Comparison of Feeding Functional Morphology Development in North American Esocids

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For Andi and Lynika
Pike, three inches long, perfect
Pike in all parts, green tigering the gold.
Killers from the egg: the malevolent aged grin.
They dance on the surface among the flies.
Or move, stunned by their own grandeur,
Over a bed of emerald, silhouette
Of submarine delicacy and horror.
A hundred feet long in their world…

-Ted Hughes
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii  
LIST OF TABLES viii  
LIST OF FIGURES ix  
CHAPTER ONE. INTRODUCTION 1  
CHAPTER TWO. COMPARISON OF LOWER JAW FUNCTIONAL MORPHOLOGY IN NORTH AMERICAN ESOCID SPECIES 14  
CHAPTER THREE. COMPARISON OF GEOMETRIC MORPHOMETRICS DURING DEVELOPMENT IN NORTH AMERICAN ESOCID SPECIES 40  
CHAPTER FOUR. DISCUSSION 52  
APPENDIX 57  
REFERENCE LIST 59  
VITA 65
LIST OF TABLES

Table 1. Coordinate data points collected for analysis on MandibLever 4.0. 29
Table 2. Mean MA for jaw opening and jaw closing for each North American esocid species. 32
Table 3. Coordinate data points collected for lateral cranial shape analysis. 43
LIST OF FIGURES

Figure 1. Photos of *Esox lucius*. 2

Figure 2. Proposed phylogenetic relationships of Esocids, based on literature. 5

Figure 3. Worldwide distribution of *Esox lucius*. 7

Figure 4. Distribution of *Esox lucius* and *Esox masquinongy* in the United States and its territories. 9

Figure 5. Distribution of *Esox niger*, *Esox americanus americanus*, and *Esox americanus vermiculatus* in the United States and its territories. 10

Figure 6. First order levers. 16

Figure 7. Photograph of *E. lucius* specimen (LUF EL 54A) with relevant structures labeled. 17

Figure 8. Second order levers. 19

Figure 9. Third order levers. 20

Figure 10. Photograph of *E. lucius* specimen (LUF EL 64B with A2 section of section of *abductor mandibulae* muscle reflected to reveal A3 section. 24

Figure 11. Radiograph of *E. lucius* specimen (USNM 201266 B). 26

Figure 12. Photograph of *E. masquinongy* specimen (LUC EM52Cβ) with posterior end of A2 subsection of *adductor mandibulae* pulled away to reveal A3 subsection and its tendon. 30

Figure 13. Mean MA for jaw opening for each North American species or subspecies in study. 33

Figure 14. MA for jaw opening (IOP.MA) graphed against size category. 34
Figure 15. Mechanical advantage for A2’s contribution to jaw closing graphed against size category for E. lucius and E. masquinongy.

Figure 16. Mechanical advantage for A2 and Aω’s collective contribution to jaw closing graphed against size category for E. lucius and E. masquinongy.

Figure 17. Mechanical advantage for A3’s contribution to jaw closing graphed against size category for E. lucius and E. masquinongy.

Figure 18. Landmarks used in lateral cranial study marked by red points on a specimen of E. masquinongy.

Figure 19. Bar graph of variance in shape difference distributed across principal components.

Figure 20. Scatter plot of specimens of E. lucius and E. masquinongy along principal components 1 and 2 with 95% frequency ellipses around each group.

Figure 21. Scatter plot of group two, middle size, specimens of E. lucius and E. masquinongy along principal components 1 and 2 with 95% frequency ellipses around each group.

Figure 22. Wireframe graphs of group two, middle size, specimens of E. lucius and E. masquinongy along principal component 1.

Figure 23. Transformation grid with lollipop diagram of group two, middle size, specimens of E. lucius and E. masquinongy along principal component 1.
CHAPTER ONE
INTRODUCTION

Esocidae is a family of freshwater fishes within order Esociformes known for its long, cylindrical bodies, duck-bill snouts, and an impressive number of sharp teeth (Figure 1). It is partly because of these characteristics, along with their wide geographic distribution, that these fishes have maintained a long-lasting and prominent place in many Northern Hemisphere cultures and in scientific text. Perhaps poet Ted Hughes best captured the appeal of esocids in his poem “Pike,” quoted in the epigraph of this thesis; these fishes certainly are a “silhouette of submarine delicacy and horror” (Hughes 1960). These fish have inspired the cuisine of ancient Rome, filled the bellies of tribes of the Great Lakes area (Cleland 1982), and support a large number of recreational fisheries across the modern world, while simultaneously serving as a model organism for studying basal euteleost evolution (Pospialova et al. 2019), piscivory characteristics, or successfully rearing wild fish stocks for recreational fisheries (Burdi and Grande 2010, McCormick et al. 2020).

Taxonomic classification

The order Esociformes comprises two families: Esocidae (pikes and pickerels) and the Umbridae (mudminnows). The phylogenetic history of Esociformes has had a long and sorted past. For example, Berg (1948) placed the Esocoidei [Dalliidae (Dallia), Umbridae (Novumbra and Umbra), Esocidae (Esox)] within the order Clupeiformes. Greenwood et al. (1966) placed Esociformes in the Protacanthopterygii closely related to the smelts, and salmonids. Williams
Figure 1. Photos of *Esox lucius*. a) Lateral cranial view and b) lateral full body view (Photos taken by Cheryl Theile at Tennessee Aquarium).
(1987) considered osmeroids and argentinoids (marine smelts) as the primitive sister group to neoteleosts with salmonids and esocoids as close relatives.

Ishiguro et al. (2003) investigate the relationships between basal euteleosts, using molecular data, selecting 12 protein coding mitochondrial genes and 22 tRNA genes from 34 species across 27 families and 9 orders. Two members of the order Esociformes were tested, *Dallia pectoralis* (then classified in the family Umbridae) and *Esox lucius* from the family Esocidae. Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses resulted in trees with similar topography. Both trees maintained *D. pectoralis* and *E. lucius* as members of Esociformes and identified Salmoniformes as the appropriate sister group. Also, both of these orders had been identified as members of the superorder Protacanthopterygii. The Maximum Parsimony and Maximum Likelihood analyses both suggested that this superorder is actually a paraphyletic group by placing the order Alepocephaloidea within the Otocephala and the four other orders of Protacanthopterygii (Esociformes, Salmoniformes, Argentinoidea, and Osmeroidei) as basal euteleosts. Later work by Near et al. (2012) supported the placement of Esociformes and Salmoniformes as a clade within Euteleostei and was further strengthened by the work of Campbell et al. (2013).

López et al. (2004) examined esociform interordinal relationships based exclusively on molecular data (i.e., nuclear RAG1 mitochondrial gene 12S and 16S (mt rRNA)) and recovered the monophyletic families Esocidae and Umbridae and supported the placement of Salmoniformes as sister group to the esociforms. Within family Esocidae, the pikes (*Esox lucius*, *Esox reichertii*, and *Esox masquinongy*) were grouped together, with the pickerels (*Esox americanus* and *Esox niger*) forming their own clade as a sister group to the pikes. This aligns
with the subdivision of genus *Esox* into two subgenra, *Esox* and *Kenoza*, with *Esox* containing the pikes and *Kenoza* containing the pickerels (Nelson et al. 2016) (Figure 2).

Grande et al. (2004) took a closer look and focused on the relationships of all of the extant species and subspecies within the family Esocidae classified at the time of publication. While López et al. (2004) looked at the five species in family Esocidae (and its only genus *Esox*), Grande et al. (2004) considered both of the subspecies of *E. americanus*, *E. americanus americanus* and *E. americanus vermiculatus*. Two members of family Umbridae were included as an outgroup, *Novumbra hubbsi* and *Umbra limi*. Also, this study included 38 morphological characters in its analysis in addition to nuclear and mitochondrial genetic data (cytochrome *b* and an intron portion of nuclear gene RAG1).

