VARIABLE GROWTH AND LONGEVITY OF YELLOW BULLHEAD (*AMEIURUS NATALIS*) IN SOUTH FLORIDA

REVISED FINAL REPORT BY

DEBRA J. MURIE AND DARYL C. PARKYN

Department of Fisheries and Aquatic Sciences, Institute of Food and Agricultural Sciences,
University of Florida, 7922 NW 71st Street, Gainesville, Florida 32653, U. S. A

WILLIAM F. LOFTUS

U. S. Geological Survey, Florida Integrated Science Center, Water and Restoration Studies,
Everglades National Park Field Station, 40001 S. R. 9336, Homestead, Florida, 33034 U. S. A.

LEO G. NICO

U. S. Geological Survey, Florida Integrated Science Center, 7920 NW 71st Street, Gainesville,
Florida, 32653 U. S. A.

October 2006

Abstract.– To determine age and growth of yellow bullheads (*Ameiurus natalis),* we collected fish from canals and marshes in southern Florida from April 2000 to January 2001, and from Oct 2003 to February 2005. Fish ranged from 97 to 312 mm in total length and from 13.4 to 538.1 g in wet mass. We aged 144 yellow bullheads using cross-sections of pectoral spines and a subsample of 55 fish were aged using sanded and polished cross-sections of lapillar otoliths. Pectoral-spine age estimates had relatively high precision between two experienced readers (average percent error [APE] = 6.1%, CV = 8.6%), with 65% of ages having perfect agreement and 92% agreeing within ±1 year. Lapillar-otolith age estimates provided inadequate precision with high APE (16.8%) and CV (23.7%), and low percent agreement (25% perfect agreement and 61% within ±1 year). Annuli in lapilli were not clearly demarcated and were not evident in all cross-sections of the otoliths; moreover, longitudinal sections of lapilli were unreadable. Precision estimates in comparing ages between pectoral spines and otoliths also indicated aging difficulties, with an APE of 20.6% and a CV of 29.1%. Determining ages by using cross-sections of lapilli was therefore not reliable for yellow bullheads in southern Florida, and so we did not use otolith age estimates as input into the growth model. The pectoral-spine aging method was validated using oxytetracycline (OTC)-injected yellow bullheads, which were found to deposit one complete annulus (one translucent and one opaque zone) over a 12-month period. Yellow bullheads from south Florida ranged in age from 1-12 years based on aging using pectoral spines; and therefore had a maximum age that was double that previously reported for any other yellow bullhead population. Yellow bullheads grew relatively rapidly in their first 3 years, but after age 5 their growth slowed and approached an asymptote of ~214 mm total length. Yellow bullheads in south Florida grew relatively slowly and were smaller compared to yellow bullheads from other populations throughout the United States.

Introduction

In southern Florida, a major effort is underway to restore historical functions of the ecosystem by altering hydrological management. This restoration process employs ecological-simulation models, such as the Across Trophic Level System Simulation (ATLSS), to guide and evaluate restoration alternatives. Because fishes are major components of wetland food webs in southern Florida through their roles as prey and predators, they have been recognized as key indicators for measuring restoration success (Loftus et al. 2001; Trexler et al. 2001). Fish life-history traits, such as reproduction, feeding ecology, and age and growth, must be known to build effective models of the responses of individuals, populations, and communities to abiotic and biotic conditions. To contribute to models to be used in restoration, we are studying age and growth and population dynamics of native and non-native fishes in south Florida, including the indigenous yellow bullhead, *Ameiurus natalis*.

The yellow bullhead is the most abundant ictalurid catfish in southern Florida (Loftus and Kushlan 1987; Nelson and Loftus, 1996). The species is omnivorous as well as predatory (Scott and Crossman 1973; Becker 1983; Laerm and Freeman 1986; Sheldon and Meffe 1993; Loftus 2000; Ross 2001), and appears to have variable growth rates depending on environmental conditions (Schoffman 1955; Ross 2001; Boschung and Mayden 2004). Information is scarce for the yellow bullhead throughout its southeastern United States range and, in particular, there is no information on the age and growth of the species in southern Florida. The lack of information on life-history parameters severely limits our ability to predict effects on yellow bullhead population demographics or resiliency in relation to changes in environmental conditions.

Although some catfishes, such as flathead catfish *Pylodictis olivaris* (Nash and Irwin 1999) and channel catfish *Ictalurus punctatus* (Buckmeier et al. 2002), have been aged using both pectoral-fin spines and otoliths, yellow bullheads have only been successfully aged using pectoral-fin spines (see Jenkins et al. 1952; Schoffman 1955; Finnell et al. 1956). There may be biases in using pectoral-fin spines versus otoliths for aging catfishes and bullheads, most notably by underestimating ages of older fish when using spines (Nash and Irwin 1999). Therefore, one of our objectives was to compare pectoral-spine ages against ages obtained using otoliths. Another factor that influences this southernmost population of yellow bullheads in North America is the sub-tropical temperature and rainfall regime. Under those conditions, the fish might deposit more than one annulus each year (Brothers 1979) because of a presumed longer and continuous growing season and other climatic cues. Because yellow bullheads have not been aged in southern Florida, it was critical that we evaluate the aging method to validate the accuracy of age and growth estimates in the southern Florida population.

The goal of our study was to model the age and growth of yellow bullheads from southern Florida and to compare our results qualitatively with growth estimates for yellow bullhead populations from other parts of its geographical range. This comparative review, with inclusion of Florida data, will provide a more complete understanding of the variation and limits of yellow bullhead growth.

Methods

Fish Collections

Yellow bullheads were collected in freshwater boxcut canals along the Atlantic Coastal Ridge of southern Florida, and from freshwater slough wetlands within the southern Everglades (Fig. 1). The first series of yellow bullhead specimens were collected during electrofishing sampling by boat in three canals: Tamiami Canal (C-4), Snake Creek Canal (C-9), and Canal L-

31W. The canals have marginal submerged and emergent vegetation, and limestone sides and bottoms, with sand or marl sediment over the rock. This fieldwork was conducted in April, July, October, and December 2000, and in January 2001. During each sampling period a 1-km segment of canal was sampled (ultimately covering a continuous reach of 4 km within each of the three canals samples). Few yellow bullhead specimens were taken in these deep, open-water canals. We obtained additional specimens during intensive bimonthly sampling for yellow bullheads over the period October 2003 to February 2005 at routine fish-monitoring sites in the Everglades marsh (Fig. 1). During this latter period, yellow bullheads were collected using an airboat-mounted electrofishing system along 100-m transects in spikerush-dominated *Eleocharis* spp. marshes. These marshes are less than 1 m deep, have peat substrates, and are densely vegetated (Loftus and Kushlan 1987). Samples were also collected during 5-min electrofishing bouts in open-water alligator ponds within the wetlands. Alligator holes are the deepest, open water habitats in the natural freshwater Everglades, and are created and maintained by the American alligator *Alligator mississipiensis* (Gunderson and Loftus 1993).

We measured the yellow bullheads for total length (TL) and/or standard length (SL) (± 1 mm) and weighed each individual (± 0.00 g). Total lengths were determined for all fish either through direct measurement or from a predictive regression based on TL as a function of SL (TL = 1.2034[SL] + 2.9847; $r^2 = 0.99$; L. G. Nico, unpublished data). Yellow bullheads were placed on ice and transported to the laboratory where each fish was decapitated posterior to the pectoral girdle and frozen until processed. Fish were later processed for aging structures by removing both pectoral spines at the articulation, which were then cleaned by immersion in hot water to remove skin and tissue and stored dry. The largest pair of otoliths in this species, the lapilli, were removed, cleaned, and stored dry. Sex was not determined in most of the yellow bullheads collected.

Pectoral Spine Processing and Aging

We measured the cleaned pectoral spines for length from mid-central of the ventral process to the distal end of the spine using digital calipers (Ultra-Cal Mark III, Fred V. Fowler Company, Newton, MA). Spine length was regressed on fish TL to determine if the growth of the aging structure was correlated with fish growth. We then prepared the pectoral spines for aging by modifying methods given in Crumpton et al. (1984) and Nash and Irwin (1999). Specifically, the left pectoral spine was mounted on a fully frosted slide using adhesive (Superglue, Loctite Corporation, Rocky Hill, CT). Spines were then cross-sectioned in ~0.3-mm increments using a Buehler Isomet 1000 Variable-Speed Saw (Buehler®, IL) with a diamond-wafering blade (7.6 cm diameter X 0.15 mm wide). Sectioning started at the ventral process, continued past the articulating process, and was completed when sections distal to the basal recess were obtained (~5-8 sections depending on the size of the spine). This allowed proximal sections to be examined for the appearance of annuli that may be occluded by the lumen of sections more distal to the articulating process (Buckmeier et al. 2002). We washed the sections in distilled water, air-dried them, and permanently mounted them on labeled slides using Flo-Texx® (Lerner Laboratories, New Haven, CT). We viewed the spines using a Leica MZ12 stereomicroscope with reflected light while the spines were submerged under water on a black background. We also examined the spines with transmitted light (7-50X), using a narrowband green filter (wavelength 550 nm) to enhance contrast. Annuli were discernible as alternating opaque and translucent zones. We enumerated the translucent zones for the age estimates. We also noted the condition of the growth on the edge of each of the aging structures as to the zone type (translucent versus opaque), and the amount of growth based on: a) zone forming on edge; b) zone of growth equivalent to <⅓ of the last completed annulus; c) zone of growth equivalent to ⅓ - ⅔ of the last completed annulus; and d) zone of growth > ⅔ of the last completed annulus. This was necessary so that we could obtain comparative age estimates between readers and between structures (see comparative aging).

Otolith Processing and Aging

We measured the left lapillar otolith for length and width (± 0.01 mm) using a digital image analysis system (MOTIC Instruments Inc., Richmond, British Columbia). Each pair of otoliths was weighed (± 0.0001 g). If either of the two otoliths from an individual fish was broken, the mass was determined for the whole otolith and multiplied by two. Regressions were determined for otolith length and width as a function of fish TL, and otolith mass as a function of fish mass.

