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An Early Pliocene Fish Assemblage from the Southern Appalachians: Ichthyofauna of the Gray
Fossil Site

A thesis
presented to
the faculty of the Department of Geosciences
East Tennessee State University

In partial fulfillment
of the requirements for the degree
Master of Science in Geosciences, Paleontology

by
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August 2023

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Keywords: *Lepomis*, *Micropterus*, Centrarchidae, ichthyology, paleoecology

ABSTRACT

An Early Pliocene Fish Assemblage from the Southern Appalachians: Ichthyofauna of the Gray

Fossil Site

by

Shayleigh E. Maden

The Gray Fossil Site (GFS) preserves an early Pliocene sinkhole pond that accumulated a rich flora and vertebrate fauna. Fossils of fish are incredibly common at GFS, representing thousands of specimens recovered to date. Comparison with extant species reveals a depauperate freshwater fish fauna consisting of only two species: *Lepomis* sp. and *Micropterus* sp. The absence of fishes that are ubiquitous in modern streams suggests no perennial surface hydrologic connection between the Gray Fossil Site and the nearby Holston River. Limited opportunities for dispersal into the pond and deleterious conditions such as eutrophy and high competition may have resulted in this extremely low diversity. Examination of articulated and partially articulated GFS fish specimens show mild to moderate degrees of post-mortem disarticulation, suggesting slowed decay likely attributable to cool water temperatures and/or anoxic conditions within the hypolimnion. Preliminary examination of osteological thin sections suggests slow growth in GFS fishes.

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CHAPTER 1. INTRODUCTION

Southeastern Freshwater Fish Diversity

The southeastern United States is a hotspot of freshwater fish diversity, particularly for a temperate region (Lévêque et al. 2008; Elkins et al. 2019). More than 500 fish species inhabit the Mississippi, Tennessee, and South Atlantic-Gulf river basins, and the southern Appalachians are home to more than 350 endemic species (Lévêque et al. 2008). This diversity is thought to be due in part to the age and course of the Mississippi River, which drains much of the southeast. The Mississippi River has been draining the interior of North America since at least the Late Jurassic (Coleman 1988; Coleman et al. 1998) and has maintained a generally north-to-south course for this entire duration (Smith 1981). This allowed the river to act as a refugium for species during periods of sea level change and glaciation – tributaries that were isolated by such events were later recolonized by fishes surviving downriver (Smith 1981). Heterogeneity of habitats (Lévêque et al. 2008; Stokes et al. 2023) and topography (Badgley et al. 2017) in the southeastern U.S. are likely also a proximal source of freshwater fish diversity in the region as it results in suitable habitat for species with varying tolerances for temperature, gradient, substrate, etc. (Coblentz and Riitters 2004). Perhaps most importantly, this region escaped the Pleistocene glaciation that left many northern ichthyofaunae depauperate following the Last Glacial Maximum (Lévêque et al. 2008).

This has led to an understanding that the freshwater fish fauna of eastern North America (east of the Rocky Mountains) has remained relatively stable over the course of the Cenozoic, experiencing no major extinctions (Smith 1981). The presence of numerous basal ray-finned fishes such as paddlefish (Polyodontidae), gar (Lepisosteidae), and bowfin (Amiidae) supports this hypothesis (Smith 1981). The fossil record of eastern North America appears to provide

support as well – nearly all material identifiable to genus level has been assigned to genera still present in the region today (Uyeno & Miller 1963). Certainty in this stability is, however, undermined by the paucity of pre-Pleistocene freshwater fish fossils from eastern North America.

Cenozoic Fossil Record of Freshwater Fish in the Southeastern U.S

By far the largest contribution to the fossil record of freshwater fish in the southeastern United States comes from the late Pleistocene and early Holocene, primarily from cave deposits (e.g. Dickinson 1986; Jacquemin et al. 2016). These deposits seem to preserve an ichthyofauna nearly identical to that of the modern day, save for a handful of now-extralimital taxa and the recently extinct harelip sucker, *Moxostoma lacerum* (Etnier and Starnes 1993).

Freshwater fish are well-documented from a number of localities in Florida, including the late Miocene – early Pliocene Montbrook site (Hulbert 2018; Steadman and Takano 2019), early Pliocene Palmetto Fauna (Smith et al., 2014), and early Pleistocene Leisey Shell Pit (Scudder et al. 1995). Outside of Florida, however, the pre-Pleistocene freshwater fish record in the eastern U.S. is much sparser. Only three sites (Table 1) outside of Florida in the eastern U.S. preserve freshwater ichthyofaunae with only two located in the southeast.

Table 1 Pre-Pleistocene fossil records of freshwater fish from the eastern U.S. outside of Florida

Site	Age	Fish Represented	Reference
Pipe Creek Sinkhole, IN	~ 5 Ma (Farlow et al. 2001)	Ictaluridae, Centrarchidae	R. Stearley, pers. comm., Jun. 8 2023
Mauvilla Local Fauna, AL	7.3 – 6.8 Ma (Hulbert and Whitmore 2006)	<i>Aplodonitus grunniens</i> , <i>Ictalurus</i> , Lepisosteidae, unidentified teleosts	Ebersole and Jacquemin 2018
Gray Fossil Site, TN	4.9-4.5 Ma (Samuels et al. 2018)	Centrarchidae	Woodward 2011

Family Centrarchidae

The Gray Fossil Site ichthyofauna is dominated by centrarchids. Centrarchids, also known as freshwater sunfishes, are a speciose family of freshwater fish native to North America (Warren 2009). While widely introduced to freshwater habitats throughout North America and globally, their native range is mostly limited to warm, slow-moving waters in eastern North America with their greatest diversity occurring in the southeastern United States (Warren 2009; Page and Burr 2011). They are best known for the bright coloration of breeding males, their complex courtship and nesting behaviors, and their popularity in sportfishing (Warren 2009). Family Centrarchidae consists of eight accepted genera, four of which (*Ambloplites*, *Lepomis*, *Pomoxis*, and *Micropterus*) occur in the southern Appalachians (Etnier and Starnes 1993; Near and Koppelman 2009). Of these, *Lepomis* is by far the most diverse, containing 13 accepted species, though there is evidence for widespread polytypism (Birmingham and Avise 1986; Harris et al. 2005).

Centrarchids vary in body shape between highly laterally compressed, deep bodied fishes (e.g., *Lepomis*, *Pomoxis*) and more elongate and streamlined fishes (e.g., *Micropterus*) (Page and Burr 2011). Feeding habits vary among centrarchids, though all can be considered predators of invertebrates and other vertebrates (Collar and Wainwright 2009). Smaller species, like *Lepomis*, feed extensively on aquatic invertebrates such as insect larvae, crustaceans, and mollusks (Warren 2009). These species are often abundant within their habitats and form an important food source for larger, more piscivorous fish (Warren 2009). Larger centrarchids, particularly those in the genera *Pomoxis* (crappie) and *Micropterus* (black basses), are predators of other fishes, larger invertebrates such as crayfish, and even terrestrial vertebrates like frogs and rodents (Warren 2009; Page and Burr 2011). These fish are often top predators within their environment (Warren 2009).

The oldest record of centrarchids comes from the Late Eocene/Early Oligocene, ~35 mya (Cope 1883, Smith and Miller 1985, Near and Koppelman 2009). These early species belong to the extinct genera *Plioplarchus* and *Boreocentrarchus* and have yet to be formally described; little is known of their morphology or proposed ecology (Cavender 1986). All other centrarchid fossils belong to extant genera (Near and Koppelman 2009). Several extinct centrarchid species have been described, all from the Mississippi River drainage basin save for three species of *Archoplites* from the western U.S. and *Micropterus relictus* from western Mexico (Near and Koppelman 2009). The first record of extant species in the fossil record comes from the Rexroad local fauna of Kansas and the Sand Draw local fauna of Nebraska, both dated to ~3.4 Ma (Smith 1962; Smith and Lundberg 1972). Outside of Florida, there are as yet no published pre-Pleistocene records of centrarchids east of the Mississippi River.

Geologic Setting

The Gray Fossil Site (GFS) in Washington County, Tennessee is an early Pliocene locality dating to ca. 4.9-4.5 Ma based on relative dating of small mammals (Samuels et al. 2018; Samuels and Schap, 2021). Located in the southern Appalachians, GFS is the only site of its age in the Appalachian region. The site is interpreted as a series of collapsed sinkhole basins within the Cambro-Ordovician Knox Group carbonates which plugged, filled with water, and created a large (~4.5 acre) pond or series of ponds that preserve an exceptional assemblage of fossils within lacustrine sediments (Wallace and Wang 2004; Shunk et al. 2006; Whitelaw et al. 2008; Shunk et al. 2009). A diverse flora and vertebrate fauna are preserved from the site, including abundant mammals, fish, amphibians, reptiles, and numerous plant taxa (Parmalee et al. 2002; Wallace and Wang 2004; Desantis and Wallace 2008; Boardman and Schubert 2011; Mead et al. 2012; Ochoa et al. 2012; Worobiec et al. 2013; Jasinski and Moscato 2015; Jasinski 2018, 2023; Siegert and Hermsen 2020; Hermsen 2021; Quirk and Hermsen 2021).

Faunal and floral evidence indicate a closed oak-hickory forest surrounding the site (Desantis and Wallace 2008; Ochoa et al. 2012, 2016), contrasting starkly with the more open, grass-dominated environments which predominated in the late Cenozoic of North America (Graham 1999; Jacobs et al. 1999; Hulbert et al. 2009; Edwards et al. 2010; Strömberg 2011). Isotopic and palynofloral analyses suggest a paleoclimate that was warmer and experienced more precipitation and less seasonality than east Tennessee today (Desantis and Wallace 2008, Reichgelt et al. 2023). Ecometric estimates of paleoclimate based on small mammal teeth also point to warmer and wetter conditions (Schap et al. 2021).

The presence of perennial aquatic habitat at GFS is well supported by sedimentological, floral, and faunal evidence. The sinkhole fill consists of graded sediments overlain by

fossiliferous rhythmic clays (Shunk et al. 2006). These clays are interpreted as accumulated sediments from a low-energy, poorly oxygenated lacustrine system with no evidence of periodic drying (Shunk et al. 2009). GFS rhythmites are thought to be the result of cyclical, possibly seasonal, increases in precipitation which increased the inflow of sediment and organics into the sinkhole pond (Shunk et al. 2009). Components of the GFS flora also indicate riparian or marshy habitat, such as tupelo, cypress, and multiple species of algae (Brandon 2013; Noll 2013; Worobiec et al. 2013). Abundant aquatic and semiaquatic vertebrates recovered from the site include salamanders, turtles, fish, ducks, and tapirs (Hulbert et al. 2009; Boardman and Schubert 2011; Steadman 2011; Woodward 2011; Bourque and Schubert 2015; Jasinski 2018). Other aquatic and semiaquatic taxa such as beavers, desmans, and alligators are recovered with less frequency (Parmalee et al. 2002; Mead et al. 2012; Oberg and Samuels 2022).

CHAPTER 2. METHODOLOGY

Specimen Collection and Material Analyzed

The majority of GFS fish material is recovered through wet-screening of excavated sediments using 1.7 mm mesh. This material thus presents as disarticulated, fragmentary, and often isolated elements. Each screened sediment sample represents ~ 5 gallons of excavated sediment from an individual 1 m. by 1 m. square. Some samples contain multiple paired elements of approximately the same size that are referable to the same taxon (e.g., left and right premaxillae with left and right dentaries of *Lepomis*), these are treated as associated elements. While some elements can be confidently referred to individual species, most isolated components of a fish skeleton (i.e. vertebrae, ribs, dorsal spines) do not contain morphological features sufficient for diagnosis below the family level. Systematic paleontology results focus on material that is taxonomically diagnostic to the genus level.

Osteological Comparison

Morphological comparisons with extant centrarchid skeletal material were used to identify GFS fish material. Anatomical terminology follows Rojo (1991). A list of comparative specimens can be found in Table 1. Comparative material came from the University of Florida Environmental Archaeological collection (UF), Cornell University Museum of Vertebrates (CUMV), and the East Tennessee State University Museum of Natural History zoology collection (ETMNH-Z). Images of isolated elements were taken using a Dino-Lite Edge MZ4815 digital microscope and DinoCapture 2.0 (v. 1.5.43) imaging software. Articulated and semi-articulated specimens were photographed using a Nikon D3100 DSLR camera.

