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Reproductive Demographics and Early Life History of the Shovelnose Sturgeon (SCAPHIRHYNCHUS PLATORYNCHUS)

Robert E. Colombo
Eastern Illinois University, recolombo@eiu.edu

REPRODUCTIVE DEMOGRAPHICS AND EARLY LIFE HISTORY OF THE SHOVELNOSE STURGEON (*SCAPHIRHYNCHUS PLATORYNCHUS*)

by

Robert E. Colombo

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Due to the collapse of the historic caviar fisheries in the Volga River and Caspian Sea, the demand on domestically produced caviar has increased. To supply the increased demand, the harvest of the shovelnose sturgeon *Scaphirhynchus platorynchus* has increased. Because caviar fisheries preferentially harvest females, information regarding the sexual demographics of these populations is essential for effective fisheries management. To date, knowledge regarding the sex ratios and reproductive development of the shovelnose sturgeon population in the Middle Mississippi River is lacking. In Chapter 1, I describe this problem in detail and in subsequent chapters I explain how I addressed this problem using a combination of field and laboratory techniques.

The shovelnose sturgeon and other acipenserids have no known sexually dimorphic traits, making field identification of sex difficult. Thus, in Chapter 2, I developed a non-surgical method for the determination of sex of shovelnose sturgeon. Ultrasound imaging was used to determine the sex of shovelnose sturgeon (n=51). Overall, ultrasound imaging provided an effective method for determining sex, with 86% correct identification of all individuals examined. Ultrasound was ineffective at determining the sex of post spawn females, with 60% being incorrectly identified as males. This method would allow managers to quickly identify sex in the field so that the sex ratio of the shovelnose sturgeon populations could be tracked and population dynamics can be modeled.

Understanding of the sequence of gonadal development allows for the determination of spawning stock size in a given year. This information is currently lacking for the shovelnose sturgeon. To address this, I developed a guide for shovelnose sturgeon reproductive development in Chapter 3. Shovelnose sturgeon were sampled seasonally from the Middle Mississippi River to account for all stages of reproductive development. I found that stages of gonadal development of the shovelnose sturgeon were both grossly and histologically similar to those of other sturgeon species. This sample allowed me to estimate the sex ratio in the population using an unbiased approach. The shovelnose sturgeon population in the MMR did not differ from 1: 1.

The shovelnose sturgeon has been extirpated from several portions of its historic range. Conservation aquaculture can be used to reestablish shovelnose sturgeon populations to these regions. This requires detailed information about early life history which is currently unavailable. In Chapter 4, I described the stages of development of embryonic and larval shovelnose sturgeon to resolve this issue, giving culturists the ability to track the development of their spawn using proper development as an indicator of success. Additionally, otoliths provide little use in determining hatch date in the acipenserids. Using this information, managers will be able to determine hatch date using stage of development and river temperature.

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CHAPTER ONE

GENERAL INTRODUCTION

The shovelnose sturgeon *Scaphirhynchus platorynchus* is commercially exploited in seven of 24 states where it occurs in the Mississippi River basin (Keenlyne 1997). Throughout much of its range, its status is unknown or its abundance has declined (Keenlyne 1997). Various species of sturgeon have been harvested in the United States since the 1800's, primarily to produce roe for caviar (Boreman 1997). The reduction of historic sturgeon fisheries in the Caspian Sea and Volga River (Khodorevskaya et al. 1997) and the restrictions on importation of caviar into the United States (Gnam 1998) will undoubtedly lead to increased exploitation and possibly poaching of domestic sturgeons (Secor et al. 2002).

Conservation of the shovelnose sturgeon will therefore require an increase in information about its life history and population status. Management of its stocks will be particularly difficult given that females are likely to be harvested preferentially for their roe. Given this, females within an exploited population should be considered the effective management unit (i.e., stock). The fecundity of females controls population growth of many fish species including shovelnose sturgeon (Quist et al. 2002). Because females are preferentially harvested, information on the sex ratio of these populations is essential to effective fisheries management (Fabrizio and Richards 1996). Shovelnose sturgeon, as with other acipenscerids, have no apparent external sexually dimorphic traits, thereby making field identification of sex difficult (Conte et al. 1988).

Three methods of determining the sex in sturgeon do exist. In the first, a small incision is made in the ventral body wall of the fish and sex is determined by directly

inspecting the gonads (Conte et al. 1988). This method is highly invasive and difficult under field conditions. The second method uses blood plasma indicators and has only been verified for the white sturgeon (Webb et al. 2002). Although this method is accurate, it is expensive and species-specific requiring baseline data for each species examined. Recently, ultrasonic imaging has been used to effectively determine the sex of the stellate sturgeon *Acipenser stellatus* (Moghim et al. 2002).

In addition to sex ratio, understanding the reproductive demographics of shovelnose sturgeon is important. The sequence of gonadal development provides information about timing and periodicity of spawning, allowing conservationists to make predictions about population growth. Numerous investigators have developed indices of gonadal development for sturgeon species including Atlantic sturgeon *Acipenser oxyrinchus* (Van Eenennaam et al. 1996), white sturgeon *Acipenser transmontanus* (Conte et al. 1988), Russian sturgeon *Acipenser gueldenstaedtii* (Dettlaff and Ginsberg 1985), lake sturgeon *Acipenser fulvescens* (Bruch et al. 2001) and the hybrid bester (Amiri et al. 1996a,b). Little information is currently available regarding the stages of gonadal development in shovelnose sturgeon.

Because the shovelnose sturgeon has been extirpated from several states in the U.S. (Keenlyne 1997), efforts to reintroduce this species will require hatchery supplementation. Knowledge of the sex ratio and reproductive demographics of natural shovelnose sturgeon populations coupled with information of early life history will be critical for successful artificial propagation. Conservation aquaculture has been used to enhance current stocks and reintroduce several species of sturgeon to their historic ranges (Chebanov and Savelyeva 1999; Schram et al. 1999; St Pierre 1999; Chebanov et al.

2002; Ireland et al. 2002). Efforts are currently underway by the U. S. Fish and Wildlife to supplement and reintroduce shovelnose sturgeon populations in Ohio and West Virginia (G. Conover, U.S. Fish and Wildlife, *pers. comm.*). Information about the embryological development of the shovelnose sturgeon would assist in this process.

In the following section I will provide more detailed information on the techniques that I will employed to resolve the missing information on the reproductive demographics and early life history of the shovelnose sturgeon.

TECHNIQUES

Non-surgical Sex Determination

Ultrasound is a potential non-invasive tool for sex determination of shovelnose sturgeon. Although portable ultrasonographic equipment is initially expensive ($7000 – $12000 USD) the equipment can be used on numerous species (Pacific herring *Clupea harengus pallasi*: Bonar et al. 1989; striped bass *Morone saxatilis*: Blythe et al.1994; benthic marine fishes: Martin-Robichaud and Rommens 2001) and provides immediate noninvasive results. Moghim et al. (2002) used ultrasonic imaging to determine the sex of the stellate sturgeon *Acipenser stellatus*; however, this study focused exclusively on sturgeon sampled during a spawning migration. In this case, all stages of sturgeon gonadal development were not present. Ultrasonography proved to be 97.2 % accurate in sexing stellate sturgeon (Moghim et al. 2002). To date, no information is currently available on the sexing of shovelnose sturgeon using this non-invasive imaging technique. The effectiveness of this technique was evaluated in Chapter 2.

Stages of Gonadal Development

Little information is currently available on the stages of gonadal development in shovelnose sturgeon. Moos (1978) studied the reproductive development of the shovelnose sturgeon in the Missouri River using dissection and histology. However, his criteria were different than those used in other studies on sturgeon. For example, many of his stages were based on sampling time during the year rather than the standard structural characteristics used by other studies. He identified six stages of female development and five stages of male development. Additionally, stages of gonadal development were assigned to shovelnose sturgeon sampled in the Missouri River using the color of the gonad; no histology accompanied the assignment of the stages (Carlson and Pflieger 1981). His approach does not provide insight into the development of either the spermatozoa or the oocytes in the gonads which is critical to the understanding of reproductive development.

Both dissection and histology must be used when characterizing the stages of gonadal development for a species. Bruch et al. (2001) used these methods to develop a field guide for lake sturgeon gonadal development. The authors identified seven stages for female development and four for male development. These stages were directly related to the structural assignments used for other species of sturgeon (Dettlaff and Ginsberg 1985; Conte et al. 1988; Amiri et al. 1996a,b; Van Eenennaam et al. 1996). This provides an adequate framework that could be applied to the gonadal development of the shovelnose sturgeon. In Chapter 3, I described the sequence of gonadal development of the shovelnose sturgeon using both gross anatomy and histology thereby

providing a guide for managers to assess the reproductive development of their population.

Early Embryology

Understanding of the sequence of gonadal development provides population level information essential for the management and conservation of a species; however, quantifying the developmental stages of oocytes in the ovaries allows for development of proper aquaculture techniques. During maturation, sturgeon oocytes undergo changes that ultimately determine whether the oocytes are able to become activated and fertilized (i.e. become ripe). As an oocyte reaches its final stage before maturation, the nucleus or germinal vesicle migrates towards the animal hemisphere of the oocyte (Conte et al. 1988; Dettlaff et al. 1993). When boiled in Ringer's solution, the oocyte can be sectioned with a razor along the animal-vegetal axis and examined under a microscope. The distance from the inner border of the chorion to the edge of the germinal vesicle (GV) and the total egg diameter are measured. The distance from chorion to GV divided by the oocyte diameter is the polarization index (PI) (Dettlaff et al. 1993). For white sturgeon, a PI ratio of <0.10 is used as a measure of a females' ability to have its oocytes fertilized (Van Eenennaam et al. 2001).

No information is currently available about the embryological development of river sturgeon. Snyder (1999) developed a character index to the genus *Scaphirynchus* larvae, but provided no information on embryology of the genus. Early development of North American sturgeons has been described for the white and lake sturgeon (Wang et al. 1985). The rate of embryologic development in both white and lake sturgeon depends

on incubation temperature (Wang et al. 1985). Additionally, the embryology of Russian sturgeon was described (Dettlaff et al 1993). No information is currently available about the embryologic development of the shovelnose sturgeon. Since conservation aquaculture is currently being employed to reintroduce the shovelnose sturgeon to its native range, this information is critical. I described the stages of embryologic development for the shovelnose sturgeon in Chapter 4, providing a guide that can aid in the development of proper aquaculture techniques.

GOALS

I will describe the reproductive demographics and early life history of the shovelnose sturgeon in the Middle Mississippi River. In doing so I will provide managers with techniques that will allow the rapid determination of reproductive demographics in other populations of shovelnose sturgeon.

OBJECTIVES

1. Develop a non-invasive method for sexing of shovelnose sturgeon.
2. Develop a field guide to the stages of shovelnose sturgeon gonadal development.
3. Determine the fecundity of mature female shovelnose sturgeon.
4. Describe the early embryology of shovelnose sturgeon.

CHAPTER TWO

USE OF ULTRASOUND IMAGING TO DETERMINE SEX OF SHOVELNOSE STURGEON[1]

ABSTRACT

During November 2002, 51 shovelnose sturgeon *Scaphirhynchus platorynchus* were sexed by ultrasound imaging using a portable ultrasonograph. I identified males with 96% accuracy (n = 25) and females with 80% accuracy (n = 25); one hermaphroditic individual was misidentified as a male. Overall, ultrasound imaging was 86% accurate (n =51). Sex in post-spawn females was difficult to determine, with 60% misidentified as males (n = 5). Ultrasonography is an effective non-invasive method for sex determination that can be applied to other species of Acipenseriformes. Modern portable equipment expands its utility to field studies.

INTRODUCTION

The shovelnose sturgeon *Scaphirhynchus platorynchus* is commercially exploited in seven of 24 states where it occurs in the Mississippi River basin (Keenlyne 1997). Throughout much of its range, its status is unknown or has declined (Keenlyne 1997). Various species of sturgeon have been harvested in the United States since the 1800's primarily to harvest roe for caviar production (Boreman 1997). The reduction of historic sturgeon fisheries in the Caspian Sea and Volga River (Khodorevskaya et al. 1997) and the restrictions on importation of caviar into the United States of America (Gnam 1998)

[1] Colombo, R. E., P. S. Wills and J.E. Garvey. 2004. Use of ultrasound imaging to determine sex in the Shovelnose Sturgeon. North American Journal of Fisheries Management 24: 322-326.

will undoubtedly lead to increased exploitation and possibly poaching of domestic sturgeons (Secor et al. 2002).

Conservation of shovelnose sturgeon populations under increased exploitation will require an increase in information about life history and population status from the current state. Management of its stocks will be particularly difficult given that females are likely to be harvested preferentially for their roe. Given this, females within an exploited population should be considered the effective management unit (i.e., stock). In general, information on the sex ratio of a species in which females are preferentially harvested is essential to effective fisheries management (Fabrizio and Richards 1996). Therefore, effective methods for determination of sex in a field setting would greatly improve a fisheries manager's ability to collect vital population data.

Shovelnose sturgeon, as with other acipenscerids, have no apparent external sexually dimorphic traits, thereby making field identification of sex difficult (Conte et al. 1988). Two methods for determining the sex in sturgeon do exist. In the first, a small incision is made in the ventral body wall of the fish and sex is determined by directly inspecting the gonads (Conte et al. 1988). This method is highly invasive and difficult under field conditions. The second method uses blood plasma indicators and has only been verified for the white sturgeon *Acipenser transmontanus* (Webb et al. 2002). Although this method is accurate, it is expensive and species-specific requiring baseline data for each species examined. Another potential tool is ultrasound, which been used effectively for sex determination in several species of fishes (Pacific herring *Clupea harengus pallasi*: Bonar et al. 1989; striped bass *Morone saxatilis*: Blythe et al.1994; benthic marine fishes: Martin-Robichaud and Rommens 2001). Although, portable

ultrasonographic equipment is initially expensive (7000 – 12000 USD) the equipment can be used on numerous species and provides immediate, noninvasive results.

Ultrasound imaging has been shown to be effective for determining the sex of the stellate sturgeon *Acipenser stellatus* (Moghim et al. 2002). This study focused exclusively on sturgeon sampled during a spawning migration; therefore, all stages of sturgeon gonadal development were not present. Ultrasonography was 97.2 % accurate in sexing stellate sturgeon, suggesting that this technique should be robust for shovelnose sturgeon.

I sought to determine whether ultrasonography can be used to determine sex of shovelnose sturgeon. If effective, ultrasound imaging would provide a quick method for determining the sex of shovelnose sturgeon and potentially other species of Acipenseriformes in the field.

METHODS

Fifty-one shovelnose sturgeon ranging in size from 444 to 714 mm fork length (mean 618.4 mm fork length; SD=56.0) were collected by gillnets (90 m long, 5 cm bar mesh) set overnight between river kilometer 188 and 228 of the Middle Mississippi River during November 4 through 7, 2002. Prior to examination, fish were euthanized with quinaldine sulfate. Gonads were imaged using a Sonosite 180plus vet® portable ultrasonograph with a 38 mm 5 MHz linear transducer (L38 probe). This unit is suitable for field use due to it durability and portability. The transducer was placed on the left side of the fish above the 3rd and 4th ventral scutes anterior to the pelvic fins to produce an image in partial transverse section. This location provided reliable images with easily identifiable landmark organs (e.g., the posterior end of the swim bladder). Ultrasound

transmission jelly was used between the probe surface and the fish's skin to improve imaging. A consensus sex determination was obtained by four observers for each fish. Accuracy of ultrasound derived sex determinations were verified by visual examination of gonads. Dissected fish were assigned a stage of gonad development using the criteria developed by Bruch et al. (2001) for lake sturgeon *Acipenser fulvescens*. A digital picture and an ultrasound image of the gonads were captured for each fish. I used a Student's t-test (α =0.05) to compare differences in length between sexes.

RESULTS AND DISCUSSION

This portable ultrasound equipment produced easily captured high resolution images that enabled easy recognition of internal landmark organs and gonads. These images could be readily identified by any reader who had some prior knowledge of sturgeon gonad anatomy. Female shovelnose sturgeon were identified by having discernable ovarian folds (Figure 1 a, b) or by the presence of readily discernable oocytes (Figure 1 c, d). The testes of shovelnose sturgeon imaged as a smooth grey region that varied in lightness, depending on relative maturity, with more mature gonad images being lighter (Figure 1 e, f).

Overall, the accuracy of sex determination using ultrasound was 86% (n=51), with 96% of males (n = 25) and 80% of females (n = 25) being identified correctly (Table 1). Mean fork lengths of males (mean = 608.9; SD = 51.5) and females (mean = 624.1; SD = 59.4) did not differ (Student's t-test; p=0.149). We were able to identify immature and mature shovelnose sturgeon gonads of both female (stages Fv – FIV) and male (stages Mv – MII) individuals (Table 1). One individual identified as a male was grossly determined to be a hermaphrodite (710 mm fork length; 1420 g). The presence of a

hermaphrodite is not particularly surprising given that the frequency of hermaphroditic shovelnose sturgeon in the Middle Mississippi River was previously reported to be 3% (Carlson et al. 1985).

Although ultrasound was effective in determining the sex of both immature (Fv – FIII) and mature (FIV) female shovelnose sturgeon (Table 1), those that were post-spawn (i.e., having recrudescent ovaries) were difficult to discern from males (Figure 1g, h). Three of the five post-spawn females (FVI) examined were misidentified as males, primarily due to the overall lack of defined tissue structure (Table 1). This effect was also noted in *Gadus morhua* (Karlsen and Holm 1994) and in *Morone saxatilis* (Blythe et al.1994). Shovelnose sturgeon are believed to spawn in the late spring (Keenlyne 1997), accounting for the large number of post-spawn females in our fall sample. Since post-spawn females were the most difficult to identify, the accuracy of ultrasound for sexing shovelnose sturgeon would increase at times of the year when this stage of fish is less likely to be present (i.e., Spring).

Ultrasound imaging of shovelnose sturgeon gonads is a viable technique for sex determination. With further study, the technique could be refined such that morphological differences between stages of gonadal development, similar to those used for lake sturgeon *Acipenser fulvescens* (Bruch et al. 2001), could be detected, and then used to evaluate temporal patterns of gonadal development at the population level. Our results were similar to those presented by Moghim et al. (2002) for stellate sturgeon. Because of the similarity of gonadal structure in various species of Acipenseriformes (Dadswell 1979, Conte et al. 1988; Dettlaff et al. 1993; Van Eenennaam and Doroshov

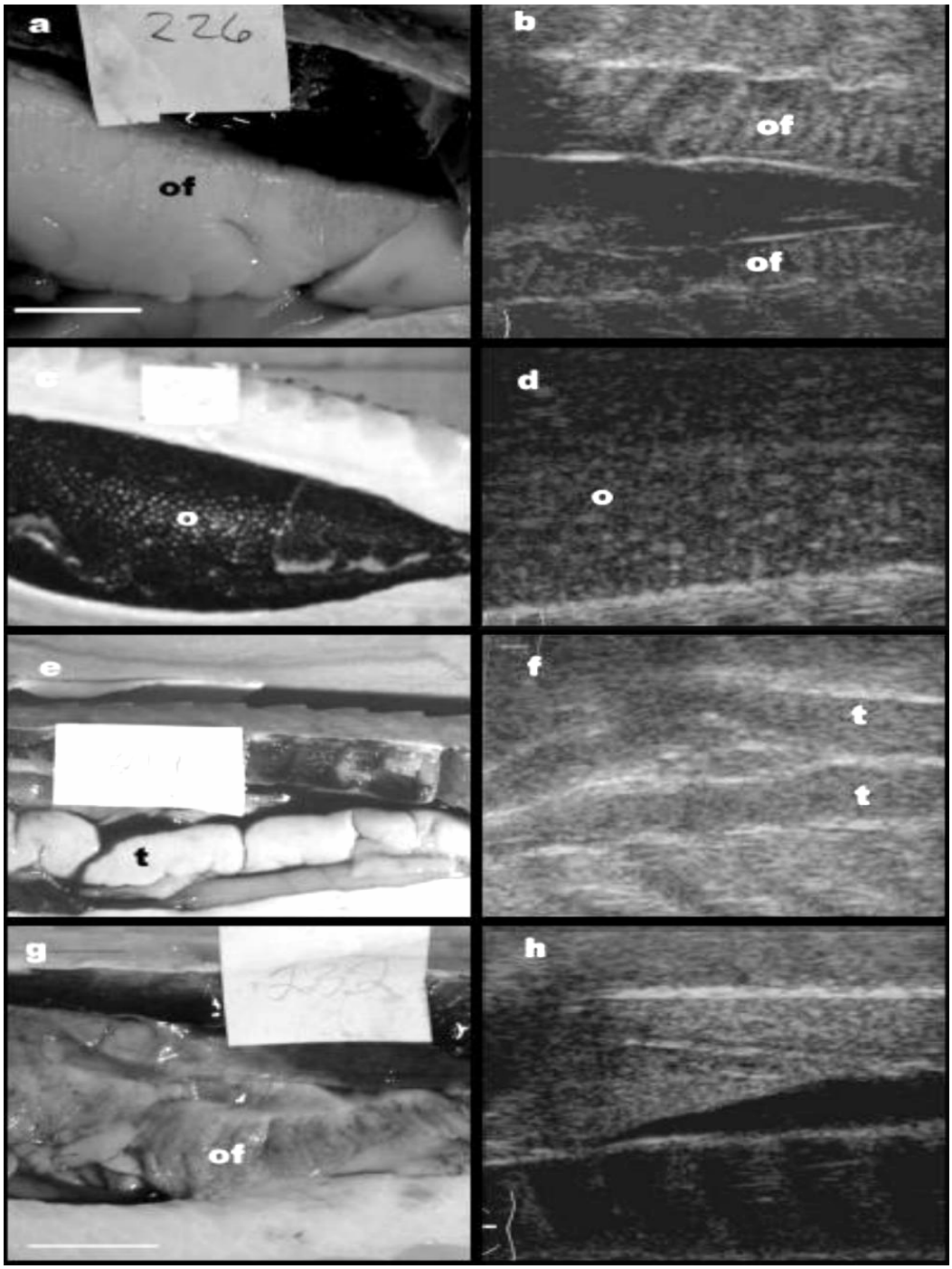
1998; Bruch et al. 2001), this noninvasive technique could be directly applied to endangered sturgeons such as the pallid sturgeon *S. albus*.

Table 1. Percent agreement of ultrasound sex determination by stage of gonadal development for the shovelnose sturgeon. Stages based on Bruch et al. (2001).

Sex	Stage	Description	Number correctly identified with ultrasound (n)	Total (n)	Percent agreement
Female	Fv	Virgin female - small ovarian folds	3	4	75
	FI	Ovarian folds	9	10	90
	FII	Small white oocytes	2	2	100
	FIII	Yellow eggs	1	1	100
	FIV	Black eggs	3	3	100
	FV	Spawning female	0	0	N/A
	FVI	Post-spawn - translucent ovary	2	5	40
	Total		20	25	80
Male	Mv	Virgin male - ribbon like testis in fat	1	1	100
	MI	Tubular testis in testicular fat	16	17	94
	MII	Large testis with small amount of fat	7	7	100
	Total		24	25	96
Total			44	51[a]	86

[a] Includes 1 hermaphroditic individual misidentified as a male

Figure 1. Ultrasound and dissected images of shovelnose sturgeon gonads sampled from the Middle Mississippi River during November 2003. (a) dissected undeveloped female; (b) ultrasound image of an undeveloped ovary, note ovarian folds; (c) dissected mature ovary, note black eggs; (d) ultrasound image of a mature ovary, note oocytes (~2 mm); (e) dissected mature testis, (f) ultrasound image of a mature testis; (g) dissected post-spawn ovary; and (h) ultrasound image of a post-spawn ovary (of – ovarian fold, t – testis, o – oocyte).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CHAPTER THREE

A GUIDE TO THE GONADAL DEVELOPMENT OF THE SHOVELNOSE STURGEON

ABSTRACT

The demand for domestically produced caviar has led to increased harvest of the shovelnose sturgeon. To determine whether these populations can withstand increased harvest, detailed information regarding sexual demographics is needed. I sexed 308 shovelnose sturgeon sampled in the Middle Mississippi River (River km 0 - 322) during September 2001 through December 2003 to develop a guide to reproductive development. Using dissection and histology, I identified three of the four stages developed for male lake sturgeon gonadal development in shovelnose sturgeon and all seven of the stages of female gonadal development. A total of seven intersexual fish were sampled coinciding with a 2% intersexuality rate, which is similar to other studies conducted on this population. Female fecundity was positively related to body weight. However, no relationship between fork length and fecundity occurred. The sex ratio of the shovelnose sturgeon in the MMR did not differ from one to one. This guide will allow for the rapid inspection of the gonad for sex and stage of development for the shovelnose sturgeon.

INTRODUCTION

The collapse of the historic caviar fisheries in the Volga River and Caspian Sea has increased demand for domestically produced caviar. However, reductions in the

stocks of sturgeon in the United States since the early 1900's have left few commercially viable populations. The only sturgeon species currently viable for commercial harvest in the US is the shovelnose sturgeon *Scaphirhynchus platorynchus*. This has led to increased harvest of the shovelnose sturgeon in the Mississippi River. This is exemplified by an increase in Illinois commercial harvest of shovelnose sturgeon caviar from 82.5 kg in 1997 (Williamson 1998) to 2388.6 kg in 2001 (Maher 2002). With increased pressure on this fishery, information on the life history, population demographics and sexual demographics of the shovelnose sturgeon is crucial to proper fisheries management.

The shovelnose sturgeon is small-bodied and matures early, likely allowing populations to sustain higher levels of harvest than most other chondrostean species (Morrow et al. 1998). In order to model the amount of harvest that is sustainable for maintaining population growth, several parameters are needed, including population size, age specific mortality and maturation, and fecundity.

