
Body Size Estimation and Identification of Twelve Fish Species Using Cleithrum Bones

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Abstract: Diet evaluations are conducted to understand predator-prey dynamics of fish communities. However, unless prey items are extracted from fish immediately after consumption, items can be observed at various stages of decomposition due to digestion. Thus, the ability to accurately measure or identify prey fish is difficult. Fortunately, some skeletal structures, such as the cleithrum bone, are not easily digested and remain in fish stomachs. Cleithra have been used to estimate the total length of a fish by determining the linear relationship between the total length, horizontal length, or vertical height of a cleithrum against known-sized fish from which the structure was taken. We used linear regression to develop equations to estimate body size for twelve common forage species found in Oklahoma reservoirs using cleithrum bones. The relationships between total fish length:cleithrum length ($r^2= 0.94-0.99$), total fish length:horizontal cleithrum length ($r^2= 0.90-0.98$), and total fish length:vertical cleithrum length ($r^2= 0.88-0.98$) were significant. Additionally, we also described cleithrum characteristics for each of the twelve fish species, such that fish can be identified even when prey items are heavily digested. When used collectively, the regression equations and diagnostic features of cleithra will provide a more accurate description of fish diets and a better understanding of predator-prey relationships.

Introduction

Dietary analysis is an important aspect to understanding predator-prey dynamics in fish communities. Diets are typically evaluated using stomach content analysis, which relies on identification of prey remains, but depending on digestion rate, prey items can be in various stages of decomposition making it difficult to accurately identify prey items, or get an accurate measure of their length and weight (Hansel et al. 1988, Scharf et al. 1998, Tarkan et al. 2007, Snow et al. 2017). Accurately identifying and measuring prey items is critical when

attempting to understand bioenergetics, feeding ecology, predator consumption rates (Hansel et al.1988, Scharf et al. 1998, Snow et al. 2017), predation influences on fish recruitment (Ball and Weber 2018), and when managing fisheries at the community level (Knight et al. 1984), so overcoming issues associated with digested prey items is important to thoroughly describe fish diets.

Skeletal remains (cleithra, dentaries, operculum bones, otoliths, pharyngeal arches, and vertebrae) found in stomach contents have been used to identify different prey species, and reconstruct length and weight of prey items in both marine and freshwater systems (Hansel et

al. 1988, Scharf et al. 1998, Radke et al. 2000, Dietrich et al. 2006, Tarkan et al. 2007, Snow et al. 2017, Yazicioglu et al. 2017, Assis et al. 2018). Cleithra (bones associated with the pectoral girdle) are often used because they are one of the largest and most robust bones in the skeletal system of a fish, persist in diets because they are not easily digested, and are morphologically distinct (Figure 1; Hansel et al. 1988). In addition, they are one of the first diagnostic bones to form during fish development, making this structure useful for young prey fish (Hansel et al. 1988). A linear relationship exists between cleithrum dimensions and fish size, which allows for back-calculation of fish total length and weight using a cleithrum measurement (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Dietrich et al. 2006, Snow et al. 2017).

Because cleithra are useful for reconstruction of fish size and identification of fish species, the objectives of this study were to evaluate the linear relationship between cleithra dimensions and fish size (length and weight) for twelve common prey fish species in Oklahoma aquatic systems. Further, we will describe diagnostic features of cleithra to aid in identification of these species. This information will benefit future diet studies in Oklahoma, or other systems where these prey species are common, by allowing for a more comprehensive description of fish diets.

Methods

A total of 737 fish were collected from twelve species, which consisted of 30 Black Crappie (*Pomoxis nigromaculatus*), 88 Bluegill (*Lepomis macrochirus*), 89 Gizzard Shad (*Dorosoma cepedianum*), 30 Golden

Shiners (*Notemigonus crysoleucas*), 78 Green Sunfish (*Lepomis cyanellus*), 75 Inland Silversides (*Menidia beryllina*), 62 Largemouth Bass (*Micropterus salmoides*), 86 Longear Sunfish (*Lepomis megalotis*), 36 Red Shiners (*Cyprinella lutrensis*), 79 Redear Sunfish (*Lepomis microlophus*), 47 saugeye (female Walleye [*Sander vitreus*] and male Sauger [*S. canadensis*], and 37 White Crappie (*Pomoxis annularis*). Fish were collected opportunistically during fall 2017 through spring 2019 using boat electrofishing, seining, or fyke netting from eight reservoirs in Oklahoma (Table 1). Once fish were collected, they were put on ice and transported to the Oklahoma Fishery Research Laboratory in Norman, Oklahoma, where they were frozen until processing.

