Annulus Validation, Time of Formation, and Mean Length at Age of Three Sunfish Species in North Central Florida

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Abstract: The visible opaque bands in transverse sections of sagittal otoliths from bluegill (*Lepomis macrochirus*), redear sunfish (*L. microlophus*), and redbreast sunfish (*L. auritus*) were verified as annual rings. The verification was done using intraperitoneal injections of oxytetracycline followed by otolith examination after the fish were at liberty for 1 year in a north central Florida pond. Injections of 75 mg/kg wet body weight resulted in the best rate of fluorescent ring formation. Saggital otoliths from bluegill and redear sunfish populations in Newnans Lake Florida were sampled monthly to determine the time of annulus formation. Annulus formation occurred for bluegill between March and July and for redear sunfish between February and July. Baseline data for length at age I to IV are presented for bluegill, redear sunfish, and redbreast sunfish populations in 58 lakes, 46 lakes, and 13 streams, respectively.

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Assessment and management of fish populations often requires determination of the age and growth of fish stocks. Age and growth information is specifically used to define year class strength, mortality rates, survival, and recruitment. In temperate climates, age determination is accomplished with the use of scales. Incorrect ages from scales, however, are prevalent in southern latitudes and in power plant cooling lakes (Taubert and Tranquilli 1982, Heidinger and Clodfelter 1987). In these situations the use of otoliths (mainly sagittae) has been verified for aging many freshwater sportfish including largemouth bass (*Micropterus salmoides*) (Taubert and Tranquilli 1982, Hoyer et al. 1985), black crappie (*Pomoxis nigromaculatus*) (Schramm and Doerzbacher 1982), white crappie (*P. annularis*) (Maceina and Betsill 1987) smallmouth bass (*M. dolomieui*), striped bass (*Morone saxatilis*), walleye (*Stizostedion vitreum*) (Heidinger and Clodfelter 1987), and bluegill (Schramm 1989).

Bluegill, redear sunfish, and redbreast sunfish, are major sportfish in Florida as well as in other subtropical areas. Schramm (1989) has verified that an opaque band in the otolith can be considered an annulus for age-1 bluegill. However, Schramm's sampling of wild populations was minimal, nor does verification exist for redear sunfish and redbreast sunfish. The time of annulus formation for sunfish species in Florida has also not been determined, which is an important component of age determination. Therefore, the objectives of this study were to: 1) verify the findings of Schramm (1989) for bluegill, 2) validate the use of otoliths for aging redear sunfish and redbreast sunfish, 3) determine the time of annulus formation for bluegill and redear sunfish, and 4) list the means and ranges of length at age for bluegill, redear sunfish, and redbreast sunfish from 58 lakes, 46 lakes, and 13 streams in Florida, respectively, as baseline data for future studies. The validation procedure also allowed us to examine 3 rates of oxytetracycline (OTC) injection and suggest a rate to use for the formation of fluorescent rings in *Lepomis* species.

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Methods

Annulus Validation

Three trials were conducted to verify that the visible opaque bands on otoliths of bluegill, redear sunfish, and redbreast sunfish were annual marks. In December 1987, 259 bluegill, 180 redear sunfish, and 27 redbreast sunfish were collected by electrofishing in Alligator Creek and Lake Rowell, Bradford County, Florida. In July 1989, 70 bluegill, 34 redear sunfish, and 47 redbreast sunfish were collected from the same 2 locations. The fish ranged from 80 to 240 mm total length (TL) and were transported in live wells to a 0.1-ha pond near Gainesville, Florida. The pond was treated with rotenone to eliminate all fish prior to each stocking. The fish collected in December 1987 received intraperitoneal injections of 25 mg/kg wet body weight (Trial 1) of oxytetracycline (OTC). The dosage was similar to that recommended by McFarlane and Beamish (1987) for the spiny dogfish (*Squalas acanthias*). The fish collected in July 1989 were divided into 2 groups by species, receiving intraperitoneal injections of 75 mg OTC/kg wet body weight (Trial 2) and 150 mg OTC/kg wet body weight (Trial 3).