Table 2 List of specimens used in osteological comparison. (* See remarks in *Micropterus* systematic paleontology)

Taxon	Common Name	Specimens	n
<i>Ambloplites ariommus</i>	Shadow bass	UF 49273	1
<i>Ambloplites rupestris</i>	Rock bass	CUMV 71484, 72920; ETVP 7053	3
<i>Enneacanthus obesus</i>	Banded sunfish	UF 20178	3
<i>Pomoxis nigromaculatus</i>	Black crappie	CUMV 28251; UF 12246-S, 32055	3
<i>Micropterus dolomieu</i>	Smallmouth bass	CUMV 72897, 72890; ETVP 10431; UF 20188	4
<i>Micropterus salmoides</i> *	Largemouth bass	CUMV 73779, 71812; UF 26999	3
<i>Lepomis auritus</i>	Redbreast sunfish	UF 26990	3
<i>Lepomis gibbosus</i>	Pumpkinseed	CUMV 71816, 72851	2
<i>Lepomis gulosus</i>	Warmouth	CUMV 74789; UF 26992	2
<i>Lepomis macrochirus</i>	Bluegill	CUMV 71493, 71507; UF 26994	3
<i>Lepomis marginatus</i>	Dollar sunfish	UF 32050	1
<i>Lepomis megalotis</i>	Longear sunfish	UF 26995	1
<i>Lepomis microlophus</i>	Redear sunfish	UF 32252	1
<i>Lepomis punctatus</i>	Spotted sunfish	UF 20520	1

Osteohistology

Ten atlantes were selected for osteological thin sectioning, all derived from the same excavation area (TP2-2004, “Rhino Pit”) and excavated within two consecutive months of the 2007 field season. Preparation of thin sections followed Lamm (2013). Individual atlantes were glued to cured Silmar® resin blocks using liquid cyanoacrylate. Labels indicating specimen number and atlas orientation were also glued to the resin blocks. Individual atlantes and

associated labels were then fully embedded in Silmar® resin. Embedding containers were then placed in a Fisher Isotemp® vacuum oven at -28 hg for ~10 minutes to ensure specimen impregnation with resin. Specimens were then refrigerated overnight at 3-5°C to slow curing and further encourage resin impregnation. Embedding containers were then placed under a fume hood until cured.

Cured blocks were trimmed using a diamond blade tile saw and wafered on a model 65-C Ingram Thin Section Saw/Grinder. Five specimens were sectioned along their sagittal axis; the remaining five were sectioned along their transverse axis. Before wafering, labels were drawn on specimens and on the outside of resin blocks to provide reference for the orientation of the atlas in each block. Wafers were adhered to acrylic slides using cyanoacrylate and ground to appropriate thickness using a Buehler Ecomet II grinder polisher. A layer of Permaslip® liquid coverslip material was added to the exposed surface of the atlantes to improve visibility and prevent cracking.

Three-dimensional Imaging of Specimens

ETMNH 7286, a semi-articulated specimen, as well as all 10 atlantes used in osteohistological analysis were scanned using a Skyscan model 1273 X-ray computed tomography scanner at East Tennessee State University. Scans were sectioned and 3-D models produced using ORS Dragonfly software (v. 2020.2) (Dragonfly 2020).

CHAPTER 3. RESULTS

Systematic Paleontology

CLASS Actinopterygii Klein, 1885

ORDER Perciformes Bleeker, 1863

FAMILY Centrarchidae Bleeker, 1859

GENUS *Micropterus* Lacepède, 1802

Micropterus sp.

Figures 1-4

Referred specimens: ETMNH 36469– Sediment block containing quadrate, scales, impressions of cranial bones; ETMNH 35765 – left and right premaxillae, left dentary and left articular, left hyomandibular, right quadrate (figured); nasals, cleithrum, vertebrae (not figured);

ETMNH 7064 – right dentary;

ETMNH 7159 – left dentary;

ETMNH 33666 – third suprapharyngeal, infrapharyngeal



Fig. 1 Impression of articulated *Micropterus* sp. specimen (ETMNH 36469) from Gray Fossil Site. Scale = 5 cm

Micropterus sp. Description

ETMNH 36469 (Fig. 1) is the only articulated GFS fish specimen readily referable to *Micropterus*. This specimen is a sediment block containing a quadrate, partial preopercle, and numerous scales. Impressions of other cranial bones, including the dentary, articular, premaxilla, maxilla, and opercle, are preserved and clearly outline the skull of the fish (Fig. 2). A few faint impressions of vertebrae are present, as well as slight impressions below the opercular region which may represent branchiostegal rays.



Fig. 2 Detail and skull outline in impression of *Micropterus* sp. specimen (ETMNH 36469) from Gray Fossil Site

ETMNH 35765 was excavated from a single jacketed skeleton and comprises multiple associated elements from a single individual. ETMNH 36469 is a sediment block containing a quadrate, partial preopercle, scales, and impressions of many cranial bones.

Premaxilla. The left premaxilla of ETMNH 35765 (Fig. 3A-B) is relatively complete but teeth, most of the ascending process, and the end of the element posterior to the anterior margin of the postmaxillary process are not preserved. The right premaxilla (Fig. 3C-D) consists of only the anterior end of the element with no teeth, but ascending and articular processes are intact. The tooth row consists of ~5 rows of small, evenly distributed pedicels with no lateral row of larger teeth. On the anterior end of the premaxilla, the tooth row extends dorsally up the medial side of the element, nearly to the base of the ascending and articular processes. There is no pronounced shelf above the tooth row on the medial or lateral faces of the element.

Articular processes of both left and right premaxillae of ETMNH 35765 have slight chipping along the dorsal margin. There is a small foramen on the lateral face of the articular process. Medially, the ventral portion of both articular processes bear a robust, nearly hemispherical tuberosity (Fig. 3D). The intact ascending process of the right premaxilla is rugose, slightly flattened, and slightly curved posteromedially. Both premaxillae exhibit mild rugosity on the lateral faces of the ascending and articular processes.

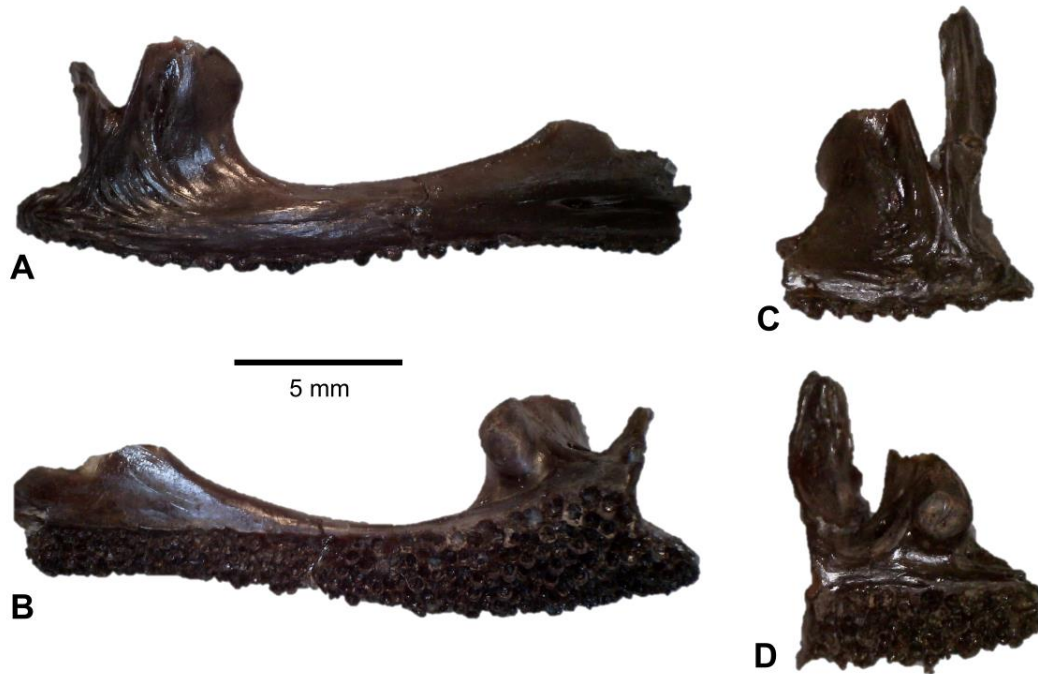


Fig. 3 Premaxillae of *Micropterus* sp. (ETMNH 35765) from Gray Fossil Site. A) Left premaxilla, lingual view. B) Left premaxilla, labial view. C) Right premaxilla, lingual view. D) Right premaxilla, labial view. Scale = 5 mm

Dentary. The left dentary of ETMNH 35765 (Fig. 4D) is largely intact, though the coronoid process and posterior portion of the tooth row are not preserved. The ventral limb is intact and has been prepared affixed to the left articular. This element lacks teeth; as in the premaxillae, the size of tooth pedicels is homogenous across the tooth row with no evidence of a lateral row of larger teeth. The tooth row itself is slightly convex anteriorly with a small horizontal sulcus extending across much of the lateral face of the dentary just ventral to the tooth row. Two to three small foramina sit within this sulcus.

Three prominent foramina are located on the lateral face of the dentary (Fig. 4D). One small foramen is located on the absolute anterior extent of the dentary, near the mandibular

symphysis; another slightly larger “mental” foramen is located just dorsal to where the ventral limb of the dentary begins to descend. The third and largest foramen is posterior and slightly dorsal to the second. The ventral limb of the dentary forms a deep, anteriorly convex arc anterior to its articulation with the articular.

Articular/Angular. The left dentary of ETMNH 35765 (Fig. 4D) is prepared affixed to the left dentary of the specimen. The retroarticular (also called the angular) was also affixed to the articular during preparation. This element is almost entirely intact, missing only the anteriormost end of the anterior process with some slight chipping just anterior to the quadrate facet. Overlap of the dentary over the anterior portion of the articular obscures the lateral aspect of the inferior crest, but no other prominent features are obscured. This is exceptional preservation for GFS articulars; typically, the delicate anterior portion of the element is damaged and only the quadrate facet is preserved.

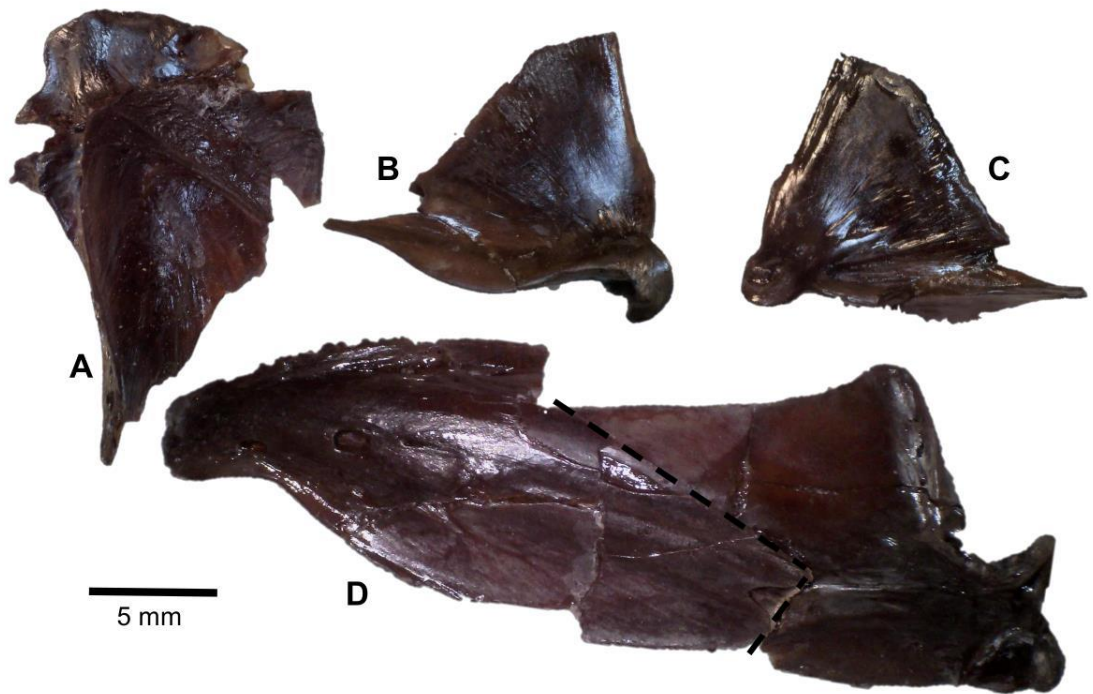


Fig. 4 Hyomandibular, quadrate, dentary, and articular/angular of *Micropterus* sp. (ETMNH 35765) from Gray Fossil Site. A) Left hyomandibular, lateral view. B) Right quadrate, medial view. C) Right quadrate, lateral view. D) Left dentary and articular with retroarticular, lateral view. Dashed line denotes separation between dentary and articular. Scale = 5 mm

The quadrate facet forms a simple saddle-shaped articulation with no additional crests or sulci within the facet itself. The postarticular process is tall and broad, reaching nearly a third the height of the coronoid process. The prearticular fossa is shallow and does not extend far past the coronoid process. The anterodorsal margin of the anterior process rises smoothly to meet the coronoid process, forming a slightly rounded triangle along the dorsal margin of the element.