Information on the methodology for aging yellow bullhead using otoliths was not available, so we tried various methods of processing the otoliths initially. The process of mounting and sanding one half of the otolith to the core, although successful for 4-year old channel catfish (Buckmeier et al. 2002), did not produce visible annuli in our yellow bullhead otoliths. Nor could we consistently find annuli in thin longitudinal-sections produced by sanding on both sides of the core of the otolith, as outlined by Crumpton et al. (1984) for brown bullhead *Ameiurus nebulosus*, white catfish *Ameiurus catus*, and channel catfish. We found that thin cross-sectioning of the otolith, as has been used successfully to age other ictalurid species (Crumpton et al. 1984; Nash and Irwin 1999), resulted in visible annuli more consistently and we therefore chose this method to age our yellow bullheads. We mounted the left otolith of each pair on a fully frosted glass slide using Crystalbond cement (Buehler, Lake Bluff, IL), then cross-sectioned it through the core (Fig. 2) using a Buehler Isomet 1000 Variable-Speed Saw fit with a diamond wafering blade (7.6 cm diameter X 0.15 mm wide) at 275 rpm. One thin section of ~0.3 mm width was obtained from each otolith. We secured this section to a partially frosted slide with Crystalbond, sanded it to the core using 400-grit and 600-grit wet/dry carborundum sandpaper, then polished it with aluminum oxide powder. Sometimes it was necessary to flip a section and sand/polish the opposite side to expose the core. The section was then covered either with immersion oil or water and viewed against a black background under reflected light (20-75X) using fiber optic light pipes. To be consistent with our procedure of annuli enumeration in the pectoral-spine sections, we also enumerated annuli in otolith sections by counting the translucent zones. Similarly, the growth observed at the edge of the aging structure was also recorded as to zone type and amount. Because of the initial difficulty in recognizing discrete annuli in cross-sectioned otoliths, we aged a random, age-stratified (based on pectoral-spine ages) subsample of 55 fish for comparison to pectoral-spine ages.

Comparative Aging

All aging structures were read by a primary reader (DJM), and an age-stratified subset was read independently by a second reader (DCP); both readers had previous experience in aging both fin rays/spines and otoliths. If the age estimates disagreed between the readers, the structure was aged independently by the primary reader (DJM) again. We used the age estimate in further analysis if at least two out of the three age estimates agreed. During aging, the readers did not know the size of the fish or the previous age estimates from either reader or aging structure. Comparative age estimates between readers and between structures were obtained by assigning an age (annulus) class to each individual fish based on the aging convention of using a birthdate of January 1st (Chilton and Beamish 1982), with the condition of the growth zone at the margin of the aging structure taken into consideration. This was necessary to be able to assign comparative age (annuli) classes to individual fish. For example, a fish collected after January 1st, and aged by one reader as having three translucent zones, with the ultimate translucent zone forming on the edge, would be assigned to the same annulus class (3) as the same fish aged by a second reader that was recorded as having two translucent zones and a large amount of opaque zone on the edge (i.e., it would be expected that the third translucent zone was late in forming but would form imminently). This method of assigning age (annuli) classes ensured that fish born into the same cohort would be assigned into the same age (annuli) class, despite potential differences in their growth rates (Murie and Parkyn 2005).

Aging precision between readers was estimated by calculating: 1) the percent agreement between two independent readers (number of fish aged by Reader1 and Reader2 that do not differ, expressed as a percentage of all fish aged by both readers); 2) the average percent error (APE) (Chilton and Beamish 1982); 3) the coefficient of variation (CV) (Kimura and Lyons 1991); and 4) the concordance correlation coefficient (ρ_c) (Lin 1989, 2000). Percent agreement is not "age independent", and as such is only a general indication of aging precision. The APE and CV methods of estimating aging precision are considered to be "age independent" and therefore provide better precision estimates over the range of ages observed for the species. The lower the APE or CV value, the greater the precision. While Lin's concordance correlation coefficient has been considered superior to comparisons using CV, paired t-tests, and regression, and hence was a more powerful and robust method of assessing reproducibility (precision) of aging (Lin 1989, 2000), CV was included for the purposes of comparability with other studies. As with a regression coefficient, ρ_c values range from 0 (no reproducibility) to 1.0 (perfect reproducibility). We also assessed comparative aging of pectoral spines versus sectioned otoliths using plots of paired age estimates (Campana et al. 1995; Nash and Irwin 1999). A line denoting the ideal 1:1 relationship was used for reference.

Validation of Aging Method with Chemical Tagging

While collections of yellow bullheads were attempted bimonthly in 2003, some months produced few or no fish when populations dispersed due to hydrological conditions in the wetlands. Consequently, in 2004, we relied on a chemical tagging method to validate the aging method instead of using marginal-increment analysis (i.e., measuring the growth increment each month over a 12-month period) (Murie and Parkyn 2005). To conduct this analysis, 22 yellow bullheads were collected from Shark River Slough on 23 February 2004 and acclimated to captivity in an outdoor mesocosm facility in Everglades National Park, Florida. Two or three fish were housed in each of nine 750-L plastic tanks fitted with airstones and sponge filters, in which they were exposed to natural light/dark regimes. Well water was used to make weekly water changes in the tanks, when the filters were also rinsed. Fish were fed ~1-2% of wet body mass per day with commercial catfish pellets, supplemented with frozen bait fish and live earthworms. After one week in captivity, fish were weighed, measured, anesthetized with tricaine methanesulphate (MS-222), and injected interperitoneally with oxytetracycline (OTC) dissolved in distilled water at a concentration of 50 mg kg^{-1} body weight (Murie and Parkyn 2005). Fish were also injected with a PIT tag for identification of individuals. Two yellow bullheads were sacrificed 15 days subsequent to injection to verify the uptake of fluorescing

OTC in the surface layer of their otoliths and pectoral spines. The injected and tagged yellow bullheads were then maintained in the outdoor tanks for a period of 12 months. At the end of this period, survivors were sacrificed to recover their otoliths and pectoral spines.

Significant mortality of the captive yellow bullheads occurred over the course of the year that they were held (March 2004-2005) because a drought and several hurricanes interrupted water supply and power to the tanks, affecting water quality. The three yellow bullheads remaining at the completion of the 12-month period were sacrificed on 7 March 2005. The fish were measured, weighed, and their lapilli and pectoral spines extracted. Pectoral spines and otoliths were wrapped in aluminum foil to prevent exposure to white light and were individually identified via the fish's PIT tag. Spines and otoliths were processed identically to all others except that the exposure of spine or otolith sections to white light was reduced by storing them in total darkness to prevent the OTC mark from fading (Beamish et al. 1983).

To evaluate the deposition of annuli relative to the OTC mark in the pectoral spines and otoliths, two images were taken of each of the sections under identical magnification and position: one image under exposure to white light and one image when exposed to UV light. To facilitate comparison of the OTC-mark and the annuli, the two images were then superimposed on one another using Adobe Photoshop (Adobe Systems Incorporated, Version 9.0) (Murie and Parkyn 2005). This composite image was then evaluated for the presence and number of annuli that were observed after the OTC mark in the section. The number of complete annuli, or pairs of translucent and opaque zones formed after the OTC-mark, indicated the number of annuli formed in a 12-month period.

Validation of First Annulus through Length Frequencies

We also used a length-frequency histogram to validate the presence and position of the first annulus for aging. Based on a spawning period for yellow bullhead that ranged from April through June (Harlan and Speaker 1956; Scott and Crossman 1973; Boschung and Mayden 2004), it was expected that fish captured in the spring (just prior to the potential spawning period) that contributed to the first mode in the length-frequency distribution would be comprised of yellow bullheads born the previous year that should be almost 1 year old at the time of their capture. The ages of these fish in the first mode were therefore compared to the expectation that they should have been correctly assigned to age (annuli) class 1.

Growth of Yellow Bullhead

The wet mass of yellow bullheads collected from the marshes was regressed as a function of their TL to determine the length-related increase in mass. Sizes of aging structures were regressed on fish size to determine the relationships between the growth of the aging structure and the growth of the fish, including: 1) pectoral spine length as a function of fish TL; 2) otolith length and width as a function of fish TL; and 3) the mass of the pair of otoliths as a function of fish mass.

The von Bertalanffy growth equation (Ricker 1975) was used to model fish TL at age for yellow bullheads using non-linear regression analysis (SAS Institute Inc., 1999):

$$L_t = L_\infty\,(1\text{-e}^{-k\,[t - t_0]})$$

where L_t is the predicted TL (mm) at time t (age, in years), L_∞ is the estimate of average maximum length (asymptotic length) (mm), k is the Brody growth coefficient, and t_o is the theoretical age (years) when fish length would be 0. For growth analysis, individual fish were assigned an absolute age based on a spawn date of 1 June, which was an assumed median value for reported spawning in yellow bullhead that ranged from April through June. This period of the year also corresponded to the completion of the translucent zone in the annulus of yellow bullhead. Absolute ages therefore allowed correction for the time of year that the fish were sampled without the need for back-calculation.

Results

Fish Collections

In total, 144 yellow bullheads were collected from southern Florida (Fig. 1). Few (n = 8) yellow bullheads were collected from the canals in 2000 and 2001. Most (n = 136) fish were collected from marshes and alligator ponds from 2003-2005, with the majority of these collected during April 2004 (n = 97); fewer fish were collected in February 2004 (n = 3), August 2004 (n = 5) and October 2004 (n = 7) 2004, October 2003 (n = 6), and February 2005 (n = 18). Yellow bullheads ranged from 97 to 298 mm TL (Fig. 3) with the majority (>90%) between 100 and 230 mm TL.