All fish were collected from the ponds 12 months after stocking by applying 2 mg/L of rotenone, and the sagittal otoliths of each fish were removed for analyses, sectioned transversely in the dorsoventral plane (Hoyer et al. 1985) and examined in transmitted blue light (450–490 nm excitation and >515 nm emission) at 100X magnification.

Time of Annulus Formation

Bluegill and redear sunfish were collected monthly by electrofishing from Newnans Lake, near Gainesville, Florida, between August 1989 and July 1990 (Mantini 1991). Monthly sample sizes ranged from 19–170 mm and 18–51 mm for bluegill and redear sunfish, respectively. Immediately upon collection, fish were put on ice and returned to the laboratory.

Fish were measured (TL) to the nearest mm and sagittal otoliths were removed and cleared in 100% glycerin for 1 week prior to viewing (Hoyer et al. 1985). Whole otoliths were viewed with a binocular dissecting scope equipped with an ocular micrometer and a source of reflected light. Magnification ranged from 10X to 150X. Maximum otolith radius and the distance from the approximate center to each annulus were measured on the distal surface of the otolith (Schramm and Doerzbacher 1982). Under reflected light, annuli appear as opaque zones.

Time of annulus formation of bluegill and redear sunfish was determined by noting monthly proportions of otoliths with completely formed annuli (Coleman et al. 1985, Crawford et al. 1989). Marginal increments, the distance from the outermost annulus to the otolith margin, were measured. The marginal increment distance was minimal at time of annulus formation (Gregory and Jow 1976, Schramm and Doerzbacher 1982, Maceina and Betsill 1987, Thorogood 1987).

Monthly surface water temperatures were recorded from Newnans Lake, as well as fresh gonad weights from all female bluegill and redear sunfish. This was done in order to define spawning condition of the fish and water temperature during annulus formation. The gonadosomatic index (GSI) was calculated to estimate time of spawn for each species. GSI was fresh gonadal weight expressed as a percentage of the total body weight.

Mean Length at Age for Bluegill, Redear Sunfish, and Redbreast Sunfish in Florida

Canfield and Hoyer (1992) sampled fish populations from 60 Florida lakes with rotenone, gillnets, and electrofishing between 1986 and 1990. Individual fish lengths were measured and otoliths (sagittae) were collected on a subsample of bluegill and redear sunfish collected in blocknets, gillnets, and electrofishing. The subsample consisted of 10 fish from each 40 mm TL size group. Length at age estimates were determined for bluegill and redear sunfish in 58 and 46 Florida lakes, respectively. Whole otoliths were measured according to the methods of Hoyer et al. (1985). Samples were collected in 2 different years for 3 lakes.

Canfield and Hoyer (1988) sampled fish populations in 13 Florida streams with electrofishing between 1984 and 1986. Individual lengths and otoliths (sagittae) were collected on a subsample of redbreast sunfish collected from each stream. The subsample consisted of 10 fish from each 20 mm TL size group. Length at age estimates were determined for redbreast sunfish using whole otoliths according to the methods of Hoyer et al. (1985). Back-calculations for data from each study were made with the Lee method and the intercept values from the following fish total length and otolith radius regressions for all bluegill (N = 1,096), all redear sunfish (N = 775), and all redbreast sunfish (N = 560) collected:

bluegill length (mm) = −32 + 63 x otolith radius (mm) R^2 = 0.87
redear sunfish length (mm) = −35 + 67 x otolith radius (mm) R^2 = 0.87
redbreast sunfish length (mm) = −17 + 57 x otolith radius (mm) R^2 = 0.88

Length at age for bluegill, redear sunfish, and redbreast sunfish were only determined to age IV because of the possibility of missing annuli while reading whole otoliths (Hoyer et al. 1985).