When processing samples, each fish was measured for total length (TL; nearest mm) weighed (nearest g), and both cleithra removed (Figure 1). Cleithra were cleaned by placing them into a beaker filled with water and boiled on a hot plate (Thermolyne Type 1900; 107.2°C). Cleithra were removed from small fish (<50 mm) by boiling them whole. Similarly, fish with fragile cleithra (Gizzard Shad and Inland Silverside) were cut into a section that encapsulated the cleithra and boiled, which lessened the risk of damaging diagnostic features of the cleithra. Cleithra were boiled until they could be easily cleaned (30 to 90 sec, depending on size). Cleaned cleithra were placed into an envelope to dry and stored until measuring.

Cleithra were measured (nearest .01mm) under a dissecting scope, using AmScope 3.7 software. If cleithra were too large to be measured under the microscope, a digital caliper

Table 1. Sampling locations of the twelve fish species collected for cleithra evaluation.

Species	Lakes						
	Arcadia	Dahlgren	Elmer	New Spiro	Pawhuska	Sparks	Stilwell City Thunderbird
Black Crappie (<i>Pomoxis nigromaculatus</i>)				X	X		X
Bluegill (<i>Lepomis macrochirus</i>)			X	X	X	X	X
Gizzard Shad (<i>Dorosoma cepedianum</i>)	X		X	X			
Golden Shiner (<i>Notemigonus crysoleucas</i>)					X		X
Green Sunfish (<i>Lepomis cyanellus</i>)		X	X		X		
Inland Silverside (<i>Menidia extensa</i>)	X			X			X
Largemouth Bass (<i>Micropterus salmoides</i>)	X		X	X	X	X	X
Longear Sunfish (<i>Lepomis megalotis</i>)	X				X		X
Red Shiner (<i>Cyprinella lutrensis</i>)	X						
Redear Sunfish (<i>Lepomis microlophus</i>)			X	X	X	X	X
Saugeye: female Walleye [<i>Sander vitreus</i>] and male Sauger [<i>S. canadensis</i>]	X					X	
White Crappie (<i>Pomoxis annularis</i>)			X	X		X	

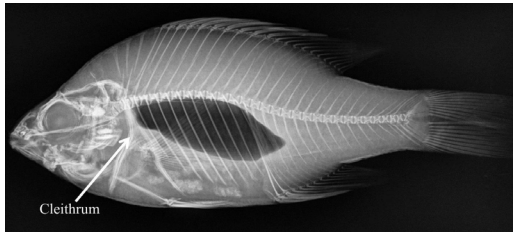


Figure 1. X-ray image showing the location of the cleithrum in a Redear Sunfish.

(Griffon Corporation, New York, NY) was used to measure cleithra (± 0.03 mm). Three measurements: cleithrum length (CL), vertical length (VL), and horizontal length (HL), were recorded for each cleithrum from the inside lateral view (ILV) side for both the left and right cleithrum (Figure 2). Only CL could be measured for Gizzard Shad and Inland Silversides because of the shape of their cleithra. If a cleithrum was damaged any measurement that would have been affected by the damage was not taken. For example, if a spine was broken neither CL nor VL were measured.

