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Diet and growth of larval and juvenile grass pickerel, Esox americanus vermiculatus, and central mudminnow, Umbra limi, in the Green Bottom Wildlife Management Area, Cabell County, West Virginia

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Diet and growth of larval and juvenile grass pickerel, *Esox americanus vermiculatus*, and central mudminnow, *Umbra limi*, in the Green Bottom Wildlife Management Area, Cabell County, West Virginia

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ABSTRACT

The grass pickerel, *Esox americanus vermiculatus*, is listed as Undetermined on the Vertebrate Species List of Concern in West Virginia. The central mudminnow, *Umbra limi*, is a disjunct population in Green Bottom Swamp. The lentic, vegetated areas that are required for spawning for the grass pickerel and the central mudminnow have been reduced by residential, agricultural, and industrial developments. Green Bottom Swamp, a naturally occurring wetland of 58 ha, and a nearby mitigated wetland of 29 ha, provides spawning habitat for the grass pickerel and the mudminnow. Fishes were collected in beds of *Potamogetan crispus* and *Ceratophyllum demursum* from the old swamp. In December 1994, 15 pickerel were collected from beds of *Polygonum* sp. in the mitigated wetland. Between April 1995 and July 1996, 65 pickerel (6.67-101.45 mm) and 155 mudminnows (3.89-36.97 mm) were collected from the old swamp. Growth and diet of these fish were determined. Stomach contents of each fish were analyzed, assigned a point value ranging from 0 (empty gut) to 30 (full gut), and expressed as percent frequency of food items per stomach. Cladocerans and copepods were the most abundant In larval pickerel and mudminnows. In juvenile mudminnows, items ranged from larval chironomids and ostracods to cladocerans, while pickerel consumed naiadal stages of large aquatic insects (odonates and corixids) and small fish. Horn, Morisita, and percent similarity indices were used to determine whether a dietary overlap occurred between the phases of each fish. Yolk-sac larvae (YSL) and post yolk-sac larvae (PYSL) of pickerel (Horn = 0.778, Morisita = 0.666, percent similarity = 56.0) and mudminnows (Horn = 0.797, Morisita = 0.750, percent similarity = 57.0) were the most similar. A dietary overlap between fishes was also ascertained. Pickerel PYSL and mudminnow PYSL were similar with values of Horn = 0.900,

Morisita = 0.872, and percent similarity = 70.0. Daily growth (mm/day) was calculated. Using back-calculated values from a linear regression (r = 0.9257), daily growth for the pickerel was determined to be 1.10 mm/day compared to only 0.35 mm/day for mudminnows. A pilot study was conducted on the grass pickerel and mudminnow with electrophoretic techniques and a LDH stain to determine if different isozymes were utilized during periods of low dissolved oxygen. Pickerel head and muscle tissue yielded two homotetramers, LDH-B and LDH-A, and one hetrotetramer, LDH-A_2B_2. The heart tissue displayed only one homotetramer, LDH-B. All mudminnow tissues expressed a single homopolymer, therefore, a single locus expressed for LDH could not be identified. The pI values for stained bands were calculated using linear regression analysis. Fish collected during this period showed no shift in LDH-A or LDH-B even though dissolved oxygen levels varied from 1.33 mg/L to 3.00 mg/L.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere thanks to Dr. Tarter for accepting me as a graduate student and providing me with countless hours of guidance and wisdom. I would also like to thank Dr. Pauley and Dr. Little for consenting to serve on my thesis committee and for their suggestions in the preparation of this manuscript. Much thanks is given to everyone who helped with field collections in the cold and heat: Andrea Henry, Matt Wooten, Eric Wilhelm, Kevin Brittingham, Rob Hood, Jennifer Aderman, and Gail Perrine. Without their help, I would have had fewer fish in the study. Thanks goes to Tim Hayes who collected larval fish and helped with the gut analysis. I would like to thank Wes Gladwell, Dr. Little, and Jason Barnett for dedicating their time to help with the electrophoretic experiment. Without them I would never have been able to do the pilot study. Special thanks goes to my boyfriend, Paul Grimmett for helping with field collections and for the encouragement he provided throughout all phases of my research. I would also like to thank my son Jonathan, for always believing in me and helping me when he could. Finally I would like to thank my parents, Bill and Theresa Moore, for helping me in anyway possible when I needed it.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Green Bottom Wildlife Management Area (GBWMA) is a naturally occurring wetland that serves as a nursery for larval fish including the grass pickerel (*Esox americanus vermiculatus*) and central mudminnow (*Umbra limi*). Larvae of both species inhabit in water with abundant submerged or emergent vegetation. These areas are often subject to hypoxia (reduced oxygen levels) during the summer and fall months. Grass pickerel and the mudminnow are physostomous fishes that are able to utilize both atmospheric and dissolved oxygen.

Pickerel are northern-hemisphere freshwater fishes that are usually classified in the order Salmoniformes. They are considered to be efficient predators that control centrarchid populations (Etnier and Starnes, 1993). The grass pickerel may be a rare fish in West Virginia whose status is listed as Undetermined on the Vertebrate Species of Concern in West Virginia (West Virginia Nongame Wildlife and Natural Heritage Program Cooperative Research Projects, 1995) .

Mudminnows make up a small family of strictly freshwater northern-hemisphere fishes. They are considered closely related to the pike family. *Umbra limi* has some economic value as a bait fish (Etnier and Starnes, 1993).

While the diet of the adult pickerel and mudminnow has been studied extensively, little is known about the feeding habits and growth rates of the larval fish and juvenile fish. The grass pickerel is a highly piscivorous fish that preys on the young of later-spawning species (Wallus et al., 1990). A study in Connecticut found that the diet of the grass pickerel under 15 cm consisted of crustaceans, algae, insects, plants, and fish (Buss, 1962). In Oklahoma, grass pickerel from 12 - 49 mm in length fed on copepods, cladocerans, chironomids, and ephemeropterans. In the next size range (50 - 99 mm), diet is composed mainly of fish along with amphipods, decapods, chironomids, and odonates (Ming, 1968). Ming (1968) and Crossman (1962) found that aquatic vegetation, leaves, filamentous algae, and sand grains were ingested by accident. Kleinart and Mraz (1966) noted that the stomach contents of grass pickerel 9.0 - 15.0 mm long contained cladocerans, copepods, and ostracods.

Diet of the central mudminnow is similar to the younger stages of the grass pickerel. *The central mudminnow* is considered a carnivorous species, although it does ingest plant material. According to Peckham and Dineen (1957), important food items of the young fish are small crustaceans (ostracods, cladocerans, and copepods). Aquatic invertebrates were the major food items along with mollusks, cladocerans, ostracods, and copepods for all age classes of the mudminnow (Martin-Bergmann and Gee, 1985; Colgan and Silburt, 1984; Scott and Crossman, 1973). Mudminnows (1 year old +) were shown to consume several species of fish (Chilton et al., 1984).

Daily larval growth rate in the grass pickerel has been documented; in Oklahoma, the spring-spawned grass pickerel grew at a rate of 0.60 mm/day during the period from March to June. The winter-spawned pickerel grew 0.25 mm/day during the winter months and 0.66 mm after March. In Michigan, grass pickerel grew at a rate of 0.96 mm/day for a 30-day period early in their first summer (Wallus et al., 1990). In West Virginia, grass pickerel grew 0.55 mm/day in an oxbow lake in Wayne County (Evans, 1972).

Hubbs and Lagler (1943) collected a fall spawn of the grass pickerel in a tributary of Fleming Creek, Washtenaw County, Michigan. Twelve of the fall-spawned fish averaged only 27.7 mm in length in comparison with spring-spawned fish that averaged 113.5 mm. The fall spawn was attributed to unusually warm weather in October.

No information on larval growth has been found pertaining to the central mudminnow. Wallus et al. (1990) determined the lengths of each life history phase (yolk-sac 5-8 mm; post yolk-sac 8-19 mm; juvenile 20-46 mm). Although size ranges have been referred to by several sources, none have indicated calculated growth rates for the larval and juvenile stage.

The isozymes of lactic acid dehydrogenase (LDH) have been utilized as genetic markers to examine gene expression and diversity in numerous teleost species (Eckroat, 1975). LDH has been used to identify species from eggs of marine and hybrid species (Mork et al., 1983). Vonwyl and Fischberg (1980) stated that in most vertebrates, the enzyme LDH is a tetramer composed of two subunits A and B. In most fishes, the occurrence of an additional C locus, which probably arose by the duplication of the B locus, could be demonstrated. Eckroat (1975) analyzed extracts of skeletal muscle of the grass pickerel electrophoretically and found that it exhibits homotetramers of A and B and also a heterotetramer, A_2B_2. This same pattern has also been observed in the Amazon fishes *Mylossoma duriventris* and *Colossoma macropomum* (Fonseca de Almeida-Val et al., 1990). It has been illustrated that the B homopolymer migrates to the more acidic or anodal end of the gel, whereas the A homopolymer electrophoreses to the more basic or cathodal end (Beck et al., 1984; Clayton and Gee, 1969).

The major objectives of my study were: (1) to investigate the feeding habits and growth rates of larval and juvenile grass pickerel and central mudminnow and (2) to determine the lactic acid dehydrogenase (LDH) isozyme patterns involved with hypoxic conditions in grass pickerel and the central mudminnow.

CHAPTER II

TAXONOMY AND DISTRIBUTION

Taxonomy

Esox americanus contains two subspecies, *E. a. americanus* Gmelin (redfin pickerel) and *E. a. vermiculatus* LeSueur (grass pickerel). These two species are the smallest in the family Esocidae (pike), rarely exceeding 38 cm in length. The grass pickerel was named *vermiculatus* due to worm-like appearance of the markings on its body (Buss, 1962). *Esox a. vermiculatus* also has a distinctive teardrop on its cheek (Fig. 1a). The grass pickerel is also commonly referred to as a mud pickerel because it prefers clear water with muddy bottoms. Other common names for the grass pickerel include: little pickerel, pickerel, grass pickerel, and mud pike (Crossman, 1962).

Umbra limi (central mudminnow) belongs in the family Umbridae. This species of *Umbra* is approximately 10 cm in length as an adult and is characterized by vertical bar of dark pigment (Wallus et al., 1990) (Fig. 1b). The central mudminnow is often referred to as the western mudminnow, Mississippi mudminnow, mudfish, dogfish, and mudminnow (Scott and Crossman, 1973). Members of Umbridae are considered to be close relatives of the family Esocidae , although they superficially do not resemble them.

Distribution

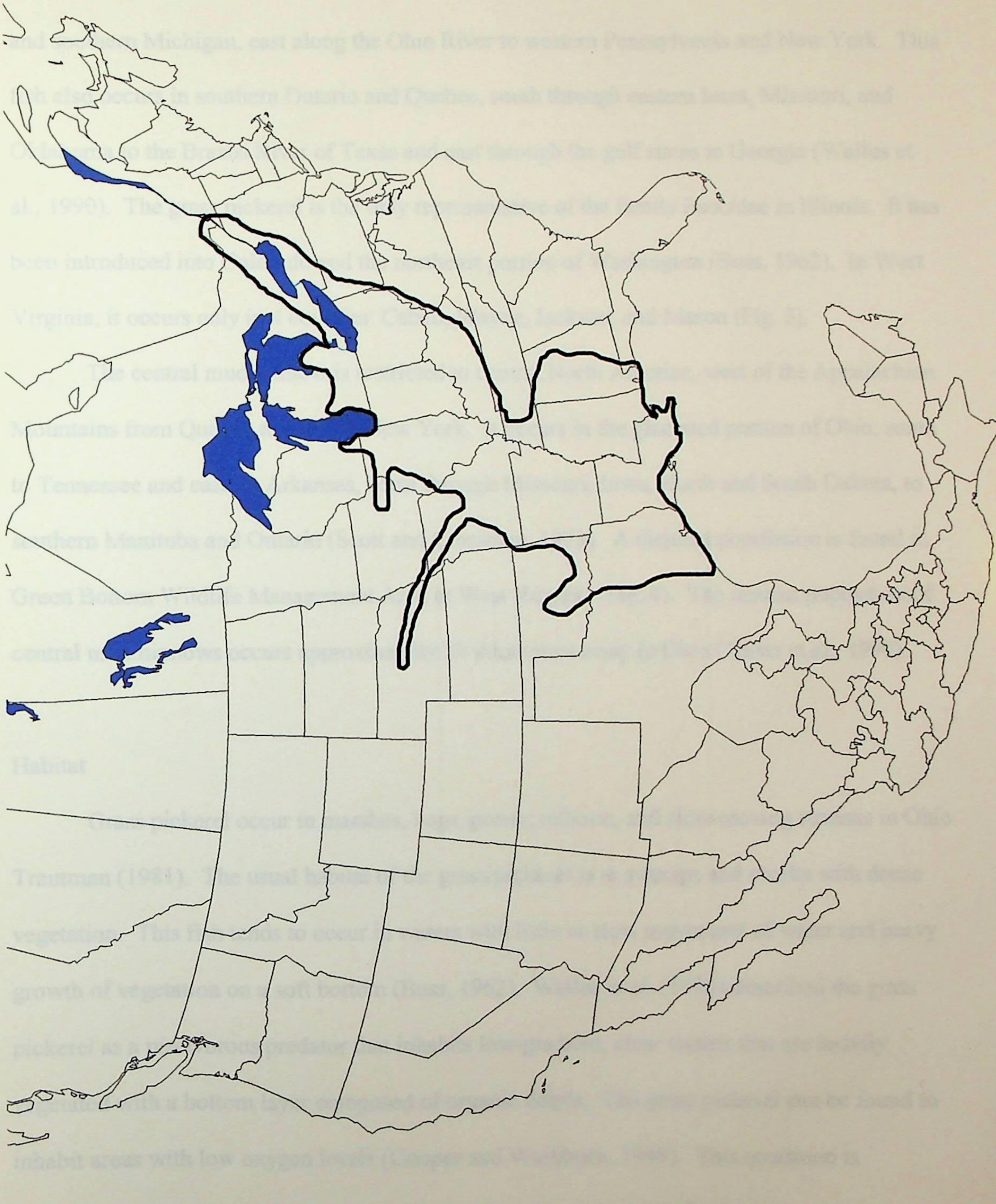
The grass pickerel is considered to be the western subspecies of *Esox americanus* (Fig. 2). It occurs throughout the Mississippi drainage. The grass pickerel is found in Wisconsin

Figure 1. (a) Photograph of grass pickerel, *Esox americanus vermiculatus* (b) Photograph of central mudminnow, *Umbra limi*

(a)

(b)

Figure 2. North American distribution of the grass pickerel.



and southern Michigan, east along the Ohio River to western Pennsylvania and New York. This fish also occurs 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). The grass pickerel is the only representative of the family Esocidae in Illinois. It has been introduced into Colorado and the northeast portion of Washington (Buss, 1962). In West Virginia, it occurs only in 4 counties: Cabell, Wayne, Jackson, and Mason (Fig. 3).