Results and Discussion

Oxytetracycline Injections

Survival rates of fish injected with 150 mg/kg body weight were only 3% for bluegill, 7% for redear sunfish and 14% for redbreast sunfish, which were less than the survival rates for fish in the 25 and 75 mg/kg body weight trials (Table 1). These data are from small samples and are subject to large errors; however, they suggest that intraperitoneal injections of OTC ≥150 mg/kg body weight may increase the mortality of bluegill, redear sunfish, and redbreast sunfish. Although OTC was injected, these findings are similar to those of Schmitt (1984) who observed 100% mortality of the Australian species (*Hypoatherina tropicalis*) and (*Spratelloides delicatulus*) after immersion into a maximum level of OTC (400 mg/liter) and only 10% mortality after immersion into a minimum level of OTC (50 mg/liter).

Several studies have indicated success marking otoliths with a wide range of OTC concentrations; Simoneaux and Warlen (1987) used 13–20 mg/kg body weight in Atlantic menhaden (*Brevoortia tyrannus*), McFarlane and Beamish (1987) used 25 mg/kg body weight in the spiny dogfish, and Beamish et al. (1983) used 50 mg/kg body weight in sablefish (*Anoplopoma fimbra*). The otoliths of bluegill, redear sunfish, and redbreast sunfish injected with 25 mg/kg body weight of OTC showed no fluorescent rings in sectioned view (Table 1). Species injected with 75 mg/kg body weight of OTC showed between 40% and 50% frequency of occurrence of a fluorescent ring (Table 1), while 80% of the fish recovered from the ponds and injected with 150 mg/kg body weight of OTC had a fluorescent ring (Table 1). Thus, our study found that intraperitoneal injections of 150 mg/kg wet body weight of bluegill, redear sunfish, and redbreast sunfish yielded the highest incidence of fluorescent rings on otoliths. OTC injections of ≥150 mg/kg, however, may have increased mortality of these species. Injections of ≤25 mg/kg of OTC were inadequate concentrations for fixing fluorescent rings on otoliths of bluegill, redear sunfish, and redbreast sunfish. Thus, we suggest that injections of 75 mg OTC/kg wet body weight may be a satisfactory level for the formation of fluorescent rings on otoliths of Lepomis species.

When fish were recovered and otoliths examined, the number of opaque bands that formed beyond the OTC rings in all but 1 case was equal to 1 year. The 1

Table 1. Number of bluegill, redear sunfish, and redbreast sunfish stocked into a 0.1-ha pond after intraperitoneal injections of 25, 75, and 150 mg/kg wet body weight of oxytetracycline (OTC). The number of fish recovered after 1 year at liberty and the number recovered showing a fluorescent ring are listed with the corresponding percentage of the total fish stocked or corresponding percentage of fish recovered in parentheses.

	Bluegill	Redear sunfish	Redbreast sunfish
Trial 1	25 mg/kg (30 Dec 87 to 29 Dec 88)		
Fish stocked	259	180	27
Fish recovered	41 (16%)	50 (28%)	9 (33%)
Fish showing fluorescence	0 (0%)	0 (0%)	0 (0%)
Trial 2	75 mg/kg (31 Jul 89 to 16 Jul 90)		
Fish stocked	38	20	26
Fish recovered	4 (10%)	7 (35%)	10 (38%)
Fish showing fluorescence	2 (50%)	3 (43%)	4 (40%)
Trial 3	150 mg/kg (31 Jul 89 to 16 Jul 90)		
Fish stocked	32	14	21
Fish recovered	1 (3%)	1 (7%)	3 (14%)
Fish showing fluorescence	1 (100%)	1 (100%)	2 (67%)

exception (bluegill no. 3, Table 2) had not yet formed an annulus beyond the OTC ring. It was, however, still mid-summer (July) and an annulus could have been laid down later. The bluegill data are similar to data collected by Schramm (1989) who used monthly marginal increments to validate the opaque band of age-1 bluegill as an annulus. Therefore, these data validate that the formation of opaque bands found on otoliths of bluegill, redear sunfish, and redbreast sunfish are an annual event (Table 2) and can be used for aging of these species in Florida as well as in other subtropical areas.