Linear regression models relating fish total length and cleithrum measurements were

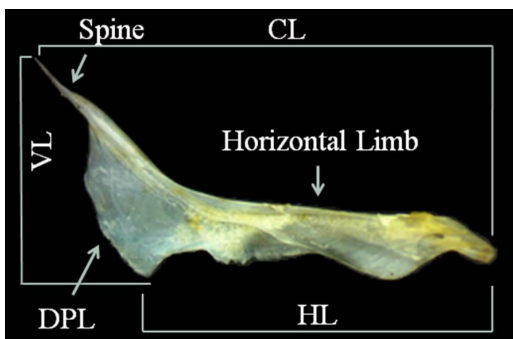


Figure 2. Photograph of a Redear Sunfish cleithrum with labels describing morphological features and measurements taken for regression analysis. Cleithra are viewed from the inside lateral view. Cleithrum length (CL) is measured from tip of spine to posterior end of the horizontal limb. Vertical length (VL) is measured from tip of spine to posterior end of the vertical limb, also known as the dorsoposterior lobe (DPL). Horizontal length (HL) is measured from the posterior end of the DPL to the posterior end of the horizontal limb.

calculated using Microsoft Excel. Models were developed for the left and right cleithra, using all three cleithrum measurements. A paired t-test was used to compare the predicted total fish length from the right and left cleithrum for each measurement (CL, VL, and HL) and species. Test outcomes were considered significant at $P \leq 0.05$. If t-tests were not statistically different, the left and right cleithra were pooled for regression analysis. Each correlation was tested for strength using r^2 values and mean percent prediction error $[(\text{Observed} - \text{Predicted})/\text{Predicted} * 100]$ for each model and averaging the percent prediction error for each observation (Wood 2005, Scharf et al. 1998, Snow et al. 2017). Lastly, an exponential equation was developed to predict fish weight using the observed length of the fish.

Results

Estimation of Original Prey Length

Linear regression models indicated significant relationships between fish total length and all cleithrum measurements ($P < 0.001$; Table 2- 4). The predicted fish lengths using measurements from the left and right cleithrum for each species were not significantly different ($P > 0.05$), so samples were pooled. On average, fish total length:CL (left, right, and pooled) had the strongest linear relationships ($r^2=0.95-0.99$; Table 2), although strong relationships existed for fish total length: HL ($r^2 = 0.90-0.98$; Table 3) and fish total length: VL ($r^2 = 0.88-0.98$; Table 3). The mean percent predictive error range was smallest for measurements of CL (-0.9-0.26%; Table 1), followed by HL (-1.79-0.52%; Table 3) and VL (-2.72-1.74%; Table 4). The exponential equations relating length and weight were highly correlated ($r^2 \geq 0.91$; Table 5).

Cleithrum Morphology

The cleithra were diagnostic for all species studied, although some are more difficult to differentiate than others (Figure 3). Cleithra morphology is very similar across species in the family Centrarchidae. In general, members of this family have cleithra with a short spine at the tip of the vertical limb, an elongated horizontal limb, and an unserrated dorsoposterior lobe (DPL; Hansel et al. 1988). Distinguishing among