Pectoral spine processing and aging

In general, pectoral spine sections were clearer to read using reflected light with focused fiberoptic-light pipes rather than with transmitted light. For transmitted light to be effective, the sections needed to be very thin (<0.3 mm), either through sectioning or through sanding. However, such thin sections increased the difficulty of enumerating annuli along on the edge of the otoliths in older fish. We obtained ages using sections through the articulating process distal to the basal recess. Sections taken above the basal recess consistently showed fewer annuli, both in the central-lumen area and on the edge of the structure; therefore these were not used for aging.

Pectoral spines of yellow bullhead showed distinct translucent and opaque zones (Fig. 4a, b), corresponding to annuli ranging from 1 to 12. The oldest fish aged was estimated to have 12 annuli and was 220 mm TL and 132 g, whereas the largest fish aged was 298 mm TL, 287 g, and had 5 annuli. Yellow bullheads collected in February were evenly divided between having a translucent (52%) versus opaque (48%) zone at the edge of the pectoral spine. The majority of fish collected in April had a translucent zone at the edge (87%), whereas 100% of fish in August and 85% of fish in October had an opaque zone at the edge.

Otolith processing and aging

Similar to pectoral-spine sections, cross-sections of otoliths of yellow bullhead appeared to be easier to age using reflected light instead of transmitted light. Some otoliths had relatively well-demarcated opaque and translucent zones (Fig. 5a, b); however, most otoliths were difficult to age regardless of preparation method or lighting modification. In particular, unlike pectoral spine sections, annuli in otolith sections could not be followed around the complete structure and only were observed in partial sections of the otolith. In particular, annuli within the area of the macular hump of the otolith were completely indistinct (Fig. 5b). This made it difficult to ascertain whether an annulus was complete or a false annulus (i.e., a check). Differentiating between opaque and translucent zones near the edge of the section also became more difficult as the size of the otolith and age of the fish increased.

Comparative aging

In total, 65% of two independent age readings (n = 75) of pectoral-spine sections of yellow bullhead were in 100% agreement, with 27% differing in ±1 yr (Fig. 6a); all other readings differed by ±2 years (8%). Other measures of precision also indicated relatively low variability with CV = 8.6% and APE = 6.1%. Lin's concordance correlation was high with $\rho_c = 0.95$, indicating that the two independent readers were substantially similar, and that any differences between the two independent readings did not increase with age in yellow bullhead.

Relative to the initial 55-fish subsample, otolith ages estimated by two independent readers differed considerably. Only 25% were in 100% agreement, with 61% within ±1 year (Fig. 6b); one otolith could not be aged at all. Precision estimates for reading otolith cross-sections were low, with CV = 23.7% and APE = 16.8%. Lin's concordance correlation was also relatively low with $\rho_c = 0.503$, indicating that the two independent readers were not able to consistently estimate similar ages for the same individual yellow bullhead.

Similarly, precision estimates between aging structures were also low, with CV = 29.1% and APE = 20.6%. In relation to pectoral-spine ages, sectioned otoliths appeared to overestimate the age of younger yellow bullheads (<5 yrs by spine ages) and underestimate the age of older bullheads (>5 yrs by spine ages) (Fig. 6c). Based on the lack of precision in using otoliths to age yellow bullhead, and lack of comparison with pectoral-spine ages, age estimates derived from otoliths were only used for comparative purposes and were not used in further growth analyses.

Validation of aging method through chemical tagging

Three yellow bullhead initially injected with OTC on 9 March 2004 were sacrificed on 17 or 24 March 2004. Our examination of their calcium-bearing structures for the presence of fluorescing

OTC demonstrated that they had incorporated the OTC. Of the remaining 19 OTC-injected yellow bullhead, only three survived 12 months. We sacrificed these on 7 March 2005 and examined sections of their pectoral spines and otoliths for a fluorescent mark. One yellow bullhead that had not grown during the previous 12 months showed no evidence of OTC-fluorescence in its pectoral spine sections. The other two yellow bullheads that had grown during the 12 months in captivity (both ~13-14 mm growth) showed deposition of the OTC-mark in their pectoral spines, followed by one translucent and one opaque zone (Fig. 7). Based on chemical tagging, it was therefore apparent that only one complete annulus was deposited in a 12-month period in pectoral spines of yellow bullhead from south Florida. Validation of the otolith method of aging yellow bullhead could not be completed due to the difficulty in enumerating discrete translucent and opaque zones in otolith cross-sections.

Validation of first annulus through length frequencies

Yellow bullheads collected in February and April that comprised the first mode in the length frequency distribution were presumed to be fish that were close to their first birthday based on spawning date. These fish ranged in size from >95 to ~120 mm TL (Fig. 3). Of the 15 yellow bullheads in this length range, 80% (n = 12) had been assigned an age of 1 and 20% (n = 3) had been assigned an age of 2. Although a small sample size, this indicated that the first annulus had been recognized and interpreted correctly in the age assignments for the majority of the age estimates.

Growth of yellow bullhead

Pectoral-spine length was related to fish TL ($R^2 = 0.90$, $n = 95$), with larger yellow bullheads having longer pectoral spines (Fig. 8a). Pectoral spine growth was not linear, however, and the rate of growth decreased as fish TL increased. Similarly, both otolith length and otolith width were related to fish TL ($R^2 = 0.93$ and 0.83, respectively), but the rate of growth in the otoliths was non-linear with an increase in fish size (Fig. 8b). The mass of the otolith pair as a function of fish mass was also non-linear ($R^2 = 0.88$) (Fig. 8c). The mass of yellow bullheads was also significantly related to their TL ($R^2 = 0.97$, $P < 0.0001$), and was close to being isometric, with a power exponent of 2.9 (Fig. 9).

Based on aging precision, only ages obtained from pectoral spines were used to model the growth of yellow bullhead. Total length of yellow bullheads as a function of absolute age (Fig. 10) was adequately modeled using a von Bertalanffy growth equation, as indicated by model convergence, $P < 0.0001$, and general fit and was described by:

$$L_t = 213.8\ (1\text{-}e^{-0.4288[t + 1.0755]})$$

where L_t is the predicted TL (mm) at time t (age, in years). In general, yellow bullheads increased in length quickly in their first 3 years, after which their growth rate decreased substantially. After age 5 yellow bullhead growth attained a plateau that was similar to the asymptotic length estimated by the von Bertalanffy model (214 mm). Most notably, growth of yellow bullheads was extremely variable within and between all age classes. This variability was also notable in the relationship between pectoral-spine age and spine length ($R^2 = 0.48$) (Fig. 11), which indicated that fish age could not be reliably predicted from spine length.

Discussion

Aging yellow bullhead using pectoral-spine sections was relatively precise, as long as sections distal to the basal recess were used for age assignment to prevent under-aging of individuals. Levels of aging precision obtained for yellow bullheads while using pectoral spines (CV = 8.6%, APE = 6.1%) was comparable to that obtained in a majority of studies that aged fish using otoliths, where the median value for the CV was 7.6% and for D (equivalent to average percent error when otoliths are aged twice) was 5.5% (Campana 2001). In addition, our study provided the first validation of pectoral-spine aging in yellow bullhead, demonstrating that one complete annulus (one opaque zone and one translucent zone) was deposited each year. Although not conclusive for all yellow bullheads injected with OTC because of the small sample size, the validation study was successful in demonstrating that a 7-year old yellow bullhead deposited one complete annulus in a 12-month period (Fig. 7). It would be beneficial to conduct further validation studies that encompass the range of ages observed in yellow bullhead from south Florida.

Precision in aging yellow bullheads using sections from otoliths was unacceptably low (CV = 23.7%, APE = 16.8%) relative to using pectoral spines. Otoliths in ictalurids can be problematic for aging because the sagittae are not the largest otolith pair as they are in the majority of other fishes. In yellow bullheads, as in channel catfish (Barbour and Kollmar 2003) and armoured catfish *Hoplosternum littorale* (Ponton et al. 2001), the lapilli are larger than the asterici and wider than the sagittae, which are thin and fragile compared to the lapilli (pers. obs.). Most aging of teleosts using otoliths, however, has developed around using the sagittal otolith for aging because of its usually larger size and relatively clearly demarcated growth zones. In yellow bullheads, difficulties in enumerating annuli were exacerbated by the morphology of the lapilli. Unlike the sagittae and the asterisci, the lapilli are orientated horizontally in the utriculus, are dorso-ventrally flattened (instead of laterally flattened as in the sagittae and asterisci), and rest with the ventral surface in contact with the macular bed (Assis 2005). The lapilli therefore have a distinguishing, relatively large, macular hump (*gibbus maculae*) on their ventral surface (Assis 2005), and it is this area of the lapillus that was unreadable in cross-section. The macular hump created a discontinuity in the ventral part of the otolith section, making it impossible to trace annuli around the entire section. This undoubtedly led to problems in recognizing annuli versus false annuli or "checks" in the cross-sections. The South American pimelodid catfish *Hypophthalmus edentatus* have been successfully aged using asterisci, with CVs ranging from 3.5-18.4% depending on the age class (Ambrósio et al. 2003). Crumpton et al. (1984) were unsuccessful using otoliths to age brown bullhead, channel catfish, and white catfish. However, more recently, Nash and Irwin (1999) successfully aged flathead catfish using sagittal otoliths with 85% between-reader agreement. Testing a variety of modifications in the aging method, Buckmeier et al. (2002) aged 1-4 year old channel catfish using sectioned sagittae also with 85% between-reader agreement. Their method could be considered to have been validated for 2-4 year old catfish since the fish used in their study were initially obtained from an aquaculture facility, grown-out for a period of years, and were therefore of known age. Although yellow bullhead lapilli used in our study were small (2-4 mm length) but similar in shape to the lapillar (*sic* sagittal) otolith illustrated in Buckmeier et al. (2002), the majority of cross-sections produced for aging yellow bullhead using the same methods were not adequate. Without further development of otolith aging in yellow bullhead, pectoral spines would continue to be the aging structure of preference. An added advantage to using a pectoral spine for aging is that, unlike the use of otoliths, the fish would not have to be sacrificed to obtain a spine if it was desired to return the fish alive to the water.

