ABSTRACT

NEAL, JASON WESLEY. Live Fast and Die Young: On the Growth and Mortality of Largemouth Bass in Puerto Rico. (Under the direction of Dr. Richard L. Noble)

Largemouth bass (*Micropterus salmoides*) have been widely introduced into freshwater systems around the world. In Puerto Rico, this species presents a management challenge to natural resource agents who wish to promote it as a sportfish because growth and survival are unlike that observed in its native temperate regions. Juvenile growth is linear and rapid (≥1 mm/day), attributed in part to a continuous growing season near optimum temperature year-round. Upon maturation, growth rate slows to near 0 mm/day, and few fish surpassing age 3.

This dissertation hypothesized that the slow growth of adult fish results from excessive energy allocation to reproduction. Largemouth bass in Puerto Rico reach sexual maturity in 1 year, spawn over a six-month period, and individual fish spawn multiple times. The diversion of energy from growth to reproduction causes growth rates to decline, and the risk of disease, parasites, predation, or other means of natural mortality increases. I used three approaches to address this hypothesis: (1) empirical assessment of population dynamics, (2) theoretical modeling of bioenergetics processes, and (3) direct experimentation to compare reproductive and non-reproductive largemouth bass.

Adult mortality strongly coincided with the reproductive period (January-June), and limited mortality occurred thereafter. Fish condition varied seasonally and with size, and was generally lowest in November just before the reproductive period, making these fish more susceptible to spawning related mortality. Condition declined with increasing
age, suggesting a cumulative effect with no recovery period. Overall, empirical data on largemouth bass population dynamics supported the reproductive energetics hypothesis.

Bioenergetics simulation using a conservative mean daily ration of 2% body weight predicted that a non-reproductive, 500-g largemouth bass would grow to 1,140 g in six months (182 d), the maximum spawning season duration. The actual size from tagging studies was 740 g, yielding a 400-g discrepancy between observed and predicted weight. This discrepancy in observed and predicted growth was explained for females using a range of spawning frequency-magnitude combinations, and for males by accounting for lost consumption.

To experimentally test the reproductive energetics hypothesis, techniques for artificially propagating largemouth bass and inducing triploidy are discussed. I validated erythrocyte cell length as a ploidy verification technique using known ploidy largemouth bass. Erythrocyte cell length 99% confidence intervals ranged 14.43-16.66 µm for triploids, and 10.23-13.62 µm for diploids. Erythrocyte length correctly distinguished 100% of known-status largemouth bass (n=22) using a sample of 100 erythrocytes per individual.

Growth, condition, and reproductive development of diploid and triploid largemouth bass were compared through age 1 in Lucchetti Reservoir. Growth rates up to the size of maturity (275 mm) were similar for both groups, and maturity was not reached until midway into the spawning season, preventing extensive spawning of diploid bass, and resulting in growth rates similar to triploid bass. Diploid largemouth bass exhibited higher GSI values than triploids, and no triploid females had GSI values
consistent with maturation, suggesting that the triploids do not invest significant energy into reproductive development.

As a result of this study, more comprehensive management of largemouth bass is possible. I refined techniques to produce triploid largemouth bass, and demonstrated the reduced reproductive investment of these sterile fish. Further research using triploids is needed to determine the efficacy of triploidy as a management option, particularly to determine if accelerated adult growth rates are possible. Specific research needs and management recommendations are discussed along with ecological implications of this research.
LIVE FAST AND DIE YOUNG: ON THE GROWTH AND MORTALITY OF LARGEMOUTH BASS IN PUERTO RICO

By

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BIOGRAPHY

Wes Neal was born in Virginia on 23 October 1972. He graduated from Alleghany High School in 1990, and completed his Bachelor of Science in Forestry and Wildlife Sciences at Virginia Tech in 1994. His Master of Science in Fisheries and Wildlife Sciences thesis entitled “Hybrid Striped Bass Characteristics and Community Impacts in Farm Ponds: Understanding a Supplemental Predator in Established Systems” was completed in December 1996. The following spring he became a technician on a fisheries research project stationed in Boquerón, Puerto Rico. This position led to the appointment as the lead research biologist on the project and the pursuit of his Doctor of Philosophy in Zoology.
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# Table of Contents

List of Tables. ................................................................. vi
List of Figures. ............................................................... vii
Preface. ........................................................................... x

## CHAPTER 1

The Relationship of Seasonal Patterns in Condition and Mortality of Largemouth Bass to Reproductive Periodicity

Introduction. ................................................................. 1
Methods. ........................................................................ 2
  *Study Site.* ................................................................. 2
  *Defining Seasonal Changes.* ......................................... 4
Results. .......................................................................... 8
Discussion. ..................................................................... 18
Literature Cited. ............................................................. 23

## CHAPTER 2

The Energetics of Reproduction and Growth

Introduction. ................................................................. 27
Methods. ........................................................................ 29
  *Prey Energy and Consumption.* .................................. 29
  *Modeling Reproductive Energetics.* ......................... 31
Results and Discussion. .................................................. 32
  *Largemouth Bass Diet.* .............................................. 32
  *Prey Energy Densities.* .............................................. 36
  *Energetics of Reproduction.* ...................................... 36
Literature Cited. ............................................................. 48

## CHAPTER 3

The Development of Technologies for Triploid Production and Verification

Introduction. ................................................................. 53
Methods. ........................................................................ 55
  *Triploid Production.* ................................................. 55
  *Verification of Ploidy.* .............................................. 57
Results. .......................................................................... 59
  *Propagation and Induction.* ....................................... 59
  *Erythrocyte Length.* .................................................. 61
  *Verification Using Erythrocytes.* ............................... 63
Discussion. ..................................................................... 66
Literature Cited. ............................................................. 70
CHAPTER 4

Field Comparison of Diploid and Triploid Largemouth Bass During the First Two Years in Lucchetti Reservoir, Puerto Rico

- Introduction: 74
- Methods: 76
- Results: 79
- Discussion: 85
- Literature Cited: 90

CHAPTER 5

Synthesis of Research on Reproductive Energetics, Growth, and Mortality of Tropical Largemouth Bass

- Discussion of Findings: 94
- Research Implications: 101
  - A Temperate Fish in the Tropics: 101
  - Global Implications: 104
- Literature Cited: 109

APPENDIX

Timing of Pressure Treatment to Induce Triploidy and Dependence on Water Temperature

- Timing Study: 113
List of Tables

CHAPTER 2
The Energetics of Reproduction and Growth

Table 2.1 – Mean and diel proportions of largemouth bass (>150 mm) diet by weight of prey items collected seasonally using electrofishing. . . . . . . . . . . . . . . 33

Table 2.2 – Mean energy densities in cal/g wet weight (value given in joules in parentheses) and standard error (SE) for primary prey species during spring and fall. . . . 37

CHAPTER 3
The Development of Technologies for Triploid Production and Verification

Table 3.1 – Stocking and ploidy statistics of control and pressure-treated largemouth bass stocked in Lucchetti Reservoir. . . . . . . . . . . . . . . . . . . . . . . . . . . . . 60

CHAPTER 4
Field Comparison of Diploid and Triploid Largemouth Bass During the First Two Years in Lucchetti Reservoir, Puerto Rico

Table 4.1 – Descriptive statistics of two experimental groups of control and treated largemouth bass stocked into Lucchetti Reservoir, Puerto Rico. . . . . . . . . . . . . 77

APPENDIX
Timing of Pressure Treatments to Induce Triploidy and Dependence on Water Temperature

Table A.1 – Triploidy induction success of 20 time-temperature combinations. . . . . 115
List of Figures

CHAPTER 1

The Relationship of Seasonal Patterns in Condition and Mortality of Largemouth Bass to Reproductive Periodicity

Figure 1.1 – Map of Puerto Rico streams and impoundments (Bottom). The enlarged area (Top) shows Lucchetti Reservoir, and impoundment of the Yauco River. 3

Figure 1.2 – Water level dynamics in Lucchetti Reservoir, Puerto Rico. 5

Figure 1.3 – Catch per unit effort (fish/hr) of largemouth bass during day and night electrofishing from May 1998 to February 2001. 9

Figure 1.4 – Mean seasonal catch per unit effort of largemouth bass averaged for three years of sampling in Lucchetti Reservoir. 9

Figure 1.5 – Initial cohort delineations and age designations for largemouth bass collected May 1998. 10

Figure 1.6 – Catch per unit effort (fish/hr) of largemouth bass from May 1998 to February 2001. 11

Figure 1.7 – Age 1+ catch per unit effort (fish/hr) during 2000 quarterly samples. 13

Figure 1.8 – Catch per unit effort (fish/hr) of adult largemouth bass in 2000 using electrofishing. 14

Figure 1.9 – Timing of natural and total mortalities of age-1 and age-2 largemouth bass determined using biotelemetry methods in Lucchetti Reservoir, Puerto Rico. 14

Figure 1.10 – Mean relative weight of largemouth bass collected every three months from May 1998 to February 2001 from Lucchetti Reservoir. 16

Figure 1.11 – Change in condition with season and age for annual largemouth bass cohorts hatched 1996 to 1999 and sampled May 1998 to February 2001. 17
CHAPTER 2

The Energetics of Reproduction and Growth

Figure 2.1 – Contributions of prey items by weight collected from largemouth bass stomachs in Lucchetti Reservoir, May 1998 to February 2001. ............... 34

Figure 2.2 – Occurrence of stomachs containing food during diel samples of largemouth bass in Lucchetti Reservoir, May 1998 to February 2001. ............... 34

Figure 2.3 – Specific weight (weight of prey/weight of predator) of prey during diel sampling of largemouth bass in Lucchetti Reservoir, may 1998 to February 2001. ............... 35

Figure 2.4 – Bioenergetics model of temperature-dependent growth rate (solid line) based on observed growth of microtagged largemouth bass and temperature variations (dashed line) in Lucchetti Reservoir. ............... 39

Figure 2.5 – Bioenergetics model of temperature-dependent growth rate (solid line) based mean daily ration of 2% body weight and temperature variations (dashed line) in Lucchetti Reservoir. ............... 39

Figure 2.6 – Daily maintenance and spawning energy requirements for a 500-g largemouth bass female with up to 20 spawning events. ............... 41

Figure 2.7 – Estimated growth of a 500-g largemouth bass female consuming a mean daily ration of 2% body weight, and which engages in a range of spawning frequencies (1-20 events) and magnitudes (1-10% of body weight is lost in the form of gametes per event). ............... 43

Figure 2.8 – Estimated growth of a 500-g largemouth bass male consuming a mean Daily ration ranging 1.5% to 2.5% body weight, and constant swimming speed ranging 0 to 0.5 m/s. ............... 46

CHAPTER 3

The Development of Technologies for Triploid Production and Verification

Figure 3.1 – Largemouth bass triploid verification model developed from known-status diploid (n=10) and triploid (n=10) fish. ............... 62

Figure 3.2 – Frequency distribution of erythrocyte cell lengths (n=100) from 22 microtagged largemouth bass collected in Lucchetti Reservoir. ............... 64
Figure 3.3 – Mean erythrocyte length (based on n=100), model ploidy decision, and ploidy status of 22 microtagged largemouth bass recollected from Lucchetti Reservoir, Puerto Rico.

Figure 3.4 – Logistic regression model predictions of ploidy status using mean erythrocyte length (based on 100 erythrocytes per individual). Actual ploidy status of the 22 microtagged largemouth bass is given.

CHAPTER 4

Field Comparison of Diploid and Triploid Largemouth Bass During the First Two Years in Lucchetti Reservoir, Puerto Rico

Figure 4.1 – Growth in length of diploid (solid circles) and triploid (open circles) largemouth bass stocked into Lucchetti Reservoir on 5 May 2000 (Group 1, Top), and on 30 June 2000 (Group 2, Bottom).