The central mudminnow is restricted to central North America, west of the Appalachian Mountains from Quebec to western New York. It occurs in the glaciated portion of Ohio, south to Tennessee and eastern Arkansas, north through Missouri, Iowa, North and South Dakota, to southern Manitoba and Ontario (Scott and Crossman, 1973). A disjunct population is found in Green Bottom Wildlife Management Area in West Virginia (Fig. 4). The nearest population of central mudminnows occurs approximately 50 kilometers away in Ohio (Tarter et al., 1990).

Habitat

Grass pickerel occur in marshes, bogs, ponds, oxbows, and slow-moving streams in Ohio Trautman (1981). The usual habitat of the grass pickerel is in swamps and creeks with dense vegetation. This fish tends to occur in waters with little or slow movement of water and heavy growth of vegetation on a soft bottom (Buss, 1962). Wallus et al. (1990) described the grass pickerel as a piscivorous predator that inhabits low-gradient, clear waters that are heavily vegetated with a bottom layer composed of organic debris. The grass pickerel can be found to inhabit areas with low oxygen levels (Cooper and Washburn, 1949). This condition is known to occur in swamps and other areas that become stagnant in the summer season.

Figure 3. Distribution of the grass pickerel in West Virginia.

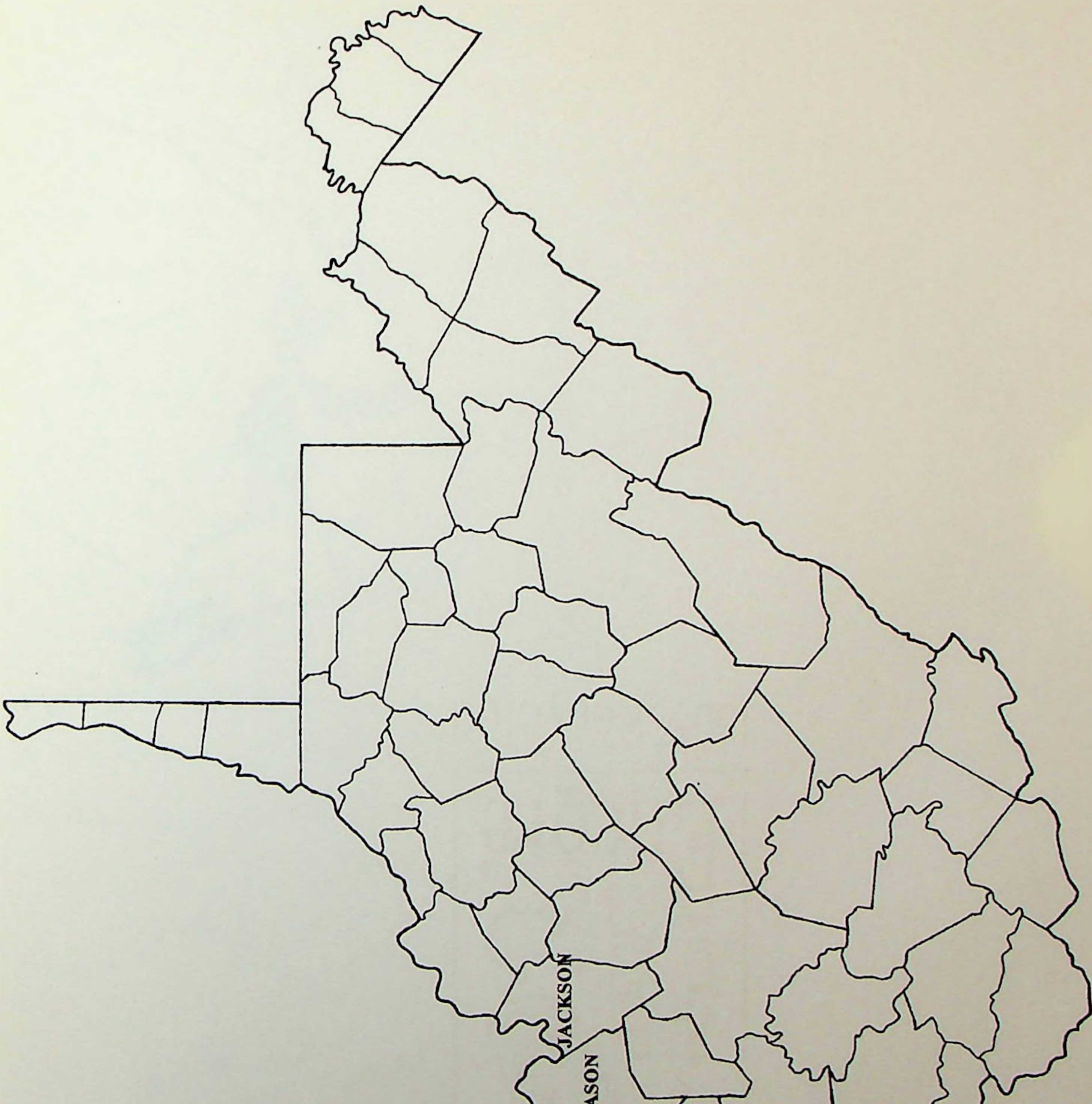
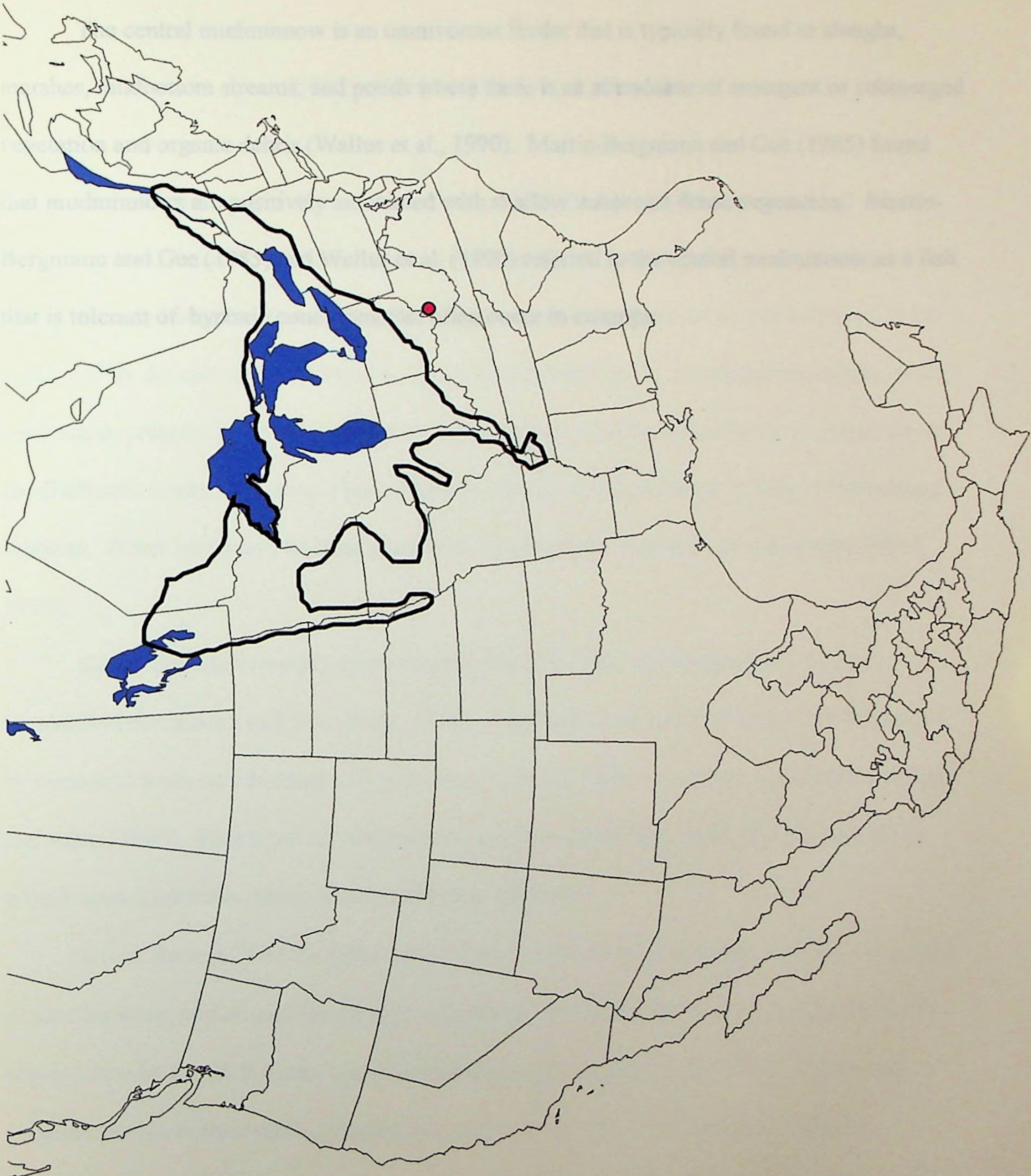

JACKSON
MASON
CABELL
WAYNE

Figure 4. North American distribution of the central mudminnow.



The central mudminnow is an omnivorous feeder that is typically found in sloughs, marshes, mudbottom streams, and ponds where there is an abundance of emergent or submerged vegetation and organic debris (Wallus et al., 1990). Martin-Bergmann and Gee (1985) found that mudminnows are positively associated with shallow water and dense vegetation. Martin-Bergmann and Gee (1985) and Wallus et al. (1990) referred to the central mudminnow as a fish that is tolerant of hypoxic conditions that often occur in swamps.

CHAPTER III

DESCRIPTION OF THE STUDY SITE

Green Bottom Wildlife Management Area (GBWMA) is a naturally occurring wetland located on State Route 2, 26.7 km northeast of Huntington, West Virginia, along the Cabell and Mason County Line (Evans and Allen, 1995). The wetland is bordered on the north by the Ohio River and on the east and west by open, grassy farmlands (Fig. 5). A mitigation project (29 ha) has been in progress since 1990 to replace wetlands that were destroyed by the construction of the Gallipolis Locks and Dam. This mitigation area occurs on the western side of the existing wetland. Water levels will be controlled with a preservation weir and a levee system (Wirts, 1993).

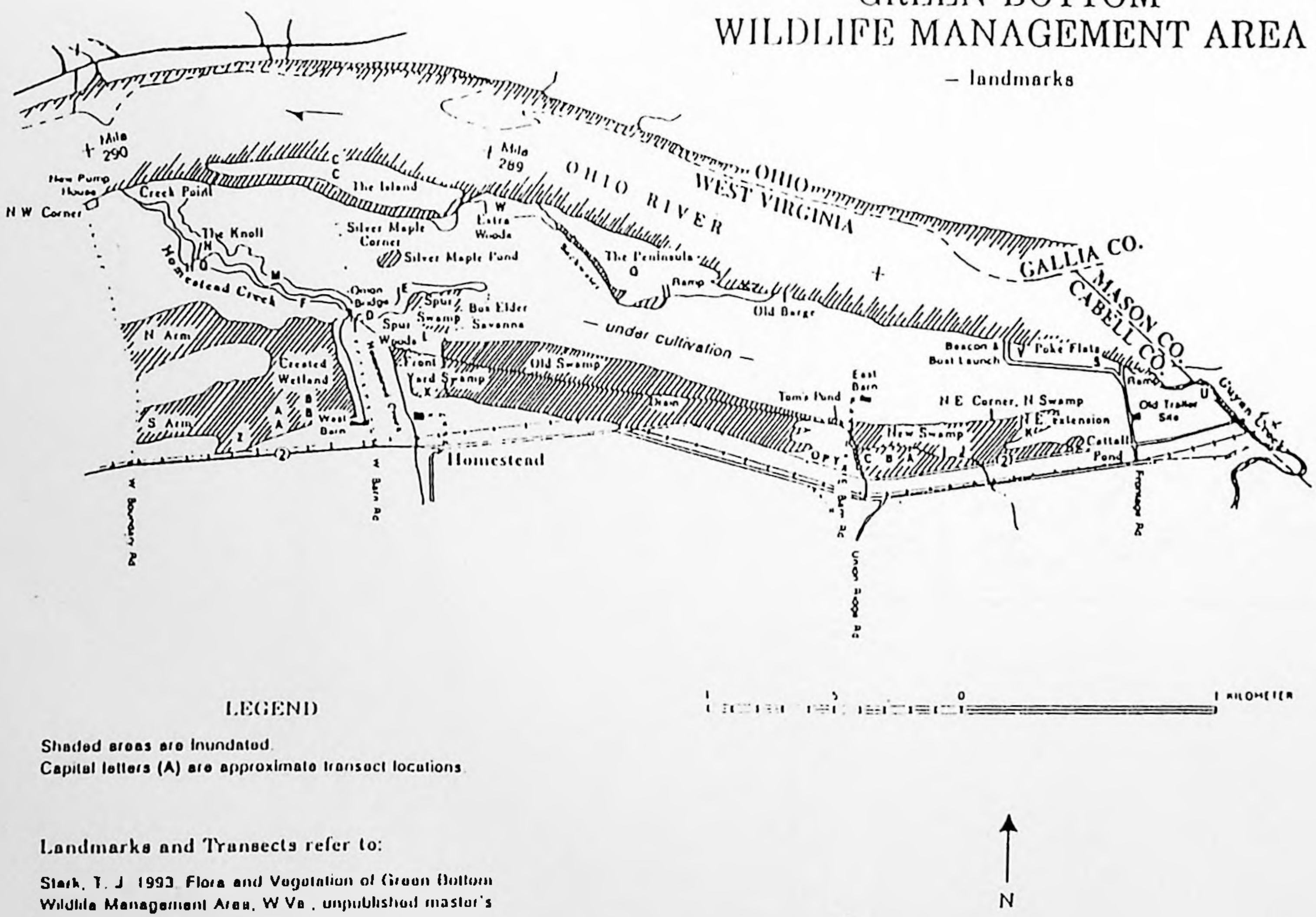
Green Bottom Swamp is approximately 60 ha in size, with water depth varying seasonally between 0.5 to 2.5 m (Furry, 1978). Hydrology and wetland acreage are influenced by terrestrial input and drainage controls, such as beaver dams and human construction (Evans and Allen, 1995). Four types of wetlands are present in GBWMA: seasonally flooded flats, inland open freshwater, shrub swamp, and wooded swamp.

Green Bottom Wildlife Management Area has an atypical occurrence of *Acer negundo*, *A. saccharinum*, and *A. saccharum* in standing water due to the beaver activity and the levee construction by the U. S. Army Corps of Engineers (Evans and Allen, 1995). The swamp contains mainly buttonbush, *Cephalanthus occidentalis*, with marsh mallow, *Hibiscus mocheutos*, also being present. Three genera of duckweed, *Lemna minor*, *Spirodela polyrhiza*, and *Wolffia* sp., cover the majority of the surface water during the spring, summer, and fall

Figure 5. 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.

