

**Appendix A.** Age Determination of Smallmouth Bass Sampled from the Connecticut River in 2000

## **Age Determination of Smallmouth Bass Sampled from the Connecticut River in 2000**

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## Introduction

This report provides the results of the age analysis of smallmouth bass captured in the Connecticut River in the year 2000. Partners in the project included the New England Interstate Water Pollution Control Commission (NEIWCCC), EPA-New England (EPA-NE), US Fish and Wildlife Service (USFWS), US Geological Survey (USGS), CT Department of Environmental Protection (CTDEP), CT Fish and Game (CTF&G), MA Department of Environmental Protection (MADEP), NH Department of Environmental Services (NHDES), NH Fish and Game (NHF&G), VT Department of Environmental Conservation (VTDEC), VT Fish and Game (VTF&G) and the University of Connecticut Environmental Research Institute (ERI). Following sampling, representatives of EPA-New England delivered aging structures (otoliths and/or scales) from a number of fish species to the USFWS for age analysis. It was determined by both agencies that the best use of the limited resources available was to focus on the aging of smallmouth bass specimens. Of the eight sampling reaches (Table 1) in the original study, smallmouth bass were sampled in seven. It was decided that otoliths should be used as the primary aging structure due to their relatively higher level of accuracy in age determination when compared to scales. Of the seven reaches, otoliths were available for most smallmouth bass samples from Reaches 1,2,3 and 5. Scales were available from the smallmouth bass specimens from all seven reaches in which they were sampled. The aging of the smallmouth bass samples from Reach 7 was of particular interest to EPA-NE due to the elevated mercury found in some fish from this Reach. In evaluating all considerations (financial limitations, aging structure availability and mercury levels found in specimens from particular Reaches) it was mutually determined to limit the scope of the study to aging the smallmouth bass specimens from Reaches 1,2,3,5 and 7. The age analysis and final report were completed by the USFWS, Central New England Fishery Resources Office, Nashua, New Hampshire.

Table 1. Description of Sampling Reaches on the Connecticut River- 2000.

Site Identification Code	Reach Description
CT1	Reach 1 - Haddam CT to Enfield, CT
CT2	Reach 2 - Enfield CT to Holyoke, MA
CT3	Reach 3 - Holyoke Dam to Turner Falls Dam, MA
CT4	Reach 4 - Turner Fall Dam, MA to Vernon Dam, VT
CT5	Reach 5 - Vernon Dam, VT to Claremont, NH
CT6	Reach 6 - Wilder Dam in Lebanon/Hanover, NH to Moore Dam
CT7	Reach 7 - Moore Dam Littleton, NH to Canaan, VT Dam
CT8	Reach 8 - Above Canaan, VT Dam to Stewartstown/Clarksville, NH

The report which follows is divided into three sections. The first section is the methods section. This section contains the specific steps used for preparing and analyzing the body structures (otolith<sup>1</sup> and/or scales) used to age the specimens. The second section summarizes the data. The data section is subdivided into five subsections; one subsection for each of the five Reaches in which age analyses were performed for this report. Each of the data subsections contain a table listing morphometric<sup>2</sup> data collected during field sampling, along with the age determinations made using scales and/or otoliths. Following the data table for each subsection is an overview of observations made during the aging process that are specific for that subsection (Reach). The last section is a summary. The summary contains a composite graph of length-age relationships determined for all of the Reaches analyzed in this study and a narrative statement comparing this relationship between reaches. The summary section also contains a statement about the limitations of this study along with the potential impacts of these limitations on the validity of the results.

## **A. Methods**

In the year 2000, EPA-NE together with other federal and state agencies performed fish sampling in eight reaches of the Connecticut River (Table 1). Fifteen smallmouth bass were sampled in seven of these eight Reaches. A combination of scales and otoliths were delivered to staff biologists at the U.S. Fish and Wildlife Service, Central New England Fishery Resources Office for use as aging structures. Age determinations were conducted on smallmouth bass specimens from five (Reaches 1,2,3,5 and 7) of the eight sampling reaches. Scale samples were taken for aging purposes for all specimens. Otolith samples were also taken from many of the specimens from Reaches 1,2,3 and 5.