Quadrate. Only the right quadrate of ETMNH 35765 (Fig. 4B-C) is represented, but it is in excellent condition with only slight chipping along its ventromedial margin. A single quadrate is represented in ETMNH 36469 (Fig. 4) and is in similar condition. The bone is generally triangular and flattened over most of its extent save for the anteroventral end which is more robust. This anteroventral end consists of two condyles which articulate with the quadrate facet of the articular, with the lateral condyle slightly larger and extending more ventrally than the medial condyle. The preopercular process extends well beyond the posterior margin of the body of the quadrate and the process itself is slightly convex ventrally. The symplectic incisure between the preopercular process and the body of the quadrate is narrow and extends into a shallow groove which extends nearly all the way to the lateral and medial condyles.

Hyomandibular. The hyomandibular is a flattened, irregularly shaped bone with numerous facets which form part of the jaw suspensorium. Preservation of the hyomandibular of ETMNH 35765 (Fig. 4A) is typical of hyomandibula from GFS – the margin of the bone is highly damaged, with only the central portion of the bone alongside the preopercular groove intact. Despite this damage, it is evident that the dorsal half of the element is broad and nearly square in shape and tapers sharply ventrally. On this specimen, only the sphenotic and pterotic facets on the more robust dorsal margin of the element are present with all other facets along the margin are missing due to damage. These facets are directly adjacent to one another and generally ovate in shape. A prominent L-shaped ridge on the lateral face of the element just anterior to the preopercular groove is also chipped along its lateral margin but is relatively intact and distinctive. The ventral limb of the element, including the sphenotic facet, is not preserved.

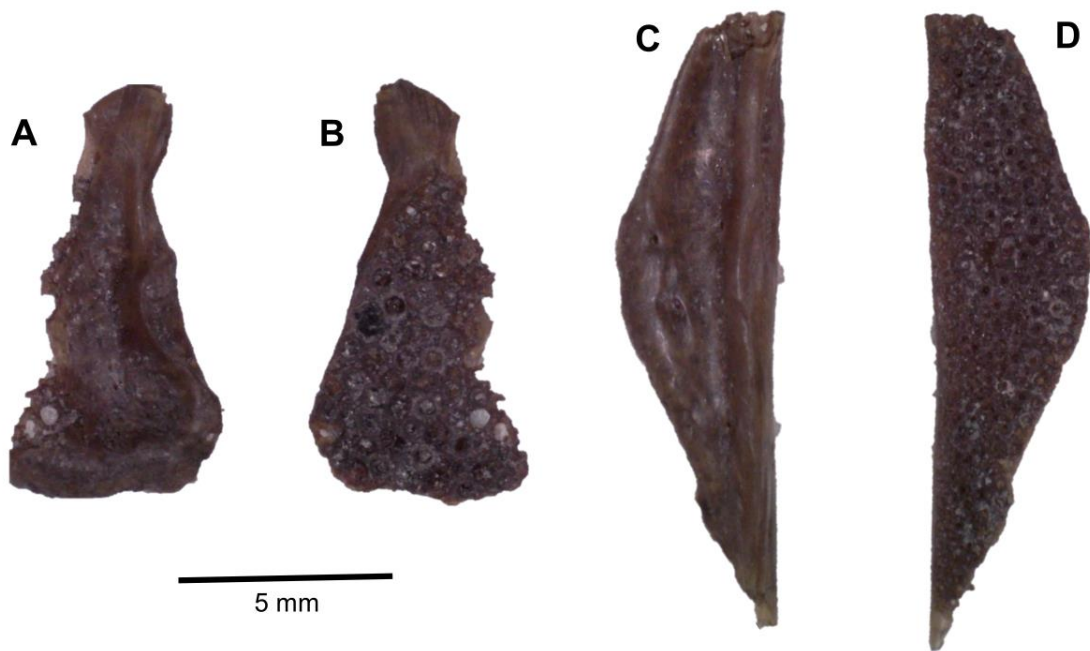


Fig. 5 Third suprapharyngeal and infrapharyngeal of *Micropterus* sp. (ETMNH 33666) from Gray Fossil Site. A) Third suprapharyngeal, dorsal view. B) Third suprapharyngeal, ventral view. C) Infrapharyngeal, ventral view. D) Infrapharyngeal, dorsal view. Scale = 5 mm

Third suprapharyngeal. The third suprapharyngeal (Fig. 5A-B) is a flat dentigerous bone, loosely triangular in shape. The dorsal surface is concave centrally and a small lip of bone follows the dorsolateral margin of the element. The ventral surface bears a tooth patch covering all of the element save for a small projection extending posteriorly. The tooth patch consists of 4-6 rows of pedicels with those near the center of the element slightly larger than the pedicels around its margin. No *Micropterus* suprapharyngeals have been recovered with teeth still attached.

Infrapharyngeal. The infrapharyngeal (Fig. 5C-D) is an elongate, nearly rod-like bone. Its dorsal surface bears a large tooth patch that covers virtually all of the element. At its widest point, this tooth patch bears 6-7 rows of pedicels; at its narrowest, 1-3 rows of pedicels. Pedicels on the infrapharyngeal are significantly smaller than those of the suprapharyngeals. Posteriorly, the tooth patch ends and a very small process is present for attachment to the ceratobranchials, though this feature is almost always not preserved in GFS material. As with the suprapharyngeals, no *Micropterus* infrapharyngeals have been recovered with teeth.

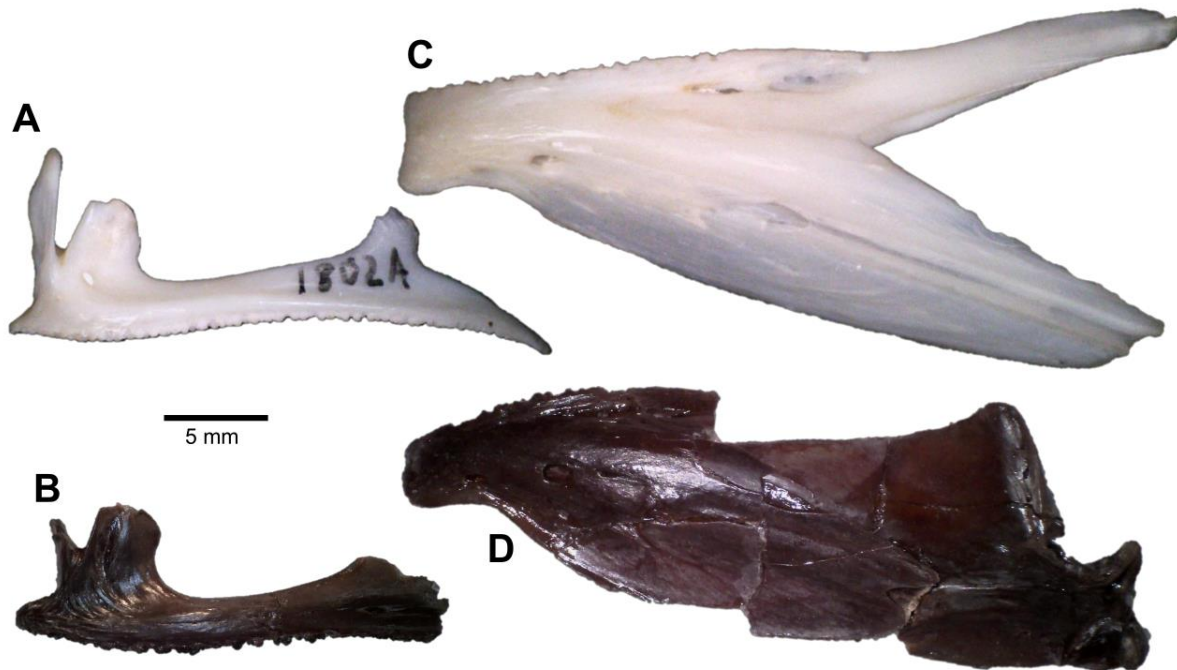


Fig. 6 Comparison between GFS *Micropterus* sp. premaxilla & dentary and *M. salmoides* premaxilla and dentary: A) UF 26999, *M. salmoides*, left premaxilla. B) ETMNH 35765, *Micropterus* sp., left premaxilla. C) UF 26999, *M. salmoides*, left dentary. D) ETMNH 35765, *Micropterus* sp., left dentary and articular/angular. Note that attachment of dentary and articular results in a slightly different orientation to C

Remarks. Numerous disarticulated *Micropterus* remains have been recovered from GFS, with premaxillae, dentaries, and pharyngeal jaws most easily referred to the genus. Remains are typically fragmentary; anterior (symphyseal) ends of premaxillae and dentaries, isolated condyles of quadrates, and isolated quadrate facets of articulars are most commonly preserved. *Micropterus* skeletal material is distinguishable from that of other centrarchid genera based on features of the premaxilla and dentary. Size of tooth pedicels on these elements is homogenous across the tooth row, distinguishing them from *Lepomis*, *Ambloplites*, and *Pomoxis* which have a

row of larger teeth on the labial margin of the dentary and premaxilla. Features of GFS *Micropterus* premaxillae strongly resemble *M. salmoides* and *M. floridanus*, including a dorsal expansion of the tooth row on the anteromedial face of the element (Fig. 3B) and the presence of a small foramen between the ascending and articular processes (Fig. 7).

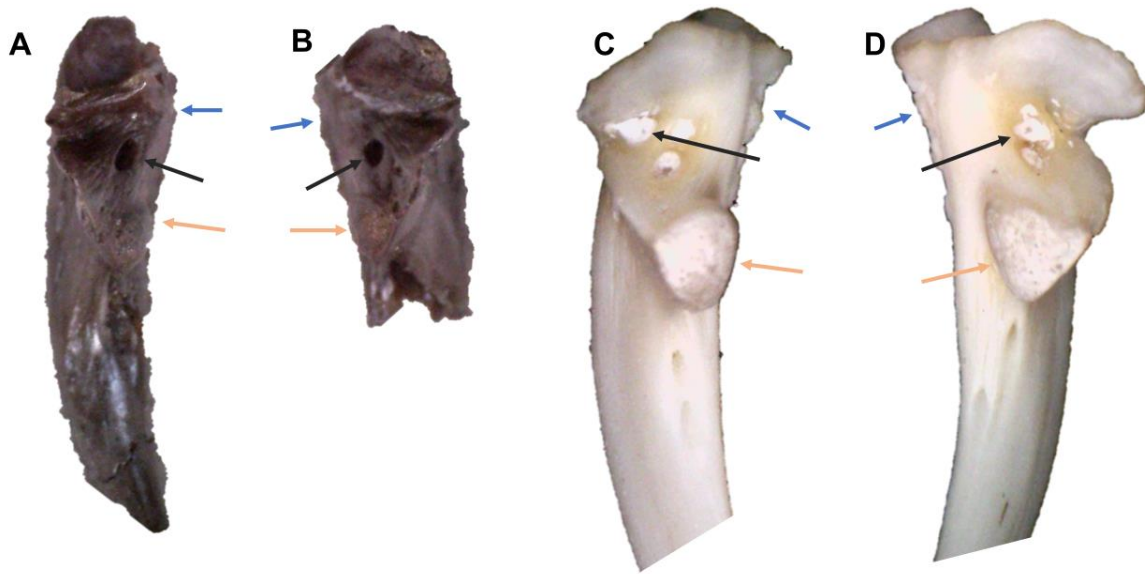


Fig. 7 Dorsal view of *Micropterus* premaxillae showing foramina between ascending and articular processes. A) ETMNH 35765, *Micropterus* sp., left premaxilla. B) ETMNH 35765, *Micropterus* sp., right premaxilla. C) UF 26999, *M. salmoides*, left premaxilla. D) UF 26999, *M. salmoides*, right premaxilla. Blue arrows indicate ascending process, orange arrows indicate articular process, black arrows indicate foramen

Features of the dentary also resemble *M. salmoides* and contrast with *M. dolomieu*: a slightly convex tooth row, presence of a horizontal sulcus just ventral to the tooth row, and positioning of lateral foramina are all consistent with *M. salmoides*/*M. floridanus* and not *M. dolomieu*. However, other features in GFS *Micropterus* dentaries differ from *M. salmoides* and

M. floridanus: 1) lateral foramina larger; 2) anterior end of the element shallower dorsoventrally; 3) body of the ventral process deeper. These latter two features more closely resemble *M. dolomieu*.