Figure 6. Collecting site at Green Bottom Swamp located in front of General Jenkin's House.

seasons. Many species of *Carex* thrive in and around the swamp in the soft, marshy soils (Fig. 6).

Green Bottom Wildlife Management Area provides excellent habitat for several amphibians, reptiles, mammals, and birds. Many aquatic insects, such as dragonflies, damselflies, and mayflies, are present in the sedges and rushes of the swamp. Several species of fish are known to occur in the wetland. GBWMA has the only reproducing population of bowfin (*Amia calva*) in West Virginia. The central mudminnow (*Umbra limi*) is a disjunct population that survives in Green Bottom Swamp (Daniels, 1993). Other species of fish known to occur in Green Bottom Swamp are: grass pickerel (*Esox americanus vermiculatus*), bluegill (*Lepomis machrochirus*), green sunfish (*Lepomis cyanellus*), largemouth bass (*Micropterus salmoides*), black and white crappie (*Pomoxis nigromaculatus* and *P. annularis*), carp (*Cyprinus carpio)*, and black and yellow bullheads (*Ictaluras melas and I. natalis*) (Evans and Allen, 1995).

The first photographic record of GBWMA is an aerial photograph in 1934. The photograph demonstrates that Green Bottom Swamp was drained and used as a pasture and an orchard. Since 1950, three events occurred which raised the water level and caused the swamp to redevelop: (1) the construction of a farm road, (2) the construction of State Route 2, and (3) colonization by beaver (Evans and Allen, 1995). When State Route 2 was constructed in 1975, approximately 3.24 ha of the swamp was lost. Over 85 percent of the bottom land forest was dead or dying by 1989 due to the inundation caused by the beaver.

CHAPTER IV

MATERIALS AND METHODS

Field Collections

Larval and juvenile mudminnow and grass pickerel were collected from Green Bottom Wildlife Management Area during April and May of 1995 and 1996. Grass pickerel larvae were also collected in December 1994, June 1996, and July 1996. Dipnets and seines were used to collect larval and juvenile fish. Upon capture, all fish were placed in chlorotone to prevent regurgitation. In the laboratory, fish were transferred into 70 percent ethanol for preservation. Mudminnow and grass pickerel collected on May 22 and 23, 1996 were placed on dry ice to slow protein degradation for electrophoretic analysis .

Water Quality and Temperature

At the beginning of each month, water quality and temperature (°C) were determined. Temperature was measured using a Taylor minimum-maximum thermometer. The following water quality parameters were measured using a Hach chemical kit (Model 36-WR): dissolved oxygen (mg/L), alkalinity (mg/L $CaCO_3$), carbon dioxide (mg/L), hardness (mg/L), and pH.

Laboratory Studies

Grass pickerel and mudminnow were divided into the following early life phases: (1) yolk-sac larvae (YSL), (2) post yolk-sac larvae (PYSL), and (3) juveniles (J). The YSL phase is the phase of development from the moment of hatching to complete absorption of the yolk. The

PYSL phase begins with complete absorption of the yolk and ends when a minimum adult complement of rays is present in all fins and the median finfold is completely absorbed. The J phase begins when an adult complement of rays is present in all fins and the median finfold is completely absorbed (Wallus et al., 1990). The following size ranges are associated with the early life phases of the grass pickerel: (1) YSL/6-11mm; (2) PYSL/12-22mm; (3) J/23-100mm. The size ranges differ somewhat for the central mudminnow: (1) YSL/5-8mm; (2) PYSL/9-19mm; (3) J/20-46mm.

Growth

The weight (0.001 g) of each fish was recorded on a top-loading analytical balance and total length (0.01 mm) was measured using vernier calipers. Total length is the greatest dimension between the anterior projecting part of the head and the farthest tip of the caudal fin when the caudal rays are squeezed together (Hubbs and Lagler, 1958). Measurements were made shortly after preservation to disallow for error due to shrinkage or swelling of the fish tissue. Daily growth rates (mm/day) were determined for the two larval fishes using linear regression analysis.

Food Habits

Microdissecting scissors were used to excise the digestive tract from the esophagus to the beginning of the small intestine. Stomachs were then placed in 70 percent ethanol. Volume of all food items in a stomach occupied was estimated using a point method devised by Hynes (1950). In this point method, each stomach was allotted a specific number of points based on its fullness (distended - 30; full - 20; three-quarters full - 15; half full - 10; one-quarter full - 5; trace - 2; empty - 0). After the points were allotted, the stomach was opened and the food items were identified under a dissecting microscope using Merritt and Cummins (1984) and Needham and Needham (1962). The point values awarded to each type of food item were used to calculate (1) percent total volume = total number of points per given food item / total points awarded per study period, and (2) percent frequency of occurrence = number of stomachs in which an item appeared / total number of stomachs in a given study period. Diet overlap values were determined for each species and size classes using the indices of Horn (1966) and Morisita (1959).

Electrophoresis

On May 22 and 23 1996, 21 grass pickerel and twenty-five central mudminnows were collected in the old swamp in front of General Jenkin's house. Time of day, dissolved oxygen (mg/L), carbon dioxide (mg/L), and pH were recorded from each site where larval fish were collected. Fishes were immediately preserved on dry ice to slow degradation of proteins and were stored at -70°C. Each fish was measured for total length and weight. Skeletal muscle, heart, liver, brain tissue, and stomach were removed from each fish. Stomachs were preserved in 70 percent ethanol until food analysis was performed. Tissues were homogenized separately in grinding buffer and isozymes of each fish and tissue were separated on 3 mm acrylamide isoelectric focusing gels. Each gel was stained for LDH with LDH stain (Hillis et al., 1996).

CHAPTER V

RESULTS AND DISCUSSION

Field Collections

In December of 1994, 8 post yolk-sac and 8 juvenile grass pickerel were collected in the south arm of the mitigated area. This collection represents a fall spawn of the grass pickerel based on the mean length (23.88 mm) of the 16 fish collected. The grass pickerel is a spring spawner, although fall spawns have been known to occur if the temperature threshold of 9°C has been met or exceeded. In December, unusually high temperatures occurred (Fig. 7) in Green Bottom Swamp and may have initiated the fall spawn.

From 3 April 1995 to 8 July 1996, three stages of the grass pickerel were collected. The larval and juvenile fish ranged from 6.67 to 101.45 mm in length. The central mudminnow was collected from 8 April 1995 to 6 June 1996. The smallest mudminnow collected was 3.89 mm in length, while the largest mudminnow collected was 36.97 mm. Mudminnows of all three stages were collected during these dates. On 22 and 23 May 1996, 46 pickerel and mudminnows were collected for an electrophoretic experiment.

Water Quality and Temperature

Water quality parameters are listed in Table 1. Dissolved oxygen (DO) ranged from 3 mg/L in September 1995 to 10 mg/L in March and April 1995. A mean value of 6.4 mg/L for DO was calculated for 1995-96. Carbon dioxide levels in Green Bottom Swamp ranged from 10-25 mg/L, with a mean of 15.9 mg/L. Alkalinity in the swamp was at its lowest point

Figure 7. Minimum, present, and maximum temperatures recorded at Green Bottom Wildlife Management Area, Cabell County, West Virginia from December 1994 through July 1996.

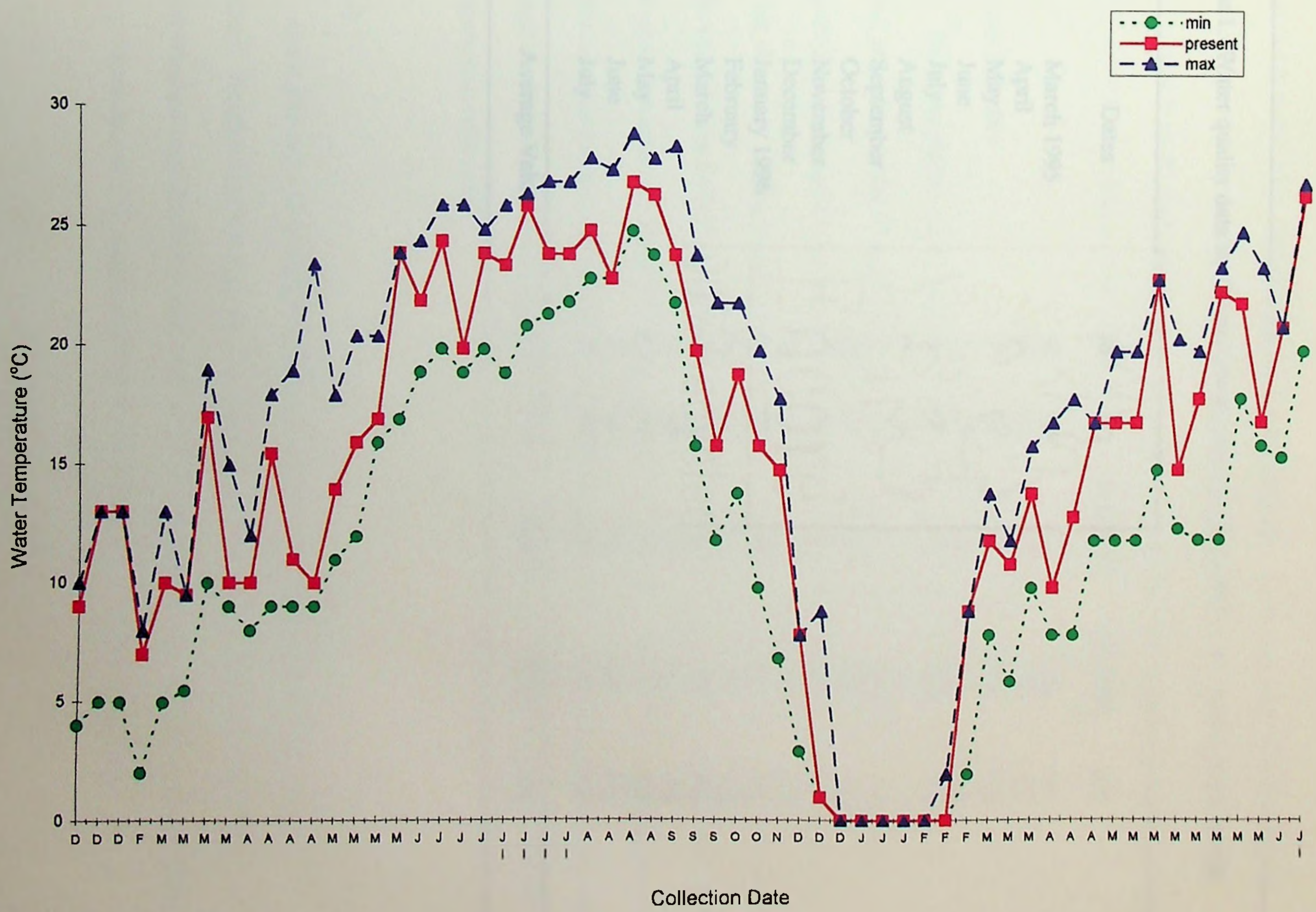

min
present
max
30
25
20
15
10
5
0
Water Temperature (°C)
D D D F M M M M A A A A M M M M J J J J J J J J A A A A S S S O O N D D D J J J F F F M M M A A A M M M M M M M M J J
Collection Date

Table 1. Water quality data for Green Bottom Swamp (1995-1996). All units in mg/L except pH.

Dates	DO	CO_2	Alkalinity	Hardness	pH
March 1995	10	10	119	119	7.1
April	10	15	51	123	7.3
May	7	15	51	123	7.6
June	5	10	140	102	7.2
July	4	15	140	119	7.2
August	4	15	51	119	7.7
September	3	15	51	153	7.8
October	5.5	20	85	119	7.8
November	5	20	85	102	7.6
December	7.5	10	51	102	7.5
January 1996	8	15	85	119	7.1
February	9	15	102	85	7.2
March	7	15	102	85	7.5
April	7	20	85	123	7.5
May	6.5	25	119	119	6.9
June	5	20	119	119	7.2
July	5	15	102	102	7.3
Average Value	6.4	15.9	90.5	113.7	7.4

(51 mg/L) in April, May, August, September, and December of 1995. Values for alkalinity reached a high of 140 mg/L in June and July of 1995; mean value was 90.5 mg/L. Hardness values ranged from 85 mg/L to 153 mg/L, with a mean of 113.7 mg/L. The pH varied only slightly, 6.9-7.8, from March 1995 to July 1996.

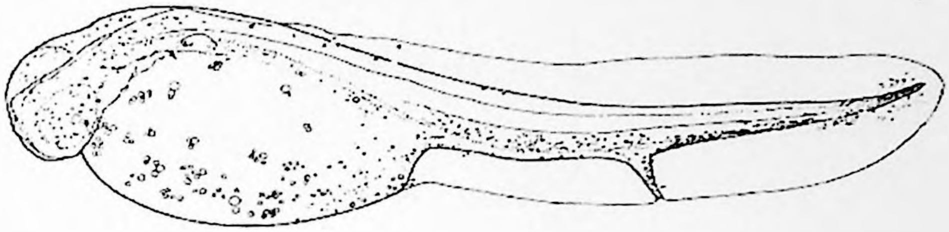
Laboratory Studies

The grass pickerel (Fig. 8a-d, Fig 9a-d) and central mudminnow (Fig. 10a-e) were divided, according to their lengths, into three early life history phases, (1) yolk-sac larvae, (2) post yolk-sac larvae, and (3) juveniles (Walllus et. al.1990). Of 77 pickerel collected, four were yolk-sac larvae, 12 were post yolk-sac larvae, and 61 were in the juvenile phase. Yolk-sac larvae lengths ranged from 6.67-7.43 mm, while the post yolk-sac larvae collected ranged from 12.69-21.99 mm. Juveniles had the largest range, 23.91-101.45 mm, in length. Nineteen mudminnows that were captured in the yolk-sac larvae phase ranged from 3.89-8.96 mm. Forty-nine post yolk-sac stage mudminnows ranged from 9.14-19.92 mm in length. Fifty-two juveniles (20.02-36.97 mm) were also collected.