Otoliths were cleaned and dried using a method of bleach soaking, distilled water rinsing, and ethanol soaking followed by air drying. Cleaned otoliths were embedded in epoxy resin and thin sectioned (15-20 microns) through the transverse plane using an Isomet low speed saw with a diamond cutting blade. The sectioned wafers were clarified using clove oil, and permanently mounted on microscopic slides with Cytoseal 280 mounting medium. The number of annuli contained on the otoliths were determined using a dissecting scope, compound microscope, or overhead projector with transmitted light. Each dark band was considered a yearly mark

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<sup>1</sup>“Otolith - Calcareous concretions in the ear capsules of bony fishes used for perception of acceleration including gravity. Also called 'ear bones', 'ear stones' or statoliths. These bones frequently show daily, seasonal or annual checks, rings or layers which can be used to determine ages. The lapillus lies in the utricle, the sagitta in the saccule, and the asteriscus in the lagena. See also otoconium and marginaria.”  
([www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=otolith](http://www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=otolith), 2004)

<sup>2</sup> “Morphometric - Relative to measurements of the shape of an individual; body proportions; the size relationship of various morphological characteristics of an animal.”  
([www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=morphometric](http://www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=morphometric), 2004)

(annulus). The date of sampling was unavailable for all specimens. Since the time of annuli formation is frequently late in the season (midsummer) for smallmouth bass in this climate area (Chandler, 1977, Vol.2). the determined age is given as the number of annuli plus one for specimens with considerable growth beyond the last annulus and the determined age is equal to the number of annuli when the outermost annulus is at the outer margin of the otolith.

Scales were cleaned and mounted between microscopic slides, and read using an overhead projector with 50X magnification. Three scales containing the most distinct annuli were identified and the positions of their focus, annuli, and margin were recorded on specially designed data sheets. Annuli were determined by the signatures formed by the circuli<sup>2</sup>, specifically, the constriction and expansion patterns in intercirculi spacing, cutting over of the circuli, and the extension of circuli into the posterior field (Lager 1952). The determined age of specimens using scales was equal to the number of annuli counted. In cases where a constriction pattern appeared on the outer margin of the scale, indicating the initiation of annulus formation, an additional year was added to the age of the specimen (see the discussion of the limitation to this study in the Summary Section).

## **B. Data/ Discussion**

The following five subsections contain the age determination data for smallmouth bass sampled from the five reaches of the Connecticut River that were analyzed in this study. The comments used in the data tables are from the following key.

### **Comment Key**

<sup>1</sup>annuli easy to discern

<sup>2</sup>annuli difficult to discern

<sup>3</sup>some annuli difficult to discern

<sup>4</sup>outer annulus forming at the scale margin

<sup>5</sup>no annulus forming at the scale margin

<sup>6</sup>might have additional annulus at scale margin

<sup>7</sup>tight inner annulus (first annulus near focus)

<sup>8</sup>tight inner two annuli

<sup>9</sup>false annulus

<sup>10</sup>might be only two years old

<sup>11</sup>might be six years old

<sup>12</sup>very strong growth pattern as indicated by intercirculi spacing

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<sup>2</sup>"Circuli - Concentric, continuous lines on fish scales and various bones."  
([www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=circuli](http://www.fishbase.org/Glossary/Glossary.cfm?TermEnglish=circuli), 2004)

<sup>13</sup> very strong growth between first and second annuli

<sup>14</sup> very strong growth between second and third annuli

<sup>15</sup> poor quality of otolith sections

<sup>16</sup> all three annulus only visible on two otolith sections

<sup>17</sup> could be only four years old

<sup>18</sup> might only have five annuli

<sup>19</sup> third and fourth annuli very close together, might be one divided annulus

<sup>20</sup> no scale sample available, age determination is estimated based on comparisons with other samples from this Reach

Subsection 1: Age Analysis of Smallmouth Bass Sampled in Reach 1 of the Connecticut River.