Based on these similarities, GFS *Micropterus* material is referred only to *Micropterus* sp. Recent phylogenomic work by Kim et al. (2022) showed that *M. salmoides* holotype material, collected from Florida, is in fact referable to the Florida bass (previously *M. floridanus* [Lesueur 1822]). As such, Kim et al. (2022) elevate the former junior synonym *M. nigricans* (Cuvier 1828) for the largemouth bass and state that *M. salmoides* should refer to the Florida bass. Given the long standing of *M. salmoides* in referring to the largemouth bass, it is here chosen to use *M. salmoides* to refer to the largemouth bass pending publication of an ICZN opinion in favor of this nomenclatural change.

GFS *Micropterus* material is referred to *Micropterus* sp. based on the aforementioned combination of features seen in the premaxilla and dentary. Further comparison with smaller and/or younger specimens of *Micropterus* species is necessary to rule out the role of ontogeny or allometry in some of the skeletal distinctions between GFS *Micropterus*, *M. salmoides*, *M. floridanus*, and *M. dolomieu*. Comparison with more recently elevated *Micropterus* species (e.g., *M. chattahoochae* Baker et al. 2013) will also serve to strengthen confidence in identification of GFS *Micropterus* material. Additionally, Kim et al. (2022) indicates divergence dates for the largemouth (*M. salmoides*) and Florida (*M. floridanus*) basses between 3.73 and 1.13 Ma, meaning GFS material predates this estimated divergence and may thus represent an ancestral stock not readily comparable to either extant species, or a distinct species unto itself.

GENUS *Lepomis* Rafinesque, 1819

Lepomis sp.

Figures 8-10

Referred specimens: ETMNH 35720 – Left and right premaxillae, right maxilla, left and right dentaries, 3rd suprapharyngeal, infrapharyngeal;

ETMNH 7295 – right premaxilla;

ETMNH 8593 – left premaxilla;

ETMNH 36505 - left premaxilla;

ETMNH 7406 – left premaxilla;

ETMNH 7386 – left dentary;

ETMNH 33666 – right premaxilla

Additional referred specimens (not figured) in Appendix A

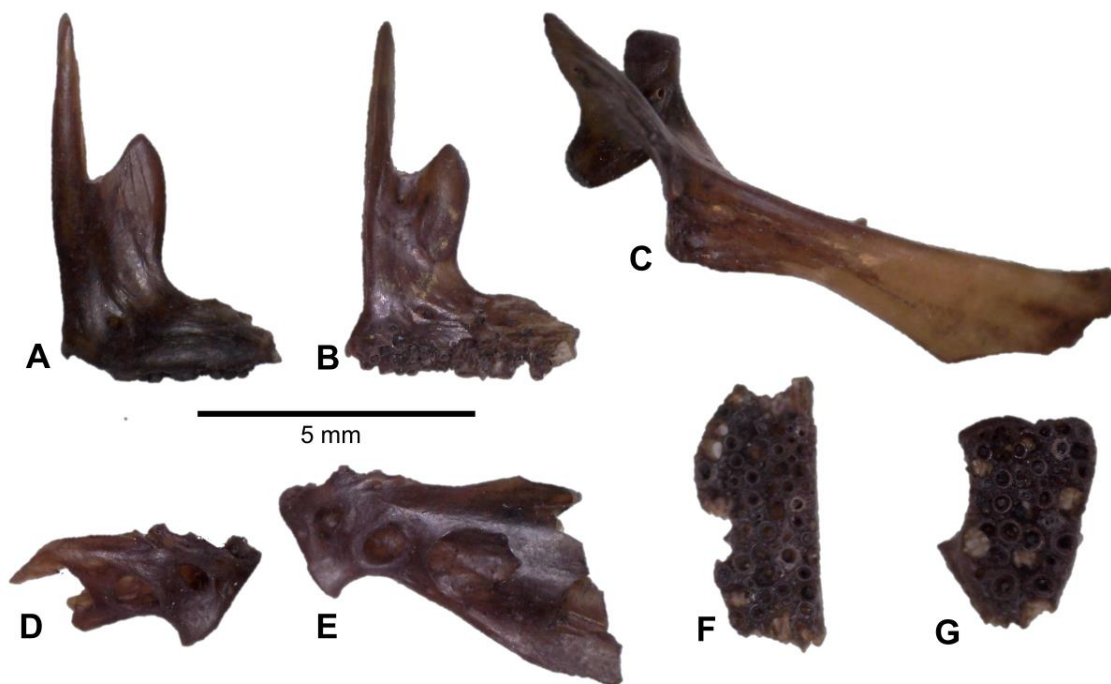


Fig. 8 *Lepomis* sp. skeletal material (ETMNH 35720) from Gray Fossil Site: A) left premaxilla, labial view. B) Right premaxilla, lingual view. C) Right maxilla, medial view. D) Right dentary,

lateral view. E) Left dentary, lateral view. F) Infrapharyngeal, dorsal view. G) Third suprapharyngeal, ventral view. Scale = 5 mm

Lepomis sp. Description

ETMNH 35720 represents associated specimens from a single sediment sample.

Premaxilla. Premaxillae of ETMNH 35720 (Fig. 8A-B) show typical preservation for GFS *Lepomis* premaxillae. The anterior end of the element is intact but broken posterior to the more robust portion of the tooth row posterior to the articular process. Ascending and articular processes are both intact, but teeth are absent. The element is slightly curved medially and the tooth row consists of 2-3 rows of small pedicels with a single row of much larger pedicels laterally.

The ascending process is straight and terminates dorsally in a point. This feature is variably cylindrical or slightly concave posteriorly in cross section. Laterally, the dorsal margin of the articular process meets the ascending process either seamlessly with a “shelf” of bone connecting the features (Fig. 9A-B) or a small sulcus which extends a short distance ventrally (Fig. 9C-D).

Articular processes of GFS *Lepomis* vary near-continuously in shape from posteriorly rounded (Fig. 9A) to sharply pointed dorsally (Fig. 9B) to leaf-shaped (Fig. 9C-D), with the last being the most common. Also variable is the position of a foramen on the lateral surface of the element ventral to and between the ascending and articular processes. This foramen is always situated within a sulcus, but the depth and position of this sulcus varies from a deep sulcus just above the tooth row (Fig. 9A-B) to a shallow sulcus more dorsally (Fig. 9C-D). Regardless of articular process conformation, all GFS *Lepomis* exhibit a similar medial surface of the articular process which bears a small ovate tuberosity. The tooth row extends only a short distance

dorsally on the medial face of the element and tooth pedicels are typically slightly smaller on this face.

The posterior portion of the premaxillary tooth row is generally not preserved in GFS material and is always broken away from the anterior portion if present. Specimens with this portion of the element preserved exhibit a short, subtriangular postmaxillary process. The caudal process is rarely preserved in GFS material.

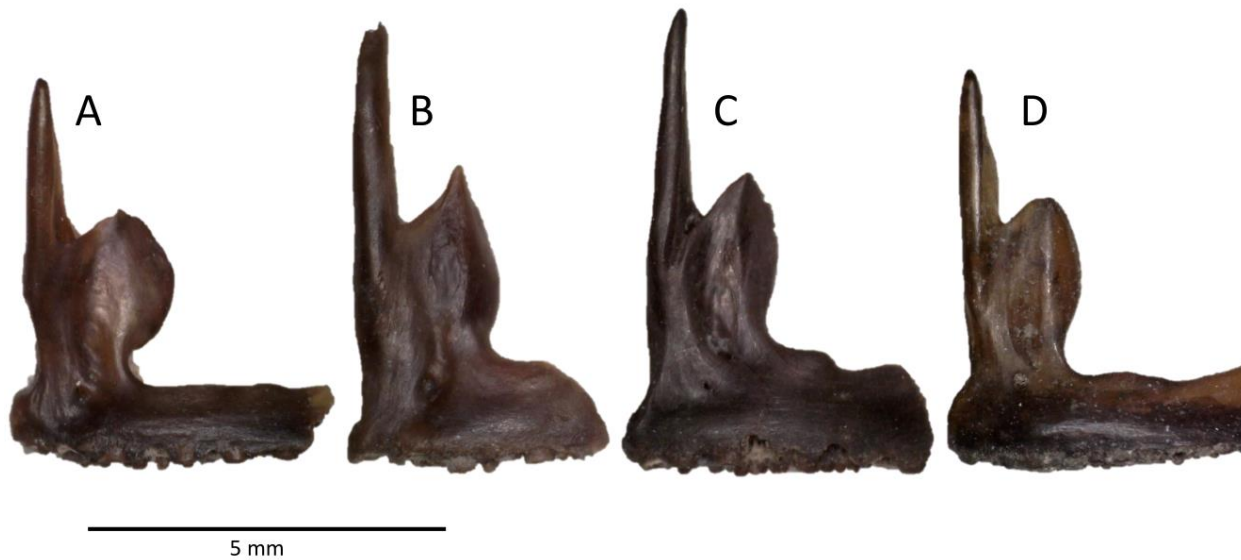


Fig. 9 Variation in *Lepomis* sp. premaxillae from Gray Fossil Site: A) ETMNH 7295, right premaxilla (mirrored). B) ETMNH 8593, left premaxilla. C) ETMNH 36505, left premaxilla. D) ETMNH 7406, left premaxilla. Scale = 5 mm

Dentary. GFS *Lepomis* dentaries (Fig. 8D-E) are generally poorly preserved. The anterior (symphyseal) end of the element is most often preserved (see Fig. 8D). The tooth row is often poorly preserved, being broken during fossilization or screening just anterior to the large mental foramen. Teeth are never preserved attached to the dentary. The dentary is short with a relatively deep ventral process. The lateral face of the dentary bears three large foramina. The anteriormost of these foramina is the smallest, while the most posterior is approximately three times the circumference of the anteriormost. Dorsal to the two posterior foramina on the lateral face and just ventral to the tooth row sits the large ovate mental foramen. A notable feature of GFS *Lepomis* dentaries is a hook-shaped projection that extends ventrally from the anterior end of the element. This feature is present on all GFS *Lepomis* dentaries, though it is variable in size.

Maxilla. GFS *Lepomis* maxillae (Fig. 8C) are often fragmentary, with the anterior portion consisting of the internal and external processes usually separated from the remaining body of the element. Intact maxillae are exceedingly short and bear no teeth. A small, nearly triangular crest is present posterior to the palatine sulcus. The posterior margin of the element, consisting of the maxillary and caudal processes, is generally square in shape when both processes are intact (note maxillary process is not preserved in Fig. 8C).

Suprapharyngeal. Third suprapharyngeals (Fig. 8G) from GFS *Lepomis* are the most commonly preserved pharyngeal elements at GFS and some of the only elements to still have teeth present when recovered. Teeth, when present, are small, slightly rounded cones. The third suprapharyngeal is roughly triangular in shape and bears 4-5 rows of teeth on its ventral surface. These elements are gracile and relatively flat dorsoventrally.

Infrapharyngeal. Infrapharyngeals (Fig. 8F) bear most of the same characteristics as third suprapharyngeals, apart from being narrower and more elongated anteroposteriorly. The dorsal surface bears 4-5 rows of teeth; as in suprapharyngeals, these teeth are small and rounded when present. Posteriorly, the tooth patch ends and there is a small rod-like process for attachment to the ceratobranchials, but this is rarely preserved in GFS material.

Remarks. *Lepomis* material represents by far the most common vertebrate material recovered from GFS sediments, occurring much more frequently than even *Micropterus*. In TP2-2004 (“Rhino Pit”) sediments, diagnostic *Lepomis* elements outnumber those of *Micropterus* by approximately 1:5; in TP1-2012 (“Tortoise Target”) sediments, this ratio increases to approximately 1:20. Anterior (symphyseal) ends of premaxillae and dentaries, maxillae, and pharyngeal jaws are the most commonly preserved diagnostic elements. *Lepomis* skeletal material can be distinguished from that of other centrarchids based on features of the premaxilla and dentary. In *Lepomis*, the ascending process is tall, gracile, and typically not angled anteriorly or posteriorly. This differs from *Micropterus*, *Pomoxis*, and *Ambloplites* wherein the ascending process is generally curved posteriorly or angled anteriorly. *Lepomis* also exhibits a much smaller tuberosity on the medial surface of the premaxillary articular process than the aforementioned genera. Dentaries of *Lepomis* are distinct in the large size of the mental foramen as well as other foramina located on the lateral surface of the dentary.