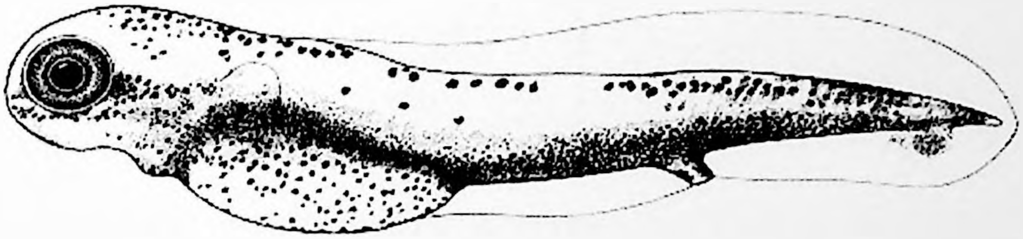
Growth

Grass pickerel and central mudminnows collected were weighed (0.001 g) in the laboratory. Weights for grass pickerel YSL phase varied from 0.001 to 0.002 g. The PYSL pickerel weighed considerably more (0.009 - 0.080 g). Juveniles had the largest range of 0.068 - 6.993 g. Mudminnow YSL weighed 0.000 - 0.002 g, while the PYSL mudminnows

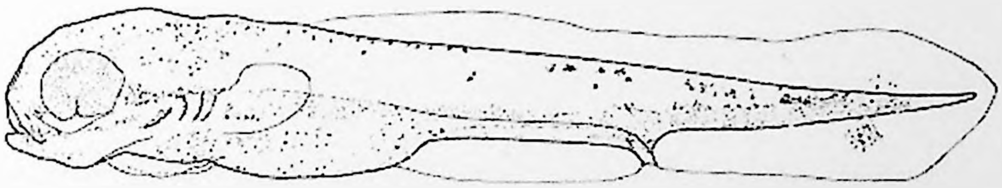
Figure 8. Development of young grass pickerel. (a) - (c) Yolk-sac larvae. (d) Post yolk-sac larvae.



(a) 6 mm



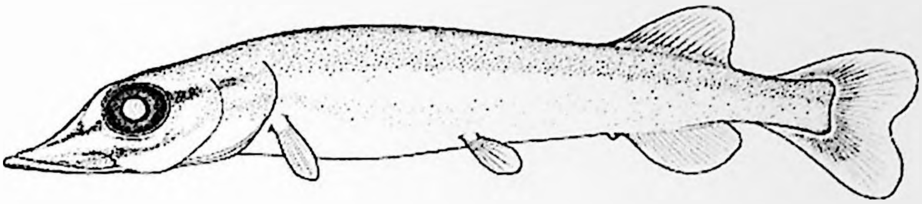
(b) 8.5 mm



(c) 10 mm

(d) 13 mm

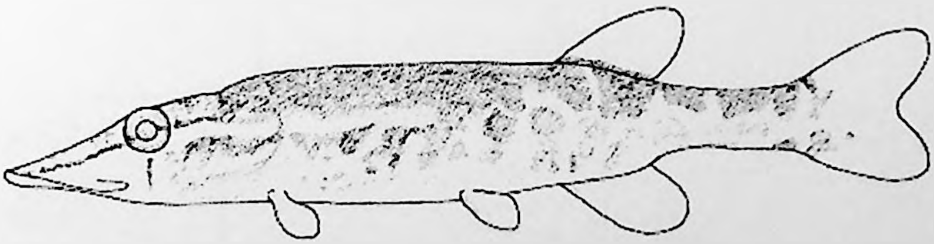
Figure 9. Development of young grass pickerel. (a) - (d) Juveniles.



(a) 25 mm

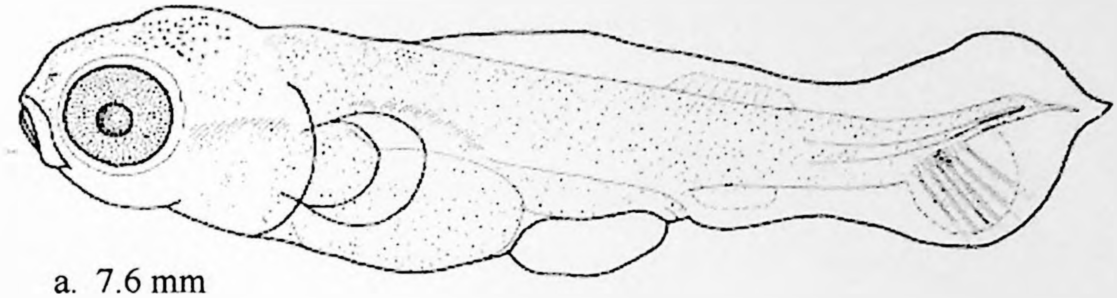
(b) 48 mm

(c) 75 mm

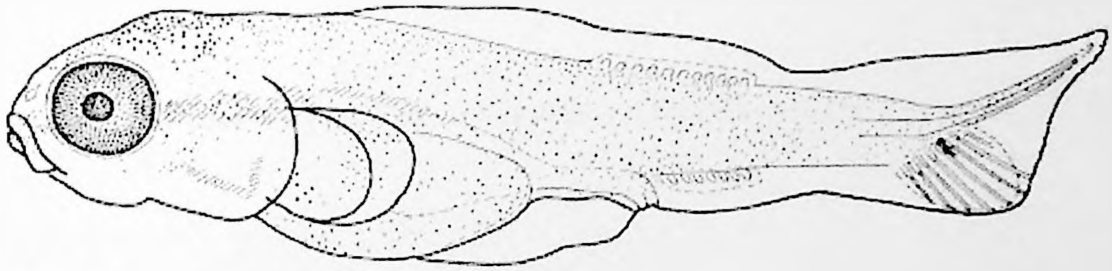


(d) 100 mm

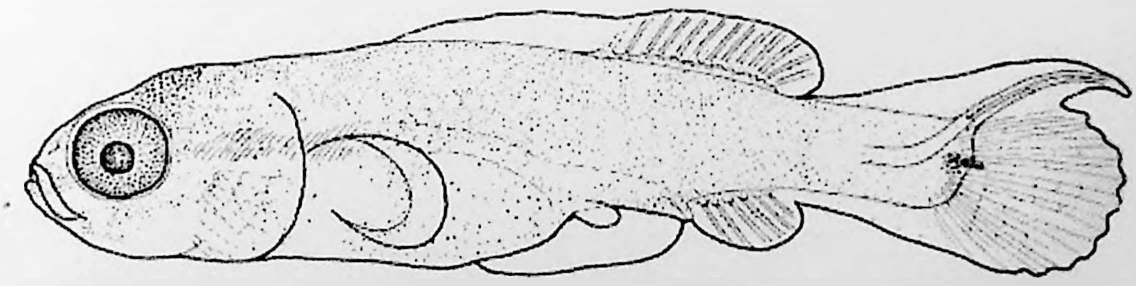
Figure 10. Development of young central mudminnows. (a) Yolk-sac larvae. (b) - (c) Post yolk-sac larvae.



a. 7.6 mm

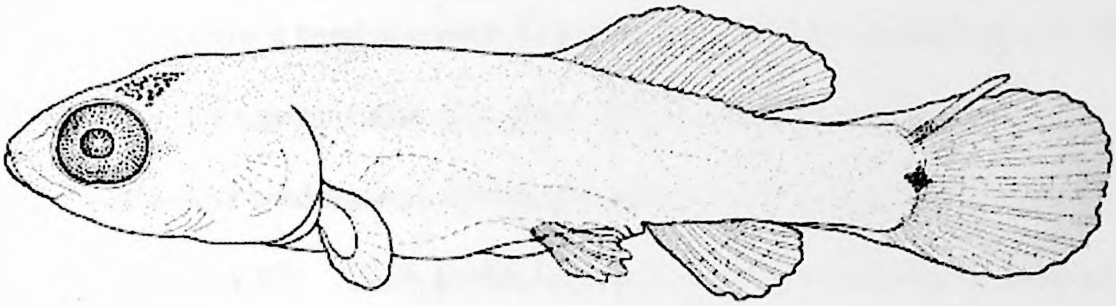


b. 9 mm

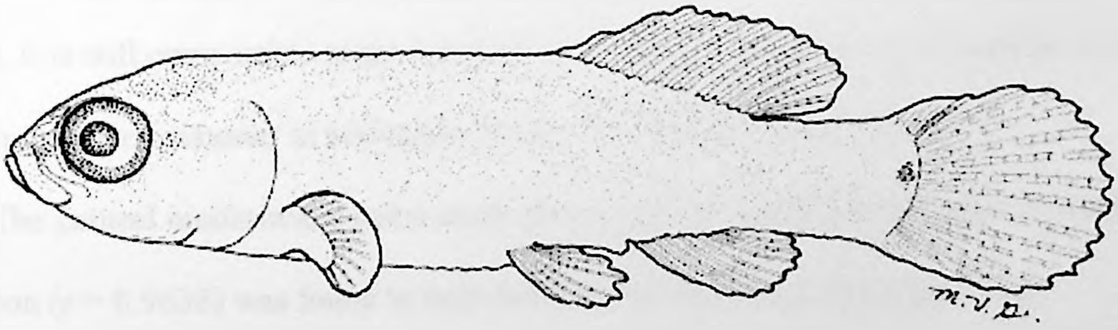


c. 12 mm

Figure 10. Development of young central mudminnows. (d) Post yolk-sace larvae. (e) Early juvenile.



d. 15.5 mm



e. 19 mm

weighed 0.005 - 0.167 g. The juvenile phase had the largest weight range, 0.045 - 0.566 g.

Growth measurements were made for each fish after preservation. Lengths were plotted against Julian days to show a trend in growth throughout the collection period (Fig. 11). The lengths were also plotted against Julian days using a linear regression analysis from Kwikstat 3.3. Julian days used are equivalent to the collection dates (e.g. collection date 4/8/95 is equivalent to Julian day 98). Daily growth rate was back-calculated using the graph (Fig. 12. and 13.).

The grass pickerel grew at a calculated daily rate of 1.10 mm/day. There is a positive correlation ($r = 0.9243$) between the length and Julian day (Fig. 12). This correlation is statistically significant (0.05 Confidence Level). Linear regression equation for the growth of grass pickerel is $Y = -92.82799 + 1.10948X$. Although the daily growth rate is high when compared to the growth rates (0.55 mm/day) of pickerel collected from an oxbow lake in West Virginia, it is still comparable to growth rates (0.96 mm/day) of pickerel collected for a 30 day period in their first summer in Michigan (Evans, 1972; Wallus et al., 1990).

The central mudminnow had a much slower growth rate of 0.35 mm/day. A positive correlation ($r = 0.9638$) was found to exist between the length and Julian day of the mudminnows (Fig. 13). This value is statistically significant using a 95 percent Confidence Level. The linear regression equation for this graph is $Y = -26.36742 + .356844X$. No calculated daily growth rate of mudminnows was found in the literature.

Food Habits

Diet analyses were performed on all three stages of grass pickerel collected from

Figure 11. Mean length (mm) of grass pickerel and central mudminnow for each Julian Day (collection date).