Table 2. Linear regression equations for predicting total length from the right cleithrum length (CLR), left cleithrum length (CLL), and pooled cleithrum length (CLP) with the related r^2 , P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R ²	P-value	% Error
Black Crappie	65-157	TL = 5.1936CLR + 11.69	30	0.97	<0.001	0.26
		TL = 5.3621CLL + 8.0177	29	0.97	<0.001	-0.8
		TL = 5.27CLP + 9.9957	59	0.97	<0.001	-0.24
Bluegill	25-203	TL = 5.1173CLR + 5.9752	80	0.98	<0.001	-0.73
		TL = 4.943CLL + 6.5757	81	0.98	<0.001	-0.9
		TL = 5.0246CLP + 6.3331	161	0.98	<0.001	-0.82
Gizzard Shad	65-217	TL = 6.8994CLR - 14.278	84	0.98	<0.001	-0.1
		TL = 6.907CLL - 14.709	82	0.99	<0.001	-0.12
		TL = 6.9028CLP - 14.484	166	0.98	<0.001	-0.11
Golden Shiner	78-221	TL = 7.3608CLR + 25.481	26	0.95	<0.001	-0.24
		TL = 7.8963CLL + 14.854	25	0.95	<0.001	-0.08
		TL = 7.6329CLP + 20.053	51	0.95	<0.001	-0.18
Green Sunfish	25-171	TL = 4.4246CLR + 11.517	75	0.98	<0.001	-0.31
		TL = 4.4922CLL + 8.8259	72	0.98	<0.001	-0.05
		TL = 4.4464CLP + 10.421	147	0.98	<0.001	-0.18
Inland Silverside	24-110	TL = 11.428CLR - 5.9938	70	0.97	<0.001	0.21
		TL = 11.453CLL - 5.0227	69	0.98	<0.001	0.11
		TL = 11.455CLP - 5.5879	139	0.97	<0.001	0.16
Largemouth Bass	65-197	TL = 5.8924CLR + 13.093	55	0.97	<0.001	-0.12
		TL = 5.8796CLL + 12.257	52	0.96	<0.001	-0.08
		TL = 5.8945CLP + 12.544	107	0.97	<0.001	-0.1
Longear Sunfish	55-142	TL = 3.8577CLR + 23.621	84	0.97	<0.001	-0.09
		TL = 4.0157CLL + 19.305	74	0.97	<0.001	-0.08
		TL = 3.9239CLP + 21.79	158	0.97	<0.001	-0.09
Red Shiner	37-75	TL = 6.6307CLR + 9.767	33	0.95	<0.001	-0.04
		TL = 6.5405CLL + 9.5064	36	0.96	<0.001	-0.02
		TL = 6.5747CLP + 9.6915	69	0.95	<0.001	-0.03
Redear Sunfish	54-183	TL = 4.5682CLR + 10.9	78	0.98	<0.001	-1.65
		TL = 4.679CLL + 7.5378	76	0.98	<0.001	-1.74
		TL = 4.6197CLP + 9.3033	154	0.98	<0.001	-1.7
Saugeye	56-130	TL = 9.2917CLR - 7.4933	43	0.99	<0.001	0
		TL = 9.2475CLL - 7.8239	43	0.98	<0.001	-0.02
		TL = 9.2709CLP - 7.6717	82	0.98	<0.001	-0.1
White Crappie	91-174	TL = 5.0061CLR + 24.1	37	0.95	<0.001	-0.05
		TL = 5.3132CLL + 17.439	35	0.94	<0.001	-0.04
		TL = 5.146CLP + 21.074	72	0.94	<0.001	-0.05

genera (*Lepomis*, *Micropterus*, and *Pomoxis*) within Centrarchidae is not difficult, but it can be challenging to distinguish species within a genus. In *Lepomis*, Bluegill and Longear Sunfish have morphologically similar cleithra, however Bluegill have a more rounded DPL, and Longear Sunfish cleithra have a more robust spine. Redear Sunfish have a long, thin spine with a rectangular-shaped DPL. Green Sunfish cleithra spines taper abruptly and have a notch in the DPL. Black and White Crappie (*Pomoxis spp.*) and Largemouth Bass (*Micropterus*) cleithra

all have thick spines, which easily distinguish them from *Lepomis spp.* However, Largemouth Bass cleithra are distinguished from Crappie by having a flatter HL. *Pomoxis spp.* are difficult to distinguish from each other using cleithra, but these species can be separated because Black Crappie have a depression at the top of the DPL where it intersects with the spine. Black Crappie cleithra also have a trapezoidal-shaped notch where the DPL transitions to the horizontal limb, whereas this notch is rounded in White Crappie cleithra. Saugeye (Percidae) cleithra are similar

