ESTIMATING LENGTH, WEIGHT, AND AGE OF CENTRAL STONEROLLER (*CAMPOSTOMA ANOMALUM*) USING BONE MEASUREMENTS

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INTRODUCTION

Identifying fish using bony structures found in stomach and fecal contents is common in dietary evaluations of piscivorous aquatic and terrestrial animals (e.g. Bald Eagle *Haliaeetus leucocephalus,* Cash et al. 1985; European Whitefish *Coregonus lavaretus,* Stich and Maier 2006; Giant Otter *Pteronura brasiliesis,* Mallea Cárdenas et al. 2015; Pike *Esox lucius,* Mann and Beaumont 1980; and Steller Sea lions *Eumetopias jubatus,* Cottrell and Trites 2002). Stomach content analysis is the most common method of understanding predator-prey interactions of fishes, however due to digestive processes, a fish may not be intact enough to get an accurate size measurement or accurate identification. The ability to estimate length, weight, or age of ingested prey species is an important step in understanding predator consumption rates (biomass of prey consumed) and size selectivity, which are needed for bioenergetics models or to understand community dynamics (Granadeiro and Silva 2000; Hansel et al. 1988; Scharf et al. 1997; Wood 2005).

Bony structures have been used to identify fish remains and fish size by both fisheries biologists and archaeologists (Hansel et al. 1988; Plug 2008). Due to problems with heavily digested prey, morphological features of fish species commonly found during diet analyses are often used for prey identification. For example, the eye diameter or caudal peduncle depth (Scharf et al. 1997), pharyngeal teeth, opercula, cleithra, and otoliths (Tarkan et al. 2007), and vertebrae (Granadeiro and Silva 2000) have been used to generate models for estimating the size of fish consumed by piscivores.

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Central Stonerollers (*Campostoma anomalum*) may be seasonally important prey for Smallmouth Bass (*Micropterus dolomieu*) in eastern Oklahoma streams based on the presence of cleithra and pharyngeal teeth in stomach contents from this species (C. Porter and R. Snow, personal observation). Dauwalter and Fisher (2008) found that Smallmouth Bass in the Baron Fork Creek, Oklahoma, consumed Central Stonerollers in the spring, comprising 12% of the fish portion of the diet. This may be a conservative estimate, however, as unidentified fish remains represented 54% of the diet samples of adult Smallmouth Bass. Furthermore, Central Stoneroller shift from pool to shallow riffle habitats when in the presence of black bass, suggesting that Central Stonerollers are avoiding predation (Harvey et al. 1988). However, this habitat shift does not eliminate all predation risks as piscivorous birds can alter the size structure and abundance of Central Stonerollers in shallow water habitats (Steinmetz et al. 2003). Because stonerollers are an important prey species for Rock Bass (*Ambloplites rupestris*), Smallmouth Bass (Probst et al. 1984, Angermeier 1992), and predatory birds (Steinmetz et al. 2003), the objective of this study was to generate accurate predictive

Nuptial male Central Stoneroller (*Campostoma anomalum*). (Photo by Konrad Schmidt)

regression equations to estimate total length, weight, and age of Central Stoneroller from pharyngeal teeth, cleithra, and otoliths (asterisci, sagittae, and lapilli). This technique would allow researchers to predict fish length, weight, and age of ingested Central Stonerollers using bones collected during diet analyses.

METHODS

We sampled Central Stonerollers from eight reaches of the Illinois River from East 648 Road bridge off of Highway 10 (36.104354, -94.783289) downstream to Cherokee Hills Byway Bridge on Highway 62 (35.926235, -94.923667). Multiple seine hauls were performed within each reach during July and August of 2015 using a 6-m long x 1.21-m high seine with 5-mm mesh. When the leading seine bridle reached 10 meters from shore, a semicircle approach was made towards the bank to entrap fish to prevent escape. Kick seining was conducted where riffles were present by placing the seine at the downstream end of the riffle. A one-pass kick method was used to dislodge substrate, thereby washing fish into the seine (Patton et al. 2000; Porter and Patton 2015). Upon capture, Central Stonerollers were measured to the nearest (mm) and 10 fish per each 10 mm length group were placed in a zip-closure plastic bag. Fish were placed on ice and transported to the Oklahoma Fisheries Research Lab, Norman, Oklahoma. Upon arrival, length (mm) and weight (g) measurements were recorded and pharyngeal teeth, cleithra, and otoliths were removed. To ensure proper identification of species, the pharyngeal tooth count (0,4–4,0) was verified (Miller and Robison 2004).