Efforts are currently underway to determine the population size and mortality of shovelnose sturgeon in the Mississippi River (Jackson 2004). However, little information is currently available on the sexual demographics and fecundity of this species, which requires a strong understanding of gonadal development. This information coupled with fecundity data allows for determination of population-level reproductive potential (Begon et al. 1996). Because shovelnose sturgeon females are preferentially harvested for caviar production, proper management also requires information regarding the sex ratio of the population. Current research has suggested that the population of shovelnose sturgeon in the Middle Mississippi River (MMR) has a skewed sex ratio with 4 males for every 1 female (Jackson 2004). This information may be biased because all specimens were

obtained from a commercial egg fisherman at a time near spring spawning. Sturgeon species collected on a spawning run have been shown to have skewed sex ratios (Van Eenennaam et al. 1996). More reliable estimates using unbiased gear during a season when sturgeon are not staging to spawn is needed to determine the actual sex ratio currently at large in the MMR.

Another problem with the current information available for shovelnose sturgeon is that the gonads of immature female and male shovelnose sturgeon are difficult to distinguish with little information available to resolve this. Moos (1978) studied the reproductive development of the shovelnose sturgeon in the Missouri River using dissection and histology. However, his criteria differed from those used in other studies on sturgeon. For example, many of his stages were based on sampling time during the year rather than the standard structural characteristics used by other studies. He identified six stages of female development and five stages of male development. Carlson and Pflieger (1981) assigned stages of gonadal development to shovelnose sturgeon sampled in the Missouri River, using the color of the gonad with no accompanying histology. This approach does not provide insight into the development of either the spermatozoa or the oocytes in the gonads, which is critical to the understanding of reproductive development.

Both dissection and histology must be used when characterizing the stages of gonadal development for a species. This information can then be used to develop a guide for gonadal development (e.g., lake sturgeon; Bruch et al. 2001). For lake sturgeon, seven developmental stages of female development and four for male development were identified (Bruch et al. 2001) that were directly related to previous structural assignments

for other species of sturgeon (Dettlaff et al. 1985; Conte et al. 1988; Amiri et al. 1996a,b; Van Eenennaam et al. 1996). These stages provide an adequate framework to produce a guide that could be applied to the gonadal development of the shovelnose sturgeon.

The purpose of this study is to describe the stages of gonadal development for the shovelnose sturgeon and determine the reproductive demographics of the population. With this information, a guide to the development of the shovelnose sturgeon will be elucidated.

METHODS

During September 2001 through December 2003, I sampled shovelnose sturgeon seasonally (based on meteorological season), at various locations on the Middle Mississippi River (River km 0-322) to obtain a representative sample of all stages of gonadal development. Sturgeon sampled with gill nets set behind wing dikes were taken back to the Southern Illinois University, Fisheries and Illinois Aquaculture Center. At the lab, I euthanized sturgeon with a lethal dose of MS-222 (tricane methonsolfate). Sturgeon were measured to the nearest millimeter fork length, weighed to the nearest gram and then dissected for gonadal evaluation. Stages of gonadal development were determined visually and a digital image was captured of the gonad. Females were assigned to 1 of 7 stages and males to 1 of 4 stages using the criteria developed for lake sturgeon (Table 2) (Bruch et al. 2001). The gonad and gonadal fat were removed and weighed for determination of the gonadal somatic index (GSI), which was calculated as the gonad and fat divided by the total weight multiplied by 100.

The gonads of a subsample of each stage were fixed in 10% neutral buffered formalin. Fixed gonads of different stages were brought to Southern Illinois University

histology lab for histological analysis. Sections through the middle of the gonad were processed using standard histological techniques and stained with hematoxylin and counterstained with eosin. I viewed histological samples under a compound microscope (40x – 630x) with a top mount digital camera and digital images of the gonad were captured.

While both dissection and histology provide a guide to the stages of development of the shovelnose sturgeon, these methods require the animal to be destroyed. In an effort to provide a nonlethal technique I compared the images captured during ultrasound sexing (see chapter 2) with the stages determined by dissection to provide an illustration of the stages of shovelnose sturgeon reproductive development. Additionally, since other authors have staged shovelnose sturgeon gonads results from this study were compared with those of Moos (1978) and Carlson and Pflieger (1985) (Table 3). This will provide a complete guide to the development of the shovelnose sturgeon.

I quantified fecundity for mature females (FIV) by removing five, 1 g samples from each ovary and counting the eggs in this subsample. A mean number was taken for each ovary and these two means were used to calculate number of eggs per ovary and per gram of fish (Keenlyne et al. 1992). To determine if fecundity was related to body size total number of eggs for both ovaries was regressed against weight and fork length of the fish.

Statistical Analysis

To determine if the ratio of males to females was different from one to one analysis of proportions was used. In addition to sex ratio it is important to determine if

the length frequency is similar between sexes. Differences in length-frequency histograms were analyzed using a Kolomogorov-Smirnov test. Differences in the mean GSI among stages was analyzed using one way ANOVA and Tukey's HSD test to control for experiment wise error rate.

RESULTS

Sex ratios

The sex of 308 shovelnose sturgeon was determined by gross examination of their gonads. Of these, 150 were identified as males and 149 were identified as females with the remainder being indeterminate or intersexual. This was not different than one to one ($X^2 = 0.000$, $P = 1.0$) Length frequency distributions did not differ between sexes (ks = 0.0859, $P = 0.5746$) (Figure 2).

Male Development

Virgin males (Mv) were identified as having a small ribbon like testis embedded in a relatively small amount of testicular fat. Mean length of Mv was 453 ± 8 mm (n = 19) and ranged from 369 mm to 500 mm, suggesting that male shovelnose sturgeon do not become reproductive until they reach approximately 500 mm fork length. Proportion of Mv males was highest in the summer and lowest in the winter (Figure 3). Mean GSI of Mv males (0.83%) was lower than the other stages of male development (ANOVA, $P < 0.05$) (Figure 4). The testes of Mv males were pink in color with white to yellow testicular fat (Figure 5a). Ultrasound images of the Mv males exhibited a small testicular material that was light in color (Figure 5b). Histologic evaluation of stage Mv male testis displayed seminiferous tubules devoid of mature spermatozoa (Figure 5c). At 250x

magnification, spermatogonia can be seen embedded within the seminiferous tubules (Figure 5d).

Males of stage one (MI) were similar in appearance to males of stage Mv with the chief difference being the amount of testicular fat. This is exemplified by the difference in mean GSI between stage Mv and MI males with MI males being significantly greater (ANOVA, $P < 0.05$) (Figure 4). Stage MI males comprised 42% (n = 57) of the sample and this proportion was similar among all seasons (Figure 3). Mean size of stage MI males was 593 ± 7 mm and ranged between 493 and 715 mm. The testes of stage MI males were yellow in color and larger with more testicular fat than Mv males (Figure 6a). Individuals of this stage were easily sexed using ultrasound with 100% (n = 8) being correctly identified as males. The testes of stage one males appeared as smooth light gray structures in the ultrasound images (Figure 6b). Histologic analysis of MI males appeared similar to the virgin males. The seminiferous tubules are devoid of developed sperm (Figure 6c) with numerous spermatogonia dispersed in a homogenous fashion (Figure 6d).

Males of stage two (MII) are fully developed and will release sperm when stripped during the spawning season. Stage MII males comprised 44% (n = 60) of the sample, and were present in all seasons (Figure 3). Mean size of MII shovelnose was 614 ± 6 mm and ranged from 499 to 719 mm. The chief difference between MII and MI males is the amount of testicular fat. The mean GSI of MII males was greater than that of stage Mv and MI (ANOVA, $P < 0.05$) (Figure 4) males due to an increase in the size of the testis from MI to MII. I found MII males to have reduced amount of testicular fat present surrounding the testis (Figure 7a). The testes of dissected MII males were white

in appearance with a pink undertone (Figure 7a). Ultrasound imaging was effective in identifying the sex of stage MII males with 100% being correctly identified as males. The testes of MII males appeared as smooth dark gray structures in the ultrasound images (Figure 7b). Histological analysis of this stage displayed the presence of mature spermatozoa packed into the seminiferous tubules (Figure 7c). The spermatozoon of the shovelnose sturgeon is similar to that of other sturgeon having an elongated head (Figure 7d).

Female Development

Virgin females (Fv) had a mean length of 508 ± 8 mm fork length (range 400 – 600 mm) and comprised 18% (n = 25) of the population of female shovelnose sturgeon (Figure 8). Mean GSI of stage Fv shovelnose sturgeon was low due to a low amount of ovarian fat (Figure 9). Virgin female shovelnose sturgeon were grossly characterized as having a yellow gonad with orderly, well-formed ovarian folds and no atretic oocytes from previous spawnings (Figure 10a). Additionally, the ovaries of these females did not have grossly discernable oocytes. In general, Fv females were difficult to distinguish from males using ultrasound due to the small size of the ovary. However, I was able to identify ovarian folds in 3 of 4 virgin females (Figure 10b). Histologically, Fv female ovaries contained nests of pre-meiotic oogonia that stained lightly acidophilic (Figure 10d). Some post-meiosis I or primary oocytes could be seen in the follicular epithelium of virgin female. These early oocytes contained a large central nucleus (germinal vesicle) and stained strongly basophilic (Figure 10c).

Stage one (FI) females comprised 40% (n = 61) of all the female sturgeon sexed (Figure 8). Stage FI females had a mean length of 604 ± 7 mm and ranged between 511 and 734 mm. Mean GSI remained low in the FI sturgeon but was variable due to large differences in the amount of ovarian fat (Figure 9). Ovaries of FI females were similar in color to the ovaries of the virgin females. The differences arose in the presence of a large amount of ovarian fat, presence of atretic oocytes, and ragged structure of the ovarian folds (Figure 11a). Oocytes were not discernable in FI females. These fish were easily identified as females using ultrasound imaging due to prominent ovarian folds (Figure 11b).

Histologically the ovaries of FI females contained many small oocytes that stained strongly basophilic (Figure 11c). These primary oocytes had migrated to the walls of the ovigerous lamellae. The nuclei (germinal vesicle) of these previtellogenic oocytes were centrally located and contained numerous provitelline nucleoli (Figure 11d). The oogonial nests present in Fv females were no longer present as meiosis I and formation of the primary oocytes was complete (Figure 11c).

Stage two (FII) females comprised 16% (n = 24) of the female population of shovelnose sturgeon (Figure 8). Both the mean length (608 ± 8 mm) and range (535-695 mm) were similar to FI females. Stage FII females differed grossly from FI females due to visually identifiable oocytes. Also, there was a large increase in mean GSI from stage FI to FII (Figure 9). Clear to yellow oocytes were seen distributed throughout the ovary of an FII female (Figure 12a). The ovaries of FII females were characterized by a large amount of ovarian fat with small un-pigmented oocytes giving the ovary a salt and pepper like appearance. Although, ultrasound was efficient at distinguishing the females of stage

FII from males due to the clearly identifiable ovarian mass, oocytes were not clearly identifiable in the ultrasound image, making distinction between FI and FII Females difficult (Figure 12b).

Histological examination revealed the beginning of yolk formation (vitellogeneses) in FII females. This was evident by the appearance of two distinct zones of yolk formation in the cytoplasm (Figure 12c). The lightly acidophilic internal zone contained large yolk vacuoles (macroplatelets) (Figure 12c) and the strongly acidophilic external zone appeared smooth microscopically with small yolk vacuoles (Figure 12d). The germinal vesicles of these oocytes contained abundant euvitelline nucleoli that stained strongly basophilic (Figure 12d). The cell wall showed the primordium of the zona radiata as a slightly acidophilic band inside the follicular envelope (Figure 12e).

The yellow egg female (Stage FIII) was relatively rare in my sample accounting for only 7.5% (n = 11) of the females in the population (Figure 8). The mean length of FIII females was 606 ± 17 mm and ranged between 555 and 735 mm. The amount of ovarian fat was generally lower than that of the FII females. However, the mean GSI was relatively high (Figure 9).

The ovary of FIII females was easily identified grossly by the presence of large yellow and light green oocytes (Figure 13a). The FIII females occurred in each season of the year with the proportion of occurrence similar throughout the year (Figure 8). The ovaries of the FIII females were easily identified by ultrasound imagery (Figure 13b). Although, the ovarian folds of were not distinguishable the relatively large oocytes were distinguishable.

Histological examination of the ovaries of FIII females showed the presence of large oocytes with a large central nucleus (Figure 13c), the cellular matrix was composed of both micro and macroplatelets distributed in a two layer fashion. The large macroplatelets were distributed adjacent to the nucleus and the microplatelets form a region close to the cell membrane (Figure 13d). The euvitelline nucleoli now stained slightly acidophilic (Figure 13d). The cell membrane of the oocyte had become more complex with the presence of two distinct zona radiata that stained acidophilic (Figure 13e). There were many striations apparent in the zona radiata giving it a columnar like appearance (Figure 13e).

Black egg stage FIV females comprised 12.8% (n = 19) of the population of females and were present in all seasons except the summer suggesting that shovelnose sturgeon spawn during spring (Figure 8). Mean length of FIV females was 655 ± 9 mm and ranged between from 569 to 713 mm. Grossly, the ovaries of a FIV female were unmistakable due to the presence of large black oocytes (Figure 14a). The mean GSI of 18.9 for FIV females was greater than that of any other stage (Figure 9). Oocytes of the FIV females were easily identified using images captured with ultrasound (Figure 14b).

Melanophores were present inside the cell membrane of the oocytes of the FIV female (Figure 14e) giving the oocytes their characteristic black color. The germinal vesicle and the microplatelets had migrated into the animal hemisphere of the oocytes (Figure 14c). The macroplatelets coalesced in the vegetal hemisphere (Figure 14d). The cell membrane had segregated into three distinct zona radiata (Figure 14e). All three layers of the zona radiata contained striations and stained lightly acidophilic (Figure 14e).

Only one spawning female was obtained during the study. This individual was caught by a commercial fisherman at a water temperature of 19°C. The spawning female had freely expressed oocytes when the abdomen was stroked using the Bruch Stroke (Bruch et al. 2001). Because the female was in the possession of the commercial fisherman no additional data was available.

Spent females (FVI) comprised 5% (n = 8) of the total catch of females (Figure 8). The mean length of FVI females was 638 ± 11 mm and ranged between 597 and 705 mm. The FVI females were present during three of the four seasons, and no spent females were caught in the summer. This was due to the low catch rates of shovelnose sturgeon during summer of the year. Mean GSI of FIV females was similar to that of Fv females (Figure 9). The gross appearance of ovaries of spent females varied. Most were pink to translucent in color with few atretic follicles (n = 5) (Figure 15a), while others had many atretic oocytes suggesting follicular atresia (n = 3) (Figure 15b). Ultrasound imaging did not prove to be an effective method for distinguishing spent females from males with 75% (N = 4) being identified as males (Figure 15c). This is due to the general lack of structure of the ovary of a stage FVI female.

Histological examination of the stage FVI females revealed the presence of both atretic oocytes and the developing oogonia of the next clutch of eggs (Figure 15d). At high magnification, atretic oocytes stained lightly acidophilic, and were identified as having an irregular shape with degradation of the cellular wall (Figure 15e). The developing primary oocytes for the next clutch of eggs were similar to oocytes of stage FI (Figure 15d).

I was able to compare the gonadal designation asserted by other authors with those that were outlined here. The staging designation used here agreed with those used by Carlson and Pflieger (1985) and Moos (1978) with few exceptions (Table 3). Those exceptions arose from differences in the methods for stage designation.

Fecundity

The mean fecundity of FIV female shovelnose sturgeon was 30767 ± 2143 eggs (n = 13), or 23.6 ± 1.26 eggs per gram of fish weight. The fecundity of females was positively related to the weight of the female with (number of eggs = 30.24 x body weight – 8392, $P = 0.013$, $r^2 = 0.4466$) (Figure 16). However, there was no relationship between fork length of the female and fecundity (number of eggs = 146.37 x fork length – 66176, $P = 0.053$, $r^2 = 0.2337$) (Figure 17).

Intersexuality

A total of seven intersexual sturgeon were sampled during this study. Because all of the identified intersexual fish were predominantly male some obscure hermaphrodites may have been missed (Figure 18a). The intersex fish I identified all had patches of oocytes embedded in the testicular tissue (Figure 18a). Only one intersexual fish was scanned with ultrasound and it was misidentified as a male with the only structure evident being the testis (Figure 18b). Histological analysis of these fish confirmed the presence of both male and female tissues (Figure 18c). The male tissue had reduced reproductive output with most of the seminiferous tubules being devoid of spermatozoa (Figure 18d).

The only visible oocytes were primary oocytes similar to those of a FI female (Figure 18c).

DISCUSSION

Male development followed the patterns of development seen in other species of sturgeon. I identified three stages of male development coinciding with those identified for lake sturgeon (Bruch et al. 2001), the white sturgeon (Conte et al. 1988) and the Russian sturgeon (Dettlaff et al. 1993). One stage of males not present in my sample that was described for other species of sturgeon was the spent males. Spent males were also rarely seen in the lake sturgeon due to the relatively fast turnaround of testicular material (Bruch et al 2001). The authors found one spent males by chance after examining over 1000 specimens. In the MMR, all three stages of development were identified in all seasons with running ripe male stage MII individuals being only found in the spring.

In the testes the spermatozoa develop similarly to other species of fishes (Groman 1982; Grizzle and Rogers 1976; Yasutake and Wales 1983). The development of the spermatozoa begins as premeiotic undifferentiated spermatogonia embedded in the seminiferous tubules. After completion of meiosis, the sturgeon becomes a stage MII male with its seminiferous tubules packed with fully developed spermatozoa. The chief difference in sperm development of the shovelnose sturgeon compared to the teleosts is the presence of an enzymatic acrosome at the apex of the spermatozoa cap. This acrosome is present in the other species of acipenscerids, however, is not in most other groups of fishes (Dettlaff and Vassetzky 1991).

The female shovelnose sturgeon, like the males, developed similarly to other sturgeon species. I identified six of the seven stages of female sturgeon gonadal

development described for lake sturgeon (Bruch et al. 2001). Using those stages identified for lake sturgeon I was able to fit designations of shovelnose sturgeon gonadal

Although the structure of the ovary of the acipenserids is different from teleosts, the development of the oocytes is similar (Grizzle and Rogers1976; Groman1982; Yasutake and Wales 1983). In the ovary, the development of female germ cells begins with mitosis of oogonia in oogonial nests. On completion of meiosis I oogonia become primary oocytes. As maturation in the gonad continues, the primary oocytes become larger and migrate to the walls of the ovigerous lamella. The primary oocytes are similar in appearance to the primary oocytes of other fishes. As the size of the oocytes increases the oocytes undergo vitellogeneses, leading to the deposition of fat vacuoles inside the cytoplasm. After completion of vittelogenesis the oocyte is now considered a secondary oocyte. The yolk deposition in the sturgeon differs from other fish species in that the large yolk vacuoles form a ring adjacent to the germinal vesicles. These fat vacuoles eventually coalesce in the vegetal hemisphere of the mature oocytes. In fishes such as the striped bass *Morone saxatilis*, the deposition of fat occurs in regions near the cell membrane (Groman 1982). The unequal distribution of yolk in the shovelnose sturgeon oocytes is a characteristic of the acipenserids (Conte et al 1988; Dettlaff and Vassetzky 1991; Dettlaff et al. 1993, Bruch et al. 2001). The final stage of maturation in the secondary oocytes involves the laying down of the pigment melanin adjacent to the zona radiata giving the oocyte its characteristic black color. The germinal vesicle then migrates into the animal hemisphere. Because the oocytes and spermatozoa develop similarly to other species of sturgeon, we can use conventional staging guidelines for this species.

Length at maturity differed for male and female shovelnose sturgeon, with males becoming mature at 500 mm and females at approximately 570 mm. However, there was considerable overlap in length between FI and Fv females attributable to differing reproductive cycles among individuals. Once length at maturity is reached, neither the male nor female shovelnose sturgeon appear to spawn every year. This is supported by the presence of large stage MI (605 mm) males and stage FI (629 mm) females present in the spring. Protracted spawning is common among the fishes in the order Chondrostei, with some species requiring as much as 7 years to complete a cycle of development (Bruch et al. 2001).

Although the duration of each stage can't be surmised from the design of this study, the female shovelnose sturgeon seem to require 3 to 4 years to complete the cycle from spent (FVI) to black egg (FIV). This is corroborated by the length of time that females remain in the spent condition. I found a number of females with recrudescent ovaries in both the fall and winter seasons suggesting that they do not become stage FI until the spring following spawning. Additionally, stage FI females greater than 700 mm were found in the fall, summer, and winter which imply that females may require up to one year to pass through stage FI. The stage FII females were sampled in three of the four seasons with none being caught in the spring, signifying that stage FI females become FII in the summer and remain FII until the following spring at which time they become FIII (yellow egg females). Females of stage FIII were found in all seasons, implying a year to develop into FIV. The stage FIII females become FIV during the second fall. These black egg females are the most desirable to commercial fishers because they are the caviar producers that will spawn during the following year.

Numerous stage FVI shovelnose sturgeon were found undergoing follicular atresia. This is not surprising because follicular atresia has been found to occur in other species of sturgeon (Conte et al. 1988). Since there is a large energetic cost for developing oocytes in some circumstances a female may reabsorb her eggs instead of releasing them. Male shovelnose sturgeon seem to be on a protracted spawning schedule as well. This can be surmised by the presence of large stage MI males in the spring. However, no time between stages can be determined to because there were only three stages of male development.

The images produced using ultrasound seem to have utility in the staging of shovelnose sturgeon gonads. Although we have not examined the fidelity of this equipment for staging, these preliminary results are promising. The only stage at which ultrasound was not effective was FVI. However, with further study this may be resolved by identifying those structures that may be indicative of the stage.

I identified a total of seven intersex fish in my sample, resulting in a 2% intersexuality rate in the population of shovelnose sturgeon in the middle Mississippi river. This amount of intersexuality is consistent with the rate of 3% found by Carlson et al. (1985) for shovelnose sturgeon and 1% for Atlantic sturgeon found by Van Eenennaam et al. (1996). Research is currently underway to determine if the intersexuality can be attributed to increased estrogen mimicking chemicals found in the Middle Mississippi River (B. Koch, Southern Illinois University, *personal communication*).

This guide presented here provides a guide that allows for the comparison of reproductive development between sturgeon species. This was lacking in the previous

studies. This guide also used both dissection and histology, providing better insight into the development of oocytes and spermatocytes in the gonadal tissue which was lacking in the staging designation developed by Carlson and Pflieger (1985). Furthermore, contrary to Moos' (1978) study, season was not used as an indicator, allowing the structural attributes of the gonad to be the sole determinant of stage thereby reducing the bias of the inspector. I have also provided a complete guide by incorporating ultrasound images of each stage, which has been lacking from every other study of sturgeon gonadal development. This provides managers with a quick and reliable method to assess the reproductive demographics of populations of shovelnose sturgeon.

With increased demand on our native sturgeon stocks, maintaining adequate natural female stock sizes is vital to maintaining population size. With a one to one sex ratio and equivalent length frequency distributions, it appears that the commercial fishery for shovelnose sturgeon is not affecting the sexual demographics of shovelnose sturgeon in the MMR at this time. Maintaining this ratio is important to successful management of this species. Additionally, since the shovelnose sturgeon displays protracted spawning it is important to maintain a proportion of mature females that can maintain population levels. The percentage of mature females (~12%) and fecundity data I presented coupled with population demographics will allow for the modeling of the population of shovelnose sturgeon in the Middle Mississippi River allowing managers to determine optimum sustainable yields for this species.

Table 2. Stages of gonadal development of the Lake Sturgeon (Bruch et al. 2001)

Sex	Stage	Description
Male	Mv	Virgin male; ribbon like testis embedded in testicular fat
	MI	Tubular testis in fat
	MII	Large testis in reduced amount of fat
	MIII	Spent male
Female	Fv	Virgin female, small well ordered ovarian folds
	FI	Ovarian folds
	FII	Small white to yellow oocytes
	FIII	Yellow eggs
	FIV	Black eggs
	FV	Spawning female
	FVI	Spent female, translucent ovary with atretic oocytes

Table 3. Comparison of stages of gonadal development for the shovelnose sturgeon.

Description	This Study	Carlson and Pflieger 1985	Moos 1978
Small pink testis with small amount of ovarian fat	Mv	N/A	I
Yellow testis large amount of testicular fat	MI	1	II and III
Large pink testis	MII	2 and 3	IV and V
Well ordered ovarian folds small amount of ovarian fat	Fv	N/A	I
Ovarian folds large amount of ovarian fat	FI	1	II
Clear to yellow small oocytes	FII	2	III
Yellow and light green oocytes	FIII	3	III
Large black oocytes	FIV	4 and 5	IV
Spawning female	FV	5	V
Translucent ovary	FVI	6	VI

Figure 2. Length-frequency histogram of (a) female and (b) male shovelnose sturgeon sampled in the Middle Mississippi River (River km 0 - 322) during September 2001 through December 2003.