**Table 2.** Whole body weight, total length and age of Smallmouth Bass Sampled in Reach 1.

Field Sample Number	Whole Body Weight (gm)	Total Length (cm)	Age Determination from Scales			Age Determination from Otoliths			Reconciled Age Determination
			# of annuli	age	comments	# of annuli	age	comments	
CT1-SMB-01	401.9	31.4	3	3	1	2	3		3
CT1-SMB-02	382.8	30.8	2	3	10	2	3		3
CT1-SMB-03	349.9	30.1	3	3	1	NA	NA		3
CT1-SMB-04	354.6	30.4	3	3	4	2	3		3
CT1-SMB-05	482.8	33	3	3	1,12	2	3		3
CT1-SMB-06	371.8	30.1	3	3	4	2	3		3
CT1-SMB-07	398.2	31.3	2	3	4	2	3		3
CT1-SMB-08	379.5	29.8	2	3	4	2	2		3
CT1-SMB-09	481.3	32.3	2	3	5	2	3		3
CT1-SMB-10	338.4	29.4	3	3	4,12	2	3		3
CT1-SMB-11	396.4	30.9	4	4	9	3	4		4
CT1-SMB-12	651.1	37.6	3	3	2	2	3	13	3
CT1-SMB-13	463.9	33.2	3	4	5	NA	NA		4
CT1-SMB-14	534.8	33.6	4	4		3	4		4
CT1-SMB-15	421.7	31	2	3	5	2	3		3

Summary Reach 1: The majority of scale samples either had a complete annulus near the outer scale margin or where in the process of forming one. Overall the scale annuli were very distinct and intercirculi spacing indicated fairly good growth. The inner annulus was not tight to the focus and was easy to discern.



Subsection 2: Age Analysis of Smallmouth Bass Sampled in Reach 2 of the Connecticut River.

**Table 3.** Whole body weight, total length and age of Smallmouth Bass Sampled in Reach 2.

Field Sample Number	Whole Body Weight (gm)	Total Length (cm)	Age Determination from Scales			Age Determination from Otoliths			Reconciled Age Determination
			# of annuli	age	comments	# of annuli	age	comments	
CT2-SMB-01	760.6	38.2	5	5		NA	NA		5
CT2-SMB-02	930.3	38.8	5	5		4	5		5
CT2-SMB-03	780.1	35.5	5	5	14	4	5		5
CT2-SMB-04	472.4	31	3	3	4	NA	NA		3
CT2-SMB-05	359.6	30.5	3	4	4	NA	NA		4
CT2-SMB-06	402.6	30.2	3	4		NA	NA		3
CT2-SMB-07	431.5	30.9	4	4		NA	NA		4
CT2-SMB-08	373.7	30.7	3	3		3	3		3
CT2-SMB-09	296.6	27.9	2	2	4	2	2		2
CT2-SMB-10	342.1	29.1	3	3		NA	NA		3
CT2-SMB-11	388.7	30.4	3	4		NA	NA		4
CT2-SMB-12	288.3	28.2	2	2		2	2		2
CT2-SMB-13	330.4	28.8	3	4	4	NA	NA		4
CT2-SMB-14	392.9	30.4	3	4		3	3		4
CT2-SMB-15	260.2	27.0	3	3	1	2	2		3

Summary Reach 2: Many scales had an annulus near their outer margin indicating that the current year's annulus had already been formed. Therefore an additional year was not added to the number of annuli when determining the age of the fish from scales.

Subsection 3: Age Analysis of Smallmouth Bass Sampled in Reach 3 of the Connecticut River.