Maximum Parsimony trees were constructed from each dataset (morphological, mitochondrial, nuclear DNA) separately, while Maximum Likelihood trees were generated for the molecular datasets. Although the mitochondrial trees suggested past hybridization between the pickerels *E. americanus americanus* and *E. niger*, the morphological and RAG1 trees were congruent, and the relationships seen in these trees corroborate the results of López et al. (2004).

Since the Grande et al. (2004) study, two additional species of esocids have been identified. First, the Southern Pike (*Esox cisalpinus*, Bianco and Delmastro 2011) (junior synonym= *Esox flaviae*, Lucentini et al. 2011), was identified in central and northern Italy. More recently, the Aquitanian Pike (*Esox aquitanicus*, Denys et al. 2014) was identified in west to southwest France, in the Charente River to the Adour River drainages. During the identification of *E. aquitanicus*, a Bayesian tree was produced using CO1 for 140 specimens and a neighbor-joining tree used a portion of CO1 for 136 specimens (including historical specimens). Both of
these trees placed *E. lucius* and *E. cisalpinus* in a clade together with *E. aquitanicus* as a sister group to this clade. The muskellunge, *E. masquinongy*, in turn, was a sister group to the clade formed by *E. lucius*, *E. cisalpinus*, and *E. aquitanicus*. Thus, the relationship among these *Esox* species can be viewed as (((*E. lucius*, *E. cisalpinus*) *E. aquitanicus*) *E. masquinongy*). The placement of the pickerels (*E. niger*, *E. americanus americanus*, and *E. americanus vermiculatus*) was similar to that of Grande et al. (2004) and López et al. (2004) in that they again formed their own sister group to the pikes listed above (Figure 2).

**Geographic distribution**

Of the seven currently identified species of genus *Esox*, three are native to Europe, two to Asia, and four (including two subspecies) are native to North America. The Northern Pike, *E. lucius*, is circumpolar (Figure 3), and is therefore found in all three of these continents (Nelson et al. 2016, Froese and Pauly 2018). As previously described, the Southern Pike, *E. cisalpinus*, is native to Italy and the Aquitanian Pike, *E. aquitanicus*, is native to regions of France (Bianco and Delmastro 2011, Denys et al. 2014). In addition to *E. lucius*, the Amur Pike, *E. reichertii*, is found in Asia, specifically the Amur River basin in northeastern Asia (Nelson et al. 2016). North America is unique in the fact that, in addition to the pikes *E. lucius* and *E. masquinongy*, is home to members of the subgenus *Kenoza*: the pickerels *E. niger*, *E. americanus americanus*, and *E. americanus vermiculatus* (Nelson et al. 2016).

Within North America, *E. lucius* is distributed in northern regions, primarily Canada, Alaska, the northern portions of the Midwest, and upstate New York of the United States of America. The other North American pike, the muskellunge, *E. masquinongy*, has an overlapping distribution in the Great Lakes region, from there reaching up into central Canada and spilling
through Ohio and as far south as northern Georgia. While the overlapping portions of the native
distribution of these species is limited to the Great Lakes region, artificial stocking efforts have
pushed the distribution of both species slightly south and west of their native habitats (Figure 4)
(Fuller and Neilson 2018). The pickerels generally have a much more southern distribution. The
Grass Pickerel, *E. americans vermiculatus*, reaches the farthest north, spreading from as far
south as Florida and Texas up to the Great Lakes region (Figure 5) (Fuller 2018). The remaining
pickerels, the Redfin Pickerel *E. americanus americanus* and the Chain Pickerel *E. niger*, have a
distribution limited to the East Coast region and the Southeast, with *E. niger* spreading slightly
farther north and west than *E. americanus americanus* (Figure 5) (Fuller et al. 2018, Fuller and
Sheehy 2018).
Across all these various geographic locations, esocids tend to prefer slow moving or still water, many times with submerged vegetation or woody debris. Not only does this serve as a suitable spawning site in the spring months, but the vegetation also helps camouflage the resulting fry to avoid predators. In addition, vegetation in deeper water camouflages adults while they lie in wait and hunt their prey (Wahl and Stein 1989, Eklöv, 1997).

**Life History**

Northern pike begin spawning in early spring, generally initiated by rising temperatures, longer photoperiod, and occasionally rising waters, which results in spawning beginning sometime between mid-February to early June depending on location (Raat 1988). However, *E. lucius* has been documented spawning under ice in still frozen lakes (Forney 1968). During spawning, adults will move into tributary streams, sloughs, marshes, or the littoral zone of lakes to scatter eggs among submerged vegetation. Eggs generally hatch 10-14 days after fertilization, with the exact time dependent on water temperature. The eleuthero-embryos swim up to and stick to underwater vegetation at the site via adhesive glands and remain dependent on their yolk-sac for feeding. The larvae then release from the vegetation and begin exogenous feeding and swimming after another 10-14 days, just prior to the yolk-sac being fully exhausted (Scott and Crossman 1973, 1998, Raat 1988). The primary diet of these young fish begins as zooplankton and transitions to larger invertebrates as the fry grow, with vertebrates (primarily other fish) generally entering their diet at a size of ~6 cm TL (Nikolski 1963, Scott and Crossman 1973). It is interesting to note, however, that at a high population density some individuals may begin to attack conspecifics and become cannibals at sizes as small as ~3 cm TL (Frost and Kipling 1967, Fago 1977).
Figure 4. Distribution of *Esox lucius* and *Esox masquinongy* in the United States and its territories (Fuller and Neilson 2018). a) *Esox lucius* and b) *Esox masquinongy*. 
Figure 5. Distribution of *Esox niger*, *Esox americanus americanus*, and *Esox americanus vermiculatus* in the United States and its territories (Fuller 2018, Fuller et al. 2018, Fuller and Sheehy 2018). a) *Esox niger*, b) *Esox americanus americanus*, and c) *Esox americanus vermiculatus*. 
Muskellunge begin spawning in the spring, later than Northern pike, but also generally scatter their eggs across submerged vegetation. As in *E. lucius*, the eggs hatch at a relatively early stage of development and the eleuthero-embryos feed endogenously from their yolk-sacs while remaining still. Unlike *E. lucius*, *E. masquinongy* does not seem to have as strong an affinity to adhere to vegetation during this stage. As the yolk-sac is exhausted, *E. masquinongy* begins exogenous feeding on zooplankton, with larger invertebrates becoming increasingly important as the larvae grow. At about 3.8 cm TL, smaller than *E. lucius*, the young muskies then switch to a diet of fish and other vertebrates (Scott and Crossman 1973, 1998, Becker 1983, Bozek et al. 1999, McCormick et al. 2020).

**Predator Traits**

Both species (*Esox lucius* and *E. masquinongy*) continue their primarily piscivorous diet into adulthood, though some may occasionally consume other vertebrates such as ducklings or frogs. They hunt as ambush predators; the esocids lie in wait, hiding among vegetation, and strike out quickly to grasp their prey (Eklov and Hamrin 1989, Eklov and VanKooten 2001). Depending on the proximity of the prey, esocids will use fast starts with either C or S-shaped type strikes, with S-strikes being more common (New et al. 2001, Hale 2002, Schriefer and Hale 2003). This type of swimming is categorized as unsteady (time dependent) locomotion or body/caudal fin transient propulsion, and fishes with this type of swimming require a large ratio of muscle mass to body mass (less “dead weight” to propel through the water), a flexible body to amplify propulsive movements, large caudal fin and body area, a deep caudal peduncle accentuated with posteriorly placed dorsal and anal fins, and an anterior stabilizing body mass (Webb 1982, 1984). In addition, a large mouth gape allows for a large volume of water (and hopefully the prey as well) to be consumed during ram feeding. This helps compensate for the
reduced time to accurately position the body and jaw during such quick feeding strikes while also allowing for larger prey items to be consumed (Higham 2007). Also, large pectoral fins improve stability and drag for braking in suction feeding fish but would negatively affect the speed of ram feeders such as esocids (Higham 2007). Adult *E. lucius* and *E. masquinongy* have many of these adaptations: cylindrical, muscular bodies, little tapering of the body at the peduncle when compared to other species of fish, very posteriorly placed dorsal and anal fins, relatively small pectoral fins, and large, duck-like snouts where the jaws are filled with sharp teeth to grasp prey (Figure 1). While these traits have been studied in adults, less work has been done to study the development of these traits during ontogeny (Webb 1984, New et al. 2001, Hale 2002, Schriefer and Hale 2004, McCormick et al. 2020).