The length of the maxilla and dentary indicate that GFS *Lepomis* is a “short-jawed” species dissimilar to the “long-jawed” species *L. gulosus* and *L. cyanellus*. Likewise, the pharyngeal elements recovered from GFS are gracile, unlike the hypertrophied pharyngeals of *L. microlophus* and *L. gibbosus*. GFS *Lepomis* most closely resembles the spotted sunfish, *L. punctatus* (Fig. 10) and the longear sunfish, *L. megalotis*. Assignment to either of these species is

avoided, in part, due to the high degree of morphological variability seen in GFS *Lepomis* premaxillae. Other GFS *Lepomis* elements are more morphologically consistent, but differ from both of the aforementioned species in features of the dentary. Estimated molecular divergence dates were calculated for *Lepomis* species by Near and Kim (2021). Their results indicate that GFS may predate the divergence between several *Lepomis* lineages and thus GFS *Lepomis* may not be readily referable to an extant species.

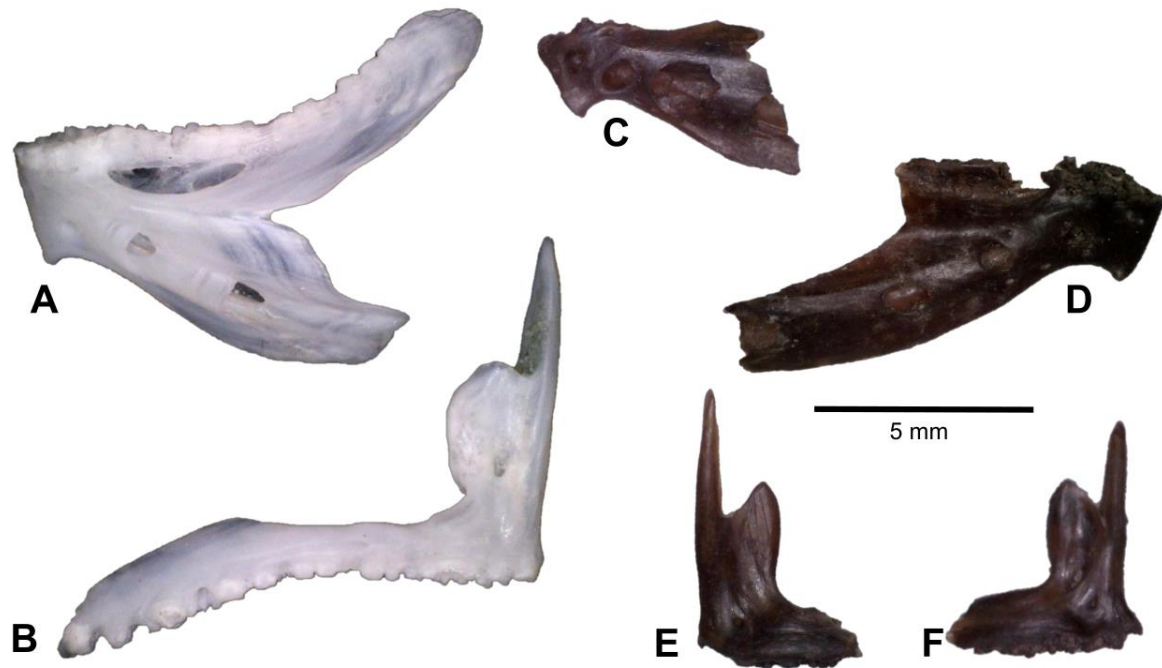


Fig. 10 *Lepomis* skeletal material comparison: A) UF 20520 *L. punctatus*, left dentary. B) UF 20520 *L. punctatus* right premaxilla. C) ETMNH 35720, *L. sp.*, left dentary. D) ETMNH 7386, *L. sp.*, right dentary. E) ETMNH 35720, *L. sp.*, left premaxilla. F) ETMNH 33666, *L. sp.*, right premaxilla.

Centrarchidae indet.

Referred specimens: ETMNH 36470 – sediment block containing semi-articulated cranial material;

ETMNH 7286 – sediment block containing articulated postcrania;

ETMNH 36467 – sediment block containing articulated postcrania;

ETMNH 36468 – sediment block containing semi-articulated postcrania;

ETMNH 36666 – otolith;

Numerous assorted isolated or fragmentary non-diagnostic elements (Appendix B)

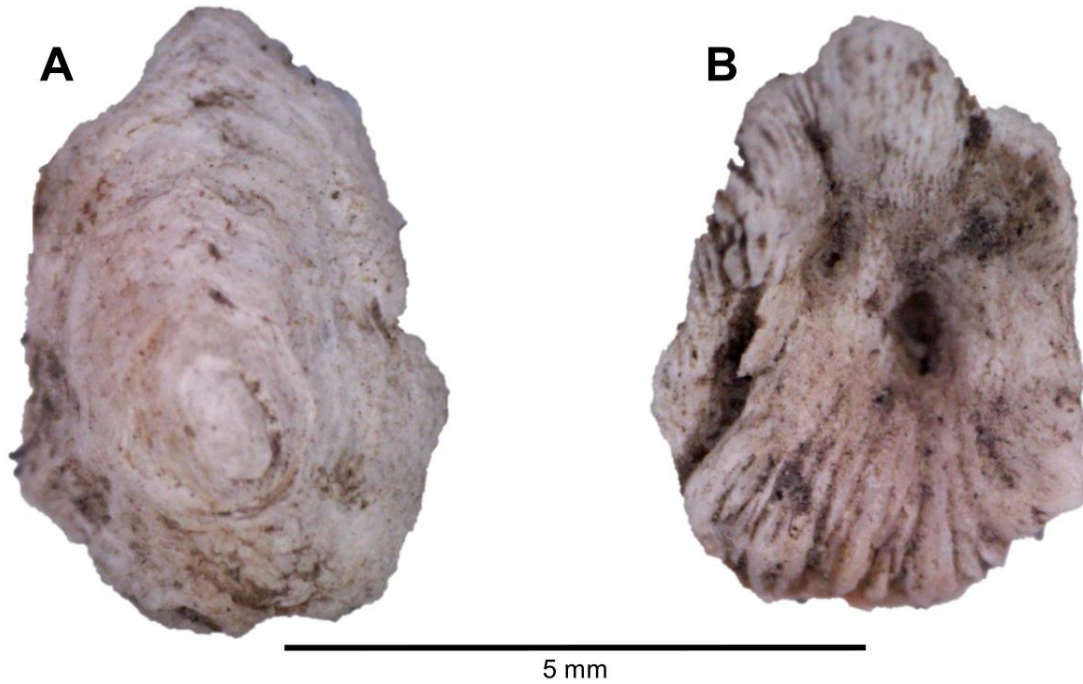


Fig. 11 Sagittal otolith from Gray Fossil Site. A) Medial view. B) Lateral view. Scale = 5 mm



Fig. 12 ETMNH 36470. Scale = 5 cm

Centrarchidae indet. Description

Non-diagnostic elements such as scales, fin rays, and vertebrae, highly fragmentary elements which cannot be referred to genus are listed in Appendix B, but are not described further. There are also several articulated/semi-articulated specimens which cannot be referred to genus, but the cranial and postcranial material within is appropriate for more detailed description.

Otolith. ETMNH 36666 (Fig. 11) is the only otolith recovered from the site to date. This element appears, based on its large size and overall conformation, to be a sagittal otolith, though the characteristic sulcus present on the medial side of sagitta is not present. The medial surface is convex and smooth without any distinctive features. Laterally, the element is concave, heavily weathered around its margin, and has a small hole near the center. The weathering evident on the lateral side coupled with delamination on the medial surface indicate that this otolith has been heavily eroded.

Articulated and Semi-articulated Specimens. While rare due to the excavation methods used at GFS, a handful of articulated specimens have been recovered. ETMNH 36470 (Fig. 12) consists of partially articulated cranial material partially enclosed within a clay block. Visible elements include an articulated parasphenoid and vomer, a partial premaxilla, and a vertebra. Other semi-articulated specimens (ETMNH 36468, Fig. 13; ETMNH 36467, Fig. 14) preserve only articulated vertebrae surrounded by disarticulated fin rays and scales.

Remarks. While ETMNH 36666 is referred here to Centrarchidae indet., the absence of the medial sulcus makes it difficult to assign the element any taxonomic identity. The sulcus and other identifying features may have been obscured by erosion. Calcitic elements from GFS are often poorly preserved; ostracods from the site are often heavily eroded and in poor condition (Wallace, personal communication with author, June 9, 2023). This may explain the paucity of ostracods recovered from the site. Another possibility is that this otolith was eroded during digestion by a larger animal.



Fig. 13 ETMNH 36468. Scale = 5 cm



Fig. 14 ETMNH 36467. Scale = 5 cm

Osteohistology

Osteohistological analysis of vertebrate tissues can aid in elucidating age, growth rate, and even seasonality of death in modern and fossil vertebrates by examining growth marks in bone thin sections. In the paleontological sphere, the bulk of this work has centered on interpreting growth rates and life history of archosaurs, particularly extinct groups (Woodward et al. 2013). Osteohistology of fishes has focused primarily on extant chondrichthyans (e.g., Goldman and Cailliet 2004; Cailliet et al. 2007). Work on osteichthyans is considerably more limited. Following is a preliminary account of results from osteohistological analysis of GFS fish atlantes. In the absence of a comparative sample of centrarchid vertebrae or atlases, these results follow established osteohistological principles as outlined in Francillon-Vieillot et al. (1990).

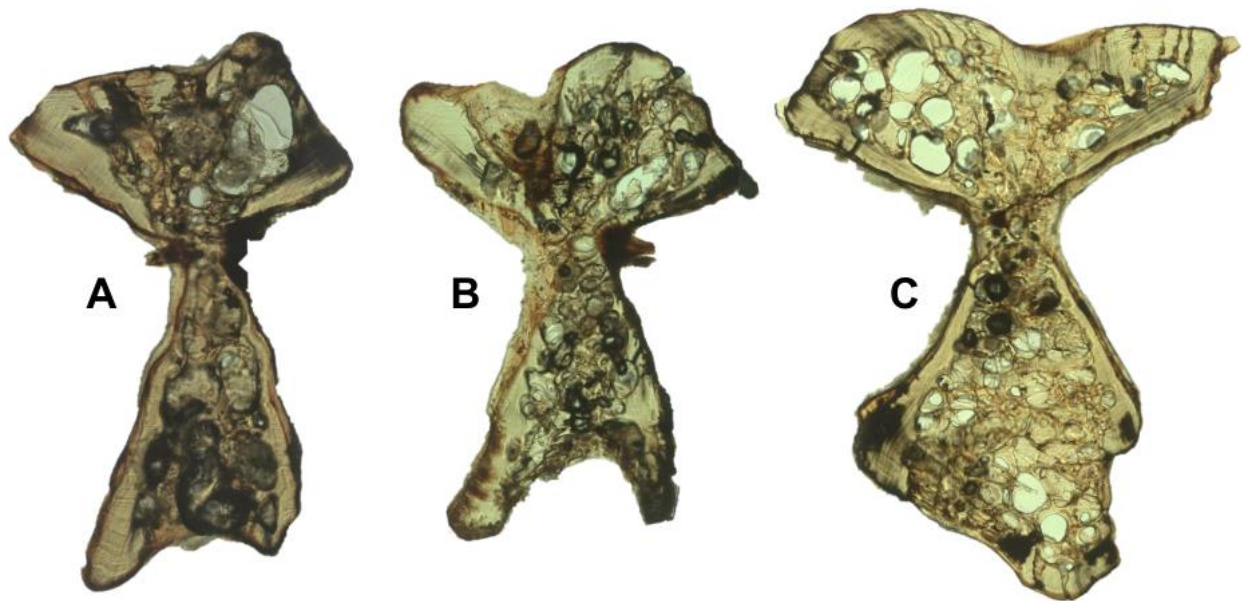


Fig. 15 Histological thin sections of fish atlantes from Gray Fossil Site. A) ETMNH 35751. B) ETMNH 35754. C) ETMNH 35752.

Ten total GFS fish atlantes were sectioned, though some had sustained damage prior or during excavation and were unsuitable for analysis. These atlantes show numerous growth marks

in thin section (Fig. 15). Some of these marks appear to be represent some kind of cyclical environmental signal (i.e., they are visually distinct and occur at regular distances from one another), while others are more narrowly spaced (Fig. 16) and do not appear to indicate long-term cyclical variation in growth rate. While unclear which, if any, of these marks represent a seasonal signal, the presence of numerous growth marks indicates that these fishes may have experienced some form of persistent metabolic stress resulting in slow growth.