Yellow bullheads in south Florida grew relatively slower and were smaller at age compared to other populations throughout the U.S. (Fig. 12). Growth in yellow bullheads was similar in Florida, Oklahoma (Jenkins et al. 1952; Finnell et al. 1956), and Washington (Wydoski and Whitney 1979) for their first 3 years, after which fish from Oklahoma and Washington achieved much larger size with increasing age. Although based on very limited data (n = 5 fish aged), only yellow bullheads specifically from Rowland Lake, Washington, were observed to have similarly slow growth in ages 4-6 (Jackson and Caromile 2000) as we observed in southern Florida. Yellow bullheads have been previously documented as having variable growth, with fish from a tributary of the Mississippi River also being much smaller at ages 2-3 (<200 mm SL, or ~ 244 mm TL) compared to yellow bullhead from Reelfoot Lake in Tennessee (Ross 2001). The growth rate reported for yellow bullhead populating Reelfoot Lake was much greater than that of yellow bullheads studied in other regions, including south Florida. For example, Reelfoot Lake bullheads that were 2-5 yr old were more than double the size of yellow bullhead of the same age from other populations (Fig. 12), and they also attained unusually large sizes, reaching a maximum of 470 mm TL (corresponding to ~1.0-1.4 kg) (Carlander 1969). Yellow bullhead in regions other than Tennessee, however, have also been documented to attain relatively larger sizes compared to south Florida. According to the International Game Fish Association, the world all-tackle angling record for yellow bullhead is 1.92 kg (4.25 lb) for a fish caught in Mormon Lake, Arizona, in 1984, although Sternberg (1987) reported an Illinois state record catch of 2.4 kg (5 lb 4 oz). In addition to their slow growth and small size, yellow bullheads in south Florida also attained a much older maximum age (12 years) (Fig. 12) than the previously reported 7 years (Scott and Crossman 1973). Given that only a moderate number of yellow bullhead from south Florida were aged in our study (n =144), it is probable that even older fish might be detected with increased sampling in south Florida.

Assuming yellow bullheads from Tennessee do not differ genetically from yellow bullheads in south Florida, then we would expect that fish from south Florida should have a similar potential for growth as fish from Tennessee. The fact that they are smaller indicates that they are limited in their growth by biotic and abiotic environmental influences. This variability in growth might be partially explained by density-dependent processes, such as relative abundance and availability of prey on a per-capita basis. Ross (2001) has observed that yellow bullhead exhibit stunted growth when living in overpopulated ponds, which would support a food limitation hypothesis. Low densities and small maximum sizes are common among aquatic animals in southern Florida, and are thought to be related to the oligotrophic nature of aquatic systems there (Turner et al. 1999; Rice et al. 2005). Limitations in available prey resources could also result from intra- and interspecific competition or interference with other predators, such as wading birds in the area. Loftus (2000) found that feeding by many fishes in the Everglades is reduced during the spring dry season, when bullheads and other fishes are concentrated in dry-season refuges like alligator holes. Densities of large fishes there are very high, prey are depleted, and water quality poor (Loftus and Kushlan 1987; Nelson and Loftus 1996). Growth of fishes would be slow under those conditions.

In general, any factors that influence the density of yellow bullhead may contribute to density-dependent growth, including habitat quality and quantity. Yellow bullhead have been observed to be most common in shallow, relatively clear-water portions of lakes, ponds and streams with heavy vegetation (Scott and Crossman 1973; Trautman 1981; Laerm and Freeman

1986; Loftus and Kushlan 1987). They also occur in relatively slow-moving waters rather than in faster-flowing rivers (Scott and Crossman 1973; Laerm and Freeman 1986), and Jackson (1996) observed that yellow bullhead were, while common in oxbow lakes, rare in the main channel of the Yockanookany River, Mississippi. Similarly, in south Florida, few yellow bullhead were collected in the deeper, box-cut canals with patchy vegetation compared to the densely vegetated, shallow, marshes.

While the tolerance of yellow bullhead to general habitat perturbation and pollution may be debatable, it is apparent that they have a negative response to loss of vegetation. Trautman (1957) observed a decrease in the abundance of yellow bullhead with aquatic vegetation loss and increased turbidity in an Ohio lake, as well as a general inverse relationship between the abundance of yellow, as well as black *Ameiurus melas* and brown bullheads, with habitat degradation (Trautman 1981). Scott and Crossman (1973) have also documented that removal of suitable habitat, such as vegetation and logs, leads to a decrease in the number of yellow bullheads, although less so than black or brown bullheads. Koonce et al. (1996) ascribed the change in yellow bullhead occurrence in Lake Erie from common in the pre-1800 period to rare in this decade as being due to habitat degradation. Simon (1991), however, considered yellow bullhead to be highly tolerant to declines in stream quality. In south Florida, this species is one of the most tolerant fishes able to survive crowding and poor water quality in isolated ponds in the dry season, when water temperatures are high, ammonia levels are high, and dissolved oxygen levels are very low (Kushlan 1974; Loftus and Kushlan 1987).

Acknowledgments

Many individuals provided help by participating in field work, maintaining captive live bullheads, processing the aging structures, and facilitating the research. For their help we extend special thanks to Jeff Herod, Kelly Johnson, Mary Brown, Eddie Leonard, and Steve Crawford. Funding was provided by G. Ronnie Best through the U.S. Geological Survey's South Florida Place-based Science Program to enable the completion of the study of south Florida fish life histories. We also appreciate assistance in the field and at the mesocosms provided by Amy Benson, John Curnutt, Chis Diel, Dan Doroshef, Brad and Krissy Dunker, Nick Flavin, Phil George, Gary Hill, Robert Lewis, Stan Howarter, Michael Perry, Eric Rolla, Rita Taylor, Michael Perry, and Susan Vincent. Our cooperators at Florida International University made this work possible by providing specimens from their wetland monitoring; in particular, we thank Dr. Joel Trexler and Brooke Shambling. Administrative assistance was provided by Christine Fadeley and Dieter Elswick of the U.S. Geological Survey-Florida Integrated Science Center, and by personnel at the Department of Fisheries and Aquatic Sciences, IFAS, University of Florida.

References

Ambrósio, A. M., L. C. Gomes, and A. A. Agostinho. 2003. Age and growth of *Hypophthalmus edentatus* (Spix), (Siluriformes, Hypophthalmidae) in the Itaipu Reservoir, Paraná, Brazil. Revista Brasileira de Zoologia 20: 183-190.

Assis, C. A. 2005. The utricular otoliths, lapilli, of teleosts: their morphology and relevance for species identification and systematics studies. Scientia Marina 69: 259-273.

Barbour, A., and R. Kollmar. 2003. Catfish otoliths. Imaging Technology Group: Image of the Week-14 October 2003. http://www.itg.uiuc.edu/exhibits/iotw/2003-10-14/

Beamish, R.J., G.A. McFarlane, and D.E. Chilton. 1983. Use of oxytetracycline and other methods to validate a method of age determination for sablefish. Pages 95-116 *in* B. R.

Melteff, editor. Proceedings of the International Sablefish Symposium. Alaska Sea Grant Report 83-8.

Becker, G. C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison.

Boschung, H. T., Jr., and R. L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington.

Brothers, E. B. 1979. Age and growth studies on tropical fishes. Pages 162-179 *in* S. B. Saila and P. M. Roedel, editors. Stock assessment for tropical small-scale fisheries. Proceedings of an international Workshop. University of Rhode Island, Rhode Island.

Buckmeier, D. L., E. R. Irwin, R. K Betsill, and J. A. Prentice. 2002. Validity of otoliths and pectoral spines for estimating ages of channel catfish. North American Journal of Fisheries Management 22: 934-942.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59 (2): 197-242.

Campana, S. E., M. C. Annand, and J. I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 124: 131-138.

Carlander, K. D. 1969. Handbook of freshwater fishery biology: Volume one. The Iowa State University Press, Ames, Iowa.

Chilton, D. E., and R. J. Beamish. 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. Canadian Special Publication of Fisheries and Aquatic Sciences 60.

Crumpton, J. E., M. M. Hale, and D. J. Renfro. 1984. Aging of three species of Florida catfish utilizing three pectoral spine sites and otoliths. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 38: 335-341.

Finnell, J. C., R. M. Jenkins, and G. E. Hall. 1956. The fishery resources of the Little River system, McCurtain County, Oklahoma. Oklahoma Fisheries Research Laboratory Report 55.

Gunderson, L. H. and W. F. Loftus. 1993. The Everglades. Pages 199-255 *in* W. H. Martin, S. G. Boyce, and A. C. Echternacht, editors. Biodiversity of the southeastern United States. John Wiley and Sons, New York.

Harlan, J. R., and E. B. Speaker. 1956. Iowa fish and fishing, 3rd edition. State Conservation Committee, State of Iowa.

Jackson, C., and S. Caromile. 2000. An assessment of the warmwater fish community in Rowland Lake, September 1999. Washington Department of Fish and Wildlife, Olympia, Washington.

Jackson, J. R. 1996. Polychlorinated biphenyl (PCB) characterization and bioaccumulation pathways in channel catfish and yellow bullhead from the Yockanookany River, Mississippi. Ph.D. Dissertation, Mississippi State University, Starkville, Mississippi.

Jenkins, R. M., E. M. Leonard, and G. E. Hall. 1952. An investigation of the fisheries resources of the Illinois River and pre-impoundment study of Tenkiller Reservoir, Oklahoma. Oklahoma Fisheries Research Laboratory Report 26.

Kimura, D. K., and J. J. Lyons. 1991. Between-reader bias and variability in the age-determination process. Fisheries Bulletin 89: 53-60.

Koonce, J. F., W.-D. N. Busch, and T. Czapla. 1996. Restoration of Lake Erie: contribution of water quality and natural resource management. Canadian Journal of Fisheries and Aquatic Sciences 53 (Supplement 1): 105-112.