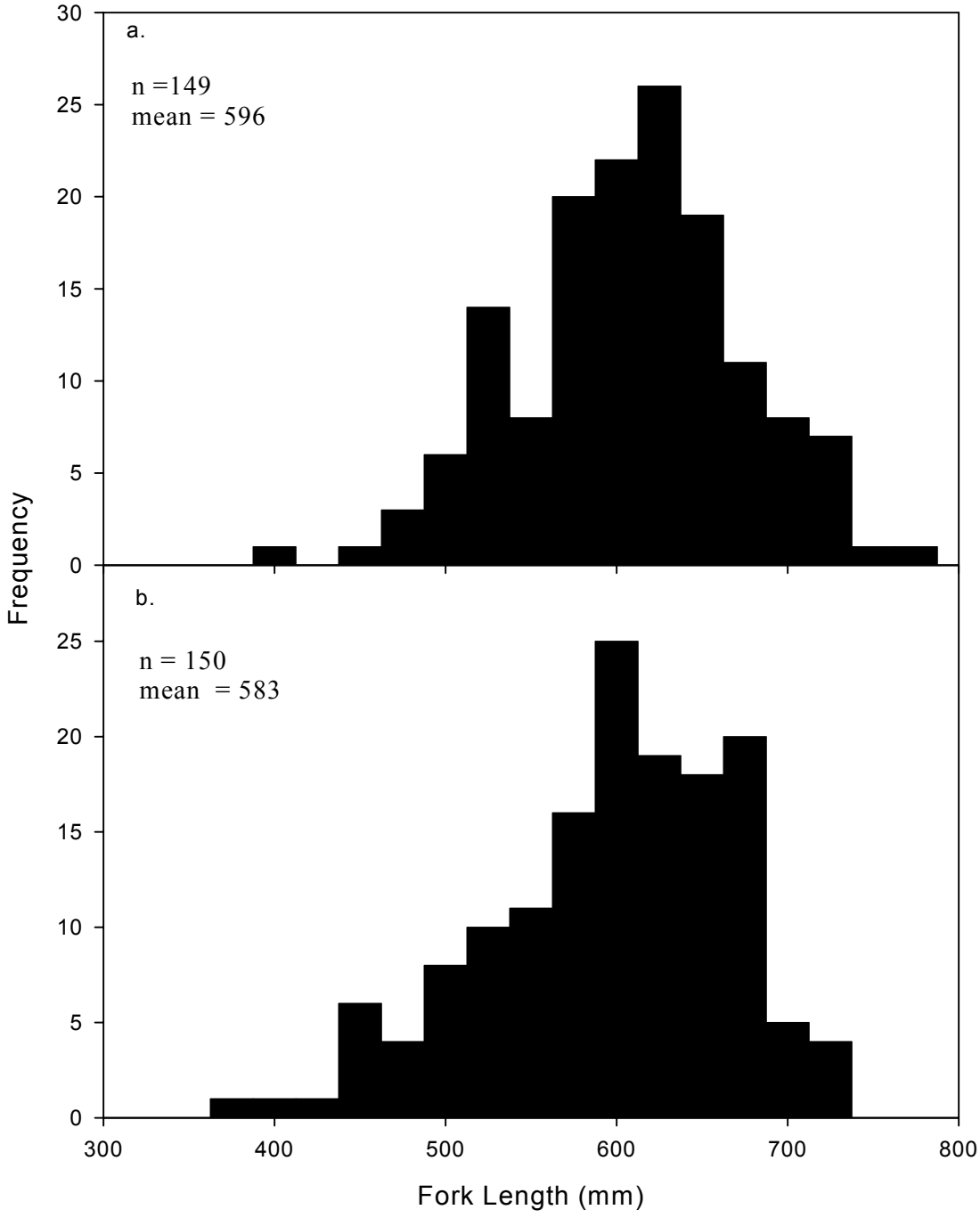

a.
n =149
mean = 596
b.
n = 150
mean = 583
Frequency
Fork Length (mm)
0
5
10
15
20
25
30
300
400
500
600
700
800

Figure 3. Proportion of occurrence of stages of male shovelnose sturgeon development by season sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003.

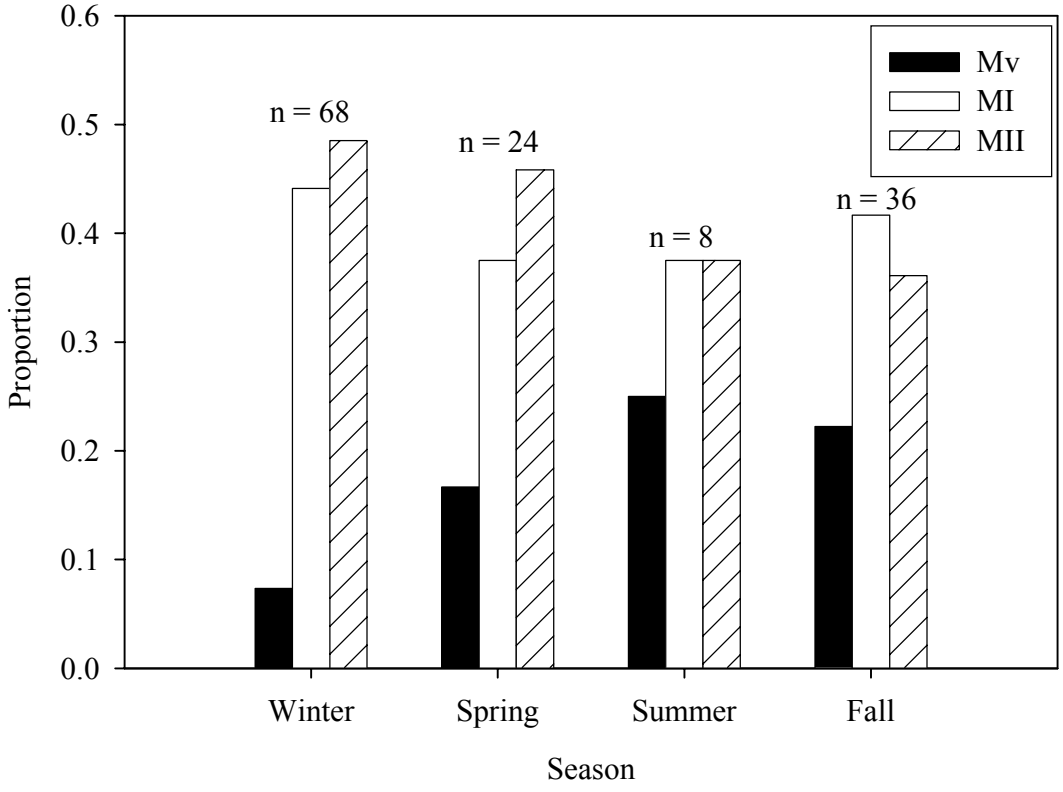

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion
n = 68
n = 24
n = 8
n = 36
Mv
MI
MII
Winter
Spring
Summer
Fall
Season

Figure 4. Mean gonadal somatic index (± SE) by stage for male shovelnose sturgeon sampled in the Middle Mississippi River (River km 0 - 322) during September 2001 through December 2003. (Different letters represent significantly different means, $P < 0.05$).

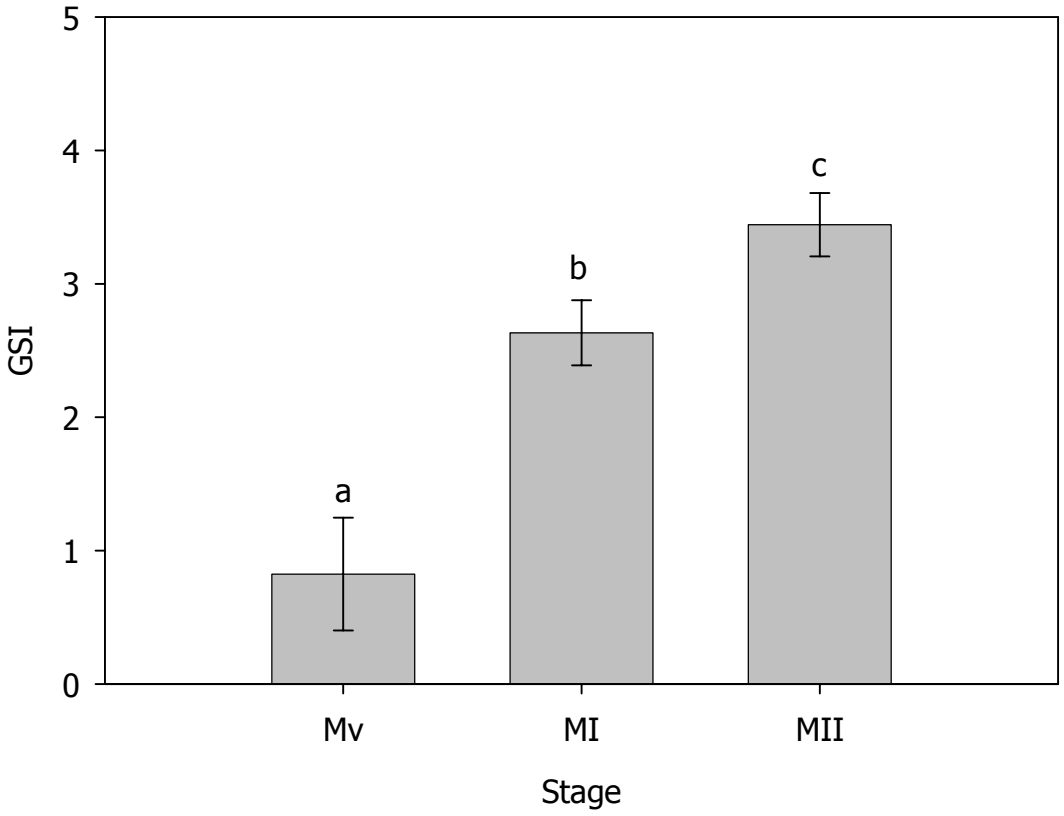

5
4
3
2
1
0
GSI
a
b
c
Mv
MI
MII
Stage

Figure 5. Example images of male shovelnose sturgeon stage Mv sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound image c. 100x magnification (scale bar = 200 um) and d. 250x magnification (scale bar = 80 um). sg – spermatogonia, st – seminiferous tubules, t – testis, and tf – testicular fat.

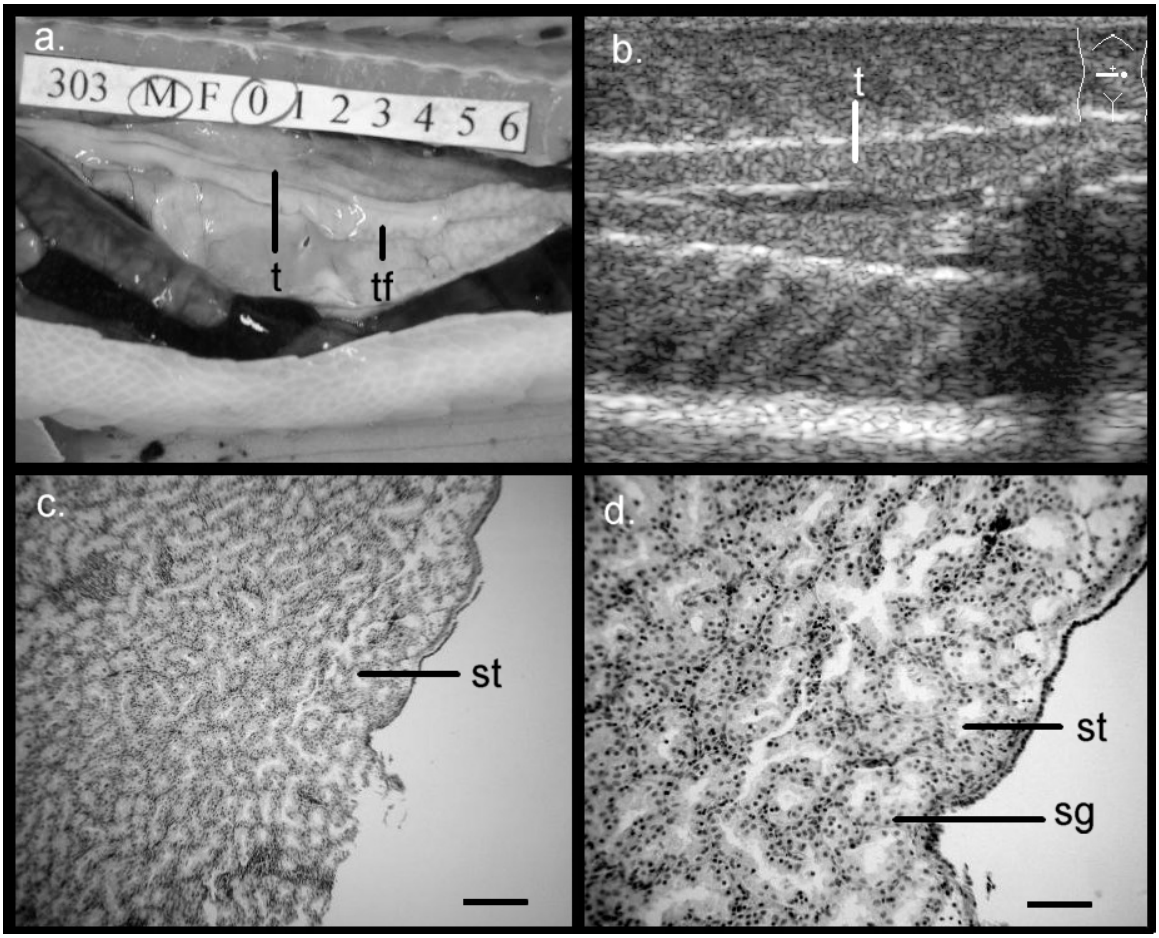

a.
303 M F 0 1 2 3 4 5 6
t
tf
b.
t
c.
st
d.
st
sg

Figure 6. Example images of male stage MI shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound image c. 100x magnification (scale bar = 200 um) and d. 250x magnification (scale bar = 80 um). sg – spermatogonia, st – seminiferous tubules, t – testis, and tf – testicular fat.

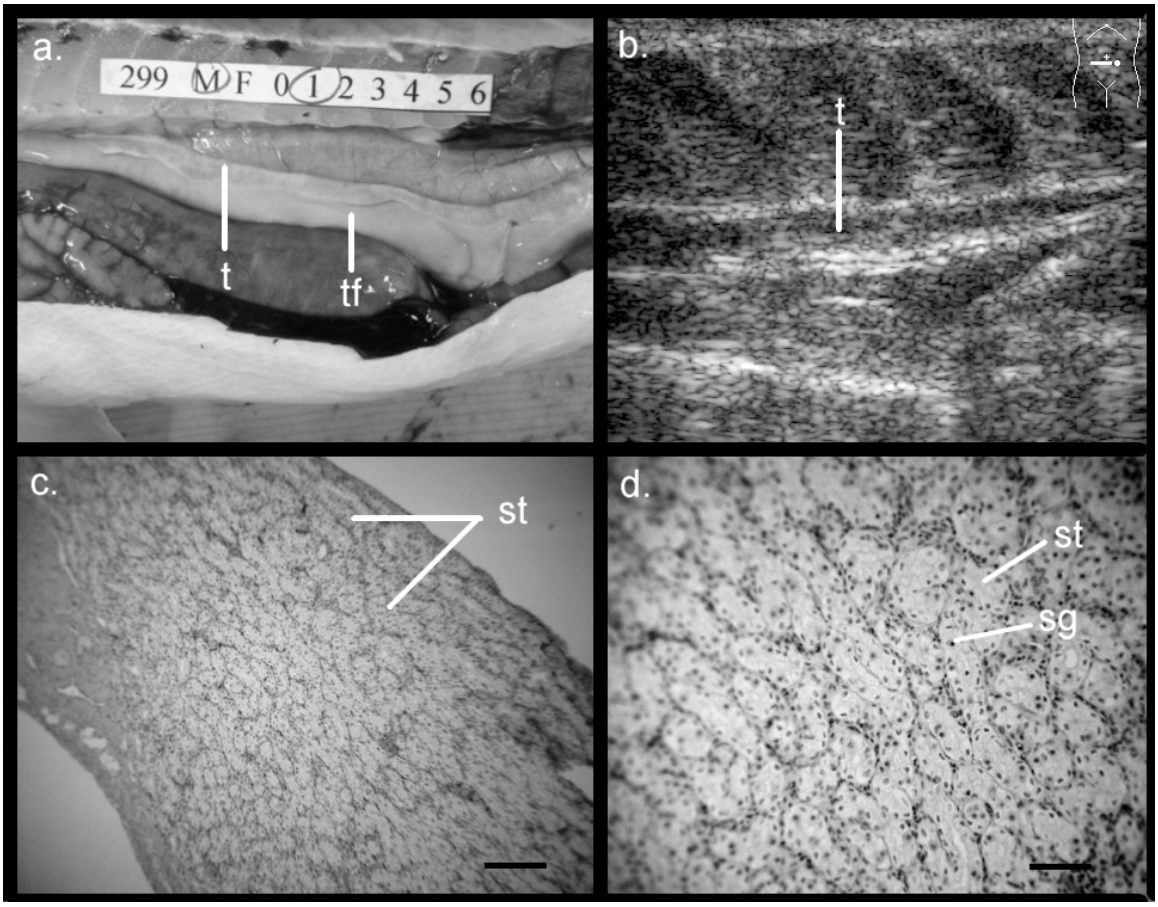

a.
299 M F 0 1 2 3 4 5 6
t
tf
b.
t
c.
st
d.
st
sg

Figure 7. Example images of male stage MII shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound image c. 100x magnification (scale bar = 200 um) and d. 250x magnification (scale bar = 80 um). sz – spermatozoa, st – seminiferous tubules, t – testis, and tf – testicular fat.

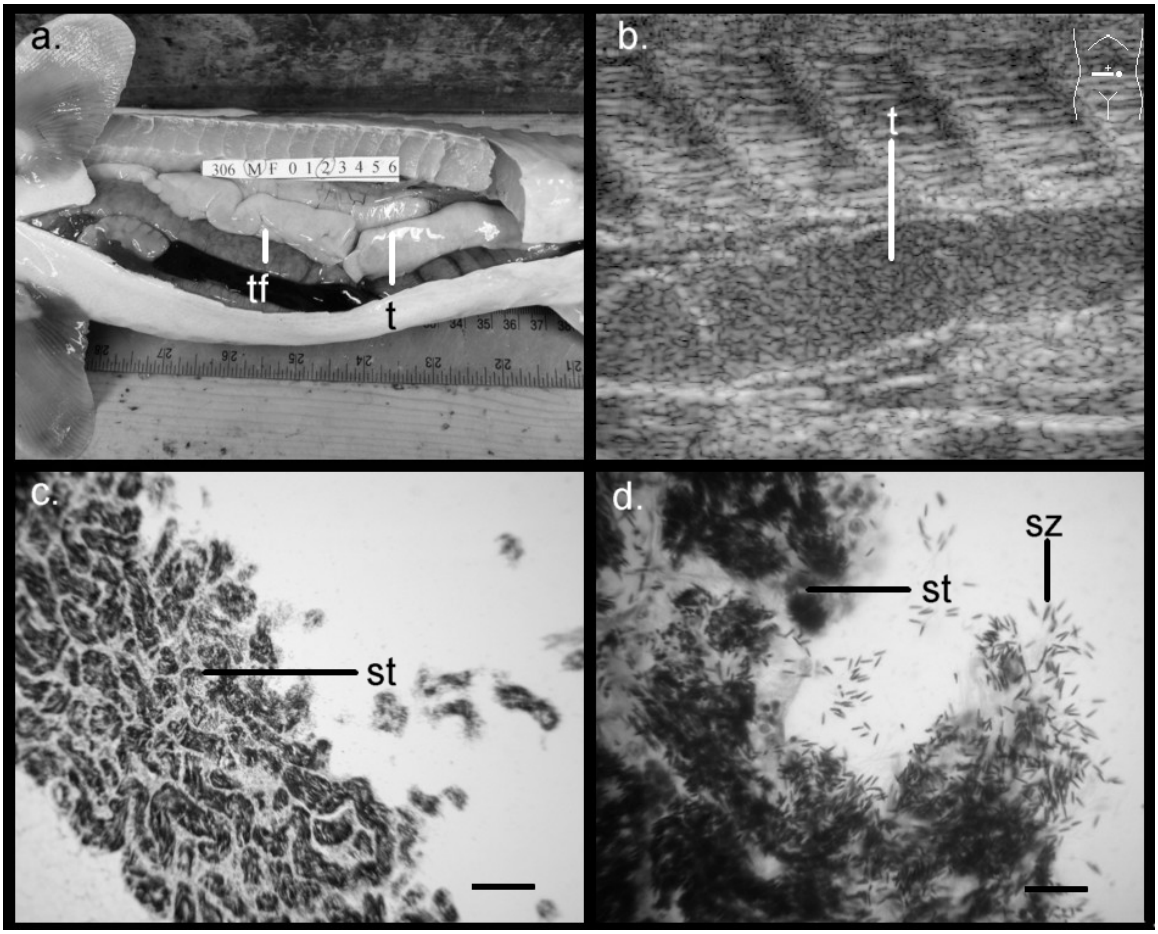

a.
306 M F 0 1 2 3 4 5 6
tf
t
b.
t
c.
st
d.
sz
st

Figure 8. Proportion of occurrence of stages of female shovelnose sturgeon development by season sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003.

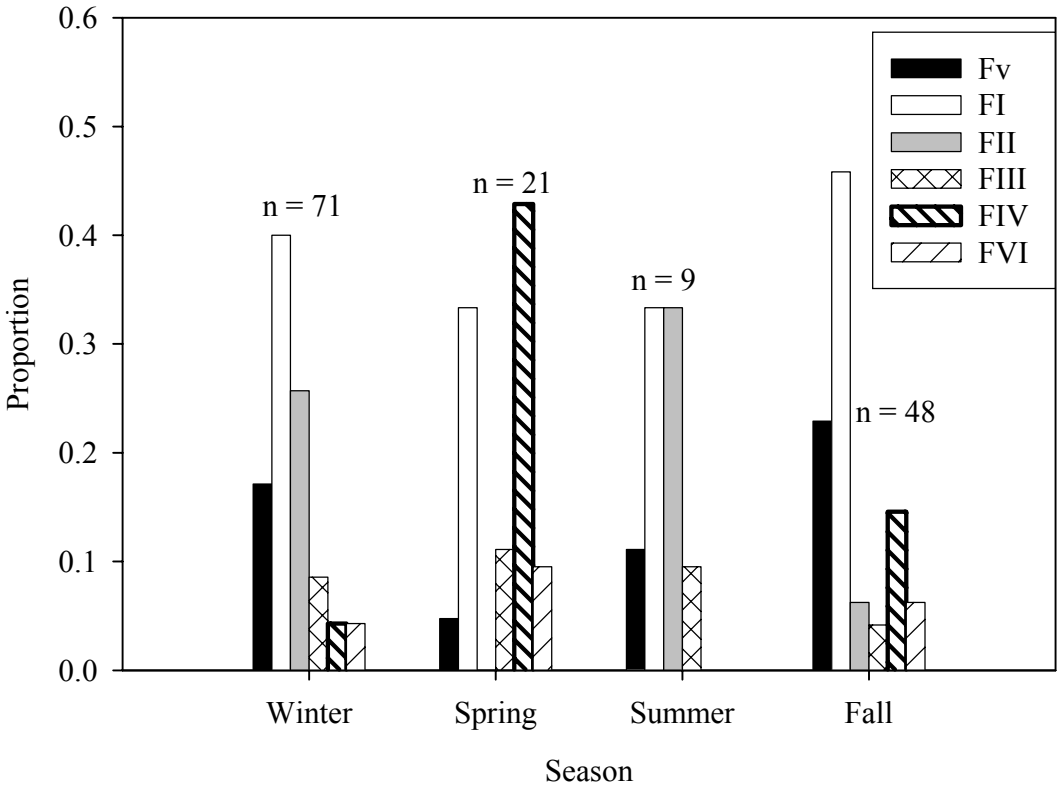

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion
n = 71
n = 21
n = 9
n = 48
Fv
FI
FII
FIII
FIV
FVI
Winter
Spring
Summer
Fall
Season

Figure 9. Mean gonadal somatic index (±SE) by stage for female shovelnose sturgeon sampled in the Middle Mississippi River (River km 0 - 322) during September 2001 through December 2003. (Different letters represent significantly different means, $P < 0.05$).

