1-1-1995

Reproductive Biology of the Grass Pickerel Esox Americanus Vermiculatus Lesueur, from the Green Bottom Wildlife Management Area, Cabell County, West Virginia

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REPRODUCTIVE BIOLOGY OF THE GRASS PICKEREL, *ESOX AMERICANUS VERMICULATUS* LESUEUR, FROM THE GREEN BOTTOM WILDLIFE MANAGEMENT AREA, CABELL COUNTY, WEST VIRGINIA

A Thesis
Presented to
the Faculty of the Graduate School
Marshall University

In Partial Fulfillment
of the Requirements for the Degree
Masters of Science

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by
Ronald Clifton Tipton
May 1995

THIS THESIS WAS ACCEPTED ON April 14, 1995
Month Day Year

as meeting the requirement for the master's degree.

Advisor Dr. Donald Fonter
Department of Biological Sciences

Leonard J. Deutsch
Dean of the Graduate School 4-85-T

ACKNOWLEDGMENTS

Involved, long term, studies require the support and cooperation of many people. Without these people goals may be left unachieved. It is with this in mind that I express my deepest gratitude to Dr. Donald C. Tarter for allowing me to do this research and for his superior guidance during the study. I must also thank Dr. Thomas K. Pauley and Dr. Dan K. Evans for graciously consenting to serve on my graduate committee. Tremendous time and effort was also donated by undergraduate and graduate students working in the lab under Dr. Tarter. Special thanks to those students; Erica Midkiff, Lee Ann Mullins, Conley Marcum, Glenn Mills, Brent Johnson, and Kevin Yokum for their help in the field. I wish to convey special thanks to Matthew Wooten, a valued friend, who was present during most of the field trips. I must also thank Katie Lee Daniels and Daniel Chaffin for generously donating their time and expertise during the histological portion of this study. I wish to also thank Dr. Joy for his generous donation of time, expertise, and photomicroscopy equipment. Perhaps of greatest personal support to me during this study has been my family. Special thanks to my father, Ron Tipton, who encouraged me to enter the field of biology. Also, special thanks to my brother, Benjamin, and his wife, Donna, for their assiduous support. Finally, words cannot adequately express my gratitude to my wife, Debra, and mother, Patty, for their unconditional love and devotion. It is to them that I dedicate this thesis.

This project was supported by the Wetlands Research Program, Waterways Experimental Station (WES), Vicksburg, Mississippi, and the Huntington District, U.S. Army Corps of Engineers: Contract Number DAWC 60-93-R-0015.

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ABSTRACT

The grass pickerel, *Esox americanus vermiculatus* LeSueur, is listed as Undetermined on the Vertebrate Species List of Concern in West Virginia. The reason for the West Virginia status is that lentic, vegetated habitats required for spawning by the grass pickerel are limited within the state and are being reduced by agricultural, residential, and industrial developments. Green Bottom Swamp, a naturally occurring wetland of 58 hectares, provides habitat for the grass pickerel. A proposed habitat alteration to add marshland by building dykes prompted this study. Information from this investigation will establish a baseline for reproductive activities of the grass pickerel prior to potential habitat perturbation at Green Bottom Swamp. The spawning act was not observed during the reproductive period. It was thought to have occurred in aquatic vegetation associated with the buttonbush (*Cephalanthus occidentalis*) community in late February to early March. One ripe female containing primary (mature), secondary, and tertiary eggs was collected on 26 February 1993. The threshold temperature for spawning is approximately 9 °C. No yolk-sac larvae were found during the post spawning period. On 4 April 1995, 3 post yolk-sac larvae (18.0, 12.0, & 11.0 mm TL) were collected. One juvenile grass pickerel (24.2 mm TL) was collected on 10 March 1993 and 43 juveniles (22.9 to 67.5 mm TL; mean = 37.3 mm TL) were found on 21 April 1993. The nursery area for juveniles was beds of *Potamogeton crispus* (curly pondweed). The estimated number of

primary (mature) eggs was 3,167 (N = 1; 280 mm TL). The gonosomatic index (GSI) reached a maximum in late February (ripe female 4.31% and spent female 0.34%). Egg diameters (N = 50) ranged from 1.40 to 2.15 mm (mean = 1.79 mm). The estimated incubation time for egg development was 8-12 days. Seasonal gonad histology confirmed trends exhibited in seasonal GSI. Based on seasonal GSI, histology, and young - of - the - year, spawning occurred in late February to early March. Morphometrics varied significantly ($p < 0.05$) for length of pectoral ray, length of pelvic ray, and head length. Greater lengths were attributed to males. Meristics did not vary significantly ($p < 0.05$) between the sexes. Sex ratio did not deviate significantly ($p < 0.05$) from the expected 1:1 ratio.

CHAPTER I
INTRODUCTION

Objectives

The grass pickerel, *Esox americanus vermiculatus* LeSueur, is listed as Undetermined on the Vertebrate Species of Concern List in West Virginia (WVDNR, 1987). The reason for the West Virginia status is the lack of data and habitat destruction of lentic, vegetated spawning areas by agricultural, residential, and industrial developments (i.e., via pond and lake construction and habitat drainages and fillings).

Grass pickerel require flooded vegetation for spawning and nursery areas for their young - of - the - year (YOY). Green Bottom Swamp, a naturally occurring wetland, provides excellent habitat for their spawning activities. This type of habitat is rare, accounting for less than one percent of the state's total surface area, in the otherwise mountainous West Virginia. Additionally, personnel at the U. S. Army Corps of Engineers, Huntington District, are attempting to add new marshland (mitigation site) by building dykes at the downstream end of the property and pumping water from the Ohio River to fill them. Data from this investigation will provide information aiding in management strategies relating to grass pickerel. Additionally, little spawning or developmental information is available on the grass pickerel throughout its range (Wallus et al., 1990). The objectives of this investigation were:

(1) to elucidate the spawning activities of the grass pickerel in the Green Bottom Swamp,

(2) to establish baseline data for managing the reproductive activities of the local population,

(3) to determine any reproductive activities in the Green Bottom Mitigated Wetland, and

(4) to enhance the data base on the undetermined status of the grass pickerel in West Virginia.

Study Area

The Green Bottom Wildlife Management Area (GBWMA) is a 338 hectare area along the Ohio River in Cabell County, West Virginia (Figure 1) (Stark, 1993). Green Bottom Swamp is comprised of 58 hectares. The site is located in the Greenup Navigational Pool at approximately 82° 14'00"W, 35° 00'30"N. Terrestrial runoff and drainage controls, such as beaver dams and human construction, are constantly influencing the hydrology and wetland acreage of the swamp. There are four wetland types in GBWMA: (1) seasonally flooded flats, (2) inland open freshwater, (3) shrub swamp, and (4) wooded swamp. The U. S. Army Corps of Engineers, Huntington District, is currently constructing 29 ha of wetland habitat (mitigated area).

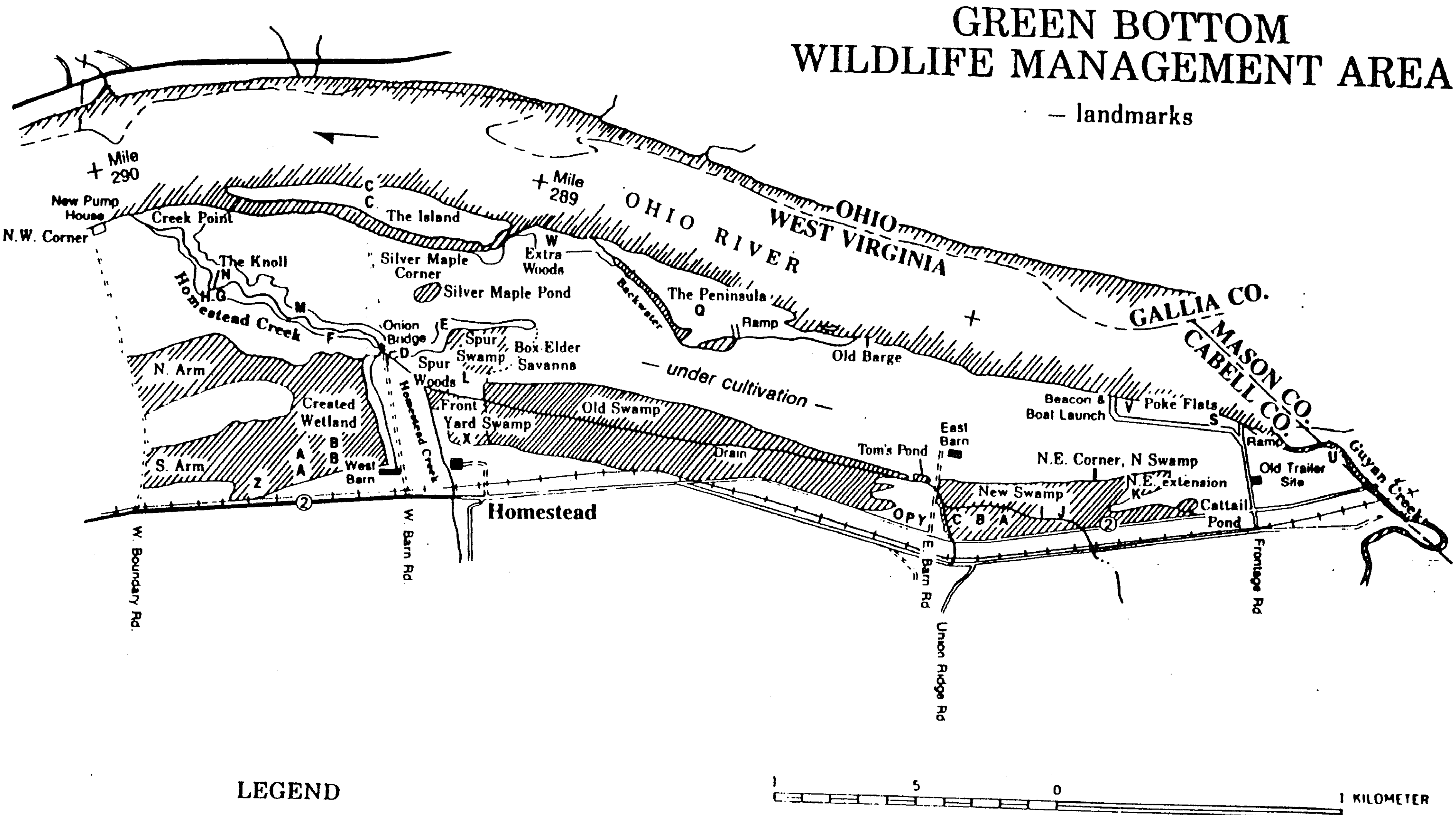
Cephalanthus occidentalis (buttonbush), a large wetland shrub, dominates the deepest and wettest areas, while *Lemna minor*, *Spirodela polyrhiza* and *Wolffia sp.* (duckweed) cover the surface water. *Carex spp.* (sedge) and *Juncus spp.* (rush) thrive in the swamp on the soft, marsh soil.

The GBWMA was purchased by the U. S. Army Corps of Engineers, Huntington District, to mitigate wetland loss incurred by placement of the Gallipolis Locks and Dams. The overall mitigation plan recommended the acquisition and management of the area for wildlife, hunting and fishing, as well as bird watching, photography, and nature study (WVDNR, 1991).

Figure 1. Map of Green Bottom Wildlife Management Area, Cabell County, West Virginia (Stark, 1993).

GREEN BOTTOM WILDLIFE MANAGEMENT AREA

— landmarks



LEGEND

Shaded areas are inundated.
Capital letters (**A**) are approximate transect locations.

Landmarks and Transects refer to:

Stark, T. J. 1993. Flora and Vegetation of Green Bottom Wildlife Management Area, W.Va., unpublished master's thesis, Marshall University, Huntington, W.Va.