Kushlan, J. A. 1974. Effects of a natural fish kill on the water quality, plankton, and fish population of a pond in the Big Cypress Swamp, Florida. Transactions of the American Fisheries Society 103: 235-243.

Laerm, J., and B. J. Freeman. 1986. Fishes of the Okefenokee Swamp. The University of Georgia Press, Athens.

Lin, L. I-K. 2000. A note on the concordance correlation coefficient. Biometrics 56: 324-325.

Lin, L. I-K. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45: 255-268.

Loftus, W. F. 2000. Accumulation and fate of mercury in an Everglades aquatic food web. Ph.D. Dissertation, Florida International University, Miami, Florida.

Loftus, W. F., L. Nico, J. Trexler, J. Herod, and T. Connert. 2001. Influence of hydrology on life-history parameters of common freshwater fishes from southern Florida. Pages 119-120 *in* U.S. Geological Survey Program on the South Florida Ecosystem: 2000 Proceedings. Presentations made at the Greater Everglades Ecosystem Restoration (GEER) Conference, December 11-15, 2000. Naples, Florida.

Loftus, W. F., and J. A. Kushlan. 1987. Freshwater fishes of southern Florida. Bulletin of the Florida State Museum, Biological Sciences 31: 147-344.

Murie, D. J., and D. C. Parkyn. 2005. Age and growth of white grunt (*Haemulon plumieri*): a comparison of two populations along the Florida west coast. Bulletin of Marine Science 76(1): 73-93.

Nash, M. K., and E. R. Irwin. 1999. Use of otoliths versus pectoral spines for aging adult flathead catfish. American Fisheries Society Symposium 24: 309-36.

Nelson, C. M., and W. F. Loftus. 1996. Effects of high-water conditions on fish communities in Everglades alligator ponds. Pages 89-101 *in* T. V. Armentano, editor. Proceedings of the 1996 conference: ecological assessment of the 1994-1995 high water conditions in the southern Everglades. Florida International University, Miami, FL, 22-23 August 1996.

Ponton, D., J. H. Mol, and J. Panfili. 2001. Use of otolith microincrements for estimating the age and growth of young armoured catfish *Hoplosternum littorale*. Journal of Fish Biology 58: 1274-1285.

Rice, K. G., F. J. Mazzotti, and L. A. Brandt. 2005. Status of the American alligator (*Alligator mississippiensis*). Pages 145-153 *in* W. E. Meshaka and K. J. Babbitt, editors. Status and sonservation of Florida amphibians and reptiles. Krieger Publishing., Melbourne, Florida.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 382p.

Ross, S. T. 2001. Inland fishes of Mississippi. University Press of Mississippi, Jackson, Mississippi.

SAS Institute Inc. 1999. SAS for Windows, release 8.01. SAS Institute, Cary, North Carolina.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Canadian Fisheries Research Board Bulletin 184: 595-598.

Sheldon, A. L., and G. K. Meffe. 1993. Multivariate analysis of feeding relationships of fishes in blackwater streams. Environmental Biology of Fishes 37: 161-171.

Schoffman, R. J. 1955. Age and rate of growth of the yellow bullhead in Reelfoot Lake, Tennessee. Journal of the Tennessee Academy of Science 30:4-7.

Simon, T. P. 1991. Development of index of biotic integrity expectations for the ecoregions of Indiana. I. Central corn belt plain. U. S. Environmental Protection Agency, Region V, Environmental Sciences Division Monitoring and Quality Assurance Branch: Ambient Monitoring Section. EPA 905/9-91-025. Chicago, Illinois.

Sternberg, D. 1987. Freshwater game fish in North America. Prentice Hall Press, New York.

Trautman, M. B. 1957. The fishes of Ohio. Ohio State University Press, Columbus.

Trautman, M. B. 1981. The fishes of Ohio, revised edition. Ohio State University Press, Columbus.

Trexler, J. C., W. F. Loftus, F. Jordan, J. H. Chick, K. L. Kandl, T. C. McElroy, and O. L. Bass, Jr. 2001. Ecological scale and its implications for freshwater fishes in the Florida Everglades. Pages 153-181 *in* J. W. Porter and K. G. Porter, editors. The Everglades, Florida Bay, and coral reefs of the Florida Keys: an ecosystem sourcebook. CRC Press, Boca Raton, Florida.

Turner, A. M., J. C. Trexler, F. Jordan, S. J. Slack, P. Geddes, J. Chick, and W. F. Loftus. 1999. Targeting ecosystem features for conservation: standing crops in the Florida Everglades. Conservation Biology 13: 898-911.

Wydoski, R. S., and R. R. Whitney. 1979. Inland fishes of Washington. University of Washington Press, Seattle.

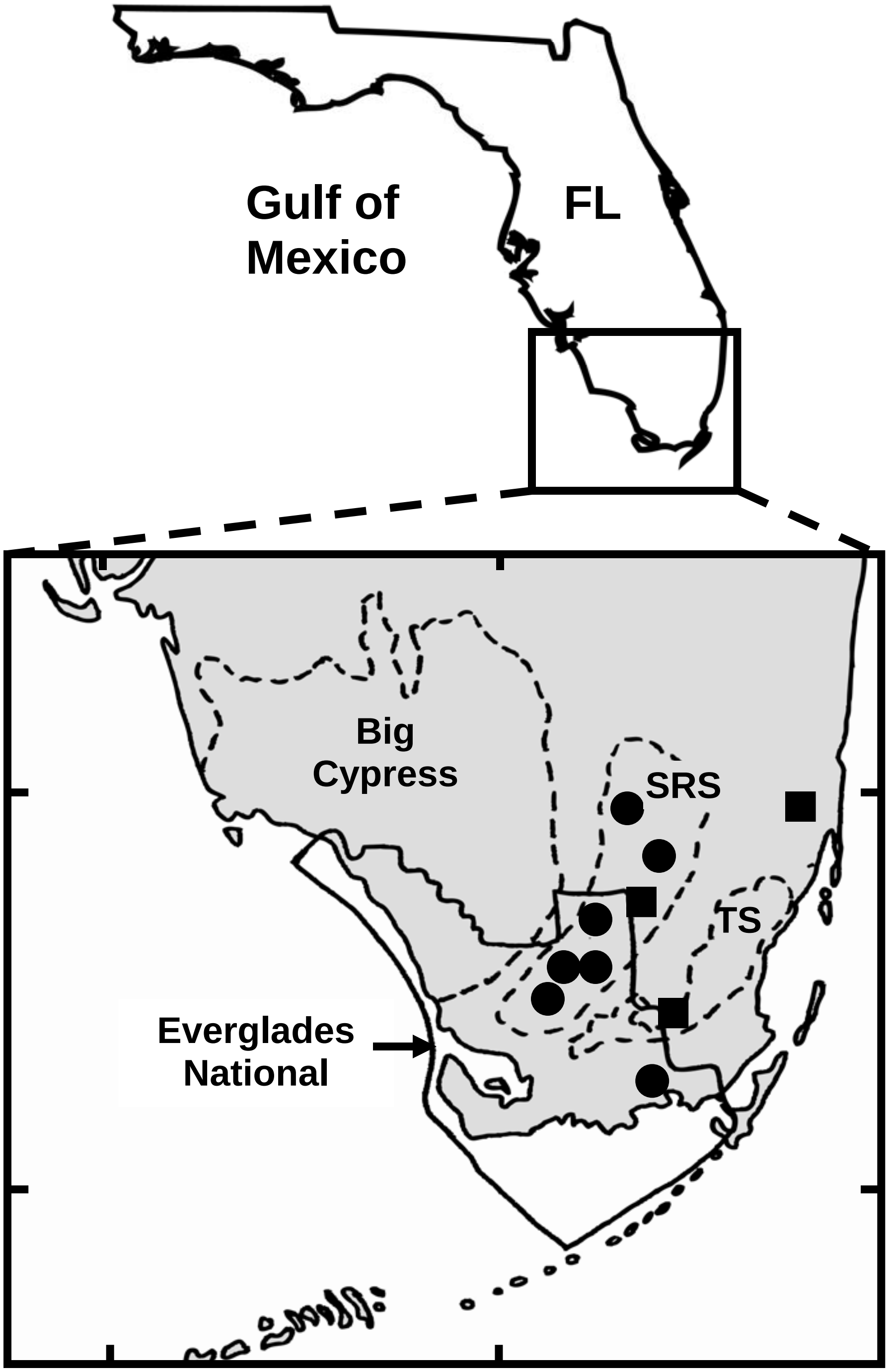

FIGURE 1.─ Sampling sites for yellow bullhead in marshes and alligator holes (filled circles) and canals (filled squares) in southern Florida, where SRS = Shark River Slough and TS = Taylor Slough.

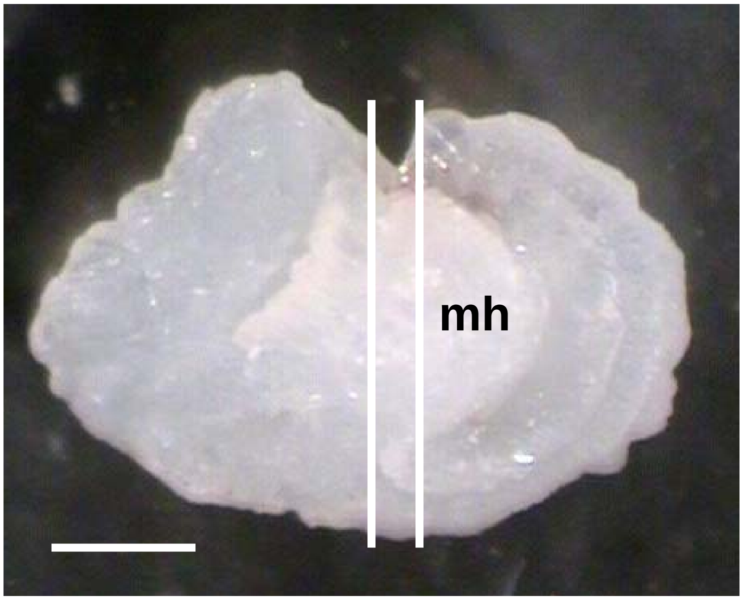

FIGURE 2.─ Ventral surface of the lapillus showing the location of the cross-section used for aging yellow bullhead. The macular hump (mh) on the ventral surface of the lapillus is also notable. Scale bar = 1 mm.