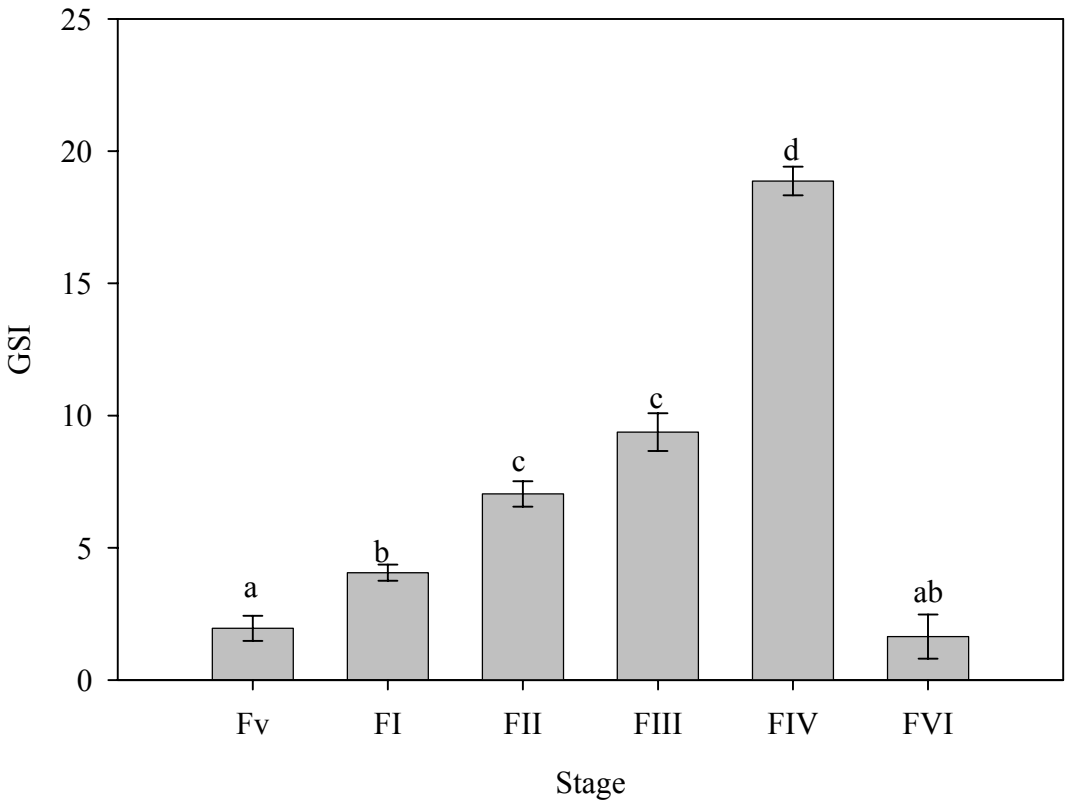

25
20
15
10
5
0
GSI
a
b
c
c
d
ab
Fv
FI
FII
FIII
FIV
FVI
Stage

Figure 10. Example images of female shovelnose sturgeon stage Fv sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 100x magnification (scale bar = 200 um) and d. 250x magnification (scale bar = 80 um) with oc –oocyte, of – ovarian folds, and og – oogonia

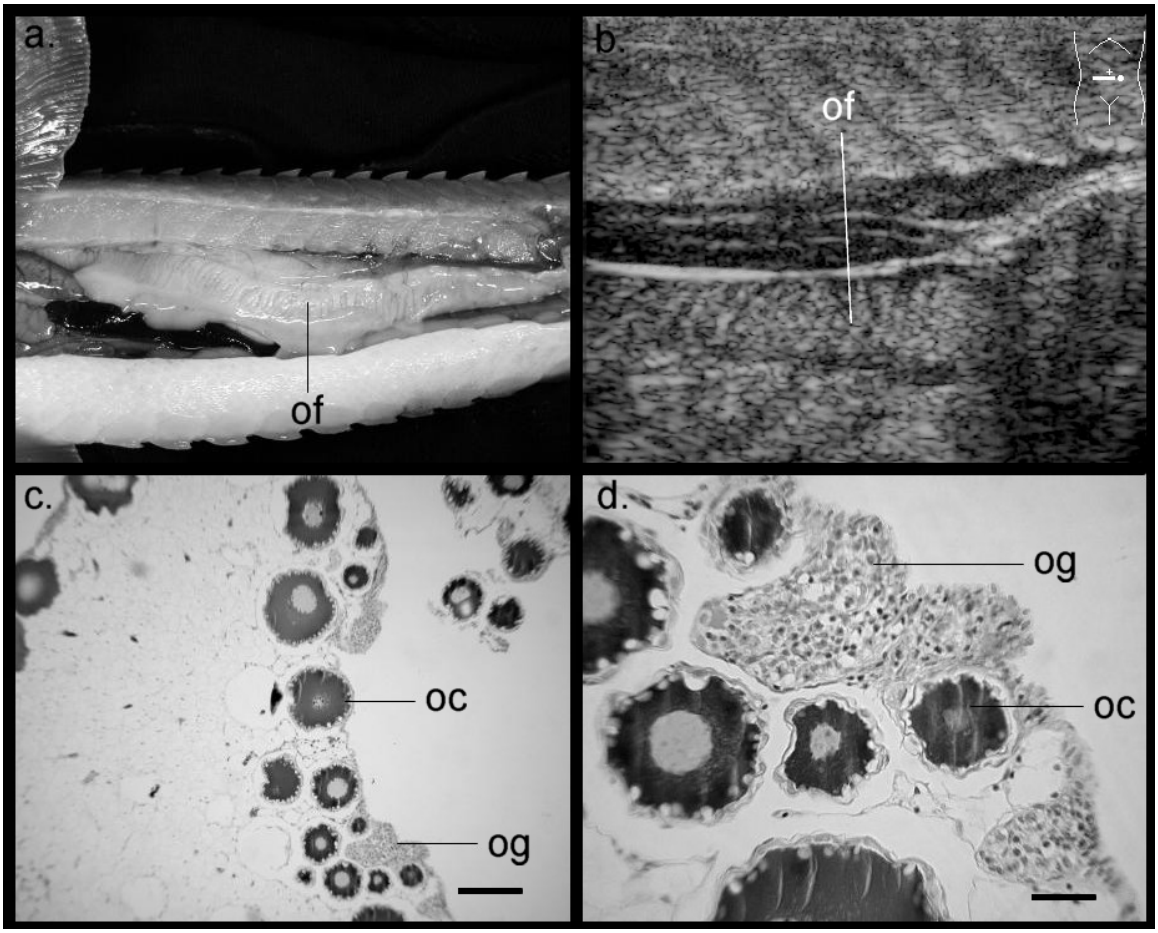

a.
of
b.
of
c.
oc
og
d.
og
oc

Figure 11. Example images of female stage FI shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 100x magnification (scale bar = 200 um) and d. 250x magnification (scale bar = 80 um). oc –oocyte, of – ovarian folds, f – follicular envelope, gv –germinal vesicle, and pn – provitelline nucleoli.

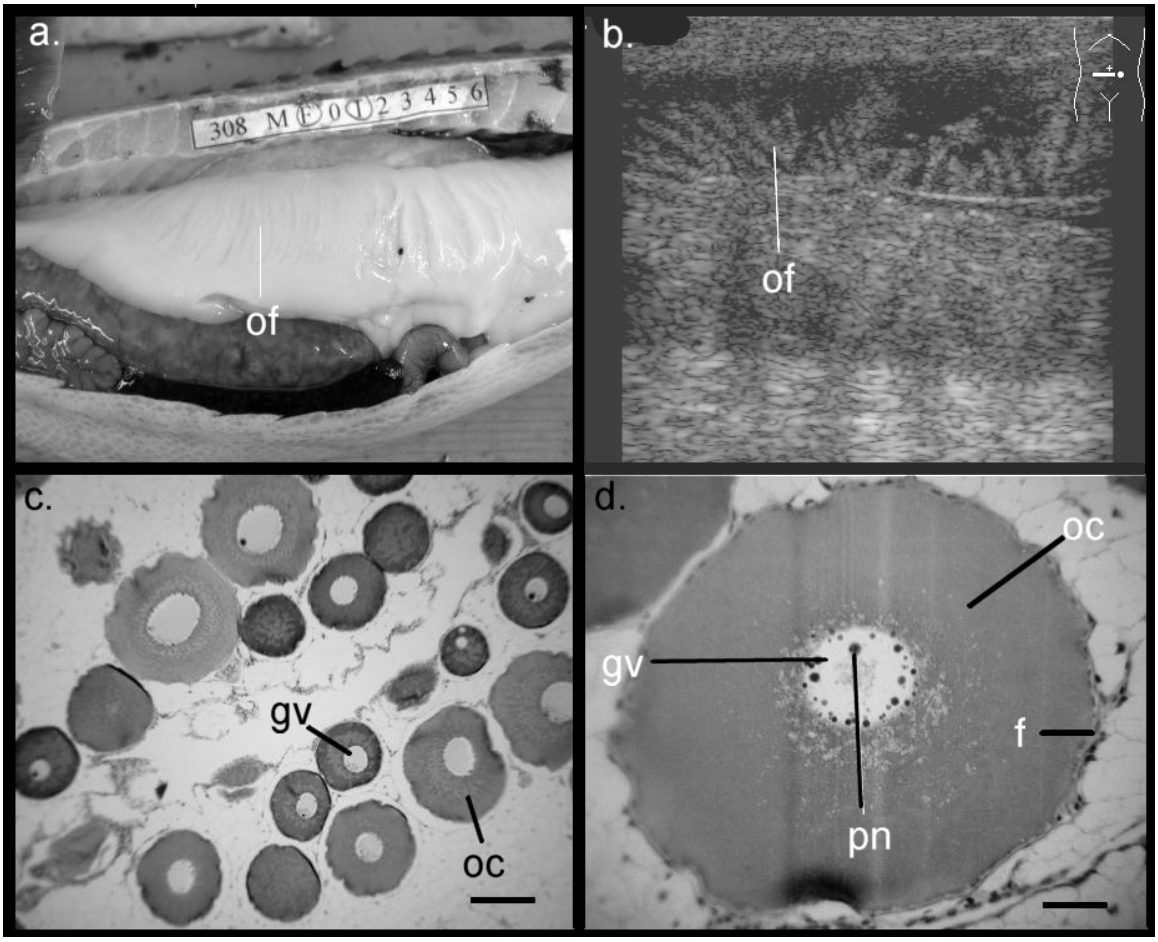

a.
308 M F 0 1 2 3 4 5 6
of
b.
of
c.
gv
oc
d.
oc
gv
f
pn

Figure 12. Example images of female shovelnose sturgeon stage FII sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 100x magnification (scale bar = 500 um) and d. 250x magnification (scale bar = 80 um) e. 630x magnification (scale bar = 32 um. oc –oocyte, of – ovarian folds, om – ovarian mass, en – euvitelline nucleoli, f – follicular envelope, gv –germinal vesicle, pz – primordial zona radiata, and yv – yolk vacuoles.

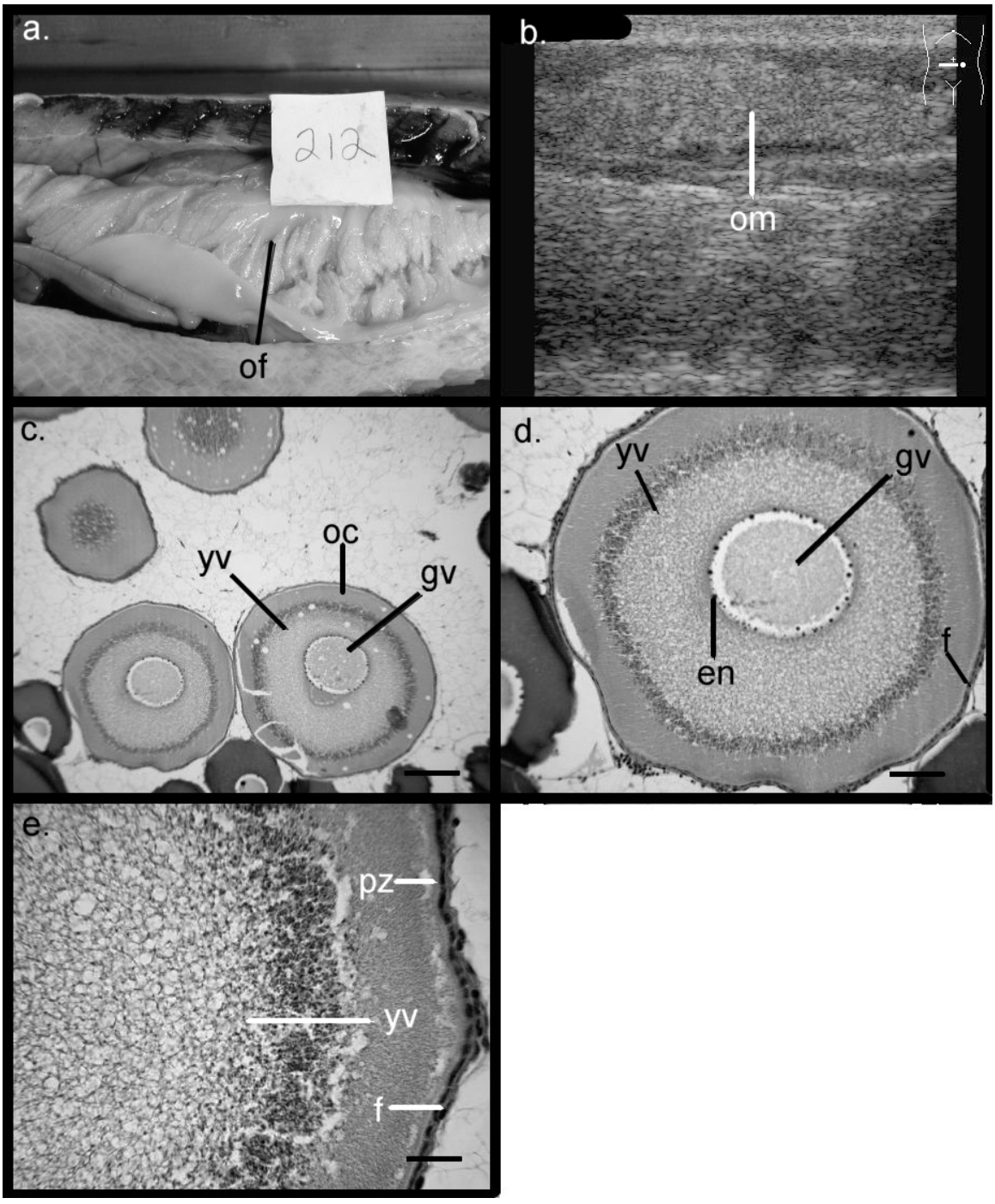

a.
212
of
b.
om
c.
yv
oc
gv
d.
yv
gv
en
f
e.
pz
yv
f

Figure 13. Example images of female shovelnose sturgeon stage FIII sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 40x magnification (scale bar = 500 um) and d. 250x magnification (scale bar = 80 um) e. 630x magnification (scale bar = 32 um). oc –oocyte, om – ovarian mass, en – euvitelline nucleoli, f – follicular envelope, yv – yolk vacuoles, and zr –zona radiata.

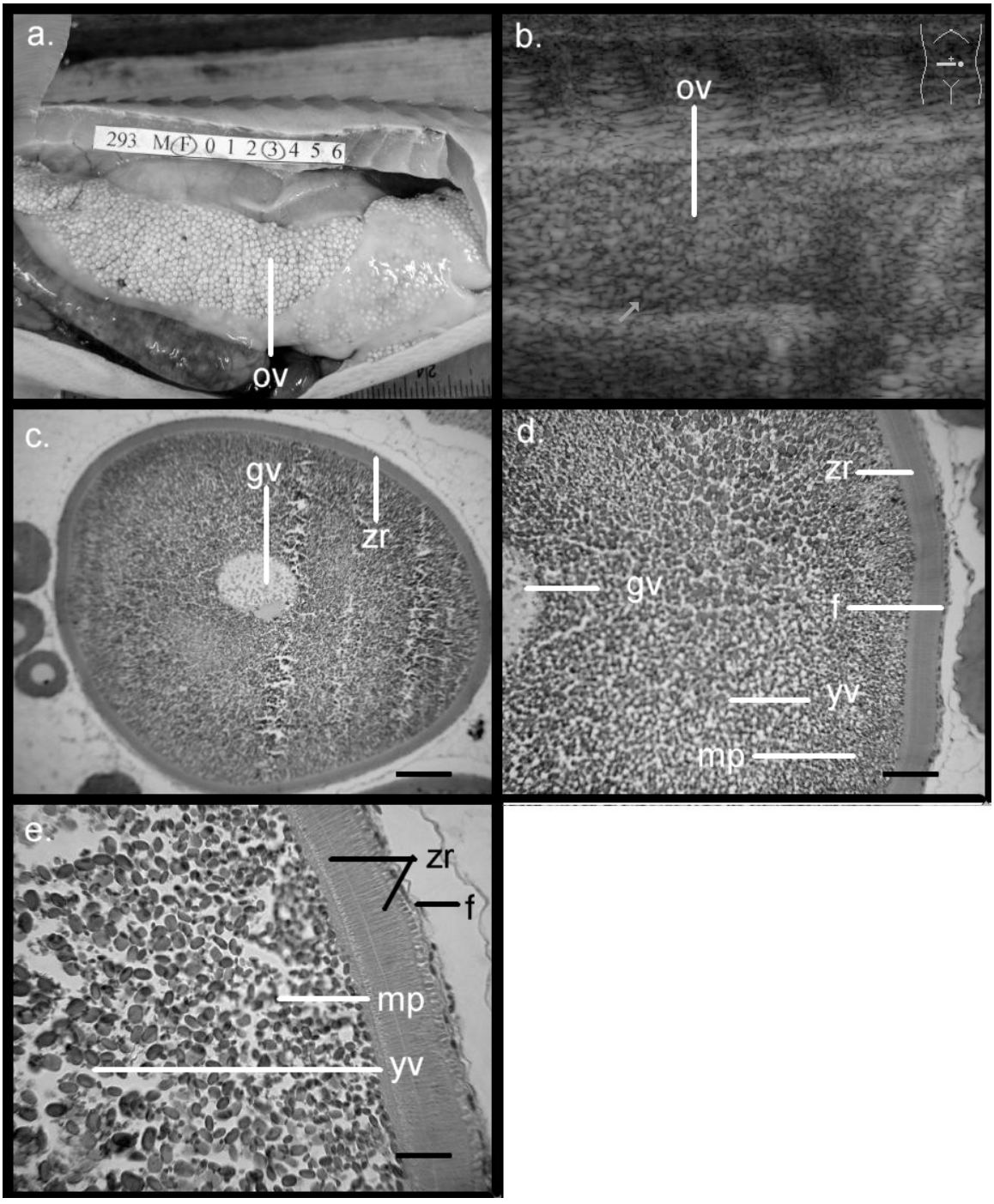

a.
293 M(F) 0 1 2(3)4 5 6
ov
b.
ov
c.
gv
zr
d.
zr
gv
f
yv
mp
e.
zr
f
mp
yv

Figure 14. Example images of female shovelnose sturgeon stage FIV sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 40x magnification (scale bar = 500 um) and d. 250x magnification (scale bar = 80 um) e. 630x magnification (scale bar = 32 um). an – animal hemisphere, oc –oocyte, f – follicular envelope, gv – germinal vesicle, ml – melanophores, vg – vegetal hemisphere, yv – yolk vacuoles, and zr –zona radiata.

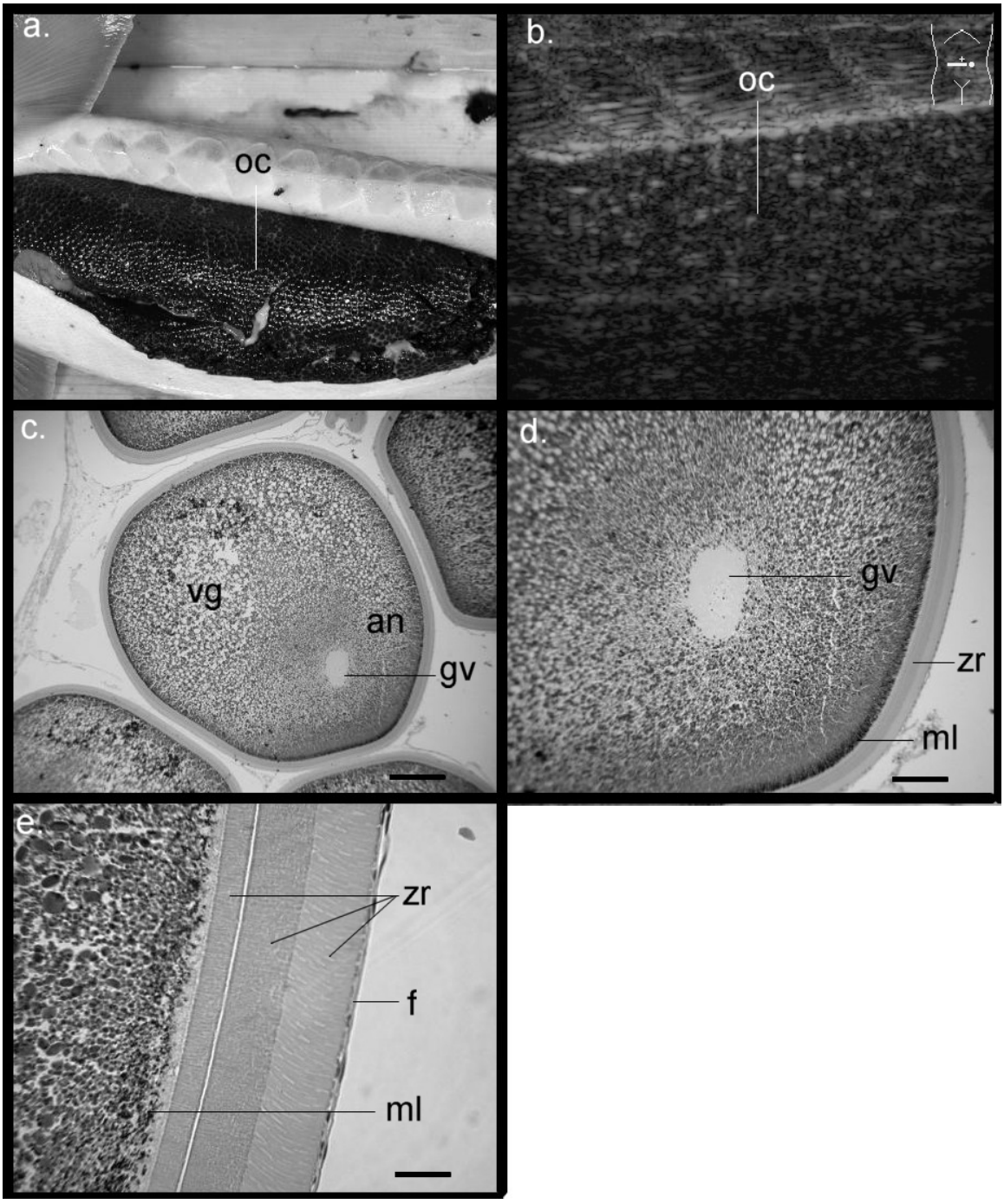

a.
oc
b.
oc
c.
vg
an
gv
d.
gv
zr
ml
e.
zr
f
ml

Figure 15. Example images of female shovelnose sturgeon stage FVI sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 100x magnification (scale bar = 500 um) and d. 250x magnification (scale bar = 80 um). ao – atretic oocytes, oc – developing oocytes, and pf – post-ovulatory follicle.

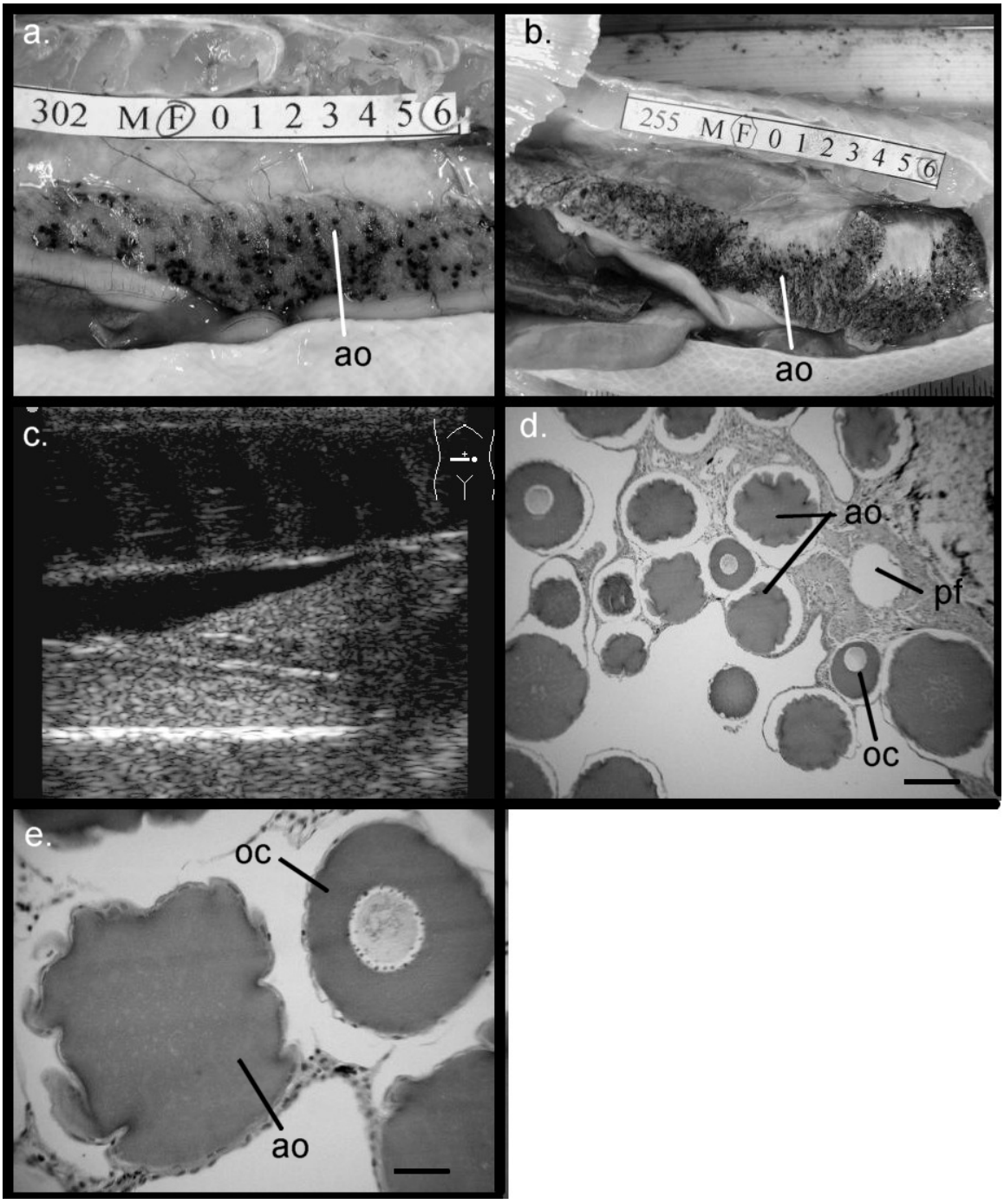

a.
302 M F 0 1 2 3 4 5 6
ao
b.
255 M F 0 1 2 3 4 5 6
ao
c.
d.
ao
pf
oc
e.
oc
ao

Figure 16. Relationship between weight and fecundity of stage FIV female shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003 ($y = 30.24 x – 8392$, $P = 0.01$, $r2 = 0.333$).