CHAPTER II
TAXONOMY AND DISTRIBUTION

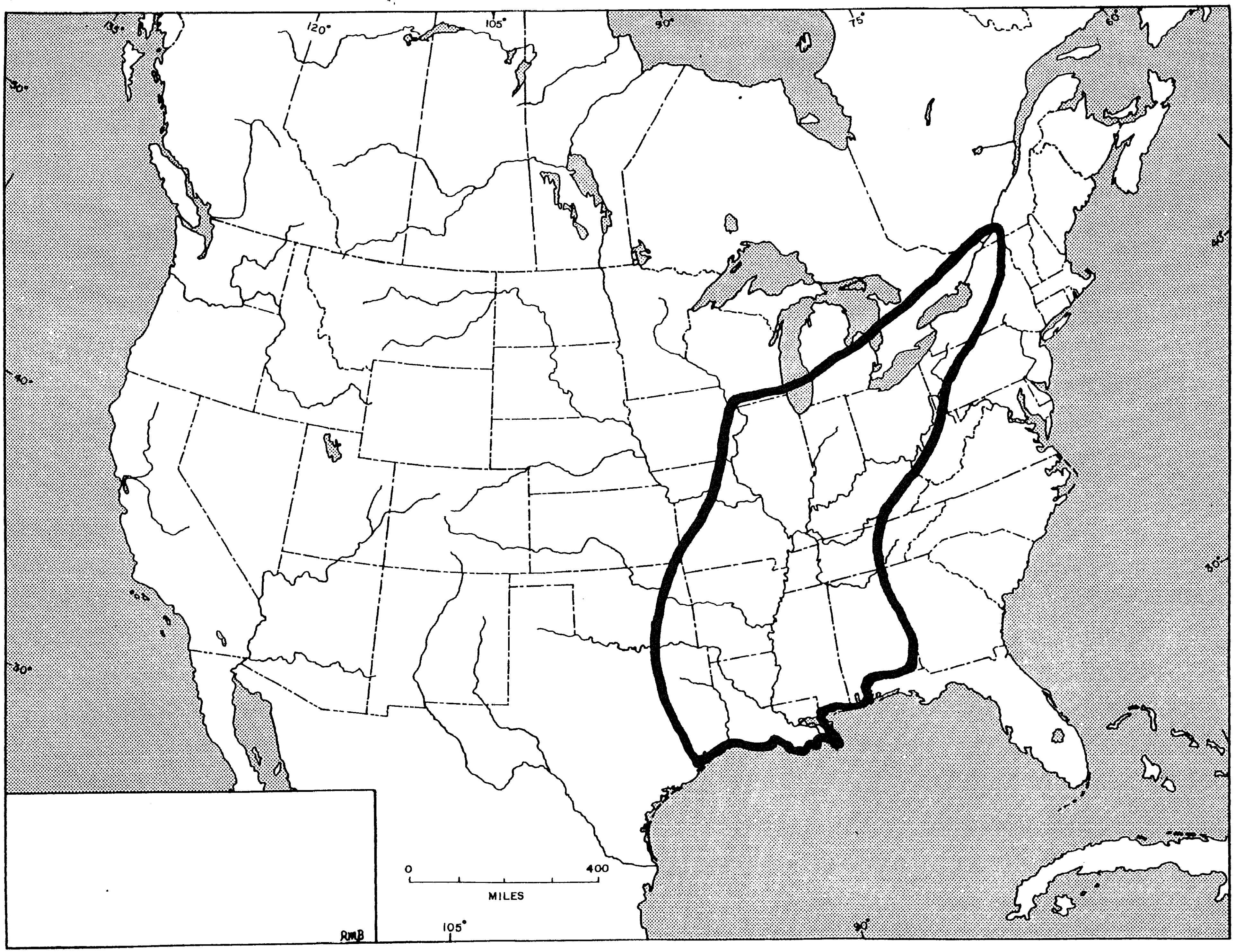
Taxonomy

The family Esocidae contains a single genus *Esox* which includes five extant species (Nikolski, 1961): *Esox reicherti* Dybowski (Amur pike), *E. masquinongy* Mitchell (muskellunge), *E. lucius* L. (northern pike), *E. niger* LeSueur (chain pickerel) and *E. americanus* Gmelin (redfin pickerel). *Esox americanus* was reported to include two subspecies, *E. a. americanus* Gmelin (redfin pickerel) and *E. a. vermiculatus* LeSueur (grass pickerel) (Legendre, 1954). Legendre's classification was accepted in the American Fishery Society Special Publication No. 6 (1970) and is the basis for the subspecific designation in this report.

Distribution

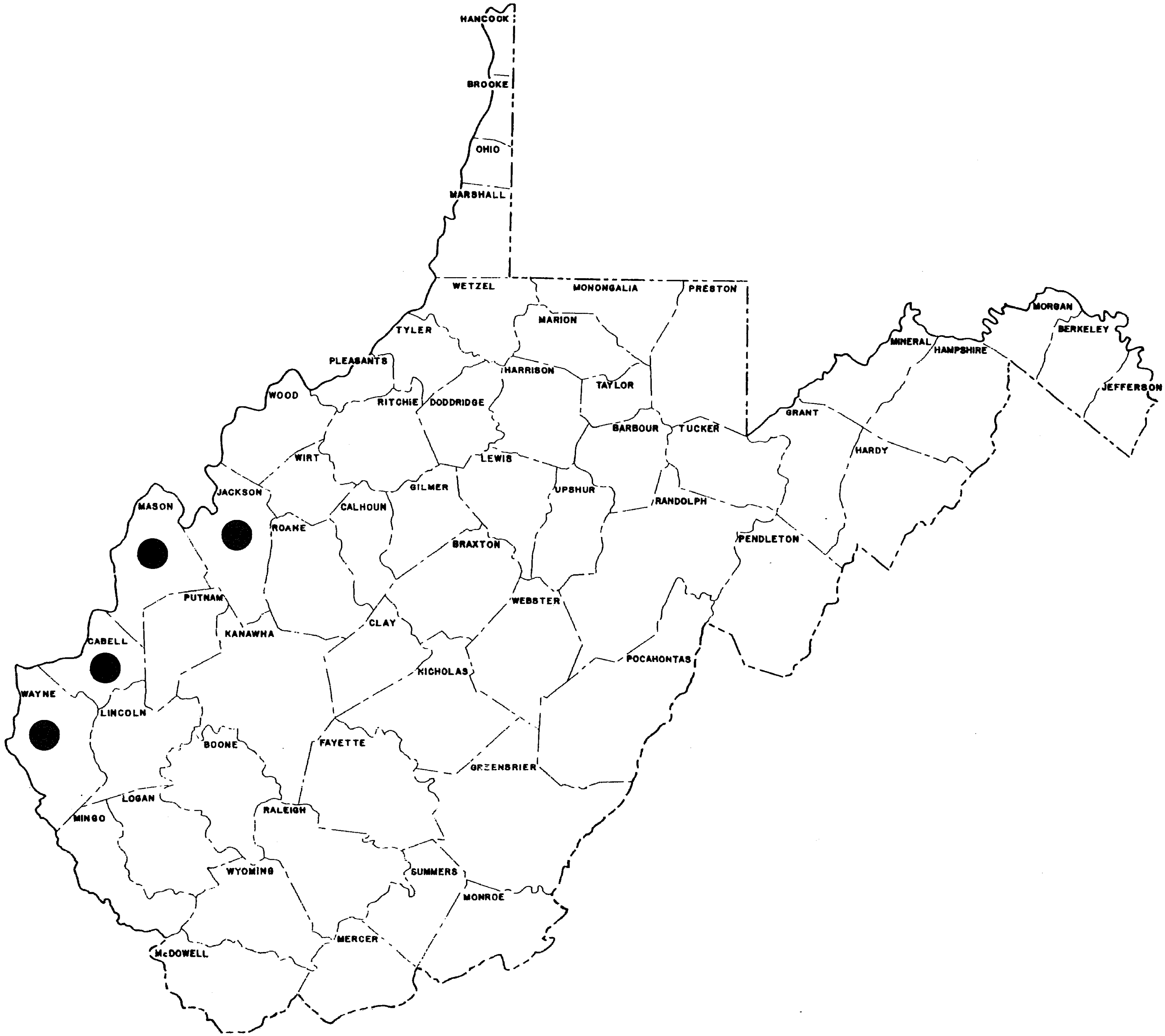
The grass pickerel occurs throughout the Mississippi River drainage, from Wisconsin and southern Michigan, east along the Ohio River to western Pennsylvania and New York; in southern Ontario and Quebec; south through eastern Iowa, Missouri, and Oklahoma to the Brazos River of Texas; and east through the gulf states to Georgia (Wallus et al., 1990) (Figure 2). In West Virginia, the distribution is limited to four counties (Cabell, Jackson, Mason, and Wayne) along the Ohio River (Figure 3).

Figure 2. North American distribution of the grass pickerel.



120°
105°
90°
75°
60°
40°
30°
0
400
MILES
105°
90°
RMB

Figure 3. West Virginia distribution of the grass pickerel.


HANCOCK
BROOKE
OHIO
MARSHALL
WETZEL
MONONGALIA
PRESTON
MARION
TYLER
PLEASANTS
HARRISON
TAYLOR
MORGAN
BERKELEY
MINERAL
HAMPSHIRE
JEFFERSON
WOOD
RITCHIE
DODDRIDGE
BARBOUR
TUCKER
GRANT
HARDY
WIRT
LEWIS
JACKSON
GILMER
UPSHUR
RANDOLPH
MASON
CALHOUN
ROANE
PENDLETON
BRAXTON
PUTNAM
WEBSTER
CLAY
CABELL
KANAWHA
POCAHONTAS
NICHOLAS
WAYNE
LINCOLN
BOONE
FAYETTE
GREENBRIER
LOGAN
MINGO
RALEIGH
WYOMING
SUMMERS
MONROE
MERCER
McDOWELL

Habitat

McClane (1965) reported that the grass pickerel prefers shallow, low gradient environments with a soft bottom covered with a heavy growth of aquatic vegetation. Trautman (1981) reported the grass pickerel to occur in marshes, bogs, ponds, oxbows, and slow moving streams in Ohio. Pflieger (1975) reported that the grass pickerel's preferred habitat is clear, slow moving and heavily vegetated waters such as marshes, back channels, oxbows, and bogs. Highest population densities are found in shallow, weedy areas with plants such as leafy liverworts, pondweeds, water lilies, filamentous algae, and broadleaf cattails (Becker, 1983).

CHAPTER III

MATERIALS AND METHODS

Field Studies

Collections

Seasonal collections of grass pickerel were made on the following dates (number of fish in parenthesis): Winter/26 February 1993 (2); Spring/21 April 1993 (48); Summer/20 July 1993 (21); and Fall/20 October 1993 (16). Methods of collection included trapping, electrofishing, and seining. Seining was the most successful method. The predatory nature of grass pickerel and the vast unrestricted area of the swamp rendered the use of traps and electrofishing inefficient. Upon collection pickerel were stored on ice and later preserved in 10 percent formalin solution.

Water Quality and Temperature

On each seasonal collection date, temperature (°C) and the following water quality parameters were measured using a Hach chemical kit (Model 36-WR): dissolved oxygen (mg/L); alkalinity (mg/L $CaCO_3$); CO_2 (mg/L); total hardness (mg/L $CaCO_3$); total acidity (mg/L); and pH. Temperatures were measured with a maximum - minimum thermometer.

Spawning

In an attempt to observe spawning activities, 21 trips were made to the swamp between 28 February and 18 April 1994. Grass pickerel of breeding age were not collected during February and

April of 1994. Further attempts were made to observe spawning actives between 17 February and 4 April 1995. On 4 April 1995 several young - of - the - year individual grass pickerel were collected from the Green Bottom Wildlife Management Area.

