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Morphological Development of the AxialSkeletons of Esox Lucius and Esox Masquinongy (Euteleostei: Esociforms), with Comparisons in Developmental and Mineralization Rates.

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MORPHOLOGICAL DEVELOPMENT OF THE AXIAL SKELETONS OF ESOX LUCIUS AND ESOX MASQUINONGY (EUTELEOSTEI: ESOCIFORMS), WITH COMPARISONS IN DEVELOPMENTAL AND MINERALIZATION RATES.

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

PROGRAM IN BIOLOGY

BY
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ABSTRACT

Developmental morphology of the axial skeleton of *Esox lucius* (i.e., northern pike and type species) and *E. masquinongy* (i.e., muskellunge) was investigated. More than 1,000 specimens were examined ranging in size from approximately 10 mm notochordal length (NL) post-hatching juveniles to over 80 mm standard length (SL) foraging sub-adults. Results show that regardless of individual variation, the relative sequence of bone formation and mineralization is consistent between the two species. This consistent developmental pattern enabled construction of an ontogenetic staging scheme of eight developmental stages, each characterized by one defining criterion. The first appearance in cartilage and/or first sign of mineralization of each axial skeleton bone was plotted against time and age for each species and compared. Observed variation in bone development (e.g., number of epurals) inconsistent with the published literature is discussed. Based on the entire developmental study, *Esox lucius* grows in size faster than *E. masquinongy*, but its axial skeleton develops and mineralizes slower. For example, at 25 mm SL, the axial skeleton of *E. masquinongy* is 55% mineralized, while *E. lucius* is only 25% mineralized. *Esox masquinongy* at this point however, is 1000 hrs old, while *E. lucius* is only 700 hrs. These results suggest that *E. masquinongy* has adapted a developmental strategy whereby more energy is put into skeletal development than growth in size. This strategy may reflect *E. masquinongy’s* early
foraging behavior. Unlike *E. lucius*, *E. masquinongy* absorbs its yolk sac earlier in life, and becomes an active predator just a few days after hatching. A well mineralized axial skeleton with developed dentition would facilitate this early predacious behavior.
CHAPTER ONE

INTRODUCTION

The Esocidae (e.g., pikes and pickerels) is a group of freshwater fishes related to the salmons (e.g., Salmonidae) whose extant members are widely distributed in lakes and streams of the northern hemisphere (Liem et al. 2001). The Esocidae includes one genus (*Esox*), with five extant species: *Esox lucius* (northern pike), *E. reichertii* (Armur pike), *E. masquinongy* (muskellunge), *E. niger* (chain pickerel), and *E. americanus* which is divided into two subspecies: *E. americanus americanus* (redfin pickerel), and *E. a. vermiculatus* (grass pickerel) (Grande 1999). Based on morphological (Nelson 1972; Sytchevskaya 1976) and molecular data (Grande et al. 2004), these *Esox* species are grouped into two subgenera: *Kenoza* (Jordan and Evermann 1896) the pickerels, and *Esox* (Linnaeus 1758) the pikes.

The northern pike, *E. lucius*, has the most widespread natural distribution of any completely freshwater fish (Helfman et al. 1997), and has a circumpolar distribution across the northern portions of North America, Europe, and Asia. Its ability to live in cold-water environments might account for this distribution pattern (Helfman et al. 1997). *Esox masquinongy*, *E. niger* and *E. americanus* are also found in North America with ranges that often extend into the southern United States and overlap with *E. lucius* in the north. *Esox reichertii* has a distribution restricted to the Amur River basin of northeastern Asia (Grande et al. 2004). In addition, all species live in areas where
sympatry occurs and hybridization among the species has been demonstrated among all taxa (Grande et al. 2004). Although extant *Esox* species are now restricted to the Northern Hemisphere, the fossil record indicates a once broader distribution for the group (Wilson et al. 1992). The earliest fossil is known from the Early Paleocene (Tiffanian) of western Canada (Wilson 1980). More recently a fossil esocid, closely related to *E. lucius*, was described from the Eocene beds of the Huangxian Formation, China (Chang & Zhou 2002).

**Esocid Economic, Scientific and Ecological Importance**

Esocids have both economic and scientific importance. They are of substantial economic importance because they are fished commercially and for sport in North America and Europe. The U.S. Fish and Wildlife Service stocks larger esocids (*E. masquinongy* and *E. lucius*) in lakes and streams. Both species prefer shallow, weedy, and clear lakes and marshes, but they are also known to inhabit slow streams. They are both efficient predators known to consume approximately three percent of their weight per day. Adults feed largely on other fish as well as frogs, crayfish, mice, muskrats, and ducklings. *Esox lucius* spawns in flooded areas of vegetation in early spring at temperatures of 2.2 – 2.8 degrees Celsius. Females deposit up to 100,000 eggs that stick to flooded vegetation and hatch 12 - 14 days later. Young remain in shallow nursery areas feeding on zooplankton before converting to a fish diet. By fall fry reach a length of 15 centimeters or more and at the end of their third year measure 43 – 58 centimeters when they reach maturity (Smith 2002). *Esox lucius* is one of the largest predators of the northern waters. Its adult length and weight varies between 46 and 76 centimeters and
2.27 and 5.44 kilograms, but has been known to exceed one meter and weigh over 18 kilograms. The body color of *E. lucius* is highly variable, depending on the waters from which it is found. The dorsal and lateral sides are predominantly dark green to brown with irregular rows of yellow and white spots; the ventral side is generally much lighter and eyes are a brilliant yellow. The body is long and narrow with a lateral line scale count of 119 to 128. The dorsal fin position has a fin count of 16-19 soft rays. The head is long and flat with a pointed, elongated snout often resembling a duckbill and strong jaws with numerous sharp teeth. Cheeks are fully scaled but the lower half of each opercle is scaleless, and the caudal fin is rounded (Smith 2002).

The muskellunge (*E. masquinongy*) is the largest predator within the pike family and is the top predator among freshwater fishes of the Great Lakes region (Smith 2002). Adults can reach over a meter in length, and weigh over 22 kilograms. The body is streamlined with a long flat head, pointed elongated snout (i.e., duckbill-like) and strong jaws with numerous sharp teeth. The posterior sections of the jaws and suspensorium are fully scaled covering bones such as the opercle, interopercle, subopercle, preopercle and parts of the hyomandibula and pterygoid bones. Muskies, like northern pike, also vary in color, markings and fin tip structure depending on the clarity and color of their home waters. It is believed that the body color of each species changes according to the season or the environment (Tommelleri & Eberie 1990). The dorsal surface, head and dorsolateral sides primarily range from iridescent green-gold to light brown and the ventral surface is cream-colored with small gray or brown spots. There are three common muskellunge pattern variations: fish categorized as clear are solid in color with
any type of dark markings only on the posterior section of their body; spotted fish have
dark circular markings over its entire body and fins; and barred fish are characterized by
vertical dark markings along the entire length of its body. Clear and barred muskellunge
have caudal fins with rounded tips, whereas spotted muskies have more pointed caudal
fin tips (Smith 2002).

Although fishery biologists often describe different species of *Esox* exclusively on
the basis of color patterns, these species are not taxonomically valid. An uncommon
variant of *E. lucius* exists called the “silver pike”. These fish lack rows of spots and
appear silver, white or silvery-blue in color (Smith 2002). This pike was originally
thought to be more closely related to *E. masquinongy* because it did not have the
characteristic body patterns of *E. lucius*. Yet, upon further examination, it was
determined that the silver pike was more closely related to *E. lucius* because it had
similar internal and external characteristics (i.e., scale and lateral line pore pattern). In
summary, it must be noted that the silver pike is not simply a hybrid or subspecies of *E.
lucius*, but rather a color morph of *E. lucius* that occurs in scattered populations. There is
however, a recognized hybrid species that exists called the “tiger muskie”. The hybrid
gets its name from its distinct, dark vertical bars, although some large individuals display
very little barring. The tiger muskie is considered a sterile (although no tests have
confirmed this sterility) hybrid cross between *E. masquinongy* and *E. lucius*. Although
this hybrid can occur in the wild where both species coexist, the vast majority of hybrids
are bred in captivity and are released into the environment (Crossman & Scott 1973).
Esocid Evolutionary Placement among Euteleosts

The evolutionary (i.e., systematic) placement of *Esox* within Euteleostei and even in relation to the Salmoniformes has been controversial at best. *Esox* was traditionally placed within Salmoniformes and usually placed as basal (most primitive within the order). However in 1991, Begle split Salmoniformes and made *Esox* the sister group to the ostariophysans (e.g., carps, minnows, suckers and catfish). After reevaluating Begle’s (1991) work, Johnston and Patterson (1996) placed *Esox* exclusively into the Esociformes and then placed Esociformes as the sister to all higher fishes (i.e., the Neoteleostei). Recent data (Lopez in prep) suggests that Begel’s original classification might in part be correct. Where exactly *Esox* fits within Euteleostei is therefore still debatable. A better understanding of the paleontology and developmental morphology of this group might provide phylogenetic clues.

The Structure of the Axial Skeleton

“Although considerable work has been published on species composition, phylogenetics and biogeography of *Esox* (e.g., Nelson 1972, Crossman, 1966, Crossman 1978, Rab & Crossman 1994, Johnson & Patterson 1996, Grande et al. 2004), little has been published on the morphological development of these fishes. Of the few studies that have been published (e.g., Pehrson 1944, Jollie 1975 and Patterson & Johnson 1995), they are narrow in focus, and do not contain large enough sample sizes to account for individual variation. Pehrson (1944) for example, studied the early development of the latero-sensory canals in *Esox lucius*, whereas Jollie (1975) examined the comparative ossification in the skulls of *E. lucius* and *E. americanus* mostly in relation to bones of the
cranial lateral line system. Although Jollie’s (1975) work set a good developmental foundation, his work did not examine the post-cranial skeleton. In addition, to date virtually no developmental information has been published on *E. masquinongy*” (Burdi and Grande 2010, p 411-412). Therefore, a complete developmental scheme of esocid skeletal anatomy is needed.

Because a description of the developmental morphology of the esocid axial skeleton is lacking in the literature, it is important to begin by defining the axial skeleton in general terms and then specifically in *Esox*. The axial skeleton consists of three main components: the skull (Appendix A, red and light/dark blue), vertebral column (Appendix A, pink, purple, orange, and dark green), and median fins (i.e. dorsal, anal and caudal) (Appendix A, yellow and light green). In addition, the entire skeleton can be divided into the cranial portion, which is comprised of all the bones of the skull, and the postcranial portion, which includes all the vertebral column structures and the median fins. These portions together serve many functional roles such as providing shape, supporting body mass and withstanding the stress of locomotion.

*The Skull and Visceral Skeleton*

The skull is made up of three subdivisions: the chondrocranium, splanchnocranium and dermocranium (Appendix A, red and dark blue). The chondrocranium, also known as the braincase or neurocranium (Grande and Bemis 1998), consists of cartilage and cartilage replacement bone that encases the sense organs (e.g., inner ear and nose) and the brain ventrally, caudally and in part laterally (e.g., basioccipital, exoccipital, supraoccipital, prootic, basisphenoid) (Liem *et al.* 2001).
During development the chondrocranium is formed by a combination of mesodermal sclerotome and neural crest cells (Liem et al. 2001).

The splanchnocranium (composed of cartilage or cartilage replacement bone) consists of the gill arches that support the gills and the jaws (Appendix A, light blue). The splanchnocranium is thought to be derived evolutionarily from ancestral visceral arches. It is hypothesized that the first of seven visceral arches of an ancestral jawless fish (Agnatha) gave rise to the jaws (i.e., the Mandibular Arch). As demonstrated in developing chondrichthyan (first jawed fishes), the upper jaw, derived from the mandibular arch, is called the palatoquadrate cartilage and the lower jaw is called the Mandibular or Meckel’s cartilage. The dorsal part of the second ancestral gill arch (hyoid arch) is called the hyomandibular cartilage and suspends the jaws to the chondrocranium. The remaining five visceral arches give rise to the branchial arches that support the gills. *Esox* and most bony fish exhibit a hyostylic jaw suspension because the upper jaw loses any major direct connection with the chondrocranium, and the both jaws are supported solely by the hyomandibula (derived from the upper hyoid arch). In more advanced vertebrates, parts of the splanchnocranium are modified to form derived structures such middle ear ossicles and parts of the hyoid apparatus and pharyngeal cartilage. The splanchnocranium of *Esox* contains bones that are derived from portions of the palatoquadrate (e.g., pterygoid series) and mandibular cartilages (e.g., anguloarticular) (Figure 7).

The dermatocranium is made up of the dermal bones that encase the chondrocranium and splanchnocranium and contribute to the protective structure of the
braincase, jaws, as well as skeletal elements of the mouth (e.g., teeth) (Appendix A, red and light blue). According to Liem et al (2001), there are six basic groups of dermal bones that make up the dermatocranium: the dermal roof makes up the dorsal and lateral sides of the skull (e.g., in *Esox*, frontals and parietals) (Figure 6); the palatal series consists of the bones that make up the roof of the mouth and encloses most of the ventral surface of the palatoquadrate cartilage except for the opening that allows for passage of jaw muscles from the chondrocranium (e.g., in *Esox*, premaxilla and dermal toothed component of the palatine (dermopalatine)) (Figure 7); the parasphenoid makes up the ventral surface of the skull (Figure 6) the lower jaw series encloses the mandibular cartilage and joins with the articular bone (e.g., in *Esox*, dentary) (Figure 7); the opercular series laterally covers the branchial region (e.g., in *Esox*, the opercle) (Figure 7); and the gular series which covers the ventral branchial region (e.g. the gular series predominately found in lower actinopterygians (i.e., *Amia calva*) and is therefore not present in *Esox*).

The Vertebral Column and Median Fins

The primary function of the cranial skeleton is to protect and support the brain and associated sensory organs. In contrast, the postcranial skeleton is used less for protection but more for support of the body and for locomotion. The postcranial skeleton can be divided into two regions: the axial skeleton (Appendix A, pink, purple, orange, light/dark green, yellow, and light green) includes the vertebral column, associated ribs, and arches and the median fins; and the appendicular skeleton, which is comprised of the paired fins and girdles. This study focused on the axial skeleton of *Esox*, thus the following description is focused on that morphological assemblage.
The vertebral column is the basic support structure of the body and develops from individual units called vertebrae, which in gnathostomes, and more specifically teleost fishes, replaces the notochord during development. According to Schultze and Arratia (1988), the potential vertebral column, and specifically each individual centrum, in teleosts begins with the formation of chordacentra. Chordacentra result from a direct mineralization from within the notochordal sheath after segmentation. In *Esox*, mineralization begins simultaneously from the dorsal and ventral sides of the perspective abdominal centra, eventually forming a ring (in ural centra, mineralization begins in the ventral region and moves dorsally). At this time other potential vertebral elements such as the cartilaginous precursors of neural and haemal arches (i.e., basidorsals and basiventrals) begin to form on the dorsal and ventral surfaces of the chordacentra, respectively. Arcocentra then start to ossify and form over the perichondral basidorsals and basiventrals, and eventually autocentra (a secondary mineralization of each centrum from outside of the notochordal sheath) begin to form as direct perichordal ossifications around the chordacentrum. In some fishes (e.g., *Esox*), this enclosing by the autocentra during development makes the arcocentra and autocentra indistinguishable. Membrane bone is deposited on arches and spines to form points of attachment for intermuscular bones and muscles. Resulting vertebrae are thus composites of centra and associated structures such as neural spines and neural and haemal arches that develop independently and from multiple processes.