Fig. 16 Detail of growth marks in ETMNH 35754. Arrows indicate prominent growth marks

CHAPTER 4. DISCUSSION

Gray Fossil Site Ichthyofauna

This study showed that the Gray Fossil Site ichthyofauna is exceedingly depauperate and consists of only two species in two genera within the same family (Centrarchidae). This contrasts sharply with the diversity of modern ichthyofauna in east Tennessee (Etnier and Starnes 1993). The GFS ichthyofauna also contrasts with other pre-Pleistocene freshwater fish communities in the eastern United States where at least two, if not several, families of fish are represented (Table 1). Both genera represented at GFS are common in east Tennessee today (Etnier and Starnes 1993, Page and Burr 2011).

Micropterus

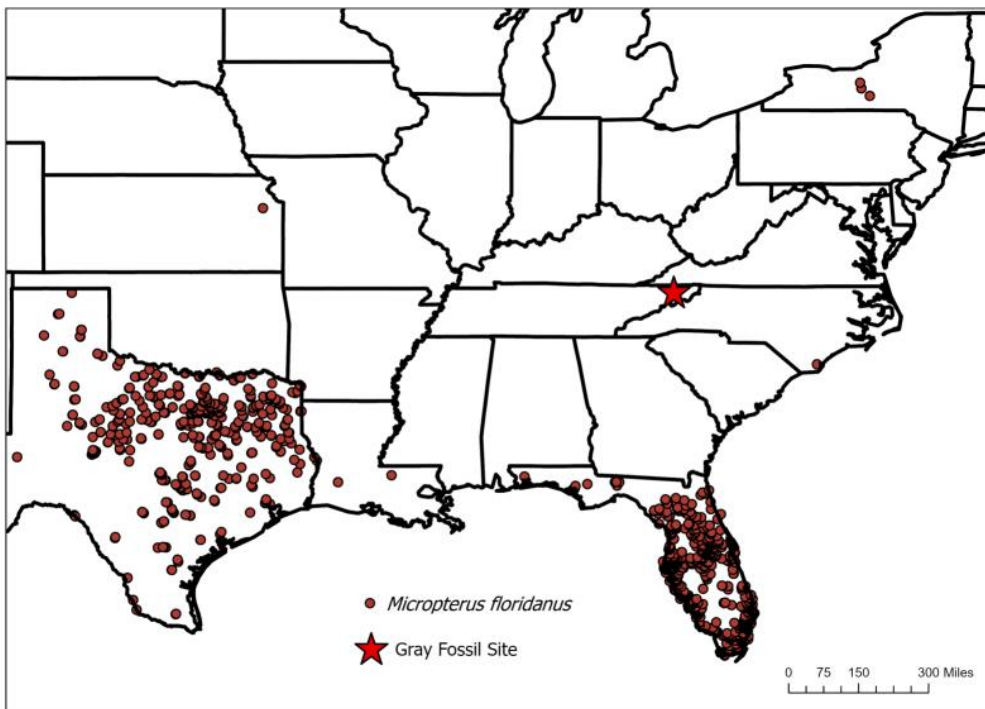
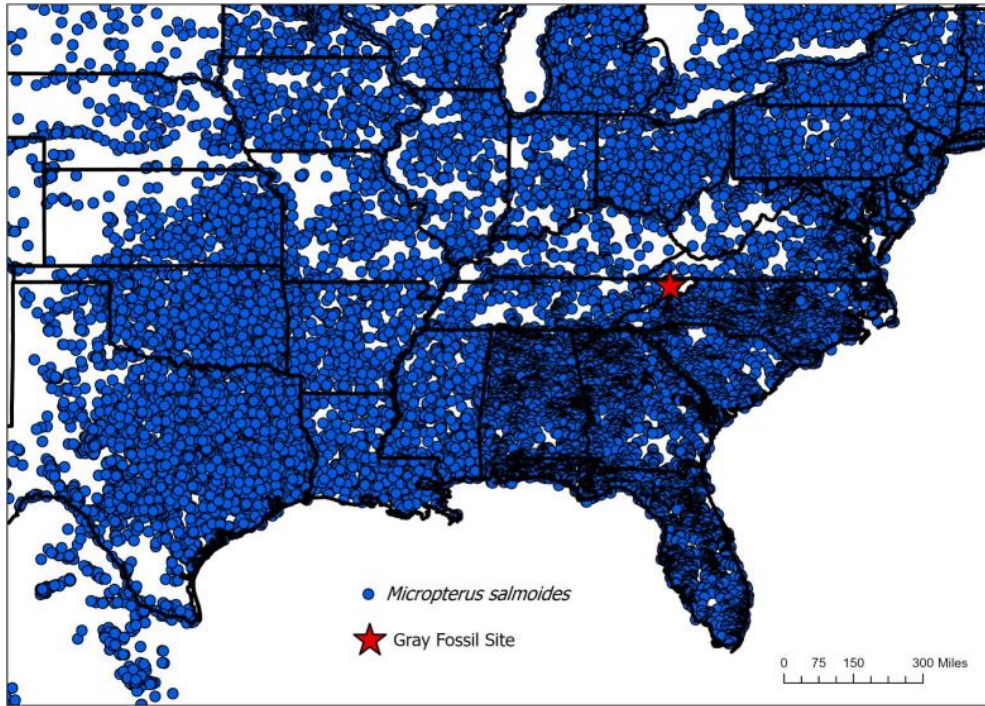


Figure 17. Maps of modern distribution of *Micropterus salmoides* and *M. floridanus*. Occurrence data via GBIF, basemap via ESRI. Note that distribution overlap in Florida is due to records which predate the split between *M. salmoides* and *M. floridanus*

Micropterus, the black basses, are a diverse genus of centrarchid (Collar and Wainwright 2009). Fishes of this genus are more elongate and have larger mouths than other centrarchids, and are the largest and most piscivorous members of the family (Collar et al. 2009; Warren 2009). These fish are predators of smaller fishes, crayfish, and even terrestrial vertebrates (Warren 2009). Black basses are found in a wide variety of aquatic habitats, ranging from headwater streams to isolated backwaters to large lakes and river systems (Etnier and Starnes 1993; Page and Burr 2011). While deeper areas may be utilized during times of temperature stress or to evade predators, most *Micropterus* species forage and build nests in relatively shallow water (2-4 m depth) (Warren 2009). The closely related species *M. salmoides* and *M. floridanus* are among the largest and most piscivorous *Micropterus* species (Warren 2009), and widely distributed (Fig. 17). Both species are also more tolerant of suboptimal water conditions than other members of the genus, being well adapted for survival in warm, eutrophic conditions (Warren 2009).

Micropterus sp. at the Gray Fossil Site likely fed extensively on the smaller *Lepomis* species also present at the site. Terrestrial vertebrates such as frogs, salamanders, and small mammals documented from the site likely provided an additional food source for these fish. *Micropterus* also feed on crayfish (families Astacidae and Cambaridae) when available, but thus far no evidence of crayfish in the form of bioturbation or their readily preserved gastric mills has been documented from GFS. Though the largest GFS *Micropterus* specimens are considerably smaller than the upper size limits for the genus, the presence of larger individuals like ETMNH

35765 suggests that the paleosinkhole pond was suitable enough habitat for these fish to survive for multiple years. Despite this, all GFS *Micropterus* recovered thus far are considerably smaller than the upper size limit for extant species - ETMNH 35765, one of the largest specimens collected thus far, is estimated at 22.6 cm TL using equations for largemouth bass based on cleithrum length (Jeter et al. 2019).

Juvenile *Micropterus* feed on microcrustaceans, incorporating aquatic insects and small fishes into their diet as they increase in size (Warren 2009). This indicates that the paleosinkhole pond at GFS supported a substantial enough population of these organisms to support both fish species present at the site. The preference modern *Micropterus* and *Lepomis* exhibit for constructing nests in shallow water and the abundance of fish material at GFS strongly suggest that certain areas of the sinkhole pond were shallow (~0.5-4 m) to allow both fish species to spawn.

Lepomis

Lepomis, the common sunfishes or bream, is a speciose genus of small, deep-bodied fishes that are primarily invertivores, feeding extensively on zooplankton, aquatic insect larvae, microcrustaceans, and occasionally on small fishes (Collar and Wainwright 2009; Warren 2009). Some species are specialist invertivores, like the mollusk-feeding *L. microlophus* and the zooplankton specialist *L. macrochirus* (Collar and Wainwright 2009). Like *Micropterus*, fishes of this genus occupy a variety of aquatic habitats (Warren 2009) and many *Lepomis* species are distributed across the eastern United States. Foraging generally occurs near shore or cover such as aquatic vegetation or debris and nest construction generally takes place in shallow (<2 m) waters (Warren 2009).

GFS *Lepomis* are “short-jawed” and thus likely did not exhibit levels of piscivory comparable to modern *L. cyanellus*. Pharyngeal jaws of GFS *Lepomis* also do not exhibit the robust, hypertrophied morphology associated with durophagy seen in *L. gibbosus* and *L. macrochirus*. However, Wainwright et al. (1991) observed that *L. gibbosus* raised in a setting with few aquatic snails had smaller and more gracile pharyngeals than those raised in a setting with abundant snails. Aquatic snails have thus far not been documented from GFS. Given that most modern *Lepomis* are generalist invertivores and GFS *Lepomis* shows no evidence of a specialist diet, GFS *Lepomis* likely fed on aquatic insects, microcrustaceans, and zooplankton within the sinkhole pond.

GFS Fish Assemblage

The depauperate GFS ichthyofauna contrasts with modern and fossil diversity of freshwater fish in east Tennessee. A number of factors may have contributed to this lack of diversity. First, as a sinkhole pond, GFS was likely isolated from other waterways. Shunk et al. (2006, 2009) suggested that GFS rhythmites may have been caused by seasonally variable inflow into the pond in the form of a small stream or streams. Smith (2003) also observed the presence of extralimital sediment grains within the deposit, some derived from as far away as 50 km in the neighboring Blue Ridge. While sediment was clearly being introduced to the sinkhole basin by fluvial action, the absence of fossils of stream fishes such as minnows and chubs (family Cyprinidae), even as isolated or fragmentary remains, strongly suggests the paleosinkhole pond was not perennially connected to another waterway. Fluvial inputs into the stream may have taken the form of ephemeral streams which developed during periods of high precipitation. This provided limited opportunities for fish dispersal into the pond and may also explain the absence of crayfish and aquatic snails.

Dispersal of fish into hydrologically isolated bodies of freshwater is well-established, but only recently was evidence of a non-anthropogenic method of dispersal established. Silva et al. (2019) and Lovas-Kiss et al. (2020) found that a small percentage of fish eggs can pass unharmed through the digestive system of waterfowl. Given the abundance of fossil duck material recovered from GFS (Steadman 2011; S. Grantham, personal communication with author, Jun 8, 2023) and the shallow depth at which centrarchids construct their nests (Warren 2009), this seems a probable explanation for the introduction of fish into this isolated sinkhole pond.

Another explanation for the absence of ubiquitous fishes, such as cyprinids and darters, from GFS is the presence of more piscivorous centrarchids. Piscivorous fish with large adult body sizes exert a significant pressure through predation and competition on fish with small adult body sizes, being observed to completely exclude them from some lake assemblages (Tonn and Magnuson 1982; Robinson and Tonn 1989). It is possible that other fish species, if introduced, were unable to maintain a viable population due to predation and competition from an already-established population of centrarchids.

Taphonomy

Taphonomy of fishes has been used extensively in interpreting paleoecology of aquatic settings, particularly lacustrine and marine deposits. As animals that live their entire lives within an aquatic medium, their remains can aid in interpretation of energy, temperature, depth, and presence of scavengers in an aquatic deposit (Schäfer 1962; Elder 1985; Elder and Smith 1988).

All sediments excavated from the site are wet-screened by hand using 1.7 mm mesh. This method yields an exceptional quantity of microfossils but makes more delicate fossils susceptible to fragmentation and even disintegration due to the weight of sediment being shaken in the

screen. As such, the vast majority of GFS fish material consists of disarticulated, often fragmentary remains. However, several articulated specimens have been collected (Fig. 1-2, Fig. 12-14). Even disarticulated material collected from screening often contains associated elements such as multiple paired bones from a single individual. Both these facts seem to indicate that GFS fish are sometimes preserved as articulated or semi-articulated skeletons prior to excavation.

The nature of the fish skeleton means it is exceedingly prone to postmortem damage (Rojo 1991). All bones, save for those of the neurocranium, are loosely articulated with one another and are easily separated over the course of decay and scavenging (Schäfer, 1962). Thus, the presence of articulated and semi-articulated fish from GFS indicates somewhat unusual conditions within the pond and furthermore suggests that alternate excavation methods could produce articulated skeletons of not only fish, but also other microfauna.