Larvae and Juveniles

Several attempts were made to collect larvae (10 - 21 mm TL) and juveniles (> 22 mm TL) in the field during March and April (1993-1995). Long-handled dredges with a fine mesh were used to sample young - of - the - year in submerged aquatic vegetation.

Laboratory Studies

Fecundity

The number of eggs was estimated using the volumetric method (Lagler, 1956). A sample of eggs was blotted dry on a paper towel. The volume of the sample was obtained by water displacement in a 50 ml burette (0.1 ml increments). Similarly the volume of the entire lot of eggs, including the sample, was obtained in a burette. The total number of eggs was estimated by the following equation:

$$X:n = V:v$$

where X = unknown total number of eggs in the lot, n = number counted in the sample, V = total displaced volume of all eggs, and v = volume of the sample.

Sexual Maturity

One sexually mature female (280 mm TL) was collected during the study on 26 February 1993. An additional spent female (240 mm TL) was collected on 26 February 1993. No sexually mature males were encountered. Total lengths were measured with a standard fish measuring board.

Gonosomatic Index (GSI)

In order to determine the seasonal GSI, gonads were removed from 32 grass pickerel. They were blotted dry on a paper towel and weighed on a Mettler balance (nearest 0.001g). Similarly the grass pickerel were blotted dry, and weighed on the balance (nearest 0.01 g). The GSI was determined by dividing the gonad weight by the body weight (X100). Sex was determined based on gonad morphology of specimens collected in the fall and winter seasons. Juveniles collected during the spring and summer seasons could not be visually sexed, due to their small size and lack of development, and were therefore grouped together.

Egg Diameters and Incubation

In order to determine the egg diameters, 50 mature eggs were removed from an ovary. Eggs were separated and measured with a dial vernier caliper (nearest 0.001 mm). Based on primary eggs in the sexually mature female and the appearance of larval fish in the field, the length of the incubation period was estimated for the local population.

Seasonal Histology

Histological sections of testes and ovaries were prepared for comparison with seasonal GSI. Representative individuals from each season provided either testicular or ovarian tissue. Tissue samples were then dehydrated in a butanol series followed by embedding in paraffin (Brauer, 1961; Johansen, 1940). Embedded tissues were then cross - sectioned (10 μm) with a sliding blade microtome (American Optical Co. Model 820) equipped with a razor blade. Sectioned tissues were mounted onto slides and stained with saffranin and fast green (Holbrook, 1975; Johansen, 1940). One male and one female from each season exhibiting the greatest development was selected and photographed under magnification (100X, 250X, and 400X) using Zeiss photomicroscopy equipment. Seasonal comparisons were made of oocyte and spermatozoa development.

Morphometrics

In order to determine sexual dimorphism, measurements were made on 10 individuals of each sex. Dividers and a metal millimeter ruler (nearest 0.1 mm) were used to obtain measurements. Measurements pertaining to the head were divided by head length. All other measurements taken, except standard length, were divided by standard length. Standard length values were obtained by dividing the measurement by total length. Manipulation of the values in this manner was necessary to compensate for differing age and growth within the sample group. Statistical analysis included ANOVA and t - tests ($p < 0.05$) and were performed with KWIKSTAT

3.3 computer software. Measurements taken were based on Hubbs and Lagler (1958):

Total Length - The greatest dimension between the most anteriorly projecting part of the head and the farthest tip of the caudal fin when the caudal rays are squeezed together.

Standard Length - The distance from the most anterior part of the head backward to the end of the vertebral column or caudal base.

Body Depth - The greatest dimension, exclusive of fleshy or scaly structures, which pertain to the fin bases.

Depth of Caudal Peduncle - The least depth of the caudal area before the caudal base.

Length of Caudal Peduncle - The oblique distance between the end of the anal base and the hidden base of the middle caudal ray.

Predorsal Length - The distance from the tip of the snout or upper lip to the structural base of the first dorsal ray.

Length of Dorsal/Anal Base - The greatest overall basal length, extending from the structural base of the first ray to the point where the membrane behind the last ray contacts the body.

Length of Anal Ray - The distance from the structural base of the longest ray to its tip.

Length of Pelvic Ray - The distance from the extreme base of the uppermost, outermost, or anteriormost ray to farthest tip of the fin, filaments, if any, included.

Length of Pectoral Ray - The distance from the middle of the base of the fin (if the longest ray is at or near the middle of the fin) to the farthest tip of the fin.

Head Length - The distance from the most anterior point on the snout or upper lip to the most distant part of the opercular membrane.

Depth of Head - The distance from the midline at the occiput vertically downward to the ventral contour of the head or breast.

Head Width - The greatest dimension when the opercles, if dilated, are forced into a reasonably normal position.

Snout Length - The distance from the most anterior point on the snout or upper lip to the front margin of the orbit.

Postorbital Length of Head - The greatest distance between the orbit and the membranous opercular margin.

Height of Cheek - The least distance from the orbit downward to the lower edge of the anterior arm of the preopercle.

Length of Cheek - The distance from the most posterior point of the preorbital (lachrymal) horizontally backward to the caudal margin of the preopercle, including spines if present approximately along this horizontal.

Interorbital Widths - 1) Least fleshy width - the dividers are not squeezed at all or 2) Least bony width - the points are pressed tightly against the bone so as to eliminate the thickness of the flesh overlying the bony rims.

Length of Orbit - The greatest distance between the free orbital rims; often oblique.

Length of Upper Jaw - The distance from the anteriormost point of the premaxillary to the posteriormost point of the maxillary.

Width of Gape - The greatest transverse distance across the opening of the mouth.

Meristics

Counts of scales and rays were performed on 10 individuals of each sex to determine any sexual dimorphism in grass pickerel. Counts included lateral line scales, scale rows above and below the lateral line, and scale rows around the caudal peduncle. Ray counts included the dorsal, pectoral, pelvic, and anal rays (Hubbs and Lagler, 1958). Sexual dimorphism was determined statistically using ANOVA and t - tests ($p < 0.05$). KWIKSTAT 3.3 computer software was used for statistical analysis of results.

Sex Ratio

A chi-square test was used to determine any deviation from the expected 1:1 sex ratio (0.05 confidence level).

Spawning

Several attempts were made during the spawning season (1994 & 1995) to collect mature males and females for observation on spawning behavior in a "Living Stream". No adults were found for laboratory observations of spawning activities.

CHAPTER IV
RESULTS AND DISCUSSION
Field Studies

Water Quality and Temperature

Water quality parameters are summarized in Table 1. The water temperature in Green Bottom Swamp fluctuated from 0.0 to 27.8 °C, winter (February) and Summer (July), respectively; the mean was 13.9 °C. Ice covered the area for portions of January and February. Dissolved oxygen values ranged from 2 to 6 mg/L, summer and winter, respectively; the mean was 4.3 mg/L. Total acidity was zero in all seasons. Carbon dioxide values ranged from 10 to 20 mg/L, fall and spring/winter, respectively; the mean was 16.3 mg/L. Total alkalinity values ranged from 51 to 102 mg/L $CaCO_3$, summer and spring, respectively; the mean was 85.0 mg/L $CaCO_3$. Total hardness values ranged from 102 to 123 mg/L $CaCO_3$; the mean was 11.6 mg/L $CaCO_3$. The pH values ranged from 6.5 to 8.0, fall and winter, respectively; the mean was 7.1.

Spawning

Spawning Act - No grass pickerel were observed in the act of spawning during the study period (1993 - 1995). Apparently, the spawning activities are secretive. Collections of naturally spawned eggs have not been reported from the Ohio River drainage (Wallus et al., 1990). Attempts made to observe spawning behavior during the

Table 1. Seasonal water quality and temperature data from the Green Bottom Wildlife Management Area, Cabell County, West Virginia (1993).

	SEASONS			
PARAMETERS	Winter (Feb.)	Spring (April)	Summer (July)	Fall (Oct.)
Temperature (°C)	0.0	17.8	27.8	10.0
Dissolved oxygen (mg/L)	6.0	4.0	2.0	5.0
Total acidity (mg/L)	0.0	0.0	0.0	0.0
Carbon dioxide (mg/L)	20.0	20.0	15.0	10.0
Alkalinity (mg/L $CaCO_3$)	102.0	119.0	51.0	68.0
Total hardness (mg/L $CaCO_3$)	119.0	123.0	119.0	102.0
pH	8.0	7.2	6.7	6.5

spawning season in 1995 failed in spite of numerous efforts during both daylight and nighttime hours.

According to Raney (1959), single females are accompanied by several males during the spawning act. Eggs and milt are ejected at intervals by lashing of the caudal fin. Eggs are broadcast over vegetation (Crossman, 1962). Scott (1954) and Ming (1968) reported that nest building and parental care of young - of - the - year does not occur.

Early spawning is thought to be an adaptation for highly piscivorous fishes. Larval and juvenile grass pickerel are thought to feed on the larvae of later-spawning fishes.

<u>Location</u> - Spawning is suspected to occur in the aquatic vegetation associated with buttonbush. Generally, grass pickerel spawn over aquatic vegetation, moss, leaves, and twigs in sloughs, temporary flood plains, marshes, and shallow vegetated pools of tributary streams (Kleinert and Mraz, 1966; Crossman, 1962; and Scott and Crossman, 1973). Sometimes grass pickerel migrated from lakes into small stream for spawning activities (McNamara, 1937).

<u>Season</u> - Based on young - of - the - year growth and development and water temperature data, spawning has been determined to occur in late February to early March in Green Bottom Swamp. One gravid female was collected, in a trap, on 26 February 1993. The female was ripe and contained primary, secondary, and tertiary eggs. Another female was collected, in a trap, on 26 February 1993. The female was spent and contained secondary and tertiary eggs and only a few primary eggs. This

suggests that the two females were collected during the spawning period.

McNamara (1937) reported that the grass pickerel is one of the earliest fish to spawn in spring. Larval collections from the Big Sandy River in Tennessee in early April suggested a late February/March spawn (Wallus et al., 1990). Ming (1968) reported that grass pickerel spawned in late February/March in Oklahoma, while Kleinert and Mraz (1966) noted a mid-March to mid-April spawning season in Wisconsin. Evans (1972) collected pickerel fry (12) from an oxbow lake near Shoals, West Virginia, on 9 May 1971. He suggested spawning occurred in April. On 4 April 1995, 3 post yolk - sac larvae (10 - 21 mm TL) were collected from the Green Bottom Wildlife Management Area. Total lengths for the larvae were 18.00 mm, 12.00 mm, and 9.00 mm. Based on field temperatures and growth and development data (Ming, 1968; Kleinert & Mraz, 1966), spawning was estimated to have occurred during the last week of February 1995.

A second, low-intensity spawning period was reported in summer or fall in Michigan (Hubbs and Lagler 1943). On 9 December 1994, 12 young - of - the - year grass pickerel were sampled from the dense cover of *Potamogeton crispus* in the mitigated wetland area (GBWMA). Total lengths of the pickerel ranged from 13.00 to 31.00 mm (mean = 23.80 mm TL). Collection of these individuals is the first evidence of a fall spawn at the Green Bottom Wildlife Management Area.

<u>Temperature</u> - Based on field data, the threshold temperature for spawning at Green Bottom Swamp is approximately 9 °C. This

temperature was first recorded on 25 February 1994 and again on 6 March 1994.

Crossman (1962) reported that grass pickerel spawned between 7.8-12.2 °C in Ontario, Canada. In Wisconsin, grass pickerel spawned at 4.4-11.7 °C (Kleinert and Mraz, 1966), while they spawned at < 18.3 °C in Oklahoma (Ming, 1968). In other studies in Canada, grass pickerel spawned between 4-12 °C (Scott and Crossman, 1973).