For this project, I have divided the *Esox* vertebral column into the three regions: anterior, abdominal and caudal. The anterior region (Appendix A, pink) is comprised of
the anterior most centra that attach to the back of the skull and do not bear pleural ribs. Some researchers refer to these centra as cervical vertebrae because they form anterior to *Hoxc6* expression and they lack ribs (Bird & Mabee 2003). The abdominal region (Appendix A, purple) is positioned posterior to the anterior centra, and consists of those centra that articulate with pleural ribs. The caudal region (Appendix A, orange) is comprised of the centra and associated elements that support the caudal fin. Caudal centra articulate ventrally with the haemal arches. Within the caudal fin region (Appendix A, light green), modified centra include the preural centrum 1 (associated with the parhypural), ural centrum 1 (associated with the first and often second hypurals), and ural centrum 2 (associated with the third, fourth and fifth hypurals).

The function of fins is to prevent the body from pitching and rolling, and to slow forward motion. With the exclusion of the caudal fin, fins are generally composed of pterygiophores that in the case of paired fins articulate the fin to its respective girdle, and lepidotrichia (dermal bundles of actinotrichia) or fin rays. Fins are subdivided into two types: the median fins which are composed of the dorsal, anal and caudal fins; and the paired fins which are composed of the paired pectoral and pelvic fins. It should be noted that this project will not deal with the paired fins as they are part of the appendicular skeleton.

**Cartilage and Bone Development**

This study relies on an understanding of the structure, development and differences between cartilage and bone and the various types of bone. The teleost axial skeleton consists of varying degrees of both bone and cartilage. Both tissues add rigidity
(cartilage adds flexibility) to the hydrostatic skeleton thus resisting compression and telescoping as a fish moves through water. According to Liem et al. (2001 p. 187), cartilage develops “when mesenchyme cells transform into chondroblasts and begin to secrete and deposit an extracellular matrix. The mature chondroblasts or chondrocytes lie in small spaces within the matrix called lacunae. Cartilage grows on its surface by the recruitment of chondroblasts, and interstitially by the mitotic division of chondrocytes. Daughter chondrocytes separate and synthesize more matrix”. Cartilage is a major constituent of the embryonic and young vertebrate skeleton and it is converted largely to bone during maturation. It is highly flexible and can change drastically under stress but snaps back into its original shape. According to Liem et al. (2001 p. 188), “later in embryonic development of the majority of vertebrate species, nearly all of the cartilage is replaced by bone, but some cartilage persists where its effortless growth, smoothness or elasticity are particularly important qualities”. In teleosts, many of the skull bones (e.g., hyomandibula, neural and haemal arches and fin supports) are pre-formed in hyaline cartilage and are found in areas below or not in contact with the skin.

Bone is the primary skeletal tissue of most adult vertebrates. It is vascular, mineralized, dense connective tissue that is hard, resilient and capable of slowly changing in structure as forces on the body change during an organism’s life (Liem et al. 2001 p. 188). “Bone develops when osteoblasts produce a matrix of polysaccharides and many collagen fibers. The binding of calcium phosphate to the collagen fibers then calcifies bone”. There are two major types of bone in fishes: dermal and chondral. Dermal bones tend to be superficial bones (e.g., frontals, parietals, structures that give support for
sensory canals) that lie in or just beneath the skin and develop from the direct deposition of bone in connective tissue. They usually develop after endochondral bones, and usually contain “ornamental ridges” that provide places for skin connections (Liem et al. 2001). Chondral bone is bone that forms within cartilage thus replacing it (often called replacement bone). These bones usually develop first and during development are reinforced with dermal bone. In some cases we find three other types of bone: composite, membrane and perichondral. Composite bone results from the fusion of two distinct bones usually one dermal and one chondral bone (e.g., anguloarticular, pterotic). Membrane bone develops within membranous tissue without previous cartilage formation (e.g., intercalar). Finally, perichondral bone is pre-formed within the notochordal sheath (e.g., chordacentra, epurals).

**Fish Development**

Fish development can be divided into five major periods: embryonic, larval, juvenile, adult and senescent periods. The embryonic period occurs when the developing individual is entirely dependent on nutrition provided by the yolk sac. This period begins at fertilization and can in turn be divided into three phases. The first phase, the cleavage phase, is the interval between the first cell division and the appearance of recognizable predecessors of organ systems, but especially the neural plate (Moyle & Cech 2000). The second phase, the early embryo phase, is the interval when the embryo becomes recognizable as a vertebrate because the major organ systems begin to appear, to hatching. The extent of embryo development at hatching varies among species and within species depending on environmental conditions. In Esox, the diameter of newly
spawned eggs ranges from 2.5 to 3 mm. Embryos are 7.5 to 10 mm in length and are able to swim after hatching, but stay on the sediment for some time. *Esox* eggs and new hatchlings (which stay inactive, attached to vegetation for their first few days of life) fall prey in large numbers to larger pike, perch, minnows, waterfowl, aquatic mammals, and insects. The third phase, the late embryo phase, begins when the embryo is free of the egg membranes. During this phase the embryo becomes very fishlike but still relies on its yolk sac for nutrition (Moyle & Cech 2000). In *Esox*, the entire embryonic stage lasts about 5 to 16 days and is primarily dependent on water temperature (19 and 10 degrees respectively).

The larval period begins with the ability of the fish to capture food. This period ends when the axial skeleton is formed and the embryonic median fin-fold is gone. The survival rate of free swimming *Esox* larva to a length of 75 mm is very small due to their vulnerability to predation. The juvenile period begins when the organ systems and fins are fully formed. They often possess distinctive color patterns that are influenced by the habitats they occupy (see above for color and spot patterns in *Esox*). The juvenile period lasts until the gonads become mature and, as a result of their incessant feeding habit, young *Esox* during this period grow rapidly in both length and weight. The adult period occurs once the gonads are mature, secondary reproductive structures develop, color patterns change and spawning behavior begins. Female *Esox* become sexually mature on average at age three or four years and 30 centimeters, and males at two to three years and 19 centimeters. After reaching sexual maturity, *Esox* continues to gain weight, although more slowly. The senescent period occurs when growth has stopped and the gonads are
not producing gametes. This period lasts for different amounts of time in different fish (Moyle & Cech 2000). In general *Esox* have an average life span of 10 to 12 years, but can be as old as 30 years. Life expectancy and growth are greatly dependent on environmental conditions.

*Fish Axial Skeletal Developmental Work Conducted*

The use of zebrafish (*Danio rerio*) as a vertebrate model organism is extremely popular and, as a result, a wealth of genetic and early developmental data have resulted. However, the specific morphology, individual bone mineralization patterns, and sequence of bone mineralization differ tremendously from other fishes. For example, in zebrafish, centra formation follows an anterior to posterior sequence, whereas in *Esox*, centra formation begins anteriorly (Appendix A, pink), then posteriorly (Appendix A, light green), and then finally convening in the abdominal region (Appendix A, purple). The formation of individual centra in ostariophysans also appears to be different from euteleosts. Centra formation is regulated by Hox gene expression, but different types of centra (abdominal, caudal, ural etc.) seem not be regulated by the same groupings of Hox genes (Bird and Mabee 2003). For example, in the zebrafish the Hoxc6 gene is associated with rib-bearing centra and the anterior limit of the Hoxc6 gene expression is between vertebrae 2 and 3, whereas the Hox c10a2 gene in *Esox* is associated with centra 13-16. Little is known about Hox gene regulation of the caudal fin. There may also be different groupings of Hox genes that code for specific types of centra in different species across euteleosts. It is for this reason that zebrafish developmental studies concerning the skeleton (e.g., Cubbage & Mabee 1996, and Bird & Mabee 2004, among others) cannot
be applied across fish species, and zebrafish may not be an appropriate model for all fishes.

Although few fish developmental studies on the axial skeleton on species other than zebrafish have been conducted, these have set a good foundation for this study of esocid fishes. Schultze & Arratia’s (1989, 1992) studies on ontogenetic development and the timing and sequence of specific skeletal structures provided a good starting point. Their study of basal teleost fishes produced an ontogenetic pattern of centrum and caudal skeleton formation to be tested across teleosts. This pattern of development was tested and used specifically in this study of *Esox* (e.g., formation of autocentra, arcocentra, chordacentra, etc.). In addition, Schultze & Arratia (1989, 1992) examined the sequence and origin of specific caudal fin elements (e.g., the timing of ossification of both dermal and chondral bones), using salmonids as examples of basal teleosts which can also be tested across teleosts including *Esox* (e.g., timing of chondral hypurals versus other dermal caudal elements).

The staging criteria employed by Shardo (1995) and Grande & Shardo (2002) was used in this study to ensure a precise methodology of comparing development across species. Both studies create a detailed staging scheme for teleost fishes where each stage of development is defined by a specific morphological structure (e.g., defining criterion), and additional concurrent structures that are more variable in terms of ontogenetic timing or first appearance during development. Because age and length do not correspond to a level of development across species, this method of staging allows for comparison with other fish species and was used to create a staging scheme for *Esox* as well.
Purpose

Because a description of the developmental morphology of the esocid axial skeleton is lacking in the literature the purpose of this study was to create a complete developmental scheme of esocid axial skeletal anatomy (e.g., cranial and post-cranial). This was accomplished by examining in detail the developmental morphology of the axial skeleton of *Esox lucius* (type species of the genus) and compare it with that of *Esox masquinongy*. In addition, the relative timing and rates of development of bone ossifications in the axial skeleton of *E. lucius* and *E. masquinongy* were determined. Thus, this thesis has two components: 1) A descriptive component detailing the development of the axial skeleton of *E. lucius*; 2) A more comparative component examining the developmental rates of bone ossification in *E. lucius* and *E. masquinongy*. Differences in developmental timing between species were evaluated and possible causal explanations are discussed. The final component of this thesis is a glossary of terms (Appendix B) that defines each individual component (morphological structure) of the axial skeleton in *Esox*, as well as specific terms necessary for understanding fish morphological development in general. This glossary of terms serves not only to aid in the understanding of esocid skeletal structure, but also as an aid in understanding fish morphological development for future researchers and students of fish development.

This thesis was expanded in a collaborative, published study by Burdi & Grande (2010). Based on the staging scheme of *Esox lucius* described above, the second author examined the developmental series of *E. masquinongy* and created a separate staging scheme for this species. This allowed for the comparison of the developmental
morphology of the entire axial skeleton of *E. lucius* and *E. masquinongy*. Grande also added, in the published work, a detailed description of both supraneurals and intermuscular bones, which was beyond the scope of this thesis. “Over 500 specimens of each species were examined for this [collaborative] study, thus providing an adequate sample size to assess inter and intraspecific variation in bone development. The [collaborative] study is timely and has widespread significance because *Esox lucius* (type species of the genus) and *E. masquinongy* are of importance both scientifically and commercially. They are of substantial economic importance as a sport and commercial fishery, and are stocked into northern streams and lakes by the U.S. Fish and Wildlife Service. As a result, significant hybridization has been reported (Crossman & Buss 1965, Casselman *et al.* 1986, Tomelleri & Eberle 1990). It is therefore imperative to better understand wild-type developmental morphology and developmental patterns before examining hybrids. In addition, although the recent distribution of *Esox* is fairly restricted, fossil representatives are found throughout the Northern Hemisphere (Grande 1999) and China (Chang & Zhou 2002). Thus, a better understanding of the development of these two key *Esox* species may provide insight into basic c teleost development and provide insight into the morphology of the many, often fragmented, fossil esocids that are in need of assessment and possible redescription (Grande 1999)” (Burdi & Grande 2010 p. 412).
CHAPTER TWO

METHODS

Materials

The Illinois Department of Natural Resources, Jake Wolf Memorial Fish Hatchery, Topeka, IL, provided developmental material of both *Esox lucius* and *Esox masquinongy*. Collections were made by fish hatchery staff beginning in late February 2003 and continued through late June 2003. Three to five specimens of each species were sampled three times a day (i.e., morning, afternoon, and evening) until ossification of skull and vertebral column was complete, and the fish were ready to be released into the wild. Adult fish of each species (see below) were cleared and stained to provide comparisons with developmental material. Based on examination of these specimens, the axial skeleton of *E. lucius* is completely ossified at about 80 - 90 mm SL.

For each species, fish samples were taken from nine spawns or clutches and pooled to obtain a complete developmental series (Bird & Mabee 2003). Using multiple spawns allows for the examination of the relative timing of certain structures by minimizing genetic differences. Spawns for each species were fertilized on the same initial date and time, and were grown under the same environmental conditions that mimic their respective natural environments. *Esox lucius* spawns in early spring at water temperatures between 1.1º - 4.4º C, whereas *E. masquinongy* spawns later in spring at slightly warmer water temperatures between 8.8º - 13.3º C. Females of both species

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deposit up to 100,000 eggs scattered at random, and hatching occurs 12 - 14 days later. Specimens were placed in vials of 10% buffered formalin for three days and then transferred to 70% ethyl alcohol. The time of fertilization was recorded for each spawn (*E. lucius* 3/10/03, 2:30 p.m. and *E. masquinongy* 3/25/03, 11:50 a.m.), and sampling began one hour and ten minutes after fertilization for *E. lucius* (3/10/03, 3:40 p.m.), and four hours and ten minutes for *E. masquinongy* (3/25/03, 4:00 p.m.). Sampling immediately following fertilization was not imperative because this study was not concerned with early cleavage stages, but rather with axial skeleton formation and the identification of endochondral and dermal bones.

A total of 980 specimens of *Esox lucius* and 1500 specimens of *E. masquinongy* were collected and examined for this thesis. From these specimens, data were collected from approximately 500 specimens of *E. lucius* and 600 specimens of *E. masquinongy* for the collaborative study. All fish were deposited in the fish collection at Loyola University Chicago (LUD 081089 = *E. lucius*; LUD 070278 = *E. masquinongy*). Figured specimens were assigned individual Loyola University (LUD.F) catalogue numbers.

**Additional Comparative Material**

*Esox lucius*: 22 spec. (SL: 60 - 400 mm): FMNH 142, 144, 3160, 4007, 6304, 6460, 6724, 7406, 10064, 18090, 43024, 75232, 79584, 91381 (alcohol, c&s), FMNH 32734, 9760, 9964, 73641 (dried skeletons); LUF 09808, 09809, 09811, 09825 (alcohol, c&s).


*Esox reichertii*: 6 spec. (SL: 65 – 225 mm): CU 64227, 64228, 64229, 64231 (alcohol, c&s); FMNH 109221 (alcohol).