To remove the pharyngeal teeth (Figure 1 D, E, and F and Figure 2) and cleithra (Figure 1 A, B, and C), individuals were laterally positioned under a dissecting scope (4-40x depending on size). The operculum was removed with surgical scissors, and the gill structures removed with forceps to expose the pharyngeal teeth. After removal, the pharyngeal teeth were soaked in a 3:1 diluted bleach solution for two minutes. During that time cleithra were removed. (Clethera are the paired bones of the pectoral girdle that form the frame of the body wall directly posterior to the opercular cavity [Scharf et al. 1998]). Once the cleithrum was located, forceps were used to pry the bone loose from connective tissues beginning at the ventral edge and moving dorsally towards the spine. After all the connective tissue was severed, the cleithrum was carefully removed so as not to damage the structure. Removed cleithra were were placed in a 3:1 bleach solution for two minutes. At this time the pharyngeal teeth were picked clean of flesh under a dissecting microscope, and rinsed with water until clean. The same process was used to clean the cleithra. After cleaning, the structures were placed in a vial and dried before measuring.

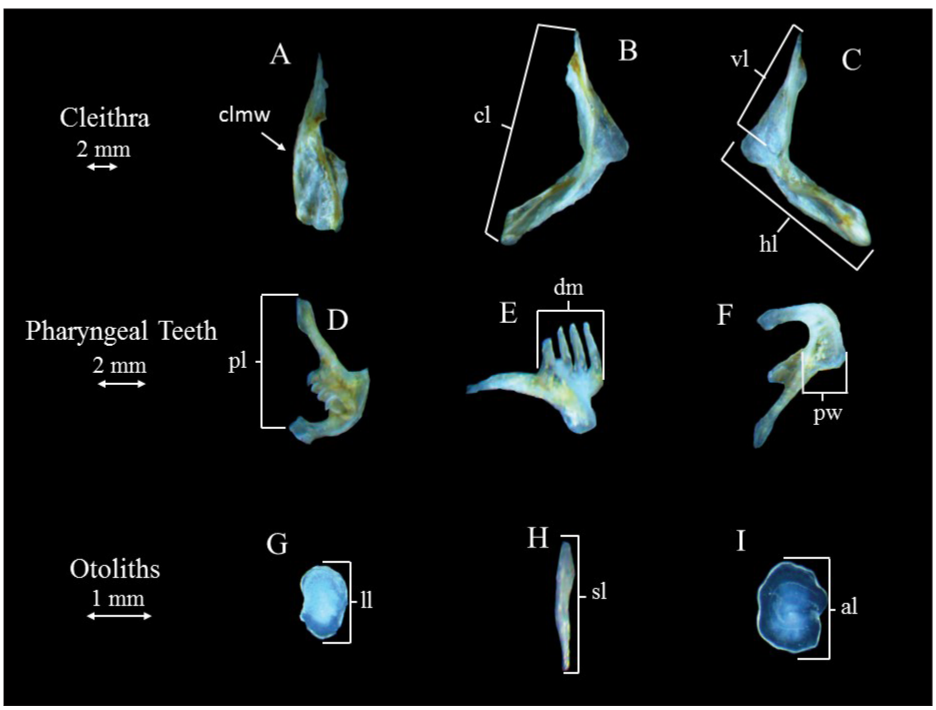

Figure 1. Structures taken from a Central Stoneroller (*Campostoma anomalum*) measuring 113 mm TL for estimating original length in mm. Cleithra are viewed from three orientations (A) anterior view of the cleithrum medial wing [clmw], (B) distal lateral view of the cleithra arch length (cl) and (C) mesial lateral view for measuring vertical [vl] and horizontal [hl] length. Pharyngeal teeth (D) are viewed dorsally to measure the pharyngeal arch length [pl], (E) dentary measurement [dm] is taken in a mesial lateral view, and (F) the pharyngeal width is measured when the pharyngeal teeth are viewed ventrally. The otoliths (G) lapillus, (H) sagitta, and (I) asteriscus are measured at their longest axis [ll, sl, al]