Figure 4.2 – Reproductive development of diploid and triploid largemouth bass females (Top) and males (Bottom) as determined using the gonadosomatic index (GSI).

Figure 4.3 – Relative weight ($W_r$) of diploid and triploid largemouth bass stocked in Lucchetti Reservoir.

Figure 4.4 – Water level dynamics of Lucchetti Reservoir in 2000.

CHAPTER 5

Synthesis of Research on Reproductive Energetics and the Growth and Mortality of Tropical Largemouth Bass

Figure 5.1 – Estimated growth in weight lost due to spawning for a 500-g largemouth bass over a six-month spawning period.

Figure 5.2 – Length distribution of largemouth bass in Carite Reservoir during the marking period of a Petersen-type population estimate.

Figure 5.3 – Record weights (as of December 2001) of largemouth bass recognized for the 48 contiguous states, Hawaii, Puerto Rico, and Mexico.
INTRODUCTION TO THE LARGEMOUTH BASS DILEMMA IN PUERTO RICO

Largemouth bass (*Micropterus salmoides*) have been widely introduced into freshwater systems around the world. This ecologically and economically important piscivore can reach 23 years of age (Green and Heidinger 1994) and over 10 kg (24.25 lbs) in weight within its native range (IGFA 2003). In Florida, one study reported that the ages of trophy largemouth bass (4.5 kg and larger) ranged from 4.0 to 16.5 years, with a mean of 9.7 years (Crawford et al. 1996). That report concluded that accelerated growth and longevity were important for trophy largemouth bass production.

In Puerto Rico, largemouth bass were successfully introduced in 1946 (Erdman 1984). Today, all of the island’s major reservoirs with the exception of Guajataca Reservoir and Cerrillos Reservoir contain self-sustaining largemouth bass populations with a mixture of Florida (*M. s. floridanus*) and northern (*M. s. salmoides*) alleles. The genetic composition of these “intergrade” populations is strongly skewed towards the Florida subspecies (Neal and Noble 2002). In Guajataca Reservoir and Cerrillos Reservoir, populations of pure Florida largemouth bass have become established (Neal et al. 1999).

Many studies have examined the reproduction, population dynamics, and growth of largemouth bass in Puerto Rico and other tropical systems. Unlike in temperate regions, tropical largemouth bass have a prolonged spawning season with multiple-clutch, group synchronous spawning (Dadzie and Aloo 1990; Gran 1995; Waters 1999).
The duration of the spawning season in Puerto Rico can be as long as 6.5 months (Neal et al. 1999), and additional spawning can occur outside of the recognized spawning season when water level dynamics are suitable (Churchill et al. 1995; Ozen 2002). Although photoperiod and water temperature may influence the onset of primary reproductive development, the timing of spawning episodes appears to be directed by hydrodynamics (Ozen and Noble 2000). Whereas water level can fluctuate erratically, numerous spawning episodes are possible throughout the spawning season.

Growth of juvenile bass can be rapid in Puerto Rico. Continuously warm water temperatures result in a year-round growing season (Churchill et al. 1995), and prey for young fish appear consistently abundant in many reservoirs (Alicea et al. 1997; Stancil et al. 1997; Neal et al. 1999). In intensively studied Lucchetti Reservoir, juvenile largemouth bass have exhibited growth rates of up to 1.5 mm/d until they reach maturity, and intraspecific competition appears minimal, restricted to the largest year-classes (Neal et al., in press). With growth rates generally about 1 mm/d, all largemouth bass reach maturity (at about 275 mm total length; Gran 1995) by age 1, and in some cases earlier.

The rapid growth that is characteristic of juvenile largemouth bass in Puerto Rico quickly declines when individuals reach maturity. Growth rates have been reported at 0.20 mm/d for age-1 largemouth bass in Lucchetti Reservoir, and fish age 2 and older displayed much slower growth that approaches 0 mm/d by age 3 (Neal et al., in press). This occurred despite the abundant prey and continuous growing season available to adult bass. In fact, Ashe et al. (1998) determined that despite a three-fold variation in adult largemouth bass population size from 1994 to 1998, relative weights of Lucchetti Reservoir largemouth bass were consistently close to 100, suggesting that stock size
rarely approaches carrying capacity in this reservoir. However, uncommonly large year-
classes can experience food limitations, and may exhibit lower relative weights until 
mortality reduces the cohort size (Ozen 2002).

In addition to the cessation of growth of largemouth bass over 2 years of age, the 
longevity of these fish appears to be severely reduced. Several studies estimated annual 
mortality of adult largemouth bass to be 55-64% (Waters 1999; Neal and Lopez-Clayton 
2001), and one study found that less than 1% of telemetered age-1 and age-2 largemouth 
bass reached age 3 (La Plata Reservoir; Lilyestrom et al. 1994). Although annual fishing 
mortality has been estimated to be 27-29% (Waters 1999; Neal and Lopez-Clayton 2001), 
harvest alone cannot explain why most populations are composed primarily of only three 
year classes (Neal et al. 1997). This truncated size structure makes largemouth bass 
populations in Puerto Rico highly vulnerable to variations in year-class strength (Neal et 
al. 2002).

The Puerto Rico Paradox

Management officials, researchers, and fishermen have frequently asked the 
question: Why don’t largemouth bass, with unlimited prey and year-round warm water 
temperatures, grow fast and survive to trophy status in Puerto Rico? Based on general 
characteristics of largemouth bass biology, fish in Puerto Rico should be growing as fast 
or faster than fish in the temperate United States. The preferred water temperature at 
which an individual largemouth bass consumes the maximum quantity of prey is 27.5°C 
(Rice et al. 1993). Puerto Rico reservoir water temperatures tend to range 22-30°C, with 
much of the year at or near optimum water temperature (Churchill et al. 1995). Because
prey for largemouth bass are generally abundant, largemouth bass could theoretically consume at or near the maximum rate for most of the year, yielding rapid growth. Yet, at maturity, growth rates slow to near zero.

One hypothesis suggested to explain the paradox is that the intergraded genetics of largemouth bass found in most island reservoirs is sub-optimal for the tropical climate. The Florida subspecies, native to southern Florida, has rapid growth and reaches large size in sub-tropical environments including South Florida and Mexico. Hence, Florida largemouth may be superior to intergrade largemouth bass in the tropical climate of Puerto Rico. Neal and Noble (2002) tested this by simultaneously stocking fingerlings of both genetic groups, and monitoring their growth and survival for 5 years. Recapture rates of each group were equivalent for the first 2 years of the study (ages 0 and 1), indicating similar mortality rates. However, during the third year (age 2), 76% of the recaptures were Florida fish, indicating higher mortality of intergrade bass after age 1. At ages 3 and 4, only Florida largemouth bass were caught in samples. Although Florida largemouth bass demonstrated increased longevity, superiority in growth was not apparent. Observed growth rates of the two strains were nearly identical up to age 2, and although Florida fish were sampled for two additional years, their growth rates remained very low. Hence, genetic management using the Florida subspecies would likely improve largemouth bass survival, but these older fish would probably not substantially alter size distributions.

An alternative hypothesis for the slow growth and reduced longevity of Puerto Rico largemouth bass is that reproduction is energetically restrictive. Largemouth bass in Lucchetti Reservoir reach sexual maturity in 1 year. Unlike largemouth bass in temperate
regions, spawning occurs during a six-month period beginning in mid-winter, and individual bass spawn multiple times each season (Gran 1995; Waters 1999). Reproduction is generally an energetically expensive undertaking, and energy must be diverted from other processes such as growth and maintenance (Wootton 1985). For some species, the investment in reproduction may be at the expense of bodily maintenance causing accelerated senescence (Calow 1978). This could lead to quantitative reduction in the somatic biomass and a qualitative deterioration of the soma (Shevchenko 1972; Roff 1983), and may reduce the capacity of the immune system. Hence, the protracted reproductive period in Lucchetti Reservoir may be partially responsible for both the slow growth and high mortality of adult largemouth bass.

**My Approach**

This dissertation was undertaken to address the hypothesis that the slow growth of adult largemouth bass in Lucchetti Reservoir is the result of excessive energy reallocation to gamete production and associated reproductive behavior. I used three approaches to study this hypothesis – analysis of seasonal population dynamics, bioenergetics modeling, and direct experimentation using reproductive and non-reproductive largemouth bass. In Chapter 1, I examine largemouth bass population dynamics in Lucchetti Reservoir with emphasis on the seasonality of mortality. In Chapter 2, I describe theoretical modeling used to estimate the energetic cost of multiple spawning, which I used to approximate the amount of somatic weight gain forgone for gametic production. In Chapter 3, I describe the refinement of technologies to produce and verify triploid (sterile) largemouth bass. Chapter 4 compares growth, condition, and
reproductive development of fertile (diploid) and sterile (triploid) largemouth bass. A synthesis of research findings, discussion of ecological and management implications, and future research directions is given in Chapter 5.
Literature Cited


CHAPTER 1

THE RELATIONSHIP OF SEASONAL PATTERNS IN CONDITION AND MORTALITY OF LARGEMOUTH BASS TO REPRODUCTIVE PERIODICITY

The largemouth bass (Micropterus salmoides) was successfully introduced into Puerto Rico in 1946 (Erdman 1984). This species is considered the principal target fish of Puerto Rico reservoir sport fisheries (Corujo-Flores 1989, 1990) and is found in all major island reservoirs (Neal et al. 1999). However, the slow growth and high mortality that largemouth bass experience in Puerto Rico presents an unusual management challenge to natural resource agents who wish to promote it (e.g., Churchill et al. 1995; Neal and Lopez-Clayton 2001). Whereas growth rates and age determine fish size (Crawford et al. 1996), reservoirs in Puerto Rico are limited in their ability to provide trophy-fishing opportunities.

The extended reproduction period of largemouth bass in Puerto Rico may be a regulating factor in their growth and mortality. Gran (1995) demonstrated that female largemouth bass in Lucchetti Reservoir participated in multiple, partial spawning events during each reproductive season. Similarly, male largemouth bass have been shown to return multiple times to spawning areas as water levels allow, suggesting multiple spawning events per season (Waters 1999). Reproduction can be energetically expensive, and often energy must be diverted from growth and maintenance to support gametic development, behavioral modifications, and even development of secondary sexual characteristics (Wooton 1985). Whereas spawning seasons can last up to 6.5
months in Lucchetti Reservoir, it is conceivable that largemouth bass sacrifice growth opportunities for half of the year in order to support reproductive effort.

In this chapter, I present data that evaluate the hypothesis that extended spawning reduces growth and survival. If this hypothesis is valid, seasonal patterns in condition and mortality should be dependent on the reproductive cycle. Reproduction generally occurs from January to June (Ozen 2002), and the bulk of spawning activity is usually from January to April. Hence, the first four months of the year should demand the greatest energy resources. I assessed condition over a three-year period in Lucchetti Reservoir, and compared mortality estimates based on seasonal changes in catch rates and radio telemetry (Waters 1999).