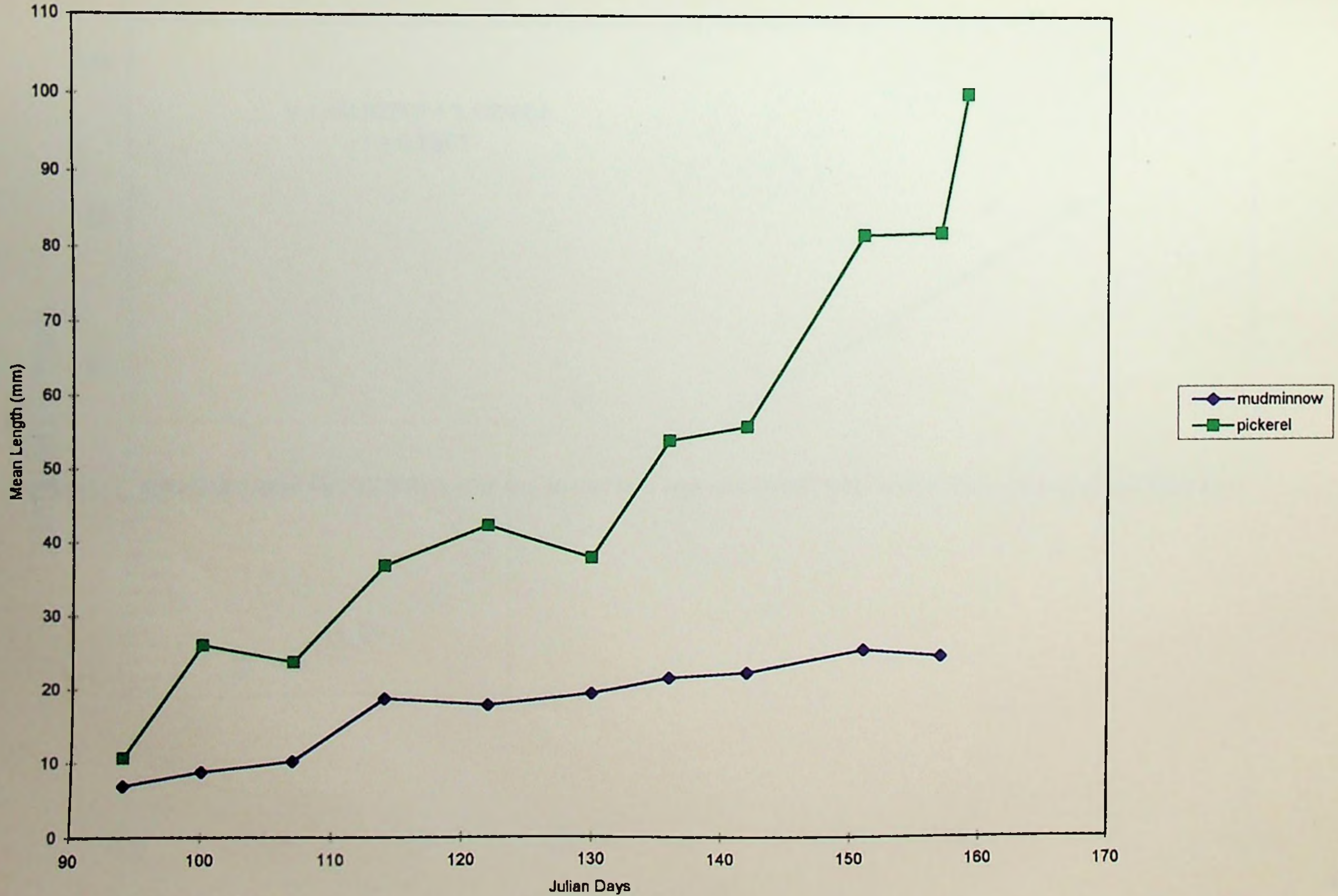
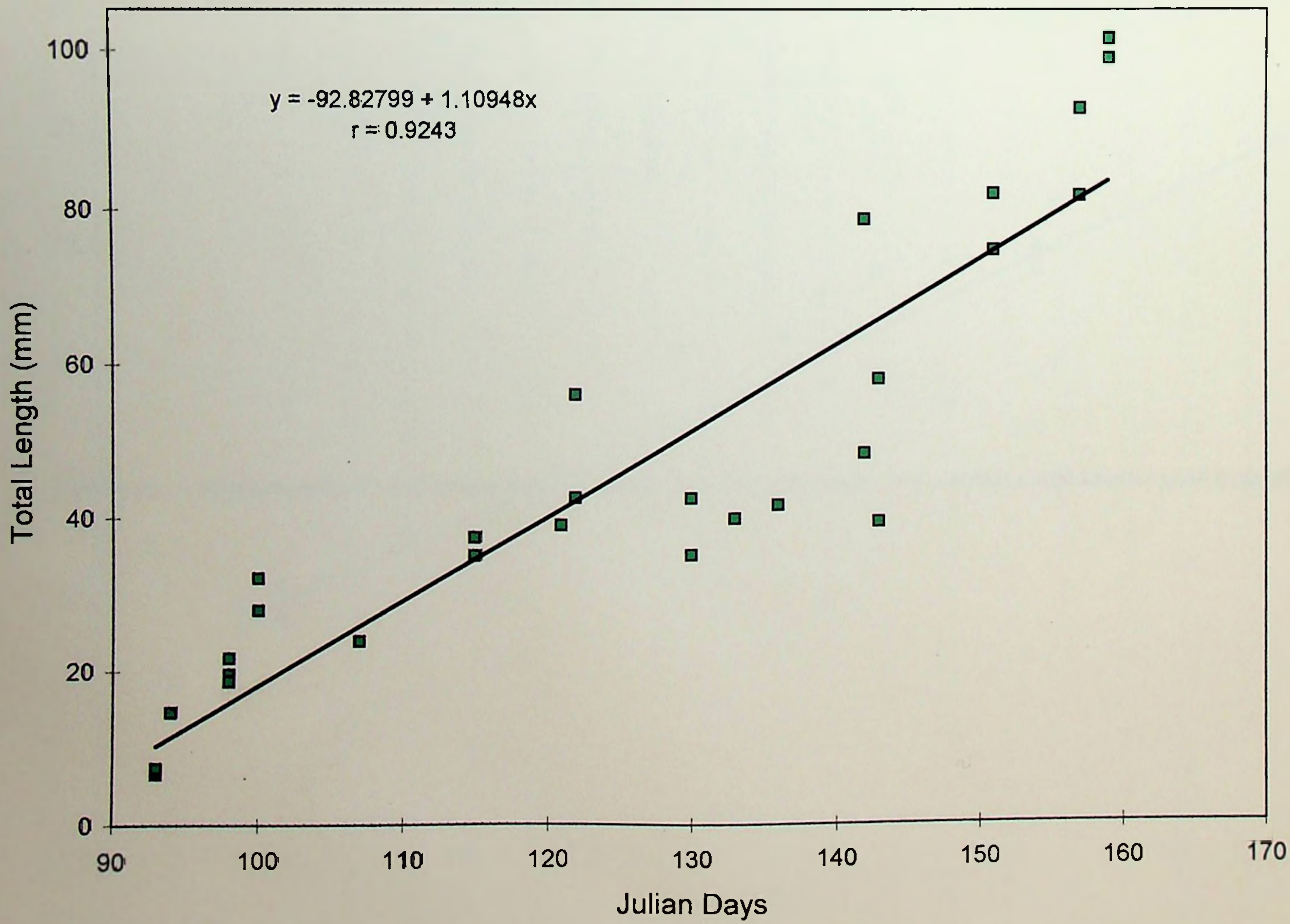
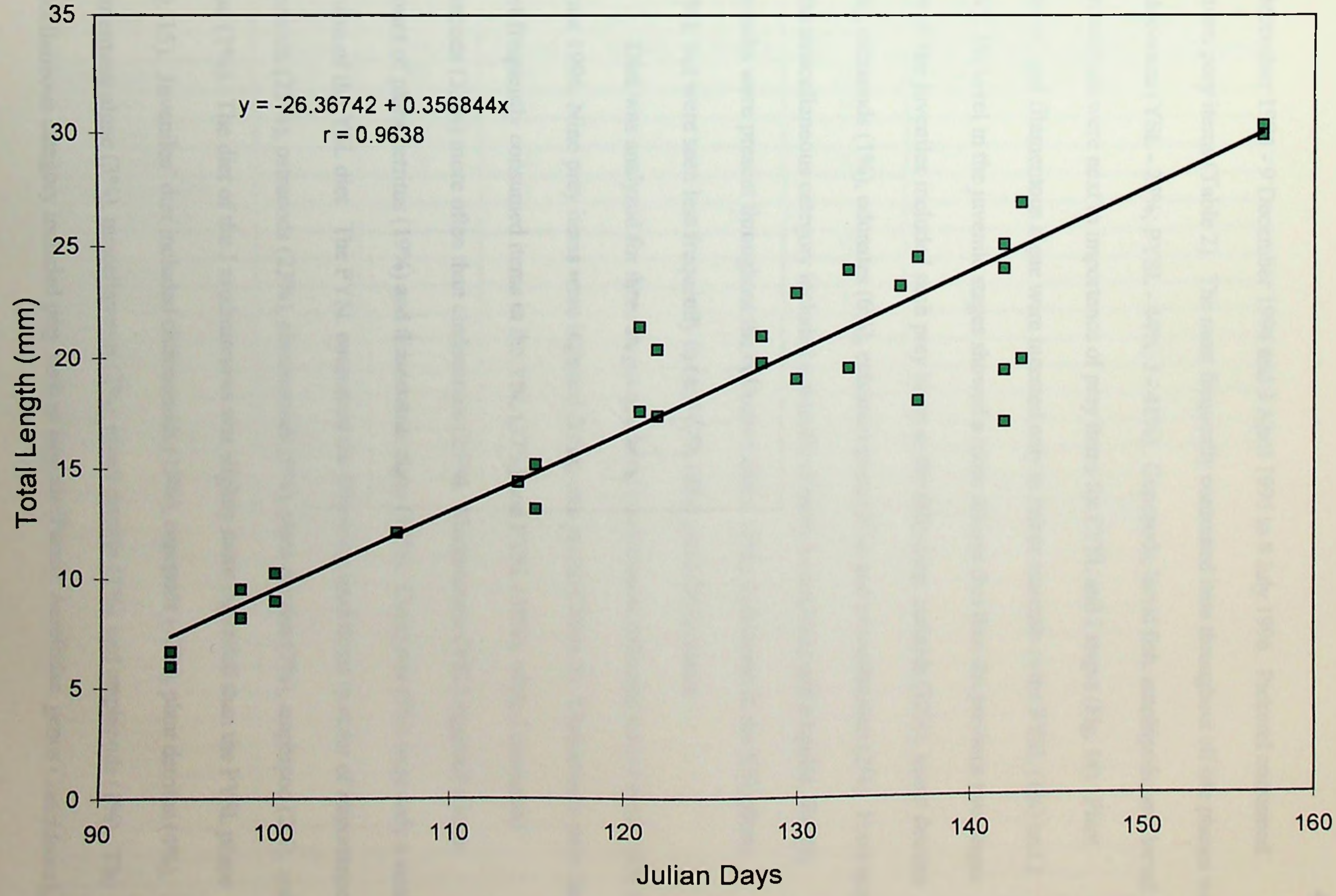

Mean Length (mm)
Julian Days
mudminnow
pickerel

Figure 12. Calculated daily growth rate of yolk-sac larvae, post yolk-sac larvae, and juvenile grass pickerel from GBWMA.



y = -92.82799 + 1.10948x
r = 0.9243
Total Length (mm)
Julian Days
0
20
40
60
80
100
90
100
110
120
130
140
150
160
170

Figure 13. Calculated daily growth rate of yolk-sac larvae, post yolk-sac larvae, and juvenile mudminnows from GBWMA.


y = -26.36742 + 0.356844x
r = 0.9638
Total Length (mm)
35
30
25
20
15
10
5
0
90
100
110
120
130
140
150
160
Julian Days

2 December 1994 - 9 December 1994 and 3 April 1995 to 8 July 1996. Pickerel consumed thirteen prey items (Table 2). The most frequently consumed item throughout all life phases was cladocerans (YSL - 25%; PYSL - 44%; J - 41%). Copepods, larval fish, amphipods, and larval chironomids were next in importance of prey items for PYSL and J stages (Fig. 14). Plant material and filamentous algae were ingested only in minor amounts in the PYSL (1%) and J (3%). Pickerel in the juvenile stages showed a more diverse diet than the previous two stages. Diet of the juveniles included such prey items as the following: corixids (12%), insect detritus (2%), ostracods (1%), odonates (6%), ephemeroptera (1%), and miscellaneous (2%). Food items in the miscellaneous category included a stonefly (Family Leuctridae) and a tipulid. Empty stomachs were present throughout the collection dates. They dominated in the YSL phase (75%), but were seen less frequently in the PYSL (8%) and J (3%) phases.

Diet was analyzed for three stages of central mudminnow collected from 4 April 1996 to 6 June 1996. Nine prey items were ingested during this period (Table 2). Cladocerans were the most frequently consumed items in the YSL (37%) and PYSL (38%), while J consumed ostracods (33%) more often than cladocerans (25%). Mudminnow (YSL) ingested a large amount of plant detritus (19%) and filamentous algae (17%). Copepods (5%) were only a small portion of the YSL diet. The PYSL consumed the following food items in order of importance: copepods (25%), ostracods (23%), chironomids (6%), plant detritus (3%), amphipod (2%), and algae (1%). The diet of the J mudminnows was slightly more expanded than the PYSL phase (Fig. 15). Juveniles' diet included chironomids (15%), copepods (12%), plant detritus (4%), filamentous algae (3%), miscellaneous (2%), insect detritus (1%), and amphipods (1%). The miscellaneous category included prey such as isopods (Family Aesellidae, genus *Caecidotea*),

Table 2. Prey items taken by grass pickerel and central mudminnow from GBWMA from December 1994 to July 1996.

Prey Item	12/2/94	12/7/94	12/9/94	4/3/95	4/4/95	4/8/95	4/4/96	4/17/96	
	Pickerel	Pickerel	Pickerel	Pickerel	Pickerel	Pickerel	Mudminnow	Pickerel	Mudminnow
Cladocerans	70	65	54	25		26	48	25	40
Copepods		15	39			17	7	75	41
Ostracods									4
Chironomids		20	4			2			
Filamentous algae			1				21		1
Empty stomach				75	100				14
Fish						33			
Amphipods						20			
Plant detritus						2	24		
Insect									
Miscellaneous									
Corixids									
Odonates									
Ephemeropterans	30		2						

Prey Item	4/25/96		5/2/96		5/10/96		5/13/96	
	Pickerel	Mudminnow	Pickerel	Mudminnow	Pickerel	Mudminnow	Pickerel	Mudminnow
Cladocerans	54	27	25	41	75	69	45	35
Copepods	7	24		12	3	5	5	18
Ostracods		29		29	2	24		41
Chironomids	18	8	25	6	3	1	50	4
Filamentous algae		2	3	3		1		
Empty stomach								
Fish	9		42					
Amphipods	3			7				
Plant detritus	8	10		2	17			1
Insect detritus	1							
Miscellaneous			5					1
Corixids								
Odonates								
Ephemeropterans								

Table 2 continued. Prey items taken by grass pickerel and central mudminnow from GBWMA from December 1994 to July 1996.

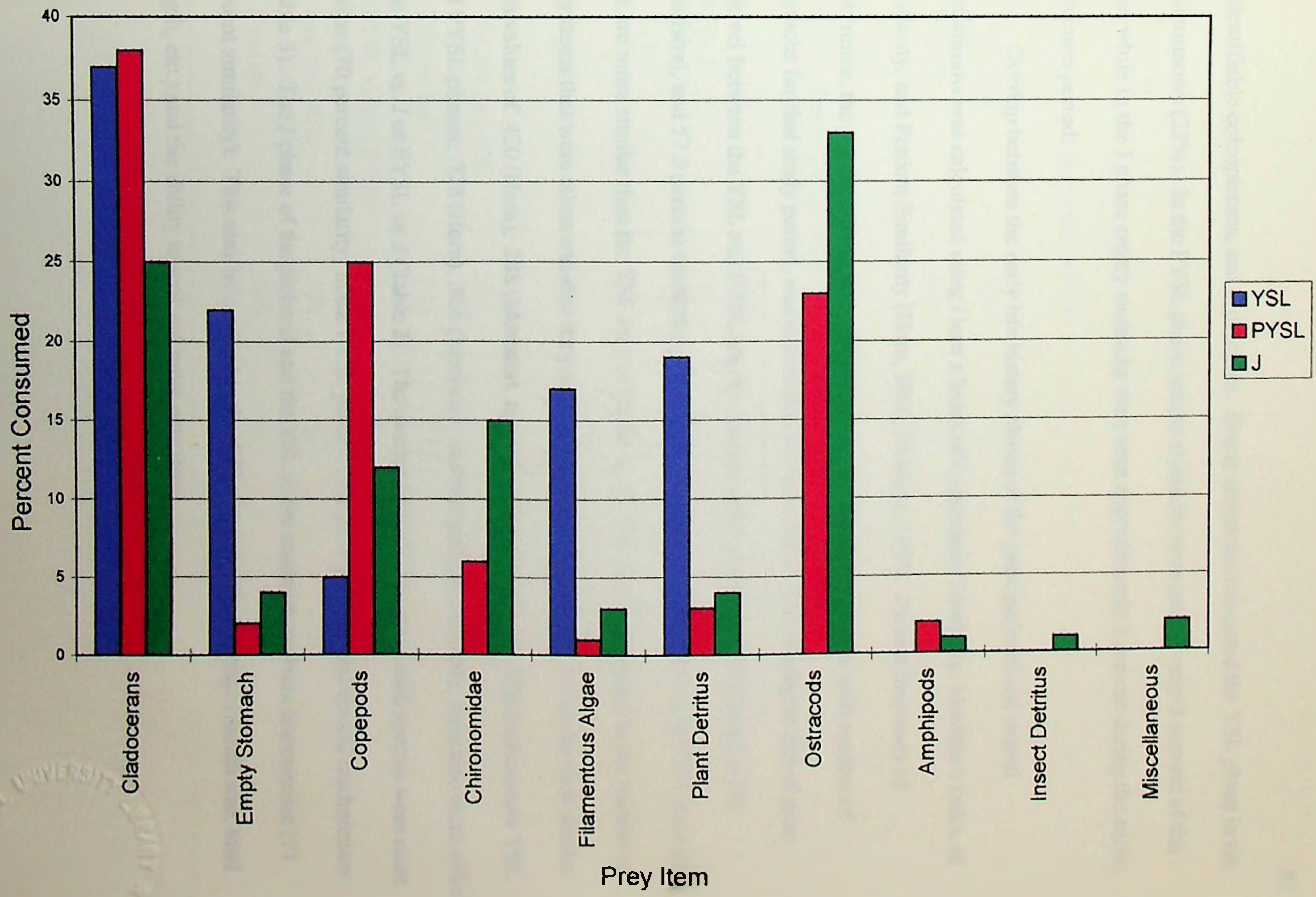
Prey Item	5/16/96		5/17/96		5/22/96		5/23/96	
	Pickerel	Mudminnow	Pickerel	Mudminnow	Pickerel	Mudminnow	Pickerel	Mudminnow
Cladocerans	58	13		15	59	49	46	14
Copepods	3	2		18		7		8
Ostracods	3	85		48	3	13	1	48
Chironomids	12		25	9	12	15	15	21
Filamentous algae			5					
Empty stomach						13	5	6
Fish					25		15	
Amphipods	22			2				
Plant detritus				5	1	3	1	2
Insect detritus	2		20	3			4	1
Miscellaneous							1	
Corixids			45				10	
Odonates			5				2	
Ephemeropterans								

Prey Item	5/31/96		6/6/96		7/8/96
	Pickerel	Mudminnow	Pickerel	Mudminnow	Pickerel
Cladocerans	25	9	4	10	7
Copepods		6		4	
Ostracods		26		30	
Chironomids	9	38	3	23	5
Filamentous algae		11	1		3
Empty stomach	10				
Fish			20		45
Amphipods					
Plant detritus	3	4		12	
Insect detritus	5				3
Miscellaneous		6	1	21	
Corixids	45		19		
Odonates	3		52		37
Ephemeropterans					

Figure 14. Early life phase comparison of the percent frequency of occurrence of prey items of the grass pickerel, GBWMA, Cabell County, West Virginia.