Otoliths (asterisci, sagittae, and lapilli) (Figure 1 (G, H, and I) were removed following removal of cleithra and pharyngeal teeth. To do so, fish were placed dorsal side down and the lower jaw structure was cut in half using surgical scissors. Fish were bi-laterally pinned through the remaining jaw and cheek structure to keep the specimen in place. Forceps were used to remove excess tissue revealing the ventral side of the braincase. The asterisci and sagittae can be visually identified through translucent bone structures of the ventral brain case, which were dissected to carefully expose and remove otolith pairs. After the brain matter was removed the lapilli were then visible and removed. Otoliths were cleaned of any excess tissue or blood and placed in a vial and dried before measuring.

Once all structures were dry, each was measured in (mm) using ToupCam (Toup Tek Photonic, Zhejieng, P. R. China) com-

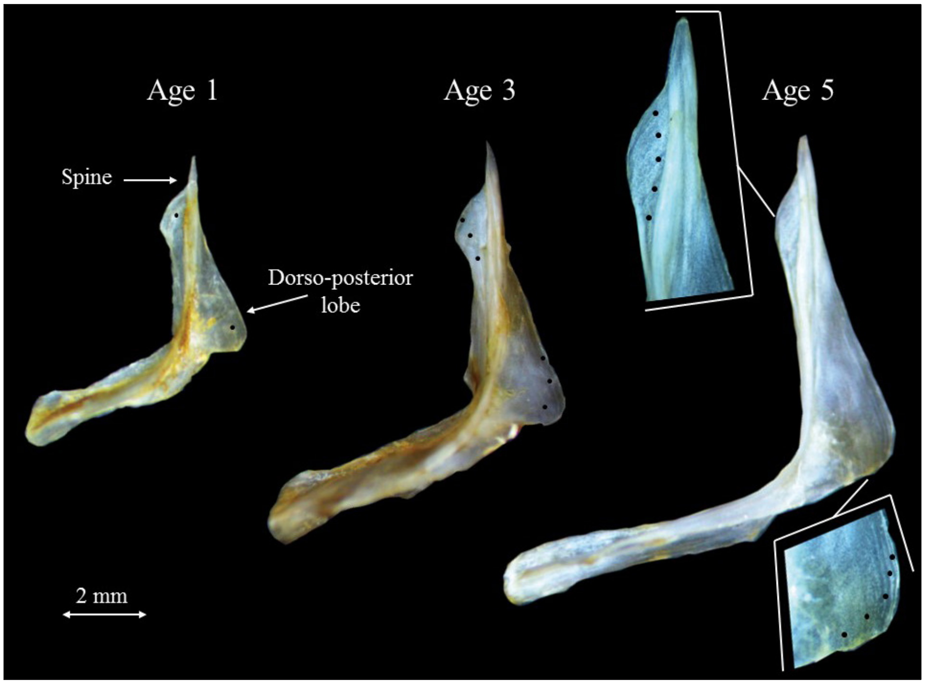

Figure 2. Lateral view of cleithra estimated to be age-1 (71mm), age-3 (93mm), and age-5 (104mm) from Central Stoneroller (*Campostoma anomalum*) collected from the Illinois River in Cherokee County, Oklahoma in 2015. Age estimates were taken from the spine and dorsoposterior lobe region of the cleithra. Black dots represent location of each annulus.

puter software and camera attached to a dissecting scope. Cleithra measurements were taken from the horizontal limb (hl), vertical limb (vl), and cleithra arch length (cl) in a lateral view (Figure 1). The pharyngeal arch length (pl) was measured in an anterior view, while the pharyngeal arch width (pw) was measured in a posterior view. The dentary measurement (dm) of the pharyngeal teeth was recorded in a lateral view. All otoliths were measured along their longest axis (Figure 1).