METHODS

Study Site: Lucchetti Reservoir

I chose Lucchetti Reservoir, a 108-ha impoundment in the mountain region of southwestern Puerto Rico, as the study lake for my research. The lake is divided into four embayments, two major and two minor arms, corresponding to its four primary tributaries (Figure 1.1). The lake basin is located in a subtropical moist forest with a mean annual precipitation of 198 cm (Churchill et al. 1995). The Lucchetti Reservoir watershed is comprised of 45.1 km² of largely agricultural land that has been developed from the tropical forest landscape. The original capacity of the lake at spill level (174 m above sea level) was 20.4 million m³. The maximum depth is 22.2 m (Neal et al. 1999), and retention time is 0.66 years, an above-average value for Puerto Rico reservoirs (Pérez Santos 1994).
Figure 1.1: Shaded relief map of Puerto Rico (Bottom; courtesy of United States Geological Survey). The enlarged area (Top) shows Lucchetti Reservoir, an impoundment of the Yauco River. The Department of Natural and Environmental Resources (DNER) boat ramp and management station is displayed.
The reservoir is characterized by steeply sloping shorelines with a mixture of rock and clay substrates representing several distinct habitat types. Water level can vary as much as 17 m annually (Figure 1.2), causing dramatic differences in shoreline distance, surface area, and available habitat (Neal et al. 2001). Conversely, variation in mean daily temperature of surface water is low, typically fluctuating about 5 °C annually (Gran 1995). This reservoir has been categorized from mesotrophic (Pérez Santos 1994) to eutrophic (Cham and Carvajal-Zamora 1981) on the basis of nutrients, physical limnology, chlorophyll $a$, and phytoplankton biomass data. The reservoir contains sunfishes *Lepomis* spp., tilapias *Tilapia* spp., catfishes Ictaluridae, threadfin shad *Dorosoma petenense*, armored catfish *Liposarcus multiradiatus*, and largemouth bass.

Previous sport fish investigations in Puerto Rico focused on this impoundment, and therefore an extensive database is available (Churchill et al. 1995; Neal et al. 1999).

**Defining Seasonal Changes in Population Demographics**

I used a boom-mounted electrofishing unit at 7-8 A and 60 pps DC to collect largemouth bass every 3 months from May 1998 to February 2001. Nine representative electrofishing sites were identified, and six sites were electrofished during each quarterly sample. Three sites each were randomly selected for day (9 am – 3 pm) and night (9 pm – 3 am) diel electrofishing. Diel sampling was conducted to collect diet information described in Chapter 2. I collected all sizes of largemouth bass, and each was measured for total length (TL, mm) and weighed (g).

Acquiring accurate age information is difficult in Puerto Rico due to lack of valid age indicators (Neal et al. 1997), so I used length distributions to differentiate age classes
Figure 1.2: Water level dynamics in Lucchetti Reservoir, Puerto Rico. Maximum depth is presented as reported by Neal et al. (1999), and is substantially less than maximum depths reported previously. Values are given in meters above sea level (m asl). Dotted lines indicate missing data.
during each quarter. I visually delineated length modes (age classes) based on the assumption that cohort size distributions were normally distributed, and I also used previous and subsequent sample data, reported growth rates from independent studies, and personal experience to define cohort boundaries. When distinct length modes were present, overlapping distributions were divided at the primary shared length bin, which was defined as the length category between two age classes with the ratio of the two ages closest to 1:1 assuming normal distributions. Distribution “tails” beyond the primary shared length bin were ignored. Ages (yr) were assigned conventionally based on a January 1 hatch date (DeVries and Frie 1996).

For the 1998 quarterly samples, insufficient information was available to separate length data further than a bimodal structure (two age classes). The initial age 0 and age 1 cohorts defined in 1998 were tracked until 2001 and 2000, respectively, and additional recruitment to the population occurred in 1999 and 2000. Rapid growth of younger fish and slow growth of older fish in Lucchetti Reservoir often resulted in a unimodal distribution containing two or more age classes. I used a juvenile (age 0) growth rate of 1 mm/day to project growth for distinct juvenile length modes, and assumed that growth slowed to the same rate as older fish when multiple age populations became unimodal distributions. Delineations of age classes under these conditions using the methods defined above are admittedly imprecise.

Mortality rates between sample periods were estimated for adult largemouth bass using changes in catch per unit effort. Whereas catch per effort data from electrofishing are subject to many biases in sampling efficiency (Reynolds 1996), telemetry data from adult largemouth bass in Lucchetti Reservoir originally collected by Waters (1999) were
analyzed for seasonal patterns in natural and total mortality. In his study, 50 (6 censored from study at onset) largemouth bass were implanted with ultrasonic tags, and were relocated every 3 to 9 days for up to 18 months. Transmitters remaining in the reservoir without appreciable movement were considered natural mortalities, and all tag disappearances excluding possible tag failures and losses during flooding were considered fishing mortalities. The seasonal distribution of natural and fishing mortalities of telemetered largemouth bass was compared to estimates based on changes in catch per unit effort. Relative weight ($W_r$) served as an estimate of largemouth bass condition for fish 150 mm TL or larger, and was based on the standard weight ($W_s$) equation of Wege and Anderson (1978).

I compared catch rates between day and night samples using a paired t-test, and seasonal variations in catch rates were determined using One-way Repeated Measures ANOVA over the 3-year period. I used the Kruskal-Wallis One-Way ANOVA on Ranks for comparison of condition among size classes, and Dunn’s Method as the multiple comparison procedure to isolate difference among individual size groups. Power, a measure of the sensitivity of a statistical test for detecting differences between groups, is presented for all tests not significant at $\alpha = 0.05$, and power values below 0.80 indicated that sample size may have been insufficient. All statistics were calculated using SigmaStat 2.03© computer software.
RESULTS

Diel sampling conducted quarterly demonstrated consistent differences in catch rates between day and night electrofishing (Figure 1.3). Nighttime electrofishing (mean = 126.2 fish/hr, SE = 15.2) collected an average of 39% more largemouth bass per unit time than daytime electrofishing (mean = 90.6 fish/hr, SE = 11.9), and was higher for 11 out of 12 sampling periods ($t_{11} = 3.595, P = 0.004$). Electrofishing catch rates varied seasonally (Figure 1.4), but these differences were not significant for either day ($F_{3, 6} = 2.525, P = 0.154$) or night ($F_{3, 6} = 0.732, P = 0.570$). However, the power of these tests was only 0.231 and 0.050, respectively, and therefore the probability of a statistical type II error ($\beta$) is high.

Diel catch data from day and night sampling were pooled for creation of length distributions, and age classes were delineated (e.g., Figure 1.5) for all 12 quarterly samples (Figure 1.6). The initial length distribution of May 1998 was composed of three obvious length modes. The first mode peaked at 80 mm total length, and was considered age-0 based on normal juvenile growth rates of about 1 mm/day (Neal and Noble 2002) and spawning occurring primarily from mid-January to mid-March in 1998 (Ozen 2002). The second mode peaked at 200 mm total length and extended up to 260 mm. These fish were considered age 1 because age-0 fish would have required growth rates in excess of 2 mm/day to reach 200+ mm since January. Hence, the third mode was considered age 2. No information was available to differentiate any older fish in the upper tail of the assigned age-2 distribution.

Age-0 and age-1 length modes quickly merged in 1998, consistent with fast age-0 growth and slow age-1 growth. Additional recruitment to the age-0 cohort was observed
Figure 1.3: Catch per unit effort (fish/hr) of largemouth bass during day and night electrofishing from May 1998 to February 2001.

Figure 1.4: Mean seasonal catch per unit effort of largemouth bass averaged for three years (May 1998-February 2001) of sampling in Lucchetti Reservoir. Error bars are one standard error.
Figure 1.5: Initial cohort delineations and age designations for largemouth bass collected May 1998. Age classes are estimated visually by halving the primary shared length class.
**Figure 1.6**: Catch per unit effort (fish/hour) of largemouth bass from May 1998 to February 2001. Age classes are estimated visually by halving the primary shared length class. Age class catch per unit effort is given above individual modes.
in November 1998, and by February 1999 the length distribution was comprised of 2
modes containing 3 age classes (age 1, 2, and 3). Between February and May 1999, catch
per unit effort and relative abundance of the age-3 cohort declined sharply (28.4 fish/hr to
11.0 fish/hr and 45.2% to 13.7% relative abundance), suggesting high mortality during
this period. The 1999 age-0 recruitment became apparent in the August sample, and
these fast-growing fish quickly reached sizes equivalent to older largemouth bass,
yielding a unimodal distribution comprised of 4 age groups by February 2000. The same
occurred in 2001, when the strong 2000 year-class merged with older age groups by

To avoid the obvious difficulties of age determination from multi-age unimodal
length distributions, I chose an individual adult mode to estimate mortality without regard
to the exact age composition. The February 2000 unimodal population was
distinguishable for an entire year, so I focused on this cohort (Figure 1.7). Although
likely comprised of three or more age classes, the majority of these fish were of sizes
considered to be mature. Hence, mortality estimates between sample periods should be
representative of overall adult seasonal mortality.

Catch per unit effort of adult largemouth bass was high in the February sample (149
fish/hr), and dropped to less than half (72 fish/hr) in May (Figure 1.8). Hence, if it is
assumed that this decline was solely related to mortality and not due to biases associated
with catchability, the estimate of total mortality from February to May was about 52%.
Catch per unit effort was relatively stable from May to November, suggesting low
mortality during that period. The same adult cohort was more difficult to delineate in
February 2001, but catch rates appeared to be about 30 fish/hr based on age classes
Figure 1.7: Age 1+ catch per unit effort (fish/hr) during 2000 quarterly samples. Total catch per unit effort and mean total length (mm) are presented.
Figure 1.8: Catch per unit effort (fish/hour) of adult largemouth in 2000 using electrofishing.

Figure 1.9: Seasonal proportions of natural and fishing mortalities of age-1 and age-2 largemouth bass determined using biotelemetry methods in Lucchetti Reservoir, Puerto Rico. The number of fish considered to be mortalities are presented above each bar. Relocation efforts occurred 3-9 days apart. Data are adapted from Waters (1999) telemetry study ($N_{\text{initial}} = 44$; 18-month duration).
estimated in Figure 1.6. Hence, mortality from November to February appeared very high (60%) based on differences in catch rates during the two sampling events.

Data from Waters (1999) showed a similar pattern in natural and fishing mortality (Figure 1.9). The highest mortality rates observed occurred from January to April (11.8% natural, 19.6% fishing) and from May to August (15.5% natural, 25.0% fishing) with only 1 fishing mortality (5% of tagged fish at large) recorded for September through December. No natural mortalities were observed from September to December.

Largemouth bass condition as measured using relative weight varied among size classes and among seasons. Overall mean relative weight was lower for larger size classes, and differences in condition were significant ($H_3 = 34.56, P < 0.001$) for all comparisons except for the two smallest and the two largest size classes (Figure 1.10). Relative weights varied seasonally, and lowest condition was consistently observed during November (Figure 1.11). For the largemouth bass cohort spawned in 1997, a trend toward decreasing relative weight with increasing age was apparent, and lowest mean condition was observed at older ages (age 3-4), consistent with high mortality rates. Similar patterns in condition were observed for 1996 and 1999 cohorts. The year-class produced in 1998 was large (Ozen 2002) and had low mean relative weight during age 0 and age 1, likely a result of uncommonly high intraspecific competition for this reservoir. Relative weight increased by age 2, then began to decline consistent with other year-classes.
Figure 1.10: Mean relative weight of largemouth bass collected every three months from May 1998 to February 2001 from Lucchetti Reservoir. Error bars are one standard error, floating bars indicate significant differences, and numbers above bars are sample size. Length bins under a common line are not significantly different.
Figure 1.11: Change in condition with season and age for annual largemouth bass cohorts hatched 1996 to 1999 and sampled May 1998 to February 2001. Cohorts are defined in Figure 1.6, and ages are presented for each cohort based on January 1 birthdate.
DISCUSSION

Analyses of length distributions over the three years of this study demonstrated the difficulty created by lack of accurate age information for largemouth bass in Puerto Rico. Previous research demonstrated that otoliths were not accurate indicators of age using annuli, and suggested length-frequency analysis as a viable alternative to the use of hard structures (Neal et al. 1997). However, the previous study did not account for unimodal distributions containing multiple year-classes, which are produced by differential growth rates among year classes. Hence, annual cohorts are often impossible to separate using normal distribution methods such as the Bhattacharya technique (Ozen 2002). The Bhattacharya method for splitting a composite distribution into separate normal distributions requires an uncontaminated (clean) slope of a normal distribution (Sparre and Venema 1998). Uncontaminated slopes were often unavailable in my seasonal data.