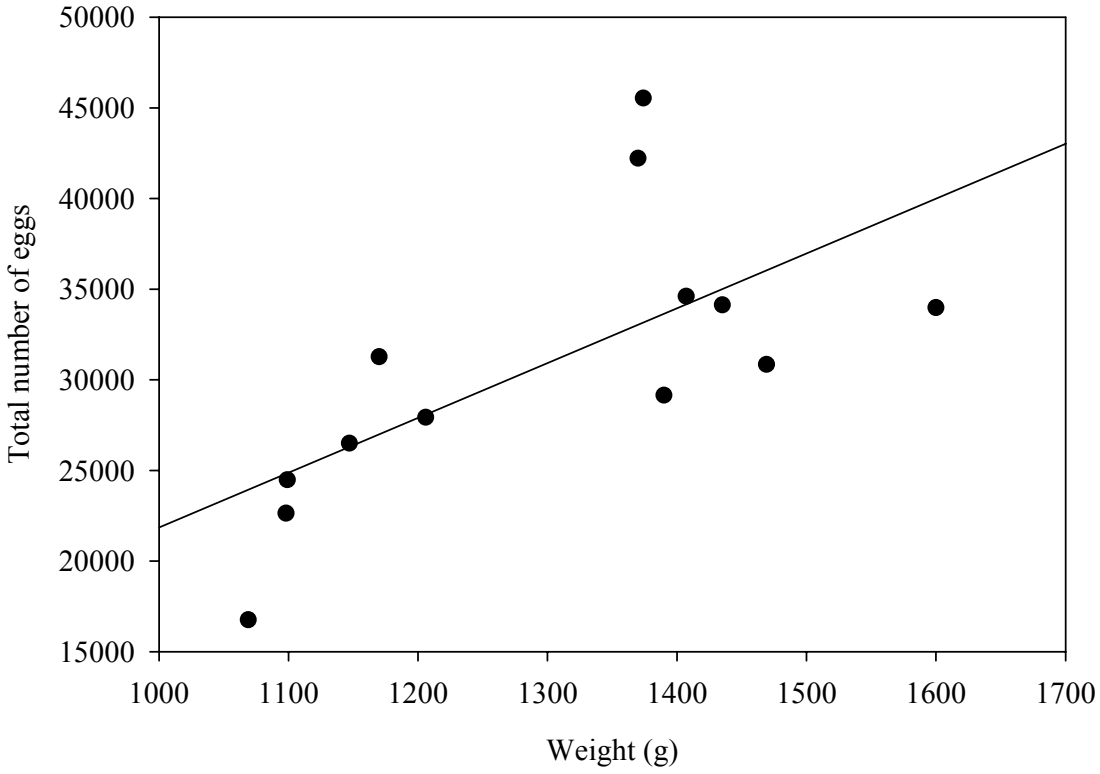

50000
45000
40000
35000
30000
25000
20000
15000
Total number of eggs
1000
1100
1200
1300
1400
1500
1600
1700
Weight (g)

Figure 17. Relationship between fork length and fecundity of stage FIV female shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003 ($y = 146.37 x – 66176$, $P = 0.053$, $r^2 = 0.2337$).

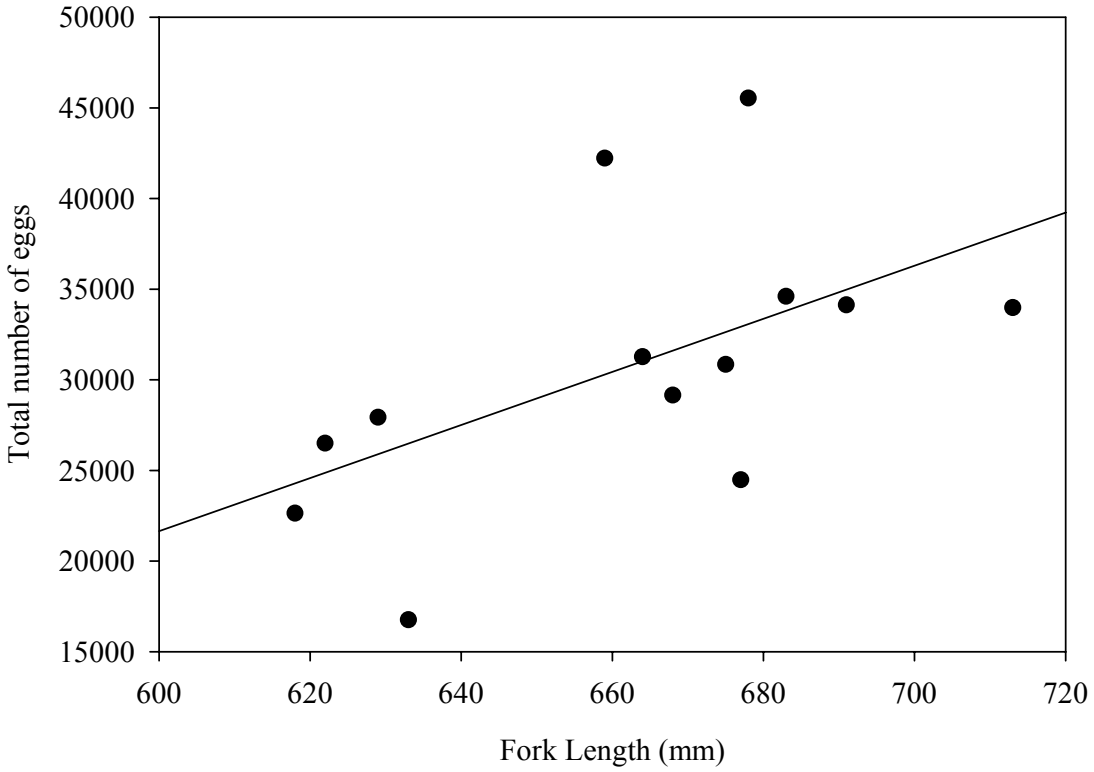
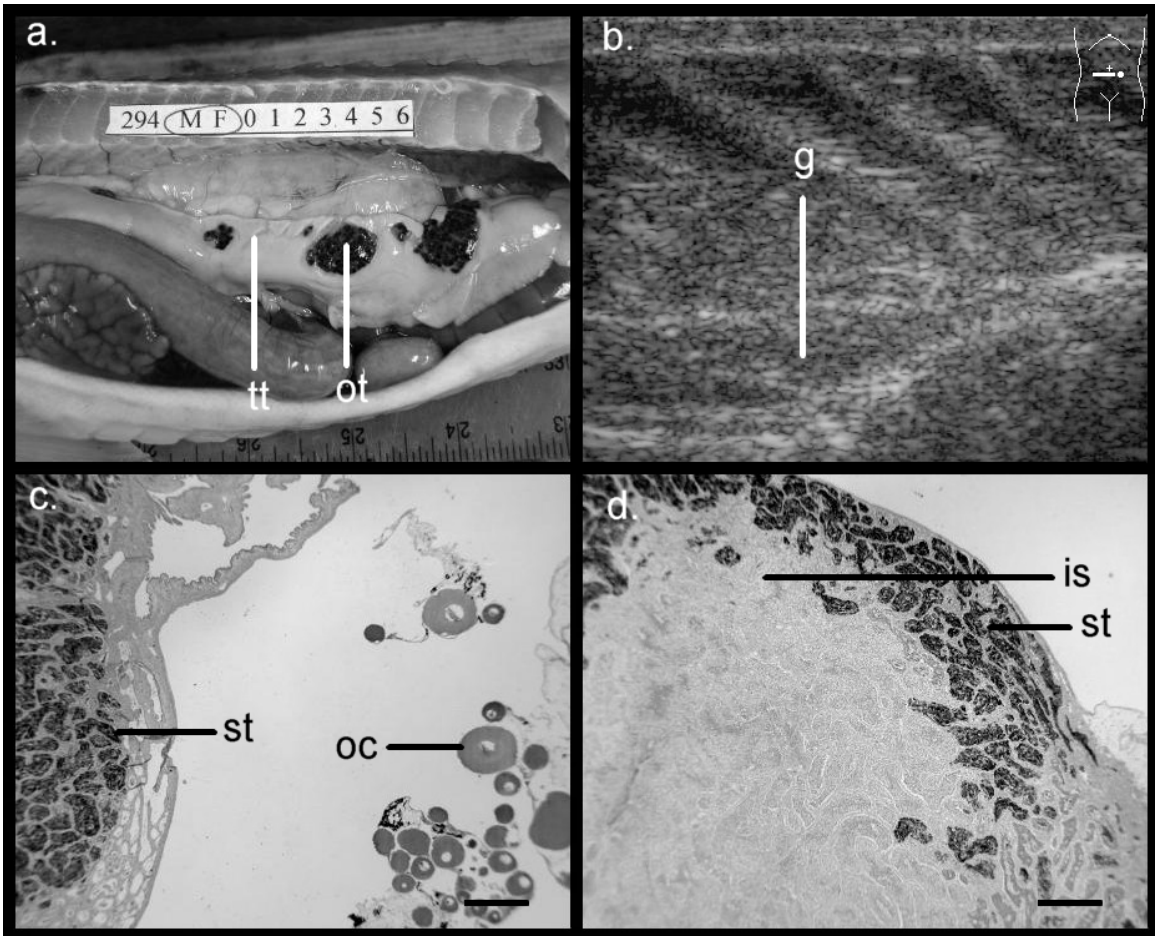

50000
45000
40000
35000
30000
25000
20000
15000
Total number of eggs
600
620
640
660
680
700
720
Fork Length (mm)

Figure 18. Example images of intersexual shovelnose sturgeon sampled from the Middle Mississippi River (River km 0 -322) during September 2001 through December 2003. a. dissected, b. ultrasound, c 100x magnification (scale bar = 500 um) and d. 250x magnification (scale bar = 80 um) oc –oocyte, ot –ovarian tissue, g – gonad, is – seminiferous tubules devoid of sperm, st – seminiferous tubules, and tt – testicular tissue.


a.
294 M F 0 1 2 3 4 5 6
tt
ot
b.
g
c.
st
oc
d.
is
st

CHAPTER FOUR

A GUIDE TO THE EMBRYONIC DEVELPOPMENT OF THE SHOVELNOSE STURGEON

ABSTRACT

I described the sequence and timing of embryologic development of shovelnose sturgeon (n = 850) reared at a constant temperature (20 ± 0.5^{o}C). Artificially inseminated, fertilized eggs were held in a recirculating system. Embryos were subsampled hourly for the first 48 hours of development and every three hours subsequently. Embryos were viewed and imaged under a stereo microscope at 35x magnification. Developmental patterns were consistent with other Acipenserid species, displaying holoblastic cleavage. The first cleavage furrow appeared two hours post-fertilization and cleavage was completed after seven hours. Blastulation concluded at sixteen hours when the dorsal blastopore lip formed. The slitlike blastopore appeared at 29 hours signifying the completion of gastrulation. At 33 hours, the rudiments of the excretory system emerged, followed by closing of the neural tube at 36 hours and formation of the s-shaped heart at 60 hours. The body continued to elongate and mass hatch occurred at 102 hours. After hatch, larvae swam into the water column and drifted in the flow for approximately two days after which the larvae became positively rheotaxic. After excretion of the pigment plug, the larvae began exogenous feeding and other structures continue to develop. Metamorphosis was completed after 26 days of development. Because shovelnose sturgeon show developmental patterns similar to those of other sturgeon, we can use this species as a model for the closely related pallid and Alabama sturgeon.

INTRODUCTION

Currently, the only sturgeon species that provides a commercial fishery in the US is the shovelnose sturgeon *Scaphirhynchus platorynchus*. This species is commercially harvested in seven of the 24 states in which it occurs (Keenlyne 1997) and harvest has been increasing. In Illinois, for example, commercial harvest of shovelnose sturgeon caviar increased from 82.5 kg in 1997 (Williamson 1998) to 2388.6 kg in 2001 (Maher 2002). Aquaculture would mitigate this increased pressure on natural stocks of shovelnose sturgeon by providing an alternate source of caviar production.

In addition to providing caviar production, aquaculture techniques can be used to aid conservationists. Conservation aquaculture has been used to enhance current stocks and reintroduce several species of sturgeon to their historic ranges (Chebanov and Savelyeva 1999; Schram et al. 1999; St Pierre 1999; Chebanov et al. 2002; Ireland et al. 2002). Efforts are currently underway by the U. S. Fish and Wildlife to supplement and reintroduce shovelnose sturgeon populations in Ohio and West Virginia (G. Conover, U.S. Fish and Wildlife Service, *pers. comm.*). Information about the embryological development of the shovelnose sturgeon would assist in this process.

Currently, information regarding the development of shovelnose sturgeon embryos and larvae is lacking. Snyder (2002) described the morphological differences between the shovelnose and pallid sturgeon, but provided no information about the embryologic development of the group. Furthermore, the developing larvae were not maintained at a specific temperature throughout development so duration of a stage could not be ascertained. The embryologic development of several species of sturgeon has

been described (Conte et al. 1988; Dettlaff and Vassetzky 1991; Dettlaff et al. 1993), but no studies have included the genus *Scaphirhynchus*.

I sought to describe the embryological development of the shovelnose sturgeon to facilitate development of proper aquaculture techniques for this species. This information would be directly applicable to the endangered pallid sturgeon *S. albus* to enhance the culture processes currently being used. For field biologists sampling young of year sturgeon, information regarding time to developmental stage would allow determination of time and temperature of spawning.

METHODS

Artificial Spawning

Shovelnose sturgeon caught in the spring from the Mississippi River at Grand Tower, IL were transported to Logan Hollow fish farm and acclimated to system temperature (18 ± 1ºC). Fish were sexed by either the presence of milt when the abdomen was palpated (males) or the presence of oocytes when catheterized (females). Fish that did not produce milt or eggs were considered undetermined were discarded. I removed approximately 10 oocytes from each female for determination of oocyte readiness. Oocyte condition was determined using the Polarization Index (PI) (Van Eenennaam et al. 2002).

The PI method consisted of boiling the oocytes in Ringer solution for 5 to 8 minutes. These oocytes were then cooled and placed in 10% neutral buffered formalin for 24 hours. Each oocyte was then sectioned through its animal and vegetal hemisphere. Digital images were taken under a dissecting microscope and measurements of oocyte diameter and distance from the chorion to the closest border of the germinal vesicle (GV)

was taken using Scion Image 4.0.2 image analysis software. This ratio of the distance from chorion to germinal vesicle to total oocyte diameter is the polarization index (PI). A fish with a PI of less then 0.10 is considered to have a migrated germinal vesicle suggesting a mature ovary.

To induce the sturgeon to reach final maturation a synthetic hormone was used (Ovaprim®). Ripe males were injected with Ovaprim® 24 hours preceding semen collection at a dose of 0.25 ml/kg. Ripe females were injected once at 24 hours with a 10% dose of 0.50 mg/kg and with a resolving dose (remaining 90%) at 9 hours before insemination. Eggs were removed by the Bruch stroke (Bruch et al. 2001) and Caesarian section (Conte et al. 1988). Eggs were placed in a stainless steel bowl. Milt was extracted into a vial by stroking the abdomen while using a sperm aspirator. This milt was held on ice until use. The dosage was 10 ml of sperm per 1 kg of eggs (Hochleithner and Gessner 2001) and the sperm was diluted with water at 1: 200 to avoid polyspermy. The sperm and eggs were mixed for approximately three minutes. The eggs were then rinsed with freshwater until they became adhesive. To remove the adhesiveness of the eggs, the eggs were mixed with Fullers earth and water for 20 minutes. These fertilized eggs were then added to McDonald incubation jars and incubated at 20±1°C in a temperature controlled closed system.

Embryologic and Larval Development

A sample of 10 developing oocytes was removed every hour for the first 48 hours, and then every three hours until hatch. The embryos were viewed and imaged under a dissecting microscope at 20-35x magnification. The developing embryos were assigned a

stage coinciding with those determined for the Russian sturgeon *Acipenser guldenstadti* embryo development (Table 4) (Dettlaff and Vassetzky 1993).

Hatched larvae were kept in floating baskets in a recirculating system at a constant 20±1°C for 11 days. Larvae were then placed in a larger recirculating system for grow out due to poor water quality in the smaller recirculating system. A sample of three to five larvae were removed daily until the larvae had undergone metamorphosis to their adult form.

To standardize the time to developmental stage over varying temperatures, I first calculated the number of degree hours over 10ºC to provide a standard with utility to aquaculture. This temperature was used because it was the lowest reported temperature at which sturgeon underwent mitosis (Shelton et al. 1997). Secondly, I described development as it related to the number of mitotic intervals within each stage (Dettlaff and Vassetzky 1993). The Dettlaff unit (τ_0) provides a temperature independent time to developmental stage (Dettlaff and Vassetzky 1993). For shovelnose sturgeon reared at 20ºC τ_0 = 45 minutes (Shelton et al. 1997). To determine the number of mitotic intervals to the onset of a stage the time at 20ºC (τ_n) was divided by τ_0.

RESULTS

Artificial Spawning

A total of 10 female shovelnose sturgeon were assessed for ripeness using the PI. Of these only two were considered ready for injection. Both females and four males were injected with Ovaprim®. After 24 hours, only one female had ovulated, with the other developing poor quality overripe eggs. Milt from two males was mixed with the oocytes of the successfully ovulated eggs and placed in the recirculating system.

Cleavage

The mature prefertilized oocytes of the shovelnose sturgeon were black and grey with the animal hemisphere being grey and the vegetal hemisphere being black (Table 4, Figure 19a). Upon fertilization, a light spot formed at the apex of the animal hemisphere (Table 4, Figure 19b). After one mitotic interval, the polar fertilization spot disappeared (Table 4, Figure 19c), which was then followed by the formation of an eccentric crescent band in the animal hemisphere (Table 4, Figure 19d). The first cleavage furrow was formed after 2.5 mitotic intervals (Table 4, Figure 19e). This cleavage furrow did not cleave through the entire longitudinal axis but was confined in the animal hemisphere (Table 4, Figure 19f). The second cleavage furrow occurred after 4.0 mitotic intervals and bisected the first cleavage furrow at its midpoint forming four equal blastomeres (Table 4, Figure 20a). The second cleavage furrow also did not divide the vegetal hemisphere (Table 4, Figure 20b). After 5.0 mitotic intervals, the embryo reached the eight cell stage (Table 4, Figure 20c,d) and cleavage had begun in the vegetal hemisphere (Table 4, Figure 20c). This infiltrating furrow did not cleave through the entire vegetal hemisphere. After 6.5 mitotic intervals cleavage in the animal hemisphere formed blastomeres of differing sizes (Table 4, Figure 20e), while the vegetal hemisphere was divided completely by the cleavage furrows (Figure 20f). As cleavage continued, small blastomeres proliferated in the animal hemisphere, along with continued division of the vegetal hemisphere (Table 4, Figure 21a-d). Eight hours post fertilization (10.5 mitotic intervals) the cleavage cavity became visible in the animal hemisphere (Figure 21e) and individual blastomeres were difficult to distinguish.

Blastulation

Blastulation in the shovelnose sturgeon occurred between nine and 11 hours at 20°C (Table 4). Early blastula was characterized by the presence of small blastomeres visible under low power magnification (Table 4, Figure 22a,c). The vegetal hemisphere contained large blastomeres with distinct small blastomeres along the equator (Table 4, Figure 22a,b). At the apex of the animal hemisphere, the primordial cleavage cavity (blastocoel) was seen as an invagination through the blastula roof (Table 4, Figure 22a,c). The late blastula was smooth in appearance. Individual blastomeres in the animal hemisphere that were distinguishable in the early blastula were no longer evident at low power magnification (Table 4, Figure 22d). The blastocoel was larger and more evident (Table 4, Figure 22e). The transition zone along the equator still contained distinguishable blastomeres (Table 4, Figure 22e) and large blastomeres were present within the vegetal hemisphere (Table 4, Figure 22f).

Gastrulation

The onset of gastrulation occurred at 17 hours and was complete by 29 hours post fertilization (Table 4, Table 4). At the onset of gastrulation a band formed between the animal and vegetal hemisphere just below the equator (Table 4, Figure 23a.b). The dorsal blastopore lip formed one hour after the onset of gastrulation, forming in the position previously occupied by the pigment band (Table 4, Figure 23c,d). The blastocoel which appeared as an indentation of the pole can be seen through the thin cell layer at the animal pole (Table 4, Figure 23d). Five hours after the onset of gastrulation, two-thirds of the embryo was covered by animal material (epiboly) and the blastopore

had closed to a ring (Table 4, Figure 23e,f). The blastocoel was still evident through the roof of the embryo (Table 4, Figure 23f). The primitive gut or archenteron was now present and could be seen as a dark region on the dorsal surface of the embryo (Table 4, Figure 23e). As gastrulation continued, epiboly covered more than two-thirds of the embryo. A large yolk plug was now evident (Table 4, Figure 24a). The blastocoel took a ring like appearance on the ventral surface of the embryo (Table 4, Figure 24b). At 27 hours post fertilization, epiboly covered most of the embryo and the blastopore size declined (Table 4, Figure 24c), the blastocoel appeared only as a dark spot on the ventral surface of the embryo (Table 4, Figure 24d). Gastrulation was completed with the formation of the slit like blastopore after 29 hours of development (Table 4, Figure 24e).

Neurulation Preceding the Onset of the Heartbeat

Neurulation began 29 hours post fertilization (Table 4). At the ventral surface of the embryo, the neural plate was present and raised (Table 4, Figure 24f), and the neural groove was present. The neural folds appeared in the head region and the neural groove became wider after 31 hours of development (Table 4, Figure 25a,b). After 33 hours of development, the excretory rudiments were present as cords running parallel to the neural groove (Table 4, Figure 25c). In the head region, the neural folds thickened and rose, approaching each other (Table 4, Figure 25d). As neurulation continued, the rudimentary excretory system elongated and became more distinct. The neural tube began to close in the caudal region (Table 4, Figure 25e). At the head region, the neural groove had closed at its most anterior part, which is the sight of the prosencephalon (Table 4, Figure 25f). After 36 hours of development, the neural tube fully closed and the suture became clearly

evident (Figure 26a,b). In the head region, the neural tube began to form into different brain regions (Figure 26b). As neurulation continued, the eye protrusions formed and the anterior portion of the excretory system thickened (Figure 26c,d). Additionally, the first pair of visceral arches appeared as two arms emanating from the metencephalon (Table 4, Figure 26d). In the trunk region, the tail began to thicken at its most caudal point (Table 4, Figure 26c). After 45 hours of development, the eye rudiments were clearly visible at the most cranial point of the head region as was the second visceral arch (Table 4, Figure 26f). Additionally, the lateral plates were evident and were becoming close in proximity (Table 4, Figure 26f). The tail region continued to thicken, although it had not separated from the yolk sac membrane (Table 4, Figure 26e). In the middle region of the embryo, the pronephros was now present as tubes running perpendicular to the neural tube and the first somites appeared (Table 4, Figure 26e,f).

At 52 hours post fertilization, the lateral plates had fused cranial to the prosencephalon, forming the rudimentary heart (Table 4, Figure 27b). The tail had separated from the yolk sac membrane and begun to lengthen (Table 4, Figure 27a). In the head region, the myelencephalon cavity was strongly pronounced as a triangular structure in the hindbrain, and the third visceral arch was evident (Table 4, Figure 27b). The head region began to separate at 54 hours post fertilization (Table 4, Figure 27d). In the caudal region, the tail continued to elongate (Table 4, Figure 27c). At 54 hours post fertilization, the somites were strongly pronounced and covered the entire body of the embryo (Table 4, Figure 27e). The heart was now present as a short tube (Table 4, Figure 27f), and the eye rudiments were present as paired slits on the head of the embryo (Table

4, Figure 27f), which marked the beginning of eye cup formation. The head had begun to thicken and separate from the membrane of the yolk sac.

Embryo Development from the Onset of the Heartbeat until Hatch

At 60 hours post-fertilization the tail continued to elongate and the rudiments of the fin fold appeared (Table 4, Figure 28a). Cranial to the cephalic region, the heart became s-shaped and began to beat (Table 4, Figure 28b). The head continued to deepen but was still associated with the yolk sac membrane (Table 4, Figure 28b). The tail continued to lengthen at 63 hours post-fertilization and the embryo became bent (Table 4, Figure 28c). The hatching gland was now pronounced on the ventral surface of the head (Table 4, Figure 28c) and the olfactory sacs were present as circular structures at the most cranial part of the head. The dorsal view of the cranial end, showed that heart had increased in size and the distinctions between brain regions were difficult to discern (Table 4, Figure 28d).

At 81 hours post-fertilization, the tail end had reached the level of the heart and the fin fold became clearly evident (Figure 28e,f). The eye cups were evident in the area rostral to the olfactory sacs and the hatching gland had thickened at the ventral base of the head (Table 4, Figure 28e). Furthermore, the cranial most part of the head had now separated from the yolk sac membrane (Table 4, Figure 28e). Visual distinction of the brain regions became almost impossible as the head had deepened appreciably (Table 4, Figure 28f). Additionally, the pronephros became difficult to distinguish (Table 4, Figure 28e). After 87 hours of development, the embryo was moving within the envelope (Table 4, Figure 29a). The tail had now reached the head end of the embryo and the fin fold was

formed (Table 4, Figure 29b). In the cranial region of the embryo, the head continued to separate from the yolk sac membrane (Table 4, Figure 29a,b).

Hatching of advanced embryos began at 93 hours post fertilization (29c,d). The advanced embryos were clear with no pigment in the eye cups (29d). The yolk sac was yellow and the pigment plug was not fully formed (Table 4, Figure 29d). Mass hatch occurred at 102 hours post fertilization, these prolarvae appeared similar to the advanced embryos (Table 4, Figure 29e,f). However, these prolarvae had a well developed yolk plug (Table 4, Figure 29f). Upon hatch, these prolarvae swam up into the water column and maintained this position.

Larval Development

Eighteen hours post-hatch the mouth rudiment of the prolarvae appeared. However, there was no pigment present on the body of the prolarvae (Figure 30a,b). At 24 hours post-hatch, pigmentation appeared in both the eye cup and along the bodies of the sturgeon (Figure 30c,d). Muscle segments were clearly seen along the bodies of the prolarvae (Figure 30c). Two days post hatch, the prolarvae were noticeably more pigmented in both the cranial and caudal sections of their bodies (Figure 30e). Additionally, the rudiments of the barbels were present and the gill arches and filaments appeared (Figure 30f). Furthermore, the yolk sac was now separated by a furrow creating two distinct regions (Figure 30e). The pectoral fin buds were present as nubs along the dorsal surface of the yolk sac (Figure 30e).

Three days post-hatch, the eyes of the prolarvae became fully pigmented and the mouths had fully formed (Figure 31a,b). The pectoral fins were now discernable.