Ages for Central Stonerollers were determined by counting the number of annuli visible on both the lapilli and asterisci otoliths; however, no agreement could be reached between readers for either of these structures. For this reason, all age estimations were evaluated using the cleithra. Visible annuli were counted on the spine and posterior portion of the horizontal limb of the cleithrum (Figure 2). Cleithra were read with magnification depending on size (4–40x), either in natural light or illumination from below using a fiber-optic light source with a dark background (Oele et al. 2015). Cleithra were read independently three times in random order without reference to previous age estimation, and no two counts were made consecutively (Miller and Storck 1982; DiCenzo and Bettoli 1995; Sakaris and Irwin 2013). Age estimation for each aging structure was done without knowledge of fish length or weight.

Linear regression was used to test the relationships between each bone structure measurement and the total length of the fish, which were used to generate predictive equations. The strength of the correlation was judged by both the r^2 value, P-value, and by calculating mean percent error (Observed – Predicted/Predicted*100) for each model (Scharf et al. 1997; Wood 2005). Length-weight and length-age regressions were was conducted using estimated length from each structure. Mean length at age (using directly measured lengths and cleithra-derived age) was calculated for each age group. All statistical analyses were conducted using Microsoft Excel.

RESULTS

A total of 73 Central Stonerollers ranging from 27–127mm total length was collected from the Illinois River for analyses. The regression equations correlating measurements from pharyngeal teeth, cleithra, and otoliths to total length (Figure 3) were all significant (sagitta $P = 0.04$ and all other structures $P < 0.01$; Table 1). The r^2 values for the total length predictive equations ranged from 0.62 to 0.98, and the mean percent predictive error ranged from -0.17 to 3.0 (Table 1).

The best fit was obtained with the pharyngeal teeth dentary measurement, which had an $r^2 = 0.98$. Fish in this sample ranged from 0–5 years of age based on estimates from cleithra (Table 2). Mean length at age was 27 mm, 68 mm, 89 mm, 102 mm, 115 mm, and 104 mm for fish age 0 through 5, respectively. The relationship between weight and length was described by the equation weight = 0.000006*length$^{3.1028}$ (Table 3).

DISCUSSION

We found measurements from pharyngeal teeth, cleithra, and otoliths accurately predict the original total length of Central Stonerollers. Although significant, caution should be used if total length is being estimated with the sagittal otolith due to the low r^2. This is probably the result of inaccuracies in measurements of the sagittal otoliths that were broken due to the long, thin structure of this bone, which makes it very fragile. Using total length to estimate weight and age will give researchers or managers the ability to better understand size and age structure of Central Stoneroller populations being preyed upon. These methods have been widely used for other species, including European Perch (*Perca fluviatilis;* Polat et al. 2015); Bluefish (*Pomatomus saltatrix*; Scharf et al. 1997); and 24 species of fish collected in the Columbia River (Hansel et al. 1988). These results have allowed researchers to better understand predator and prey relationships and prey consumption rates, and led to development of more robust energetic models

Table 1. Equations for predicting the total length (mm) of Central Stonerollers (*Campostoma anomalum*) from each of nine bony structures with corresponding coefficient of determination r^2, *P*-value, and mean predictive errors (%PE) for each model.