Table 3. Linear regression equations for predicting total length from the right cleithrum horizontal length right (HLR), left cleithrum horizontal length (HLL), and pooled cleithrum horizontal length (HLP) with the related r^2 , P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R ²	P-value	% Error
Black Crappie	65-157	TL = 7.0368HLR + 14.961	30	0.96	<0.001	0.52
		TL = 7.2972HLL + 11.538	29	0.97	<0.001	-0.63
		TL = 7.1591HLP + 13.349	59	0.96	<0.001	-0.03
Bluegill	25-203	TL = 7.3123HLR + 6.582	81	0.97	<0.001	-0.8
		TL = 7.213HLL + 6.1171	84	0.98	<0.001	-0.7
		TL = 7.2608HLP + 6.3529	165	0.98	<0.001	-0.75
Golden Shiner	78-221	TL = 10.526HLR + 27.791	26	0.94	<0.001	-0.28
		TL = 11.186HLL + 18.841	26	0.94	<0.001	-0.22
		TL = 10.868HLP + 23.151	52	0.94	<0.001	-0.26
Green Sunfish	25-171	TL = 6.2311HLR + 12.158	76	0.98	<0.001	-0.32
		TL = 6.0848HLL + 13.279	74	0.98	<0.001	-0.13
		TL = 6.1593HLP + 12.695	150	0.98	<0.001	-0.23
Largemouth Bass	65-197	TL = 7.3355HLR + 10.661	58	0.97	<0.001	-0.07
		TL = 7.2502HLL + 12.198	53	0.97	<0.001	-0.05
		TL = 7.2985HLP + 11.356	111	0.97	<0.001	-0.06
Longear Sunfish	55-142	TL = 5.2714HLR + 23.683	84	0.96	<0.001	-0.09
		TL = 5.3853HLL + 20.84	74	0.96	<0.001	-0.09
		TL = 5.3163HLP + 22.498	158	0.96	<0.001	-0.09
Red Shiner	37-75	TL = 8.5844HLR + 12.419	32	0.90	<0.001	-0.06
		TL = 8.4246HLL + 12.171	36	0.92	<0.001	-0.04
		TL = 8.4773HLP + 12.413	70	0.91	<0.001	-0.05
Redear Sunfish	54-183	TL = 6.4133HLR + 14.566	79	0.98	<0.001	-1.79
		TL = 6.7244HLL + 8.3065	75	0.98	<0.001	-1.48
		TL = 6.5484HLP + 11.747	154	0.98	<0.001	-1.68
Saugeye	56-130	TL = 9.0398HLR + 3.4672	43	0.98	<0.001	0.02
		TL = 8.8001HLL + 6.9112	43	0.98	<0.001	0.03
		TL = 8.914HLP + 5.2417	82	0.98	<0.001	0.03
White Crappie	91-174	TL = 6.9388HLR + 27.71	37	0.93	<0.001	-0.07
		TL = 7.1534HLL + 24.612	35	0.91	<0.001	-0.05
		TL = 7.0395HLP + 26.263	72	0.92	<0.001	-0.06

in shape to those of centrarchids, but differ in that they have a short, wide DPL that can be serrated (Figure 3; Traynor et al. 2010).

Gizzard Shad and Inland Silverside have very distinct cleithra compared to all other fish evaluated in this study. Gizzard Shad cleithra are fragile and have a distinct sickle-shape with a large medial process. Inland silverside cleithra are claw shaped with holes in the DPL. Cleithra

in Cyprinidae can be very difficult to distinguish among species, but in general, they have cleithra with an expanded lateral shelf, and some will have a hook-like process on the anterior end of the horizontal limb (Traynor et al. 2010). In this study, only Golden Shiners and Red Shiners were common enough in reservoirs to include. Cleithra of both species are L-shaped, with a hook-like process on the anterior end of the horizontal limb, but the Golden Shiner has a