*Esox niger*: 7 spec. (SL: 110 – 180 mm): FMNH 21811 (c&s); LUF 082291 - 082293 (c&s).
Esox americanus: 1 spec. (SL: 113 mm): FMNH 31768 (c&s).

Esox americanus americanus: 3 spec. (SL: 100 – 115 mm): UAMF 10424, 24-288-3-14 (c&s).

Esox americanus vermiculatus: 1 spec. (SL: 113 mm): FMNH 7187 (c&s).

**Specimen Preparation**

Representative specimens of each developmental stage were photographed before preparation to record external phenotypic characteristics (e.g., pigmentation patterns). To examine skeletal morphology, specimens were prepared using a modified version of Dingerkus & Uhler (1977). This technique stains bone red with alizarin red, cartilage blue with alcian blue and renders the flesh transparent with the enzyme trypsin. Because potassium hydroxide (KOH) is caustic to fragile larval specimens, an ethyl alcohol series was substituted for the KOH step. Additional specimens were stained for bone in the event that the alcian blue cartilage stain inhibited the alizarin red uptake. Cleared and stained specimens were stored in 90% glycerin.

Specimens were examined, dissected and drawn using a Wild MZ8 dissecting microscope with drawing attachment and video capturing system. When necessary, larval specimens were examined using an Olympus BH3 compound microscope with Nomarski Optics. Alcohol and cleared and stained specimens were video captured using an Olympus SZX12 dissecting microscope and a Retiga 2000R imaging fast 1394 camera. High magnification digital photographs were then taken of all the bones. All digital images will eventually be deposited and archived in Morphobank. This will allow the ichthyological community access to the material used in this study. The procedure used for scoring a particular bone as either cartilaginous or mineralized followed Bird &
Mabee (2003). Specimens were scored within two days of staining. Cartilaginous structures were scored as present based upon visual examination of alcian blue staining of chondrocytes. The earliest sign of mineralization was based upon the uptake of alizarin red stain. It should be noted that bone cells will have most likely formed earlier than this record of red uptake; however there may not have been enough cells formed in order to stain with alizarin red. Specific measurements were taken from each fish examined: Total length (TL) from the tip of the snout to the tip of the caudal fin; Notochordal length (NL) from before hypural formation (i.e., from the tip of the snout to the end of the notochord); and standard length (SL) from the tip of the snout to the posterior margin of the hypurals.

**Developmental Staging**

Early ontogeny consists of growth and a sequence of developmental changes over time. These rates of growth and developmental change are not necessarily constant or correlated with each other. Thus, age or length does not consistently correspond to a level of development, particularly among different species (Shardo 1995; Grande & Shardo 2002). For these reasons, comparative morphological data were divided into morphological stages. These stages are characterized by structures (e.g., the formation of hypural 1 or the formation of four hypurals) that act as the defining criterion for that stage (Shardo 1995, Grande & Shardo 2002). Defining characters are relatively consistent in their relative timing of development (e.g., hypural 1 forms before hypural 2, hypural 2 forms before hypural 3, etc.), and the sequence of appearance of these skeletal elements is independent of size and age (Arratia & Schultze 1992). Additional, more variable characters that occur along with defining criteria, but are not necessarily linked, are
called concurrent features (Shardo 1995). Concurrent features have developmental timings that are more variable (i.e., development may vary with certain ecological conditions such as water temperature), and in some cases can be seen in multiple morphological stages (e.g., epural 1 forms along with hypural 5, but can also form with hypural 6) (Grande & Shardo 2002). This method of developmental staging allows for easy comparisons with other species, regardless of the length or age of the individuals being compared.

Developmental Timing or Rates of Development

To assess rates of mineralization, a one-way analysis of variance (ANOVA) was used (Systat 12) to examine differences between Esox species with respect to body size (SL) and age (hrs) at two developmental endpoints: the formation of three hypurals in the caudal fin skeleton (the point of 50% hypural ossification), and the presence of the first anterior twenty mineralized centra (the point when 50% of centra are mineralized). The lengths and ages of all fish at these two stages were recorded (3 hypurals: N = 10 E. lucius, N = 12 E. masquinongy; 20 centra: N = 30 E. lucius, N = 47 E. masquinongy). No data transformations were needed.

Four individual one-way ANOVA tests were performed: 1) to test for differences in Standard Length between Species (E. lucius and E. masquinongy) when three hypurals were present; 2) to test for differences in Age between Species (E. lucius and E. masquinongy) when three hypurals were present; 3) to test for differences in Standard Length between Species (E. lucius and E. masquinongy) when twenty vertebra centra were formed in the axial skeleton; 4) to test for differences in Age between Species (E.
lucius and E. masquinongy) when twenty vertebra centra were formed in the axial skeleton.

Glossary of Terms

Standardization of biological terms has become a major emphasis among morphologists and systematists. To help facilitate a discussion of appropriate morphological definitions and homology, as glossary of terms was constructed based on this study. Each morphological structure entry includes the following: a detailed definition of the structure; the bone type (e.g. dermal, chondral, composite, etc.); if it is a single or paired bone; the region of the axial skeleton it is located (e.g. skull, vertebral column etc.); a list of synonymous terms found in the literature; and a list of authors who use the same definition in the literature (Appendix B).

Abbreviations used in figures

aa, anguloarticular; boc, basioccipital; c&s, cleared and stained; den, dentary; ds, dermosphenotic; ect, ectopterygoid; ent, entopterygoid; ep, epiotic; exo, exoccipital; fr, frontal; hrs, hours; hy, hyomandibula; in, intercalar; int, interhyal; io, infraorbital; iop, interopercle; la, lacrimal; Let: lateral ethmoid; mx, maxilla; met, mesethmoid; mtg, metapterygoid; n, nasal; NL, notochordal length; op, opercle; pa, parietal; pal, palatine; par, parasphenoid; pmx, premaxilla; pop, preopercle; pro, prootic; pt, pterotic; pts, pterosphenoid; pu2, preural centrum 2; q, quadrate; ret, retroarticular; SL, standard length; smx, supramaxilla; so, supraorbital; soc, supraoccipital; sop, subopercle; sph, sphenotic; sym, symplectic; v, vomer; vl, vertebra 1.
Institutional Abbreviations

CU, Cornell University, Ithaca, NY; **FMNH**, Field Museum of Natural History, Chicago, IL; **LUD**, Loyola University Development Collection, Chicago, IL; **LUD. F.**, Loyola University Development Collection, figured specimen, Chicago, IL.
CHAPTER THREE

RESULTS

Overview

This thesis was expanded in a collaborative, published study by Burdi & Grande (2010). Based on the staging scheme of *Esox lucius* described above, the second author examined the developmental series of *E. masquinongy* and created a separate staging scheme for this species. This allowed for the comparison of the developmental morphology of the entire axial skeleton of both *E. lucius* and *E. masquinongy*. Grande also added, in the published work, a detailed description of both supraneurals and intermuscular bones, which was beyond the scope of this thesis. The following is the entire collaborative study published by Burdi and Grande (2010) showing the results generated.
Morphological development of the axial skeletons of *Esox lucius* and *Esox masquinongy* (Euteleostei: Esociformes), with comparisons in developmental and mineralization rates

Amanda Burdi and Terry Grande

Abstract

The developmental morphology of the axial skeleton of *Esox lucius* (i.e., Northern Pike and type species of the genus) and *E. masquinongy* (i.e., Muskellunge) was investigated. More than 1,000 specimens were examined ranging in size from about 10 mm notochordal length (NL) post-hatching juveniles, to over 80 mm standard length (SL) foraging sub-adults. Results show that regardless of individual variation, the relative sequence of bone formation and mineralization is consistent between the two species. This consistent developmental pattern enabled us to construct an ontogenetic staging scheme of eight developmental stages, each characterized by one defining criterion. The first appearance in cartilage and/or first sign of mineralization of each axial skeleton bone was plotted against time and age for each species and compared. Observed variation in bone development (e.g., number of epurals) inconsistent with the published literature is discussed.

Based on our developmental study, *Esox lucius* grows in size faster than *E. masquinongy*, but its axial skeleton develops and mineralizes more slowly. For example, at 25 mm SL, the axial skeleton of *E. masquinongy* is 55 % mineralized, while *E. lucius* is only 25 % mineralized. *Esox masquinongy* at this size however, is 1000 hours old, while *E. lucius* is only 700 hours. These results suggest that *E. masquinongy* has adapted a developmental strategy whereby more emphasis is put into skeletal development than into growth in size. This strategy may reflect the early foraging behavior of *E. masquinongy*. Unlike *E. lucius*, *E. masquinongy* absorbs its yolk sac earlier in life, and becomes an active predator just a few days after hatching. A well mineralized axial skeleton with developed dentition would facilitate this early predacious behavior.

Introduction

The genus *Esox*, family Esocidae, known for its duck-billed snout, elongated body and voracious predacious behavior, consists of five extant freshwater species distributed within North America, Europe and Asia (Grande 1999). Based on morphological (Nelson 1972, Sychevskaya 1976) and molecular data (Grande et al. 2004), these *Esox* species are divided into two subgenera: *Kenozia*, the pickereels, and *Esox*, the pikes. *Kenozia* consists of *E. vagen* and *E. americ anus* distributed throughout the eastern and central parts of North America. The subgenus *Esox* consists of *E. lucius* (Northern Pike) with a circumpolar distribution in the eastern and central United States and throughout Canada, Europe and into Asia, *E. masquinongy* (muskie or Muskellunge) distributed in eastern and central North America, and *E. reichertii* (Amur Pike) with a distribution restricted to the Amur River basin of northeastern Asia (Grande et al. 2004).

Although considerable work has been published on the species composition, phylogenetics and biogeography of the group (e.g., Nelson 1972; Crossman 1966, 1978; Ráb & Crossman 1994; Johnson & Patterson 1996; Grande et al. 2004), little has been published on the morphological development of these...
fishes. The few studies that have (e.g., Pebrson 1944, Jollie 1975, Patterson & Johnson 1995) are narrow in focus, and do not contain a large enough sample size to account for individual variation. Pehrson (1944) for example, studied the early development of the lateral-sensory canals in E. lucius, while Jollie (1975) examined the comparative ossification in the skulls of E. lucius and E. americanus, mostly in relation to bones of the cranial lateral line system. Although Jollie’s (1975) work set a good developmental foundation, the post-cranial skeleton was not addressed in his work. In addition, to date virtually no developmental information has been published on E. masquinongy.

This study addresses the comparative developmental morphology of the entire axial skeleton of both Esox lucius and E. masquinongy. Over 500 specimens of each species were examined for this study, thus providing an adequate sample size to assess inter- and intraspecific variation in bone development. This study is timely and has widespread significance since E. lucius (type species of the genus) and E. masquinongy are of both scientific and commercial importance. They are of substantial economic importance as a sport and commercial fishery, and are stocked into northern streams and lakes by the U.S. Fisheries Department. As a result, significant hybridization has been reported (Crossman & Buss 1965, Casselman et al. 1986, Tomsell & Eberle 1990). It is therefore imperative to better understand wild-type developmental morphology and developmental patterns before examining hybrids. In addition, although the Recent distribution of Esox is fairly restricted, fossil representatives are found throughout the Northern Hemisphere (Grande 1999) and China (Chang & Zhou 2002). Thus, a better understanding of the development of these two key Esox species may provide insight into the morphology of the many, often fragmented, fossil escids that are in need of assessment and possible redescription (Grande 1999).

Materials and methods

Materials

Developmental material of both Esox masquinongy and E. lucius was obtained from the Illinois Department of Natural Resources, Lake Wolf Memorial Fish Hatchery. Collections were made by the fish hatchery staff beginning in late February 2003 and continued through late June 2003. Three to five specimens of each species were sampled three times a day (i.e., morning, afternoon, and evening) until ossification of skull and vertebral column was complete, and the fish were ready to be released into the wild. Based on the examination of comparative museum specimens (see below), the axial skeleton of E. lucius is completely ossified at about 80–90 mm SL. For each species, fish samples were taken from nine spawns or clutches and pooled. Spawns for each species were fertilized on the same initial date and time, and were grown under the same environmental conditions that mimic their respective natural environments. Esox lucius spawns in early spring at water temperatures between 1.11 and 4.45 °C, while E. masquinongy spawns in later spring at slightly warmer water temperatures between 8.89 and 13.33 °C. Females of both species deposit up to 100,000 eggs scattered at random, and hatching occurs 12-14 days later.

Specimens from multiple spawns were pooled to obtain a complete developemental series (Bird & MaBee 2003). Using multiple spawns allows for the examination of the relative timing of certain structures, not genetic differences. Specimens were placed in vials of 10 % buffered formalin for three days and then transferred to 70 % ethyl alcohol. The time of fertilization was recorded for each spawn (Esox lucius 3/10/03, 2:30 p.m. and E. masquinongy 3/25/03, 11:50 a.m.), and sampling began one hour and ten minutes after fertilization for E. lucius (3/10/03, 3:40 p.m.), and four hours and ten minutes for E. masquinongy (3/25/03, 0:40 p.m.). Sampling immediately following fertilization was not imperative since this study is concerned with relative axial skeleton formation and the identification of endochondral and dermal bones, not early cleavage stages. A total of 980 specimens of E. lucius and 1500 specimens of E. masquinongy were collected for this study ranging from newly fertilized eggs to foraging sub-adults. Approximately 500 specimens of E. lucius and 600 specimens of E. masquinongy were examined for this study. All fish were deposited in the fish collection at Loyola University Chicago (LUD 081089 = E. lucius; LUD 070278 = E. masquinongy). Figured specimens were assigned individual Loyola University (LUD.F) catalogue numbers.

Additional comparative material

Esox lucius: 22 spec. (SL: 60–400 mm): FMNH 142, 144, 3160, 4007, 6304, 6460, 6724, 7406, 10064, 18090, 43024, 75232, 79584, 91381 (alcohol, c&s, cleared and stained), FMNH 32734, 9760, 9964, 73641 (dried skeletons); LUF 09808, 09809, 09811, 09825 (alcohol, c&s).
Esox masquinongy: 15 spec. (SL: 65-145 mm); FMNH: 85991; CU 9118, 19154 (c&s).
Esox reichertii: 6 spec. (SL: 65-225 mm); CU 64227, 64228, 64229, 64231 (alcohol, c&s); FMNH 109221 (alcohol).
Esox niger: 7 spec. (SL: 110-180 mm); FMNH 21811 (c&s); LUF 082291-082293 (c&s).
Esox americanus: 1 spec. (SL: 113 mm); FMNH 31768 (c&s).
Esox americanus americanus: 3 spec. (SL: 100-115 mm); FMNH 10424, 24-288-3-14 (c&s).
Esox americanus vermiculatus: 1 spec. (SL: 113 mm); FMNH 7187 (c&s).

Institutional abbreviations: CU, Cornell University, Ithaca, NY; FMNH, Field Museum of Natural History, Chicago, IL; LUD, Loyola University Development Collection, Chicago, IL; LUD.F, Loyola University, Development Collection, figured specimen, Chicago, IL; LUF, Loyola University, fish collection, Chicago, IL.