The absence of well-defined length distributions hampered analysis using the Bhattacharya technique. However, a composite separation method based on halving length modes (where appropriate), and using growth rates, previous and subsequent distributions, and personal experience produced delineations of year-classes for the purpose of seasonal comparison. Although this method was somewhat subjective, it was useful at providing general age structures. Delineation of older age-classes from single length modes was especially subjective, but my delineations closely matched size-at-age data from previous tagging studies. For example, Neal and Noble (2002) found that age-2 through age-4 largemouth bass averaged (annually) 384, 414, and 429 mm total length, respectively. Similar size-at-age values were determined using my composite method.
Analysis of the adult mode present between February and November 2000 without considering age structure (contained at least 3 year classes) indicated that catch per unit effort of adult largemouth bass declined over 50% between February and May 2000, and remained relatively stable for the remainder of the year. This suggests that mortality was high during the spawning period, and relatively insignificant thereafter.

Although electrofishing is a principle technique for collecting largemouth bass, electrofishing efficiency can often vary over time. Differences have been reported between day and night (diel) electrofishing catch rates (e.g., Witt and Campbell 1959; this study) and size distributions (Dumont and Dennis 1997), and seasonal differences in largemouth bass catch rates have been attributed to changes in physiochemical parameters, habitat, or behavioral changes (Edwards et al. 1997). However, the seasonal mortality indicated by catch per effort is supported by Waters (1999) telemetry studies, which found that more that two-thirds of natural and fishing mortality occurred from January to April, and the remainder occurred May to August.

The incidence of mortality corresponds to the observed spawning season in Lucchetti Reservoir. Gran (1995) found that gonad development in 1994 began in late-January, peaked in April, and returned to pre-spawn levels by early-June. Assessment of hatching periodicities determined that spawning begins as early as December (Ozen 2002), but the interval of January to June was consistently the period of greatest reproductive activity. Hence, the majority of mortality occurred during the spawning season, and the rest occurred immediately following spawning. It is possible that behavioral changes and habitat utilization led to higher fishing mortality during spawning, but natural mortality demonstrated a similar seasonal trend. Catch-and-release
mortality is typically low from January to April (Neal and Lopez-Clayton 2001), suggesting that live-release fishing did not contribute significantly to estimates of natural mortality. Thus, seasonal mortality provides circumstantial support to the hypothesis that extended reproduction impacts survival.

The overall fishing mortality found by Waters (1999) was 29% and no difference in mortality was detected between age-1 and age-2 fish. This estimate of fishing mortality was comparable to fishing mortality estimated using creel and catch-and-release studies (27% annual fishing mortality; Neal and Lopez-Clayton 2001). Although fishing mortality is relatively high in this largemouth bass population, it does not explain the rapid loss of age 3 largemouth bass from the fishery. Conversely, the natural mortality rates found by Waters (1999) increased with age. Of the initial 44 ultrasonic-tagged largemouth bass at the beginning of the year, only 7 died of natural causes during year 1 (14%), and 4 of the remaining 15 fish died during the first six months of year 2 (27%). Unfortunately, natural mortality rate was not estimated with telemetry during age 3, but it is likely that it would have increased consistent with the observed changes in length distributions.

Analyses of seasonal variability in condition revealed that largemouth bass enter the spawning season in low condition. Sampling in November demonstrated the lowest mean relative weight, and condition values increased in February, likely in response to gonadal development. The low condition of largemouth bass going into the spawning season suggests that these fish would be more susceptible to spawning related mortality. Low values of relative weight are indicative of low energy reserves (fat and protein), and may be related to high rates of mortality (Newsome and Leduc 1975). Malnutrition of
any animal likely increases susceptibility to pathogens or other environmental stresses (Anderson and Neumann 1996), and may even increase vulnerability to angling (Forney 1980).

Condition appeared to decline with increasing age. The standard weight equation takes into account changes in body shape as largemouth bass grow (Anderson and Neumann 1996), so the low condition of larger fish is indicative of an energetic constraint in Lucchetti Reservoir that intensified with age. The decline in condition observed is consistent with the early mortality (reduced longevity) experienced in Lucchetti Reservoir. Whereas it is likely that fish with lower condition values die first and are not available to be sampled, the observed change in condition may be artificially high. Calow (1978) hypothesized that investment in reproductive production may be at the expense of somatic maintenance, causing accelerated senescence. Although senility is generally not apparent in iteroparous fish populations (Beverton and Holt 1959), the mortality following spawning in semelparous species might be due to accelerated senescence (Larsen 1973). Whereas the reproductive strategy of largemouth bass in Puerto Rico has shifted from the temperate strategy of short spawning periods over many years to the tropical strategy of extended spawning over few years, it is possible that reproductive investment is accelerating senescence in this species.

The research presented in this chapter elucidates several important characteristics of largemouth bass populations in Puerto Rico. First, it is often difficult to obtain reliable age information on largemouth bass using conventional techniques, but a combination of techniques can provide some insight into the age structure of a population. Second, both natural and fishing mortality coincide with spawning season, indicating that spawning
activity may increase vulnerability to angling and natural mortality vectors. Furthermore, natural mortality rate appears to increase with age. Third, condition prior to spawning is low, increasing the likelihood of spawning-related mortality. Finally, condition generally decreases with increasing age, increasing vulnerability to mortality and possibly accelerating senescence. These findings support the reproductive energetics hypothesis, which implicates extended spawning as the mechanism for slow growth and reduced longevity of largemouth bass in Puerto Rico.
Literature Cited


Life-history patterns are often adaptive to specific environments. For many species, characteristics such as growth rate and longevity may depend in part on seasonal temperature regimes. For instance, annual growth rates of smallmouth bass *Micropterus dolomieu* and walleye *Stizostedion vitreum* are positively correlated with the temperature of the habitat (Colby and Nepszy 1981). Maximum age of these species, however, may be greater in the colder part of the range where annual mortality is reduced (Pauly 1980). For largemouth bass, similar patterns in maximum size, growth rates, and longevity have been reported. Modde and Scalet (1986) found a negative linear relationship between largemouth bass maximum size and latitude, and Crawford et al. (1996) found that largemouth bass in Florida exhibited growth rates that decreased with increasing latitude. Longevity tends to increase with latitude (Carlander 1973), and the oldest largemouth bass collected was caught in New York at an estimated age of 24 years (Green and Heidinger 1994).

These studies observed largemouth bass within their native temperate latitudes where they have short spawning periods with one or a few spawning events per season (Heidinger 1975). In Puerto Rico, largemouth bass reproductive period is extended up to six months or more with individual fish spawning multiple times (Gran 1995). Concurrently, growth rate of adult largemouth bass is low and maximum age is reduced. Whereas growth rate and age determine fish size, size distributions of largemouth bass in
Puerto Rico are comprised of many small and medium-size fish with few fish exceeding 2 kg.

A fundamental assumption of life history theory is that there is a cost of reproduction to the individual (reviewed in Stearns 1976). Energy that is allocated to reproduction will not be available to energy-consuming functions such as growth and maintenance, possibly reducing survival and the opportunity to reproduce at some future time. Whereas ecological success depends on balancing the tradeoffs between immediate reproduction and future reproduction (e.g., Jennings and Philipp 1992), energy allocation decisions can determine reproductive fitness.

Energetics models have been used successfully to model the energy budget of fishes. Like all organisms, fish must consume energy in the form of food (consumption, \( C \)) to be used in the synthesis of tissues (production, \( P \)) and as fuel for metabolic processes (respiration, \( R \)). The conversion of food energy is imperfect, and some of the food resource is lost in the form of waste products (egestion and excretion, \( E \)). Whereas all organisms follow the law of thermodynamics (Kleiber 1975), the simple energy budget below can be used to describe the flow of energy within an organism.

\[
C = P + R + E
\]

Each component of the simple energy budget represents an interaction of environmental conditions with physiological processes that are described by a species-specific set of physiological parameters and associated functional equations (Hanson et al. 1997).

Bioenergetics models have been used extensively to model the effects of consumption on predator-prey dynamics and, to a lesser extent, estimate somatic growth (e.g., Stewart et al. 1981, Stewart and Ibarra 1991; Perry et al. 1995; Neal et al. 1999).
However, there is a surprising paucity in the application of these models to simulate reproductive energetics and tradeoffs, especially for freshwater fish species. One explanation may be that the absolute costs of reproduction are difficult to quantify. The energetics of reproduction likely involve more than the production and maintenance of gametes (Miller 1979). They may involve development of secondary sexual characteristics (e.g., spawning coloration) and complex reproductive behavior (e.g., migration, courtship, parental care, and reduced consumption). All of these require the expenditure of energy in addition to the energy spent on production of gametes (Wootton 1985).

The terms of the energy budget for largemouth bass are well known (Rice et al. 1983), and have been successfully evaluated and applied to model largemouth bass energetics (Rice and Cochran 1984; Perry et al. 1995; Whitledge and Hayward 1997). In this chapter, I evaluate the hypothesis: The slow growth of adult largemouth bass in Lucchetti Reservoir is the result of excessive energy reallocation to gamete production. I apply a bioenergetics model to observed diet and temperature data of a largemouth bass population in Puerto Rico, and I evaluate energy expenditure and growth under a range of possible fecundities and spawning frequencies.

METHODS

Prey Energy and Consumption

I performed diel sampling once every 3 months for 3 years to collect information on largemouth bass diet. Day samples were collected from 9 a.m. to 3 p.m. at three sites, and night samples were collected from 9 p.m. to 3 a.m. at three different sites. Day and night samples were collected no more than two days apart. I established nine shoreline
sites within Lucchetti Reservoir, and randomly chose six sites to be sampled during each event. I used a boom-mounted electrofishing unit at 7-8 A and 60 pps DC, and each site was fished for up to 45 minutes. All largemouth bass were collected, measured in total length (TL), weighed (g), and stomach contents were extracted using plastic tubes (Van Den Avyle and Roussel 1980). Stomach contents were preserved in 50% ethanol and were identified, measured and weighed within 48 hours. For diet analyses, Mozambique tilapia *Tilapia mossambica* and redbreast tilapia *Tilapia rendalli* were analyzed collectively due to the difficulty of distinguishing partially digested individuals.

I collected prey species for energy density analysis during February and August electrofishing samples of the first year of sampling. These samples were frozen in water to prevent water loss and transferred to North Carolina State University where bomb calorimetry was performed. Each prey sample was measured, weighed, and dried to a stable dry weight at 55-60°C. Samples were homogenized and dried a second time to a stable dry weight, and percent dry weight for each sample was calculated. Pellets were produced from homogenized material using a Parr Pellet Press®. For prey species, all fish material was analyzed; for larger individuals, three pellets were analyzed. Pellets were weighed to the nearest 0.0001 g, and ideally ranged in size between 0.10 and 0.15 g.

Energy density estimates were obtained for four prey species – threadfin shad *Dorosoma petenense*, redbreast tilapia, bluegill *Lepomis macrochirus*, and channel catfish *Ictalurus punctatus*. For threadfin shad and redbreast tilapia, 12-22 individuals of each species were selected during each sample period for calorimetric analysis with standard Parr oxygen bomb techniques (Parr Instrument Co. 1960; Craig 1977). I analyzed 5 bluegill and 6 channel catfish juveniles for energy density, which were
available in the August sample only. A representative size range of prey was selected based on sample length distributions and observed size of consumed prey.