Structure	Equation	*n*	r^2	*P*-value	% PE
Astericus (al)	TL = 74.979(AL) + 0.6772	68	0.86	<0.01	0.17
Sagitta (sl)	TL = 32.268(SL) + 33.497	36	0.62	0.04	3.0
Lapillus (ll)	TL = 109.76(LL) – 32.494	65	0.93	<0.01	0.03
Cleithrum Vertical Limb (vl)	TL = 14.46(VL) + 12.773	73	0.86	<0.01	0.16
Cleithrum Horizontal Limb (hl)	TL = 0.0731(HL) + 0.5712	73	0.94	<0.01	-0.14
Cleithrum Arch Length (cl)	TL = 8.4624(CL) + 4.8796	73	0.90	<0.01	0.17
Primary Dentary Measurement (dm)	TL = 37.653(DM) + 7.8062	72	0.98	<0.01	0.21
Primary Pharyngeal arch width (pw)	TL = 43.09(PW) + 10.778	72	0.87	<0.01	0.15
Pharyngeal arch length (pl)	TL = 16.87(PL) + 7.4167	72	0.88	<0.01	-0.17

Table 2. Number of Central Stonerollers (*Campostoma anomalum*) per age group collected from the Illinois River in Cherokee County, Oklahoma in 2015. Mean total length (mm) is presented for each age class followed by standard error.

Age	0	1	2	3	4	5
Number	1	24	21	20	6	1
Mean Total Length (mm)	27	68.2 ± 1.5	88.9 ± 2.1	102.2 ± 1.9	114.7 ± 4.3	104

Table 3. Equations for predicting age and weight (g) of Central Stonerollers (*Campostoma anomalum*) from total length (mm) with corresponding coefficient of determination r^2, P-value, and mean predictive error (%PE) for each model.

Parameter	Equation	*n*	r^2	*P*-value	% PE
Weight	$W = .000006(TL)^{3.1028}$	73	0.97	<0.01	4.43
Age	A = 0.04731(TL) - 2.04584	73	0.79	<0.01	-5.1

in both freshwater and marine environments.

The reconstruction of original prey size from a bony structure can have limitations. Comparison of bones from smaller Central Stonerollers with those of larger individuals did not indicate differences in the structure's shape or form. However, previous research determined preservation of stomach contents in formalin can cause the breakdown of otoliths (Scharf et al. 1997) and boiling structures for cleaning purposes could cause deformation or shrinkage, which could affect the proper identification of species or under estimate original size (Scharf et al. 1998). Although structures were similar across size ranges, small individuals may be more susceptible to digestive process than structures from larger fish (Hansel et al. 1988). For this reason, special care should be used when preserving and cleaning structures to ensure accurate estimates using the regression equation.

Challenges using asterisci and lapilli for age estimation were caused by irregular growth patterns in the formation of opaque and translucent bands. Although the lapilli were clearer when heat-browned, which helped distinguish annuli, readers still could not agree during a consensus read. We therefore use cleithra as the preferred aging structure. Cleithra have been used to estimate ages of Northern Pike (*Esox lucius*) (Faust et al. 2013) and Indian Major Carp (*Labeo rohita*) (Bhat and Khan 2014); however, previous studies that have estimated age of Central Stonerollers have only used scales and asterisci (Bisping et al. 2010). Furthermore, without a validation study to determine the proper structure to estimate ages of Central Stonerollers, managers and researchers should proceed with caution when using any single structure for age estimation.

Although cleithra have not been validated for the Central Stoneroller, age estimates were similar to studies that had estimated age with scales and otoliths. Both Gunning and Lewis (1956) and Quist and Guy (2001) found Central Stonerollers up to age 3 in their study systems, whereas Lennon and Parker (1960) estimated ages of Central Stoneroller from 0 to 5 with most less than age 3. In the most recent Central Stoneroller aging study, Bisping et al. (2010) estimated mean length at age using asterisci (75.3, 95.7, 109.3, and 114 mm for ages 1–4, respectively), which were similar to our findings from cleithra age estimates. The ability to estimate age from the cleithra of Central Stonerollers would be less time-consuming, however, some time is needed for cleaning structures for age estimation.