Methods

Selected specimens of each developmental stage were photographed before preparation to record external characteristics (e.g., pigmentation patterns). To examine the skeletal morphology, specimens were prepared using a modified version of Dingerkus & Uhler (1977). Because potassium hydroxide (KOH) is caustic to fragile larval specimens, an ethyl alcohol series was substituted for the KOH step. Additional specimens were stained for bone only in the event that the alizarin blue carilage stain would inhibit the alizarin red uptake. Cleared and stained specimens were stored in 90% glycerin.

Specimens were examined, dissected, and drawn using a Wild MZ8 dissecting microscope complete with drawing attachment and video capturing system. When necessary, larval specimens were examined using an Olympus BH3 compound microscope with Nomarski Optics. Alcohol and cleared and stained specimens were video captured using an Olympus SZX12 dissecting microscope and a Retiga 2000R imaging fast 1394 camera. High magnification digital photographs were then taken of all the bones.

The procedure used for scoring a particular bone as either cartilaginous or mineralized followed Bird & Mabee (2003). Specimens were scored within two days of staining. Cartilaginous structures were scored as present based upon visual examination of alizarin blue staining of chondrocytes. The earliest sign of mineralization was based upon the uptake of alizarin red stain. Total lengths (TL) were taken from each specimen examined. Notochordal lengths (NL) were taken from specimens before hypural formation, and after that, standard lengths (SL) were measured from the tip of the snout to the posterior margin of the hypurals.

Developmental staging. Comparative morphological data were divided into stages characterized by structures (e.g., the formation of hypural 1 or the formation of four hypurals) that act as the defining criteria for that stage (Shard 1995, Grande & Shard 2002). Defining characters are relatively consistent in their timing of development (e.g., hypural 1 forms before hypural 2, hypural 2 forms before hypural 3, etc.), and the sequence of appearance of these skeletal elements is independent of size and age (Arratia & Schultze 1992). Additional, more variable characters that occur along with defining criteria, but are not necessarily linked, are defined as concurrent features (Shard 1995). Concurrent features have developmental timings that are more variable, and in some cases can be seen in multiple morphological stages (e.g., epural 1 forms along with hypural 5, but can also form with hypural 6) (Grande & Shard 2002).

This method of developmental staging allows for easy comparisons with other species, regardless of the length or age of the individuals being compared.

Developmental timing or rates of development. Staging data suggests that Esox masquinongy mineralizes at a faster rate and at a smaller size in comparison to E. lucius. To assess rates of mineralization, a one-way analysis of variance (ANOVA) was used (Systat 12), to examine differences between Esox species with respect to body size (SL) and age (hrs, hours) at two developmental end-points: the formation of three hypurals in the caudal fin skeleton (the point of 50% hypural ossification), and the presence of the first anterior twenty mineralized centra (the point when 50% of centra are mineralized). The lengths and ages of all fish at these two stages were recorded (3 hypurals: N = 10 E. lucius, N = 12 E. masquinongy; 20 centra: N = 30 E. lucius, N = 47 E. masquinongy). No data transformations were needed.
Fig. 1. First appearance and/or mineralization of individual bones within the axial skeleton. Cranial and post-cranial bones are divided by species and arranged in order from the last to form (top) to the earliest. Blue denotes cartilage, red mineralization. Specific ages noted on the x-axis for comparisons. A, *Esox lucius* cranial bone formation relative to standard length (mm). B, *Esox masquinongy* cranial bone formation relative to standard length (mm). C, *Esox lucius* post-cranial bone formation relative to standard length (mm). D, *Esox masquinongy* post-cranial bone formation relative to standard length (mm). Figure format modified from Bird & Mabee (2000).
Developmental staging

The development of *Esox lucius* and *E. masquinongy* can be divided into three major periods as described in Bemis & Grande (1992) and Shando (1995): the embryonic period (defined as the period from fertilization to hatching), the yolk sac larval period and the foraging period (identified by the absence of yolk, a developed gut and often food in the stomach and intestine). This study compares the relative sequence of bone formation in both *E. lucius* and *E. masquinongy* and not early cleavage stages, so staging in *Esox* begins with the yolk sac period. Unlike the American shad (Shando 1995), catfish (Grande & Shando 2002), and paddlefish (Bemis & Grande 1992), the yolk sac period in *Esox* is very short. Teeth begin to form in *E. masquinongy* at about 11 mm SL and shortly after that in *E. lucius*, at about 14 mm SL. Within staging periods 2-3, the yolk sac is completely absorbed and food was observed in the intestine of both species. The sequence of the first appearance of each axial element in cartilage and/or bone as a function of age and length is presented in Figure 1A-D. Although the exact time of bone formation, as indicated by the age and length of our specimens of *E. lucius*, differs from Jollie’s (1975) reports, the sequence of bone formation observed in our material and his is generally similar. Exceptions are discussed within the relevant stage.

In general, our results demonstrate that *E. masquinongy* and *E. lucius* share a common developmental pattern in the postcranial skeleton, hypurals forming first, but only after the formation of the cartilaginous
hyomandibular, dentary and quadrate bones. The first skull bones in each species to begin mineralization are the hyomandibula and dentary, followed by the maxilla, symplectic and opercle. Like the results found by Jollie (1975), the last skull bones to mineralize are the supraorbitals and dermosphenotics. Our results show that although the relative pattern of bone formation between the two species is similar, whereas the timing of bone formation and mineralization differs. For example, the hyomandibula is the first bone to form in the skull. In *E. masquinongy* it forms in cartilage at 10.3 mm SL, while in *E. lucius* it forms at 13.5 mm SL. The frontals in *E. masquinongy* begin formation at 20.6 mm SL and in *E. lucius* at 35.5 mm SL. The opercles in *E. masquinongy* form at 12.7 mm SL, while in *E. lucius* they form at 17.5 mm SL.

This study identifies eight developmental stages. The first two stages are yolk sac stages. Interestingly, *Esox lucius* retains its yolk sac well into stage three, while *E. masquinongy* absorbs its yolk sac and begins foraging much earlier, in Stage 2. Larger specimens of *E. masquinongy* at stage two were observed with food in their stomachs. Food was not observed in the stomachs of *E. lucius* at this stage.

**The length and age ranges for each stage with defining criteria**

**Stage 1:** Neural tube and notochord formed
- *E. lucius*: 11.5-12.0 mm SL; 412-428 hrs
- *E. masquinongy*: 10.3-11.4 mm SL; 506-530 hrs

**Stage 2:** Formation cartilaginous hypurals 1 and 2
- *E. lucius*: 12.0-15.5 mm SL; 428-528 hrs
- *E. masquinongy*: 10.3-15.0 mm SL; 530-697 hrs

**Stage 3:** Formation of at least 4 cartilaginous hypurals (hypurals 1-4)
- *E. lucius*: 11.0-17.5 mm SL; 467-665 hrs
- *E. masquinongy*: 17.5-18.5 mm SL; 836-883 hrs

**Stage 4:** Formation of at least 5 cartilaginous hypurals (hypurals 1-5)
- *E. lucius*: 12.5-19.0 mm SL; 570-696 hrs
- *E. masquinongy*: 17.5-23.7 mm SL; 836-1250 hrs

**Stage 5:** Formation of at least 6 hypurals (hypurals 1-6)
- *E. lucius*: 16.0-29.5 mm SL; 653-833 hrs
- *E. masquinongy*: 21.2-30.5 mm SL; 837-1496 hrs

**Stage 6:** Formation of at least 5 centra (centra 1-5)
- *E. lucius*: 29.5-35.5 mm SL; 750-834 hrs
- *E. masquinongy*: 32.9-41.2 mm SL; 1515-1580 hrs

**Stage 7:** Formation of ural centra 1 and 2
- *E. lucius*: 35.5-70.0 mm SL; 834-1275 hrs
- *E. masquinongy*: 35.0-63.0 mm SL; 1415-1690 hrs

**Stage 8:** Formation of dermosphenotic
- *E. lucius*: 70.8-82.0 mm SL; 1277-1422 hrs
- *E. masquinongy*: 62.5-81.0 mm SL; 1688-1796 hrs

**Stage 1**

Stage 1 is defined by a formed neural tube and notochord (Grande & Shardo 2002). Cartilaginous visceral gill arches including the interhyal and a cartilaginous hyomandibula are present (Fig. 2A). Also present at this stage in both species are the optic vesicles, Meckel’s cartilage, pterygoid cartilage, paraphenoid and sclerotic ring. The palatine bone is thin and just beginning to form. There are no teeth on any skeletal element. The notochord is straight, uncondstricted, and extends to the tip of the caudal fin. Hypurals, dorsal and ventral acrocentra and median fin pterygiphyses are not present in either species. Pectoral fin buds first appear and all fish at this stage exhibit a continuous fin fold.
Stage 2

This stage is characterized by the formation of cartilaginous hypurals 1 and 2. This is the first consistent benchmark in axial skeleton formation (Fig. 3A). At the onset of this stage, both species are still in the yolk sac, a larval stage of development. *Esox masquinongy*, however, absorbs its yolk sac by about 10.6 mm SL, and is foraging by the end of the stage. In many specimens examined, the cartilaginous parhypural is also present. In both species, the cleithrum is clearly mineralizing, along with at least eight anterior cartilaginous arcocentra (terminology of Schultze & Arratia 1989, Arratia & Schultze 1992; basidorsals of Grande & Bemis 1998). Fifteen arcocentra are observed in *E. lucius*, and 8 in *E. masquinongy* (Fig. 2B,C). The cartilage that eventually forms the exoccipital is also observed in both species. In *E. masquinongy* the opercle begins to mineralize at the articulation facet with the hyomandibula, and sharp pointed teeth stained with alizarin red are observed extending from the dentary (about 12),
vomer (about 6) and palatine (about 8) cartilages. In some specimens of *E. masquinongy*, tooth plates are observed on ceratobranchial 5. Teeth are observed in a few specimens of *E. lucius*, but they are far fewer in number than they are in *E. masquinongy* (e.g., vomer: 2, palatine: 3-4, dentary: 5). According to Jollie (1975) the vomer has a bilateral origin and fusion of the anlagen occurs later in development. We are unable to support Jollie’s (1975) observation based on our material. At no time in development does the vomer appear to be bilateral. The dentary at its symphysis is also beginning to mineralize in *E. masquinongy*. The hyomandibular cartilage (hyosymplectic cartilage of Grande & Bemis 1998) has begun to differentiate into the hyomandibula and the symplectic. The snout in *E. masquinongy* already has begun to elongate, taking on its characteristic duck-billed appearance. The snout of *E. lucius* remains rounded and larval in appearance.

**Stage 3**

*Four cartilaginous hypurals (hypurals 1-4) are present at this point, thus defining Stage 3 (Fig. 3B).*

By this foraging stage, cartilaginous dorsal aracoenstra have formed along the length of the notochord in *Esox lucius*, while most have formed in *E. masquinongy*. Notochordal flexure is observed in some specimens of *E. lucius*, but not *E. masquinongy*. At least ten cartilaginous ventral aracoenstra directly anterior to the parhypural are observed in both species. In *E. lucius*, three or four aracoenstra directly anterior to the parhypural have already formed haemal arches. Also in both species, dorsal and anal fin supports are obvious. In *E. lucius*, five dorsal and five anal fin radials are observed, while in *E. masquinongy*, thirteen dorsal and eleven anal fin radials are observed. In *E. lucius* the mineralizing opercular bone is now observable, and there are a few more teeth evident on the dentary, vomer and the palatine cartilages. The pterygoid cartilage is not differentiated at this point, but elongation of the palatine and dentary is already producing the characteristic *E. lucius* duck-billed skull. *Esox masquinongy*, unlike *E. lucius*, has numerous, small, needle-like teeth. At this point, the skull bones of *E. masquinongy* are considerably more mineralized than those of *E. lucius*. In *E. masquinongy*, the dentary, symplectic, palatine, maxilla, quadrate, paraphenoid and hyomandibula are clearly stained with alizarin. The supraoccipital is observable in *E. masquinongy* but not in *E. lucius*. Tooth plates are present on the epibranchials and small tooth patches are seen lining each side of ceratobranchials 3 and 4. In *E. masquinongy*, an expanded supraneural diagnostic of the genus (Grande et al. 2004) has formed in cartilage above centra 1 and 2.

**Stage 4**

*The presence of five hypurals (hypurals 1-5) in the caudal fin skeleton defines this stage (Fig. 3C).*

Notochordal flexure is observed in all specimens. In *E. lucius*, the expanded supraneural is now observable, along with the mineralizing maxilla, hyomandibula, quadrate and supraoccipital with crest. Posterior supraneurals are not observed in either species at this stage. In *E. masquinongy* (Fig. 2D), the pterygoid cartilage has begun to differentiate and distinct ectopterygoid and endopterygoid bones are observed. In addition, mineralization of the vomer, retroarticular, prootic and exoccipital bones is observed in specimens of *E. masquinongy* but not in *E. lucius*. Premaxilla, supramaxilla and pterotics are not observed in either species. Distal and proximal radials are observable in the dorsal and anal fin skeletons of *E. masquinongy*. Radial differentiation is not observed until later in *E. lucius*. In the caudal fin, the hypurals show marked elongation in both species. Two epurals are observed in *E. lucius* while three are observed in *E. masquinongy*. Actinotrichia have formed distinct caudal fin rays in *E. masquinongy* but not in *E. lucius*. Actinotrichia completely surround the dorsal and anal fins of both species at this stage.

**Stage 5**

*The presence of six hypurals (hypurals 1-6) defines this stage (Fig. 3D).*

Although the timing of the formation of hypurals 5 and 6 is close together and in some cases might be incorporated into one stage, hypural 6 always forms after hypural 5, and is associated with distinct developmental changes. We have thus put the formation of hypural 6 into its own stage for ease of comparisons. In some specimens of *E. masquinongy*, the urocentral has begun to form. In *Esox lucius*, the formation of epural 3 accompanies hypural 6 in development. Hypurals 1-5 show significant ossification in their centers. Median fins now have distal and proximal radials and all haemal and neural arches have formed and have begun to mineralize. From the hyomandibular cartilage, a cartilaginous symplectic is obvious. This differentiation occurs earlier in *E. masquinongy*, and like *E. masquinongy*, ossification of the
Fig. 3.
Caudal fin development and mineralization in *Esox masquinongy*.
A, LUD.F 2418101, 12.1 mm SL.
B, LUD.F 241803, 14.6 mm SL.
C, LUD.F 241804, 21.2 mm SL.
D, LUD.F 241807, 25.5 mm SL.
E, LUD.F. 241808, 40.0 mm SL.
F, LUD.F 241809, 41.1 mm SL.
Anterior to the left.
symplectic begins close to the center of the bone. The ceratohyal now has distinct anterior and posterior portions with a full complement of branchiostegal rays. Like those of *E. masquinongy* (Fig. 2E), the anterior acrocentra have elongated and ribs are obvious.