Time of Annulus Formation in Bluegill and Redear Sunfish

Monthly samples of Newnans Lake bluegill and redear sunfish were most complete for ages I–III; therefore, periodicity of annulus formation was determined from these data. Differences in monthly mean marginal increments and appearance of recently formed annuli indicate that annulus formation occurred for bluegill between March and July of 1990, with the lowest percentage of completed annuli occurring in April (Table 3). Similarly, annulus formation occurred for redear sunfish between February and July 1990, with the lowest percentage of fish with completed annuli occurring in May (Table 3). These data provide additional evidence that annuli formation on otoliths of bluegill and redear sunfish are an annual event.

Investigators have suggested that temperature is a major factor in the formation of annuli. In studying geographically disparate populations of largemouth bass, McCauley and Kilgour (1990) found that accumulated degree-days $>10°$ C best correlated with growth. Temperature manipulations have been used to induce annulus

Table 2. Measurements of annuli (ages I, II, III, IV, V), oxytetracycline fluorescent ring (OTC) and outer edge (OE) from sectioned otoliths of bluegill, redear sunfish, and redbreast sunfish. Measurements are in mm.

Fish species	OTC rates mg/kg	Age (actual otolith measurements in mm)						
Individual bluegill								
No. 1	75	I (0.25)	OTC (0.42)	II (0.45)	OE (0.49)			
No. 2	75	I (0.22)	OTC (0.30)	II (0.35)	OE (0.42)			
No. 3	150	I (0.25)	II (0.37)	OTC (0.50)	OE (0.57)			
Individual redear sunfish								
No. 1	75	I (0.18)	II (0.27)	III (0.35)	OTC (0.38)	IV (0.44)	OE (0.46)	
No. 2	75	I (0.18)	OTC (0.28)	II (0.31)	OE (0.36)			
No. 3	75	I (0.17)	OTC (0.23)	II (0.34)	OE (0.34)			
No. 4	150	I (0.19)	OTC (0.24)	II (0.30)	OE (0.34)			
Individual redbreast sunfish								
No. 1	75	I (0.14)	II (0.20)	III (0.30)	OTC (0.38)	IV (0.38)	OE (0.40)	
No. 2	75	I (0.20)	II (0.30)	III (0.37)	OTC (0.45)	IV (0.45)	OE (0.45)	
No. 3	75	I (0.23)	II (0.30)	III (0.36)	IV (0.45)	OTC (0.52)	V (0.55)	OE (0.60)
No. 4	75	I (0.27)	II (0.35)	III (0.42)	OTC (0.50)	IV (0.55)	OE (0.57)	
No. 5	150	I (0.13)	II (0.25)	III (0.35)	OTC (0.39)	IV (0.42)	OE (0.45)	
No. 6	150	I (0.15)	II (0.28)	III (0.35)	OTC (0.40)	IV (0.44)	OE (0.45)	

Table 3. Marginal increments (mm) and percentages of complete (% complete) outer annuli in otoliths of monthly samples for bluegill and redear sunfish populations in Newnans Lake Florida. Mean monthly gonadosomatic indices (GSI) are recorded as percentage of total body weight comprised by the gonads. Monthly surface water temperatures (° C, Temp) are also recorded. Values in parentheses represent sample sizes (*N*). NS = no sample.