**Table 4.** Whole body weight, total length and age of Smallmouth Bass Sampled in Reach 3.

Field Sample Number	Whole Body Weight (gm)	Total Length (cm)	Age Determination from Scales			Age Determination from Otoliths			Reconciled Age Determination
			# of annuli	age	comments	# of annuli	age	comments	
CT3-SMB-01	241.8	27.5	2	3		2	2		2
CT3-SMB-02	321.5	28.5	2	3	4	2	2		2
CT3-SMB-03	394.2	31.1	4	4	2	3	3		3
CT3-SMB-04	545.7	33.7	4	4	4	NA	NA		4
CT3-SMB-05	538.3	35.3	4	4	3	4	4		4
CT3-SMB-06	655.0	36.4	5	5		5	5		5
CT3-SMB-07	462.0	33.4	4	5	1	5	5		5
CT3-SMB-08	698.4	37.5	7	8	3	8	8		8
CT3-SMB-09	599.7	36.1	5	5	2	5	5		5
CT3-SMB-10	681.7	38.4	6	5		4	5		5
CT3-SMB-11	771	38.3	8	8	1	7	7		7
CT3-SMB-12	561.3	38.4	5	5		5	5		5
CT3-SMB-13	286.2	27.9	3	3	7	2	2		3
CT3-SMB-14	457.0	31.8	3	3		2	3	15	3
CT3-SMB-15	329.9	28.4	3	3		2	3		3

Summary Reach 3: Many scales had an annulus near their outer margin indicating that the current year's annulus had already been formed. Therefore an additional year was not added to the number of annuli when determining the age of the fish from scales.

Subsection 4: Age Analysis of Smallmouth Bass Sampled in Reach 5 of the Connecticut River.

**Table 5.** Whole body weight, total length and age of Smallmouth Bass Sampled in Reach 5.

Field Sample Number	Whole Body Weight (gm)	Total Length (cm)	Age Determination from Scales			Age Determination from Otoliths			Reconciled Age Determination
			# of annuli	age	comments	# of annuli	age	comments	
CT5-SMB-01	798.2	37.6	5	5		5	5		5
CT5-SMB-02	792.4	36.6	5	5		5	5		5
CT5-SMB-03	571.3	34.1	4	4	1	3	3	17	4
CT5-SMB-04	717.9	36.0	4	4	7	4	4		4
CT5-SMB-05	494.2	31.2	3	3		3	3	15	3
CT5-SMB-06	432.2	30.8	3	3		3	3		3
CT5-SMB-07	683.1	35.5	4	4	6	4	4	6	4
CT5-SMB-08	514.4	32.0	3	3	12	3	3		3
CT5-SMB-09	308.9	28.0	3	3		NA	NA		3
CT5-SMB-10	1020.9	39.2	6	6		6	6	18	6
CT5-SMB-11	657.3	35.7	5	5	8	5	5		5
CT5-SMB-12	378.4	28.4	3	3		3	3	4	3
CT5-SMB-13	782.0	37.3	6	6		5	5		5
CT5-SMB-14	395.7	29.4	3	3	4,7	3	3	16	3
CT5-SMB-15	240.6	24.5	2	2	7, 12	NA	NA		2

Summary Reach 5: Most of the scale samples in Reach 5 had annuli near their margin. Therefore the age determination from scale for specimen in this Reach is given as the same as the number of annuli.

Subsection 5: Age Analysis of Smallmouth Bass Sampled in Reach 7 of the Connecticut River.