In *E. masquinongy*, the frontal bones show a high degree of mineralization. Early in development, they begin to mineralize around the orbit and extend anteriorly, but fall short of the vomer. They remain distinctly paired and separated until late in development. Jollie (1975) argued that the frontals form without any association with sensory organs. This seems to be the case in our material as well for *E. masquinongy*. Although this is more evident in our specimens of *E. lucius*, the frontal bones develop medial to the supraorbital canal of the lateral line. Even in large, fully ossified specimens of *E. lucius*, (FMNH 9964, cranium = 185 mm) the frontals and sensory organ seem distinct. The mesethmoid, now mineralized in *E. masquinongy* (mineralized in stage 6 for *E. lucius*), run lateral to the paired frontals. Contrary to Jollie (1975), the frontals in our largest specimens are not fused. The snout is very pointed and well ossified. Premaxillaries and supramaxillaries are present in all specimens of *E. masquinongy*, but not in *E. lucius*. The complete opercular series has mineralized in *E. masquinongy* at this time.

In *E. masquinongy*, sphenotics, epiotics and parietals show mineralization. Paired basisphenoids, formed in membrane bone (Patterson 1977) are identified in a few specimens of *E. masquinongy*. According to Patterson & Johnson (1995), basisphenoids are absent in *Esox*, and homoplastically absent in ostariophysans. We however observed these very small, weakly mineralized structures positioned ventral and medial to the pterosphenoid as described in Jollie (1975) and Grande & Bernis (1998). We therefore follow Jollie (1975) for the identification of these structures. The supracipital, formed earlier in *E. masquinongy*, shows a high degree if mineralization at this stage. According to Jollie (1975), the quadrate bone in *Esox* is a composite bone formed from a fusion of the quadratojugal (cartilage bone) and the quadrate (dermal bone). The quadrate forms the ventrolateral arm of the mineralizing quadrate, and positions the symplectic between it and the quadrate proper. Although we could not definitively assess this condition in *E. lucius*, the quadrate bone in *E. masquinongy* does appear to form from distinct structures that are partially fused to each other at this stage. In older or larger specimens of *E. masquinongy*, the quadrate appears as one bone with no sign of a fusion with another element. Jollie (1975) refers to this bone as the quadratejugo-quadratojugal bone.

**Stage 6**

Stage six is defined by the early the formation of the vertebral column with at least five anterior centra (centra 1-5) present (Fig. 4).

As discussed in Arratia & Schultze (1992) and Grande & Shardo (2002), after the notochord has constricted into protocentra, chondrocentra begin to develop and thicken within the notochordal sheath of each protocone in an anterior-to-posterior direction. At this stage in development, however, only a thin mineralized surface layer is present on each protocentrum. Autoconcentra will ultimately arise from direct ossifications, surround the chondrocentra and complete the formation of the vertebral column. The chondrocentra in *Esox* mineralize simultaneously both dorsally and ventrally, eventually meeting in the middle and forming a complete ring. This pattern differs from the ventral to dorsal pattern observed by Arratia & Schultze (1992) for *Onchorhynchus*.

In specimens of *Esox lucius* of 30 mm SL, about 18 chondrocentra are formed. In specimens of *E. masquinongy* of about 32 mm SL, 18-24 centra are observed. Additional characters observed in *E. lucius* include the presence of twenty cartilaginous supranerves beginning at about vertebra 14, and mineralized premaxillary, supramaxillary, retroarticular and metapterygoid bones. Neural and haemal arches/spines, ribs, and fin supports are well mineralized. Saccular and lagena otoliths are present and all gill arch dentition is complete. In *E. masquinongy*, the skull bones continue to mineralize and the basisphenoid is observable in all specimens examined. In most specimens of *E. masquinongy*, the uro neural has also formed. Ural centra are not observed in any specimen at this stage regardless of the presence of an uro neural. The expanded supranerve is still cartilaginous in both species.

**Stage 7**

Formation of ural centra 1 and 2 in the caudal skeleton defines this stage (Fig. 3E,F).

As discussed above, the caudal skeleton in *Esox* consists of six hypurals, the parhypural that forms almost synchronously with hypurals 1 and 2, an autogenous uro neural, ural centra 1 and 2 that form in associa-
tion with the hypurals, and preural centrum 1 that forms in association with the parhypural (Fig. 3F). Three (sometimes two or four) epurals are positioned between the uroneural and the neural spine of pu2. In both species of *Esox* the uroneural forms before u1 and u2. The uroneural in *E. masquinongy* begins to form in Stage 5. Although Arratia & Schultze (1992) refer to this element as a stegural, it is very splint-like in our specimens, and shows no membranous outgrowth throughout development. Unlike stegurals described in salmonids, the uroneurals in our *Esox* specimens do not appear to be cartilaginous in origin. Pending a histological study, we refer to this element as an uroneural.

In any event, ural centrum 2 forms after the uroneural, followed by ural centrum 1. Both ural centra, like those described in Arratia & Schultze (1992), form as chordacentra from ventral mineralizations. When first formed, a considerable space is present between the two ural centra (Fig. 3E). As the ural centra mineralize, they thicken and grow laterally, obliterating the space between them. At this stage, all protocentra show signs of mineralization. The more anterior chordacentra are more heavily stained with alizarin, indicating more mineralization in relation to the more posterior ones. Chordacentra that show the lowest amount of mineralization are those in the center, parallel to, or just anterior to, the dorsal fin. Caudal chordacentra show dense mineralization.

Additional structures mineralized in *Esox lucius* during this stage include the prootics, nasals, supraorbital bones, sphenotics and lateral ethmoids. All pterygoid bones are well mineralized and the frontal bones are about two-thirds complete. The epiotics, intercals, supraoccipital and parietals are now well mineralized in *E. lucius*. In addition to the structures described for *E. lucius*, the dermosphenotic is observed in five specimens of *E. masquinongy*. According to Jollie (1975), the dermosphenotic developed very late in his specimens of *E. lucius* (90 mm). Based on our material, we agree with Jollie in the developmental timing of the bone. However, in our specimens of *E. masquinongy*, the dermosphenotic is observed in fish at 45 mm SL, considerably shorter than seen in *E. lucius* in his, or our, *E. lucius* material. Also at this stage, the lacrimal is observed in both species (*E. masquinongy* 53 mm SL, *E. lucius*: 40.0 mm SL). During lacrimal development, a thin, thread-like extension projects anteriorly from the main body. This anterior extension continues mineralizing both anteriorly and dorsoventrally, eventually forming a finger-like projection (see Stage 8). The infraorbital canal never extends forward from the body of the bone. Lastly, a mineralized and expanded supraneural is observed in some specimens of both species, although the more posterior ones remain cartilaginous.

Stage 8
This stage is defined by the mineralization of the dermosphenotic and the completion of the infraorbital series.
Stage 8 is the last stage in our series. Although the dermosphenotic was observed in a few *Esox masquinongy* specimens in Stage 7, this bone is consistently observed in both species from this point onward. Contrary to Jollie (1975), the first appearance of the dermosphenotic in our *E. lucius* specimens is at
70.0 mm SL. In Jollie's specimens, the dermosphenotic appears in *E. lucius* at 90 mm SL. Intraspecific variation in the number of infraorbitals was discussed by both Nelson (1972) and Jollie (1979). Jollie (1979) however, illustrated *E. lucius* with five small infraorbitals. Not counting the dermosphenotic and lacrimal, our specimens have two larger infraorbitals (Fig. 5A). It is possible that the condition in our specimens might represent another variation in infraorbital configuration, or a fusion of the elements illustrated by Jollie (1975). Four infraorbitals are observed in *E. masquinongy* (Fig. 5B). In both species, the infraorbital canals are not complete, and each bone looks more like a long pit with sides. The dorsal closure of the canals occurs later in development. As illustrated in Figure 5A,B, the anterior projection of the lacrimal in *E. masquinongy* is comparatively longer than in *E. lucius*, and the entire bone is narrower and more delicate.

All bones of the axial skeleton show some degree of mineralization including the posterior supraneurals. In specimens of *Esoc masquinongy* of about 65 mm SL, the supraneurals have extended posteriorly to the end of neural arch three. In larger specimens examined, the neural arch reaches the anterior margin of arch four as in Grande et al. (2004). Also at this sub-adult stage, differences in overall body shape between the two species are easily comparable. The vertebral column in *E. lucius* exhibits a dorsal curvature anterior to the dorsal fin, while the vertebral column in *E. masquinongy* is straight from head to tail. As discussed by Crossman & Casselman (1969), *E. masquinongy* differs from all other esocids in that the mid-ventral groove carrying the dorsal aorta is shifted dorsolaterally at about centrum eight. The groove shifts back to a mid-ventral position after about centrum 30. This subtle shift was also observed in specimens of *E. masquinongy* (e.g., over 180 mm SL), but not in our earlier developmental material.

In the skull, a few difference in bone structure are worthy of note (Fig. 6A-D). In dorsal aspect (Fig. 6A,C), the mesethmoids of *Esoc masquinongy* are more robust than those of *E. lucius*. Unlike *E. lucius*, the nasals in *E. masquinongy* are smaller and do not overlap with the mesethmoids. The parietals in *E. lucius* are more rounded in shape, are overlapped by the frontals anteriorly, and are widely separated from each other, while those of *E. masquinongy* are triangular, larger in similarly sized fish and obscure most of the supraoccipital dorsally. In *E. masquinongy*, the chondral component of the lateral ethmoids is still visible, but not in *E. lucius*. In ventral aspect, the vomer of *E. masquinongy* is relatively shorter in length, wider anteriorly, and less pointed at its posterior tip in comparison to *E. lucius* (Fig. 6B-D). These findings agree with those of Casselman et al. (1986) except that teeth in our specimens of *E. masquinongy*
Fig. 6.
Skulls of *Esox lucius*, dorsal view (A), LUD.F 241811, 78.4 mm SL; *Esox lucius*, ventral view (B), LUD.F 241811; *Esox masquinongy*, dorsal view (C), LUD.F 241812, 81.0 mm SL; *Esox masquinongy*, ventral view (D), LUD.F 241812. Anterior to top. Abbreviations: boc, basisphenoid; ep, epiotic; exo, exoccipital; fr, frontal; in, intercalar; l.et, lateral ethmoid; met, mesethmoid; n, nasal; pa, parietal; par, parasphenoid; pro, prootic; pt, pterotic; pts, pterosphenoid; so, supraorbital; soc, supraoccipital; sph, sphenotic; v, vomer.
Fig. 7.
Suspensorium of *Esox lucius*, lateral view (A), LUD.F 241811, 78.4 mm SL; *Esox lucius*, medial view (B), LUD.F 241811; *Esox masquinongy*, lateral view (C), LUD.F 241812, 81.0 mm SL; *Esox masquinongy*, medial view (D), LUD.F 241812. Anterior to left. Abbreviations: *aa*, anguloarticular; *den*, dentary; *ect*, ectopterygoid; *ent*, entoptrygoid; *hy*, hyomandibula; *int*, interhyal; *iop*, interopercle; *mx*, maxilla; *mtg*, metapterygoid; *op*, opercle; *pal*, palatine; *pmx*, premaxilla; *pop*, preopercle; *q*, quadrato; *ret*, retroarticular; *smx*, supramaxilla; *sop*, subopercle; *sym*, symplectic.
appear to be positioned randomly, not in the single row. The paraphenoid in *E. masquinongy* appears wider than that of *E. lucius*, but the frontal bones in *E. masquinongy* are more streamlined, possibly giving the paraphenoid the appearance of wideness. Lastly, the supraoccipital bone in *E. lucius* is rounded in comparison to the more oval supraoccipital in *E. masquinongy*.

In comparison to *E. lucius*, the jaw bones and suspensorium are relatively elongate in *E. masquinongy* (Fig. 7A-D) and angled somewhat more laterally, once again giving *E. masquinongy* a more elongate-looking snout. One striking difference between the two species at this stage is the numerous teeth in the jaws of *E. masquinongy*. Not only do the jaws have more teeth, but many more of them are fang-like in appearance. Unlike that of *E. lucius*, the premaxilla in *E. masquinongy* displays a small row of teeth that extends along the expanded posterior margin of the bone. Teeth form on the premaxilla much later in *E. lucius* (beyond our developmental series), and when they do, the teeth are much smaller in size and number than in *E. masquinongy*.

Developmental timing or rates of development

**Three hypurals.** Based on the specimens examined (Fig. 8A,B) with three hypurals in the caudal fin skeleton, *E. lucius* (*n* = 10) was significantly smaller in size (Mean SL ± SE = 21.3 ± 0.38) and younger in age (Mean hrs ± SE = 688.7 ± 12.44) than *E. masquinongy* (*n* = 12) (Mean SL ± SE = 22.5 ± 0.36) (Mean hrs ± SE = 992.7 ± 16.54) (SL: *F*<sub>1,11</sub> = 5.805, *p* < 0.04) (hrs: *F*<sub>1,11</sub> = 203.645, *p* < 0.001).
Twenty centra. Our data indicate that based on the specimens examined of *Esox masquinongy* (n = 47) with 20 mineralized anterior centra, this species is significantly smaller (Mean SL ± SE = 51.0 ± 1.267) in comparison to *E. lucius* (Mean SL ± SE = 63.7 ± 3.338) at the same developmental time point (*F*₁,₁₁ = 19.367, *p* < 0.001). Interestingly, our data also show that even *E. masquinongy* is a smaller fish at this point, *E. lucius* is younger in age (Mean hrd ± SE = 1223.0 ± 46.523) than *E. masquinongy* (Mean hrd ± SE = 1637.5 ± 19.076). (*F*₁,₁₁ = 95.858, *p* < 0.001). Thus, based on these data, at the twenty centrum point, *E. masquinongy* is a smaller but older fish. This suggests that although *E. masquinongy* is growing at a slower rate, its axial skeleton is developing more rapidly in comparison to *E. lucius*.

**Discussion**

**Overview of cranial development**

Both *Esox lucius* and *E. masquinongy* are famous for their specialized skull morphologies, which are most likely correlated with their predacious foraging habits. Together with the Russian *E. reichertii*, they share several osteological, and morphometric characters that unite them in the subgenus *Esox*, and distinguish them from their sister group, the pickerels (subgenus *Kenozia*; Grande et al. 2004). *Esox lucius* and *E. masquinongy* are distinct from each other, and these differences are reflected in their developmental morphologies and patterns. As illustrated in Figure 1, cranial formation and mineralization, plus the elongation of the anterior skull bones, occurs relatively faster in *E. masquinongy*. At 20 mm SL, for example, 18 skull bones have begun to form in *E. masquinongy* compared to 11 in *E. lucius*. This pattern of rapid cranial development in *E. masquinongy* seems to hold true with respect to both chordal bones (e.g., hyomandibula, sphenotic and lateral ethmoids), and dermal bones (e.g., frontal bone elongation).

