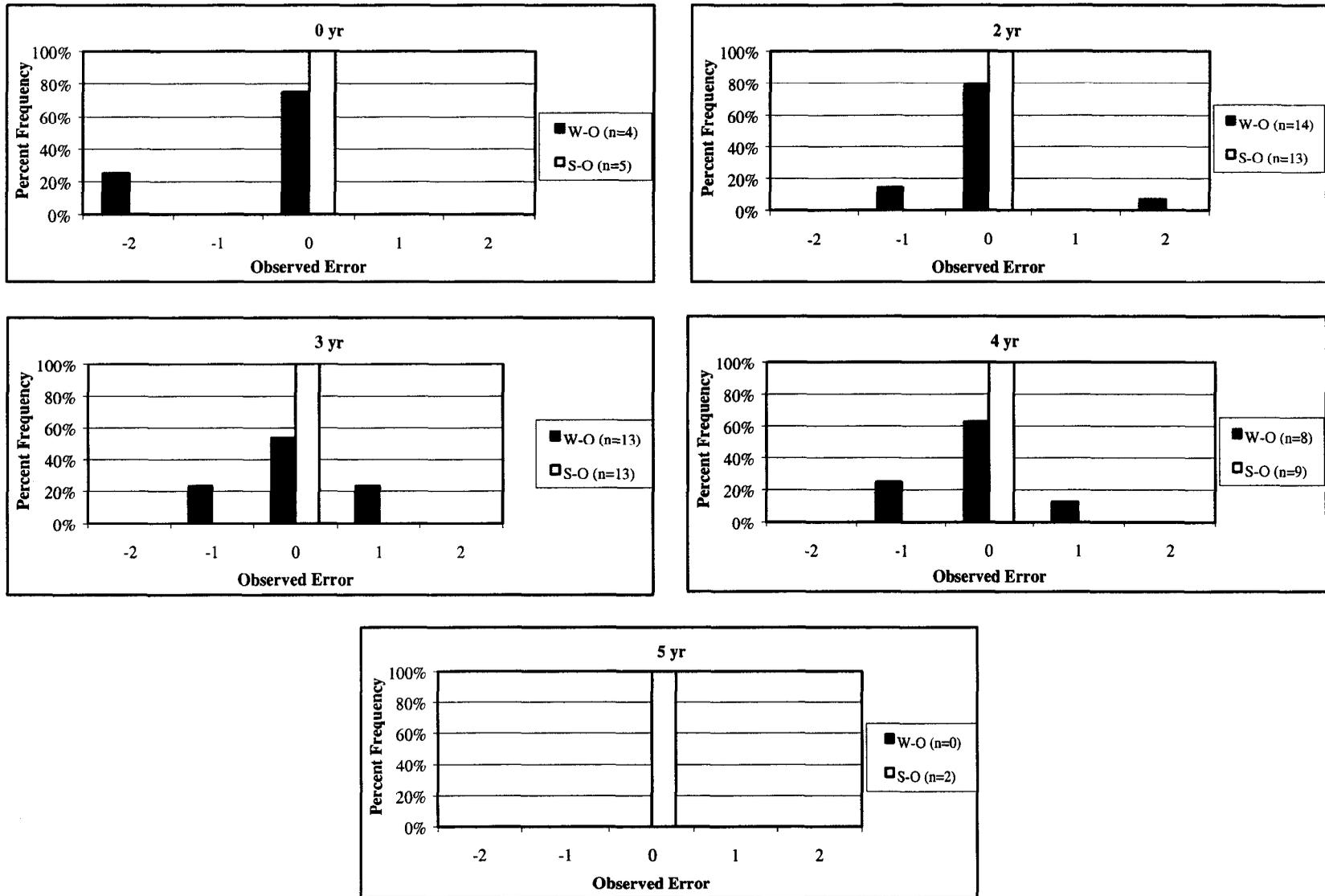


Figure 5.-Percent frequencies of observed reader errors for known ages past an OTC mark for whole (W-O) and thin-sectioned (S-O) burbot otoliths.

**Table 2.-Contingency table analysis of correct ages past the OTC marks for whole and thin-sectioned burbot otoliths.**

| Otoliths       | Correct | Incorrect | Total <sup>a</sup> |
|----------------|---------|-----------|--------------------|
| Whole          | 26      | 12        | 38                 |
| Thin-Sectioned | 38      | 0         | 38                 |

$$\chi^2 = 14.25, df = 1, P \leq 0.01$$

<sup>a</sup> Total correct/incorrect ages for otoliths where the OTC mark was visible for the whole and thin-sectioned pair.