Larvae and Juveniles

Yolk-sac larvae (6-10 mm TL) were not found during the post spawning period (1994 & 1995). Several attempts to locate them in the aquatic vegetation after the spawning period failed at GBWMA. Yolk-sac larvae of grass pickerel attach to vegetation in Canada (Scott and Crossman, 1973). Ming (1968) reported yolk-sac larvae among the leaf litter during the winter in Oklahoma. An attempt to locate yolk-sac larvae at GBWMA in 1995 was unsuccessful.

Post yolk-sac larvae (11-21 mm TL) grass pickerel were collected on 9 December 1994 from the dense cover of *Potamogeton crispus* in the mitigated wetland area at GBWMA. Total lengths of the pickerel larvae ranged from 13.00 to 21.00 mm with a mean of 19.10 mm (N = 7). On 4 April 1995, 3 post yolk-sac larvae were collected from GBWMA. Total lengths of the 3 individuals were 18.00, 12.00, and 10.30 mm Mansueti and Hardy (1967) found post yolk-sac larvae in very shallow water in roadside ditches and overflow pools of moderate to large rivers. Ming (1968) collected post yolk-sac larvae among dead leaf litter in Oklahoma.

The first juvenile (> 22 mm TL) grass pickerel (24.3 mm TL), from GBWMA, was collected in aquatic vegetation on 10 March 1993. On 21 April 1993, 43 juveniles, ranging in size from 22.9 to 67.5 mm TL (mean = 37.3 mm TL), were collected from thick beds of *Potamogeton crispus*. Five juveniles (29.50 to 31.00 mm TL) averaging 30.40 mm total length were sampled from submerged *Potamogeton crispus* on 9 December 1994. Kleinert and Mraz (1966) reported the nursery area for juveniles to be a weed-choked slough. Wallus et al. (1990) recorded juveniles in flood plain pools and sloughs of a Tennessee River tributary among dense aquatic vegetation.

Laboratory Studies

Fecundity

The fecundity of an individual female fish depends on several different factors including her size, age, and condition (Lagler et al., 1962).

Only one sexually mature female containing primary eggs was collected during the study (26 February 1993). Based on the volumetric method, the number of primary (mature) eggs was 3,167.

Crossman (1962) reported an average of 756 mature eggs from grass pickerel in Ontario, while Kleinert and Mraz (1966) noted a range of 843-4,584 mature eggs in Wisconsin. Carbine (1944) recorded 803 mature eggs (total eggs = 15,732) from a grass pickerel in Michigan.

Sexual Maturity

The ripe (280 mm TL) and spent (240 mm TL) females collected provide limited information on sexual maturity at this time.

Ming (1968) reported grass pickerel sexually mature at age one at 109 mm TL for females and 188 mm TL for males in Oklahoma. Kleinert and Mraz (1966) recorded both sexes of grass pickerel sexually mature at 108 mm in Wisconsin, while McCarraher (1960) noted sexual maturity at 102 mm in Nebraska. In Canada, the grass pickerel was sexually mature by two years of age at 141 mm TL for males and 157 mm TL for females (Crossman, 1962). Attempts made to collect additional sexually mature grass pickerel, at GBWMA, during the 1995 spawning period were not successful.

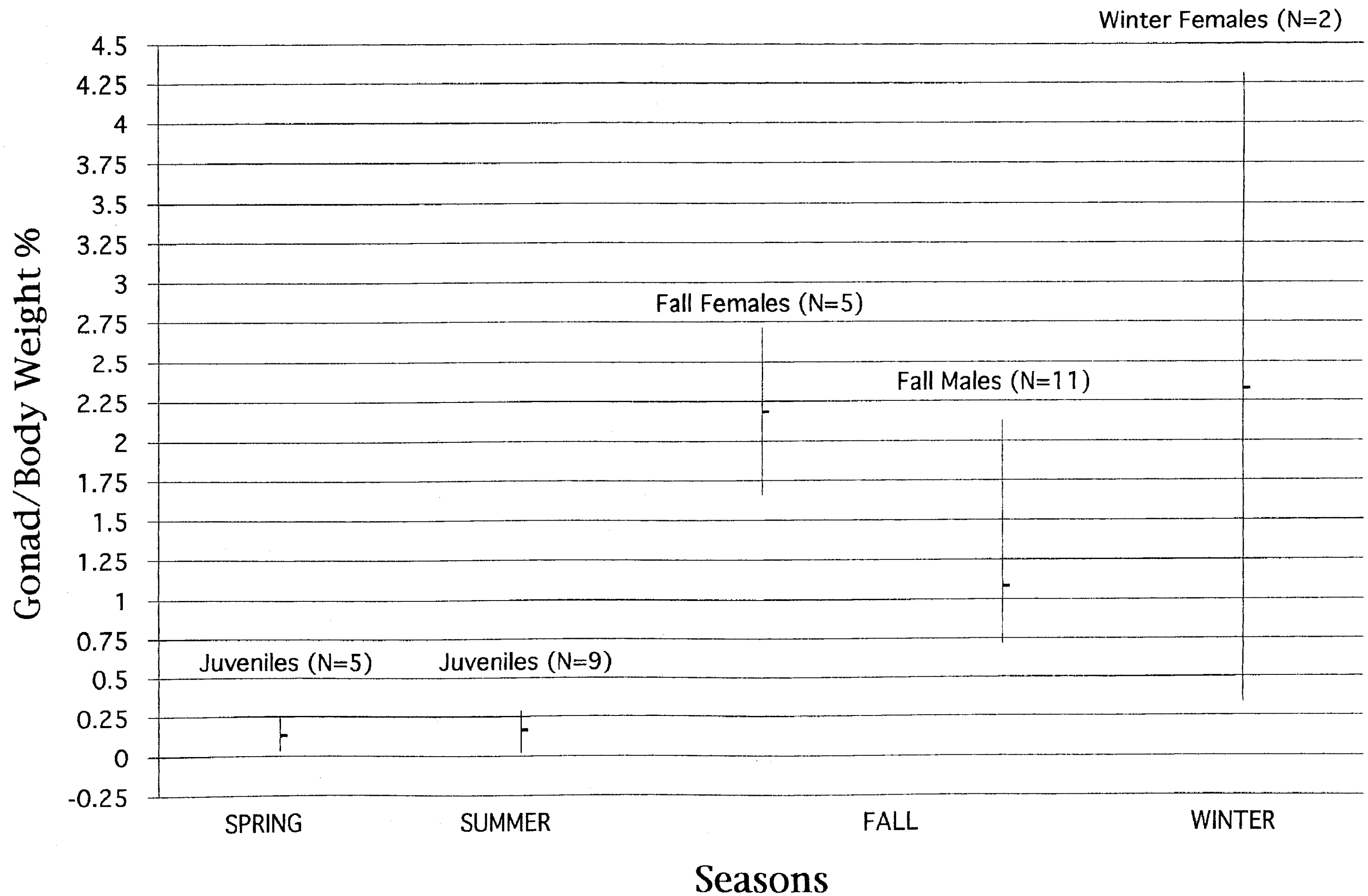
Gonosomatic Index (GSI)

Grass pickerel were divided into three groups: males (October, February), females (October, February), and juveniles (April, July). Gonads removed from grass pickerel in the spring (April) and summer (July) could not be sexed without histological preparations. Gonads could be sexed, through visual observation, in the fall (October) and winter (February) collections. In the fall season, averages of the ratios were 2.181 and 1.080 percent, females (N = 5) and males (N = 11), respectively (Table 2, Figure 4). No males were collected during the winter season. The GSI for the winter season was based on 1 ripe and 1 spent female grass pickerel. The GSI averaged 2.326 percent, for the females (Table 2, Figure 4).

Table 2. Seasonal gonosomatic indices (GSI) of grass pickerel collected from the Green Bottom Wildlife Management Area, Cabell County, West Virginia (1993). M = Male, F = Female, and J = Juvenile.

DATE	OPERCLE TAG #	SEX	BODY WT. (g.)	GONAD WT (g.)	GSI %
Feb-26	1	F	118.60	5.1147	4.313
	2	F	90.20	0.3057	0.339
Apr-21	3	J	80.49	0.5062	0.063
	4	J	82.16	0.082	0.099
	5	J	30.67	0.0775	0.253
	6	J	21.92	0.0072	0.033
	7	J	9.25	0.0182	0.197
Jul-20	8	J	42.82	0.1203	0.281
	9	J	46.75	0.118	0.252
	10	J	51.37	0.1374	0.267
	11	J	49.47	0.1205	0.244
	12	J	49.27	0.0163	0.033
	13	J	47.46	0.0148	0.031
	14	J	38.91	0.012	0.031
	15	J	36.25	0.1056	0.291
	16	J	42.90	0.0092	0.021
Oct-20	17	F	36.31	0.9863	2.716
	18	M	14.78	0.1054	0.713
	19	M	26.05	0.2387	0.916
	20	M	10.27	0.2178	2.121
	21	M	13.63	0.1216	0.892
	22	M	10.88	0.0825	0.758
	23	F	12.99	0.2871	2.21
	24	M	11.86	0.1488	1.255
	25	M	10.93	0.1132	1.036
	26	M	8.74	0.0727	0.832
	27	F	7.86	0.1699	2.162
	28	M	9.10	0.1124	1.235
	29	M	8.50	0.0928	1.092
	30	F	6.72	0.1445	2.15
	31	F	7.36	0.1226	1.666
	32	M	5.68	0.0587	1.033

Figure 4. Seasonal gonosomatic indices (GSI) of grass pickerel collected from the Green Bottom Wildlife Management Area, Cabell County, West Virginia (1993).



Winter Females (N=2)
Fall Females (N=5)
Fall Males (N=11)
Juveniles (N=5)
Juveniles (N=9)
4.5
4.25
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
-0.25
Gonad/Body Weight %
SPRING
SUMMER
FALL
WINTER
Seasons

On that basis, it was obvious that the sex products of each sex became ripe prior to the spring collection. The mean, for the spring season, was 0.242 percent (N = 5) (Table 2, Figure 4). A moderate increase in the ratio was noted in the summer season (mean = 0.161%) (N = 9) (Table 2, Figure 4). The marked increase in the ratio between gonad weight and body weight in the fall season illustrates the possibility for a fall spawn. The second GSI increase (February) was interpreted as the onset of the reproduction season. No information was found in the literature regarding the GSI and grass pickerel.

Egg Diameters and Incubation

Hickling and Rutenberg (1936) indicated that the duration of the spawning season can be ascertained accurately by recording the diameters of the eggs as they develop within the ovary.

In Green Bottom Swamp, the average diameter of eggs (N = 50) from a female pickerel collected on 26 February 1993 was 1.79 mm (range, 1.40-2.15 mm).

Kleinert and Mraz (1966) reported that mature ovarian eggs were 1.5-2.4 mm in diameter in grass pickerel from Wisconsin, while Crossman (1962) found an average of 1.4 mm in diameter from ovarian eggs in grass pickerel from Ontario. Leslie and Gorrie (1985) recorded an average of 2.8 mm (range, 2.5-3.0 mm) in diameter from grass pickerel. Fertilized eggs are transparent and amber to yellow in color, demersal, adhesive or nonadhesive (Kleinert and Mraz, 1966; Crossman, 1962; and Raney, 1959). Eggs are probably scattered among aquatic vegetation (Crossman, 1962).

The estimated incubation time for eggs developing in Green Bottom Swamp was 8-12 days at 9-10 °C. This estimation is based on a sexually mature female collected on 26 February 1993 and field collections of larval and juvenile pickerel.

Kleinert and Mraz (1966) reported eleven days (8.9 °C), 14 days (8.3 °C) and 15 days (7.8 °C) as incubation time from grass pickerel in Wisconsin. Leslie and Gorrie (1985) noted a 12 day incubation period for eggs from grass pickerel.