Table 4. Linear regression equations for predicting total length from right cleithrum vertical length (VLR), left cleithrum vertical length (VLL), and pooled cleithrum vertical length (VLP) with the related r^2 , P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R ²	P-value	% Error
Black Crappie	65-157	TL = 9.9602VLR + 24.528	30	0.96	<0.001	0.14
		TL = 9.6133VLL + 26.93	30	0.95	<0.001	1.18
		TL = 9.7827VLP + 25.752	60	0.96	<0.001	0.66
Bluegill	25-203	TL = 10.311VLR + 13.781	81	0.94	<0.001	-1.33
		TL = 10.057VLL + 13.739	83	0.96	<0.001	1.74
		TL = 10.176VLP + 13.794	163	0.95	<0.001	-1.31
Golden Shiner	78-221	TL = 9.804VLR + 37.261	26	0.88	<0.001	-0.23
		TL = 10.319VLL + 29.472	27	0.91	<0.001	-0.21
		TL = 10.077VLP + 33.129	53	0.90	<0.001	-0.22
Green Sunfish	25-171	TL = 8.0022VLR + 19.315	75	0.95	<0.001	-0.31
		TL = 8.1371VLL + 16.267	72	0.96	<0.001	0.66
		TL = 8.0425VLP + 18.079	146	0.95	<0.001	-0.19
Largemouth Bass	65-197	TL = 11.129VLR + 33.69	57	0.93	<0.001	-0.15
		TL = 11.636VLL + 27.535	60	0.94	<0.001	-0.2
		TL = 11.363VLP + 30.745	117	0.94	<0.001	-0.17
Longear Sunfish	55-142	TL = 7.5075VLR + 30.099	85	0.95	<0.001	-0.09
		TL = 7.8644VLL + 26.001	81	0.95	<0.001	-0.09
		TL = 7.6713VLP + 28.217	166	0.95	<0.001	-0.09
Red Shiner	37-75	TL = 8.0008VLR + 16.834	33	0.92	<0.001	-0.03
		TL = 7.8596VLL + 16.956	36	0.93	<0.001	-0.03
		TL = 7.9252VLP + 16.906	69	0.93	<0.001	-0.03
Redear Sunfish	54-183	TL = 8.1726VLR + 19.406	78	0.97	<0.001	-2.72
		TL = 8.4527VLL + 15.612	77	0.97	<0.001	-1.73
		TL = 8.1726VLP + 19.406	155	0.97	<0.001	-2.24
Saugeye	56-130	TL = 17.79VLR - 4.0251	45	0.96	<0.001	0.1
		TL = 17.563VLL - 4.073	44	0.98	<0.001	0.03
		TL = 17.661VLP - 3.9634	87	0.97	<0.001	0.07
White Crappie	91-174	TL = 9.43VLR + 40.056	37	0.89	<0.001	-0.01
		TL = 9.3281VLL + 39.717	37	0.94	<0.001	-0.01
		TL = 9.3692VLP + 39.975	74	0.92	<0.001	-0.01

cleithrum with a more elongated DPL, while the DPL on a Red Shiner cleithrum approximates a 90° angle, and also has a rounded anterior end on the horizontal limb (Figure 3).

Discussion

Reconstruction of original sizes and identification of prey from digested remains is essential to thoroughly characterize fish diets. While use of external features can be a quicker

method to identify a prey item (Scharf et al. 1997, Ball and Weber 2018), it may not be possible due to digestive decomposition. Numerous bony structures of fish have been used to identify the species or reconstruct the original size of the fish, including dentary, premaxilla, and maxilla bones (Hajkova et al. 2003, Wood 2005), otoliths (Tarkan et al. 2007, Snow et al. 2017, Assis et al. 2018), pharyngeal bones (Mann and Beaumont 1980, Hansel et al. 1988, Radke et al. 2000, Snow et al. 2017), opercula (Hansel et al.

1988, Scharf et al. 1998, Hajkova et al. 2003, Wood 2005), vertebrae (Trippel and Beamish 1987, Hajkova et al. 2003), and cleithra (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Snow et al. 2017). Hansel et al. (1988) found that cleithra and dentaries were found most often in stomach contents of piscivores and are the most reliable structures for identifying prey

fish. Further, cleithra measurements can be used to predict the original length of a fish (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Snow et al. 2017).

In our study, a significant linear relationship existed between the total length of a fish and the three cleithrum measurements (CL,

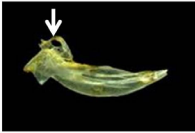
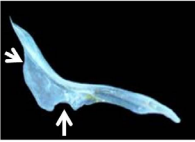