**Table 3.-Contingency table analysis of the presence and intensity of OTC marks for paired whole and thin-sectioned burbot otoliths.**

| Otoliths       | OTC Intensity |    |    |    | Total | Average |
|----------------|---------------|----|----|----|-------|---------|
|                | 1             | 2  | 3  | 4  |       |         |
| Whole          | 7             | 14 | 15 | 10 | 46    | 2.61    |
| Thin-Sectioned | 4             | 4  | 25 | 13 | 46    | 3.02    |

$$\chi^2 = 9.27, df = 3, P = 0.03$$

The OTC mark was visible in 85% of the whole burbot otoliths aged and 89% of thin-sectioned otoliths. Reinert et al. (1998) studied long-term retention and detection of OTC marks applied to hatchery-reared larval striped bass *Morone saxatilis*. They found that mark retention was independent of fish age. Visibility was not dependent on age for the Fort Knox burbot otoliths. Most researchers agree that chemical marking of otoliths produces permanent marks (Brothers 1990). Except for periods of severe anaerobic stress (Mugiya and Uchimura 1989), there is no physiological basis for resorption of otolith material (Ichii and Magiya 1983). Too much direct light can lead to fading of the OTC mark over a period of time. Lorson and Mudrak (1987) observed fading of the OTC mark in American shad fry because, until scalation occurs, the fry heads are somewhat transparent. Thus, even though the otoliths are still contained within the fish, exposure to sunlight can cause fading of the OTC mark. Burbot are elongate, robustly built fish and in the north are darkly pigmented (Scott and Crossman 1973). Thus, fading of otoliths within healthy burbot would not be a concern.

The lack of marks in a few fish could be attributed to a low dosage of OTC. McFarlane and Beamish (1987) recommended the dosage of 25 mg OTC/kg of fish. Previous studies have shown that a large dose of OTC can cause mortality (Weber and Ridgeway 1962, Kobayashi et al. 1964). Therefore, there is a trade-off between giving a fish enough OTC to imprint a distinct mark while minimizing mortality. Dosage sensitivity may also vary for species. Although it is unknown what the maximum safe dosage would be for burbot, survivability of the injected Fort Knox burbot appears high. The 1995-1999 estimates of burbot in the WSR were 825, 622, 703 and 3,609 respectively (Ott and Morris 2000). Overall, 62 total samples were collected between 1995 and 2000 (1996 samples were unavailable). Given 231 burbot were injected with OTC and approximately 27% of the OTC injected burbot were recaptured over a period of five years, it can be assumed that mortality as a result of the 25 mg/kg dosage was negligible. Also, because a high percentage of burbot otoliths clearly showed the OTC mark, this dosage can be considered sufficient for future age validation studies.

The reader observed that annuli for thin-sectioned otoliths past the OTC mark were clear and easy to observe with the ultraviolet light. Annuli were not as easy to discern for whole otoliths, and this was reflected in the percent accuracy. Evenson and Merritt (1995) compared surface readings of whole otoliths to surface break and burn methods. They found that surface reading techniques provided similar, but more precise estimates of age than did the break and burn techniques. For this study whole otoliths were compared to their thin-sectioned pairs. Viewing the thin-sectioned otoliths under a compound microscope with transmitted and ultraviolet light was very different than viewing a burned cross-section under a dissecting microscope using reflected light. Comparisons with the break and burn technique were not attempted due to the limited number of otoliths. Also, burning will destroy the OTC mark (McFarlane and Beamish 1995).

Growth in these relatively young fish (total ages estimated from 2 to 7 years) appeared to be good. Ott and Morris (2000) observed that the burbot population is healthy and the length-frequency distribution is indicative of a rapidly increasing population. Food availability for burbot production and growth in the WSR is reported to be good (B. Morris, ADF&G Habitat Division, Fairbanks, personal communication). Growth of fish as a result of favorable environmental conditions such as temperature and habitat space as well as food abundance have

a direct influence upon fish growth, which in turn are reflected in the incremental growth sequence in the otolith (Campana and Neilson 1985).

Oxytetracycline marking of burbot otoliths has been shown to be a valuable method for validation of burbot ages. One of the hardest aspects of aging burbot otoliths is ascertaining the first year mark. Future research into validating burbot ages would be to mark young of the year through injection and/or immersion into an OTC bath and validate the first year check.