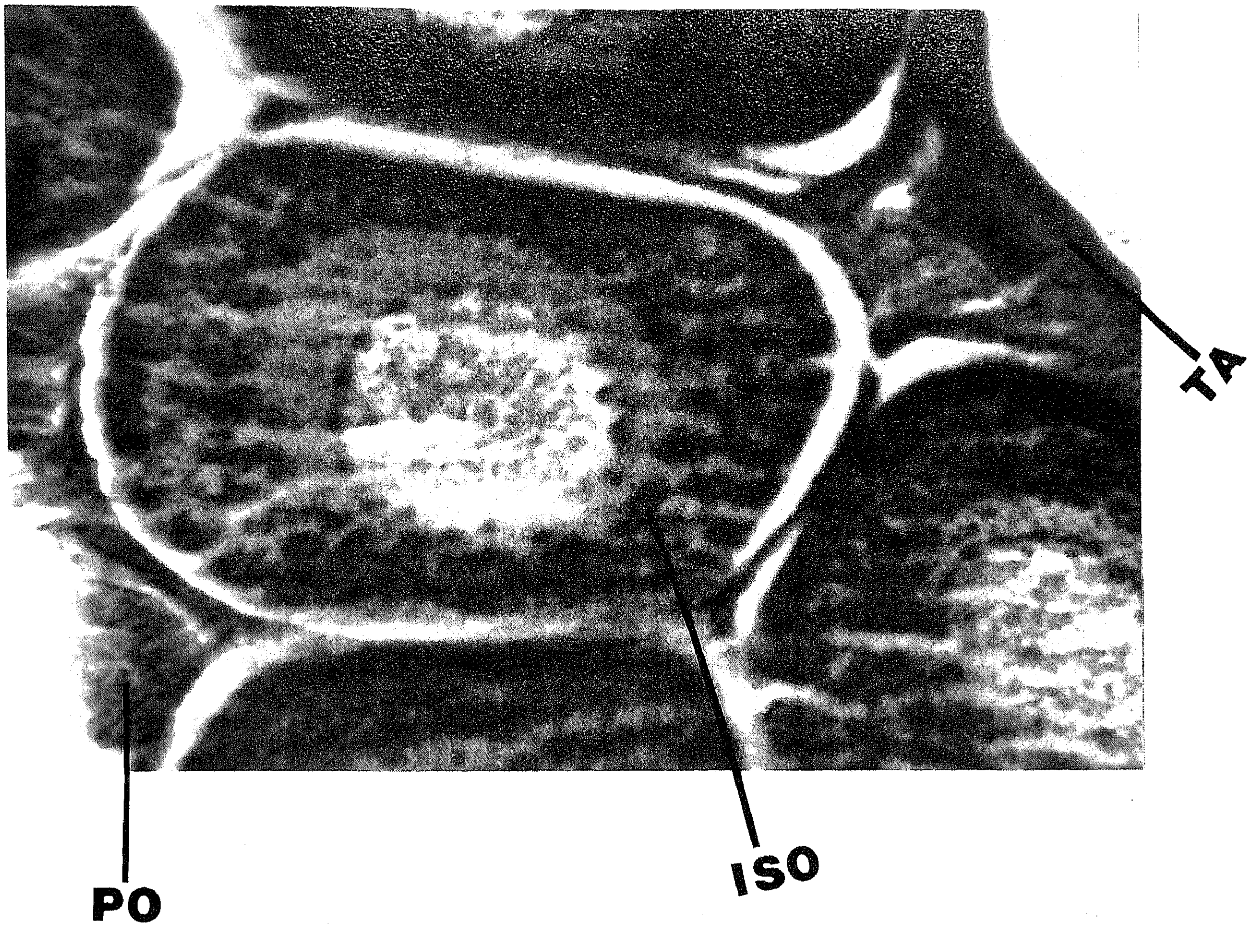
Seasonal Histology

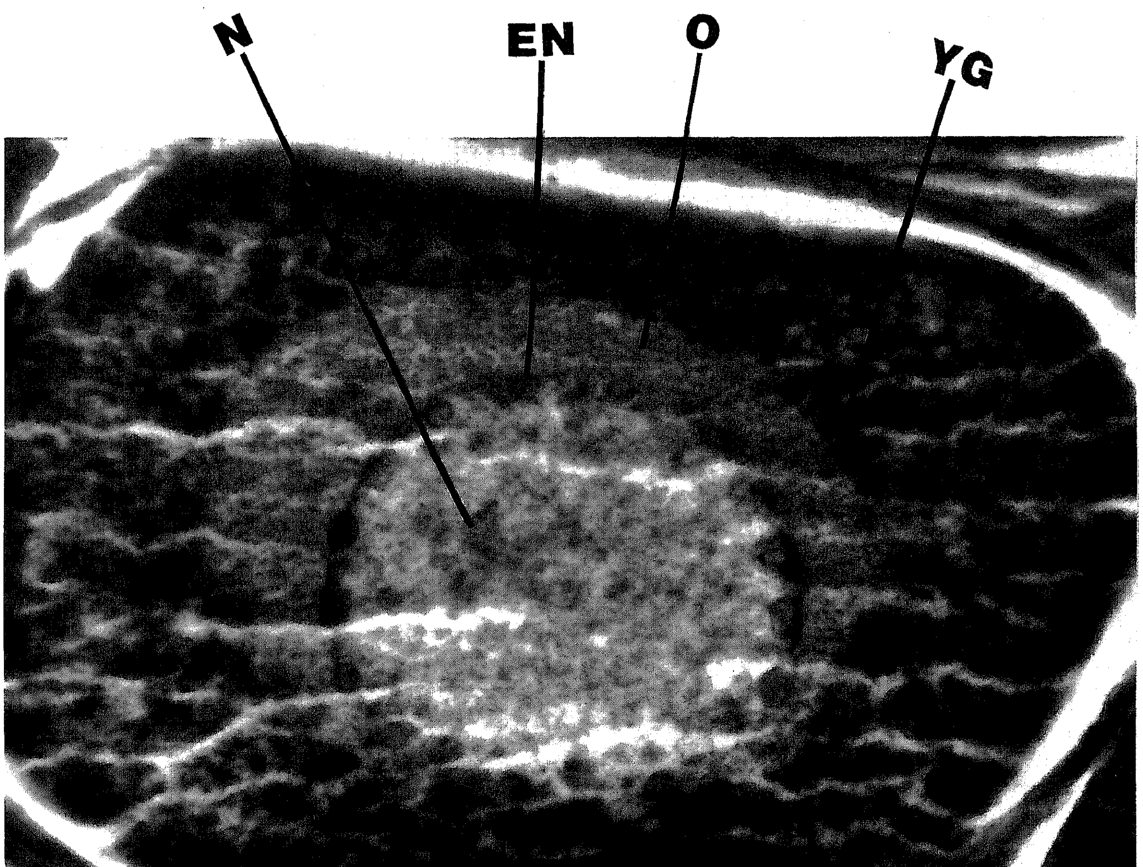
Histological sections of grass pickerel (N = 17) were prepared to determine periods of advanced gonadal development for comparison with seasonal GSI. Representatives from the spring season included three females and two males. The summer season consisted of four females and two males. The fall season consisted of two females and two males. The winter season consisted of two females only.

Summer Females - Primary and secondary immature oocytes were present in females from the summer season (20 July 1993) (Figures 5a,b). The smaller primary oocytes ranged from 35 - 126 μm in diameter (mean = 72 μm; N = 10). Immature secondary oocytes ranged from 161 - 182 μm in diameter (mean = 169 μm; N = 5). Euvitelline nucleoli (ribonucleic particles) were present within the karyoplasm. Yolk granules (protein aggregations) were evident within the ovaplasm. Fat vacuoles (oil containing vesicles) were absent from the ovaplasm. Groman (1982) suggested that the

Figure 5a. Cross-section of ovarian tissue from a grass pickerel, collected during the summer season, illustrating primary and immature secondary oocytes (250X). PO = Primary oocyte, ISO = Immature secondary oocyte, TA = Tunica albuginea.

Figure 5b. Cross-section of ovarian tissue from a grass pickerel, collected during the summer season, illustrating an immature secondary oocyte (400X). N = Nucleus, EN = Euvitelline nucleoli, O = Ovaplasm, YG = Yolk granules.


TA
PO
ISO


N
EN
O
YG

presence of euvitelline nucleoli and yolk granules coupled with the absence of fat vacuoles indicates stage-III (immature) ova.

Fall Females - Fall females (20 October 1993) showed a marked increase in oocyte size and development. Primary oocytes ranged in diameter from 70 - 294 μm (mean = 180 μm; N = 10). Mature secondary oocyte diameters ranged from 490 - 700 μm in diameter (mean = 538 μm; N = 5). Euvitelline nucleoli, yolk granules, large fat vacuoles, and a well defined vitelline membrane were evident within mature secondary oocytes (Figure 6). Histological techniques did not resolve the exact stage of fall oocyte development. Large, well defined fat vacuoles are present in stage IV - VI ova (Groman, 1982). Oocyte development in the fall female illustrates the possibility for fall spawns at GBWMA provided a male of similar developmental condition and environmental temperature parameters are met.

Winter Females - Oocytes from winter females (26 February 1993) exhibited a decrease in size and development compared to fall females (Figure 7a,b). Primary oocyte diameters ranged from 35 - 96 μm in diameter (mean = 62 μm; N = 10). Secondary oocytes ranged from 147 - 165 μm in diameter (mean = 156; N = 5). Euvitelline nucleoli were present within the karyoplasm. Yolk granules were evident in the ovaplasm. Fat vacuoles were absent from the ovaplasm. Distance between the vitelline membrane appeared to increase markedly. Due to the small sample size (N = 2), from the winter season, it is unlikely these developments are normal for the entire population at Green Bottom.

Figure 6. Cross-section of ovarian tissue from a grass pickerel, collected during the fall season, illustrating a mature secondary oocyte (100X). MSO = Mature secondary oocyte, EN = Euvitelline nucleoli, FV = Fat vacuole, VM = Vitelline membrane.