		Marginal increments					
Species	Date	Age I	Age II	Age III	% complete	GSI	Temp
Bluegill	Aug 89	0.69 (22)	0.49 (16)	0.32 (10)	100	1.76 (13)	28.6
	Sep 89	0.69 (27)	0.53 (7)	0.41 (6)	100	0.63 (17)	25.1
	Oct 89	0.74 (22)	0.55 (3)	0.32 (2)	100	0.56 (19)	18.5
	Nov 89	0.73 (11)	0.46 (9)	0.31 (4)	100	0.52 (19)	16.2
	Dec 89	0.79 (9)	0.50 (8)	0.28 (2)	100	0.50 (11)	5.8
	Jan 90	0.67 (10)	0.58 (5)	0.28 (2)	100	0.47 (16)	10.4
	Feb 90	0.87 (10)	0.63 (8)	0.31 (4)	100	0.59 (9)	15.6
	Mar 90	0.89 (28)	0.54 (22)	0.29 (2)	94	1.12 (22)	20.2
	Apr 90	0.30 (46)	0.21 (42)	0.17 (3)	33	5.22 (38)	28.0
	May 90	0.11 (21)	0.13 (45)	0.12 (15)	64	2.71 (44)	31.8
	Jun 90	0.18 (39)	0.12 (60)	0.14 (44)	88	2.60 (63)	NS
	Jul 90	0.27 (49)	0.15 (51)	0.13 (9)	98	1.41 (58)	32.0
Redear Sunfish	Aug 89	0.79 (14)	0.51 (8)	0.64 (1)	100	2.70 (10)	28.6
	Sep 89	0.75 (10)	0.54 (4)	0.22 (2)	100	0.68 (15)	25.1
	Oct 89	0.84 (13)	0.39 (5)	0.37 (3)	100	0.50 (9)	18.5
	Nov 89	0.76 (18)	0.54 (3)	0.41 (3)	100	0.71 (14)	16.2
	Dec 89	0.93 (12)	0.58 (3)	0.80 (1)	100	0.59 (6)	5.8
	Jan 90	0.62 (10)	0.44 (4)	0.33 (3)	100	0.70 (8)	10.4
	Feb 90	0.95 (15)	0.61 (5)	NS	95	0.94 (10)	15.6
	Mar 90	1.04 (4)	0.19 (3)	0.29 (1)	92	5.84 (11)	20.2
	Apr 90	0.63 (15)	0.23 (21)	0.33 (8)	53	7.57 (17)	28.0
	May 90	0.08 (13)	0.77 (21)	0.05 (7)	29	2.18 (20)	31.8
	Jun 90	0.19 (26)	0.11 (16)	0.03 (3)	80	1.73 (17)	NS
	Jul 90	0.29 (8)	0.12 (22)	0.03 (3)	82	1.63 (12)	32.0

formation on bluegill otoliths (Schramm 1989) and otoliths of green sunfish, *Lepomis cyanellus* (Taubert and Coble 1977). Under such controlled conditions, annuli were formed during periods when temperatures were <17° C and 29° C, respectively. Carlander (1977) also suggested that the completion of annulus formation proceeds from southern to northern latitudes, again suggesting that temperature may be a major factor in the formation of annuli. Supporting these observations, the largest percentages of new annuli were observed on otoliths of Newnans Lake bluegill and redear sunfish when temperatures rose from 17° C to 29° C (Table 3).

Although peak GSI values for Newnans Lake bluegill and redear sunfish indicated that major spawning occurred in April 1990 for both species (Table 3), spawning could not be solely responsible for annulus formation. Clugston (1966) observed Everglades bluegill and redear sunfish on spawning beds at temperatures from 23.8° to 26.7° C, and the season lasted 6 to 7 months. Spawning of Newnans lake bluegill and redear sunfish seemed to last 7 months, as indicated by a 5-month duration in baseline GSI values between September 1989 and February 1990 (Table 3).

Mean Length at Age for Bluegill, Redear Sunfish, and Redbreast Sunfish in Florida

The mean calculated length at ages I to IV for bluegill from 58 north Florida lakes were 61, 125, 165, and 193 mm TL, respectively (Table 4). There is a great deal of variation in the length at age for bluegill among the different Florida lakes, with ages I to IV ranging from 30 to 115, 83 to 180, 108 to 211, and 130 to 234 mm TL, respectively. Many factors likely contribute to this variation, including both density dependent and density independent factors (Carlander 1977). The mean length at ages I to IV for bluegills from Florida, however, are larger then the mean of regional means (age I = 53 mm TL, age II = 95 mm TL, age III = 128 mm TL, and age IV = 153 mm TL) reported by Carlander (1977) for mostly northern lakes in the United States. These data support the suggestion of Carlander (1977) that although there is great variation within regions, the regional averages are usually higher in southern latitudes where growing seasons are longer.