I used a paired t-test to assess diel differences in feeding as measured by proportion of stomachs containing food, and a one-way ANOVA for repeated measures to test for seasonal differences. A paired t-test was used to test for diel differences in stomach fullness as measured by prey specific weight (wet weight of stomach contents/wet weight of predator). For seasonal and size-related changes in energy density which failed the test of normality (Kolmogorov-Smirnov test), I used the Mann-Whitney test. Power (target power = 0.80), a measure of the sensitivity of a statistical test for detecting differences between groups, is presented for all tests not significant at $\alpha = 0.05$.

All statistics were calculated using SigmaStat 2.03© computer software.

Modeling Reproductive Energetics

Seasonal diet proportions and energy density data were adapted for input into the bioenergetics model package of Hanson et al. (1997) using largemouth bass physiological parameters presented by Rice et al. (1983). Temperature data were adapted from Gran (1995), and ranged from 23.3 to 29.1 °C. Empirical growth data were obtained from microtagging studies in Lucchetti Reservoir (Neal et al. 2002), which indicated that tagged largemouth bass reached maturity at about 300 g (start of age 1), and weighed 850 g and 1,100 g at the beginning of age 2 and age 3, respectively. Specific model designs and inputs are explained in the results and discussion. Conversions of energy to somatic growth assume the energy content of 1 g of largemouth bass somatic mass is 1,000 cal (Rice et al. 1983).
RESULTS AND DISCUSSION

Largemouth Bass Diet

Threadfin shad dominated largemouth bass diet in every sample with the exception of August 1998 (Table 2.1). Threadfin shad were especially important to the diet during winter and spring sampling (February and May), consistent with the primary reproduction and recruitment period of threadfin shad reported for Puerto Rico reservoirs (Stancil et al. 1997). Spiny-rayed species increased in the diet during summer and fall (August and November), presumably due to post-spawning recruitment of these prey species (Alicea et al. 1997). Overall, tilapia were the next most consumed prey complex, and other fish and non-fish prey occasionally occurred in largemouth bass stomachs (Figure 2.1).

I found significant ($t_{11} = 4.376, P = 0.001$) differences in diel occurrence of empty stomachs for largemouth bass (Figure 2.2). More empty stomachs were found at night on 11 out of 12 sample periods, with no statistically significant seasonal pattern (Day: $F_{3,6} = 2.212, P = 0.187$, power = 0.191; Night: $F_{3,6} = 0.655, P = 0.609$, power = 0.050). Also, the degree of stomach contents digestion from samples collected during the day tended to be less than for stomachs sampled at night. Items appeared more digested at night, and more items were unidentifiable at night than at day (5% and 0.5%, respectively). The specific weight of prey items (wet weight of prey/wet weight of predator) was also higher during the day (mean = 0.0058) than at night (mean = 0.0037, $t_{11} = 4.008, P = 0.002$), suggesting greater stomach fullness during the day (Figure 2.3).
Table 2.1: Mean and diel proportions of largemouth bass (>150 mm) diet by weight of prey items collected seasonally using electrofishing. Species codes are as follows: threadfin shad TFS, tilapia TIL (redbreast and Mozambique tilapia combined), bluegill BLG, largemouth bass LMB, armored catfish ARC, channel catfish CCF, and unidentifiable fish UNID FISH.

<table>
<thead>
<tr>
<th>Month-year time of day</th>
<th>Number examined</th>
<th>Percent empty</th>
<th>Proportion of Diet by Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TFS</td>
</tr>
<tr>
<td>May-98 day</td>
<td>102</td>
<td>55.7</td>
<td>0.52</td>
</tr>
<tr>
<td>night</td>
<td>59</td>
<td>67.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Aug-98 day</td>
<td>144</td>
<td>68.1</td>
<td>0.08</td>
</tr>
<tr>
<td>night</td>
<td>55</td>
<td>47.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Nov-98 day</td>
<td>138</td>
<td>49.3</td>
<td>0.76</td>
</tr>
<tr>
<td>night</td>
<td>73</td>
<td>61.3</td>
<td>0.84</td>
</tr>
<tr>
<td>Feb-99 day</td>
<td>121</td>
<td>49.6</td>
<td>0.92</td>
</tr>
<tr>
<td>night</td>
<td>56</td>
<td>55.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Aug-99 day</td>
<td>133</td>
<td>35.3</td>
<td>0.71</td>
</tr>
<tr>
<td>night</td>
<td>61</td>
<td>23.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Nov-99 day</td>
<td>104</td>
<td>37.5</td>
<td>0.77</td>
</tr>
<tr>
<td>night</td>
<td>56</td>
<td>33.3</td>
<td>0.86</td>
</tr>
<tr>
<td>Feb-00 day</td>
<td>133</td>
<td>45.8</td>
<td>0.98</td>
</tr>
<tr>
<td>night</td>
<td>44</td>
<td>27.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Aug-00 day</td>
<td>72</td>
<td>40.3</td>
<td>0.42</td>
</tr>
<tr>
<td>night</td>
<td>44</td>
<td>16.7</td>
<td>0.45</td>
</tr>
<tr>
<td>Nov-00 day</td>
<td>71</td>
<td>40.6</td>
<td>0.64</td>
</tr>
<tr>
<td>night</td>
<td>35</td>
<td>39.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Feb-01 day</td>
<td>50</td>
<td>44.5</td>
<td>0.96</td>
</tr>
<tr>
<td>night</td>
<td>26</td>
<td>43.3</td>
<td>0.99</td>
</tr>
<tr>
<td>May-01 day</td>
<td>24</td>
<td>45.7</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Figure 2.1: Contributions of prey items by weight collected from largemouth bass stomachs in Lucchetti Reservoir, May 1998 to February 2001.

Figure 2.2: Occurrence of stomachs containing food during diel samples of largemouth bass in Lucchetti Reservoir, May 1998 to February 2001.
Figure 2.3: Specific weight (weight of prey/weight of predator) of prey during diel sampling of largemouth bass in Lucchetti Reservoir, May 1998 to February 2001.
Prey Energy Densities

Since threadfin shad and tilapia comprised the primary diet of largemouth bass, I focused primarily on threadfin shad and redbreast tilapia for energy density analyses (Table 2.2). Both species had higher energy densities during spring than during fall (threadfin shad: 1172.8 and 845.5, respectively; Mann-Whitney rank sum test $T = 342$, $P < 0.001$; redbreast tilapia: 1032.2 and 913.6, respectively; $T = 277$, $P < 0.001$). Larger threadfin shad (>60 mm TL) tended to have higher energy densities than smaller threadfin shad, although these differences were not significant for spring or fall ($P = 0.220$ and $P = 0.667$, respectively). The power of these tests was low, however, so conclusions regarding size-specific variation in energy density were not possible. However, energy densities of several clupeid species (including threadfin shad) from North Carolina reservoirs are positively correlated with length (unpublished data, J. Thompson, N. C. State University)

Limited energy density data for bluegill and channel catfish were obtained. Both species displayed higher energy densities than threadfin shad, contrary to studies of Miranda and Muncy (1989), which reported similar values of energy density of shads and sunfishes. The weights of individual bluegill and channel catfish analyzed were up to $30 \times$ that of threadfin shad. Energy density increases as threadfin shad grow (J. Thompson, unpublished data), and likely account for the observed differences.

Energetics of Reproduction

*Observed Growth Assuming No Reproduction*

Previous tagging studies (Neal et al. 2002) determined that largemouth bass in Lucchetti Reservoir were about 300 g at the beginning of age 1, and grew to 850 g by the
Table 2.2: Mean energy densities in cal/g wet weight (value given in joules in parentheses) and standard error (SE) for primary prey species during spring and fall. Caloric densities are presented for bluegill and channel catfish for fall only.

<table>
<thead>
<tr>
<th>Species/size class</th>
<th>Spring</th>
<th></th>
<th></th>
<th>Fall</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SE)</td>
<td>SE (SE)</td>
<td>n</td>
<td>Mean (SE)</td>
<td>SE (SE)</td>
</tr>
<tr>
<td>Threadfin shad</td>
<td>12</td>
<td>1172.8 (4909.4)</td>
<td>64.6 (270.4)</td>
<td>22</td>
<td>845.5 (3539.3)</td>
<td>10.9 (45.6)</td>
</tr>
<tr>
<td>&lt; 60 mm TL</td>
<td>5</td>
<td>1076.0 (4504.0)</td>
<td>55.3 (231.4)</td>
<td>12</td>
<td>841.2 (3521.3)</td>
<td>14.8 (61.8)</td>
</tr>
<tr>
<td>&gt; 60 mm TL</td>
<td>7</td>
<td>1242.0 (5199.0)</td>
<td>98.7 (413.2)</td>
<td>10</td>
<td>850.7 (3560.9)</td>
<td>18.7 (78.2)</td>
</tr>
<tr>
<td>Redbreast tilapia</td>
<td>12</td>
<td>1032.2 (4320.8)</td>
<td>15.9 (66.6)</td>
<td>18</td>
<td>913.6 (3820.0)</td>
<td>22.2 (92.8)</td>
</tr>
<tr>
<td>&lt; 85 mm TL</td>
<td>7</td>
<td>1031.6 (4318.2)</td>
<td>16.1 (67.6)</td>
<td>8</td>
<td>923.7 (3866.7)</td>
<td>37.4 (156.7)</td>
</tr>
<tr>
<td>&gt; 85 mm TL</td>
<td>5</td>
<td>1033.1 (4324.6)</td>
<td>33.5 (140.3)</td>
<td>10</td>
<td>903.7 (3782.7)</td>
<td>27.9 (116.7)</td>
</tr>
<tr>
<td>Bluegill</td>
<td>6</td>
<td>1244.9 (5211.3)</td>
<td>48.5 (202.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60 mm TL</td>
<td>3</td>
<td>1265.2 (5296.3)</td>
<td>93.1 (389.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 60 mm TL</td>
<td>3</td>
<td>1224.6 (5126.3)</td>
<td>51.6 (215.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Channel catfish</td>
<td>5</td>
<td>1227.8 (5139.4)</td>
<td>28.9 (120.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
beginning of age 2, and about 1,100 g by the beginning of age 3. I used these size-at-age data combined with observed prey proportions and energy densities to estimate minimum consumption required for observed growth in the absence of reproductive output.

Mean specific consumption rate of age-1 largemouth bass growing from 300 g to 850 g over 365 days was 0.0170 g/g/d, or a daily ration of 1.70% body weight and a p-value (proportion of maximum consumption) of 0.426. At age 2, growth from 850 g to 1,100 g in 365 days was achieved with a mean specific consumption rate of 0.0114 g/g/d (daily ration = 1.14%, p-value = 0.347). Growth rate was highest when water temperatures were equal to or less than 27 °C; higher temperatures resulted in slower growth for age-1 largemouth bass and negative growth for age-2 fish (Figure 2.4).

The predicted daily rations based on observed growth were lower than those expected in a system where the prey base is not limiting (Ashe et al. 1998). In Lake Rebecca, Minnesota, Cochran and Adelman (1982) estimated largemouth bass mean daily rations using median stomach content weight and evacuation rates. Mean daily rations ranged from 0.03% to 9.85%, with an overall mean of 1.91%. During the warmest months (July-August), which were similar to year-round water temperatures in Puerto Rico, mean daily ration was 2.65% of body weight. Given the potential that largemouth bass in Lucchetti Reservoir actually feed at a daily ration higher than that predicted based on observed growth, the model was repeated using a daily ration representative of rations observed under similar prey and temperature conditions. I used a daily ration of 2.00% as a conservative estimate of potential consumption to model an age-1 largemouth bass (300 g) for 730 days (to age 3) based on observed temperatures, prey proportions and energy densities. Modeled at this consumption level, largemouth
**Figure 2.4:** Bioenergetics model of temperature-dependent growth rate (solid line) based on observed growth of microtagged largemouth bass and temperature variations (dashed line) in Lucchetti Reservoir.