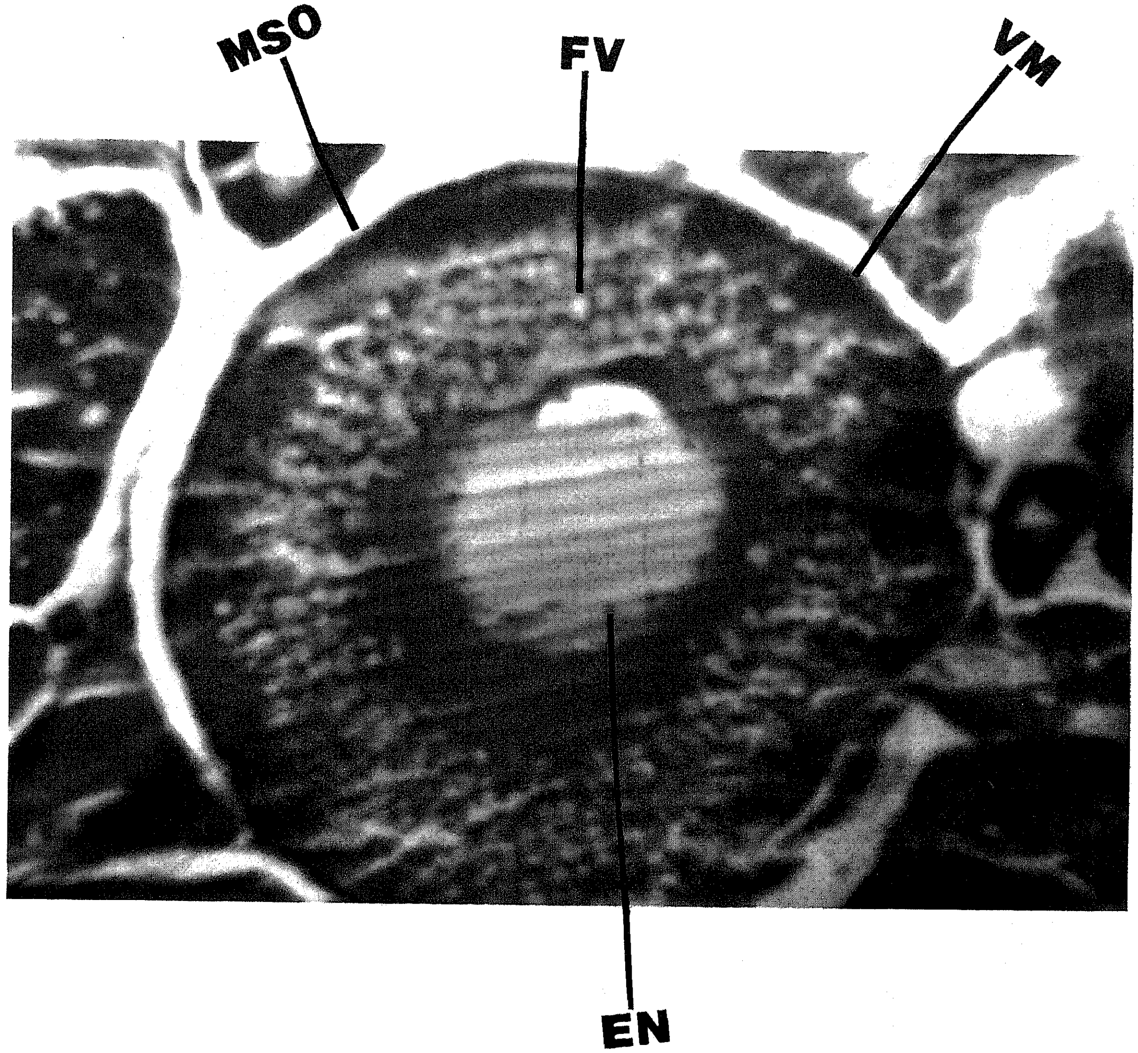
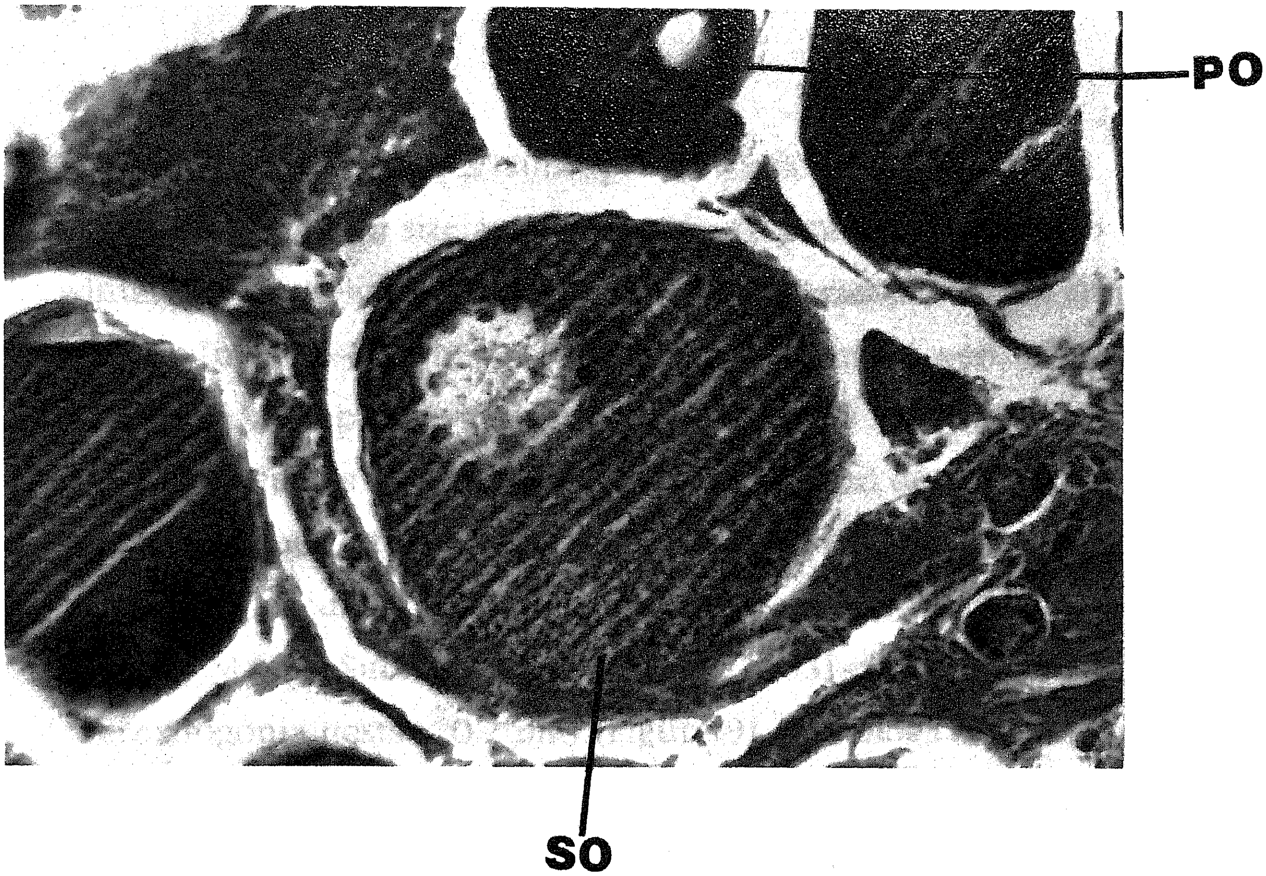
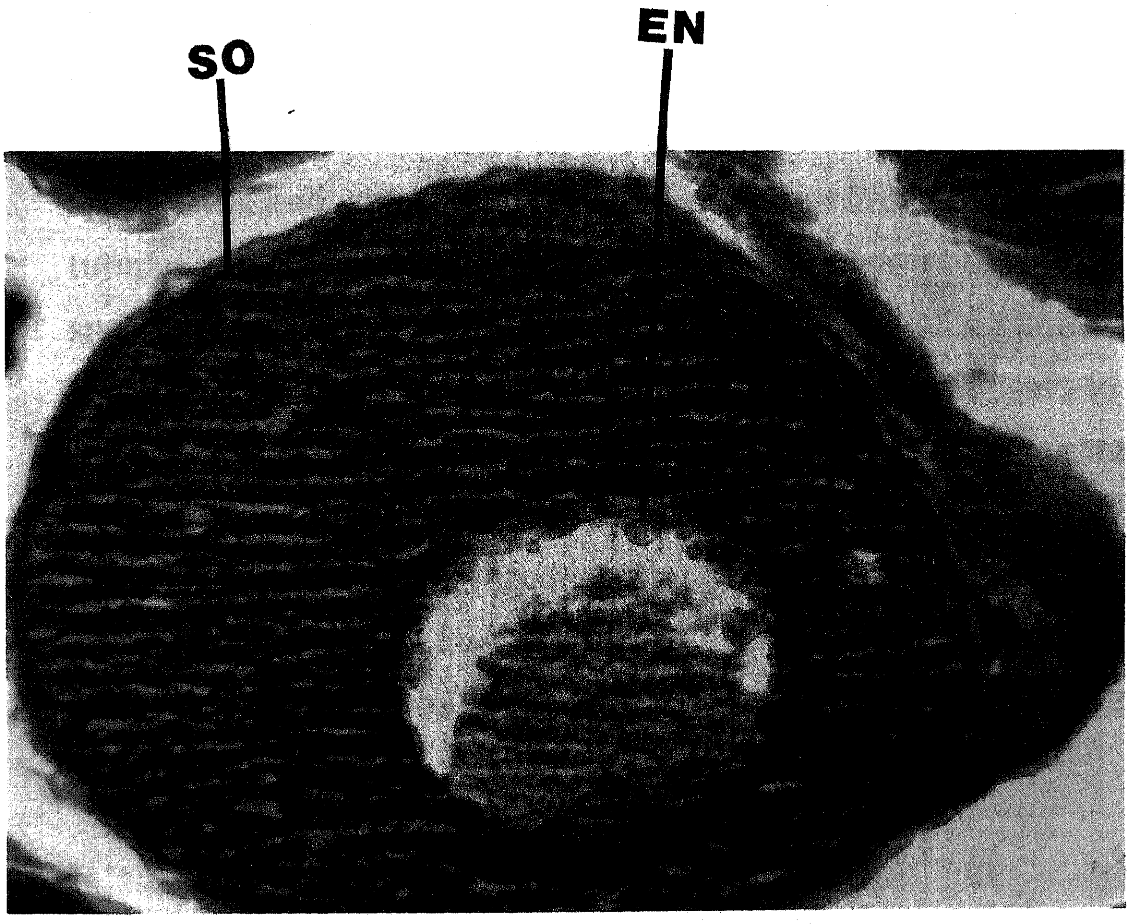

MSO
FV
VM
EN

Figure 7a. Cross-section of ovarian tissue from a grass pickerel, collected during the winter season, illustrating primary and secondary oocytes (250X). PO = Primary oocyte, SO = Secondary oocyte.

Figure 7b. Cross-section of ovarian tissue from a grass pickerel, collected during the winter season, illustrating a secondary oocyte (400X). SO = Secondary oocyte, EN = Euvitelline nucleoli.


PO
SO


SO
EN

Spring Females - Oocytes from spring females (21 April 1993) exhibited decreased size and similar development compared to winter females (Figure 8). Primary oocyte diameters ranged from 53 - 70 μm (mean = 60 μm; N = 5). Euvitelline nucleoli were present within the karyoplasm. Yolk granules were evident in the ovaplasm while fat vacuoles were absent. Reduced size and development of oocytes is expected for 21 April 1993 collections because spawning has already occurred.

Summer Males - Summer males (20 July 1993) exhibited spermatogonia nests (10.2 μm) (Figure 9). Histological techniques could not distinguish primary and secondary stages of development within the nests. However, contrasts in staining as well as the presence of large interstitial spaces in some regions suggests that different stages of development occur together within the testis.

Fall Males - Fall males (20 October 1993) exhibited a marked increase in testicular development compared to the summer season (Figure 10). Spermatogonia nests (10.2 μm) and secondary spermatocytes (@ 1.13 μm) were present within the gonads. Spermatids and spermatozoa could not be resolved. Seminiferous tubules were well defined. Groman (1982) suggested that spermatogenesis can occur along the entire length of seminiferous tubules in adult males. Male testicular development appears to be correlated with ovarian development exhibited by females from the fall season in Green Bottom Swamp.

Winter Males - No winter males were present in the collections.

Spring Males - Spring males (21 April 1993) exhibited testicular development similar to that of fall males (Figure 11). Seminiferous

Figure 8. Cross-section of ovarian tissue from a grass pickerel, collected during the spring season, illustrating primary and secondary oocytes (250X). PO = Primary oocyte, SO = Secondary oocyte, YG = Yolk granules.