**Table 6.** Whole body weight, total length and age of Smallmouth Bass Sampled in Reach 7.

Field Sample Number	Whole Body Weight (gm)	Total Length (cm)	Age Determination from Scales			Age Determination from Otoliths			Reconciled Age Determination
			# of annuli	age	comments	# of annuli	age	comments	
CT7-SMB-01	486.1	33.2	4	5	4,7	NA	NA		NA
CT7-SMB-02	353.4	29.7	4	5	4	NA	NA		NA
CT7-SMB-03	384.4	31.5	5	5	4	NA	NA		NA
CT7-SMB-04	285.4	28.6	4	4	4	NA	NA		NA
CT7-SMB-05	506.3	33.6	5	5	4	NA	NA		NA
CT7-SMB-06	404.7	32.0	5	5	4	NA	NA		NA
CT7-SMB-07	371.5	30.8	5	5	4,11	NA	NA		NA
CT7-SMB-08	425.5	31.8	5	5	4	NA	NA		NA
CT7-SMB-09	410.8	30.8	5	5	4	NA	NA		NA
CT7-SMB-10	523.3	33.1	5	5	4, 19	NA	NA		NA
CT7-SMB-11	305.7	28.0	5	5	1,4	NA	NA		NA
CT7-SMB-12	269.1	28.3	3	3	1, 5	NA	NA		NA
CT7-SMB-13	327.1	29.1	4	4	4	NA	NA		NA
CT7-SMB-14	523.0	33.3	5	5	2	NA	NA		NA
CT7-SMB-15	397.4	30.2	NA	5	20				

Summary Reach 7: Most of the scale samples in Reach 7 had annuli near their margin. Therefore the age determination from scale for specimen in this Reach is given as the same as the number of annuli. Several scale sample had third and forth annuli that were in very close proximity to one another and might actually be one divided annulus.

## Summary

Otolith annuli formation was both distinct and consistent. No annuli were found near the margin. The date listed on the scale envelopes is July 10, 2000. EPA-NE staff indicated that this date most likely indicates the processing date and not the date of capture. For both scales and otoliths it was expected that if the current year's annulus had formed it would be located near the margin of the aging structure. In structures in which an annulus was not found near the margin (all otoliths and some scales) a year was added to the number of annuli in determining the age of the specimen. Scales provided a less clear picture with some scales containing annuli formation near the margin and others not having this characteristic. Without knowing the date of capture it was particularly hard determine whether or not to add an extra year above the number of annuli found when determining the age of these fish. In general, if there was considerable growth between the last annulus and the margin then a year was added to the number of annuli in determining the specimens age. If the outermost annuli was moderately close to the scale margin than the age was determined to be equal to the number of annuli. In addition, there is a possibility that there is an inner first year annulus that was uniformly not added to all specimens. Having samples of smaller size classes from this Reach could have helped elucidate the anticipated location of the first annulus.

Figure One below shows a composite of the age-length relationship of smallmouth bass from all sampled reaches. This figure was prepared so that the reader could visually compare fish sampled from various reaches. For example, the figure shows that the age variation for fish from Reach 1 is much less than for Reach 3.

**Limitations:** There were several limitations in this study that made conditions less than ideal for the most accurate determination of age for the sampled smallmouth bass. Two primary limitations were: (1) the date of capture for the specimens was not available and (2) specimens for younger age classes were not available. Specimen samples (scale envelopes and otolith vials) had dates of processing but not dates of capture.

Limitation due to missing date of capture. By convention, all fish in the northern hemisphere are assigned birthdate on January 1 (DeVries, D.R., and R.V. Frie 1996). Smallmouth bass, in northern temperate areas frequently do not produce the current year annulus until late spring or early summer (Chandler, K.D. 1977). In determining the age of smallmouth bass taken from a spring or early summer specimen, an additional year is typically added above the number of annuli counted unless the outermost annulus is very close to the scale margin. The reason for this practice being that it is assumed that the formation of the past year's annulus is not complete.

Limitation due to lack of samples from smaller/younger fish specimens. Having samples from young-of-the- year and one-year old specimens can greatly assist in determining the placement of the inner annuli on older specimens. Although yearly fluctuation in the positions of annuli formation occurs even within a species in a given location, knowing if younger age classes have very tight inner annuli (close to the focus) can help alert a researcher of the potential presence of this type of annuli formation and the positions of annuli in younger specimens can

act as a relative benchmark in determining the presence and/or position of the inner annuli in older specimens. As fish age, inner annuli on older specimens can become obscured. Knowledge of the locations of annuli on younger specimens can help rectify this difficulty.

### **Literature Cited**

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Figure 1. Composite Age-Length Relationships of Smallmouth Bass Sampled from Five Reaches of the Connecticut River, 2000