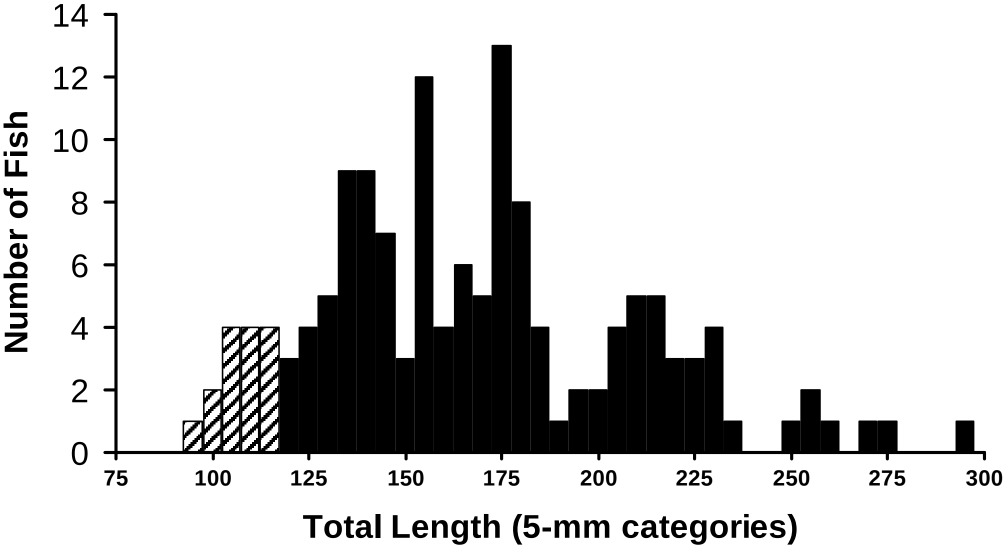

FIGURE 3.—Length frequency distribution for yellow bullheads collected from south Florida. Fish in the first length mode (hatched bars) were collected in February and April and therefore should be approaching 1-yr of age.

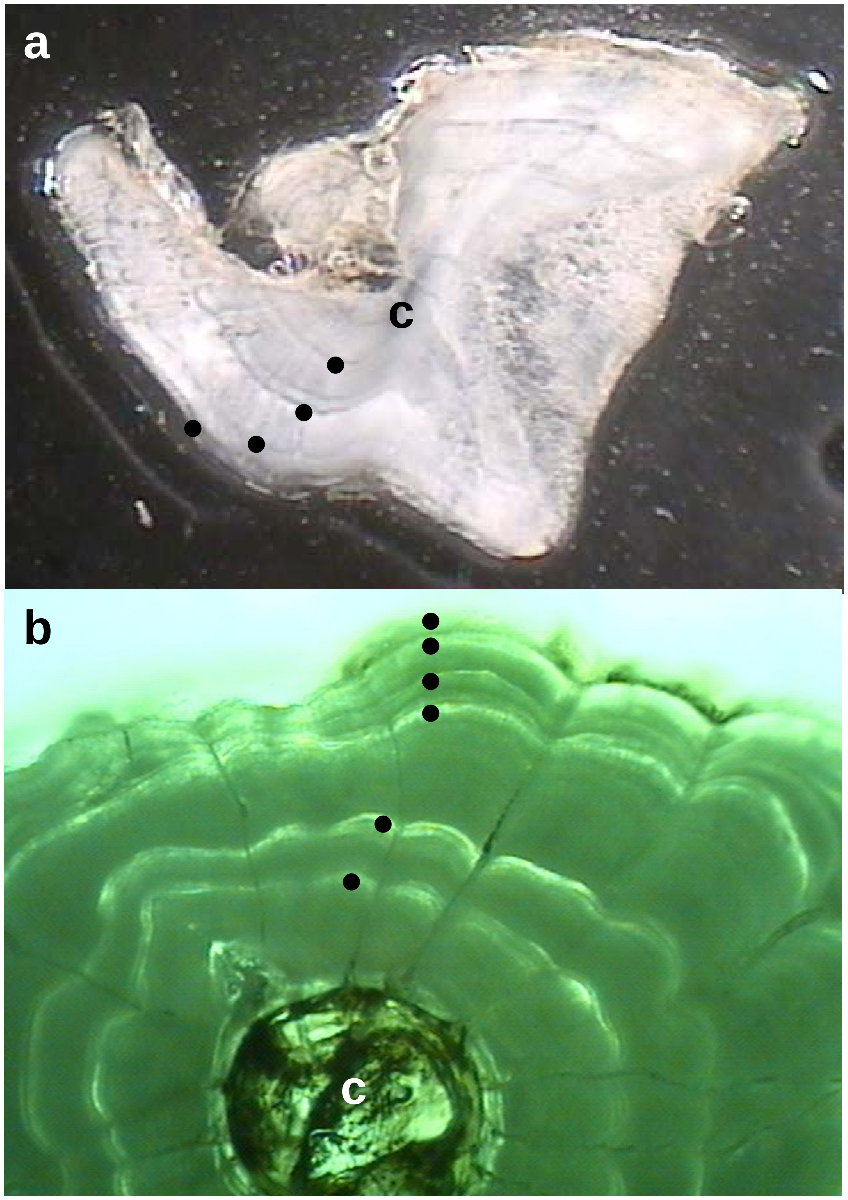

FIGURE 4.─Digital image of a cross-section through a pectoral spine from a yellow bullhead showing a) 4 annuli and b) 6 annuli. Translucent zones of annuli marked with black dots and 'C' indicates the core of the pectoral spine. Top image is taken under reflected light whereas the bottom image is taken under transmitted light, with a green filter to enhance the annuli.

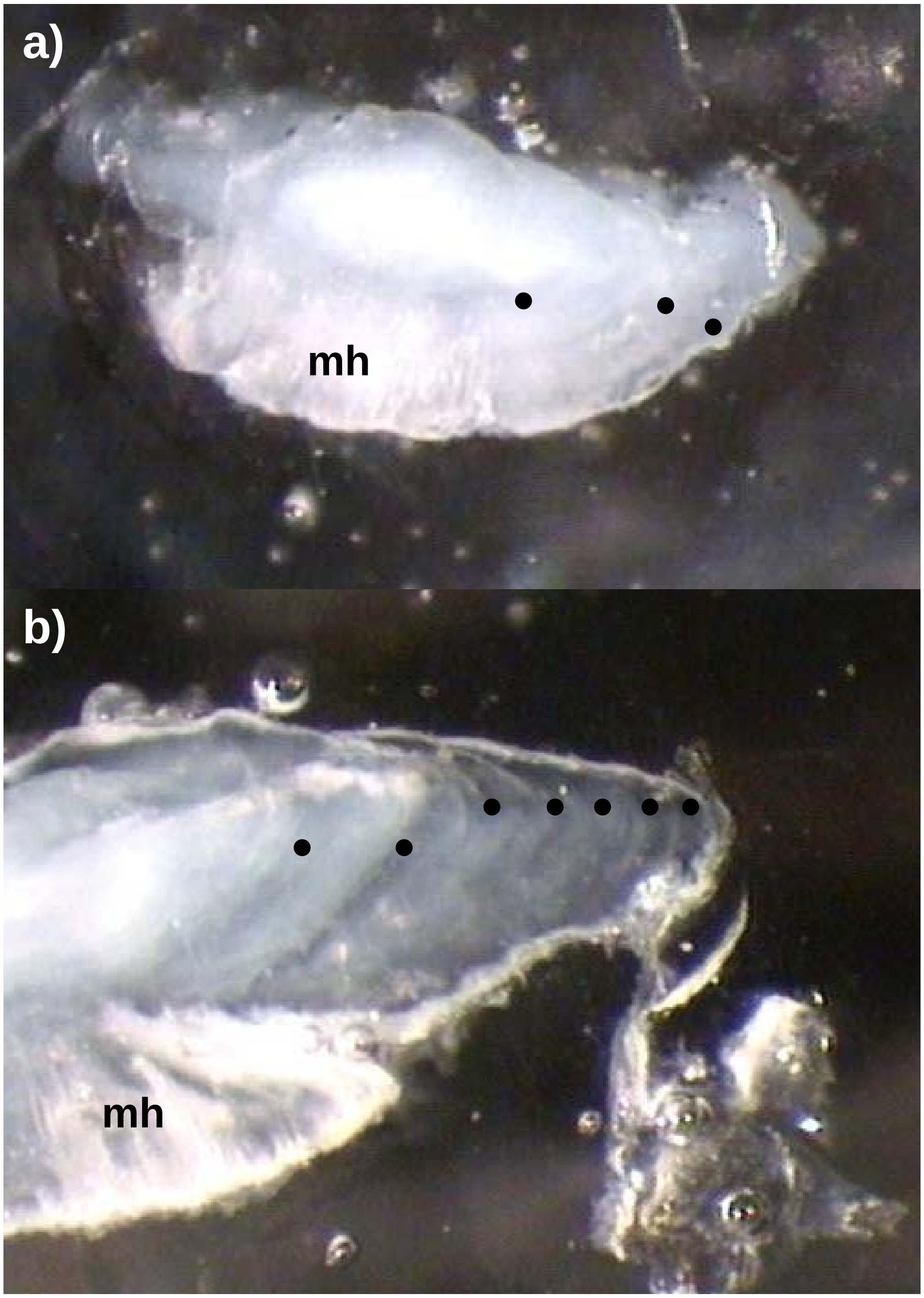

FIGURE 5.─Digital image of a sanded and polished cross-section through the lapillus of a yellow bullhead showing a) 3 annuli and b) 7 annuli. Translucent zones of annuli marked with black dots and mh = macular hump. The images have been taken while submersed and against a black background with reflected light.