The difference in skull morphology, however, can best be observed in relation to the formation of the dentition and the tooth-bearing bones. Teeth begin to form on the vomer, palatine, dentary and gill elements of *E. masquinongy* first, as opposed to the situation in *E. lucius*. Teeth in *E. masquinongy* also increase in number at a faster rate than they do in *E. lucius*. In *E. masquinongy*, teeth form first on the vomer, and then on the palatine and dentary. As a result, the mouth of *E. masquinongy* is full of teeth at a very early age. As discussed in Grande et al. (2004) and Casselman et al. (1986), the lateral edge of the vomer in *E. masquinongy* bears a pair of large fang-like teeth with smaller teeth intermixed between them. In addition, these long teeth form a single row that extends about half the length of the vomer. In *E. lucius*, the vomer consists of densely arranged teeth that extend at least two thirds the length of the vomer. Unlike the teeth in *E. masquinongy*, those in *E. lucius* are anteriorly uniform in size, and gradually decrease in size posteriorly. The palatine bones of both species also reflect this dentition pattern. *Esox masquinongy* exhibits several fang-like teeth along the medial edge, with smaller teeth intermixed. In *E. lucius* the teeth are more uniformly graded and stout, none of them resembling a fang. According to Casselman et al. (1986), the teeth on the dentary of *E. masquinongy* are robust, uniformly tapered and slightly recurved. The teeth of *E. lucius* are laterally compressed, with a prominent blade-like edge near the base. These distinctive tooth morphologies were only observed in very large specimens (e.g., FMNH 9964), and not in our developmental material. Distinctive tooth shapes must develop much later and beyond the time line of our developmental series.

Premaxillary teeth were not observed in any of our developmental material until Stage 8 for *E. masquinongy*. This suggests that teeth on the premaxilla also develop very late, and might be inconsequential to the survival of younger fish.

**Overview of post-cranial skeleton**

Historically, the development of the post-cranial axial skeleton in either of the two examined species of *Esox* has been insufficiently studied, and this is certainly so in comparison to the formation of the skull. Within this section we will discuss key elements of the post-cranial skeleton and sources of variation based on our developmental material.

The genus *Esox* is diagnosed by an interesting character (i.e., the presence of an expanded supraneural positioned above the anterior-most neural arches; Grande et al. 2004). This supraneural forms as a single cartilage early in development (stage 3 in *E. masquinongy* and later in *E. lucius* above neural arches one and two. In *E. masquinongy*, the supraneural expands posteriorly, reaching centrum four, whereas in *E. lucius* and *E. reichertii*, it expands only to the anterior limit of centrum three. Subsequent to the formation
of the anterior supraneural, more characteristic supraneurals form beginning at neural spine fourteen. According to Johnson & Patterson (1996), the first supraneural forms independently from the remaining ones. Observations from our developmental material (e.g., the large gap in time between the formation of the anterior and posterior supraneurals) support their findings.

Based on our material, two sources of variation were observed in both species of Esox. First, according to Patterson & Johnson (1995), the first pleural rib in all esocids is positioned on vertebra two (v2). This condition was reported by them in E. lucius (ROM 598 CS) and E. americanus (BMNH 1982.11.60.16), plus two specimens of Umbrà and Novumbra. We agree with Patterson & Johnson (1995) that the more common condition observed within Esox is the association between pleural rib one and v2, but we have also observed the first pleural rib extending from vertebra three (v3) in both E. lucius and E. masquinongy. Interestingly, we found the association of v3 and the first pleural rib to be more common in E. masquinongy than E. lucius. This is evident from both the developmental and adult comparative material examined (e.g., E. masquinongy, CU 9118). Also observed is that, when the pleural rib is absent from v2, its large parapophysis still forms and mineralizes. In addition, we observed the first pleural rib extending from v3 in some specimens of Kenozoa as well (e.g., E. niger, FMNH 21811). Additional study is necessary to quantify the extent of this variation among esocids.

Patterson & Johnson (1995) described the intermuscular bone arrangement for E. lucius, stating that the first four or five epineurals are broadly fused to the base of their respective neural arches, the epineurals are forked from v7-8 posteriorly, and the first three pleural ribs (v2-4) are co-ossified with the parapophysys in membrane bone, the remaining ribs ossifying in cartilage. From our research, we are able to provide a developmental perspective to the results of Patterson & Johnson (1995), and add information about the intermuscular condition in E. masquinongy. First, we agree with Patterson & Johnson (1995) that the first 4-5 epineurals are fused to their respective neural arches in E. lucius. The more common condition observed was a fusion of the first 5 epineurals. However no fusion between an epineural and the accessory neural arch was observed. Epineurals in our material branched from about v12 posteriorly. In E. masquinongy, only the first two epineurals (v1-2) were fused to their neural arches. In five specimens examined, an epineural was fused to the accessory neural arch in the same manner seen with arches one and two. Epineurals branched from about v6 posteriorly.

As illustrated in Esox lucius (Fig. 9), all parapophyses (v2 and posteriorly) form as cartilaginous arcocentra (Stage 2). Pleural ribs form as cartilaginous extensions of these parapophyses (Stage 4). Subsequently,
the ribs detach from their respective paraphyses, and as observed in both species (about 35.0 mm SL),
begin to mineralize proximally to distally. Both the ribs and paraphyses continue mineralizing, and
in the end, the ribs remain articulated with, but not fused to their paraphyses. In *E. lucius*, however,
the first two or three ribs do not appear to separate from their paraphyses. The ribs continue to
mineralize distally and the paraphyses begin ossifying first at the articulation site with the rib, and then
more proximally. These ribs differ from the more posterior ones in that they are flattened, expanded, and
considerably shorter than the thin elongated ribs that follow. In *E. lucius* specimens of about 50.0 mm SL,
the first two or three ribs are inseparable from their paraphyses. We agree with Patterson & Johnson
(1995) that these ribs are co-ossified with their paraphyses. Co-ossification as defined here is a fusion
of two elements, in which each element is still identifiable. In the case of v2-4, the paraphyses are still
recognizable as such. However, in a complete fusion, the rib and paraphysis are not discernable as
separate elements.

Variation in co-ossification patterns within multiple specimens of *E. lucius* was observed, possibly
indicating a more complicated condition than proposed by Patterson & Johnson (1995). For example, in
specimen FMNH 9964 (dried skeleton), the right side of v2 is co-ossified, but the left side is not; the rib
and paraphysis just articulate with each other, and the paraphysis is intact. On the left side of v3,
the rib is completely fused with its paraphysis, while the right side is co-ossified. The condition in
*E. reicherti* (e.g., CLI 64229, 135 mm SL) is very similar to that of *E. lucius* except that only the first two
ribs are co-ossified. The ribs, although modified, do not look as massive as those of *E. lucius*. It should not
be surprising that the condition in *E. reicherti* resembles that of *E. lucius* as they are sister taxa (Grande
e t al. 2004). The condition in *E. masquinongy* , however, is considerably different from that in either *E. lucius*
or *E. reicherti*, in that all of the ribs separate from their respective paraphyses during development, and
no co-ossification occurs. The ribs in *E. masquinongy* are unmodified and appear thin and elongated. There
is essentially no difference between the anterior and posterior ribs in *E. masquinongy*. In some examined
specimens of *E. verniculatus* (e.g., FMNH 7187), *E. americanus* (e.g., FMNH 31768) and *E. niger* (e.g.,
FMNH 21811), the anterior ribs are neither fused nor co-ossified to their paraphyses. Co-ossification
was, however, observed in other specimens of *E. americanus* (Arratia pers. comm.).

The subgenus *Esox* is diagnosed in part by the presence of three epurals (Grande et al. 2004). Variation
in the number of epurals was, however, observed in both species. Although the presence of three
epurals is the most common condition, two epurals (diagnostic number for the subgenus *Kenosa* ), and
even four epurals were often observed. Variation in salmonid caudal skeleton morphology was addressed
by Arratia & Schultz (1992) with respect to ural centra formation. Fish raised under hatchery condi-
tions consistently had two ural centra, while wild caught fish often had more. It is possible that there
is a higher level of plasticity in caudal fin formation among lower euteleosts than originally thought.
Additional study is necessary to investigate skeletal variation among lower euteleosts; we hope that this
paper provides insight into the subject.

Overview of Comparative Developmental Rates

Results from this study (i.e., ANOVA analyses of targeted postcranial elements and observations of species’
cranial morphologies) indicate that, compared to related species, *Esox masquinongy* devotes relatively more
energy to skeleton formation and relatively less to increasing body size. *Esox lucius* on the other hand,
spends its developmental time growing larger in comparison to *E. masquinongy*. Developmental strategies
vary from species to species, and even among closely related ones such as *E. lucius* and *E. masquinongy*.
Considering the postcranial axial skeleton, based on our ANOVA study, once the caudal skeleton has
begun to form, the axial skeleton of *E. masquinongy* mineralizes faster in comparison to that of *E. lucius*,
despite its slower growth rate. At the experimental end point of 20 centra formed, results indicate that
each individual of *E. masquinongy* is an older but smaller fish with a more completely developed skeleton.
These results are in accord with our observations of cranial development. As illustrated in Figure 1, the
skull of *E. masquinongy* begins development first, and at 25 mm SL, each individual of *E. masquinongy* is
more developed and more mineralized than one of *E. lucius*, but older in age. When both fish are about
800 hrs old, an individual of *E. masquinongy* is a smaller fish with a more developed axial skeleton than
is an individual of *E. lucius*.

Interestingly, *E. masquinongy* ultimately lives longer than *E. lucius* (about eight and seven years respec-
tively) and eventually grows to be a larger fish (Tomelleri & Eberle 1990). These data might seem
incongruent with the results of the present study, but insight might come from understanding the early
life history stages of these species. As discussed in Mecozzi (1989a,b), both species hatch at about fourteen days after fertilization (depending upon water temperature). *Esox lucius* begins to feed on zooplankton at about ten days of age, and on small fish when they reach fingerling size (about 15.2 cm). *Esox mas-quiNumy*, on the other hand, begins to feed on zooplankton one day after hatching and feeds on small fishes within a couple of days after that. Essentially, *E. masquiNumy* becomes a predator before *E. lucius* does. It stands to reason that *E. masquiNumy* would need a more developed axial skeleton and full set of canine teeth, even at a small size, to assume this role. Assessing the validity of this hypothesis will require concerted and collaborative efforts among morphologists and fish ecologists. The present study, however, provides a developmental foundation and a testable hypothesis for future studies comparing the developmental and mineralization rates between *E. lucius* and *E. masquiNumy*.

Acknowledgments

Because of her tremendous contribution to ichthyology and fish developmental morphology, this study in *Esox* axial skeleton development is dedicated to Gloria Arratia. The second author wishes to thank Dr. Arratia for her many years of mentorship, active debate, and friendship. The goal has always been to learn from each other without agendas and with respect for each other’s opinions. It has been a fun and rewarding journey.

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References


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CHAPTER FOUR
DISCUSSION

_Esox lucius_, as well as _E. masquinongy_, are famous among anglers, ecologists and morphologists for their predacious foraging habits. To become these fierce and efficient predators, it would stand to reason that their morphology, development, and mineralization patterns must support this type of lifestyle, even very early on in their ontogeny. Staging data generated in this study show the specific sequence in which _E. lucius_’ axial skeleton mineralizes, and the collaborative study of Burdi and Grande (2010) shows the axial skeleton sequence of both _E. lucius_ and _E. masquinongy_. Several generalizations can be made based upon these results (Figure 1A, C). In general, both studies demonstrate that _E. lucius_ and _E. masquinongy_ share a common developmental pattern in the postcranial skeleton as well as the cranial skeleton (i.e. hypurals forming first, but only after the formation of the cartilaginous hyomandibula, dentary and quadrate bones). Both studies also show that although the relative pattern of bone formation between the two species is similar, the timing of bone formation and mineralization differs. For example, the hyomandibula is the first bone to form in the skull. In _E. masquinongy_ it forms in cartilage at 10.3 mm SL, whereas in _E. lucius_ it forms at 13.5 mm SL. Hypural 1, the first bone to form in the post-cranial skeleton, forms in cartilage in _E. masquinongy_ at 10.3 mm SL, whereas in _E. lucius_ it forms at 12.0 mm (Table 1). However, the caudal fin unit begins to form in cartilage first in _E. lucius_. As stated
Table 1. First appearance of dermal bone mineralization, endochondral cartilage, and endochondral mineralization of individual bones within the axial skeleton for both *Esox lucius* and *E. masquinongy* in standard length (mm). Axial skeleton categories and bone types are also noted. S, skull; J, Jaws; V, vertebral column; C, caudal fin; D, Dermal Bone; E, endochondral bone; M/B, membrane bone; D/E, compound bone; Asterisks indicate data that is not applicable; Double asterisks indicate data that is not available.

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earlier Jollie (1975) examined the comparative ossification in the skulls of *E. lucius* and the pickerel, *E. americanus*. This thesis, as well as the collaborative study of Burdi and Grande (2010), both support and refute his findings. For example, the sequence of bone formation observed in our material is generally congruent with Jollie (1975), but differences lie in the exact timing of bone formation, which in turn may reflect differences in rearing conditions (e.g., temperature). Rearing conditions of this study mimic the natural conditions of the fish, whereas Jollie’s did not. Congruent with Jollie’s (1975) findings, the first skull bones in *E. lucius* and *E. masquinongy* that begin to mineralize are the hyomandibula and dentary, followed by the maxilla, symplectic and opercle, whereas the last skull bones to mineralize are the supraorbitals and dermosphenotics. Contrary to Jollie’s (1975) results however, the dermosphenotics developed in fish of much shorter lengths; in *E. lucius* (70 mm SL), compared with his *E. lucius* material (90mm SL). Our sequence of bone formation results agree with Jollie, however the lengths at which the first sign of mineralization occurs differs greatly. In terms of specific bone mineralization patterns and fusions, similarities to Jollie’s findings were also found. He argued that the frontals form without any association with the sensory canals. In this study’s specimens of *E. lucius*, the frontal bones and their sensory canals seem distinct. The frontals also remain distinctly paired and separated until late in development. The frontals also remain unfused in the largest specimens of both species contrary to Jollie’s results. In terms of composite bones, Jollie (1975) argues that the quadrate bone (his quadratojugo-quadrate bone) in *Esox* is a composite bone formed from a fusion of the quadrate (cartilage bone) and the quadratojugal (dermal bone). This
condition could not be identified in *E. lucius*, however Burdi and Grande (2010) observed this fusion in younger or smaller specimens of *E. masquinongy*. Finally, the timing of the mineralization or fusions of the post-cranium of *E. lucius* and *E. masquinongy* could not be compared with Jollie (1975) because he did not address this aspect of the axial skeleton.

Based on the results of this thesis and the collaborative study of Burdi and Grande (2010), the bones that are the first to form and mineralize in both species are those that comprise both the jaws and the caudal fin. It would stand to reason that a well-developed and mineralized jaw early in development allows these very young fish to eat prey items that are accessible in their natural habitat, including other vulnerable *E. lucius* and *E. masquinongy*, solidifying their role as predators. A well-developed and mineralized caudal fin, on the other hand, can also allow these vulnerable *Esox* to swim faster to avoid being preyed upon by other predators including larger *Esox*. Development of the jaws and the caudal fin thus allows *E. lucius* to carry out the anthropomorphic behaviors needed for survival.