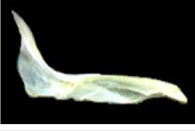
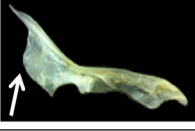
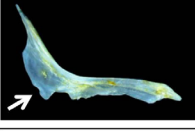
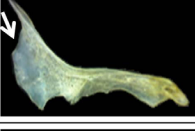
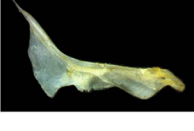
<u>Atherinidae</u>		
<p>Inland Silverside (<i>Menidia beryllina</i>)</p>		<ul style="list-style-type: none"> • Claw shaped cleithrum with holes in the DPL
<u>Centrarchidae</u>		
<p>Cleithra have a short spine at the tip of the vertical limb. The vertical limb is shorter than the horizontal limb. The dorsoposterior lobe is prominent and lacks serrations (Traynor et al. 2010).</p>		
<p>Black Crappie (<i>Pomoxis nigromaculatus</i>)</p>		<ul style="list-style-type: none"> • Small indentation in the top of the DPL (upper arrow) • The notch near the transition of the DPL and the horizontal limb is trapezoidal in shape (lower arrow)
<p>White Crappie (<i>Pomoxis annularis</i>)</p>		<ul style="list-style-type: none"> • The notch near the transition of the DPL to the horizontal limb is round in shape
<p>Largemouth Bass (<i>Micropterus salmoides</i>)</p>		<ul style="list-style-type: none"> • Flat across the horizontal limb • Thick spine
<p>Bluegill (<i>Lepomis macrochirus</i>)</p>		<ul style="list-style-type: none"> • The DPL is bulbous in shape
<p>Green Sunfish (<i>Lepomis cyanellus</i>)</p>		<ul style="list-style-type: none"> • Distinct notch on the back of the DPL • The tapering of the spine is distinguishable
<p>Longear Sunfish (<i>Lepomis megalotis</i>)</p>		<ul style="list-style-type: none"> • Very similar to a Bluegill • The top of the DPL dips slightly
<p>Redear Sunfish (<i>Lepomis microlophus</i>)</p>		<ul style="list-style-type: none"> • The DPL is slightly rectangular in shape • They have the longest, thinnest spine of all <i>Lepomis</i> species (in this study)

Figure 3. Photographs and description of cleithra diagnostic characteristics from twelve species of common forage species collected from Oklahoma reservoirs.

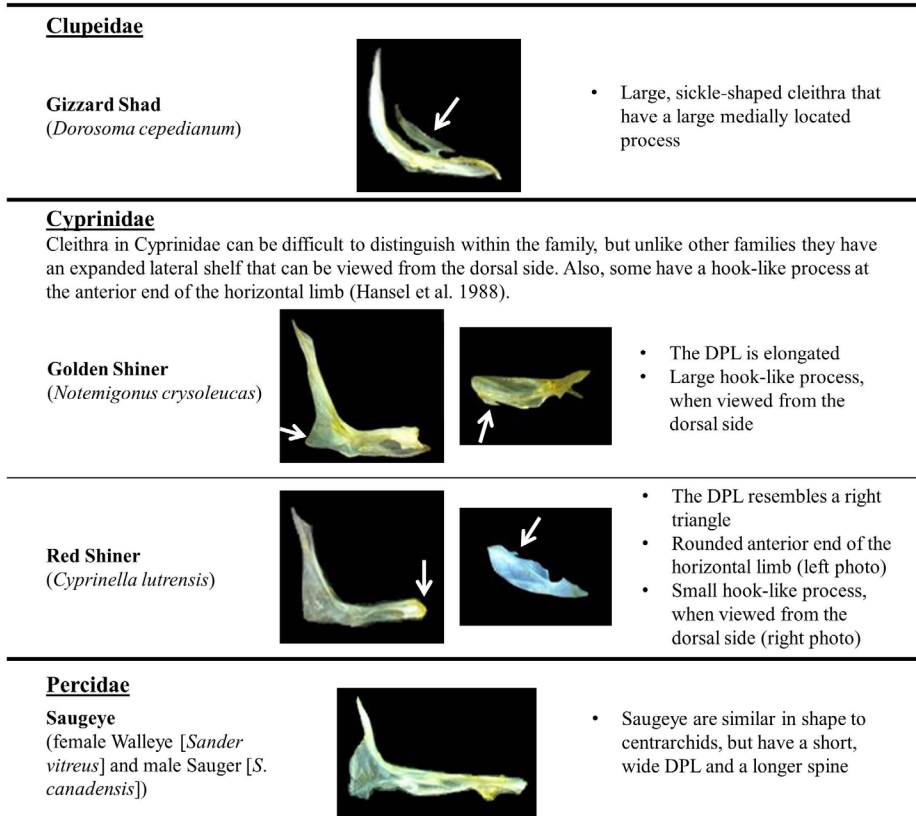


Figure 3. (Continued).