**Commercial Importance**

The members of *Esox* have long been considered prized fishes to catch, notably because of their large size, impressive teeth, and the exciting fight that they put up on the line. Therefore, esocid fishing comprised an important part of the $41.8 billion spent by the 33.1 million US recreational fishers in 2011. In particular, 31% of Minnesota’s anglers (both residents and nonresidents), and 25% of Wisconsin’s anglers fished for esocids in 2011 (U.S. Department of Interior et al. 2014). As a result of this, many states, including Illinois, artificially stock esocids through their respective Departments of Natural Resources to keep the fishery at a large enough population to accommodate these numerous anglers. However, this has resulted in expansion of *E. lucius* and *E. masquinongy* outside of their respective native ranges (Figures 4 and 5), sometimes with negative ecological and economic consequences stemming from the esocids’ voracious appetites (McMahon and Bennett 1996, Bystrom et al. 2007).
Purpose of Study

Previous research has shown that the axial skeleton of *E. masquinongy* ossifies at a faster rate than that of *E. lucius* (Burdi and Grande 2010). It was suggested that the rapid developmental pattern of *E. masquinongy* reflects the species’ ability to assume a predatory behavior early in life. However, no work has tested how the development of these skeletal features might translate into predatory functional characteristics for piscivory, including fast feeding strikes and quickly moving, large mouths. Therefore, this thesis has two main goals: (1) Explore the lower jaw functional morphology across all North American species with an emphasis on the development of *E. lucius* and *E. masquinongy*. (2) Examine the geometric morphometrics of the development of external morphology (including body shape and fin placement) of *E. lucius* and *E. masquinongy*. This thesis will test the following hypotheses within these sections:

(1) There will be a timing difference in developmental changes in jaw mechanical advantage between *E. lucius* and *E. masquinongy*. Specifically, *E. masquinongy* will decrease its mechanical advantage values earlier in development to develop faster jaw closing. Simply put, there will be a difference in the timing of mechanical advantage shifts between the species.

(2) There will be corresponding timing differences in developmental changes in cranial and body shapes as *E. masquinongy* takes on the adult piscivorous form earlier in development.
CHAPTER TWO

COMPARISON OF LOWER JAW FUNCTIONAL MORPHOLOGY IN NORTH AMERICAN ESOCID SPECIES

How an organism is able to interact within its environment is certainly a fascinating area for study. High speed cameras are often used to study kinematics (the study of the features of movement of objects) of feeding, swimming, but CT scans, dissections, and clearing and staining techniques may also be used to study the underlying functional morphology of motion. From the latter, the musculoskeletal system can then be modeled to better understand how velocity and force can be transmitted through the system and generate a given desired movement, such as maintaining position in the water column, closing the jaw, ventilating gills, etc. The resulting models can then be used to identify morphological effects on behaviors and evolutionary trends that may be used in phylogenetic studies (Westneat 1995, 2004).

Feeding mechanisms are a particularly robust and interesting area of study within functional morphology because of the high level of kineticism found in fish skulls (Schaeffer and Rosen 1961, Lauder 1982, Hulsey et al. 2005) and diversity of divisions found in cranial muscles (Wu and Shen 2004, Datovo and Vari 2014). This has resulted in various models such as the lower jaw as a simple lever (Barel 1983, Kammerer et al. 2004, Westneat 2004) and a four-bar linkage model for maxillary rotation in some fishes (Westneat 1990). Northern pike have been included in larger phylogenetic studies involving biomechanics of teleost feeding (Westneat 2004), and studies have looked at how biomechanics may change across development (Kammerer et al. 2004, Deary and Hilton 2016). However, no work has been done to explore
how the functional morphology of the lower jaw might vary between *Esox* species including changes that occur during the development of the species of this genus. I will first discuss the basics of levers below.

**Levers**

Levers are simple machines made up of a structure, like a rod, board, or bone, pivoting around a fulcrum because of a force applied to it. How these elements are oriented can be changed, and thus determines the type, or order, of the lever. The first order is the lever most people commonly think of (Figure 6a). Imagine a teeter totter (Figure 6b). The board the children sit on is the lever itself, with the central pivot point being the fulcrum. Whenever a child jumps onto their end of the teeter totter they are adding energy into the lever system and pushing that end down. Therefore, the length of the board from them to the fulcrum would be considered the in-lever arm because it is the portion of the lever from the fulcrum to the point where energy is being transferred into the system. The other child would then be moved up as the energy from the first child is transferred to them. The length of the teeter totter from that child to the fulcrum is the out-lever arm because this is the portions that energy moves through to transfer out of the system.

A first order lever can be found in the lower jaw of fish, Figures 6c and 7. In order to open the jaw, a muscle near the cranium, called the *levator operculi*, contracts and pulls on the bones of the opercular series. This force is then transferred into the lower jaw lever by the interoperculomandibular ligament, so that the back of the jaw (the retroarticular and part of the anguloarticular bone) is pulled up. The lower jaw pivots around a joint formed by the quadrate and anguloarticular bones. The remaining length of jaw, primarily the dentary bone, then swings down to open the mouth.
Figure 6. First order levers. a) Schematic of lever with fulcrum labeled as blue triangle, input into lever is red arrow, in-lever arm is red line, output from lever is yellow arrow, and out-lever arm is yellow line. b) Example of common first order lever, a seesaw or teeter totter c) Photograph of E. lucius specimen (LUD EL 54A) with example of biological first order lever used during jaw opening.
Figure 7. Photograph of *E. lucius* specimen (LUD EL 54A) with relevant structures labelled (5 mm scale bar). AA: Anguloarticular bone, A2: A2 section of *abductor mandibulae* muscle, Aω: Aω section of *abductor mandibulae* muscle, D: Dentary bone, IL: Interoperculomandibular ligament, IO: Interopercular bone, LO: *Levator operculi* muscle, O: Opercular bone, PO: Preopercular bone, RA: Retroarticular bone, SO: Subopercular bone, Q: Quadrat bone.
Second order levers, Figure 8a, rearrange elements so that the fulcrum is on one end while the input into the system is on the other end. The force applied out of the system to some resistance is then located somewhere between the in force and the fulcrum. A common example of this is a wheelbarrow, Figure 8b. The person lifting the wheelbarrow at the handles is putting force into the system and the wheelbarrow pivots at the wheel, which acts as the fulcrum. The load inside the wheelbarrow acts as resistance that the force is transferred into and moves up from the force of the person lifting the handles.

To find an example of a second order lever within the body simply look down at your own foot, Figure 8c, when you lift your heel from the ground. The ball of your foot acts as a fulcrum while your calf muscles pull on the back of your heel bone. This puts energy into your foot, which is used to elevate your heel and leg bones relative to the ball of your foot.