Even though *Esox lucius* is a fierce predator, it is often argued by ecologists and fish enthusiasts that the predatory behavior of *E. masquinongy* greatly exceeds that of *E. lucius*, especially during its early stages of life (Eddy and Underhill 1974). Comparative developmental rate data from this study and the collaborative study of Burdi and Grande (2010) also support this assessment. Results indicate that although the overall caudal fin skeleton begins to develop first in *E. lucius*, there is a change or switch in developmental strategy, in that *E. masquinongy* subsequently devotes more energy to bone formation.
and mineralization rather than to increasing body size. *Esox lucius* on the other hand, spends most of its developmental time growing larger in size. Developmental strategies vary from species to species, even among closely related ones like *Esox lucius* and *E. masquinongy*. Based on the combined developmental studies, once the caudal skeleton has begun to form, the *E. masquinongy* axial skeleton mineralizes faster in comparison to *E. lucius*, irrespective of its slower growth rate. Given the experimental end point of 20 centra formed, results indicate that *E. masquinongy* is an older but smaller fish, and more energetic emphasis is put into skeletal development than growth in size. Interestingly, *E. masquinongy* ultimately lives longer than *E. lucius* (about eight and seven years, respectively) and grows to be a larger fish. These data may seem incongruent with the results of this study, but insight might come from understanding the early life history stages of these species.

Data obtained from the Wisconsin Department of Natural Resources (1989), state that both species hatch at about fourteen days after fertilization (depending upon water temperature). *Esox lucius* begins to feed on zooplankton at about ten days of age, and on small fish when they reach fingerling size (about 15.2cm). *Esox masquinongy* on the other hand, begins to feed on zooplankton one day after hatching and feeds on small fishes within a couple of days after that. Essentially, *E. masquinongy* becomes a predator before *E. lucius* does. It stands to reason that *E. masquinongy* would need a more developed axial skeleton and full set of canine teeth, even at a small size, to assume this role. This study provides a developmental foundation and a testable hypothesis for future studies comparing the development/mineralization rates between *E. lucius* and *E.*
*masquinongy*. Possible explanations for this developmental shift include competitive exclusion in zones of sympatry resulting in niche and resource partitioning.

Now, are the developmental patterns observed in *Esox* reflective of development in other fishes? Is developmental timing a function of ecology, phylogeny or a combination of both? If the developmental patterns (e.g., endochondral then dermal bone formation, relative sequence of development of all bones, formation of individual centra, direction of centra formation) of *E. lucius* and *E. masquinongy* are compared with the developmental information of other fishes from the literature, we see some different patterns of development. For example, the specific morphology, individual bone mineralization patterns and sequence of bone mineralization of the zebrafish (*Danio rerio*) and the channel catfish (*Ictalurus punctatus*) differ tremendously from esocid fishes. Zebrafish and catfishes (both belonging to the Superorder Ostariophysi, series Otophysi) have a centra formation pattern following an anterior to posterior sequence (Grande and Shardo 2002, Bird and Mabee 2003), while in *Esox*, centra formation begins anteriorly, then posteriorly finally convening in the abdominal region. This sequence of centra formation in zebrafish and catfish may reflect Weberian apparatus formation (i.e., and otophysic connection found in all otophysan fishes) (Rosen & Greenwood 1970). According to Grande and de Pinna (2004), the Weberian apparatus consists of a series of modified anterior centra, neural arches, supraneurals and pleural ribs that connect the gasbladder to the back of the skull. This gas bladder transfers high frequency/far field sound to the Webberian ossicles where it is then transferred to the inner ear. Essentially the Weberian apparatus enables these fishes to detect approaching predators and prey.
better than most other teleosts. The Weberian apparatus is thus a major mechanism for survival, and stands to reason that it would form first during development, resulting in an anterior to posterior developmental pattern of the vertebral column. So in this case developmental timing would be a function of both ecology and phylogeny.

There are also differences seen between esocids and other teleosts in terms of individual centra formation and mineralization. According to Schultze and Arratia (1988), the presumptive vertebral column, and specifically each individual centrum, in teleosts begin with the formation of chordacentra. Chordacentra result from a direct mineralization from within the notochordal sheath after segmentation. In *Esox*, mineralization begins simultaneously from the dorsal and ventral sides of the respective abdominal centrum, eventually forming a ring. In ural centra, mineralization begins in the ventral region and moves dorsally. At this time other potential vertebral elements such as the cartilaginous precursors of neural and haemal arches (i.e., basidorsals and basiventrals) begin to form on the dorsal and ventral surfaces of the chordacentra, respectively. Arcocentra then start to ossify and form over the perichondral basidorsals and basiventrals, and eventually autocentra (a secondary mineralization of each centrum from outside of the notochordal sheath) begin to form as direct perichordal ossifications around the chordacentrum. In *Esox*, this enclosing by the autocentra during development makes the arcocentra and autocentra indistinguishable. Resulting vertebrae are thus composites of centra and associated structures such as neural spines and neural and haemal arches that develop independently and from multiple processes. According to Grande and Shardo (2002), centra formation in ostariophysans begins with chordacentra
formation from within the notochordal sheath of each protocentrum (after notochord constriction and formation of arcocentra). In these fishes mineralization of dorsal and ventral chordacentra occur simultaneously. Both ventral and dorsal pairs of chordacentra enlarge, fuse, and form a ring around each protocentrum. Autocentra will eventually surround each chordacentrum as direct ossifications completing the formation of vertebral centra. This type of centra formation is unlike esocids and salmonids in general since “chordacentra enlarge dorsally and are eventually supplanted by autocentra” (Grande & Shardo 2002). Variation in centrum formation and specifically chordacentrum formation varies among Euteleosts, however the patterns described for both esocids and ostariophysans seem to be fixed within each group. Here individual centra formation for different groups of fishes might be a function of phylogeny. The above assessment comparing the skeletal structures of Jollie’s (1975) esocids and this study’s esocid specimens is relatively straightforward because *E. lucius*, *E. masquinongy* and *E. americanus* skeletons (and all esocids skeletons in general) have similar characters and character positions. However when comparing esocids to other fishes, and when comparing teleosts in general, morphologists and phylogeneticists find it difficult to compare similar structures because similar structures are not always homologous to one another (e.g., frontals and parietals). Furthermore, even if a particular structure has an identical name in different fishes, and teleosts alike, it still does not necessarily indicate that the structures are homologous. This is the “problem of homology” discussed by Wiley (2008), Schultze (2008) and others. Wiley (2008) explains this “problem of homology” using the example of the frontal bone in
sarcopterygian and actinopterygian fishes. Jollie (1975) hypothesizes that since basal sarcopterygians lack a frontal bone, it is the parietals of sarcopterygians that are homologous with the frontal bones of actinopterygians. Ontology without a clear understanding of homology results in problematic issues for morphological, developmental and phylogenetic analyses. Even among teleosts homology questions have surfaced. For example, what morphologists have traditionally called ural centrum 1 might not be homologous across Teleostei. Developmental information suggests that the position of a particular bone relative to others is not an accurate criterion for homology.

The purpose of this thesis was to better understand the developmental morphology of *Esox lucius* and compare the development with that of *E. masquinongy*. During the course of this study it became apparent that standardization of many anatomical terms does not exist, and that hypotheses of homology were not driving forces when naming bones. This research thus provides an opportunity for a continued discussion with respect to hypotheses of homology by setting the morphological foundation for Euteleostei (Esociformes are considered basal euteleosts) Developmental data from this study will be critical in future studies of the euteleost axial skeleton (specifically the NSF-funded Euteleost Tree of Life Project of the Grande laboratory).

Finally, standardization of morphological terms (i.e., ontologies) has become a major focus among comparative biologists beginning with the formation of Zfin (zebrafish ontology) and continuing with Phenoscape (fish morphological ontology). The information provided in the glossary of terms (Appendix B) of this thesis provides a service to future researchers and students of fish development as the field moves forward.
in expanding future ontologies. This glossary defines each individual character (morphological structure) of the axial skeleton in *Esox*, as well as specific terms necessary for the understanding of fish morphological development in general. All skeletal structures defined might not be homologous across fishes (i.e. the frontal bone defined in esocids might not be homologous with the frontal of other lower fishes), but these definitions might give insight for future hypotheses of homology and defining other essential structures that are specific other species.

**Future Directions**

In terms of a global future studies, it would be interesting to continue to compare vertebral column development within a species and among euteleosts. The goal of Zfin and Phenoscape is to correlate zebrafin genetics and mutations with respective morphology. Understanding how the vertebral column develops and differences in development among axial, abdominal and caudal vertebra within a species and among euteleosts is critical. It is clear that the development of the vertebral column is regulated by different Hox gene clusters. The question becomes, is morphological development in terms of centra formation and membrane bone formation in the neural arches and spines different from region to region and if so are these differences the result of Hox gene regulation?

More specifically, in addition to providing a basis for future comparative developmental rate studies, and serving as a foundation for a better understanding of euteleost developmental morphology, the most direct and interesting sequel to this study would be one to test hypotheses presented in Burdi and Grande (2010) regarding the
differences in *Esox* skeletal development. Burdi and Grande (2010) hypothesized that although the caudal fin skeleton begins to develop first in *E. lucius*, there is a change or switch in developmental strategy, in that *E. masquinongy* subsequently devotes more energy to skeleton formation, rather than to increasing body size. *Esox lucius* on the other hand, spends its developmental time not only growing larger, but also in skeletal formation. If development is tied to predation, then the next step is to test if individual jaw elements also show the same pattern of development as seen with centra and hypural development. Preliminary data (Grande and Giampaoli in prep) of the developmental of individual jaw bones support Burdi and Grande (2010) in that the jaws of *Esox masquinongy* develop at a faster rate in comparison to *E. lucius*. Examining and comparing the jaw muscles and developing bite force among specimens of both species might give insight into whether skeletal development is correlated with jaw strength and ultimately the relative timing of predation between the two species. Because skull development should reflect bite force, if the skull of *E. masquinongy* is mineralizing faster than *E. lucius* this should be reflected in a stronger bite force earlier in *E. masquinongy*. Finally, evaluating the specific ecology and natural history of these sister species could also further support the findings of this study. Replicating this study while varying specific ecological parameters (i.e., initial water temperature at fertilization) or sampling fish from sympatric areas could provide insight into how developmental and mineralization results are affected if the ecology of these fishes changes early on in development or if these two species are competing for resources in the same environment. Assessing the validity of the hypotheses generated in the combined studies
will require concerted and collaborative efforts among developmental morphologists and fish ecologists.
APPENDIX A

GENERALIZED DIAGRAM OF THE ESOCID SKELETON
Generalized Diagram of the Esocid Axial Skeleton

Sensory canals are dark blue, the cranium is red, abdominal vertebrae with ribs are pink, supraneurals are green, caudal fin vertebrae are light green, the jaws and suspensorium are light blue, the abdominal vertebrae are purple, the dorsal and anal fin endoskeletons are yellow, and the caudal fin vertebrae are orange.
APPENDIX B

GLOSSARY OF TERMS
Age (hrs)
Definition: Age of a particular fish recorded in hours at the time of fertilization

Anguloarticular (aa)
Bone Type: composite
Number of Bones: paired
Region: lower jaws
Definition: Results from the fusion of the dermal angular and chondral articular. Both have two distinct ossification centers that form the medial portion of the lateral and medial surface of the jaws. It is positioned dorsal to the retroarticular and dentary (lateral/medial views) and articulates with the quadrate posteriorly (lateral/medial views).

Appendicular Skeleton
Definition: The section of the skeleton that includes the pectoral and pelvic girdles plus their associated fins.

Arcocentrum
Bone: ossification or mineralization that occurs over the cartilaginous basidorsal and basiventral arcuale
Region: vertebral
Definition: An ossification that forms over the perichordal basidorsals and basiventrals that gives rise to neural and haemal arches.

Autocentrum
Bone: secondary mineralization of the centrum from outside of the notochordal sheath
Region: vertebral
Definition: Arises as a direct perichordal ossification around the chordacentrum (i.e., outside of the notochordal sheath). In some fishes (e.g., Esox) the base of the arcocentrum becomes laterally enclosed by the autocentrum during development making the arcocentrum and autocentrum indistinguishable.

Axial Skeleton
Definition: The section of the skeleton comprising the skull, vertebral column and median fins.
**Basidorsal**
Bone Type: chondral  
Number of Bones: paired  
Region: vertebral  
Synonymy: neural arch anlagen Laerm (1892); basidorsal cartilage Grande & Bemis (1998)  
Definition: Cartilaginous precursor of a neural arch.

**Basioccipital (boc)**
Bone Type: chondral  
Number of Bones: median  
Region: skull  
Synonymy: occipitale basilare Segemehl (1884)  
Definition: Forms the floor of the foramen magnum, and in *Esox* articulates with the first centrum forming a monopartite occipipital condyle.

**Basiventral**
Bone Type: chondral  
Number of Bones: paired  
Region: vertebral  
Synonymy: haemal arch anlagen Laerm (1892); basiventral cartilage Grande & Bemis (1998)  
Definition: Cartilaginous precursor of a haemal arch.

**Bone**
Definition: Primary skeletal tissue of most adult vertebrates including types such as dermal, chondral, perichondral and membrane bone.

**Cartilage**
Definition: Strong, flexible connective tissue that constitutes much of the vertebrate embryonic skeleton. In teleosts, many of the skull bones (e.g., hyomandibula, neural and haemal arches and fin supports) are preformed in hyaline cartilage.

**Centrum**
Bone Type: mineralization of the notochord  
Region: vertebral  
Definition: Vertebral element that forms within or directly surrounds the notochord. (i.e., chordocentrum, arcocentrum and autocentrum).
Centrum (Abdominal)  See Precaudal Centrum or Trunk Centrum
Bone Type: mineralization of the notochord
Region: vertebral
Definition: All centra anterior to the caudal centra and posterior to the anterior centra. All pleural ribs articulate with abdominal centra.

Centrum (Anterior)
Bone Type: mineralization of the notochord
Region: vertebral
Synonymy: intercentrum Schmidt (1892); postcentrum Gadow & Abbott (1895); intervertebral body Jollie (1962); intercentral autocentrum or anterior hemicentrum Schultze & Arratia (1986), precentrum Grande & Bemis (1998)
Definition: Anterior most centra that do not bear pleural ribs. They are positioned anterior to abdominal centra.

Centrum (Caudal)
Bone Type: mineralization of the notochord
Region: vertebral
Definition: Posterior most centra which include both preural and ural centra. They are positioned posterior to abdominal centra.

Chondral Bone
Definition: Bone that forms within a cartilage precursor. In Esox, caudal fin chondral bones (i.e., hypurals,) usually develop before dermal bones.

Chordacentra
Bone: mineralization of the notochord
Region: vertebral
Definition: A direct mineralization from within the notochordal sheath after segmentation. In Esox, mineralization begins simultaneously from the dorsal and ventral sides of the perspective abdominal centra, eventually forming a ring. In ural centra, mineralization begins in the ventral region and moves dorsally.

Composite Bone
Definition: Composite bone results from the fusion of two distinct bones usually one dermal and one chondral bone; (i.e. anguloarticular, pterotic).
**Concurrent Features**
Definition: Morphological characters (structures) that are more variable in terms of ontogenetic timing or first appearance during development. In some cases Concurrent Features can be seen in multiple morphological stages.