Percent Consumed
0
10
20
30
40
50
60
70
80
Cladocerans
Empty Stomach
Copepods
Chironomidae
Filamentous Algae
Plant Detritus
Ostracods
Amphipods
Insect Detritus
Miscellaneous
Odonates
Ephemeroptera
Fish
Corixids
Prey Item
YSL
PYSL
J

Figure 15. Early life phase comparison of the percent frequency of occurrence of prey items of the central mudminnow, GBWMA, Cabell County, West Virginia.


Percent Consumed
40
35
30
25
20
15
10
5
0
YSL
PYSL
J
Cladocerans
Empty Stomach
Copepods
Chironomidae
Filamentous Algae
Plant Detritus
Ostracods
Amphipods
Insect Detritus
Miscellaneous
Prey Item

unidentifiable coleopterans, and hydrachnia. Empty stomachs dominated the YSL phase in the mudminnows (22%). In the PYSL phase, empty stomachs were present only 2 percent of the time, while in the J phase empty stomachs were seen approximately 4 percent during the entire collection period.

Overlap between the early life history phases of the grass pickerel and central mudminnow was calculated using Horn's Index of Community Similarity, Morisita's Index of Similarity, and Percent Similarity (Horn, 1966; Morisita, 1959). Percent frequency of occurrence, the number of stomachs a food item occurs in divided by the total number of stomachs for that study period, was determined for each phase. The overlap in diet of grass pickerel between the YSL and PYSL was the highest with values of 0.797 (Horn), 0.750 (Morisita), and 57.0 percent similarity (Table 3). This reveals that the YSL and PYSL have diets that are more similar than the YSL vs. J or PYSL vs. J. This can be attributed to the number of prey items that were taken and also the percent consumed. The YSL vs. J was the least similar with values of .420 (Horn), .288 (Morisita), and 24.2 percent similarity. The mudminnow YSL and PYSL phases, .778 (Horn), .666 (Morisita), and 56.0 percent similarity, were also more alike than YSL vs. J or PYSL vs. J (Table 3). The overlap between phases of each species were most similar (70 percent similarity) in the PYSL phase of the grass pickerel and central mudminnow (Table 3). The J phase of the pickerel and the YSL of the mudminnow were least similar (21 percent similarity). This may be credited to the different sizes of each stage (mouth size, total length, etc.) and the ability to seek out more prey items.

Table 3. Dietary overlap between early life history phases of each grass pickerel and central mudminnow.

Overlap in diet of the grass pickerel

	Horn	Morisita	Percent Similarity
YSL vs. PYSL	.797	.750	57.0
YSL vs. J	.420	.288	24.2
PYSL vs. J	.590	.441	34.3

Overlap in diet of the central mudminnow

	Horn	Morisita	Percent Similarity
YSL vs. PYSL	.778	.666	56.0
YSL vs. J	.680	.590	50.0
PYSL vs. J	.623	.437	34.0

Overlap in diet of the central mudminnow (m) and grass pickerel (p)

	Horn	Morisita	Percent Similarity
(p) YSL vs. (m) YSL	.436	.298	30.0
(p) YSL vs. (m) PYSL	.760	.774	61.0
(p) YSL vs. (m) J	.344	.282	17.0
(p) PYSL vs. (m) YSL	.789	.777	60.0
(p) PYSL vs. (m) PYSL	.900	.872	70.0
(p) PYSL vs. (m) J	.592	.358	37.0
(p) J vs. (m) YSL	.376	.254	21.0
(p) J vs. (m) PYSL	.548	.376	33.0
(p) J vs.(m) J	.590	.393	37.0

Electrophoresis

Twenty-one grass pickerel and 25 central mudminnows were collected between 22 and 23 May 1996. Juvenile fish were collected in the morning between 6:45 and 8:15 a.m. and in the evening between 6:50 and 8:45 p.m. to utilize the difference between the dissolved oxygen and carbon dioxide (Table 4). Dissolved oxygen (DO) was measured with the Winkler method and a meter with a probe attached to determine if a variation occurred between the methods. The DO (mg/L) varied from 1.33 - 1.64 in the a.m. to 2.40 - 3.00 in the p.m (Fig. 16). Carbon dioxide (mg/L) levels illustrated a decrease from a high of 73.4 to a low of 47.2. Air temperature during the two days ranged from 10 to 25°C. Minimum, present, and maximum water temperatures (°C) varied from 12/22.5/23.5, respectively, to 18/22/25. The pH value was measured at each site where a fish was collected and measurements ranged from 6.4 - 7.3.

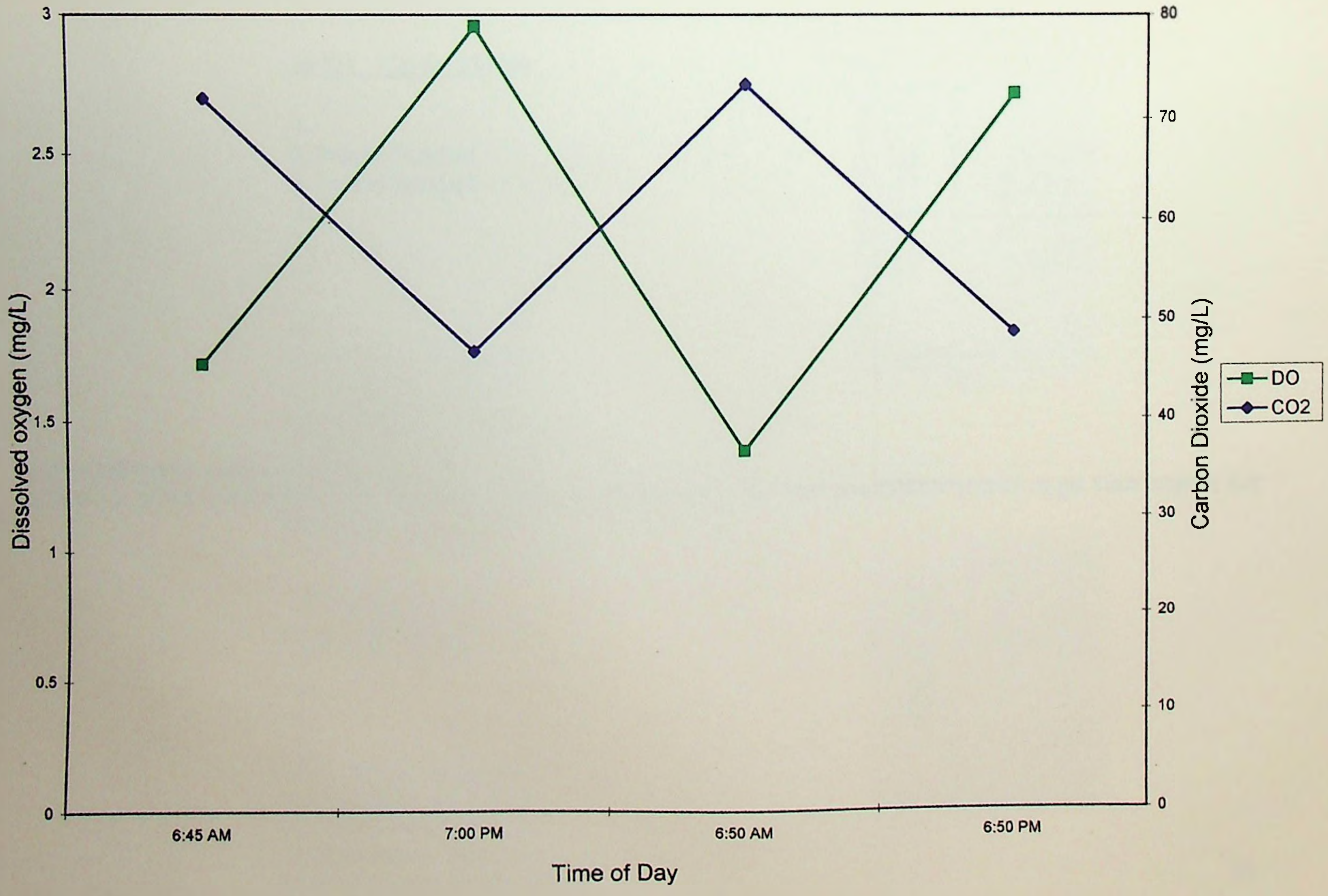
Protein standards, proteins that have established pI values for bands, were compared to LDH isozymes of pickerel and mudminnow enzyme extracts (Fig. 17). Standard pI values were plotted against the distance (pixels) of each band (Fig. 18). Using linear regression analysis ($y = -53.399x + 412.88$; $r = 0.9957$), the unknown pI values of the enzyme extracts were calculated when plotted against the distance (pixels) of each band stained for LDH. Bands that migrated toward the anode (positive side) had distances of 112, 110, and 107 pixels. These bands had the calculated pI values of 5.63, 5.67, and 5.73, respectively. Several bands migrated toward the cathode (negative side). These bands had distances of 29 pixels (7.19), 35 pixels (7.08), 59 pixels (6.63), 63 pixels (6.55), and 78 pixels (6.27).

The prominent bands that stained for LDH in the grass pickerel head and muscle tissues had pI values of 5.73, 6.27, 6.55, 6.63, 7.08, and 7.19. The band for heart tissue had a pI value

Table 4. Water quality data from Green Bottom Wildlife Management Area for 22 and 23 May 1996.

5/22/96	Winkler(DO)	Meter (DO)	Time	CO2	pH	Air Temp. (°C)	Min/Pres/Max
6:45 a.m. 18°C	1.72	1.64	6:45	72.1	7.3	14	12/22.5/23.5
		1.43	7:30		6.8		
		1.55	7:36		6.5		
		1.62	7:51		6.5		
		1.37	7:56		6.5		
		1.56	8:00		6.5		
7:00 p.m. 27°C	2.96	2.90	7:00	47.2	6.5	21	
		2.83	7:30		6.9		
		3.00	7:45		7.2		
		2.89	7:50		6.9		
		2.91	8:15		7.3		
		2.88	8:45		7.1		
5/23/96	1.38	1.33	6:50	73.4	6.5	10	18/22/25
6:50 a.m. 19°C		1.33	7:10		6.5		
		1.33	7:15		6.5		
		1.33	7:25		6.5		
		1.44	7:40		6.4		
		1.44	8:00		6.6		
		1.35	8:15		6.4		
6:50 p.m. 27°C	2.72	2.48	6:50	48.9	6.5	25	
		2.40	7:00		7.1		
		2.46	7:00		7.1		
		2.52	7:10		6.8		
		2.92	7:15		7.2		
		2.61	7:20		6.7		
		2.41	7:48		7.3		
		2.78	8:00		6.6		
		2.44	8:05		6.5		

Figure 16. Fluctuating levels of dissolved oxygen and carbon dioxide recorded on 22 and 23 May 1996, GBWMA.

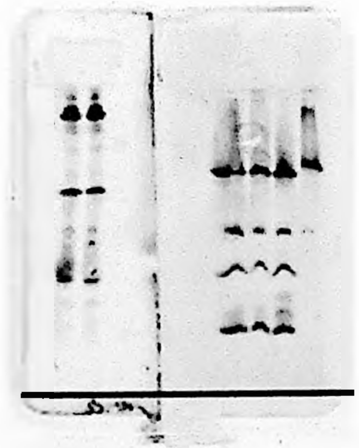


3
2.5
2
1.5
1
0.5
0
Dissolved oxygen (mg/L)
80
70
60
50
40
30
20
10
0
Carbon Dioxide (mg/L)
DO
CO2
6:45 AM
7:00 PM
6:50 AM
6:50 PM
Time of Day

Figure 17. Protein standard gels that contain protein standards with known pI values and enzyme extracts of the grass pickerel and mudminnow with unknown pI values.