Third order levers are similar to second order levers in that the fulcrum is located at one end. However, the in force and out force switch places; the out force is now at the end with the in force located somewhere between the out force and the fulcrum, Figure 9a. One example of a third order lever is a fishing rod, Figure 9b. The fulcrum is the portion nearest the fisher’s body that pivots when they pull up near the reel. That force is then transferred out of the system to pull up against the resistance of the fish on the line.

Third order levers can also be found in the lower jaw of many fish, Figures 7 and 9c. During jaw closing, the *abductor mandibulae* muscle pulls on the lower jaw along the posterior edge of the anguloarticular and dentary bones. The lower jaw pivots at the joint made by the quadrate and anguloarticular bones. Finally, the anterior tip of the dentary swings up to close the jaw.
Figure 8. Second order levers. a) Schematic of lever with fulcrum labeled as blue triangle, input into lever is red arrow, in-lever arm is red line, output from lever is yellow arrow, and out-lever arm is yellow line. b) Example of common second order lever, a wheelbarrow c) Diagram of human foot as an example of biological first order lever when lifting heel off of the ground.
Figure 9. Third order levers a) Schematic of lever with fulcrum labeled as blue triangle, input into lever is red arrow, in-lever arm is red line, output from lever is yellow arrow, and out-lever arm is yellow line. b) Example of common third order lever, a fishing pole c) Photograph of *E. lucius* specimen (LUD EL 54A) with example of biological third order lever used during jaw closing.
A few elements remain constant when considering any order of lever. The distance from the fulcrum to where force is applied to the system is always defined as the in-lever arm, while the distance from the fulcrum to where force is exiting the system and applied to some resistance is always defined as the out-lever arm. Dividing the in-lever arm by the out-lever arm calculates the mechanical advantage (MA) of the system. The mechanical advantage is the measure of how much the lever multiplies the applied force through the system. Higher MA values indicate that higher force can be transferred out of that lever system. The inverse of MA is kinematic transmission (KT), the relative speed in which the out-lever arm moves, which is calculated by dividing the out-lever arm by the in-lever arm. Therefore, a lever with a high MA would have a low KT, meaning a lever that is able to transfer a relatively large amount of force out of the system would move its out-lever arm relatively slowly. A lever with a low MA would have a high KT, meaning a lever that is able to transfer a relatively small amount of force out of the system would move its out-lever arm relatively quickly.

Levers with different MA have their own benefits for different feeding styles. Fish that eat mussels, such as round gobies, need to crack their food open by transferring a large amount of force through their jaws, so they have a short out-lever that results in a high MA. Their jaws may move relatively slowly as a result, but that’s not much of a concern; their prey are sessile. Piscivorous fish like gar, on the other hand, need fast moving jaws to quickly capture fast moving prey. Therefore, a high KT would be beneficial. This results in a low MA and what little force is applied to the prey is focused onto the prey by sharp teeth to maintain grasp of the mobile prey.

As adult ambush predators, esocids rely on a fast-feeding strike to quickly grab their prey, so a low in-lever to out-lever ratio, or MA, would be advantageous. However, these fish
experience a diet shift during their first year of life, switching from a diet of aquatic invertebrates to one of fish, so low MA may not be selected for early in their development. Between species, however, their early life history differs; *Esox masquinongy* transition to a piscivorous diet sooner than *Esox lucius*. Previous research has shown that the skeletons of these species develop at different rates. Specifically, *E. masquinongy*, though remaining smaller in body length, ossifies it’s skeleton faster than *E. lucius* (Burdi and Grande 2010), so it is possible that changes in morphology may correspond to the timing of the dietary shift in these species. Therefore, a two-part study was conducted to examine lower jaw functional morphology of Esocidae: an initial comparison of the North American species of the genus, as well as a study of changes across the development of the sister taxa *E. lucius* and *E. masquinongy*.

**Materials and Methods**

**Comparison of North American Species**

**Specimen acquisition** For comparison with my developmental series of *Esox lucius* and *E. masquinongy*, a reference collection was made of all extant North American species: *Esox lucius*, *E. masquinongy*, *E. niger*, and *E. americanus* (including both subspecies, *E. americanus americanus* and *E. a. vermiculatus*). Intact specimens were borrowed from the following museum collections: Cornell University, the Field Museum of Natural History (FMNH), Harvard Museum (MCZ), Loyola University Chicago (LUF), University of Michigan (UMMZ), and the Smithsonian National Museum of Natural History (USNM) (Appendix 1). Specimens ranged in SL from 6.0 to 25.0 cm. These specimens constituted late young of the year to adult fish but were all past the diet shift for all species.
**Confirmation of muscle attachment sites** Muscle and ligament identifications and attachment sites were confirmed by dissection of alcohol preserved specimens of *E. lucius* for the subgenus *Esox esox* and *E. americanus americanus* for *Esox kenoza* following Wilson and Williams (2010) and Wesneat (2004).

The *abductor mandibulae* muscle attaches to the lower jaw elements from the dorsal caudal (posterior) tip of the dentary and along the caudal edge of the anguloarticular for both the subgenera, *Esox esox* (pikes) and *Esox kenoza* (pickerels). This muscle is subdivided into the A2, A3 and Aω sections, all used during jaw closing, Figures 7 and 10. The Aω subdivision was included in one round of calculations for A2 because of their close association, following the protocol of Wesneat (2003).

The interoperculomandibular ligament attachment covered the caudal (posterior) edge of the retroarticular in both subgenera. Force from the *levator operculi* muscle is transferred through the opercular bones and interoperculomandibular ligament to the retroarticular to open/depress the lower jaw.

**Photography** Specimens were radiographed at FMNH. In addition, previous radiographs in the LUF collection were also included, for a total 373 specimens radiographed. Only radiographs where the cranial bones were positioned perfectly laterally with no twisting were digitized by illuminating with a lightbox and photographing with a Nikon D90, Figure 11. Fifty-six of the 373 specimens could be used for this study: 7 *E. a. americanus*, 11 *E. a. vermiculatus*, 9 *E. niger*, 17 *E. lucius*, and 12 *E. masquinongy*.

**Measurement of lever arms** Digital images of the radiographs were transferred to ImageJ64, where lengths for the in-lever arms and out-lever arm were obtained using digital
calipers. The out-lever for both jaw opening and closing was defined as the distance between the anterior most tip of the dentary bone and the quadrate anguloarticular joint. The in-lever arm for

Figure 10. Photograph of *E. lucius* specimen (LUD EL 64B) with A2 section of *abductor mandibulae* muscle reflected to reveal A3 section. Relevant structures labelled (5 mm scale bar). AA: Anguloarticular bone, A2: A2 section of *abductor mandibulae* muscle, A3: A3 section of *abductor mandibulae* muscle, Aω: Aω section of *abductor mandibulae* muscle, D: Dentary bone, H: Hyomandibular bone, IL: Interoperculomandibular ligament, IO: Interopercular bone, LO: *Levator operculi* muscle, O: Opercular bone, PO: Preopercular bone, RA: Retroarticular bone, S: Symplectic bone, SO: Subopercul ar bone, Q: Quadrate bone.
jaw opening was defined as the distance between the quadrate anguloarticular joint and the most posterior and ventral tip of the retroarticular (Figures 6 c, 7, and 11), while the in-lever arm for jaw closing was defined as the distance from the quadrate anguloarticular joint to the most posterior and dorsal tip of the dentary, the insertion point for the combined A2/ Aω subsections of the *abductor mandibulae* muscle (Figures 7, 9 c, and 11). Measurements for A3 were not included, because the in-lever for A3 could not reliably be measured from the radiographs. Each measurement was repeated three times and then averaged to minimize measurement error. Mechanical advantage (MA) was calculated from these measurements for each specimen. Opening MA was calculated as the length of the opening in-lever divided by the length of the out-lever and closing MA was calculated as the length of the closing in-lever divided by the length of the out-lever. MA for both jaw opening and jaw closing was recorded to the nearest thousandth decimal place.