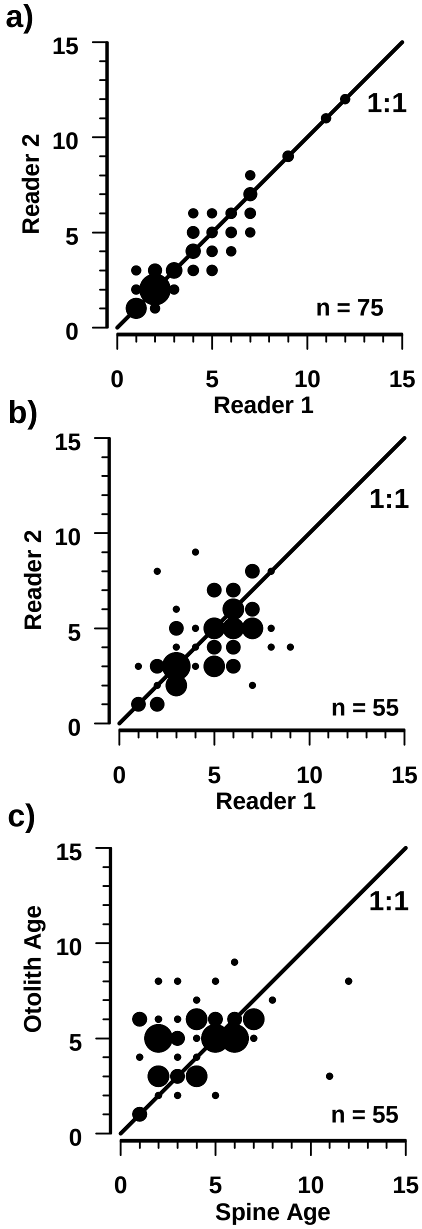

FIGURE 6.─ Scatter plot comparison of the age estimates obtained by Reader 1 versus Reader 2 using a) pectoral spines and b) otoliths, and c) age estimates obtained from otoliths versus pectoral spines. The diagonal line represents comparisons where otolith ages = spine ages. Circle size represents the relative sample size for that age combination.

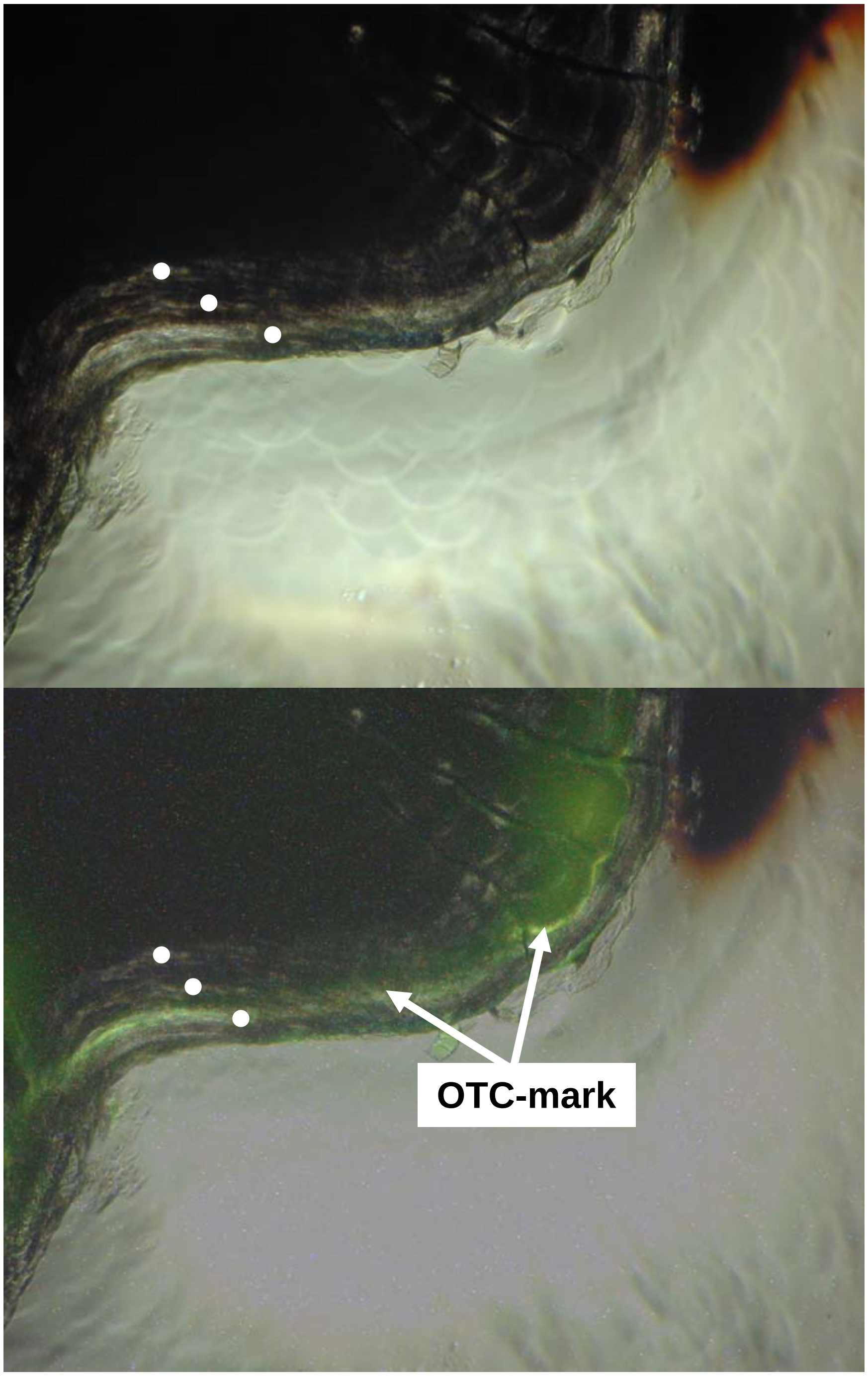

FIGURE 7.─ Thin-sectioned pectoral spine from an oxytetracycline (OTC)-injected 7-year old yellow bullhead showing the OTC-mark followed by one translucent and one opaque zone, validating that only one complete annulus is deposited in a 12-month period. The last three translucent zones at the edge of the spine are marked with white dots.

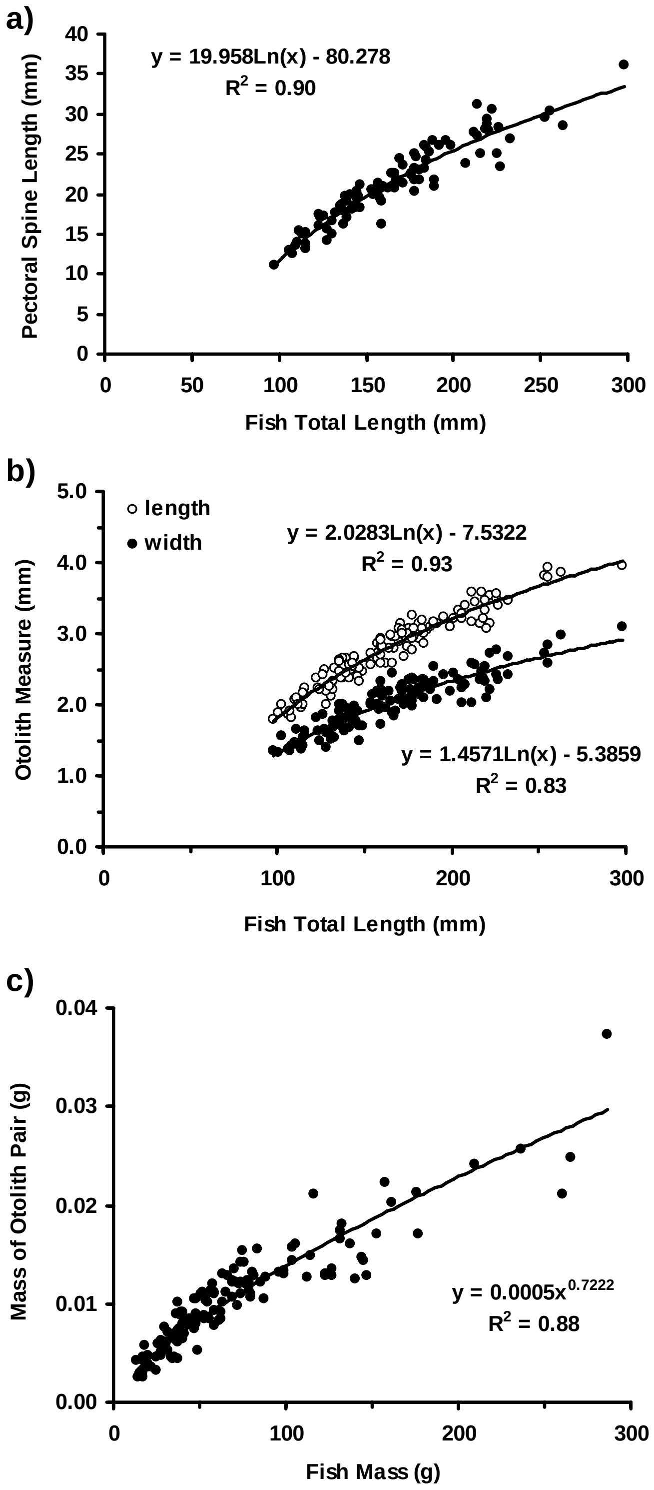

FIGURE 8.— Non-linear relationship between a) spine length and b) otolith length and width in relation to yellow bullhead total length, and c) between mass of the otolith pair in relation to fish mass.