Three factors seem to most strongly govern preservation of fish skeletons in lacustrine settings: water temperature, oxygen concentration, and presence of scavengers (Elder 1985). When water temperatures are above 16°C, fish carcasses will float due to bacterial gas production (Schäfer 1962; Elder 1985). Floating carcasses are then prone to scavenging by other fish; barring active scavenging, carcasses will become disarticulated over time as connective tissues decay and dermal bones drop to the bottom of the lake or pond (Elder 1985). This process also requires the presence of enough dissolved oxygen to support bacterial growth within the carcass – at low oxygen concentrations, carcasses will not float (Elder 1985).

Articulated GFS fish specimens, particularly ETMNH 7286 and ETMNH 36469, represent carcasses that did not experience an extended period of decay during flotation. ETMNH 36469 has impressions of the premaxilla, maxilla, and dentary in anatomical position,

though the bones themselves were evidently lost when the slab containing the specimen was split. Similarly, ETMNH 7286 was collected with the cranial region crushed, but present. This area of the specimen was unfortunately damaged prior to preparation. The anterior oral elements (premaxilla, maxilla, dentary) are loosely connected to the skull and are often the first elements to disarticulate from a floating or scavenged carcass (Elder 1985). This strongly suggests that these two specimens did not experience a prolonged period of flotation and instead were able to decay at the bottom of the pond in the absence of scavengers.

Lack of flotation in these specimens as well as other semi-articulated and associated specimens may be the result of a number of factors. Shunk et al. (2009) suggested that the sinkhole lake was poorly mixed, leading to an anoxic hypolimnion. This would also result in much cooler water within the hypolimnion. Cold, anoxic conditions both impede the growth of bacteria that would cause bloat and discourage or completely exclude invertebrate scavengers from the hypolimnion. These conditions would allow the carcass to decay slowly in a low-energy environment with very little postmortem disarticulation.

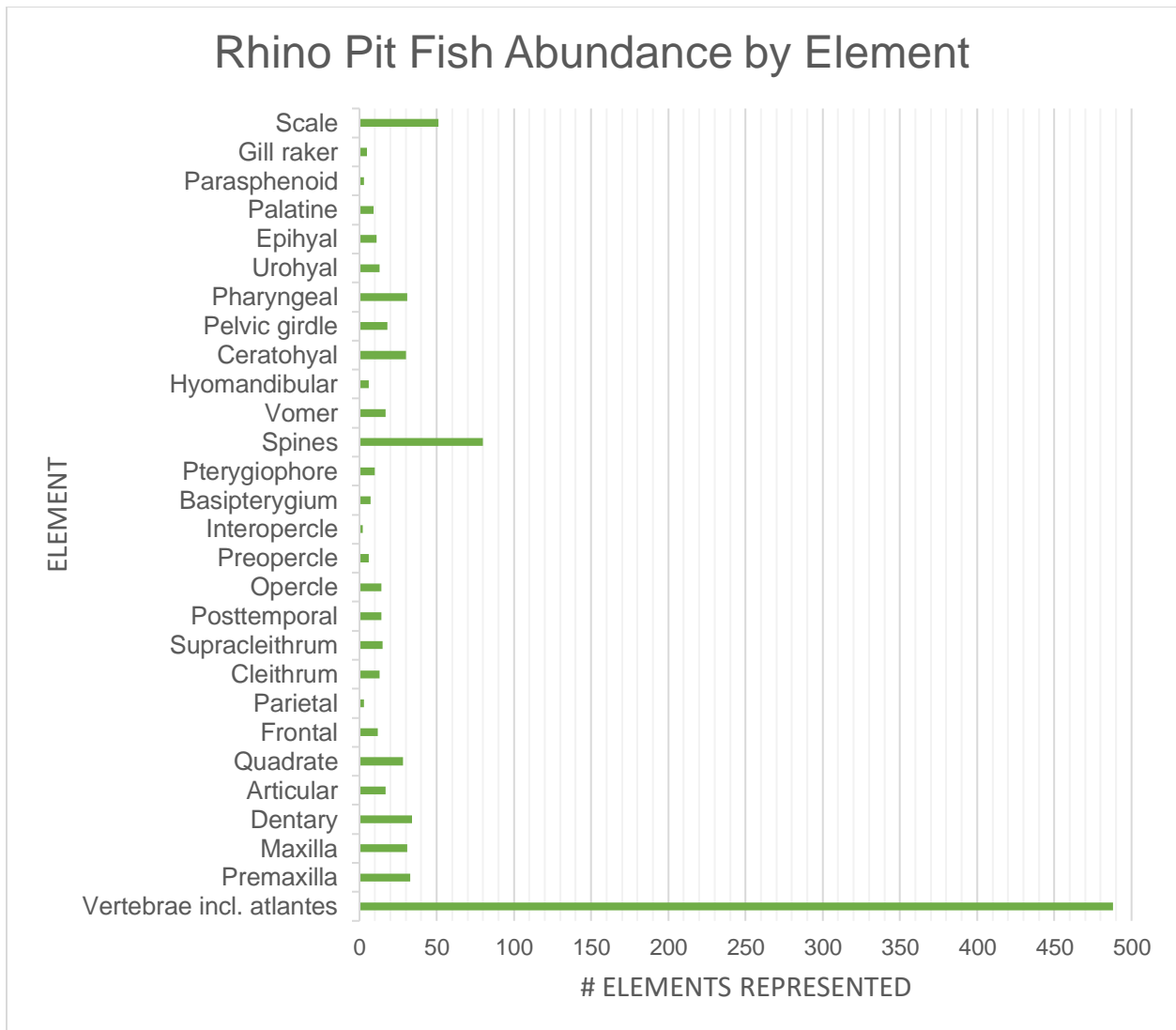


Fig. 18 Number of most common elements from 85 sediment samples collected from Gray Fossil Site TP2-2004 (“Rhino Pit”)

Vertebrae are by far the most common isolated elements recovered from the site (Fig. 18; Appendix B). Compared to scales, ribs, fin spines, and the flat bones of the cranium, vertebrae are exceedingly robust and best suited to withstand both fossilization and screening. Scales and fin spines are the next most abundant elements. Despite the number of fin spines and scales in an individual fish exceeding (and in the case of scales, vastly exceeding) the number of vertebrae in

an individual fish, these elements occur nearly 1/10th as often as vertebrae in screened sediments. Both scales and fin spines are rarely, if ever, preserved completely intact and are virtually always highly fragmented.

Bones of the opercular series are particularly poorly represented in GFS fish material. These bones are extremely flat and thin, leading to extensive breakage during fossilization and wet-screening. The opercular spine is generally the only recognizable portion of the opercle preserved intact, while the preopercle and subopercle are rarely preserved intact enough to identify. The hyomandibular and parasphenoid are poorly represented for this same reason.

CHAPTER 5. CONCLUSIONS

The Gray Fossil Site ichthyofauna is a uniquely depauperate fauna consisting of only two species. This stands in stark contrast to the diverse riverine and lacustrine faunas of modern east Tennessee. Such lack of diversity, particularly of fishes common in modern east Tennessee streams, strongly suggests GFS was isolated via surface hydrology from rivers in the area. This isolation would have provided barriers to dispersal of other fishes as well as freshwater invertebrates such as aquatic snails and crayfish, which are thus far not reported from the site. Though only a small portion of the ~4.5 acre GFS deposit has been excavated, the absence thus far of many common riparian fish and invertebrates in all excavated sediments indicates a highly unique assemblage.

Both genera represented as fossils at GFS are common in rivers, ponds, and reservoirs in modern east Tennessee. Most *Lepomis* species are generalist invertivores that feed primarily on microcrustaceans, zooplankton, and aquatic insect larvae, indicating that these would have been abundant enough at GFS to sustain a population of *Lepomis*. Fishes in the genus *Micropterus* prey on larger invertebrates, terrestrial vertebrates, and small fish. While not directly documented from the site, the abundance of these fish provides excellent evidence for the existence of numerous aquatic invertebrates in the sinkhole pond.

GFS fish material consists primarily of disarticulated and isolated elements recovered from wet-screening. However, many samples contain associated elements (e.g., paired premaxillae, quadrates, and dentaries) that are clearly derived from the same individual. This, coupled with the presence of articulated and semi-articulated fish fossils collected in situ from the site, indicates that fish skeletons are sometimes preserved relatively intact within GFS sediments. Preservation of this type is uncommon in many lacustrine environments, where fish

carcasses float due to bacterial gas production and succumb to either scavengers in the water column or slow disarticulation as the carcass continues to decay. This does not occur when water temperatures are too cool or dissolved oxygen concentrations too low for gas-producing bacteria to thrive. As a small, relatively deep pond system, the GFS palaeosinkhole was likely poorly mixed and had an anoxic hypolimnion as suggested in Shunk (2006) and Shunk (2009). These conditions would allow excellent preservation of articulated fishes and other vertebrates. This may also explain the absence of benthic freshwater invertebrates like crayfish. The relatively common occurrence of associated elements throughout all excavation areas strongly suggests that alternative methods of excavation and screening, such as splitting along varves and the already-employed method of utilizing H₂O₂ to facilitate a more gentle screening process, may yield more intact and/or articulated remains of fish and other vertebrates.

Preliminary osteohistological analysis of fish atlantes from GFS show numerous growth marks which appear to indicate slow growth. As such, the small size of GFS fishes compared to their modern relatives appears to be a product of environmental or phylogenetic causes rather than death at an early age. Continued development of comparative collections will allow more accurate assessment of GFS fish growth rates, and possibly estimation of season of death.

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APPENDICES

Appendix A: Additional Referred Specimens Not Figured

ETMNH	Taxon	Element
7155	<i>Micropterus</i>	Right premaxilla
7304	<i>Lepomis</i>	2 left premaxillae, right premaxilla
7372	<i>Lepomis</i>	Left premaxilla
7302	<i>Lepomis</i>	Right premaxilla
7093	<i>Lepomis</i>	2 left premaxillae
7309	<i>Lepomis</i>	Right premaxilla
6996	<i>Lepomis</i>	Left premaxilla
7324	<i>Lepomis</i>	Left premaxilla
7378	<i>Lepomis</i>	Right premaxilla
6982	<i>Micropterus</i>	Left premaxilla
8155	<i>Lepomis</i>	Left premaxilla
7022	<i>Lepomis</i>	2 left premaxillae
6990	<i>Lepomis</i>	Right premaxilla
7421	<i>Lepomis</i>	2 left premaxillae
8651	<i>Lepomis</i>	Right premaxilla
7042	<i>Lepomis</i>	Left premaxilla
7153	<i>Lepomis</i>	Right premaxilla
7153	<i>Micropterus</i>	Left premaxilla
7310	<i>Lepomis</i>	Right premaxilla
7359	<i>Lepomis</i>	Right premaxilla
6991	<i>Lepomis</i>	Left premaxilla, right premaxilla

7313	<i>Lepomis</i>	Right premaxilla
6981	<i>Lepomis</i>	Right premaxilla
7346	<i>Lepomis</i>	Left premaxilla
7335	<i>Lepomis</i>	Right premaxilla
7184	<i>Lepomis</i>	Right premaxilla
7415	<i>Lepomis</i>	Left premaxilla, 4 right premaxillae
12377	<i>Lepomis</i>	Left premaxilla
7303	<i>Lepomis</i>	Left premaxilla
7288	<i>Lepomis</i>	Right premaxilla
7328	<i>Lepomis</i>	Left premaxilla
7143	<i>Lepomis</i>	Right premaxilla
7101	<i>Lepomis</i>	Right premaxilla
7357	<i>Lepomis</i>	Right premaxilla
7305	<i>Lepomis</i>	Left premaxilla, right premaxilla
7122	<i>Lepomis</i>	Left premaxilla, right premaxilla
7344	<i>Lepomis</i>	Right premaxilla
7362	<i>Lepomis</i>	Left premaxilla
7299	<i>Lepomis</i>	Left premaxilla
7195	<i>Lepomis</i>	Left premaxilla
8645	<i>Lepomis</i>	Right premaxilla
7120	<i>Lepomis</i>	Left premaxilla
8558	<i>Lepomis</i>	Right premaxilla
8558	<i>Micropterus</i>	Left premaxilla
17405	<i>Lepomis</i>	Right premaxilla
7105	<i>Lepomis</i>	Right premaxilla