**Figure 2.5:** Bioenergetics model of temperature-dependent growth rate (solid line) based on mean daily ration of 2% body weight and temperature variations (dashed line) in Lucchetti Reservoir.
bass reached 2,352 g by the beginning of age 2, and were an unrealistic 33,194 g when the simulation ended at the beginning of age 3 (Figure 2.5).

*Estimating Cost of Reproduction*

Largemouth bass in Puerto Rico and elsewhere in the world do not grow at rates or to such a maximum size as suggested by Figure 2.5. Genetic restrictions and consumption limitations prevent such growth, but largemouth bass can reach sizes of 5,000 to 9,620 g. If such maximum sizes are genetically possible, and water temperatures and prey availability in Puerto Rico are not limiting, other factors must be restricting growth of largemouth bass. Whereas reproductive period is protracted and individual fish spawn multiple times per year, it is plausible that reproduction is consuming excess energy so that it is not available to growth. Multiple-spawning female largemouth bass would need to invest significant energy resources into gonadal development, and male fish would likely have prolonged behavioral modifications that use available energy and restrict consumption of new energy.

To investigate the energy requirements of reproduction for female largemouth bass, I modeled the maintenance and spawning energy required for a 500-g largemouth bass to maintain starting weight during the 6-month spawning period. Spawning of an individual largemouth bass was simulated for 1 to 20 spawning events and 1% to 10% of body weight spawned per event (Figure 2.6). The bioenergetics model only accounts for energy allocation to gonadal biomass, and does not account for behavioral changes of reproduction. Female largemouth bass undergo significant pre-spawn behavior that is unaccounted for in the model.
Figure 2.6: Daily maintenance and spawning energy requirements for a 500-g largemouth bass female with up to 20 spawning events. During each spawning event, 1 to 10% of body weight is lost in the form of gametes. Simulation period is 6 months and diet and temperature data are representative of the spawning period from January to June. Maintenance energy (the energy necessary to maintain stable body weight in the absence of reproduction) is 6,544 calories.
Maintenance energy for this simulation, defined as the energy necessary to maintain a stable weight of 500 g for the 6-month simulation in the absence of reproduction, was 6,544 cal/d. Hence, from Figure 2.6, the energetic cost of any given combination of spawning proportion (percent body weight per spawn) and spawn frequency (number of spawns per season) can be determined by subtracting 6,544 cal/d from the corresponding daily energy requirement. For example, a fish that spawns 3% of its body mass five times during the spawning season requires a total energy intake of 7,115 cal/d to stay the same size. The reproductive cost is therefore 571 cal/d over the 182-d spawning season, for a total of 104,493 cal foregone to reproduction. The energy content of 1 g of largemouth bass somatic mass is 1,000 cal (Rice et al. 1983), so this fish lost the opportunity to increase its mass by 104.5 g during the spawning season. A fish spawning 10 times at 7% body weight per spawn foregoes the opportunity to double its body mass during that time period.

The growth model based on a mean daily ration of 2% body weight (Figure 2.5) predicted that a largemouth bass weighing 500 g would increase its weight to about 1,140 g in 6 months. However, the spawning energy model suggests that energetic allotment is transferred from potential growth to reproduction as spawning frequency and magnitude increases (Figure 2.6). Given that reproduction is energetically expensive, I estimated the growth potential of a 500-g largemouth bass consuming a mean daily ration of 2% body weight and undergoing spawning frequencies ranging from 1 to 20 spawning events and in magnitude from 1% to 10% of body weight spawned per event (Figure 2.7). This model provided a matrix of possible spawning frequency-magnitude combinations that could account for observed growth in Lucchetti Reservoir.
Figure 2.7: Estimated growth of a 500-g largemouth bass female consuming a mean daily ration of 2% body weight, and engaging in a range of spawning frequencies (1-20 events) and magnitudes (1 to 10% of body weight is lost in the form of gametes per event). The model represents the 6-month spawning period, and incorporates diet and temperature data representative of the period from January to June.
The 2% MDR growth model presented in Figure 2.7 resulted in a maximum size of 1,136 g at the lowest spawning frequency-magnitude combination (1 event, 1% body weight), very close to the predicted size with no spawning (1,140 g). Actual size of a 500-g fish following a 182-day growing period in Lucchetti Reservoir was determined from previous tagging studies (Neal et al. 2002) to be about 740 g. Hence, the discrepancy between observed growth and predicted growth (at 2% MDR) was 400 g, and could be explained by 6 spawning events at 9% body weight each, 10 events at 6%, 14 events at 4%, and so forth. Higher spawning frequencies and magnitudes (e.g., 14 events at 8% per event) led to net loss of body weight in the simulation. These simulations only consider direct energy losses in the form of gametes, and do not take into account energetic costs of behavioral modifications during reproduction.

Behavioral costs are especially important to male largemouth bass, which certainly commit more energy to reproductive behavior that gamete production (Wooton 1985). There is a cost of the locomotion involved in nest construction and defense, courtship, nest fanning, and parental care (Wooton 1985). For instance, a male pupfish (*Cyprinodon*) that actively defends a reproductive territory uses twice as much energy as a male merely holding position (Feldmeth 1983). There is an energy loss associated with the cessation of foraging behavior to undergo courtship and nest guarding behaviors, and with the changes in habitat use from foraging to reproductive habitats. Waters (1998), for example, reported that largemouth bass in Lucchetti Reservoir feed extensively on threadfin shad in offshore habitats during non-reproductive periods, but move to and remain on shallow-water spawning grounds as long as water levels permit.
I estimated the behavioral costs of reproduction to male largemouth bass by adjusting metabolic activity cost to account for changes in reproductive activity, and mean daily ration to account for changes in consumption (Figure 2.8). Metabolic activity cost, which was 0.0196 in the previous models (Rice et al. 1983), ranged 0 to 0.5, and consumption ranged 1.5% to 2.5% body weight per day. Because male largemouth bass produce small amounts of sperm (see Chapter 3), no gamete costs were included in the model. Based on a mean daily ration of 2.0% (no loss in consumption from original model), the discrepancy between observed and predicted growth described previously could be explained by a metabolic activity cost of 0.25 (>12× average metabolic activity cost). If consumption is reduced concurrently with increase in activity, the activity necessary to account for observed growth is reduced as well. A reduction in mean daily ration of just 15.5% from the original model (from 2.00% to 1.69%) accounted for the difference between observed and predicted growth with no change in activity. Although an increase in activity of 1200% may be unrealistically high for spawning behavior, a 15.5% reduction in consumption seems quite plausible given the behavioral changes associated with spawning.

The theoretical perspective provided by bioenergetics simulation, combined with the discussion of behavioral costs of reproduction, suggests that the extended spawning season and associated multiple spawning events are limiting growth of largemouth bass in Puerto Rico. The additional energy requirements of gamete production and parental behavior, and associated reduced consumption during the spawning season would demand a diversion of energy away from growth and possibly maintenance. Energy limitations during spawning may also contribute in part to the high mortality observed in
Figure 2.8: Estimated growth of a 500-g largemouth bass male consuming a mean daily ration ranging 1.5% to 2.5% body weight, and constant metabolic activity cost ranging 0 to 0.5 m/s. The model represents a 6-month spawning period, and incorporates diet and temperature data representative of the period from January to June. The horizontal line at 740 g represents observed weight of a 500-g largemouth bass after 182 days in Lucchetti Reservoir (Neal et al. 2003).
adult largemouth bass populations in Puerto Rico. Exhaustion of energy reserves, malnutrition, and starvation that likely occur during spawning activity can result in reduced somatic maintenance and subsequent somatic deterioration, and can lead to reduced capacity of the immune system (Roff 1983). Furthermore, elevated stress responses have been reported for spawning fishes, and have been implicated in post-reproductive mortality (e.g., Hlavova 1992). Hence, extended spawning effort in Lucchetti Reservoir appears to demand increased energy use, reduces energy acquisition, limits growth capacity, reduces body maintenance and immune capacity, and may contribute to accelerated mortality.
Literature Cited


CHAPTER 3

THE DEVELOPMENT OF TECHNOLOGIES FOR TRIPLOID PRODUCTION
AND VERIFICATION

The largemouth bass *Micropterus salmoides* is an economically important species. Due to tradeoffs between somatic and gonadal energy partitioning, the growth potential of largemouth bass can be limited by reproductive requirements. During reproductive development, energy for somatic growth and maintenance can be reallocated to reproductive output, resulting in reduced growth after sexual maturation (Allen and Stanley 1978). Such slow growth can be counterproductive to the objectives of fisheries managers and the aquaculture industry. One solution would be to produce non-reproducing largemouth bass that invest less energy into reproductive development and output. If reproduction can be eliminated, the resulting energy surplus could supplement growth (Thorgaard and Allen 1987).

The two techniques that are currently available to mass-produce sterile fish are application of strong doses of steroid hormones and chromosomal manipulations (Stanley 1981; Donaldson and Hunter 1982; Purdom 1983; Thorgaard 1983; Yamazaki 1983; Dunham 1990; Ihssen et al 1990; Strüssmann et al. 1993). Chromosome manipulation techniques, such as triploidy induction, appear especially suitable because of the public’s acceptance of the final product over hormone-treated fish (Brown and Roberts 1982; Refstie 1982; Bye and Lincoln 1986; Strüssmann et al. 1993). In previous studies, the induction of polyploidy has proven an effective method of producing permanently sterile
fish with reduced gonad development (Purdom 1976; Wolters et al. 1982; Cassani and Caton 1986; Parsons and Meals 1997). Increasing the number of chromosome sets in fish can yield faster growth and larger ultimate size (e.g., Valenti 1975; Purdom, 1976; Wolters et al. 1982; Chrisman et al. 1983), and can have beneficial applications to fisheries management and aquaculture.

Applying high pressure to fertilized eggs has successfully produced largemouth bass triploids (Garrett et al. 1992). However, Garrett et al. (1992) pressure-treated limited numbers of fertilized eggs, and only 61 individuals from six different treatments (1-28 individuals per treatment) were analyzed for ploidy status. Also, largemouth bass are difficult to propagate artificially. Most hatchery propagation of black bass relies on natural or semi-natural reproduction in which male and female fish engage in nesting behavior in ponds or spawning tanks (Snow 1975). Whereas effective production of triploid largemouth bass requires the ability to obtain viable gametes on demand, propagation techniques need to be advanced.

Methods for mechanical induction of triploidy are not always 100% effective (Harrell et al. 1998). Hence, induction success of treatments must be verified to determine the proportion of triploids obtained. Accepted technologies for detecting polyploidy include cytological karotyping (Thorgaard 1983), staining nucleolar organizing regions (Phillips et al. 1986), particle size analysis that measures erythrocyte cell or nuclear volumes (Thorgaard 1983; Johnson et al. 1984; Wattendorf 1986; Cassani 1990), and flow cytometry analysis (Allen 1983; Johnson et al. 1984). While these techniques generally provide accurate verification of polyploidy (Harrell et al. 1998), the
high cost of equipment and associated expertise makes them unavailable to many producers and managers.

Microscopic measurements of erythrocyte dimensions have been used to verify triploidy in some fish species (e.g., Garcia-Abiado et al. 1999). The sizes of blood and other cells correlate with DNA cellular content, which in fish is primarily related to ploidy level (see review by Fange 1992). This technique is less complicated than other techniques and can be performed with minimal equipment and training. The reliability of this technique for differentiating diploids and triploids has varied among fish species, ranging from 70.8% in rainbow trout *Oncorhynchus mykiss* (Tambets et al. 1991) to 95-100% in landlocked Atlantic salmon *Salmo salar* (Benfey et al. 1984).

In this chapter, I describe the methods I used to artificially propagate largemouth bass and induce triploidy. Also, I evaluate the use of erythrocyte length measurements, by comparison with flow cytometry analysis, for differentiating diploid and triploid largemouth bass. I developed two discrimination techniques using erythrocyte cell length to assist in ploidy determination, and validated both techniques’ performance using internally tagged diploid and triploid largemouth bass.