**Data analysis** A RBANOVA was run on R (R Core Team 2017), blocking for the differences in SL among specimens, to test for variance between species for jaw opening and jaw closing MA while ruling out variance based on length of fish (Zar 2010).

**Development Series of *E. lucius* and *E. masquinongy***

**Specimen acquisition** Developmental material of *E. lucius* and *E. masquinongy* were collected for this study at the Jake Wolf Memorial Fish Hatchery in the spring and summer of 2014 to and deposited into the LUC collection. *E. lucius* were fertilized at 1:30 pm on March 18, 2014, and specimen collection began at 8:30 am on March 25, 2014. *E. masquinongy* were fertilized 1:30 pm on March 27, 2014, and specimen collection began at 8:40 am on April 5, 2014. Collection began before hatching to ensure that the point of hatching and the beginning of
Figure 11. Radiograph of *E. lucius* specimen (USNM 201266 B). A black star marks the lever fulcrum at the joint where the quadrate and anguloarticular bones meet, a green line represents one of the jaw closing in-levers, a red line represents jaw opening in-lever, and a gold line represents out-lever for both jaw opening and closing (each block equals one cm on scale bar).

Exogenous feeding would not be missed. Specimens were collected from each species three times a day until one week after their diets had been shifted from pelleted food to live fish (minnows), at 1950 hours (81 days) post fertilization (approximately 10 cm SL) for *E. lucius* and 2066 hours (86 days) post fertilization (approximately 9 cm SL) for *E. masquinongy*. This artificially controlled diet shift is past when the shift would occur in nature. The hatchery delays this diet
change to prevent cannibalism while the younger fish are stocked at higher densities in smaller tanks and runs. At this point specimens were collected once a day until they were stocked in large outdoor ponds and collection was no longer feasible. Time post fertilization was calculated to the nearest quarter hour for each sampling period. Collected specimens were fixed in 10% buffered formalin for a minimum of three days before they were rinsed and transferred to 70% EtOH for storage.

**Muscle dissections** A total of 56 specimens, 28 per species, were subsampled from the development series collected. The specimens had sizes ranging from 2.20 to 11.90 cm SL (approximately 40 to 88 days post fertilization) were separated into the following four size categories: A= 2.2–2.9 cm SL (n=16, 6 E. lucius, 10 E. masquinongy), B= 3.1–5.4 cm SL (n=16, 10 E. lucius, 6 E. masquinongy). C= 6.6–9.45 cm SL (n=13, 5 E. lucius, 8 E. masquinongy), and D= 9.6–11.9 cm SL (n=11, 7 E. lucius, 4 E. masquinongy). These were selected at sizes before the diet transition for both species (Group A), at the transition for E. masquinongy (Group B), at the transition for E. lucius (Group C), and after the transition for both species (Group D). Next, specimens were double stained and dissected to reveal lower jaw bones and sections of the adductor mandibulae muscle, following the protocol of Datovo and Vari (2014). Muscle identification and nomenclature follows Westneat (2004) and Wilson and Williams (2010). Multiple photos were taken at different focal points for the lateral view of the lower jaw for each specimen using a stereoscope and Nikon D5000. These photographs were then combined in Adobe Photoshop in a process known as focus stacking. During this process, multiple photos were aligned, and the software selects the clear portions of each photo, which are then stitched together to form one photo. This compensates for the shallow depth of field caused by the long
focal length of the camera and ensures that all jaw structures were clear and in focus. Coordinate data were then obtained from photos using the PointPicker plugin for ImageJ, with the points listed in Table 1 and diagramed in Figure 12. These data were then entered to MandibLever 4.0 (Westneat 2003). This program has been designed to use coordinate data or length measurements to model jaw closing and calculate MA, EMA, bite force, and other attributes of jaw movement. For this study, it was used to calculate MA for jaw opening (IOP.MA) and MA and EMA for A2, A3, and combined A2 and Aω subsections on all specimens.

**Data analysis** Two-way ANOVAs were calculated in R, an open-source statistics software package, for MA and EMA of each muscle subsection (R Core Team 2017). Type-III sums of squares ANOVAs were run to conservatively handle the unbalanced distribution of specimens across the size categories. Tukey multiple comparison of means were then calculated as appropriate (Zar 2010).
Table 1. Coordinate data points collected for analysis on MandibLever 4.0. The table below lists the points in the order they were placed on photographs when collecting coordinate data for MandibLever calculations.

<table>
<thead>
<tr>
<th>Point</th>
<th>Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quadrate anguloarticular joint</td>
</tr>
<tr>
<td>2</td>
<td>Distal tip of dentary</td>
</tr>
<tr>
<td>3</td>
<td>Dorsal end of retroarticular at attachment with interoperculomandibular ligament</td>
</tr>
<tr>
<td>4</td>
<td>Central origin of A2 on preopercle</td>
</tr>
<tr>
<td>5</td>
<td>Insertion of A2 onto dentary</td>
</tr>
<tr>
<td>6</td>
<td>Central origin of A3 on hyomandibula</td>
</tr>
<tr>
<td>7</td>
<td>Insertion of A3 onto medial face of anguloarticular</td>
</tr>
<tr>
<td>8</td>
<td>A3 junction of muscle and tendon</td>
</tr>
<tr>
<td>9</td>
<td>Insertion of Aω onto dentary</td>
</tr>
</tbody>
</table>
Figure 12. Photograph of *E. masquinongy* specimen (LUD EM52Cβ) with posterior end of A2 subsection of *adductor mandibulae* pulled away to reveal A3 subsection and its tendon. Gold circles mark the points marked for coordinate data for MandibLever calculations (5 mm scale bar). AA: Anguloarticular bone, A2: A2 section of *adductor mandibulae* muscle, A3: A3 section of *adductor mandibulae* muscle, Aω: Aω section of *adductor mandibulae* muscle, D: Dentary bone, H: Hyomandibular bone, IL: Interoperculomandibular ligament, IO: Interopercular bone, LO: Levator operculi muscle, O: Opercular bone, PO: Preopercular bone, RA: Retroarticular bone, S: Symplectic bone, SO: Subopercular bone, Q: Quadrate bone
Results

Comparison of North American Species

After a RBANOVA was run on jaw opening and closing MA, no significant differences were found for jaw closing. However, jaw opening MA for *E. lucius* (MA=0.118) was higher than *E. masquinongy* (MA=0.093, p<0.001), *E. a. vermiculatus* (MA=0.090, p<0.001), and *E. niger* (MA=0.098, p<0.05) (Table 2 and Figure 13). There was no significant difference between *E. lucius* and *E. a. americanus* (MA=0.101), nor were there any differences between any other species.

Comparison of Developmental Series

There was a significant difference between *E. lucius* and *E. masquinongy* for jaw opening (Figure 14, p= 5.845e-13), A2 MA (Figure 15 a, p=1.561e-05), A2 EMA (Figure 15 b, p=0.01906), A2 with Aω MA (Figure 16 a, p=7.816e-06), A2 with Aω EMA (Figure 16 b, p=0.003321), and A3 MA (Figure 17 a, p= 0.001354). There was no difference for species for A3 EMA (Figure 17 b, p= 0.27029). There was a significant difference among size categories for A2 MA (p=0.000189), A2 with Aω MA (p=0.01724), A2 with Aω EMA (p= 0.034761), and A3 MA (p= 1.388e-05), and A3 EMA (p= 0.0.03789). There was no difference for size category for jaw opening (p= 0.09767) or A2 EMA (p= 0.06547). There was a significant difference in the interaction of species and size categories for jaw opening (p= 0.03631) only.
Table 2. Mean mechanical advantage (MA) for jaw opening and jaw closing for each North American esocid species. Mean MA for jaw opening and jaw closing for each North American species or subspecies in study, including sample sizes for each. For jaw opening, *E. lucius* was significantly greater than *E. masquinongy* (p<0.001), *E. niger* (p<0.05), and *E. a. vermiculatus* (p<0.001). There were no significant differences for jaw closing.