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## **APPENDIX A**

**Appendix A.-Data files used to compare total ages between whole and thin-sectioned otoliths and accuracy in aging from known OTC marks in burbot sampled from the WSR at Fort Knox, Alaska.**

| Data File   | Description   |
|-------------|---|
| 95Data.xls  | Data file of ages past OTC marks for sectioned and whole burbot otoliths and total ages. File also contains results of analyses and comparisons between the two techniques. |
| Burboxy.xls | Data file of sampling dates and numbers of burbot collected from the WSR at Fort Knox, Alaska.  |

## **APPENDIX B**

**Appendix B.-Capture dates, ages past OTC marks, and total ages for thin-sectioned and whole otoliths sampled from burbot at the WSR at Fort Knox between 1995 and 2000.**

| Fish # | Date of Marking | Date of Capture | Actual Elapsed Time (yr) | Thin-Sectioned Otolith |                             |                    | Whole Otolith       |                             |                    |
|--------|-----------------|-----------------|--------------------------|------------------------|-----------------------------|--------------------|---------------------|-----------------------------|--------------------|
|        |                 |                 |                          | Estimated Total Age    | Estimated Age Past OTC Mark | OTC Mark Intensity | Estimated Total Age | Estimated Age Past OTC Mark | OTC Mark Intensity |
| 1      | 18-May-95       | 30-Nov-95       | Plus <sup>a</sup>        | 3                      | Plus <sup>a</sup>           | 3                  | 3                   | N/A <sup>b</sup>            | 1                  |
| 2      | 18-May-95       | 20-May-97       | 2                        | 4                      | 2                           | 3                  | 4                   | 2                           | 2                  |
| 3      | 10-May-95       | 20-May-97       | 2                        | 4                      | 2                           | 3                  | 4                   | 2                           | 2                  |
| 4      | 10-May-95       | 4-Jun-99        | 4                        | 5                      | 4                           | 3                  | 5                   | 4                           | 4                  |
| 5      | 10-May-95       | 30-Nov-95       | Plus <sup>a</sup>        | 3                      | Plus <sup>a</sup>           | 3                  | 3                   | Plus <sup>a</sup>           | 3                  |
| 6      | 18-May-95       | 30-Nov-95       | Plus <sup>a</sup>        | 3                      | Plus <sup>a</sup>           | 4                  | 2                   | Plus <sup>a</sup>           | 2                  |
| 7      | 10-May-95       | 20-May-97       | 2                        | 5                      | N/A <sup>b</sup>            | 1                  | --                  | --                          | --                 |
| 8      | 10-May-95       | 20-May-97       | 2                        | 4                      | 2                           | 3                  | 4                   | 3                           | 3                  |
| 9      | 10-May-95       | 20-May-97       | 2                        | 5                      | 2                           | 3                  | 4                   | 3                           | 2                  |
| 10     | 10-May-95       | 20-May-97       | 2                        | 5                      | 2                           | 3                  | 4                   | 2                           | 3                  |
| 11     | 18-May-95       | 20-May-97       | 2                        | 6                      | N/A <sup>b</sup>            | 1                  | 3                   | Plus <sup>a</sup>           | 2                  |
| 12     | 10-May-95       | 20-May-97       | 2                        | 4                      | 2                           | 2                  | 4                   | 2                           | 2                  |
| 13     | 18-May-95       | 20-May-97       | 2                        | 5                      | 2                           | 2                  | 3                   | 2                           | 3                  |
| 14     | 10-May-95       | 21-May-97       | 2                        | 4                      | 2                           | 4                  | 4                   | 2                           | 3                  |
| 15     | 10-May-95       | 21-May-97       | 2                        | 4                      | 2                           | 4                  | 4                   | 2                           | 2                  |
| 16     | 18-May-95       | 21-May-97       | 2                        | 5                      | 2                           | 4                  | 4                   | 2                           | 3                  |