However, no rays were distinguishable (Figure 31a). In the abdominal cavity, the yolk sac had separated into presumptive organs (Figure 31a). By the forth day after hatch, the pelvic fins had formed and the dorsal fin began to separate from the fin fold (Figure 31c). Additionally, these prolarvae have developed a negative phototaxic response. In the abdominal cavity, organs became visually evident (Figure 31d). The anal fin appeared five days post hatch as a small ridge in the fin fold (Figure 31e). In the cephalic region, the barbels elongated and separated (Figure 31f). In the sixth day post hatch, the remnants of the yolk sac was still present (Figure 32a). These prolarvae have become positively rheotaxic. After seven days of development, the yolk sac became difficult to discern (Figure 32d). The gut became more developed as segments appeared (Figure 32d). In the dorsal fin, the fin rays became evident at the origin (Figure 32c). The pigment plug was evacuated by the eighth day of development marking the transition to exogenous feeding (Figure 32f). The pectoral fin rays were now present at the margin of the fin and the body (Figure 32e).

The spiral colon was fully formed by the twelfth day of development and the pelvic fin rays appeared (Figure 33a,b). Besides increases in length no significant changes had occurred in the larvae during days 13 and 14 post hatch (Figure 33c-f). At 18 days post hatch, the rudiments of the dorsal scutes appeared as segments in the dorsal fin fold (Figure 34b). The head had elongated and took on the shape indicative of the shovelnose sturgeon (Figure 34a). After 21 days of development, the lateral scutes were present along the body of the juvenile (Figure 34c). The head continued to broaden and the barbels continued to elongate (Figure 34d). The juvenile at 26 days of development

had fully developed fins and its full complement of scutes the juvenile now closely resembles the adult (Figure 34e,f)

DISCUSSION

The shovelnose sturgeon displays holoblastic cleavage which is similar to the other species of acipenserids (Conte et al. 1988; Dettlaff and Vassetzky 1991; Dettlaff et al. 1993). This cleavage pattern is similar to that displayed by anurans. However, it differs in that the cleavage of the vegetal hemisphere is asymmetrical. This means that the embryo is not completely divided with each cleavage furrow. Holoblastic cleavage is relatively rare in fishes, with most species of fishes displaying meroblastic cleavage as found in the teleosts. In meroblastic cleavage the embryo divides on the blastodisk in the animal hemisphere. In the sturgeon, the asymmetrical cleavage of the vegetal hemisphere is due to an uneven distribution of yolk inclusions in the vegetal hemisphere (Dettlaff et al. 1993). This unequal distribution of yolk also causes the completion of cleavage in the vegetal hemisphere to occur later than that in the animal hemisphere.

The formation of a cleavage cavity in the animal hemisphere of the embryo marks the beginning of blastulation. During blastulation the blastocoel forms and individual blastocytes become undistinguishable in the animal hemisphere. In the sturgeon embryo, there is a zone of transition between the animal and vegetal hemispheres in which small blastocytes are present until blastulation is complete. Upon completion of blastulation, the blastocoel has formed in the animal hemisphere and is easily seen through the embryo roof. With the formation of the blastopore lip the embryo begins gastrulation. During gastrulation, the three germ layers arise, and the adult body plan is set as a result of the

translocation of cells. The primitive gut or archenteron is formed under the migrating cell mass.

Neurulation begins with the formation of the neural plate in the region of the presumptive head. This is followed closely by the formation of the excretory system. During neurulation, the primitive brain develops into the forebrain, midbrain, and hindbrain from most cranial to most caudal, they are the prosencephalon (telencephalon and diencephalon), mesencephalon, and the rhombencephalon (metencephalon and myelencephalon). During the later stages of neurulation, the heart forms from the fusing of the lateral plates this rudimentary heart is a straight tube which becomes s-shaped at the onset of the heart beat. The caudal half of the body separates from the yolk sac membrane and elongates forming the tail of the sturgeon. The head thickens and separates from the yolk sac membrane in the final stages before hatch.

The larvae of sturgeon contain a large amount of fat and the prolarvae hatch in a precocial condition. Upon mass hatch, the larvae swim up into the water column and remain drifting for a period of up to eight days (Kynard et al. 2002). Once this drifting is complete, they become negatively phototaxic, remaining near the bottom water column. A period of strong positive rheotaxis follows the phototaxic response, when the prolarvae orient their bodies into the flow. While the yolk sac is being absorbed, the internal organs are developing. After complete absorption of the yolk sac the pigment plug is released and exogenous feeding begins. By this time the fins are well formed, but the larvae contain no scutes and a ventral fin fold is still present. The transformation of larval juvenile sturgeon is complete with the formation of both the lateral and dorsal scutes.

With this information, fish culturists will be able to design hatchery protocols that will allow successful propagation of the shovelnose sturgeon. This allows insight about the time to formation of different stages so that they can distinguish quality embryos from those with defects. Additionally, knowledge of the stage at which first feeding occurs allows hatchery managers to better time the addition of food to the rearing raceways. This can help reduce the cost of introducing feed to prolarvae which cannot feed exogenously.

Sturgeon have small crystalline otoliths making the determination of hatch date using daily ring formation all but impossible. Therefore, hatch date can only be determined using stage and temperature data. The stage information detailed here coupled with river temperature data will allow the determination of spawning date. Once larval or embryonic sturgeon are captured, managers will be able to identify the time of fertilization based on river temperature and areas of spawning based on flow patterns. Furthermore, because the shovelnose sturgeon is the only river sturgeon not at immediate risk of extinction, information disseminated here can be used as a guide for other river sturgeons.

Although spawning habitat of the shovelnose sturgeon has yet to be determined it is assumed that they use the thalweg of large rivers to spawn (Keenlyne 1997). Several lotic species of fishes use the main channel as spawning habitat and several different strategies have evolved to disperse the embryos to suitable spawning habitats. Species such as the freshwater drum *Alpodinotus grunniens* and goldeye *Hiodon alsoides* employ buoyant eggs allowing the embryos to develop in the flow of the main channel (Pflieger 1975). Upon hatch, these larvae have relatively little yolk reserves requiring them to find

adequate nursery grounds quickly. The shovelnose sturgeon employs a different approach, the newly hatched embryos have a large yolk reserve and are capable of swimming. These embryos then drift with the current until an adequate nursery ground habitat is found. This period of drifting can last up to eight days (Kynard et al. 2002).

The female shovelnose sturgeon invests a large amount of energy into producing a large amount of yolk rich eggs (see Chapter 3). Because of this the embryos that are produced from these eggs are given a large yolk store and have the ability to maintain a position in the water column enabling the period of prolonged drift. This drift could increase recruitment by reducing intraspecific competition via dispersal or by allowing the larvae to find adequate nursery grounds.

The results of this study will allow the refinement of efficient aquaculture techniques that could be applied to help restore the shovelnose sturgeon to its entire range. Additionally, with the development of these techniques a fish farm market could be developed that would reduce fishing pressure on natural stocks of this species. This also aids in the determination of sturgeon spawning habitat by providing a guide that can be used to distinguish the *Scaphirhynchus* from other sympatric species of Chondrosteans.

Table 4. Stages of embryologic development of the shovelnose sturgeon taken from those reported for the Russian sturgeon *Acipenser guldenstadti* (Dettlaff and Vassetzky 1991). With τ_n/τ_0 equal to the time at 20ºC divided by the Dettlaff unit (τ_0)

Stage	Description	Time at 20°C (Hour)	Degree hours	τ_n/τ_0	Figure
0	Unfertilized egg	0	0	0	19a
1	Fertilization	0	0	0	19b
2	Polar spot disappeared	0.75	7.5	1.0	19c
3	Eccentric pigment accumulation	1	10	1.3	19d
4	First cleavage	2	20	2.6	19e,f
5	Four cell	3	30	4.0	20a,b
6	Eight cell, furrow into vegetal	4	40	5.3	20c,d
7	Sixteen cell, full cleavage vegetal	5	50	6.6	20e,f
8	Fifth cleavage furrow	6	60	7.9	21a,b
9	Cleavage continues to divide vegetal completely	7	70	9.2	21c,d
10	Cleavage cavity forming	8	80	10.5	21e,f
11	Early blastula	9	90	11.9	22a,b,c
12	Late blastula	11	110	14.5	22d,e,f
13	Onset of gastrulation	17	170	22.4	23a,b
14	Dorsal blastopore lip formed	18	180	23.7	23c,d
15	2/3 of embryo covered by animal material	23	230	30.3	23e,f
16	Large yolk plug	25	250	33.0	24a,b
17	Small yolk plug	27	270	35.6	24c,d
18	Gastrulation complete	29	290	38.2	24e

Table 4. continued

Stage	Description	Time at 20°C (Hour)	Degree hours	τ_n/τ_0	Figure
19	Onset of neurulation	29	290	38.2	24f
20	Wide neural plate	31	310	40.9	25a,b
21	Excretory rudiments evident	33	330	43.5	25c,d
22	Excretory rudiments elongate	35	350	46.1	25e,f
23	Neural tube closed	36	360	47.5	26a,b
24	Eye protrusions, excretory rudiments thicken	39	390	51.4	26c,d
25	Lateral plates reach the anterior of head, tail thickens	45	450	59.3	26e,f
26	Heart rudiment forming, tail rudiment separating	52	520	68.5	27a,b
27	Heart rudiment present as short tube	54	540	71.1	27c,d
28	Heart rudiment elongates	60	600	79.1	27e,f
29	Onset of heartbeat, heart S-shaped	63	630	83.0	28a,b
30	Tail begins to straighten	81	810	106.0	28c,d
31	Tail approaches heart	85	850	112.0	28e,f
32	Tail end reaches head	87	870	115.0	29a,b
33	Tail straightens fully when removed				
34	Embryo capable of movement				
35	Hatch of advanced embryos	93	930	123.0	29c,d
36	Mass hatch	102	1020	134.0	29e,f

Figure 19. Stages zero through four of shovelnose sturgeon embryologic development (Fertilization and Cleavage I) a. unfertilized oocyte (Stage 0), b. oocyte at moment of fertilization (Stage 1), c. loss of light polar spot (Stage 2), d. crescent pigment accumulation (Stage 3), e. first cleavage animal (Stage 4) and f. Stage 4 vegetal. 35x magnification, scale bar = 1mm. See Table 4 for a description of the stages.

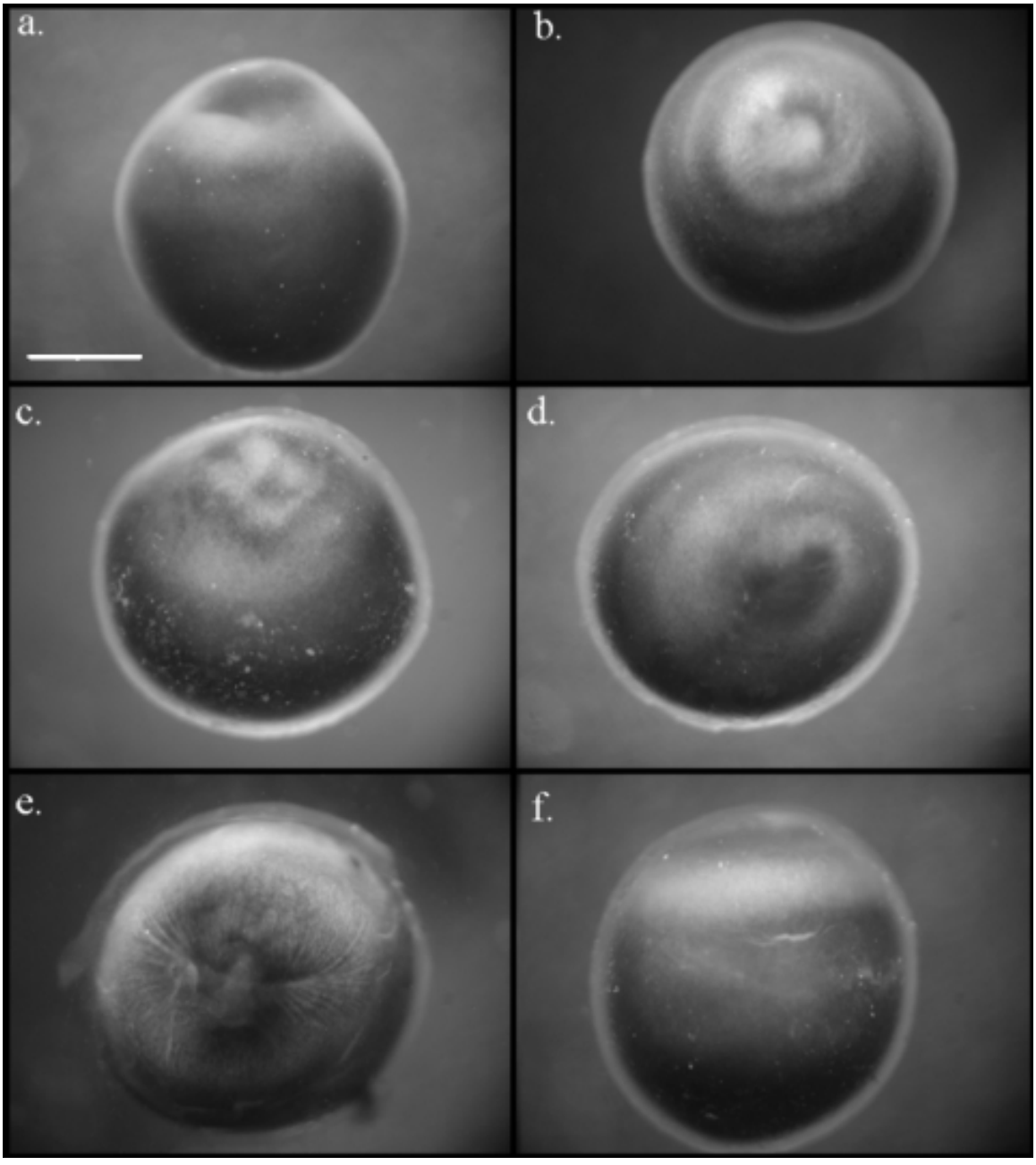

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Figure 20. Stages five through seven of shovelnose sturgeon embryologic development (Cleavage II). a. second cleavage in animal hemisphere (Stage 5), b. stage 5 side view, c. eight blastomere lateral view (Stage 6), d. Stage 6 animal view, e. 16 cell stage animal view (Stage 7), f. cleavage in vegetal hemisphere (Stage 7). 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

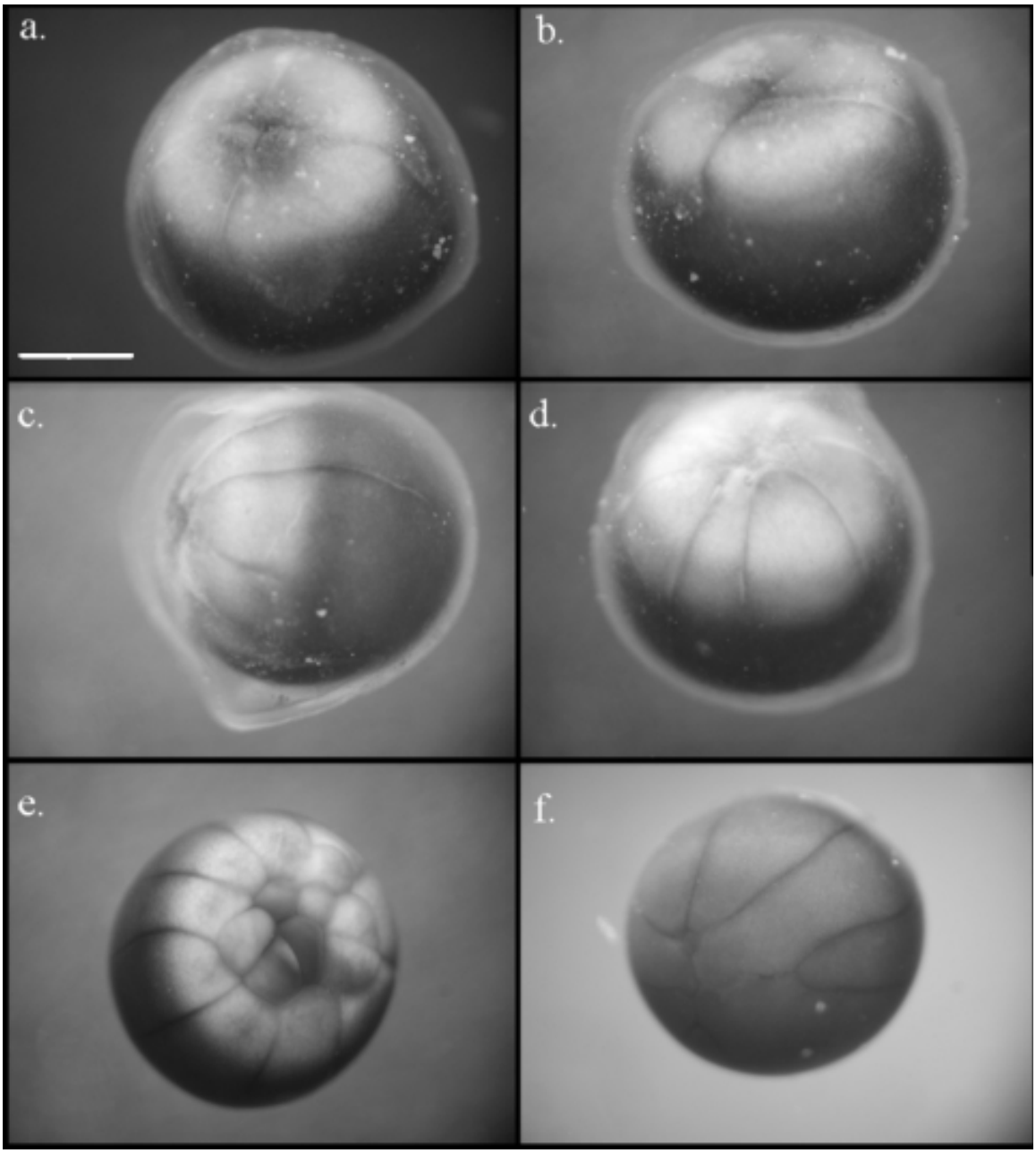
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Figure 21. Stages eight through 10 of shovelnose sturgeon embryologic development (Cleavage III). a. Fifth cleavage animal view (Stage 8), b. Stage 8 vegetal view, c. seventh cleavage animal view (Stage 9), d. Stage 9 vegetal view, e. blastocoel formation animal view (Stage 10), and f. Stage 10 vegetal view. 35x magnification scale bar = 1 mm. See Table 4 for a description of the stages.

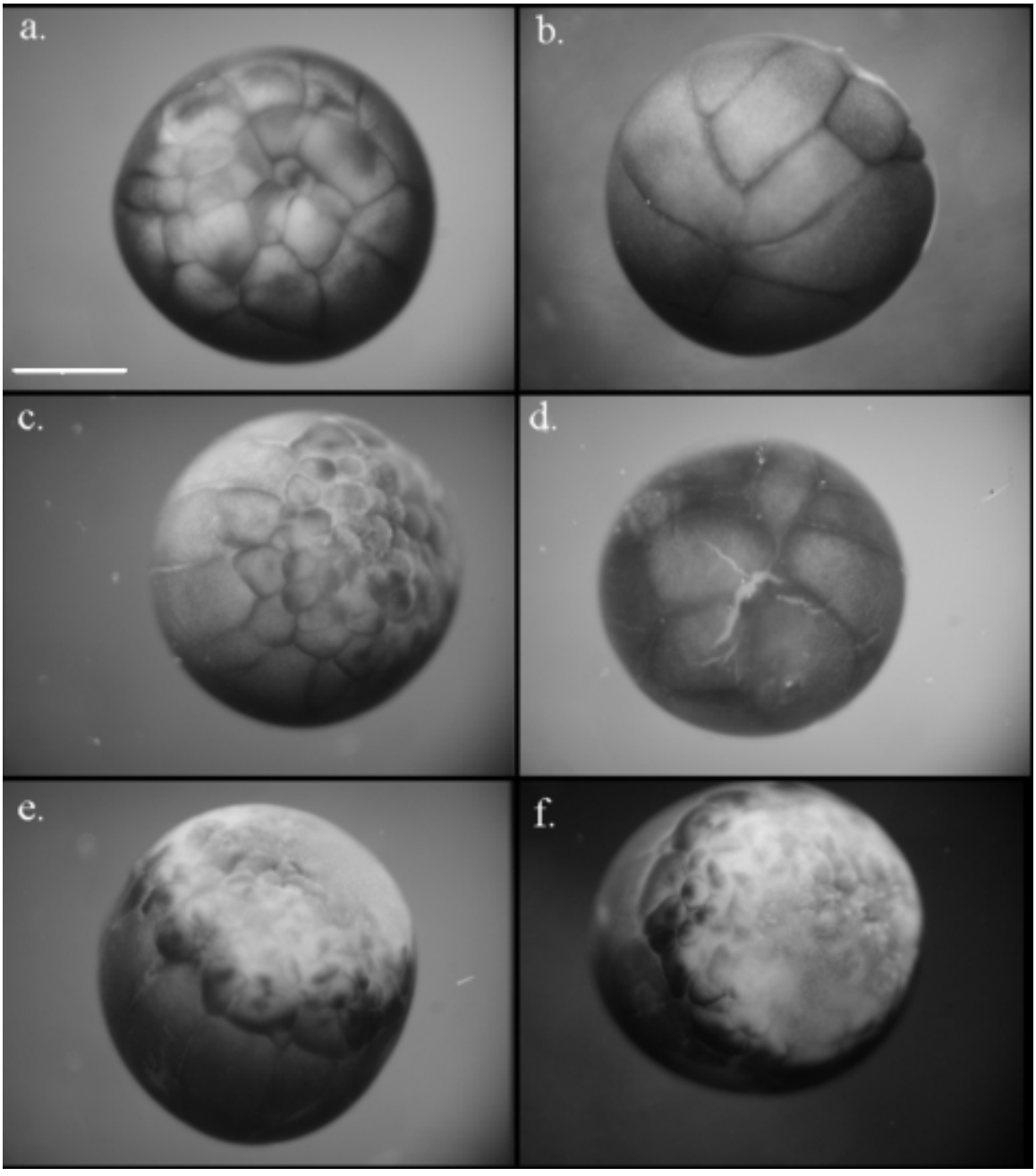
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Figure 22. Stages 11 and 12 of shovelnose sturgeon embryologic development (Blastulation). a. early blastula lateral view (Stage 11), b. Stage 11 lateral view, c. Stage 11 animal view, d. Late blastula animal view (Stage 12) e. Stage 12 lateral view, and f. Stage 12 vegetal view. 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

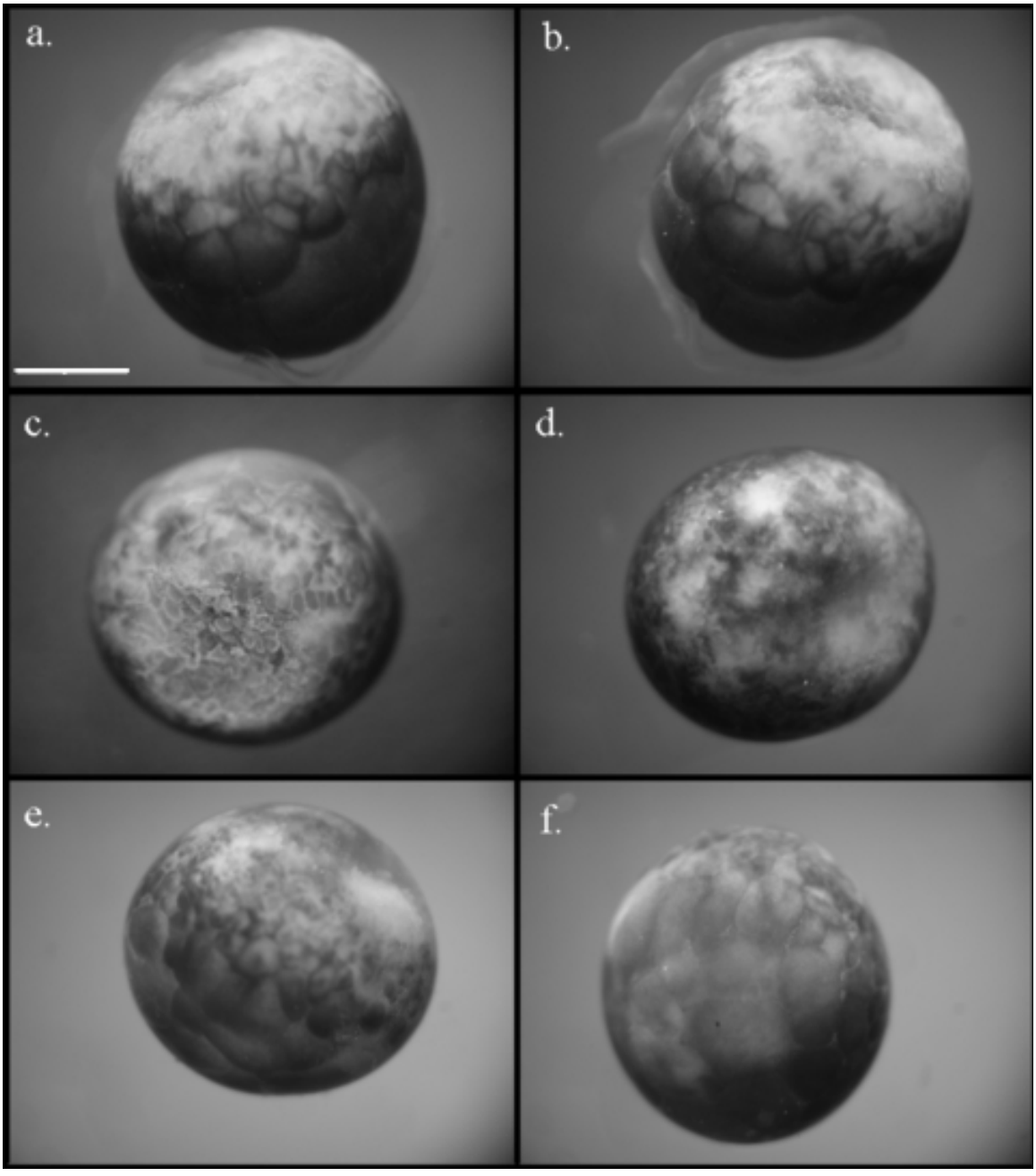
a.
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Figure 23. Stages 13 through 15 of shovelnose sturgeon embryologic development (Gastrulation I). a. onset of gastrulation lateral view (Stage 13), b. Stage 13 animal view, c. dorsal blastopore lip formation lateral view (Stage 14), d. Stage 14 animal view, e. epiboly covers 2/3 of embryo lateral view (Stage 15), and f. Stage 15 animal view. 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