**METHODS**

**Triploid Production**

Broodstock used to produce my experimental fish were collected from Lucchetti Reservoir, Puerto Rico. Males and females were sorted based on degree of reproductive development, and only fish with free-flowing gametes or advanced gonadal development were transported to the hatchery facilities at Caribe Fisheries in Lajas, Puerto Rico. Fish
with naturally free-flowing gametes were immediately spawned. Bass with advanced
gonadal development but without free-flowing gametes were artificially induced to
release gametes using hormone injections. Both males and females were injected with 5
mg/kg carp pituitary in the body muscle tissue, and with 50 µg/kg leutinizing hormone-
releasing hormone (LHRH) in the dorsal lymphatic node. Injected fish were held in
concrete tanks for spawning on the following day.

Eggs were stripped into a dry container, and milt was stripped from males and
collected via pipette. Milt was mixed with about 10 mL of 0.3% NaCl irrigation to
increase the volume, and then poured over the eggs. Fertilization was considered to be
instantaneous, although I allowed about 1 minute of fertilization time before dividing the
eggs into control and experimental groups. Eggs from the experimental group were
placed into a mesh basket, and the basket was inserted into a water-filled pressure
chamber. At 5 minutes post-fertilization, eggs were subjected to 563 kg/cm² (8,000
p.s.i.) for 1 minute (Garrett et al. 1992). No treatment was given to control eggs. Eggs
from both groups were placed on incubation mats within concrete hatching tanks at a
temperature of 23-27 °C.

Hatching began within 48 h, and swim-up and first feeding followed about 3 days
later. Fry were fed live brine shrimp *Artemia gracilis* twice daily to satiation before
being moved to a natural prey base in grow-out ponds. When juveniles were large
enough (at least 37 mm total length), they were tagged with binary coded wire tags to
differentiate treated (n = 477) and control fish (n = 487). These fish were then combined
and released into Lucchetti Reservoir to be recaptured later for validation of the
discrimination technique. A subset of each group was retained after collection from
grow-out ponds for cytometric determination of ploidy and development of the erythrocyte measurement discrimination technique.

**Verification of Ploidy**

Ploidy levels of a subset of control and treated individuals were assayed using flow cytometric analyses. Erythrocyte cells were collected from individual fish, RNAase was added to prevent RNA staining, and the DNA was stained with propidium iodide. Propidium iodide intercalates into double stranded nucleic acids, and provides a fluorescent signature. Whereas fluorescence of the cells is directly related to DNA content, triploids tend to be about 50% brighter than diploids. For each sample verified using flow cytometry, 10,000 measurements of erythrocyte fluorescence were taken. For more detailed methodology and explanation of flow cytometry, see Kerby and Harrell (1990).

I used 10 triploid individuals and 10 diploid individuals from the groups verified by flow cytometry to develop the discrimination protocol for using erythrocyte major axis length to differentiate ploidy level. Sample fish ranged in size from 17 to 25 mm total length. Blood samples from each fish were drawn and immediately analyzed. No fixative agents were used, although a small amount of 0.7% sodium chloride irrigation was used to dilute the samples. Blood smears were prepared by placing a small drop of diluted blood on a glass microscope slide and covering with a cover glass. For each fish, cell lengths were measured for 25 randomly-selected erythrocytes under 1000× magnification with oil immersion using an ocular micrometer fitted inside the eyepiece (10× magnification) of a compound microscope.
For each blood sample, I calculated the mean erythrocyte length and the 99% confidence interval for the mean (Cimino 1973). For each ploidy group, I used the lowest and highest confidence limits of mean erythrocyte length from the 10 individuals to establish the bounds of the discrimination technique. That is, if the mean erythrocyte length of a fish of unknown ploidy status fell within the boundaries of the diploid discrimination interval, I determined the fish to be diploid. The fish was determined to be triploid if mean erythrocyte length fell within the triploid discrimination interval. If mean erythrocyte length did not coincide with either the diploid or triploid discrimination interval, the fish was considered “undetermined.”

In addition to the discrimination interval technique, I created a prediction model using logistic regression. Mean erythrocyte length was the independent variable, and ploidy status was the dependent variable. The resulting equation calculated the logit value – the logarithmic of the probability that an erythrocyte mean length was triploid divided by the probability it was not. The logistic function model was designed to provide a positive (triploid) or negative (diploid) response to erythrocyte mean length based on the sign of the output number.

After I developed the original discrimination intervals and prediction model with known ploidy fish, I periodically recollected tagged bass from Lucchetti Reservoir using electrofishing. All tagged largemouth bass were measured for total length (TL, mm) and weighed (g), then transported live to the lab where erythrocyte length was used to determine ploidy. Slides were prepared using the same methods used to develop the discrimination intervals, and 100 erythrocytes were measured for each fish. I did not remove and analyze the tag to determine the treatment group of each recapture until after
ploidy was determined using the blood smear technique. This served as a blind test of the verification procedures.

I used two-tailed t-tests with a significance level of 0.05 to test for differences in erythrocyte size distributions between groups. Mean coefficient of variation (CV) was calculated from CV values of individual blood samples, and provided a comparison of the technique’s sensitivity to sample sizes for diploid and triploid samples. All statistics were calculated using SigmaStat 2.03© computer software.

RESULTS

Propagation and Induction

The use of a single treatment of carp pituitary and LHRH produced free-flowing gametes in more than half of the largemouth bass injected. Peak gamete production usually occurred within 21-24 hours following injection at water temperatures ranging 23-28 °C. Water temperature during spawning and induction ranged 23-25º C. Male largemouth bass produced a very low volume of milt, which was most effectively collected using a pipette. The quality and viability of hormone-induced eggs did not appear to be as high as that of natural-induced eggs, which appeared to exhibit slightly higher hatching success.

I successfully produced over 500 control and 500 pressure-treated fingerling largemouth bass from four female and five male largemouth bass brood fish. Cytometric verification determined that 100% of a sample (n = 23) of the pressure-treated group were triploid and 100% of a sample (n = 21) of the control group were diploid. I assumed
Table 3.1: Stocking and ploidy statistics of control and pressure-treated largemouth bass stocked in Lucchetti Reservoir on May 5, 2000. All lengths are measured in total length. Ploidy was determined using flow cytometry.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number stocked</th>
<th>Mean length (mm)</th>
<th>Standard error of mean (mm)</th>
<th>Minimum length (mm)</th>
<th>Maximum length (mm)</th>
<th>Number verified</th>
<th>Percent triploid*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>487</td>
<td>46.7</td>
<td>1.30</td>
<td>37</td>
<td>88</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Treated</td>
<td>477</td>
<td>63.3</td>
<td>1.41</td>
<td>42</td>
<td>99</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

* Based on flow cytometry of 21 control and 23 treated largemouth bass.
100% triploidy for the rest of the treated group, which were stocked into Lucchetti Reservoir in May 2000 (Table 3.1).

**Erythrocyte Length**

Similar to red blood cells of other fish, largemouth bass erythrocytes are oval and elliptical disks with a compact nucleus. Major axis lengths of triploid largemouth bass erythrocytes were significantly larger (P < 0.001) than the corresponding lengths of diploid largemouth bass red blood cells. Combined erythrocyte lengths of the original 10 triploid largemouth bass displayed a mean of 15.64 µm (SE = 0.06 µm), and individual means ranged 15.00-16.10 µm (SE ranged 0.13-0.26 µm, mean CV = 5.79%). The uppermost and lowermost 99% confidence limits for the individual means were used to produce the triploid discrimination interval, 14.43-16.66 µm.

Combined erythrocyte lengths of the original 10 diploid largemouth bass displayed a mean of 11.82 µm (SE = 0.07 µm), and individual means ranged 10.76-13.10 µm (SE ranged 0.12-0.23 µm, mean CV = 6.97%). The uppermost and lowermost 99% confidence limits for the individual means were used to produce the diploid discrimination interval, which was 10.23-13.62 µm.

Therefore, field samples with mean erythrocyte major axis lengths between 14.43 and 16.66 µm are designated triploid, and mean lengths between 10.23 and 13.62 µm are designated diploid. Field samples not coinciding with either the diploid or triploid discrimination intervals would be considered “undetermined.” These discrimination intervals were used in determining ploidy (Figure 3.1).
Figure 3.1: Largemouth bass ploidy discrimination technique developed from known-status diploid (n=10) and triploid (n=10) fish. Circles represent mean erythrocyte lengths for diploid (solid) and triploid (open) individuals, and error bars represent the 99% confidence interval for the mean. New samples coinciding with the overall confidence interval of either ploidy group would be designated as that ploidy status. Samples outside of the discrimination intervals would be considered undetermined, and should be verified using a different technique.
Logistic regression of erythrocyte length and ploidy status provided the prediction model:

$$\text{Ploidy status (+/-) = } a + b \times MEL$$

where ploidy status (the logit prediction statistic) is given as either a negative (diploid) or positive (triploid) integer, and $MEL$ is the mean erythrocyte length for a given fish sample. Coefficients $a$ and $b$ were estimated as $-196.159$ and $13.974$, respectively.

**Verification Using Erythrocytes**

I tested the technique using 22 microtagged largemouth bass recollected from Lucchetti Reservoir during a six-month period following stocking. Erythrocyte length distributions of all microtagged fish displayed two distinct modes (Figure 3.2). Means of erythrocyte measurements from individual fish accurately determined ploidy for all 22 recaptures (Figure 3.3), with no fish considered “undetermined.” Binary wire microtags verified that the actual treatment group of each recaptured largemouth bass matched the erythrocyte technique’s classification, indicating that the technique was 100% successful during the testing phase. The logistic regression equation correctly predicted ploidy status for all 22 microtagged fish as well, providing a 100% success rate (Figure 3.4).

There were no obvious changes in diploid or triploid erythrocyte major axis length as the fish grew (Figure 3.3). The initial mean erythrocyte lengths for diploid and triploid largemouth bass (11.82 and 15.64 $\mu$m, respectively) were determined from fish about 20 mm total length. Recaptured largemouth bass ranged in size from 88 to 235 mm total length, and associated mean erythrocyte lengths and standard errors fell well within the technique’s discrimination intervals for both diploid and triploid individuals.
Figure 3.2: Frequency distributions of erythrocyte cell lengths (n=100 per individual) from 22 microtagged largemouth bass collected in Lucchetti Reservoir.

Figure 3.3: Mean erythrocyte length (based on 100 erythrocytes per individual), erythrocyte discrimination intervals (short dash - diploid; long dash — triploid), and actual ploidy status of 22 microtagged largemouth bass recollected from Lucchetti Reservoir, Puerto Rico. Erythrocyte cell length is plotted against individual fish total length. Standard errors (error bars) of mean cell lengths ranged 0.05-0.18 µm.
Figure 3.4: Logistic regression model predictions of ploidy status using mean erythrocyte length (based on 100 erythrocytes per individual). Actual ploidy status of the 22 microtagged largemouth bass is given.
DISCUSSION

I was able to successfully spawn largemouth bass on demand in Puerto Rico during their natural spawning season. Natural-induced eggs appeared to be more viable, but they were not always available since the female must be collected immediately before or during the spawning event. A combination of carp pituitary and LHRH injections was successful at inducing ovulation in developing females, and provides the opportunity for greater control in the propagation process. The reduction in viability associated with hormones can be offset by the increase in available broodstock (and the available number of eggs) afforded through greater control using the hormones.