Gel #21 lot no. 234788

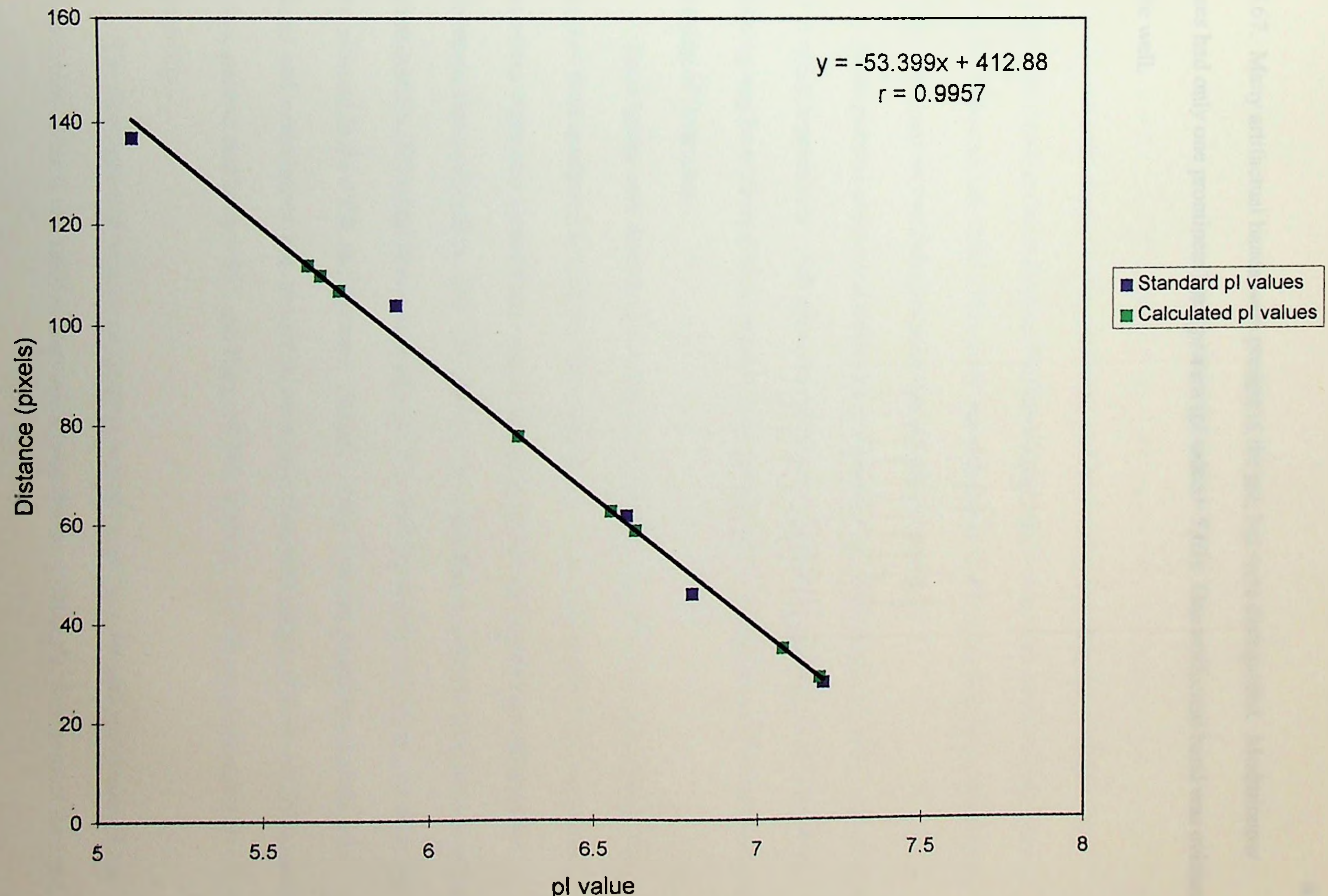
1.
2. Protein Standard
3. Protein Standard
4.
5.
6.
7.
8.
9. #138 Head (pickerel)
10. #148 Head (pickerel)
11. #162 Head (pickerel)
12. #171 Head (mudminnow)



Gel #22 lot no. 234788

1.
2. Protein Standard
3. Protein Standard
4.
5.
6.
7.
8. #148 Heart (pickerel)
9. #165 Heart (pickerel)
10. #138 Muscle (pickerel)
11. #140 Muscle (pickerel)
12. #171 Muscle (mudminnow)

Figure 18. Linear regression analysis for known (standard) and unknown (enzyme extracts) pI values.


y = -53.399x + 412.88
r = 0.9957
Standard pI values
Calculated pI values
Distance (pixels)
pI value
160
140
120
100
80
60
40
20
0
5
5.5
6
6.5
7
7.5
8

of 5.67. Many artifactual bands were present in the gel, but were disregarded. Mudminnow tissues had only one prominent band present (pI value = 5.63). One artifactual band was evident in the well.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Growth (mm/day) and dietary studies, and electrophoresis of the grass pickerel and central mudminnow at Green Bottom Wildlife Management Area, were conducted between December 1994 and July 1996. This study was undertaken to provide information about larval and juvenile stages of two fish species in Green Bottom Swamp.

Grass pickerel and central mudminnow showed a calculated daily growth rate of 1.10 and 0.35 mm/day, respectively. Fall spawning of the grass pickerel occurred in December 1994. Spawning may be attributed to the unusually high temperatures during the end of November and beginning of December.

Food habits were described by early life history phases and by collection dates. The main prey item consumed by both fish was cladocerans. Pickerel ingested plant material and filamentous algae only by accident, whereas the mudminnow consumed plant material and filamentous algae quite often. The YSL phase of the mudminnow consumed the largest amount of plant detritus (19%) and filamentous algae (17%). Fish became an important part of the diet in the pickerel in the PYSL and J phases. Dietary overlap between stages was determined. Pickerel and mudminnow YSL and PYSL were most alike with values of Horn = 0.778, Morisita = 0.666, percent similarity = 56.0 and Horn = 0.797, Morisita = 0.750, percent similarity = 57.0, respectively.

The electrophoretic experiment yielded several bands for grass pickerel tissues. Two homopolymer, LDH-B and LDH-A, and one heteropolymer, LDH-A_2B_2, were present for head and muscle tissue. The heart tissue had only one homopolymer, LDH-B. The pI values ranged from 5.63 - 7.19. The pI value is the allele designator of the only LDH-B allele found in the population. Because no difference in LDH expression was detected between head, muscle, liver, and heart tissues, the single locus expressed for LDH could not be identified. The LDH isozymes are, therefore, not identified to a specific locus. Some artifactual bands were present and were disregarded. Fish collected on May 22 and 23, 1996 showed no shift in LDH-A or LDH-B although dissolved oxygen levels varied from 1.33 to 3.00 mg/L.

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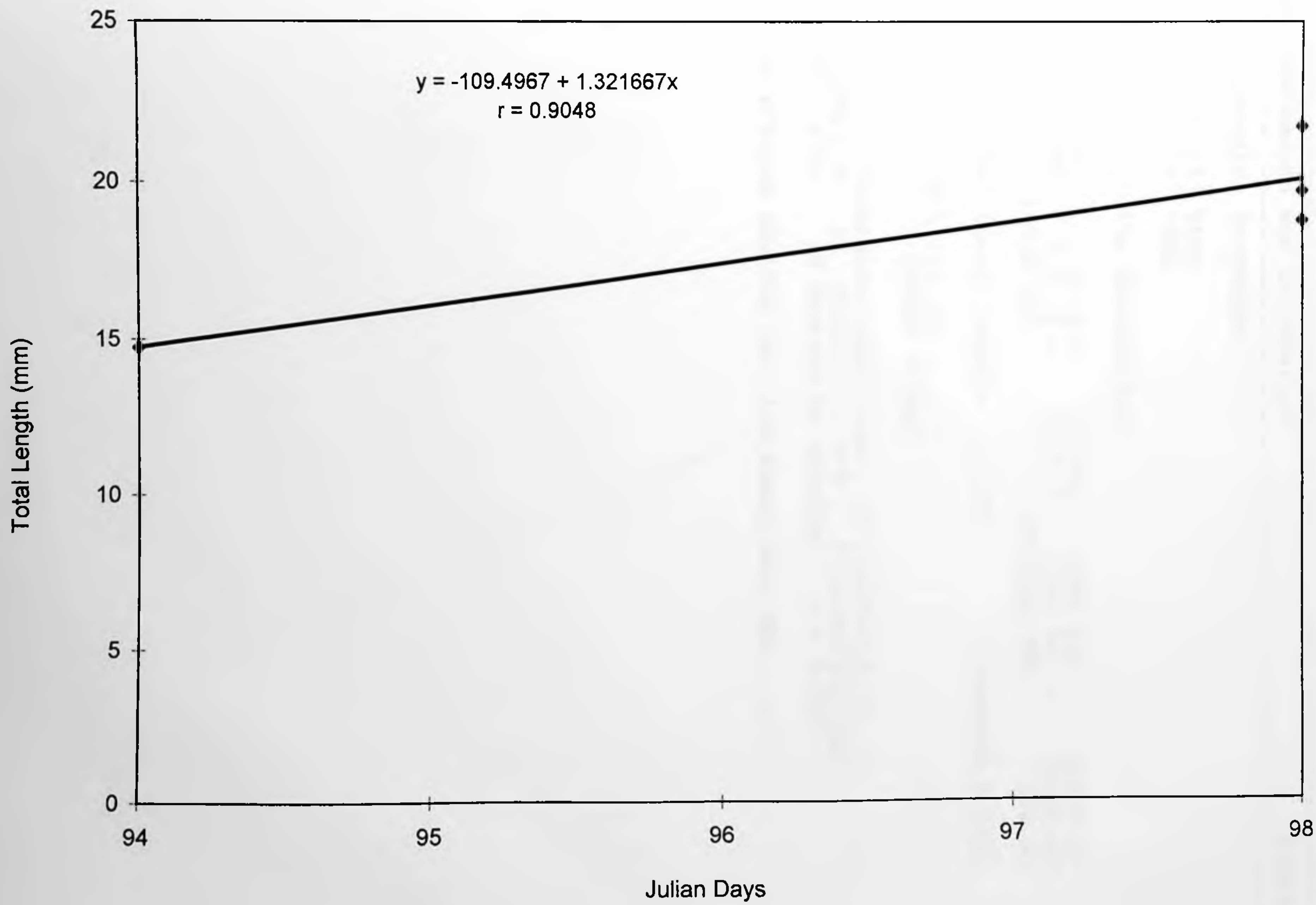
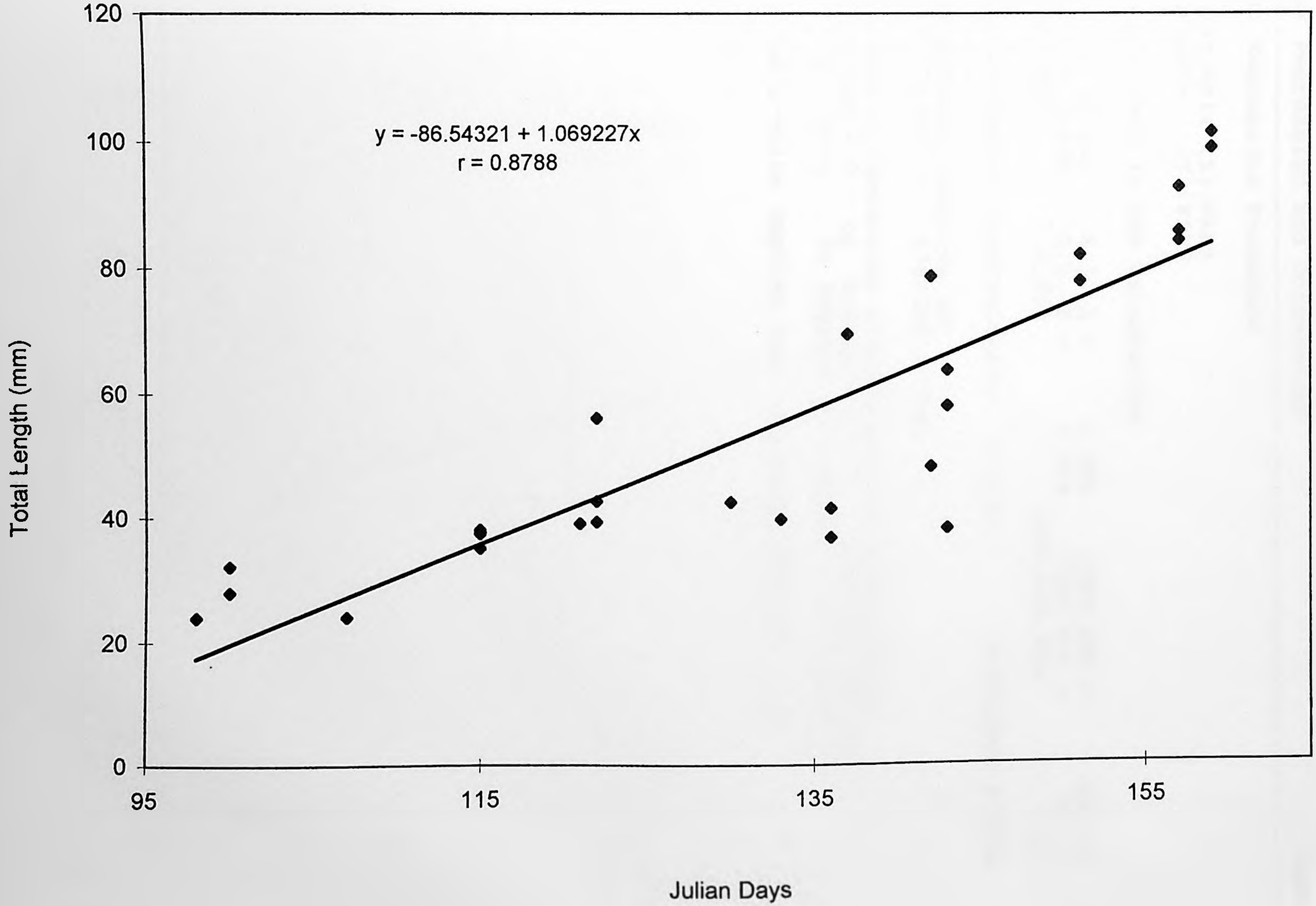
APPENDIX I

```
S'TAT                                                              04-30-1997
------------------------------------------------------------------------------
lie Linear Regression and Correlation                              PPYSL.dbf
------------------------------------------------------------------------------
lie Linear Regression Procedure

pendent Variable (X):VAR1
ndent Variable   (Y):VAR2

data points used in the calculation.

 MEAN X =     97.000    S.D. X =     2.000    CORR XSS =        12.00
 MEAN Y =     18.705    S.D. Y =     2.921    CORR YSS =        25.60
 REGRESSION MS=         20.962             RESIDUAL MS=          2.32

son's r (Correlation Coefficient)=   0.9048        R-Square= 0.8187

linear regression equation is:
 VAR2 = -109.4967  +  1.321667 * VAR1

 of hypothesis to determine significance of relationship:
 H(null): Slope = 0   or  H(null): r = 0    (two-tailed test)
 t =    3.01   with     2 degrees of freedom    p = 0.095
```