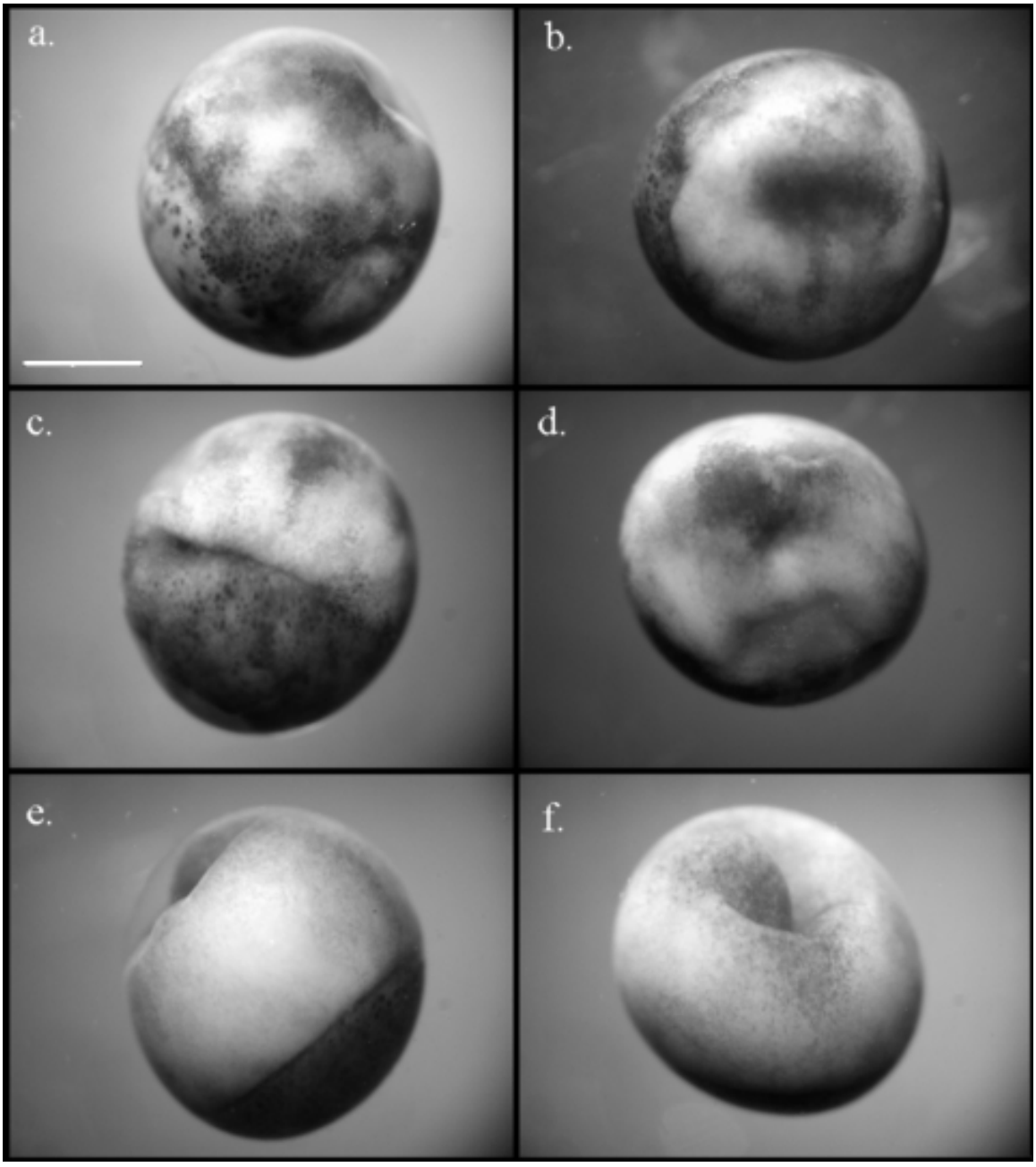
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Figure 24. Stages 16 through 19 of shovelnose sturgeon embryologic development (Gastrulation II and Neurulation I). a. Large yolk plug vegetal view (Stage 16) b. Stage 16 animal view, c. Small yolk plug vegetal view (Stage 17), d. Stage 17 animal view, e. Slitlike blastopore vegetal view (Stage 18), f. early neurula (Stage 19). 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

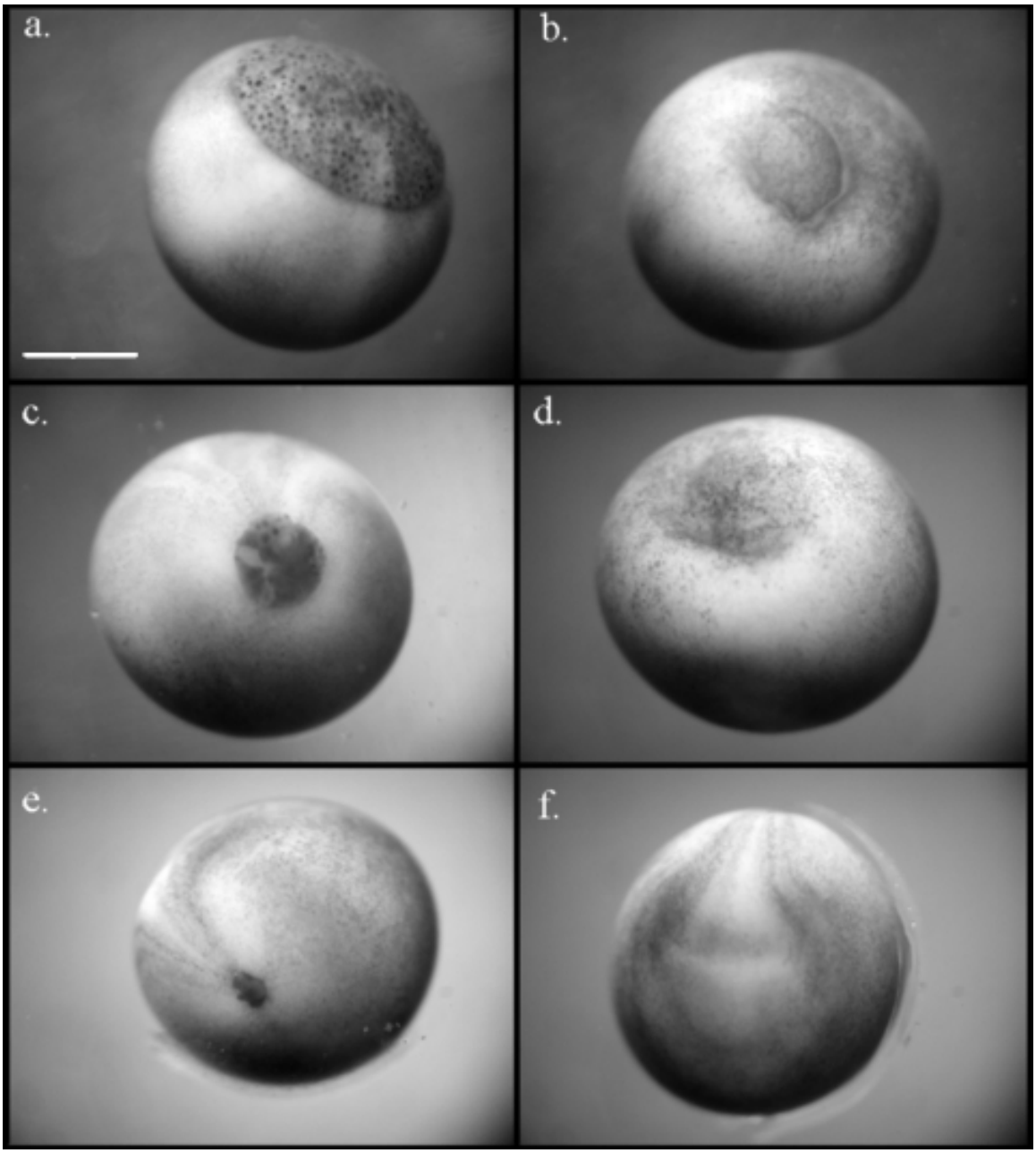
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Figure 25. Stages 20 through 22 of shovelnose sturgeon embryologic development (Neurulation II). a. wide neural plate lateral view (Stage 20), b. Stage 20 head view, c. excretory rudiments appear caudal view (Stage 21), d. Stage 21 head view, e. Late neurula tail view (Stage 22), and f. Stage 22 head view. 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

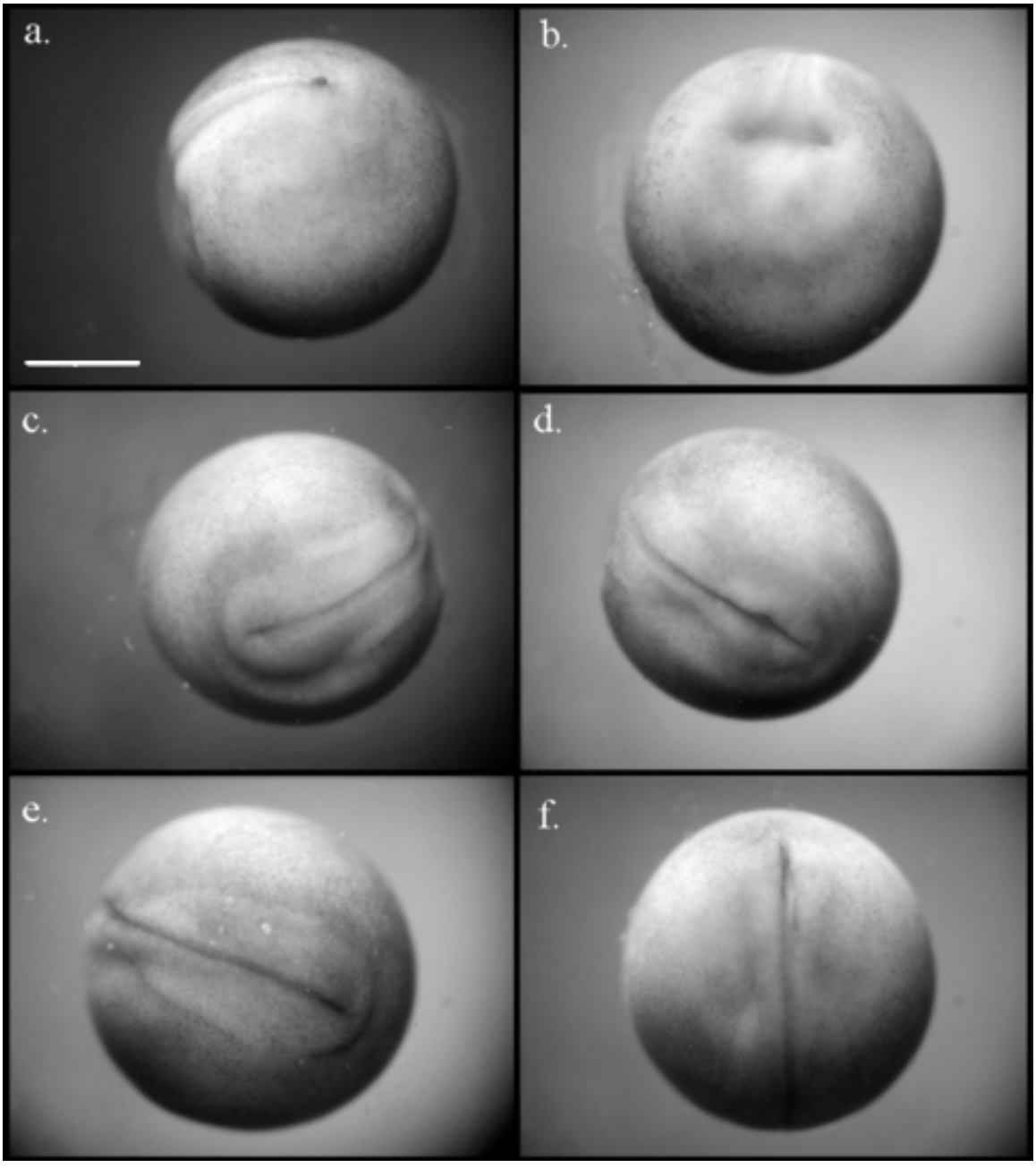
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Figure 26. Stages 23 through 25 of sturgeon embryologic development (Neurulation III) a. Closed neural tube dorsal view (Stage 23), b. neural suture head view (Stage 23), c. Thickening of excretory rudiment tail view (Stage 24), d. eye rudiments head view (Stage 24), e. Thickening of the tail view (Stage 25), and f. Lateral plates approach each other head view (Stage 25). 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

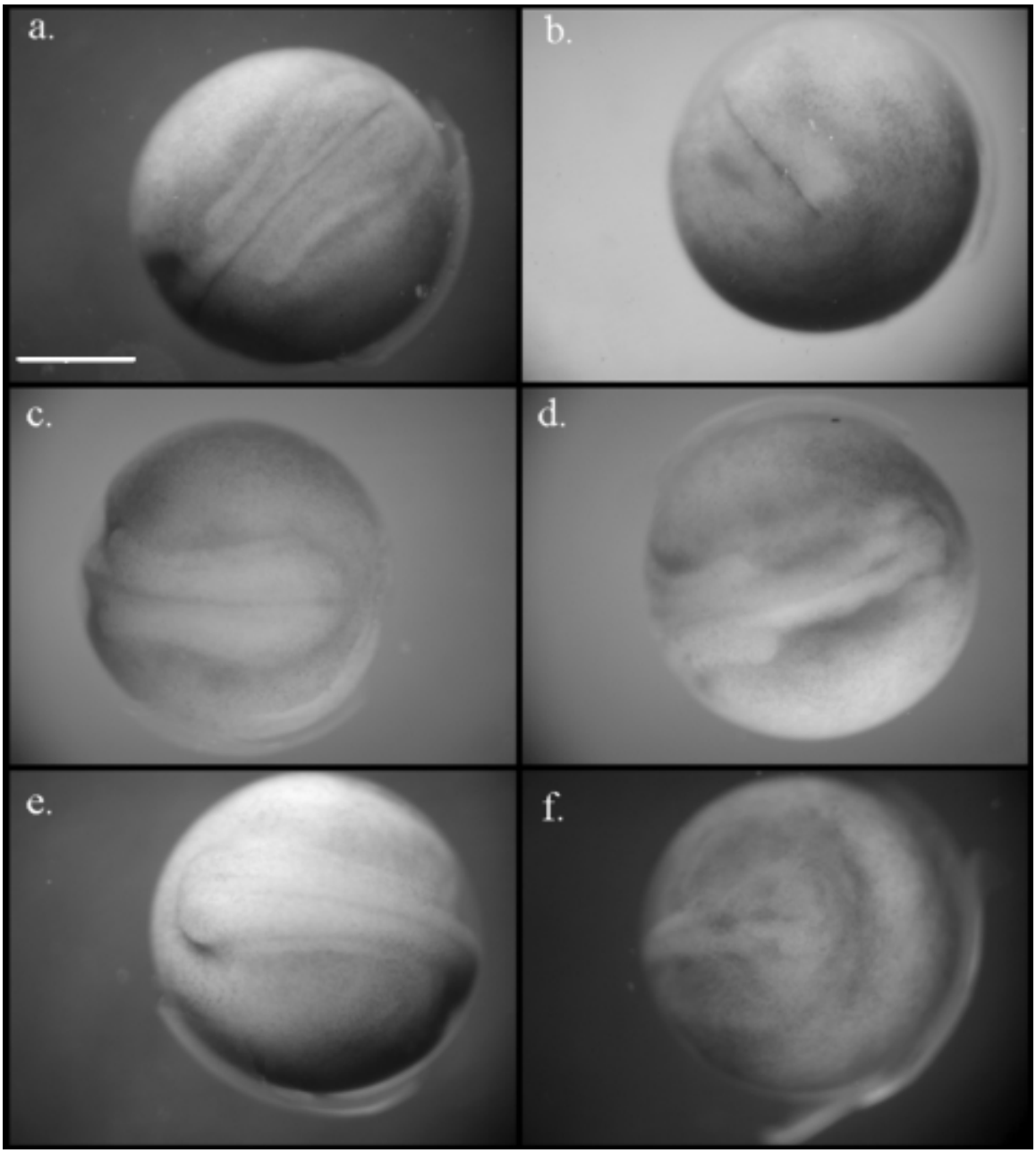
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Figure 27. Stage 26, through 29 of shovelnose sturgeon embryologic development (Heart Formation I). a. Tail separates from yolk membrane(Stage 26), b. lateral plates fuse head view (Stage 26), c. Tail elongates (Stage 27 view), d. Heart present as short tube (Stage 27 head view), e. pronephros wing like (Stage 28 dorsal view), and f. Heart present as a long tube (Stage 28 head view). 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

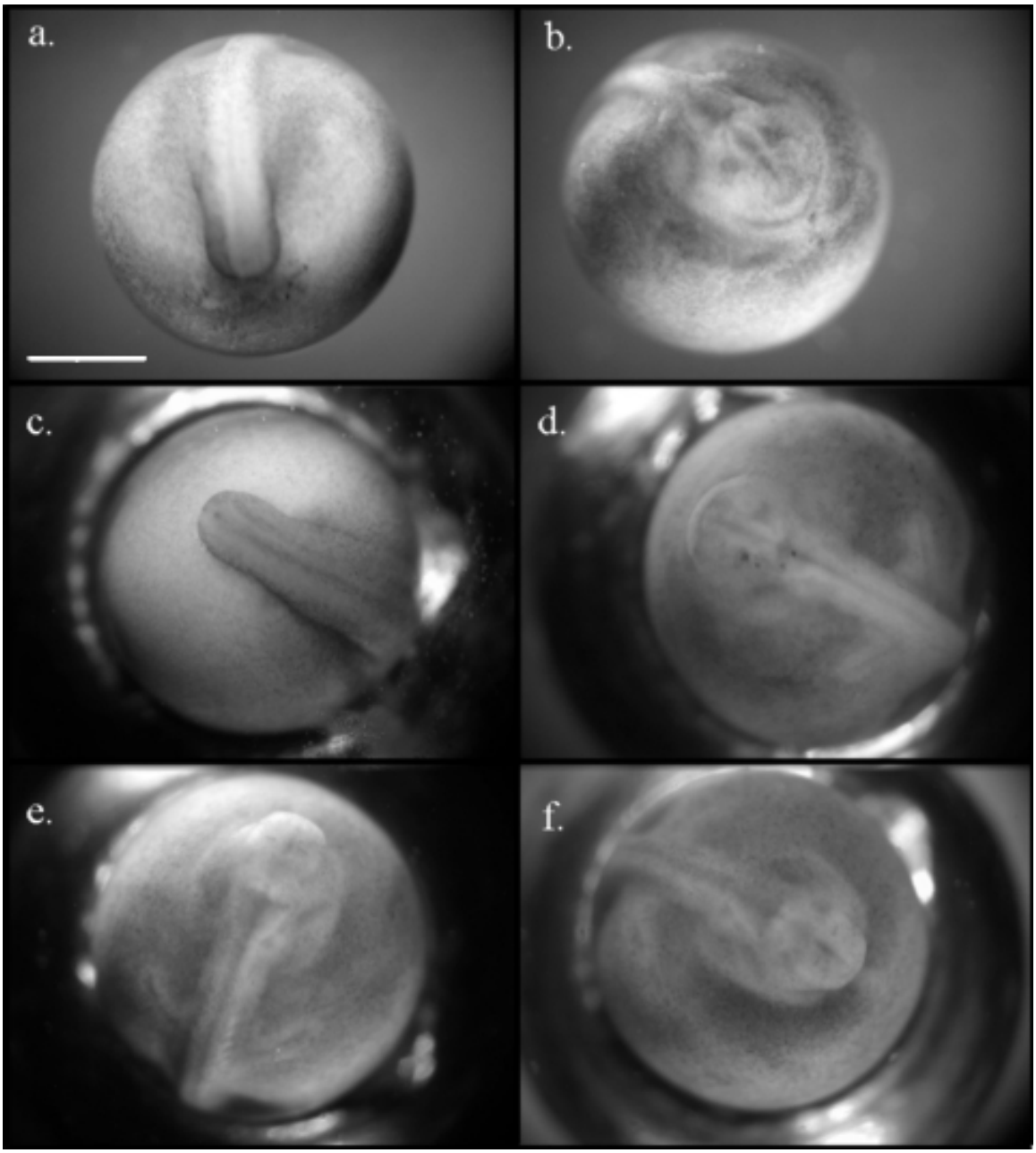
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Figure 28. Stages 29 through 31 of shovelnose sturgeon embryologic development (Heart formation II and Body development I). a. fin fold rudiment present (Stage 29 tail view), b. heart s-shaped (Stage 29 head view), c. fin fold formed (Stage 30 lateral view), d. head deepening (Stage 30 head view), e. Head separates from yolk membrane (Stage 31 lateral view), and f. Tail reaches heart (Stage 31 head view). 35x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

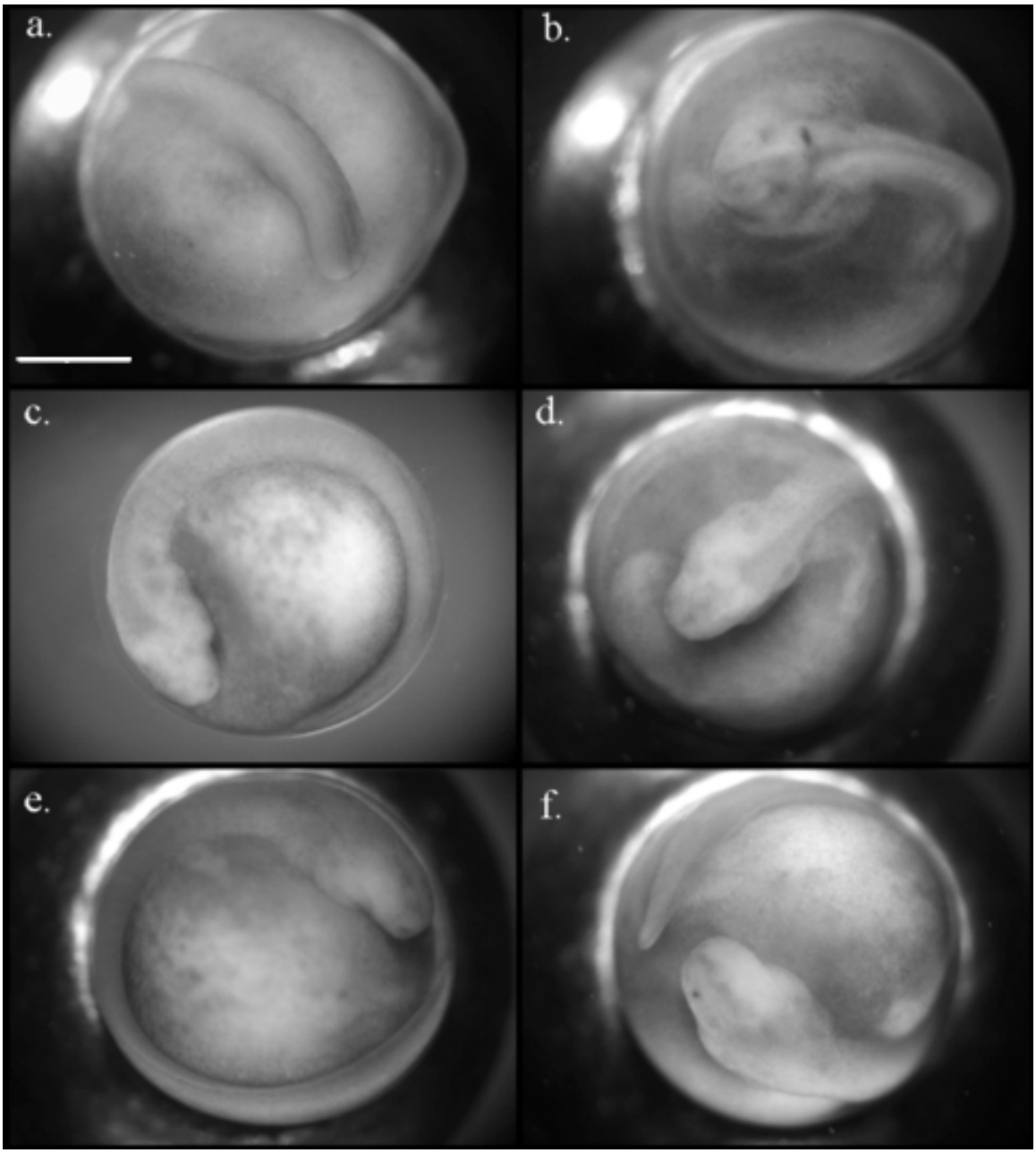
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Figure 29. Stages 32, 35, and 36 of shovelnose sturgeon development (Body elongation and hatch). a. Body bent to one side (Stage 32 dorsal view), b. Tail reaches head (Stage 32 head view), c. Developed embryo in envelope (Stage 35 head view), d. advanced embryo hatched (Stage 35 lateral view), e. Mass hatch (Stage 36 dorsal view), and F. Stage 36 (lateral view). a, b, and c 35x magnification; d, e, and f 20x magnification, scale bar = 1 mm. See Table 4 for a description of the stages.