<table>
<thead>
<tr>
<th></th>
<th>SL (cm)</th>
<th>Open MA</th>
<th>Close MA</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. a. americanus</em></td>
<td>11.45</td>
<td>0.101</td>
<td>0.299</td>
<td>7</td>
</tr>
<tr>
<td><em>E. a. vermiculatus</em></td>
<td>9.07</td>
<td>0.090</td>
<td>0.296</td>
<td>11</td>
</tr>
<tr>
<td><em>E. niger</em></td>
<td>13.91</td>
<td>0.098</td>
<td>0.297</td>
<td>9</td>
</tr>
<tr>
<td><em>E. lucius</em></td>
<td>11.38</td>
<td>0.118</td>
<td>0.283</td>
<td>17</td>
</tr>
<tr>
<td><em>E. masquinongy</em></td>
<td>12.52</td>
<td>0.093</td>
<td>0.282</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure 13. Mean Mechanical advantage (MA) for jaw opening for each North American species or subspecies in study. *E. lucius* was significantly greater than *E. masquinongy* (p<0.001), *E. niger* (p<0.05), and *E. a. vermiculatus* (p<0.001). X axis lists the different species studied, while y axis lists MA values for jaw opening. Black bars signify significant differences between the groups under the ends of the bar, while * signifies p<0.05 and *** signifies p<0.001.
Figure 14. Mechanical advantage for jaw opening (IOP.MA) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for *E. lucius* (EL) and *E. masquinongy* (EM). This graph demonstrates that there is a difference in mechanical advantage for jaw opening between the two species across the development points in this study.
Figure 15. a) Mechanical advantage for A2’s contribution to jaw closing (A2.MA) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for E. lucius (EL) an E. masquinongy (EM). b) Effective mechanical advantage for A2’s contribution to jaw closing (A2.EMA) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for E. lucius (EL) an E. masquinongy (EM). These graphs demonstrate that there is a difference in mechanical advantage at the earlier development points of this study and there is a difference between species in the mid points near diet shifts for effective mechanical advantage.
Figure 16. a) Mechanical advantage (MA) for A2 and $A_\omega$’s collective contribution to jaw closing ($A_{2Aw}.MA$) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for *E. lucius* (EL) and *E. masquinongy* (EM). b) Effective mechanical advantage for A2 and $A_\omega$’s collective contribution to jaw closing ($A_{2Aw}.EMA$) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for *E. lucius* (EL) and *E. masquinongy* (EM). These graphs demonstrate the difference in mechanical advantage for jaw closing between the two species across the development points in this study.
Figure 17. a) Mechanical advantage (MA) for A3’s contribution to jaw closing (A3.MA) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for E. lucius (EL) and E. masquinongy (EM). b) Effective mechanical advantage for A3’s contribution to jaw closing (A3.EMA) graphed against size category (A= 2.2–2.9 cm SL, B= 3.1–5.4 cm SL, C= 6.6–9.45 cm SL, and D= 9.6–11.9 cm SL) for E. lucius (EL) and E. masquinongy (EM). The second graph shows a widening difference in effective mechanical advantage for A3 jaw closing across development of the two species.
Discussion

Comparison of North American Species

The species of genus *Esox* all display a characteristic duck billed snout and are piscivorous as adults, therefore it makes sense that there would be no significant difference in jaw closing MA between species. However, this does not align with the results for jaw opening. The Northern Pike, *E. lucius*, had a significantly higher opening MA than all of the species studied, besides for the subspecies *E. a. americanus*. It is very important to note that this study does not take the action of *protractor hyoideus* muscle into consideration, which also functions in jaw depression/ opening. It would be interesting for future research to take this into consideration and see if this muscle’s action lessens or further amplifies the differences seen for MA in this study. In addition, upon visual inspection of the specimens *E. lucius* seems to generally have a wider lower jaw. This would cause a greater surface area, which would then result on increased drag forces being applied to the jaw by resistance from the water. Perhaps this increased MA could help overcome the increased drag that a wider jaw would cause during opening.

Developmental Series of *E. lucius* and *E. masquinongy*

Within this section of the study, the results for *E. masquinongy* were generally as expected; *E. masquinongy* showed a decrease of all of the metrics calculated (opening and closing MA and EMA) between groups A and B (before and at the point of diet shift). Therefore, *E. masquinongy* should experience the potential for fastest jaw opening and closing at this stage of growth. It is interesting to note, however, that in some of these metrics the MA or EMA actually increased again in groups C or D, sometimes even higher than the original value for
group A. This falls in line with observations previously made for jaw closing MA of longnose gar, *Lepisosteus osseus*, and alligator gar, *Atractosteus spatula*, which also dipped and then rebounded (Kammerer et al. 2004). While this increase may seem counterproductive (decreased speed), it is possible that the increase in MA and EMA could help the jaw overcome increased water resistance as the surface area of the jaw increases and the individual grows. However, further research is needed to test this postulation.
CHAPTER THREE

COMPARISON OF GEOMETRIC MORPHOMETRICS DURING DEVELOPMENT IN NORTH AMERICAN ESOCID SPECIES

Morphometrics is the quantitative study of shape and form. The field of morphometrics has undergone numerous changes over the past 50 years, leading to the “revolution” of geometric morphometrics in the 1980’s (Adams et al. 2004) (Rolf and Marcus 1993). Traditional morphometrics use multivariate statistical analyses of measurements such as length, height, and width while unfortunately missing other aspects of shape. However, the landmark-based approach of geometric morphometrics, used in this study, helps retain more shape information by maintaining landmarks in the original space after multivariate analysis and the ability to analyze outline data (Adams et al. 2004). The recent geometric morphometric toolkit allows for multiple forms of shape change visualization and has allowed geometric morphometrics to become applicable to several other fields (Klingenberg 2011, 2013, Adams et al. 2013, 2014). These fields have grown from phylogenetic studies and ecomorphology, to also include quantitative genetics, studies of integration and modularity, and the realm of functional morphology and biomechanics (Klingenberg and Ekau 1996, Frederich at al. 2008, Ristovska et al. 2008, Adams et al. 2013).

Currently, geometric morphometrics utilizes the Procrustes paradigm and follows the subsequent general steps when considering two-dimensional space. First, landmarks must be selected. These landmarks should have a precise location and be present on all specimens. After this, specimens must be photographed so that landmarks may be digitized into x,y coordinates.
Once these coordinates have been collected, the data may be uploaded into specific software programs for geometric morphometric analysis. Before detailed analysis can be completed, a generalized Procrustes analysis must be performed. This step allows for the removal of non-shape variations such as size and rotation. Finally, deeper analysis can be conducted through principal component analysis, multivariate analysis of variance, canonical variate analysis, etc. and results may be graphically represented on transformation grids, lollipop diagrams, or wireframe graphs (Adams et al. 2004, 2013 and Klingenberg 2013). Using these analyses and visualizations, shape change across taxonomy, development, or experimental conditions can be studied, which will be used in this thesis to study how cranial shape changes during development for *E. lucius* and *E. masquinongy* in this chapter. In addition, this technique was used in another study outside of this these to consider body shape and fin placement changes across development for a future publication.