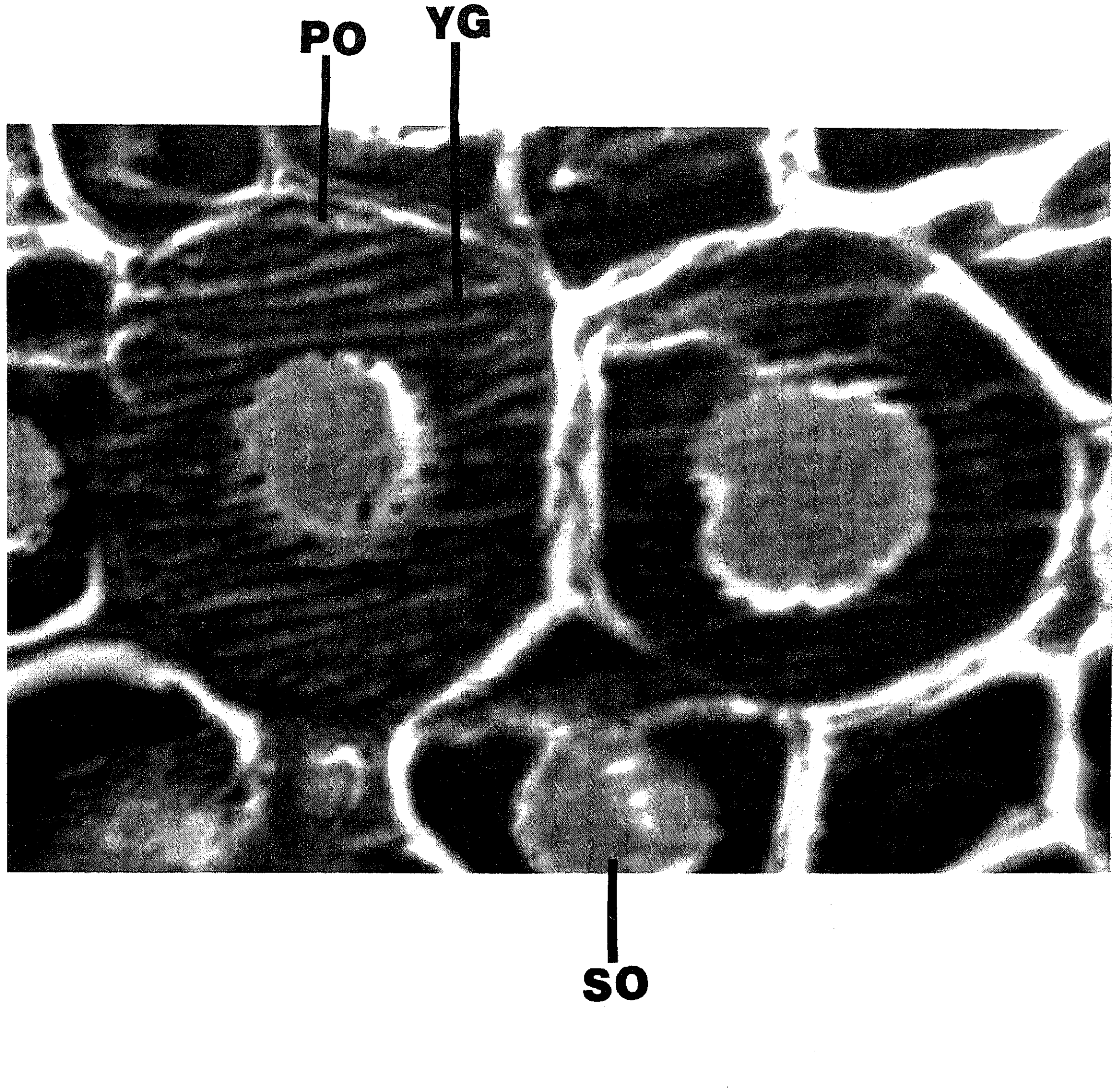

PO
YG
SO

Figure 9. Cross-section of testicular tissue from a grass pickerel, collected during the summer season, illustrating spermatogonia nests, interstitial space, and tunica albuginea (400X). SN = Spermatogonia nest, IS = Interstitial space, TA = Tunica albuginea.

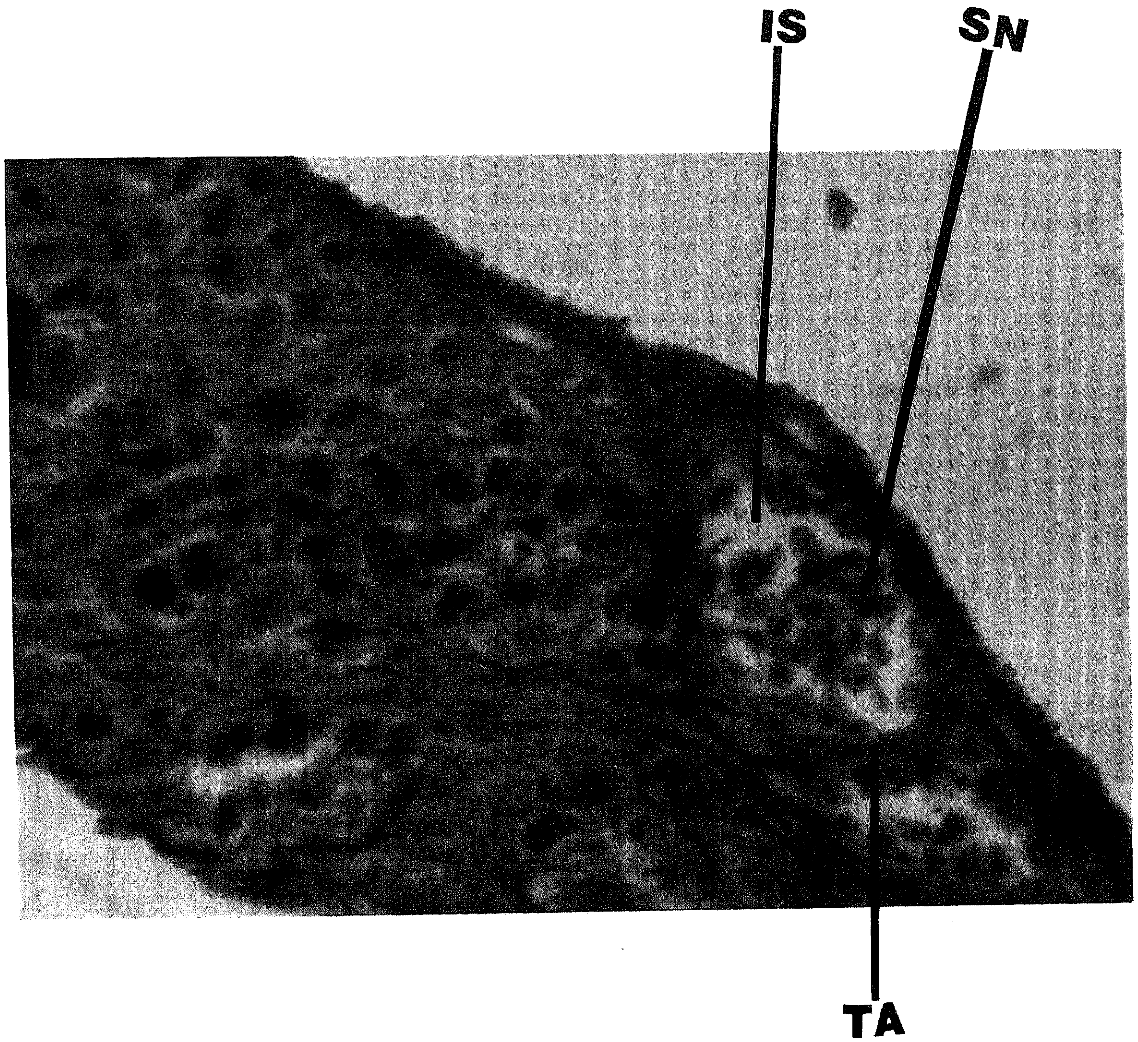

IS
SN
TA

Figure 10. Cross-section of testicular tissue from a grass pickerel, collected during the fall season, illustrating secondary spermatocytes, spermatogonia nests, and tunica albuginea (250X). SS = Secondary spermatocytes, SN = Spermatogonia Nests, and TA = Tunica albuginea.

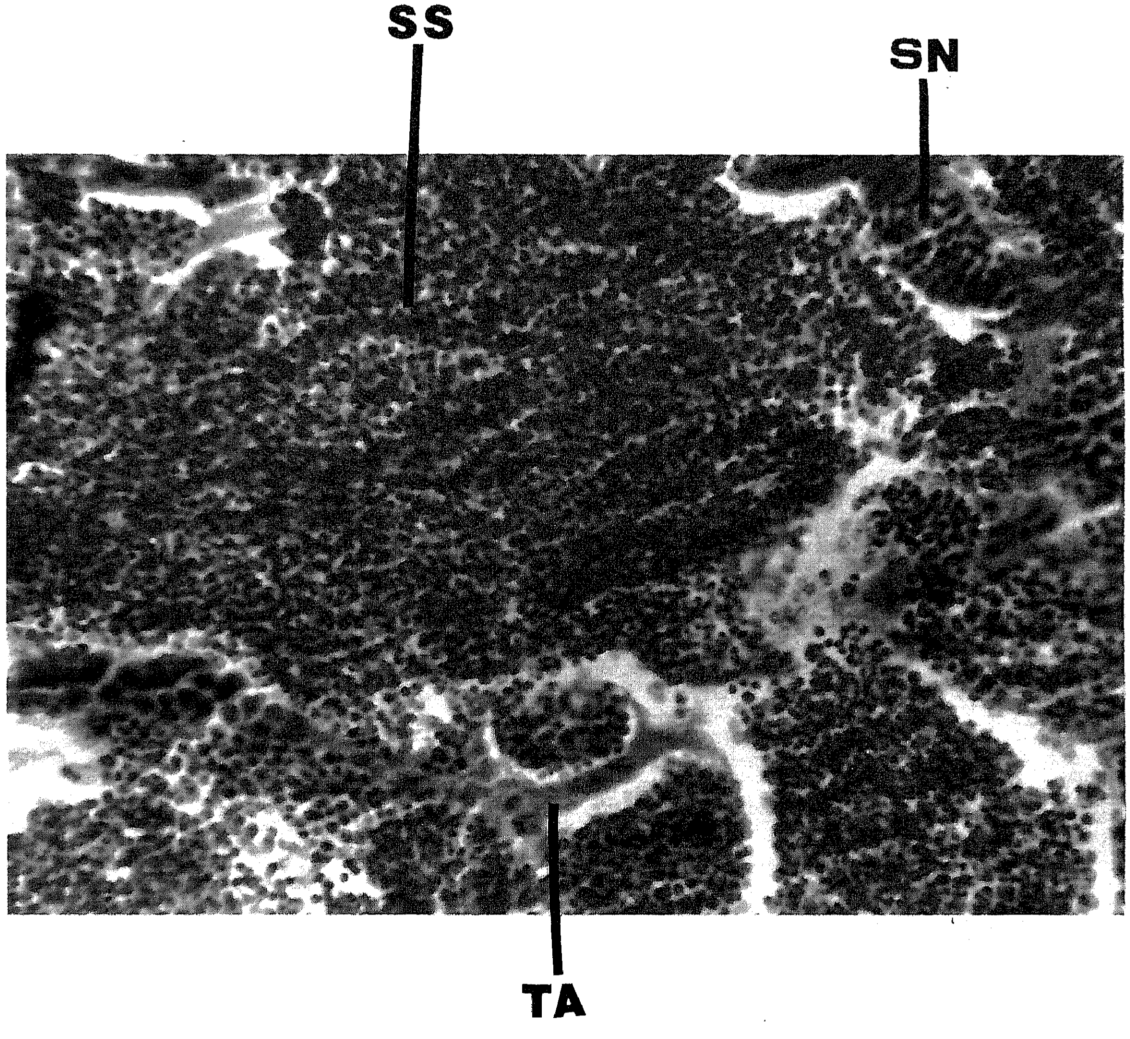
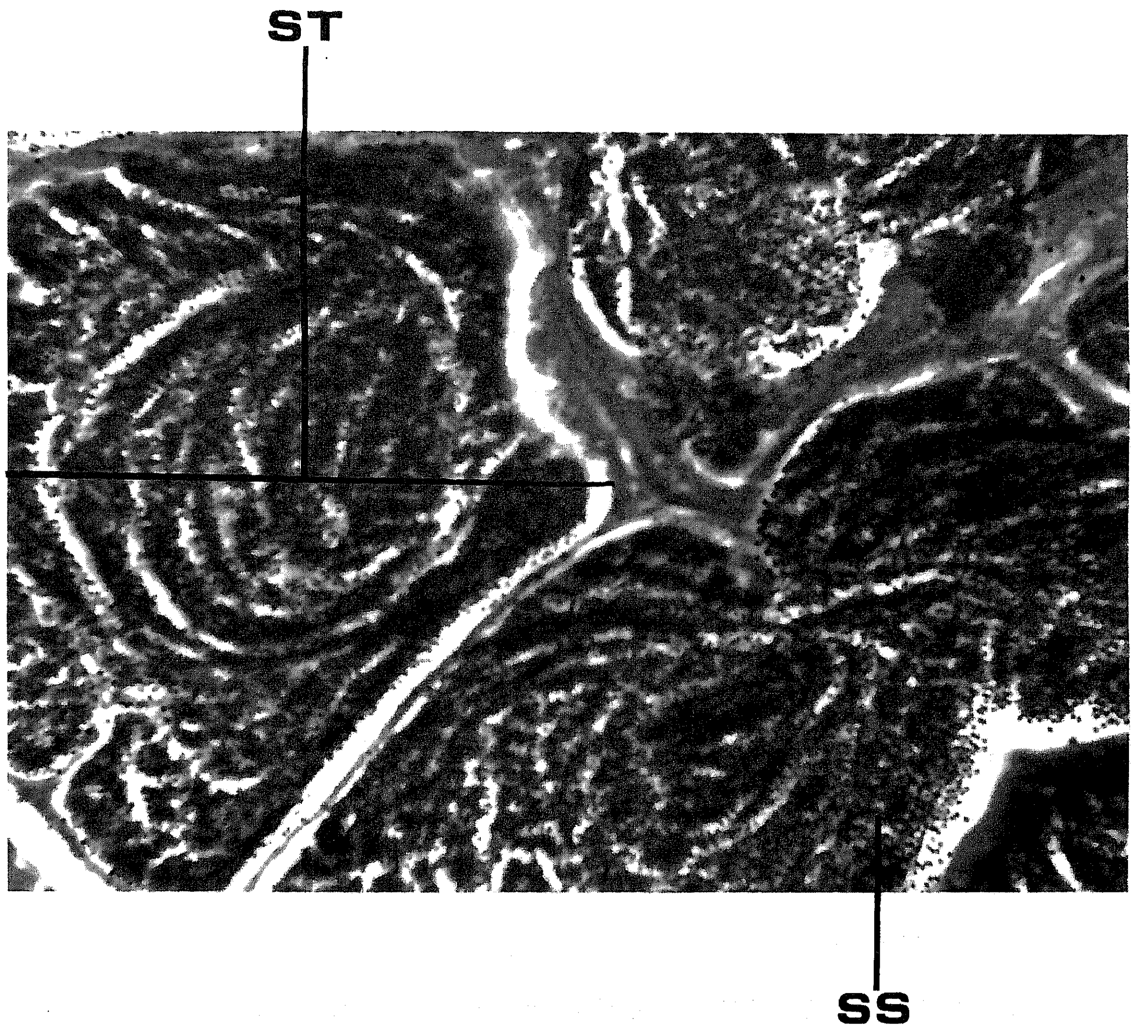