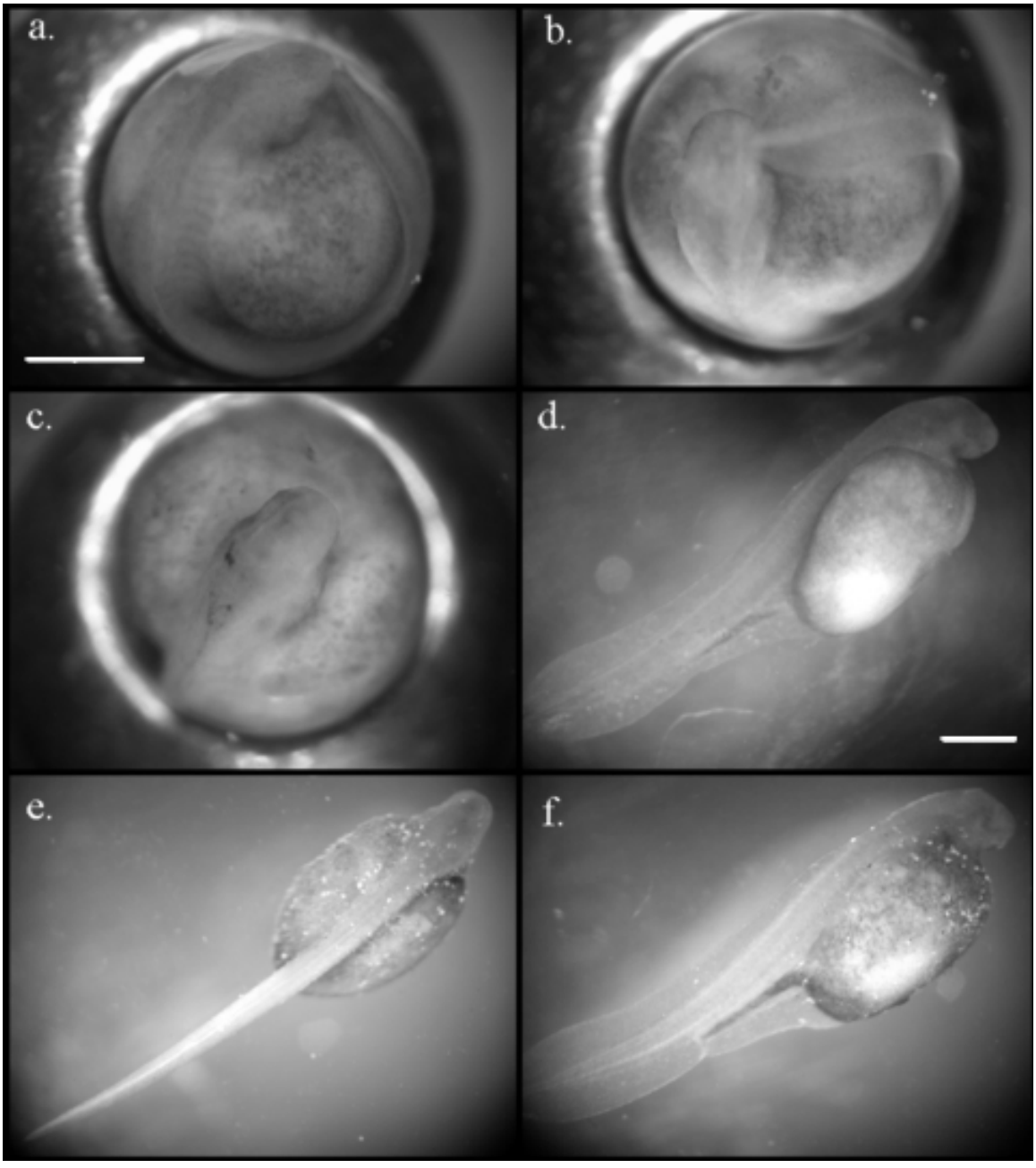
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Figure 30. Shovelnose sturgeon prolarvae; 18 hours (a, b), 24 hours (c, d) and 2 days post hatch (e, f). Magnification a = 25x, b = 45x, c,e = 20x, d,f = 35x; Scale bar = 1 mm.

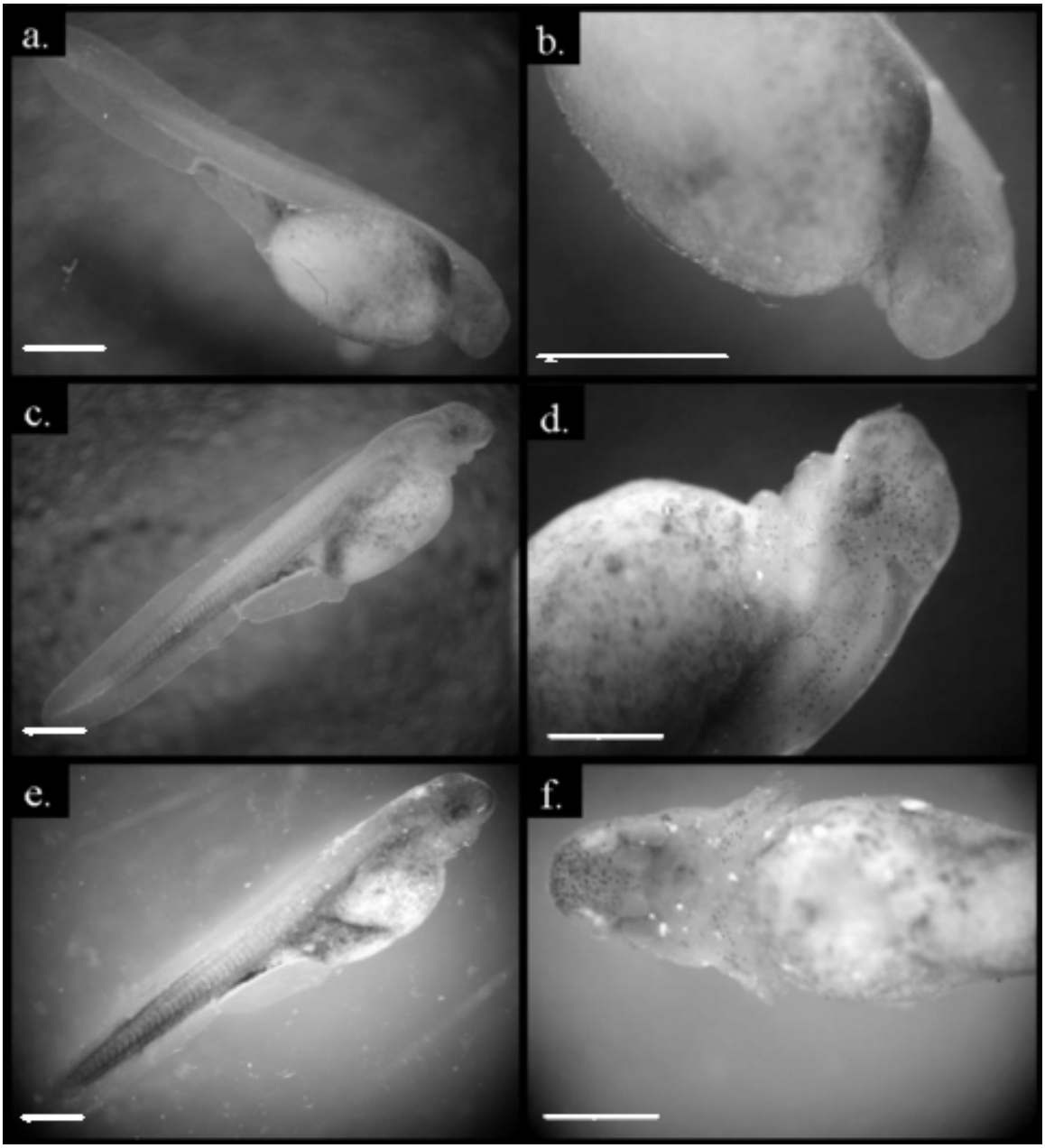
a.
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Figure 31. Shovelnose sturgeon prolarvae, 3 days (a,b), 4 days (c,d) and 5 days (e,f) post hatch. Magnification a,d,c,e = 25x, b,f = 45x; Scale bar = 1 mm.

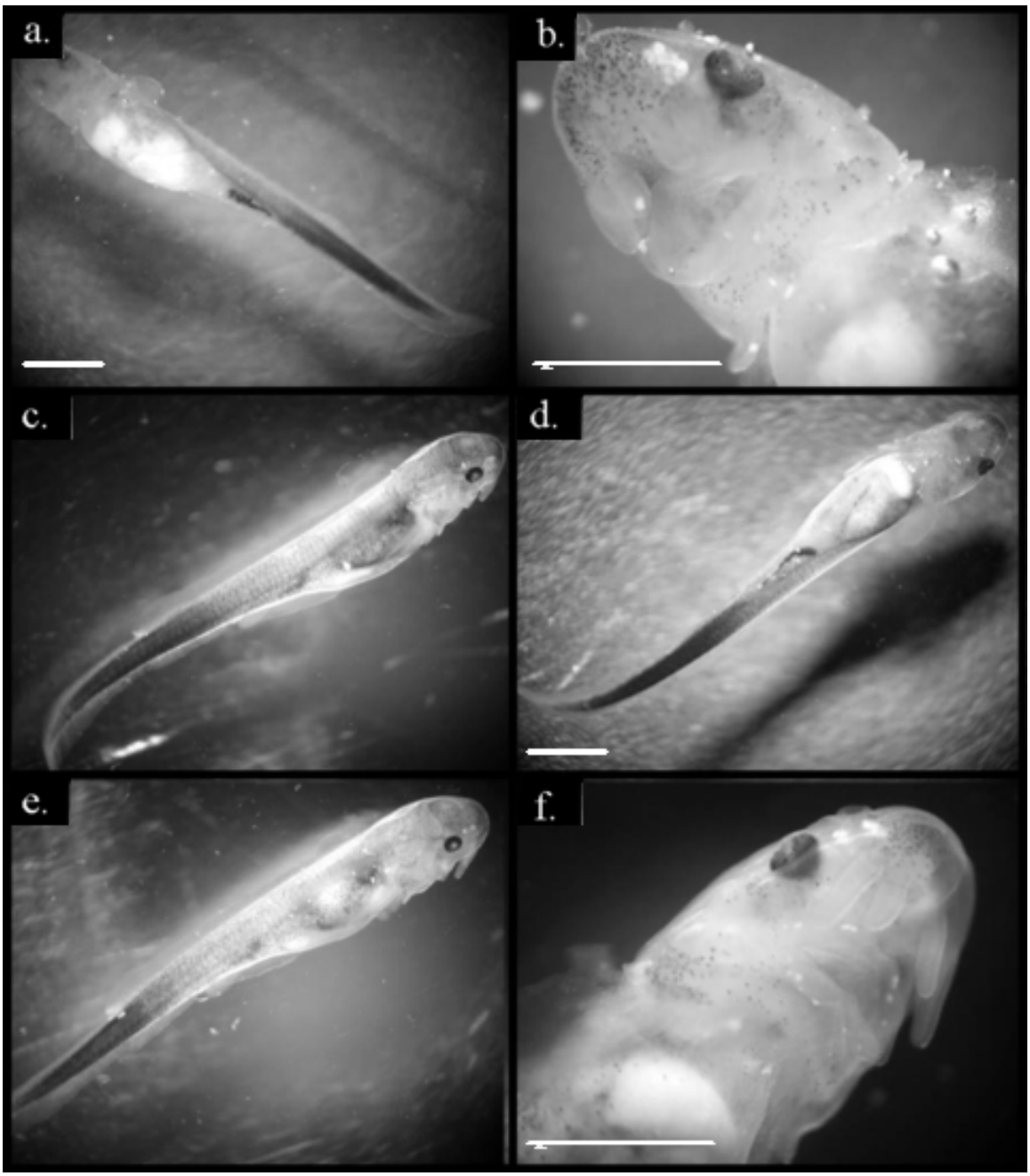
a.
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Figure 32. Shovelnose sturgeon prolarvae, 6 days (a,b), 7 days (c,d) and 8 days (e,f) post hatch. Magnification a,d,c,e,f = 25x, b = 45x; Scale bar = 1 mm.

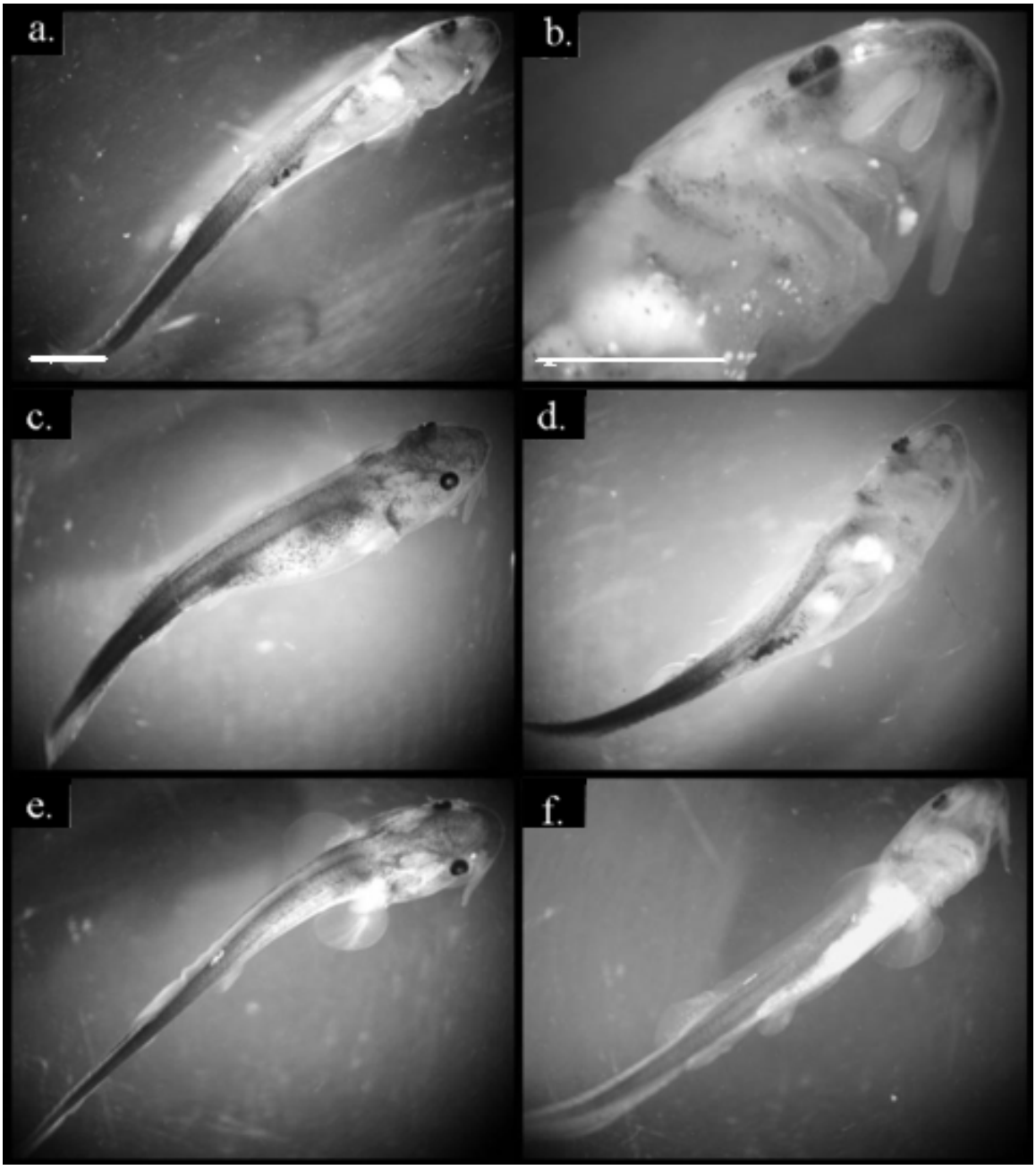
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Figure 33. Shovelnose sturgeon larvae, 13 days (a,b), 14 days (c,d) and 15 days (e,f) post hatch. Magnification 10x; scale bar = 1 mm.

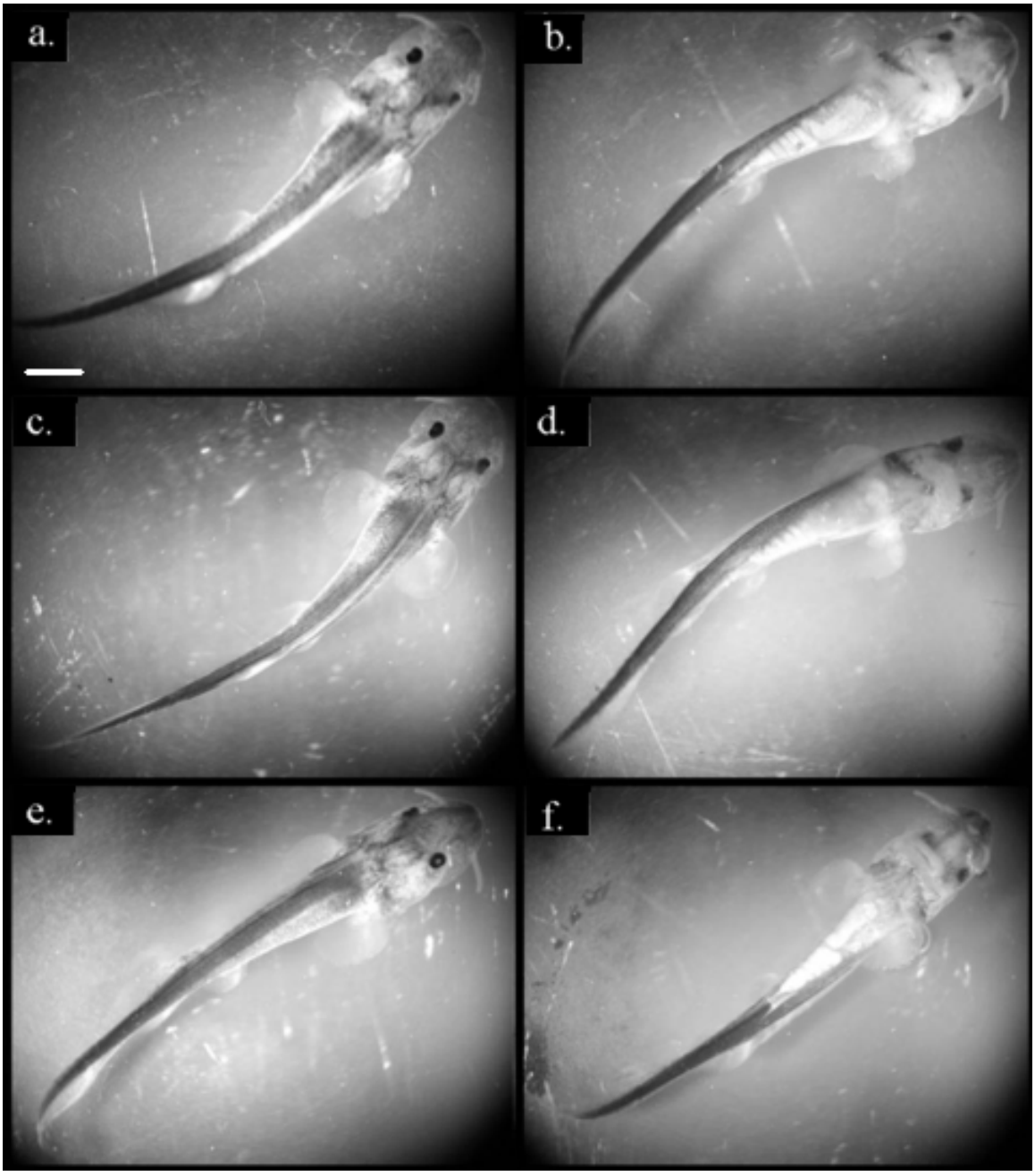
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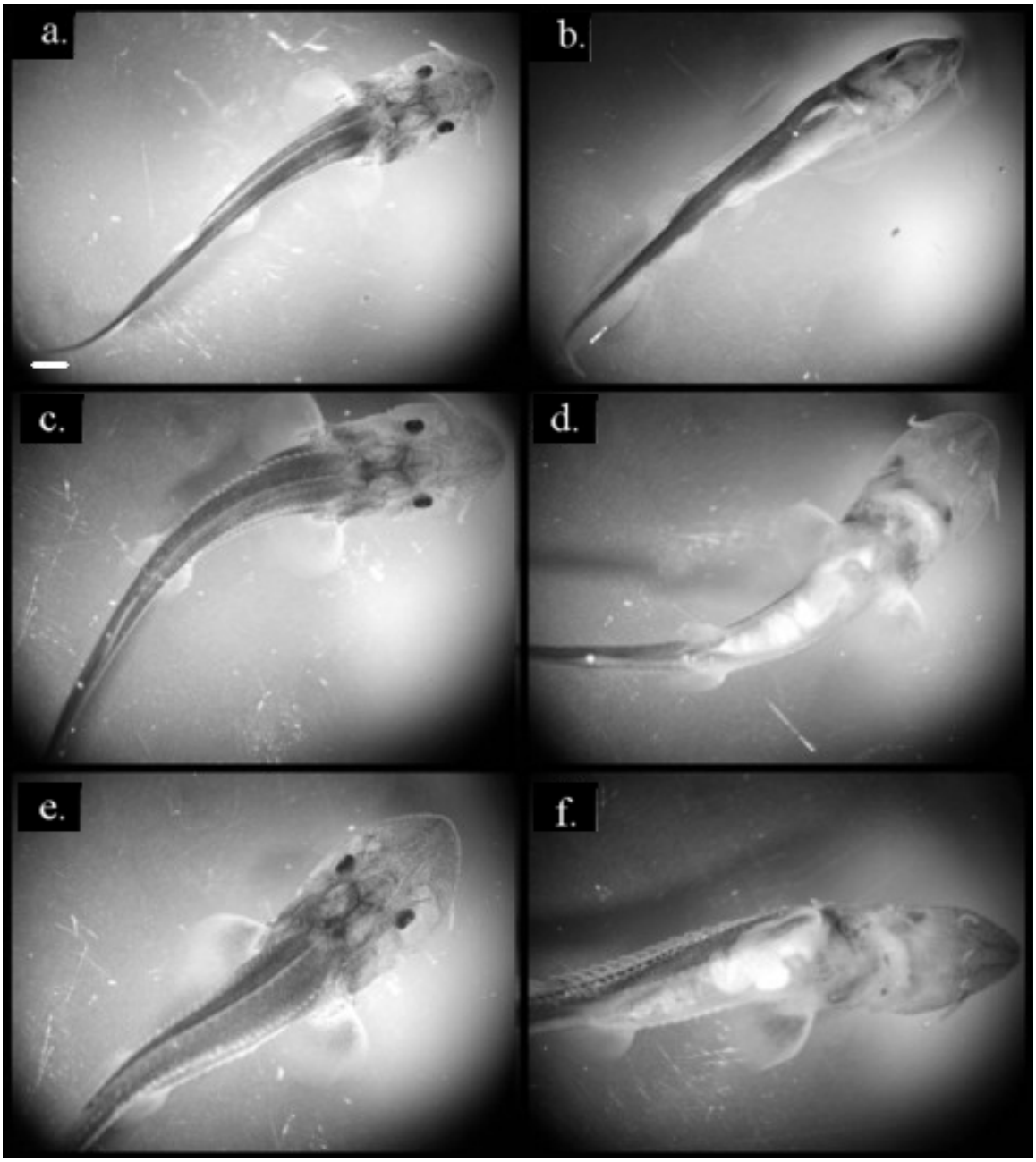
Figure 34. Shovelnose sturgeon larvae, 19 days (a,b),and juvenile, 21 days (c,d) and 27 days (e,f) post hatch. Magnification 7.5x; scale bar = 1 mm.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CONCLUSION

The shovelnose sturgeon is a commercially important fish species in the Midwestern United States. However, key pieces of life history information were lacking. To answer some of the life history questions regarding the shovelnose sturgeon, I was able to develop a non-invasive sex determination technique, and guides to the gonadal development and embryologic development of this species.

These tools will allow managers to effectively determine the sex ratios of shovelnose sturgeon in areas where commercial harvest still exists. Additionally, the guide to the reproductive development will allow the determination of the number of female spawners in a given year. This, coupled with the fecundity data that I collected, can contribute to the development of stage structured models so that population growth can be estimated. Finally, the results of my guide to embryologic development will aid culturists in the efforts currently underway to reintroduce the shovelnose sturgeon to its entire native range. For managers, the guide will allow an estimation of spawning date by using the staging data I provided coupled with river temperature data.

The major questions left unanswered for the shovelnose sturgeon and river sturgeon in general is the reproductive habitat and factors affecting recruitment. These questions need to be answered to allow for better conservation and management of these species. Although, anecdotal evidence suggests that the shovelnose sturgeon spawn over gravel in the main channel of large rivers, a project needs to determine those areas that provide adequate flow, depth, and substrate so that habitat restoration can focus on promoting the construction and maintenance of these areas. If spawning habitat is limiting, this will aid in increasing recruitment of the river sturgeon.

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VITA

Graduate School
Southern Illinois University

Robert E. Colombo Date of Birth: 05 December, 1972

110 S. Dixon Ave, Carbondale, Illinois 62901

State University of New York, College of the Environmental Science and Forestry
Bachelor of Science, Environmental and Forest Biology, May 1998

Special Honors and Awards:

Graduated magna cum laude from SUNY ESF
Who is Who in American Colleges and Universities 1998
Phi Kappa Phi National Honor Society

Thesis Title:

Reproductive Demographics and Early Life History of the Shovelnose Sturgeon (*Scaphirhynchus platorynchus*)

Major Professor: James E. Garvey

Publications:

Colombo, R.E., P.S. Wills, and J.E. Garvey. 2004. Use of ultrasound imaging to determine sex in the shovelnose sturgeon. North American Journal of Fisheries Management. 24: 322-326.