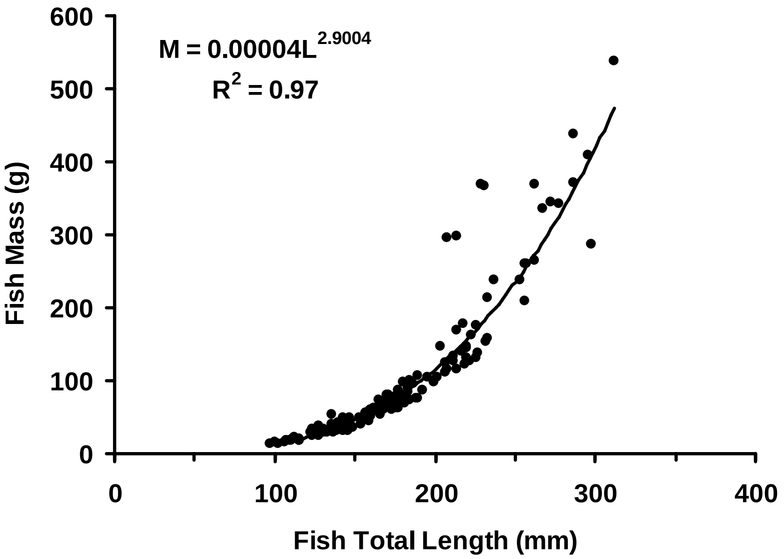

FIGURE 9.— Total body mass as a function of total length for yellow bullheads collected from south Florida.

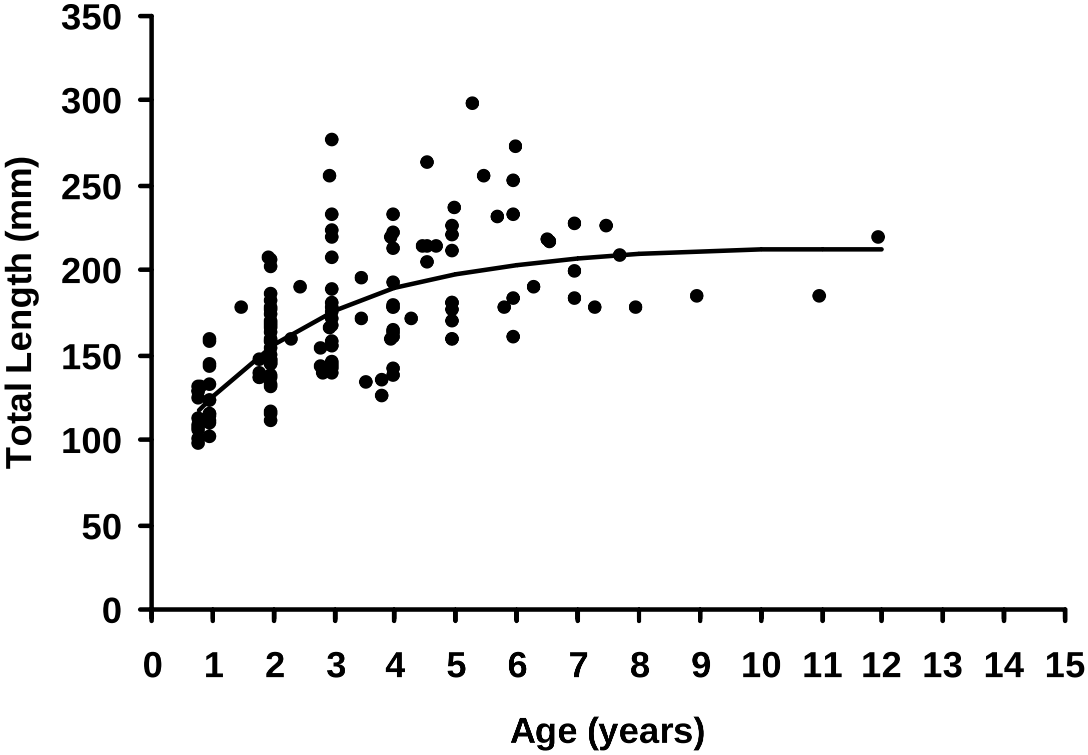

FIGURE 10.─ Total length at capture as a function of absolute age estimate (assuming a June 1st birthdate) for yellow bullhead from south Florida. Line represents estimated von Bertalanffy growth curve.

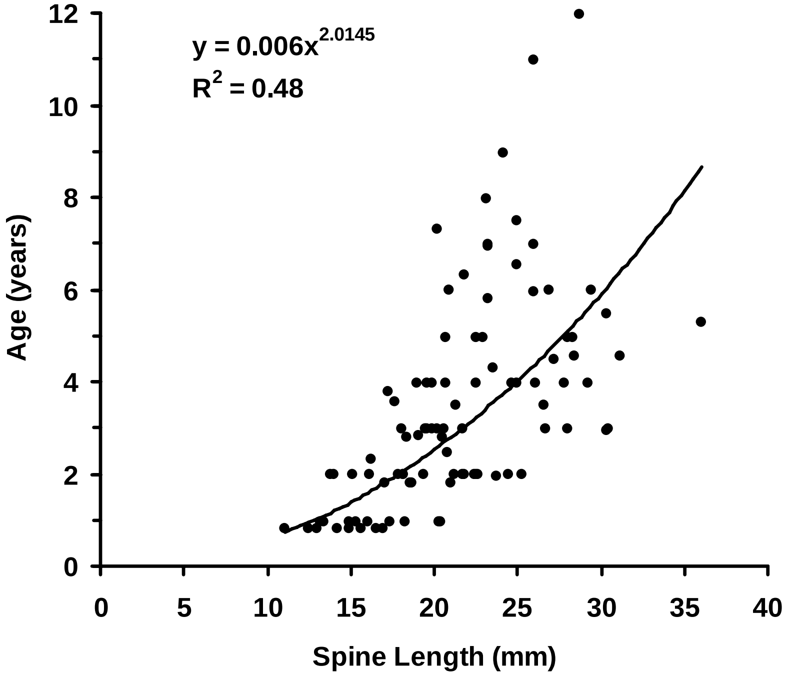

FIGURE 11.─ Non-linear relationship between absolute age estimate for yellow bullhead from south Florida as a function of pectoral spine length.

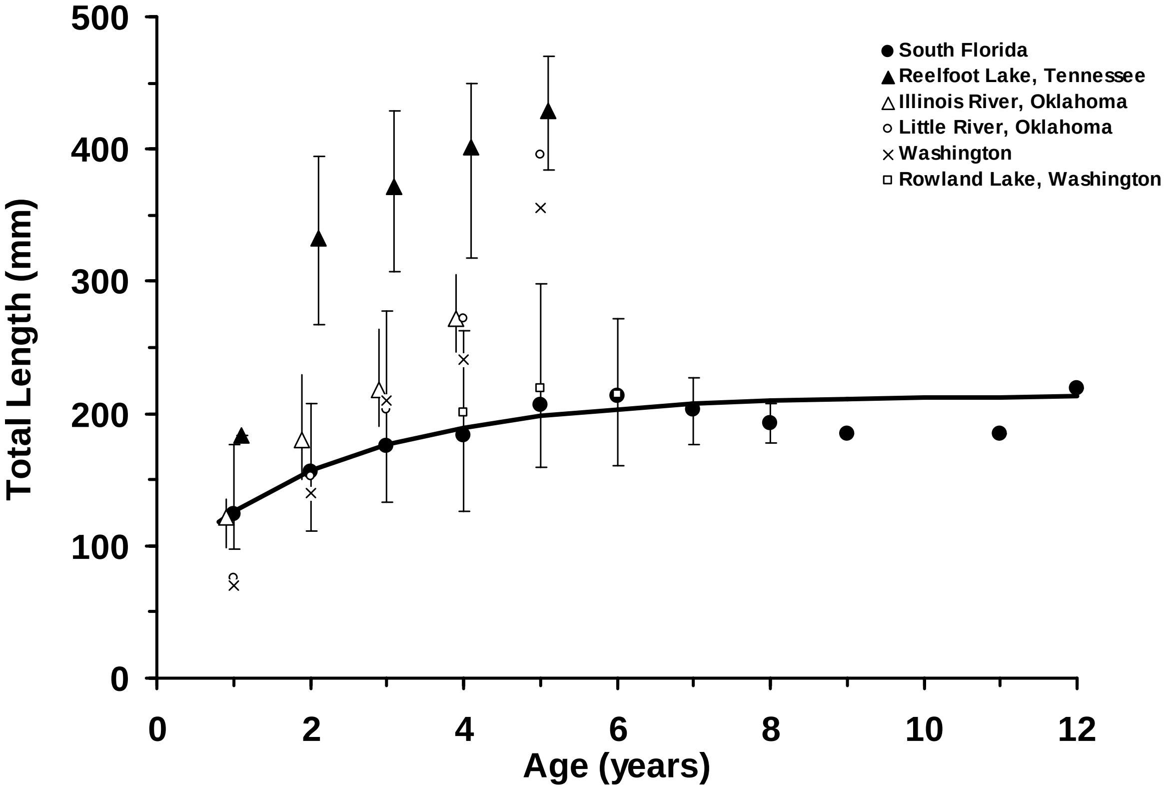

FIGURE 12.— Mean total length at observed age for yellow bullhead collected from south Florida (This Study), Illinois River and Tenkiller Reservoir, Oklahoma (Jenkins et al. 1952), Reelfoot Lake, Tennessee (Schoffman 1955), Little River, Oklahoma (Finnell et al. 1956), Washington (Wydoski and Whitney 1979) and Rowland Lake, Washington (Jackson and Carolmile 2000). Vertical bars denote the range in size of yellow bullhead at age for south Florida, Illinois River, and Reelfoot Lake and are offset from one another for clarity. Solid line represents estimated von Bertalanffy growth curve for yellow bullheads from south Florida.