SS
SN
TA

Figure 11. Cross-section testicular tissue from a grass pickerel, collected during the spring season, illustrating seminiferous tubules and secondary spermatocytes (250X). ST = Seminiferous tubule and SS = Secondary spermatocytes.


ST
SS

tubules were well defined. Secondary spermatocytes were present and densely packed within the tubules.

Morphometrics

Males (N = 10) and females (N = 10) were measured to determine any sexual dimorphism. Gonad histology was necessary to sex most of the fish measured due to their small size. Upon sex determination, measurements were taken from randomly selected fish. Of the 21 measurements, three varied significantly below the 0.05 confidence level (Table 3). The measurements were length of the pectoral ray, length of the pelvic ray, and head length (Table 3). Greater lengths were found in males.

Sexual dimorphism in nature allows for determination of gender between individuals of the reproducing age class. Female fish are often larger when compared to males of the same age class due to the size of their ovaries. Also, differences in coloration between the sexes often provide for determination of sex and reproductive condition. Differences when they occur are most pronounced during the reproduction season with males appearing brighter and more ornate than females. Observations of grass pickerel, both in the field and in the lab, did not reveal any obvious differences in size between the sexes. Coloration of individuals sampled also did not vary with season or sex. There was no mention of coloration differences between the sexes in the literature.

Statistical analysis of measurements indicated that male grass pickerel have significantly longer head length, pectoral ray length,

Table 3. Morphometric data from grass pickerel collected at the Green Bottom Wildlife Management Area, Cabell County, West, Virginia. * indicates significance < 0.05.

MEASUREMENT	SEX	MEAN	RANGE	s.d.	t p
Standard length	Male Female	.8491 .8584	.837-.822 .828-.882	.0112 .0159	1.52 .147
Body depth	Male Female	.1527 .1579	.125-.171 .132-.178	.0148 .0139	0.81 .427
Length of caudal peduncle	Male Female	.1583 .1536	.135-.183 .147-.170	.0145 .0077	0.90 .381
Depth of caudal peduncle	Male Female	.0702 .0712	.063-.082 .066-.077	.0048 .0037	0.53 .605
Predorsal length	Male Female	.7521 .7543	.724-.794 .739-.771	.0203 .0109	0.30 .769
Length of dorsal base	Male Female	.1233 .1244	.111-.139 .114-.132	.0106 .0053	0.28 .786
Length of anal base	Male Female	.1062 .1061	.088-.120 .091-.121	.0088 .0110	0.01 .990
Length of anal ray	Male Female	.1832 .1770	.107-.207 .164-.187	.0288 .0069	0.67 .513
Length of pectoral ray	Male Female	.1197 .1109	.115-.128 .102-.116	.0047 .0045	4.27 <0.05*
Length of pelvic ray	Male Female	.1351 .1231	.126-.144 .113-.132	.0060 .0057	4.60 <0.05*
Head length	Male Female	.3459 .3359	.333-.354 .330-.349	.0072 .0069	3.16 <0.05*
Head depth	Male Female	.3394 .3429	.317-.389 .317-.380	.0224 .0018	.370 .712
Head width	Male Female	.3435 .3508	.306-.371 .317-.380	.0202 .0202	0.81 .430
Snout length	Male Female	.4020 .4039	.371-.444 .380-.400	.0235 .0166	0.21 .839
Postorbital length of head	Male Female	.4508 .4621	.417-.486 .417-.500	.0208 .0289	1.01 .326
Cheek height	Male Female	.1849 .1821	.278-.357 .143-.203	.0230 .0221	0.28 .781
Cheek length	Male Female	.2947 .3032	.278-.357 .208-.333	.0411 .0347	0.50 .624
Interorbital width	Male Female	.1447 .1402	.128-.167 .117-.167	.0198 .0195	0.63 .536
Length of orbit	Male Female	.1685 .1697	.154-.190 .141-.200	.0122 .0211	0.16 .875
Length of upper jaw	Male Female	.4876 .4568	.429-.722 .433-.479	.0837 .0153	1.15 .279
Width of gape	Male Female	.3654 .3552	.231-.455 .267-.429	.0723 .0562	0.35 .729

and pelvic ray length with respect to their standard length than females. Due to the small sample size (N = 20), it is impossible to determine that these differences are consistently present in the entire population.

Meristics

Counts were taken from the same fish used during morphometric analysis. Of the 8 measurements concerning scales and rays (Table 4), none proved to vary significantly between the sexes at the 0.05 confidence level.

Sex Ratio

A chi-square test was performed on 29 grass pickerel (15 males &14 females) from the GBWMA to determine any deviation from the expected 1:1 sex ratio (Sprinthall, 1990). Results did not indicate deviation from the expected 1:1 ratio (0.05 confidence level).

Table 4. Sexual dimorphism, with respect to meristics, in grass pickerel from the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

COUNTS	SEX	MEAN	RANGE	s.d.	t p
SCALES :					
Lateral line	Male Female	39.20 39.80	35.00-46.00 36.00-45.00	3.35989 2.65832	0.44 .663
Above lateral line	Male Female	19.40 18.00	16.00-23.00 16.00-22.00	2.50333 1.88561	1.41 .175
Below lateral line	Male Female	18.40 17.70	13.00-28.00 15.00-21.00	4.45222 1.56702	0.47 .648
Around caudal peduncle	Male Female	49.70 44.20	40.00-60.00 38.00-54.00	6.86456 4.93964	2.06 .055
FINS :					
Dorsal fin rays	Male Female	13.50 13.30	12.00-15.00 12.00-14.00	0.97193 0.67495	0.53 .600
Pectoral fin rays	Male Female	13.40 13.10	11.00-15.00 12.00-14.00	1.42984 0.73786	0.59 .566
Pelvic fin rays	Male Female	9.30 9.80	8.00-10.00 8.00-11.00	0.82327 0.78881	1.39 .182
Anal rays	Male Female	12.60 12.20	11.00-14.00 11.00-15.00	1.07497 1.31656	0.74 .466

CHAPTER V
SUMMARY & CONCLUSIONS

1. Observations were made on the reproductive biology of the grass pickerel in Green Bottom Swamp, a naturally occurring wetland of 58 hectares, between 1993-95. The shallow, low gradient environment with flooded vegetation is excellent for spawning and nursery areas for young-of-the-year grass pickerel.

2. Seasonal collections were made on the following dates (number of fish in parenthesis): Winter/26 February 1993 (2); Spring/21 April 1993 (48); Summer/20 July 1993 (21); and Fall/20 October 1993 (16).

3. Actual spawning was not observed during the reproduction season but is believed to have occurred in the aquatic vegetation associated with buttonbush in late February or early March. One gravid female and one spent female was collected on 26 February 1993. The threshold temperature for spawning is approximately 9 °C.

4. No yolk-sac larvae (6 - 10 mm TL) were found during the post spawning period. On 4 April 1995, 3 post yolk-sac larvae were collected. Total lengths of the pickerel were 11 mm, 12mm, and 18 mm. Spawning was estimated to have occurred at the end of February to early March. One juvenile (24.2 mm TL)

was collected on 10 March 1993 and 43 juveniles (22.9 to 67.5 mm TL; mean = 37.3 mm TL) were found on 21 April 1993. The nursery area for the juveniles was the thick beds of *Potamogeton crispus*.

5. The estimated number of primary (mature) eggs was 3,167 (N = 1; 280 mm TL). Egg diameters (N = 50) ranged from 1.40 to 2.15 mm (mean = 1.79 mm). The estimated time for egg development was 8-12 days.

6. The gonosomatic index (GSI) reached a maximum in late February (mean = 2.2 %) (N = 2). This marked increase in the ratio between gonad weight and body weight in winter (February) was interpreted as the onset of the reproductive season. The GSI declined abruptly following spawning. The fall season exhibited another GSI increase which was interpreted as evidence for the possibility of a fall spawn. However, no fall spawn was observed in the old swamp. On 9 December 1994, 7 post yolk-sac larvae and 5 juvenile grass pickerel were collected from the mitigated wetland area. The collection is evidence of fall spawning in the mitigated wetland area.

7. Seasonal histology was performed to confirm GSI trends. The first marked increase in gonadal development of males and females was seen in the fall season. Female gonads from the winter season showed a reduction in size and development in

comparison to the fall. No winter males were collected. Gonads from the spring and summer season exhibited the least development in both males and females. Seasonal histology confirmed trends exhibited by GSI.

8. Morphometrics identified length of pectoral ray, length of pelvic ray, and head length to vary significantly ($p < 0.05$) between sexes. Each measurement was greater for males.

9. Meristics did not vary significantly ($p < 0.05$) between the sexes.

10. Chi-square analysis of sex ratio did not vary significantly ($p < 0.05$). Sex ratio did not deviate from the expected 1:1 ratio between the sexes.

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