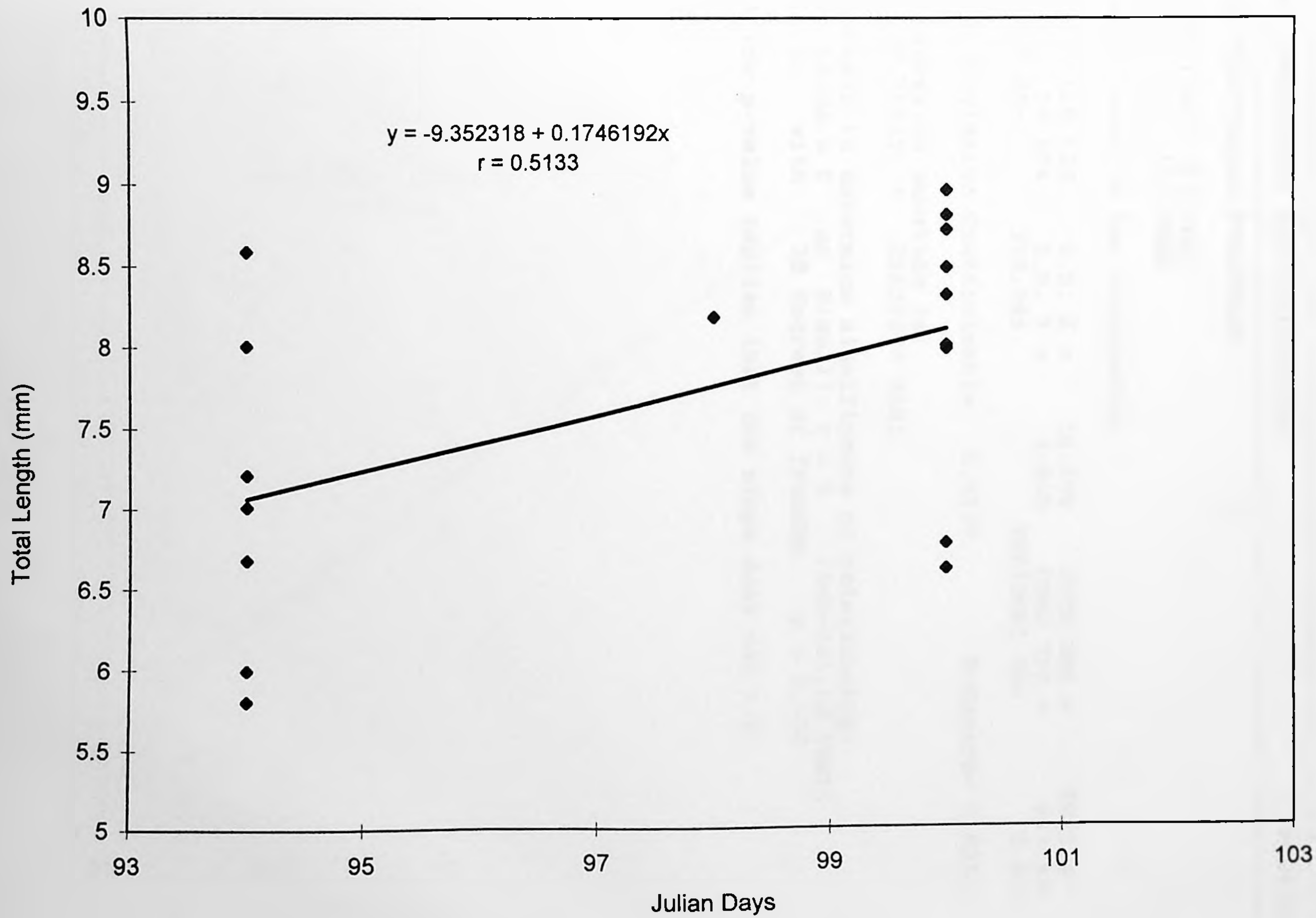

y = -109.4967 + 1.321667x
r = 0.9048
25
20
15
10
5
0
94
95
96
97
98
Total Length (mm)
Julian Days

```
;TAT                                                      04-30-1997
--------------------------------------------------------------------------
.e Linear Regression and Correlation                         PICKJ.dbf
--------------------------------------------------------------------------
.e Linear Regression Procedure

»endent Variable (X):VAR1
ıdent Variable   (Y):VAR2

data points used in the calculation.

]MEAN X =    130.867     S.D. X =     19.655     CORR XSS =     11203.47
]MEAN Y =     53.383     S.D. Y =     23.915     CORR YSS =     16585.82
:REGRESSION MS=       12808.329             RESIDUAL MS=          134.91

;on's r (Correlation Coefficient)=    0.8788        R-Square= 0.7722

.inear regression equation is:
'VAR2 = -86.54321   +   1.069227 * VAR1

‹of hypothesis to determine significance of relationship:
]H(null): Slope = 0    or  H(null): r = 0     (two-tailed test)
·t =     9.74    with     28 degrees of freedom     p = 0.000

]Note: A low p-value implies that the slope does not = 0.
```

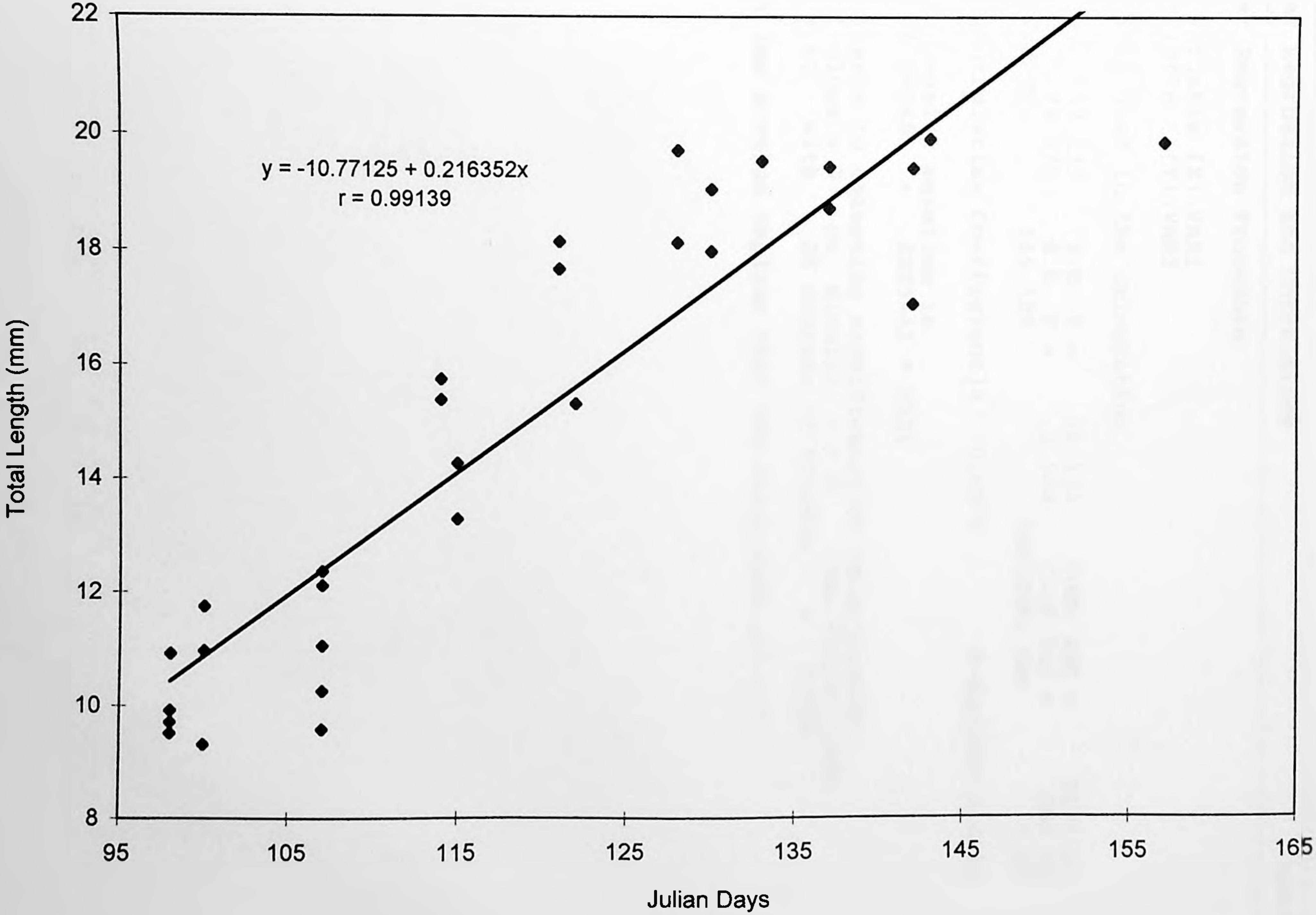


y = -86.54321 + 1.069227x
r = 0.8788
Total Length (mm)
0
20
40
60
80
100
120
Julian Days
95
115
135
155

```
STAT                                                          04-30-1997
-------------------------------------------------------------------------
.e Linear Regression and Correlation                            MUDY.dbf
-------------------------------------------------------------------------
.e Linear Regression Procedure

pendent Variable (X):VAR1
ident Variable   (Y):VAR2

 data points used in the calculation.

 MEAN X =     97.412    S.D. X =     2.980    CORR XSS =      142.12
 MEAN Y =      7.658    S.D. Y =     1.014    CORR YSS =       16.45
 REGRESSION MS=          4.333             RESIDUAL MS=         0.81

on's r (Correlation Coefficient)=   0.5133        R-Square= 0.2634

.inear regression equation is:
 VAR2 = -9.352318  +  .1746192 * VAR1

of hypothesis to determine significance of relationship:
H(null): Slope = 0   or  H(null): r = 0    (two-tailed test)
 t =     2.32   with    15 degrees of freedom    p = 0.035

Note: A low p-value implies that the slope does not = 0.
```


y = -9.352318 + 0.1746192x
r = 0.5133
Total Length (mm)
Julian Days
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
93
95
97
99
101
103

```
STAT                                                        04-30-1997
----------------------------------------------------------------------
le Linear Regression and Correlation                      MUDPYSL.dbf
----------------------------------------------------------------------
le Linear Regression Procedure

pendent Variable (X):VAR1
ndent Variable   (Y):VAR2

 data points used in the calculation.

 MEAN X =    118.533    S.D. X =    16.594    CORR XSS =     7985.47
 MEAN Y =     14.874    S.D. Y =     3.928    CORR YSS =      447.52
 REGRESSION MS=       373.785          RESIDUAL MS=             2.63

son's r (Correlation Coefficient)=   0.9139       R-Square= 0.8352

linear regression equation is:
 VAR2 = -10.77125  +  .216352 * VAR1

 of hypothesis to determine significance of relationship:
 H(null): Slope = 0   or  H(null): r = 0    (two-tailed test)
 t =   11.91   with    28 degrees of freedom    p = 0.000

 Note: A low p-value implies that the slope does not = 0.
```


y = -10.77125 + 0.216352x
r = 0.99139
Total Length (mm)
22
20
18
16
14
12
10
8
95
105
115
125
135
145
155
165
Julian Days

ɪle Linear Regression and Correlation MUDJ.dbf

ɪle Linear Regression Procedure

pendent Variable (X):VAR1
ndent Variable (Y):VAR2

data points used in the calculation.

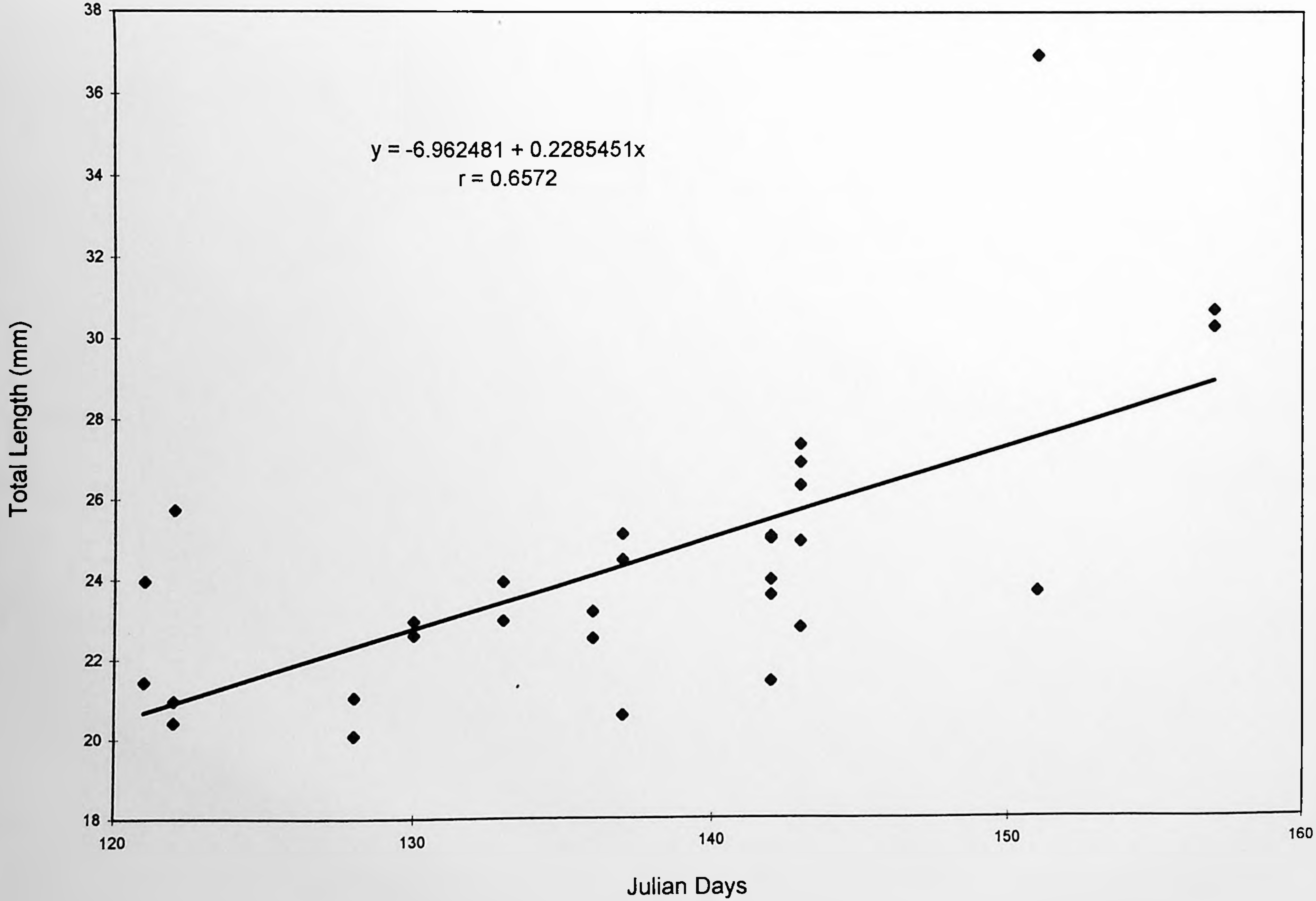
MEAN X =	137.133	S.D. X =	10.133	CORR XSS =	2977.47
MEAN Y =	24.379	S.D. Y =	3.524	CORR YSS =	360.05
REGRESSION MS=	155.522			RESIDUAL MS=	7.30

son's r (Correlation Coefficient)= 0.6572 R-Square= 0.4319

ɪlinear regression equation is:
VAR2 = -6.962481 + .2285451 * VAR1

of hypothesis to determine significance of relationship:
H(null): Slope = 0 or H(null): r = 0 (two-tailed test)
t = 4.61 with 28 degrees of freedom p = 0.000

Note: A low p-value implies that the slope does not = 0.



y = -6.962481 + 0.2285451x
r = 0.6572
Total Length (mm)
38
36
34
32
30
28
26
24
22
20
18
120
130
140
150
160
Julian Days