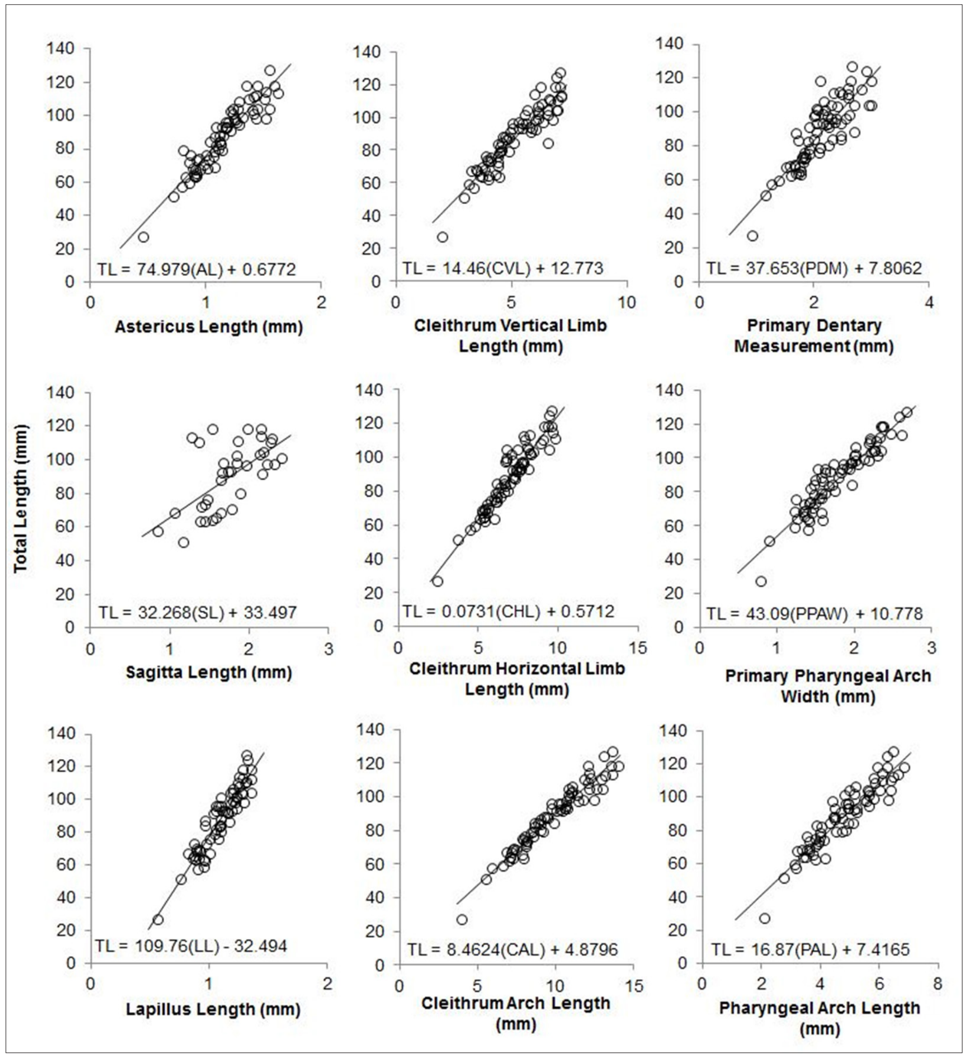

Figure 3. Total length (mm) in relation to nine bony structure measurements (mm) in Central Stonerollers (*Campostoma anomalum*). Resulting linear regression models and trendlines are shown.

To our knowledge, this information is the first of its kind for Central Stonerollers. Using measurements from cleithra, pharyngeal teeth, and otoliths is a powerful tool for estimating total length, weight, and age of Central Stonerollers, which are found throughout most of the eastern United States and occur state-wide in Oklahoma. The ability to estimate original length, weight, and age from digestion-resistant structures will lead to a better understanding of population structure of Central Stonerollers, and allow for more accurate estimates of terrestrial and aquatic predator consumption rates of this species.

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