**Materials and Methods**

**Collection of Specimens**

A subset of 90 specimens from the developmental series described in the previous chapter were used in this study. These specimens were separated into three groups according to standard length (SL) for each of the two species, *E. lucius* and *E. masquinongy*: group one contained specimens ranging from 1.7–2.7 cm, group two 3.9–4.9 cm, and group three 5.3–7.5 cm. Group one corresponds to before diet shift of both species, group two is after diet shift for *E. masquinongy* but before diet shift for *E. lucius*, and group three is after diet shift for both species. This grouping enables determination of any shape changes that correspond to earlier diet shift in *E. masquinongy*. 
Photography and Landmark Collection

Multiple photos were taken of the lateral side of the cranial region for each specimen using a stereoscope and Nikon D5000 for group one specimens, and with a Nikon D90 for groups two and three. These photographs were stacked in Adobe Photoshop to ensure that all jaw structures were clear and in focus. Coordinate data were obtained from photos using the PointPicker plugin on ImageJ (Schneider et al. 2012 and Thévenaz 2013). Landmarks used in the study are listed and diagramed in Table 3 and Figure 18.

Data Analysis

Data were imported into MorphoJ and a Procrustes Fit was performed for each data set before principal component analysis (Klingenberg 2011). As stated earlier, performing a Procrustes Fit removes variation based on size and rotation so any differences elucidated during the principal component analysis should be based on shape alone.

Results

Principal components 1 and 2 accounted for ~80% of the difference in form among the species and size groups, Figure 19, and will be the focus of the results section of this study. There was a difference along principal component one (PC1) between both species for each of the three size groups studied. In addition, each species followed a similar pattern; the smallest size group, group 1, was separate from groups 2 and 3 and the two larger groups overlapped along PC1. There was considerable overlap between the species and size groups along principal component two, Figures 20 and 21. However, while the trends are the similar for each species, *E. masquinongy* remains relatively shifted into the negative morphospace along PC1 when compared to *E. lucius.*
Table 3. Coordinate data points collected for lateral cranial shape analysis.

<table>
<thead>
<tr>
<th>Landmark Number</th>
<th>Landmark Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Midpoint of tip of lower jaw</td>
</tr>
<tr>
<td>2</td>
<td>Midpoint of tip of upper jaw</td>
</tr>
<tr>
<td>3</td>
<td>Distal point of eye</td>
</tr>
<tr>
<td>4</td>
<td>Proximal edge of eye</td>
</tr>
<tr>
<td>5</td>
<td>Dip at suture between frontal and parietal</td>
</tr>
<tr>
<td>6</td>
<td>Proximal most edge of operculum</td>
</tr>
<tr>
<td>7</td>
<td>Ventrodorsal most point of retroarticular bone/ lower jaw</td>
</tr>
<tr>
<td>8</td>
<td>Proximal tip of maxilla</td>
</tr>
</tbody>
</table>
Figure 18. Landmarks used in lateral cranial study marked by red points on a specimen of *E. masquinongy* (LUD EM48Aα)
Figure 19. Bar graph of variance in shape difference distributed across principal components. Principal components 1 and 2 account for ~80% of the differences in shape between the species and size groups.
Figure 20. Scatter plot of specimens of *E. lucius* and *E. masquinongy* along principal components 1 and 2 with 95% frequency ellipses around each group. The smallest group of *E. lucius* is 1EL, colored red, the middle size group in 2EL, green, and the largest is 3EL, dark blue. The smallest group of *E. masquinongy* is 1EM, colored orange, the middle size group in 2EM, light blue, and the largest is 3EM, pink.
Figure 21. Scatter plot of group two, middle size, specimens of *E. lucius* and *E. masquinongy* along principal components 1 and 2 with 95% frequency ellipses around each group. The *E. lucius* are colored red, while *E. masquinongy* are colored dark blue.
Group 2 highlights the size at which the two species have differing diets; *E. lucius* would still be consuming aquatic invertebrates while *E. masquinongy* would have begun feeding other fish in the wild. When comparing the two species at this size group, the two groups are separated along PC1, Figures 20 and 21. *E. masquinongy* occupies the space negative to the target/mean shape while *E. lucius* occupies the positive space along the PC1 axis. This translates to the changes in form are diagramed in Figures 22 and 23. By looking at the wireframe graph, transformation grid, and lollipop diagrams, we can see that at this size point *E. masquinongy* has a relatively longer jaw, smaller eyes, and a shallower cranium than *E. lucius* does at this size point.

**Discussion**

During development, both species trend toward a cranium shape that becomes shallower in the dorsal to ventral dimensions and more elongated in the rostral to cranial dimensions as they trend further into the negative morphospace of PC1. However, *E. masquinongy* remains further into the negative morphospace than *E. lucius*. This results in *E. masquinongy* having a greater taper from the rostrum to the cranium than *E. lucius* from the beginning of the developmental study to the end. A tapered head is a more hydrodynamic head shape that can better cut through water drag and increases efficiency while swimming (Fish 1998). It is possible that this increased swimming efficiency may allow *E. masquinongy* to feed on faster moving prey without paying as high transportation energetic costs as *E. lucius* at that size range. In addition, Figures 22 and 23 show that the relative mouth gape is larger for *E. masquinongy* that *E. lucius* across development, in size group two in particular. Many fish are considered gape restricted predators, meaning that they can only eat prey items that fit within the constraints of
their open mouth. This is because my fish do not masticate or chew their prey with their oral teeth. Instead, oral teeth many times are used to hold onto prey and keep it from escaping during manipulation into the anterior digestive tract. Therefore, prey are generally eaten whole by a number of fish, esocids included. This means that a relatively larger gape would allow *E. masquinongy* to consume prey relatively larger to its body size than *E. lucius* would at this point in development. In short, this would mean that *E. masquinongy* would have a greater size range and variety of prey that they would be able to consume.
Figure 22. Wireframe graphs of group two, middle size, specimens of *E. lucius* and *E. masquinongy* along principal component 1. Red represents target shape change along positive PC1 axis (*E. lucius*). Dark blue represents target shape change along negative PC1 axis (*E. masquinongy*) and the mean shape for both species combined is light blue.
Figure 23. Transformation grid with lollipop diagram of group two, middle size, specimens of *E. lucius* and *E. masquinongy* along principal component 1. The circle end of the lollipop diagram represents the location of landmarks on *E. masquinongy* and the end of the stick portion of the lollipop diagram represents the location of landmarks on *E. lucius*. 
CHAPTER FOUR
DISCUSSION

Previous research (Burdi and Grande 2010) has shown that the skeletal features of *E. masquinongy* ossify and develop adult morphology at a faster rate than those of *E. lucius*. This study suggested that this would aid *E. masquinongy* in developing strong predatory behaviors sooner. However, no work has tested how the development of these skeletal features may translate into predatory functional characteristics for piscivory, including fast feeding strikes and quickly moving, large mouths. Therefore, this thesis had two main sections: 1) Exploration of the lower jaw functional morphology across all North American esocid species with an emphasis on the development of *E. lucius* and *E. masquinongy*, and 2) Geometric morphometric study of the development of external morphology of *E. lucius* and *E. masquinongy*.

**Mechanical Advantage**

When comparing specimens older than young of the year, there were no significant differences between species for jaw closing MA. However, young fish of *E. lucius* and *E. masquinongy* did display differences: *E. lucius* had higher jaw closing MA in the smallest group (A), with the MA dropping to nearly similar levels as *E. masquinongy* across the remainder of the developmental series. These data suggest that *E. lucius* would have a stronger, but slower jaw closing that *E. masquinongy* early on, but eventually the gap is closed by adulthood.

When considering the main muscles responsible for jaw closing (A2, A2 combined with Aω, and A3 separately), *E. lucius* still had a higher MA. However, the pattern for how MA
values of *E. lucius* dropped in relation to *E. masquinongy* were slightly different for the muscle segments than for the overall muscle group. In fact, by the end of the developmental series the values for A3 MA seemed to be rising again for *E. lucius*. This rebound is not unheard of; Kammerer et al. (2004) saw a similar rebound in MA during development of two gar species and this inflection point was near diet shift for those species as well. It would be interesting to see how these data align with values for adult specimens of *E. lucius* in future studies. Future work should also consider the relative timing of firing of these muscles, as this could also change across development and play into prey capture.