VL, and HL). Similarly, Wood (2005) found linear relationships between dentary, maxilla, premaxilla, opercle, and cleithrum measurements and live total length of prey fish found in Bluefish diets. Hansel et al. (1988) found slightly more accurate estimates of fish length predicted using cleithrum and opercle measurements than with measurements from pharyngeal arches or dentaries for 14 species. In a comparison of boney structures to predict total length for 10 fish species, cleithrum length was the most reliable measurement for predicting live fish length (Scharf et al.1998). Snow et al. (2017) found that measurements of cleithra, pharyngeal teeth, and otoliths accurately predicted total length of Central Stonerollers. Our results suggest that cleithra measurements can be used reliably to estimate the original fish length of the twelve species evaluated in this study.

In this study, all predictive equations from the three cleithra measurements produced reliable estimates of fish total length. This suggests that even if a spine or part of the horizontal limb is broken, an accurate predicted fish length can still be attained using a measurement from the intact portion of the cleithrum. When fresh from a diet, cleithra are soft and can be easily torn, so it is important to handle them with care. Although our results suggest that all may not be lost if a structure is damaged, several considerations should be made when using cleithra dimensions to reconstruct original prey size. The cleithra used in our study were boiled and cleaned. Scharf et al. (1998) suggested that boiling to remove soft tissue might cause bones to shrink or deform if an excess of time elapses between boiling and measuring. The cleithra in this study were only boiled long enough to loosen excess tissues, given time to dry, and were measured immediately after drying. Although not used

Table 5. Exponential equations for predicting weight (W) from total length (TL), and the resulting r² value for twelve fish species.

Species	Equation	R ²
Black Crappie	$W = 0.4307e^{0.0317(TL)}$	0.98
Bluegill Sunfish	$W = 0.1019e^{0.0534(TL)}$	0.93
Gizzard Shad	$W = 0.7342e^{0.0244(TL)}$	0.96
Golden Shiner	$W = 0.7411e^{0.0244(TL)}$	0.94
Green Sunfish	$W = 0.42e^{0.0348(TL)}$	0.94
Inland Silverside	$W = 0.0431e^{0.0504(TL)}$	0.91
Largemouth Bass	$W = 0.9201e^{0.0244(TL)}$	0.97
Longear Sunfish	$W = 0.6265e^{0.0333(TL)}$	0.98
Saugeye	$W = 0.1405e^{0.0372(TL)}$	0.98
Redear Sunfish	$W = 0.479e^{0.0334(TL)}$	0.96
Red Shiner	$W = 0.0725e^{0.0581(TL)}$	0.91
White Crappie	$W = 0.838e^{0.0248(TL)}$	0.96

in this study, preservatives also alter bone dimensions if used to store stomach contents (Hansel et al. 1988, Scharf et al. 1998, Snow et al. 2017). It is imperative that cleithra are handled cautiously to ensure diagnostic features are preserved, so accurate measurements can be taken.

Significant relationships were found between the three cleithra measurements and fish total length and weight of the twelve prey species evaluated in this study. Because the linear relationships reported in this study are for fish within a particular range of sizes, caution should be taken before applying lengths to fish outside of this range, as allometric relationships may change depending on fish size (Scharf et al. 1998). Although differences among genera can be subtle, cleithrum morphology can be used to identify fish remains for the twelve fish species in this study. Identification of prey items in piscivore diets using cleithra will allow for a more accurate depiction of fish diet breadth, which is important when investigating diets of top predators. When used collectively, the regression equations and diagnostic features described in this study from cleithra will provide a more accurate description of fish diets and a better understanding of predator-prey relationships.

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