**Defining Criterion**
Definition: Structures used for staging morphological data; relatively consistent in their timing of development and thus can be used to define a particular developmental stage.

**Dentary (den)**
Bone Type: dermal
Number of Bones: paired
Region: jaws
Synonymy: dentaryinfradentary Jarvik (1980); dentosplenial Jollie (1984a, 1986),
Definition: Forms the most anterior and ventral portion of the lateral and medial surface of the jaws. It articulates posteriorly with the anguloarticular and the retroarticular, and is positioned ventral to the supramaxilla and maxilla. In *Esox* it is toothed.

**Dermal Bone**
Definition: Superficial bones that lie in or just beneath the skin and develop from the direct deposition of bone in connective tissue. In *Esox*, caudal fin dermal bones usually develop after chondral bones.

**Differentiation**
Definition: Processes whereby indifferent or unspecialized cells, tissues, become structures that attain their adult form and function.

**Entopterygoid (ent)**
Bone Type: dermal
Number of Bones: paired
Region: suspensorium
Synonymy: mesopterygoid Bridge (1877); pterygoid Jollie (1984a); endopterygoid Grande & Bemis (1998)
Definition: Forms the ventro-medial portion of the palate and the ventral wall of the orbit. It is positioned anterior and ventral to the metapterygoid (lateral view), lateral and ventral to the quadrate, and posterior to the ectopterygoid.
Epiotic (ep)
Bone Type: chondral
Number of Bones: paired
Region: skull
Synonymy: occipitale externum Sagemehl (1884); exoccipital Allis (1897a); epioccipital
Definition: Chondral bone that with the parietals and exoccipitals forms the posterio-
dorsal portion of the skull.

Epural
Bone Type: perichondral
Number of Bones: median
Region: caudal fin
Bagarinao (2009)
Definition: A detached neural spine of a preural or ural vertebra. Epurals support the
dorsal procurent rays of the caudal fin. Three epurals are present in pikes and
two in pickerels. They are numbered sequentially from anterior to posterior.

Exoccipital (exo)
Bone Type: chondral
Number of Bones: paired
Region: skull
Synonymy: occipitale laterale Sagemehl (1884); Allis (1897a), Jarvik (1980)
Definition: Together with the basioccipital forms the posterior surface or back of the
skull; It is positioned dorso-lateral to the basioccipital and ventrally to the
epiotics. It forms the lateral boarders of the foramen magnum.

First Sign of Mineralization  See First Sign of Ossification

First Sign of Ossification
Definition: First visual indication of the uptake of red stain representing the
mineralization of dermal or chondral bone, or the first sign of cartilage
formation as noted by the uptake of blue stain.
Frontal (fr)
Bone Type: dermal
Number of Bones: paired
Region: skull
Synonymy: parietal Jollie (1962)
Definition: Forms the largest portion of the dorsal skull roof. It is positioned medial to the mesethmoids, nasals, sphenotics and pterotics; It is also anterior to the parietals and the supraoccipital in dorsal aspect.

Haemal Arch
Bone Type: perichondral
Number of Bones: paired
Region: vertebral
Definition: Paired structure arising ventrally from basiventral cartilages. The dorsal aorta runs through the haemal arches.

Hyomandibula (hy)
Bone Type: chondral
Number of Bones: paired
Region: suspensorium
Synonymy: hyomandibular (adj); hyomandibula (noun)
Definition: Chondral bone that articulates with the cranium and suspends the jaw elements to the cranium. It articulates with the opercle posteriorly, the metapterygoid anteriorly and the symplectic ventrally

Hypural
Bone Type: chondral
Number of Bones: median
Region: caudal fin
Definition: Laterally flattened haemal arch/spine of a ural centrum. Hypurals support the principle caudal fin rays. They are positioned posterior to the parhypural. Hypurals are counted in sequence from ventral to dorsal with hypural 1 positioned immediately posterior to the parhypural and is the first skeletal element where the dorsal aorta (i.e., primary caudal artery), does not run through but around.
**Intercalar (in)**
Bone Type: membrane
Number of Bones: paired
Region: skull
Synonymy: opisthotic Bridge (1877), Shufeldt (1885), Regan (1923), Goodrich (1930), Romer (1962); intercalary Berg (1940)
Definition: Forms the posterior section of the ventral and dorsal surface of the skull. It is positioned lateral to the basioccipital and parasphenoid (ventral view) and ventral to the epiotics (posterior view).

**Interopercle (iop)**
Bone Type: dermal
Number of Bones: paired
Region: opercular series
Synonymy: interopercular; suboperculum
Definition: Dermal bone that makes up part of the opercular series and is positioned ventral to the opercle and posterior to the subopercle.

**Lateral Ethmoid (l.et)**
Bone Type: chondral
Number of Bones: paired
Region: skull
Synonymy: prefrontal Bridge (1877), Shufeldt (1885), (Goodrich 1930); praefrontale Sagemehl (1884); prefrontal/antorbital ossification Allis (1897a); ectethmoid Jarvik (1980)
Definition: Paired chondral bones that extend laterally from the ventral side of the frontals and dorsally from the parasphenoid.

**Maxilla (mx)**
Bone Type: dermal
Number of Bones: paired
Region: jaws
Definition: Dermal bone that forms part of the upper jaw. It is positioned posteriorly to the premaxilla and posterior-dorsally to the supramaxilla.

**Meckel’s Cartilage**
Bone Type: chondral
Region: embryonic mandibular cartilage
Definition: Cartilaginous precursor of the lower jaws.
Membrane Bone
Definition: Bone that develops within membranous tissue without previous cartilage formation; (i.e. intercalar).

Metapterygoid (mtg)
Bone Type: chondral
Number of Bones: paired
Region: suspensorium
Synonymy: dermometapterygoid (Jarvik 1980)
Definition: Chondral bone that is part of the pterygoid series, and is positioned dorsal to the entopterygoid (lateral view) and anterior to the hyomandibula.

Nasal (n)
Bone Type: dermal
Number of Bones: paired
Region: skull
Definition: Long cylindrical dermal bones positioned anterior to the lateral ethmoid, posterior to the mesethmoid and lateral to the frontals.

Neural Arch
Bone Type: chondral
Number of Bones: paired
Region: vertebral
Definition: A pair of elements surrounding the neural canal (Grande & Bemis, 1998). Neural arches form from basidorsals and are ossified by the arcocentra

Notochord
Region: embryonic
Specific Region: vertebral
Definition: Embryonic rod-like hydrostatic skeleton that extends the entire postcranial length. Notochord is replaced by the vertebral column during development.

Notochordal Length (NL)
Definition: Length of a fish from the tip of the snout to the tip of the notochord before hypural formation.
Opercle (op)
Bone Type: dermal
Number of Bones: paired
Region: opercular series
Synonymy: subopercular/suboperculum
Definition: Largest bone in the opercular series positioned posterior to the hyomandibular and is dorsal to the interopercle.

Palatine (pal)
Bone Type: composite
Number of Bones: paired
Region: palatal complex
Definition: Composite bone consisting of a toothed dermal component (dermopalatine) and a chondral component (autopalatine). The dermopalatine may or may not be fused with the autopalatine. The palatine is positioned ventral and anterior to the premaxilla. In *Esox* it is toothed.

Parasphenoid (par)
Bone Type: dermal
Number of Bones: median
Region: skull
Definition: Dermal bone that forms the base of the skull. It articulates with the vomer anteriorly, the basioccipital posteriorly and the prootics and exoccipitals laterally.

Parhypural (php)
Bone Type: chondral
Number of Bones: median
Region: caudal fin
Definition: Last haemal arch element to be penetrated by the caudal artery.
**Parietal (pa)**
Bone Type: dermal
Number of Bones: paired
Region: skull
Definition: Paired dermal bones on the dorsal surface of the skull positioned posterior to the frontals and lateral to the supraoccipital

**Perichondral Bone**
Definition: Bone that is preformed within the notochordal sheath.

**Precaudal Centrum**
See *Centrum* (Abdominal) or *Trunk Centrum*

**Premaxilla (pmx)**
Bone Type: dermal
Number of Bones: paired
Region: jaws
Synonymy: rhinopremaxillary Jarvik (1980)
Definition: Dermal bones that with the maxilla form the upper jaw. The premaxilla articulates with the maxilla posterior-ventrally and the palatine posterior-dorsally. In *Esox* the premaxilla is toothed.

**Preopercle (pop)**
Bone Type: dermal
Number of Bones: paired
Region: opercular series
Synonymy: preopercular; preoperculum
Definition: One of four dermal bones in the opercular series that carries the preopercular lateral line canal. The preopercle is positioned laterally to the hyomandibula, dorsally to the suboperculum and anteriorly to the opercle and interopercle

**Preural Centrum 1 (pu1)**
Bone Type: mineralization of the notochord
Number of Bones: median
Region: caudal fin
Definition: Caudal centrum that bears the parhypural and positioned directly anterior to ural centrum 1
Preural Centrum 2 (pu2)
Bone Type: mineralization of the notochord
Number of Bones: median
Region: caudal fin
Definition: Centrum positioned directly anterior to preural centrum 1.

Prootic (pro)
Bone Type: chondral
Number of Bones: paired
Region: skull
Synonymy: petrosum Segamehl (1884); petrosal Allis (1897a, 1897b)
Definition: Forms the posterior portion of the ventral surface of the skull. It is positioned lateral to the parasphenoid and pterotics and anterior to the exoccipitals.

Protocentra
Region: vertebral
Definition: Segments of the notochord after it invaginates. Each protocentrum will subseuqently mineralize forming chordacentra.

Pterosphenoid (pts)
Bone Type: chondral
Number of Bones: paired
Region: skull
Synonymy: alisphenoid Bridge (1877), (Snufeldt 1885), (Allis 1897a), (Regan 1923), (Jarvik 1980); posterior orbitosphenoid (Jollie 1984a)
Definition: Forms the medial portion of the ventral surface of the skull. It is positioned lateral to the parasphenoid and anterior to the prootics.

Pterotic (pt)
Bone Type: composite
Number of Bones: paired
Region: skull
Definition: Composite bone consisting of the dermal dermopterotic and a chondral component (pterotic). Forms the most posterior section of the dorsal/ventral surface of the skull. It is positioned lateral to the parietals and posterior to the frontals (dorsal view) and sphenotic (dorsal/ventral view).
Quadrate (q)
Bone Type: chondral
Number of Bones: paired
Region: suspensorium
Definition: Cheek bone that is involved in the jaw opening mechanism and in jaw suspension. It articulates dorsoventrally with the anguloarticular, dorsally with the entopterygoid, anteriorly to the ectopterygoid and posteriorly with the symplectic.

Retroarticular (ret)
Bone Type: chondral
Number of Bones: paired
Region: jaws
Definition: A chondral bone that forms from the hyosymplectic cartilage. It is positioned ventral to the anguloarticular (lateral and medial view), posterior to the dentary (lateral and medial view).

Sphenotic (sph)
Bone Type: composite
Number of Bones: paired
Region: skull
Synonymy: postfrontal Bridge (1877), Sagemehl (1884), Shufeldt (1885), Goodrich (1930); postorbital ossification (Allis 1897a)
Definition: Composite bone whose dermal portion supports the otic lateral line canal. It is positioned lateral to the frontals in dorsal view and to the pterosphenoid in ventral view. It is also anterior to the pterotic in both dorsal and ventral views.

Standard Length (SL)
Definition: Refers to the length of a fish measured from the tip of the snout to the posterior margin of the hypural plate.

Subopercle (sop)
Bone Type: dermal
Number of Bones: paired
Region: opercular series
Synonymy: subopercular, suboperculum
Definition: Opercular bone positioned ventral to the preopercle, and anterior to the interopercle
Supramaxilla (smx)
Bone Type: dermal
Number of Bones: paired
Region: jaws
Synonymy: jugal (Bridge 1877), Allis (1897a, 1898a), de Beer (1937)
Definition: Accessory jaw bone that is devoid of dentition and articulates with the posterior part of the maxilla along its ventral edge.

Supraneural
Bone Type: chondral
Region: vertebral
Definition: Rod-like structures positioned in between their corresponding neural spines of vertebrae anterior to the dorsal fin. Although the homology of supraneurals is debatable one hypothesis is that they are homologous with dorsal fin radials.

Supraoccipital (soc)
Bone Type: chondral
Number of Bones: median
Region: skull
Definition: Median chondral bone that forms the dorso-posterior portion the skull. It articulates with the parietals laterally. It also forms the dorsal margin of the foramen magnum.

Symplectic (sym)
Bone Type: chondral
Number of Bones: paired
Region suspensorium
Definition: Chondral bone formed from the ventral limb of the hyomandibular cartilage (i.e., hyosymplectic cartilage). It is positioned ventral to the metapterygoid in lateral view and articulates posteriorly to the quadrate in medial view.

Total Length (TL)
Definition: Refers to the length from the tip of the snout to the tip of the longer lobe of the caudal fin.

Trunk Centrum See Centrum (Abdominal) or Precaudal Centrum
**Uroneural (un)**
Bone Type: membrane
Number of Bones: paired
Region: caudal fin
Definition: Modified ural neural arch. The Uroneural in *Esox* is positioned to but not fused to, the posterior end of the 1st preural centrum and the entire 1st and 2nd ural centra. It is also lateral to the 6th hypural and 1st epural.

**Ural Centrum 1 (u1)**
Bone Type: mineralization of the notochord
Number of Bones: median
Region: caudal fin
Definition: Anterior most centrum that supports the 1st and often 2nd hypurals.

**Ural Centrum 2 (u2)**
Bone Type: mineralization of the notochord
Number of Bones: median
Region: caudal fin
Definition: In *Esox*, the posterior most centrum that supports the 3rd, 4th and 5th hypurals.

**Vertebra**
Bone Type: composite
Region vertebral
Definition: The individual segments that form the spinal column. Composed of a centra and associated structures such as neural spines and neural and haemal arches. Membrane bone contributes to the girth and structure of these associated structures.

**Vomer (v)**
Bone Type: dermal
Number of Bones: median
Region: skull
Definition: Forms the most anterior portion of the dorsal-ventral surface of the skull. It is positioned lateral to the mesethmoids (dorsal view) and articulates with the anterior portion of the parasphenoid. In *Esox* it is toothed.
REFERENCES


VITA

Amanda Maria (Fabiano) Burdi graduated from Loyola University Chicago in May 2000 with a B.S. in Psychology. Following a volunteer position at St. Odilo School teaching elementary math and science, Amanda decided to pursue a Master of Science degree in Biology at Loyola University Chicago, focusing on developmental morphology. Fall of 2004, she began working as a Teaching Instructor in the Biology Department at Loyola University Chicago, and from fall of 2008 – fall of 2009 she also taught Human Reproduction in Loyola University Chicago’s Natural Science Department. In the summer of 2008, she worked as a laboratory technician for Dr. Terry Grande. Amanda continues to work as a Teaching Instructor at Loyola University Chicago teaching General Biology Laboratory.