Finally, differences existed between *E. lucius* and *E. masquinongy* for jaw opening MA, both during development and in the older specimens. At all of these points, *E. lucius* had a higher MA and never closed the gap with *E. masquinongy*. This means that *E. lucius* should have a slower jaw opening across all points of its life measured in this study.

Overall, this means that this study suggests that *E. lucius* would have slower jaw opening and closing and *E. masquinongy* would have relatively faster jaw opening and closing during early development. The relative arrangement and size of its jaw structures may give *E. masquinongy* a slight competitive edge it needs to quickly capture elusive prey, such as other fish and account for its earlier diet shift.

**Geometric Morphometrics**

When considering changes in cranial shape across development for the two species, *E. masquinongy* consistently occupies more of the negative PC1 morphospace, meaning that it has a longer, shallower head that is more streamlined and has a relatively larger mouth gape compared to its body size. This would give *E. masquinongy* a competitive advantage of being able to swim quickly through the water more efficiently after fast moving prey and be able to consume a larger
variety of sized prey items in comparison to its size. This may give *E. masquinongy* a competitive advantage at this point in development and cause the species to switch diets at this point and *E. lucius* to not.

**Conclusions**

While *E. lucius* may have displayed attributes for slower moving jaws than *E. masquinongy* early in development in the first section of this thesis, the overall steep downward trend of opening and closing MA between the first and second size groups (nearly to the values calculated for the non-developmental series specimens) suggests that *E. lucius* may functionally be capable of piscivory before they make the switch in diet. But why would a species wait until later in development to shift to a higher nutrient payoff, like that found in fish prey? There may be a few possibilities to mention here: locomotion costs, potential for cannibalism, and prey availability.

Any movement requires an organism to expend energy, and fish swimming through water are no different. There are several adaptations that swimming animals use to make locomotion through the dense water environment more efficient, such as different scale designs, mucous layers, and streamlined shape (Fish 1998). The geometric morphometrics portion of this study has shown that *E. masquinongy* has a more streamlined head shape, and most likely increases its swimming efficiency across the stages of development in these studies. If this were to be supported with kinematic studies, this would mean that it would cost less energy for *E. masquinongy* to swim after more elusive prey, and it would be able to reap the reward of a higher payout of more calorically dense fish prey.

An important consideration of the diet shift for these species is the time of year in which they occur. Northern Pike hatch much earlier than Muskellunge do, therefore it is earlier in the
year when they hit the sizes of development considered in this thesis (Scott and Crossman 1973, Becker 1983, McCormick et al. 2020). In fact, Northern Pike are among the first fish to spawn in many bodies of water they inhabit. This means that the main prey items that would be available to young Northern Pike would not include other fish, so it would be difficult to switch to piscivory without resorting to cannibalism. Cannibalism is a problem frequently noted among young fish, especially, esocids, in hatchery and aquaculture settings (Scott and Crossman 1973, 1998, McCormick et al. 2020). By delaying their diet shift in the wild, Northern Pike may reduce their level of cannibalism. Muskellunge, on the other hand, hatch out later in the spring, and have more species of other fish available as prey items earlier in their development. This means that they could switch diet earlier in development without as large of a level of cannibalism that Northern Pike would incur. By this point in the growing season, Northern Pike would have grown into the larger size groups considered in this study and be able to switch diet with lower levels of cannibalism, as well.

**Future Directions**

While the biomechanical modeling performed in this thesis gives plenty of valuable insights to the functional morphology of these fishes, there certainly are limits to it. For instance, jaw opening was modeled as a simple lever, using only the force transferred into the system by the interopercular ligament. However, jaw opening also includes some action from the protractor hyoideus muscle, which connects the dentary and ventral hyoid arch. This would create another lever system that future projects can model. In addition, the relative timing for firing of the different muscles and muscle subsections was outside the scope of this study. Future work could use electromyography to rank the timing and relative contributions of each muscle and subsection to obtain an even clearer picture of what happens during jaw opening and closing in
these species. Other future considerations would be to perform kinematic studies to determine how these theoretical MA values would translate into real world movements and looking at individual bones within the skull (e.g., jaws: maxilla, dentary, palatine; skull roof: frontals; suspensorium: pterygoids; opercular series) to better understand the development of the duck-billed snout (e.g., allometric elongation of the skull) and if such development is a process of modularity.

A continuation of the geometric morphometric portion of this study is already underway; a study of fin placement and overall lateral body shape was started in conjunction with the cranial study of this thesis. This will allow for the consideration of developmental changes in other adaptations that aid fast-strike feeders such as esocids. These adaptations include caudally displaced dorsal and anal fins and anterior stabilizing body mass (Webb 1982, 1984). In addition, photos were also taken of the dorsal and ventral aspects of the fish so that shape could be considered in other planes than the lateral aspect in future research.

As technology continues to develop the ability to accurately model living organisms, the possibilities to study the movements and relationships of a variety of organisms continues to grow. These studies will not only help us better understand the form of these organisms, but how they evolved and how they continue to interact with the ever-changing environment around them. It also inspires new ideas for different fields of engineering in the form of biomimicry. We already have animals to thank for the designs of hypodermic needles, compression socks, and even polymers to better preserve frozen blood (Hargens et al. 1987, Izumi et al. 2011, Deller et al. 2014). Just imagine what could be inspired next.
APPENDIX A

LIST OF SPECIMENS
Primary Research Material

*Esox lucius* developmental series (2 – 220 mm TL): LUD EL1A–LUD EL111A, The Illinois Department of Natural Resources, Jake Wolfe Fish Hatchery, Topeka, IL.

*Esox masquinongy* developmental series (2– 150 mm TL): LUD EM1A–LUD EM82A, The Illinois Department of Natural Resources, Jake Wolfe Fish Hatchery, Topeka, IL.

Figured material: *Esox lucius*: LUD EL 54A, 64B, 90A. *E. masquinongy* specimen LUD EM48Aα, EM52Bβ

Additional Referenced 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); USNM 201266 B; LUF 09808, 09809, 09811, 09825 (alcohol, c&s)

*Esox masquinongy*: 15 spec. (SL: 65 – 145): FMNH: 85991; CU 9118, 19154 (c&s)

*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; LUF, Loyola University, Fish Collection, Chicago, IL.; USNM, United States National Museum of Natural History, Washington, D.C.
REFERENCE LIST


VITA

Cheryl Theile was born and raised in Cincinnati, Ohio. She attended Xavier University and University of Hawai‘i at Mānoa for her undergraduate education, resulting in a Bachelor of Science in Biology with a minor in Chemistry from Xavier in 2007. During her break between undergrad and graduate school she worked as a biologist and presenter for the Newport Aquarium in Newport, Kentucky, as a veterinary assistant and lab manager for PetCare Animal Hospital in Cincinnati, Ohio, and as a surgical assistant for MedVet Medical and Cancer Center for Pets in Cincinnati, Ohio.

During her time at Loyola University Chicago, Dr. Theile served as President of the Biology Graduate Student Association, teaching assistant for Comparative Anatomy lab, instructor for General Biology Labs 1 & 2, and counselor for the Biology Bootcamp. She presented portions of her thesis research at the Joint Meeting of Ichthyologists and Herpetologists in 2014 and 2016, and at the Midwest Ecology and Evolution Conference in 2015.

In addition, Dr. Theile completed her Doctorate of Veterinary Medicine at the Ohio State University College of Veterinary Medicine in Columbus, Ohio in 2022, and continues to feed her passion for ichthyology within the World Aquatic Animal Veterinary Association and the American Association of Fish Veterinarians.