7154	<i>Lepomis</i>	Left premaxilla
7427	<i>Lepomis</i>	Left premaxilla
7212	<i>Lepomis</i>	Right premaxilla
8663	<i>Lepomis</i>	Right premaxilla
8607	<i>Lepomis</i>	Left premaxilla, right premaxilla
7312	<i>Lepomis</i>	2 left premaxillae, right premaxilla
8775	<i>Lepomis</i>	Right premaxilla
7369	<i>Lepomis</i>	Right premaxilla
7104	<i>Lepomis</i>	Right premaxilla
8666	<i>Lepomis</i>	Left premaxilla
12377	<i>Lepomis</i>	2 left premaxillae, right premaxilla
8815	<i>Lepomis</i>	Right premaxilla
8687	<i>Lepomis</i>	Left premaxilla
7387	<i>Lepomis</i>	Left premaxilla, right premaxilla
7387	<i>Micropterus</i>	Right premaxilla

Appendix B: Full List of Common Elements From Selected Rhino Pit Samples

Field #	Pit	Square	Elements
051206	22004	359123	2 atlantes, 2 vertebrae, 1 pelvis, 4 bone fragments
071707	22004	355125	2 atlantes, 15 vertebrae, 1 maxilla, 1 quadrate, 1 pelvic girdle,

			1 parasphenoid, 4 spines, 1 scale, 11 bone fragments
082307	22004	362123	2 atlantes, 6 vertebrae, 1 premaxillae, 2 vomers, 1 dentaries, 2 cleithra, 1 ceratohyal, 2 frontals, 10 bone fragments
060506	22004	357122	1 atlas, 17 vertebrae, 1 maxilla, 1 dentary, 1 pelvic girdle, 2 gill rakers, 1 opercle, 1 frontal, 1 basipterygium, 2 supracleithra, 4 spines, 18 fragments
060506	22004	359122	1 atlas, 23 vertebrae, 3 quadrates, 3 dentaries, 3 ceratohyals, 12 scales, 6 spines, 5 pharyngeal jaws, 1 epihyal, 2 posttemporals, 50 bone fragments
061214	22004	361132	1 atlas, 4 vertebrae, 2 quadrates, 2 bone fragments
061406	22004	357122	1 atlas, 9 vertebrae, 3 articulars, 1 opercle, 1 parietal, 2 spines, 2 posttemporals, 11 fragments
071406	22004	359126	1 atlas, 7 vertebrae, 1 vomer, 1 maxilla, 2 spines, 1 scale, 4 fragments
071707	22004	356124	1 atlas, 21 vertebrae, 2 maxillae, 1 vomer, 1 dentary, 1 quadrate, 1 pelvic girdle, 3 spines, 32 bone fragments
072307	22004	355126/355127	1 atlas, four vertebrae, 1 spine, 1 supracleithrum
080607	22004	357124	1 atlas, 5 vertebrae, 1 maxilla, 1 ceratohyal, 1 preopercle, 1 basipterygium, 2 bone fragments
080707	22004	358124	1 atlas, 4 vertebrae, 1 premaxilla, 1 maxilla, 1 quadrate, 2 opercles, 1 pelvic girdle, 1 epihyal, 1 pterygiophore, 16 fragments
082807	22004	360125	1 atlas, 10 vertebrae, 1 premaxilla, 1 vomer, 1 quadrate, 1 opercle, 1 ceratohyal, 1 pelvic girdle, 1 supracleithrum, 30 bone fragments

091606	22004	359124	1 atlas, 13 vertebrae, 2 palatines, 1 pharyngeal jaw, 1 parietal, 1 urohyal, 2 bone fragments
092816	12015	363114	1 atlas, 8 vertebrae
101204	22004		1 atlas, 1 vertebra, 1 urohyal
091407	22004	361122	1 atlas, 9 vertebrae, 1 vomer, 1 pelvic girdle, 1 preopercle, 2 scales, 11 bone fragments
072407	22004	357126	1 atlas, 15 vertebrae, 1 premaxilla, 2 dentaries, 1 vomer, 2 pelvic girdles, 1 opercle, 1 epihyal, 1 spine, 16 bone fragments
060814	22004	361131	1 atlas, 2 vertebrae, 2 pharyngeal jaws, 1 maxilla, 5 bone fragments
101106	22004	360124	1 vertebra, 1 premaxilla, 1 maxilla, 1 dentary, 1 vomer, 1 palatine, 2 pelvic girdles, 1 gill raker, 1 posttemporal, 1 basioccipital, 1 rib, 1 pterygiophore, 2 spines, 24 bone fragments
062607	22004	361122	7 vertebrae, 2 cleithra, 1 spine, 11 bone fragments
	22004		1 articular, 1 opercle, 1 cleithrum, 1 vomer, 1 pterygiophore, 10 bone fragments
060707	22004	362126	1 premaxilla, 1 maxilla, 1 dentary, 1 spine
091906	22004	361125	1 dentary, 2 frontals, 1 ceratohyal, 2 epihyals, 1 preopercle, 1 hyomandibular, 1 supracleithrum, 1 spine, 13 bone fragments
	22004	360125	1 vertebra, 1 maxilla, 1 dentary, 1 cleithrum, 1 hyomandibular, 1 spine, 2 bone fragments
070507	22004	356126	2 vertebrae, 1 dentary, 1 vomer, 1 pelvic girdle, 1 ceratohyal, 1 posttemporal, 2 frontals, 1 spine, 6 bone fragments
100306	22004	361126	1 premaxilla (right), 1 dentary, 1 vomer, 1 pharyngeal jaw

102706	361123	361123	2 vertebrae, 1 maxilla, 1 dentary, 2 quadrates, 1 cleithrum, 2 frontals, 1 hyomandibular, 2 posttemporal, 4 spines, 1 ribs, 8 bone fragments
082707	22004	361124	3 vertebrae, 1 premaxilla, 1 pelvic girdle
041914	22004	362129	1 vertebra, 1 premaxilla, 1 hyomandibular, 1 basipterygium, 1 supracleithrum, 4 bone fragments
041914	22004	363129	8 vertebrae, 1 pharyngeal jaw, 1 supracleithrum, 3 spines, 3 bone fragments
052506	22004	359124	22 vertebrae, 4 quadrates, 1 premaxilla, 2 fin spines, 1 articular, 1 ultimate vertebra, 32 bone fragments
060106	22004	357123	28 vertebrae, 2 quadrates, 2 pharyngeal jaws, 1 urohyal, 1 spine, 1 pterygiophore, 32 bone fragments
060707	22004	361123	1 premaxilla, 1 dentary, 1 palatine, 1 ceratohyal, 1 epihyal, 2 pharyngeal jaws, 1 pelvic girdle, 1 cleithrum, 1 gill raker, 1 opercle, 2 spines, 10 bone fragments
060707	22004	361126	1 vomer
060807	22004	361127	3 vertebrae, 1 rib, 2 scales, 4 bone fragments
061214	22004	361132	1 vertebra
061314	22004	361132	9 vertebrae, 1 dentary, 1 frontal, 2 scales, 15 bone fragments
061406	22004	360124	1 premaxilla, 1 articular, 3 ceratohyals, 2 spines, 12 bone fragments, 1 quadrate
061406	22004	360124	1 premaxilla
061406	22004	360124	1 dentary (left)
061808	22004	364124	11 vertebrae, 1 vomer, 1 articular, 1 pharyngeal jaw, 1 dentary, 2 pelvic girdles, 1 ceratohyal, 3 ribs, 2 spines, 1 scale, 23 bone

			fragments
062008	22004	365126	9 vertebrae, 1 premax, 1 vomer, 1 articular, 1 pharyngeal jaw, 1 hyomandibular, 1 basipterygium, 1 pterygiophore w/spine, 5 bone fragments
062306	22004	360123	31 vertebrae, 3 dentaries, 1 premaxilla, 1 vomer, 1 maxilla, 1 pharyngeal jaw, 1 quadrate, 2 articulars, 2 ceratohyals, 1 parasphenoid, 1 gill raker, 46 bone fragments
063006	22004	358123	9 vertebrae, 1 premaxilla, 2 maxillae, 1 palatine, 2 articulars, 1 pterygiophore, 1 spine, 2 bone fragments
071707	22004	356125	1 ceratohyal
072406	22004	356123	1 pharyngeal jaw
073107	22004	ND	1 maxilla, 1 vomer, 1 ceratohyal, 1 pharyngeal jaw, 1 basipterygium, 2 pterygiophores, 2 spines, 8 bone fragments
080907	22004	361125	4 vertebrae, 1 preopercle, 1 cleithrum, 1 posttemporal, 1 spine, 7 bone fragments
080914	22004	356132	1 premaxilla (right)
081407	22004	362123	11 vertebrae, 1 premaxilla, 2 dentaries, 2 pharyngeal jaws, 1 pelvic girdle, 1 cleithrum, 6 bone fragments
082707	22004	360125	4 vertebrae, 1 premaxilla, 1 articular, 1 quadrate, 1 ceratohyal, 1 supracleithrum, 1 spine, 8 bone fragments
083007	22004	356124	5 vertebrae, 1 premaxilla, 1 maxilla, 7 bone fragments
091006	22004	360124	2 bone fragments
091106	22004	359124	22 vertebrae, 2 urohyals, 2 interopercles, 1 maxilla, 1 premaxilla, 1 opercle, 4 scales, 1 ultimate vertebra, 42 bone

			fragments
091106	22004	360124	1 dentary, 1 epihyal, 1 parietal, 5 bone fragments
091106	22004	360124	1 posttemporal, 1 ceratohyal, 5 bone fragments
091206	22004	359125	1 palatine, 1 spine, 2 bone fragments
091206	22004	360126	44 vertebrae, 3 premaxillae, 3 maxillae, 2 dentaries, 2 quadrates, 3 posttemporals, 5 urohyals, 3 pharyngeal jaws, 1 cleithrum, 1 articular, 2 ceratohyals, 1 epihyal, 1 hyomandibular, 1 parasphenoid, 8 spines, 94 bone fragments
091407	22004	361122	1 premaxilla
091906	22004	361125	1 maxilla, 1 pharyngeal jaw, 1 opercle, 1 supracleithrum, 4 bone fragments
091906	22004	361125	1 dentary
092006	22004	361125	1 ceratohyal, 1 scapula, 1 basipterygium, 1 spine, 1 rib, 8 bone fragments
092406	22004	355122	1 dentary, 3 maxillae, 1 pharyngeal jaw, 1 spine, 5 bone fragments
092506	22004	359125	1 premaxilla, 1 maxilla, 1 palatine, 1 quadrate, 1 ceratohyal, 1 scale, 6 bone fragments
092806	22004	361125	2 premaxillae, 1 dentary, 1 quadrate, 1 frontal, 1 cleithrum, 1 epihyal, 4 ribs, 6 spines, 8 scales, 19 bone fragments
093016	22004	362113	1 dentary (left)
100206	22004	360125	2 bone fragments
100306	22004	361126	1 vertebra, 1 articular, 1 palatine, 1 pharyngeal jaw, 2 urohyals, 2 pterygiophores, 3 spines, 22 bone fragments

100405	22004	ND	6 vertebrae, 1 pharyngeal jaw, 1 ceratohyal, 1 supracleithrum, 1 spine, 12 bone fragments
100506	22004	360125	1 cleithrum, 1 spine, 1 bone fragment
100906	22004	358125	25 vertebrae
101006	22004	360125	1 maxilla, 1 dentary, 1 articular, 1 urohyal, 1 opercle, 1 frontal, 3 ribs, 5 scales, 16 bone fragments
101006	22004	360126	1 dentary
101106	22004	360125	1 quadrate, 1 ceratohyal, 2 opercles, 1 preopercle, 2 spines, 15 bone fragments
101106	22004	360125	2 premaxillae, 1 opercle, 1 rib, 8 scales, 11 bone fragments
102006	22004	360127	7 vertebrae, 1 premaxilla, 1 dentary, 1 ceratohyal, 1 epihyal, 1 rib, 1 spine, 9 bone fragments
102306	22004	360123	1 premaxilla (right)
102306	22004	360124	2 maxillae, 1 articular, 1 quadrate, 2 ceratohyals, 3 supracleithra, 1 basibranchium, 3 spines, 1 scale, 14 bone fragments
102506	22004	359124	3 vertebrae, 3 bone fragments
102706	22004	361123	1 quadrate
	22004	361124	4 vertebrae, 1 premaxilla, 1 maxilla, 2 pharyngeal jaws, 1 vomer, 1 palatine, 1 pelvic girdle, 1 quadrate, 1 posttemporal, 2 supracleithra, 1 basiptyergium, 3 spines, 18 bone fragments
ND	22004		8 vertebrae, 2 maxillae, 1 premaxilla, 1 pharyngeal jaw, 1 epihyal, 3 scales, 9 bone fragments
ND	22004	ND	1 vertebra
ND	22004	ND	1 preopercle, 2 ceratohyals, 1 bone fragment

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