The mean calculated length at ages I to IV for redear sunfish from 46 north Florida lakes were 72, 138, 183, and 213 mm TL, respectively (Table 4). There is a great deal of variation in the length at age for redear sunfish among the different Florida lakes, with ages I to IV ranging from 37 to 130, 90 to 197, 125 to 242, and 137 to 319 mm TL, respectively. Carlander's (1977) back calculated length at age

Table 4. Mean length of bluegill, redear sunfish, and redbreast sunfish for age classes I–IV, back calculated from examination of otolith annuli in whole view. Length is recorded in mm total length (TL). The number of lakes or streams in which fish were examined, the mean of means, the corresponding standard error of the mean, minimum, and maximum length at age are listed by species.

Species	Age I	Age II	Age III	Age IV
Bluegill				
N lakes with samples	58	58	58	48
Mean of means	61	125	165	193
Standard deviation	17	21	23	22
Minimum	30	83	108	130
Maximum	115	180	211	234
Redear sunfish				
N lake samples	46	45	43	35
Mean of means	72	138	183	213
Standard deviation	19	24	27	33
Minimum	37	90	125	137
Maximum	130	197	242	319
Redbreast sunfish				
N stream samples	13	12	7	2
Mean of means	61	104	115	119
Standard deviation	11	20	8	4
Minimum	28	72	108	118
Maximum	93	135	139	152

data for redear sunfish is less abundant than for bluegill; however, redear sunfish length at ages I to IV ranged from 30 to 185, 79 to 221, 117 to 251, and 155 to 277 mm TL, respectively. These data suggest a great deal of overlap between Carlander's (1977) data from mostly northern lakes and the data present in this paper from Florida. Consistent with other regional studies (Lopinot 1967, Jenkins et al. 1952), redear sunfish populations in Florida appear to grow faster than bluegill populations.

The mean length at ages I to IV for redbreast sunfish from 13 north Florida streams were 61, 104, 115, and 119 mm TL, respectively (Table 4). There is a great deal of variation in the length at age for redbreast sunfish among the different Florida streams, with ages I to IV ranging from 28 to 93, 72 to 135, 108 to 139, and 118 to 152 mm TL, respectively. A limited number of studies have listed the length at age for redbreast sunfish populations. Hiranvat (1973), however, recorded the length at age for 5 populations of redbreast sunfish that averaged 41, 79, 116, and 114 mm TL for ages I to IV, respectively. These averages, which were similar to those recorded in this study for Florida streams, were from populations in North Carolina, South Carolina, Georgia, and Alabama. These data again suggest that although there is great variation in length at age for redbreast sunfish within a region, the regional averages are similar throughout the southeast United States.

Conclusions

Intraperitoneal injection of OTC verified that opaque bands of sagittal otoliths were an annual event; thus, valid aging structures for bluegill, redear sunfish, and redbreast sunfish in north central Florida. Injections of 75 mg OTC/kg body weight resulted in the most effective rate of fluorescent ring formation in the 3 Lepomid species tested.

Sampling throughout the year ensured that annuli on otoliths of bluegill and redear sunfish were not accessory growth checks, such as those caused by spawning. Using the measurements of marginal increments and appearance of newly formed annuli throughout an entire year, the time of annulus formation for bluegill and redear sunfish was determined to be between March and July for bluegill and February and July for redear sunfish.

Baseline data for calculated mean length at age I to IV was presented for bluegill, redear sunfish, and redbreast sunfish populations in 58 lakes, 46 lakes, and 13 streams, respectively. From our results, bluegill tend to grow faster in southern latitudes, when compared to literature values from northern latitudes. Redear sunfish populations appear to grow faster in length than bluegill populations, within Florida lakes. Redbreast sunfish appear to have similar growth rates throughout the southeast United States.

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