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| Fish # | Date of Marking | Date of Capture | Actual Elapsed Time (yr) | Thin-Sectioned Otolith |                             |                    | Whole Otolith       |                             |                    |
|--------|-----------------|-----------------|--------------------------|------------------------|-----------------------------|--------------------|---------------------|-----------------------------|--------------------|
|        |                 |                 |                          | Estimated Total Age    | Estimated Age Past OTC Mark | OTC Mark Intensity | Estimated Total Age | Estimated Age Past OTC Mark | OTC Mark Intensity |
| 17     | 10-May-95       | 21-May-97       | 2                        | 4                      | 2                           | 2                  | 4                   | 2                           | 3                  |
| 18     | 18-May-95       | 21-May-97       | 2                        | 5                      | 2                           | 3                  | 4                   | 2                           | 2                  |
| 19     | 18-May-95       | 30-Nov-95       | Plus <sup>a</sup>        | 4                      | Plus <sup>a</sup>           | 4                  | 3                   | Plus <sup>a</sup>           | 3                  |
| 20     | 18-May-95       | 21-May-97       | 2                        | 4                      | 2                           | 3                  | 4                   | 2                           | 2                  |
| 21     | 10-May-95       | 4-Jun-99        | 4                        | 6                      | 4                           | 3                  | 5                   | 4                           | 2                  |
| 22     | 10-May-95       | 4-Jun-99        | 4                        | 7                      | 4                           | 3                  | 6                   | 4                           | 2                  |
| 23     | 10-May-95       | 3-Jun-99        | 4                        | 6                      | 4                           | 4                  | 6                   | 4                           | 4                  |
| 24     | 18-May-95       | 3-Jun-99        | 4                        | 6                      | 4                           | 3                  | 5                   | N/A <sup>b</sup>            | 1                  |
| 25     | 10-May-95       | 7-Jun-99        | 4                        | 6                      | N/A <sup>b</sup>            | 1                  | 5                   | N/A <sup>b</sup>            | 1                  |
| 26     | 10-May-95       | 20-May-98       | 3                        | 6                      | 3                           | 3                  | 4                   | 2                           | 2                  |
| 27     | 18-May-95       | 27-May-98       | 3                        | 6                      | 3                           | 3                  | 5                   | 3                           | 3                  |
| 28     | 10-May-95       | 30-Nov-95       | Plus <sup>a</sup>        | 2                      | Plus <sup>a</sup>           | 3                  | 4                   | 2                           | 3                  |
| 29     | 18-May-95       | 5-Jun-99        | 4                        | 6                      | 4                           | 4                  | 6                   | 5                           | 2                  |
| 30     | 10-May-95       | 5-Jun-99        | 4                        | 6                      | 4                           | 3                  | 7                   | 5                           | 4                  |
| 31     | 18-May-95       | 5-Jun-99        | 4                        | 6                      | 4                           | 4                  | 6                   | 4                           | 3                  |
| 32     | 10-May-95       | 20-May-98       | 3                        | 5                      | N/A <sup>b</sup>            | 1                  | 5                   | N/A <sup>b</sup>            | 1                  |
| 33     | 18-May-95       | 21-May-98       | 3                        | 5                      | 3                           | 3                  | 4                   | 2                           | 3                  |

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| Fish # | Date of Marking | Date of Capture | Actual Elapsed Time (yr) | Thin-Sectioned Otolith |                             |                    | Whole Otolith       |                             |                    |
|--------|-----------------|-----------------|--------------------------|------------------------|-----------------------------|--------------------|---------------------|-----------------------------|--------------------|
|        |                 |                 |                          | Estimated Total Age    | Estimated Age Past OTC Mark | OTC Mark Intensity | Estimated Total Age | Estimated Age Past OTC Mark | OTC Mark Intensity |
| 34     | 18-May-95       | 27-May-98       | 3                        | 5                      | 3                           | 4                  | 5                   | 3                           | 4                  |
| 35     | 10-May-95       | 21-May-98       | 3                        | 6                      | N/A <sup>b</sup>            | 1                  | 5                   | N/A <sup>b</sup>            | 1                  |
| 36     | 10-May-95       | 21-May-98       | 3                        | 5                      | 3                           | 4                  | 5                   | 3                           | 4                  |
| 37     | 18-May-95       | 22-May-98       | 3                        | 5                      | 3                           | 4                  | 4                   | 2                           | 2                  |
| 38     | 10-May-95       | 27-May-98       | 3                        | 5                      | 3                           | 2                  | 5                   | 3                           | 4                  |
| 39     | 10-May-95       | 21-May-98       | 3                        | 5                      | 3                           | 3                  | 6                   | 4                           | 3                  |
| 40     | 10-May-95       | 21-May-98       | 3                        | 6                      | 3                           | 3                  | 6                   | 4                           | 3                  |
| 41     | 10-May-95       | 20-May-98       | 3                        | 5                      | 3                           | 3                  | 5                   | 3                           | 3                  |
| 42     | 10-May-95       | 21-May-98       | 3                        | 6                      | 3                           | 3                  | 5                   | 3                           | 4                  |
| 43     | 10-May-95       | 21-May-98       | 3                        | 6                      | 3                           | 4                  | 6                   | 4                           | 4                  |
| 44     | 10-May-95       | 21-May-98       | 3                        | 6                      | 3                           | 3                  | 6                   | 3                           | 4                  |
| 45     | 18-May-95       | 8-Jun-99        | 4                        | 7                      | 4                           | 4                  | 4                   | 3                           | 4                  |
| 46     | 18-May-95       | 2-Jun-00        | 5                        | 7                      | 5                           | 3                  | 5                   | N/A <sup>b</sup>            | 1                  |
| 47     | 18-May-95       | 2-Jun-00        | 5                        | 7                      | 5                           | 3                  | 5                   | N/A <sup>b</sup>            | 1                  |

<sup>a</sup> Most burbot were sampled in May, but a few were sampled in November. November fish showed summer plus growth past the OTC mark, but not a complete annulus.

<sup>b</sup> Whole and thin-sectioned otoliths where the OTC mark was not visible.