The approach described by Garrett et al. (1992) was successful at producing triploid largemouth bass in Puerto Rico, despite higher water temperatures during my induction procedure than during their procedures. In their study, induction was performed at 18º C, and a 1-min treatment of 8000 psi at 5-min post-fertilization yielded 100% triploidy. Whereas metabolic rate increases with temperature, the duration of the required post-fertilization time interval is expected to decrease as temperature increases. In this study, I achieved 100% induction success at temperatures ranging 23-25º C using the same post-fertilization time interval proposed by Garret et al. (1992). This was not the case during subsequent induction attempts at warmer temperatures (See Chapter 4 and Appendix).

My results with triploid largemouth bass are consistent with the findings that the incorporation of a triploid genome in fish causes a significant increase in erythrocyte cell and nucleus measurements (e.g., Benfey and Sutterlin 1984; Fange 1992; Boron 1994; Garcia-Abiado et al. 1999). Measurements of cell major axis lengths and calculation of
their respective mean were able to correctly classify 100% of diploid and triploid fish using the erythrocyte discrimination technique. The logistic regression model displayed 100% accuracy during the field test as well, despite having very high standard errors for the equation coefficients. Whereas erythrocyte size did not appear related to largemouth bass length, it appears that these techniques will be appropriate to verify triploidy for all sizes of fish.

I tested the technique using 100 measurements per fish, but sample size could be lowered depending on the desired level of accuracy. Because mean CV was similar between diploid and triploid fish, sample size requirements should be similar for each ploidy group. The overlap in cell size was minimal, but triploids tended to have a small proportion (about 2%) of erythrocytes within the primary size distribution (10-13 µm) of diploid erythrocytes. The largest diploid erythrocyte observed was 15 µm, but this size group represented only 0.09% of the entire distribution. Whereas much of the triploid distribution was 15 µm or larger, the presence of these larger cells in high numbers could alone be used to differentiate triploid and diploid largemouth bass.

The technique was not designed to differentiate mosaics, tetraploids, or other genetic arrangements. Thus, in situations where samples may contain other variations in chromosome number, I recommend using more precise techniques. However, it is reasonable to assume that further refinement of the erythrocyte length technique using other chromosomal variations could expand its applicability to differentiate haploid and tetraploid individuals as well, given the proportional change in cell size associated with chromosome number.
I have shown measurement of erythrocyte major axis length to be a viable, practical alternative for distinguishing ploidy levels in largemouth bass. Blood samples can be easily removed from small fish by severing the caudal vein with a sharp scalpel, or from biopsy of larger fish without harm. Samples can either be examined immediately or stained for examination at a later date (Cimino 1973). The simplicity of techniques and equipment make it ideal for fish managers and culturists alike, as both start-up cost and training are minimal. Measurements of cell lengths of 100 erythrocytes and calculation of the mean can be performed in about 25 min, making it a realistic alternative for small-scale studies or where immediate verification of individual fish is required. Reducing the number of measurements per sample can decrease the processing time, but accuracy may decrease as well.

Although other techniques such as flow cytometry and use of a Coulter counter can measure cells with greater speed and accuracy, the cost of equipment can be prohibitively high (up to US$33,000 for a Coulter unit). Some organizations perform analyses on a contract or per sample basis, but cost can range US$75-$120/hour excluding sample preparation, shipping, and potential loss of fish during transit. These costs could be restrictive to small-scale aquaculture operations and research projects, especially if only a small number of samples are required.

Although the results indicated that the erythrocyte technique was 100% effective during the testing phase of the study, I only evaluated 22 individuals. It is possible that a larger evaluation would have revealed some potential for misclassification. For this reason, the use of this method to identify triploid largemouth bass to be introduced into waters where diploid largemouth bass do not exist or are of different genetic composition
should be treated with caution because of possible reproduction from misclassified
diploids. In these situations, I recommend flow cytometry or using a Coulter counter to
verify ploidy until additional data are available to confirm the technique’s accuracy. For
other applications, erythrocyte length measurement appears to be a valuable technique for
distinguishing between diploid and triploid largemouth bass.
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CHAPTER 4

FIELD COMPARISON OF DIPLOID AND TRIPLOID LARGEMOUTH BASS DURING THE FIRST TWO YEARS IN LUCCHETTI RESERVOIR, PUERTO RICO

Largemouth bass exhibit slow adult growth and reduced survival in Puerto Rico, hampering efforts to create quality fisheries for this sport fish (Neal et al. 2001). The preceding chapters suggest that the observed multiple spawning events and extended reproductive period of Puerto Rico largemouth bass are energetically expensive. Hence, reproduction may draw on energetic resources needed for growth and maintenance, reducing growth rates and possibly influencing survival. If this hypothesis is correct, a solution to the poor performance of reproductively overactive largemouth bass would be to produce and stock non-reproducing (sterile) largemouth bass.

Manipulation of chromosome number, which is generally associated with sterility, has become a viable tool in aquaculture and fisheries management (Stanley 1979; Gervai et al. 1980; Thorgaard et al. 1981; Wolters et al. 1981). Polyploidy can be readily induced in many fish species by shocking eggs early in development with sharp temperature changes (up or down), increases in hydrostatic pressure, or chemical treatments (see review by Thorgaard and Allen 1987; Ihssen et al. 1990). For triploidy production, the shock must be administered shortly after fertilization. This treatment causes the egg to retain the second polar body that is normally shed, increasing the number of chromatids to three (Thorgaard 1983). The optimum time of shock
application depends on temperature, which affects the rate of embryonic development in poikilothermic species (Shelton et al. 1997).

Sterilization of fish has several possible applications, including increased growth potential (Wolters et al. 1982), creation of non-reproductive populations (Parsons and Meals 1997), and interference with reproducing populations (Parsons 1993). Particularly applicable to fish management and husbandry is the potential for faster growth, which results from the reduction in reproductive investment often observed with triploid individuals (e.g., Wolters et al. 1982; Parsons and Meals 1997). When reproductive development is reduced or foregone, energy ordinarily used for gonad development can be redirected to somatic growth, thereby increasing growth potential (Allen and Stanley 1978).

Reduced reproductive investment and increased growth rates have been demonstrated for some fish species, but many results are conflicting. Parsons (1993) found that triploid white crappie *Pomoxis annularis* had significantly lower gonadosomatic indices than their diploid counterparts, but growth data were not reported. Triploid and diploid channel catfish *Ictalurus punctatus* reared indoors differed in both gonadosomatic index and weight by 8 months of age, with triploids being significantly less sexually developed and heavier in weight (Wolters et al. 1982). When raised outdoors at high densities, however, no differences in weight were detected for diploid and triploid channel catfish, despite significantly higher gonadosomatic indices for the diploid group (Wolters et al. 1991). However, under aquaculture situations, the food is in surplus and costs of obtaining it are very low. Furthermore, gametes are typically
retained under these circumstances, and similarities in weight between diploid and triploid fish may have been related to larger gonads of diploids.

In some instances triploids displayed reduced growth rates when compared to diploids, even though gonadal development was reduced or negligible for triploids. This was the case for grass carp *Ctenopharyngodon idella* (Cassani and Caton 1986) and rainbow trout *Oncorhynchus mykiss* (Simon et al. 1993). However, Cassani and Caton (1986) examined fry and fingerling grass carp, and did not test for differences in adult growth rates, and Simon et al. (1993) studied a cold-water species that has lower temperature-dependent metabolic costs and likely only spawns once per season. Hence, it is unwise to extrapolate these results to a species like the largemouth bass, especially for Puerto Rico populations that experience year-round warm water temperatures and extended, multiple spawning.

For largemouth bass, pressure shock has been effective at inducing triploidy (Garrett et al. 1992; Chapter 3). However, field evaluations of reproductive development and growth rates have not been performed for triploid largemouth bass. If triploids do not invest as much energy into reproductive development and activity as diploids, they should have more available energy for other uses (i.e., growth). In this chapter, I compare the growth and reproductive development of diploid and triploid largemouth bass through their first reproductive season in Lucchetti Reservoir, Puerto Rico.

**METHODS**

Two separate cohorts of experimental fish were produced, tagged with binary-coded wire microtags, and released in Lucchetti Reservoir (Table 4.1). Group 1 was
Table 4.1: Descriptive statistics of two experimental groups of control and treated largemouth bass stocked into Lucchetti Reservoir, Puerto Rico. Ploidy status was verified using flow cytometry. Length data are measured as total length (mm).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Number stocked</td>
<td>487</td>
<td>477</td>
</tr>
<tr>
<td>Mean total length</td>
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<td>Standard error</td>
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<td>99</td>
</tr>
<tr>
<td>Number verified</td>
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<td>23</td>
</tr>
<tr>
<td>Percent triploid</td>
<td>0</td>
<td>100</td>
</tr>
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stocked on 5 May 2000, and Group 2 was stocked on 30 June 2000. Water levels on the
stocking dates were 170.7 m and 167.1 m above sea level, respectively. The methods
used to produce, tag, and verify diploid and triploid largemouth bass are described in
Chapter 3.

Following stocking, I periodically recaptured the experimental largemouth bass
for comparison of diploid and triploid largemouth bass up to the end of the second year at
large (age 1). To collect smaller age-0 fish, I used a 260-V DC handheld probe as
described in Jackson and Noble (1995). A boom-mounted electrofishing unit at 7-8 A
and 60 pps DC was used to collect larger fish. Sample durations and shoreline locations
varied depending on catch rates of tagged largemouth bass.

Whereas ploidy induction was not 100 percent successful in all attempts, we
performed field verification of ploidy status of recaptured bass using the erythrocyte cell
length model described in Chapter 3. Blood samples were taken from all tagged bass
collected using a large-bore syringe inserted into the blood sinuses located behind the gill
filaments. A small (< 0.5 cc) sample of blood was diluted using 0.5% NaCl solution, and
analyzed within 6 hours of collection. All microtagged bass collected were sacrificed,
placed on ice, and returned to the laboratory for analysis. Total length (mm), weight (g)
with stomach contents removed, and gonad weight (g) were recorded, and the microtag
was removed and identified.

Periodic sampling of largemouth bass in Lucchetti Reservoir provided estimates
of growth rate, condition (≥ 150 mm TL), and reproductive development. Growth rates
of individual fish were determined by dividing the difference between mean size at
stocking and individual size at recapture by the time at large. I use relative weight (W_r)
as an index of condition, which is based on the log-linear intercept and slope parameters determined for largemouth bass by Wege and Anderson (1978). To assess reproductive development, I used the gonadosomatic index (GSI), which is the gonad weight expressed as a percentage of body weight (excluding stomach contents). I used GSI threshold limits 0.2 for males and 2.0 for females to indicate sexually mature individuals (Gran 1995). Largemouth bass with values exceeding these gender-specific limits were considered reproductively mature.

I used t-tests to compare control and experimental fish whenever the data were normally distributed (Kolmogorov-Smirnov test). When the assumption of normality was not met, I used a Mann-Whitney test for comparing diploid and triploid largemouth bass. All tests used an alpha level of 0.05, and a target power of 0.80. All statistics were calculated using SigmaStat 2.03© computer software.

RESULTS

I successfully produced and stocked two groups of diploid and triploid largemouth bass fingerlings (Table 4.1). Group 1 was stocked on 5 May 2000, and was comprised of 487 diploid controls and 477 triploid experimental (treated) fish. Flow cytometry confirmed the ploidy status of sub-samples of both diploid controls (100% 2N) and triploid treated (100% 3N) largemouth bass in Group 1. Due to growth variation in the grow-out ponds prior to stocking, triploid fish in Group 1 began the experiment significantly larger than diploid bass ($t_{138} = 8.66, P < 0.001$). Diploid fish ranged 37-88 mm total length with a mean of 46.7 (SE = 1.30), and triploids ranged 42-99 mm total length with a mean of 63.3 (SE = 1.41).
Group 2 fish were tagged and stocked on 30 June 2000. Group 2 was comprised of 535 control and 537 treated largemouth bass. There was a significant difference in size between the two groups ($T = 6065$, $P < 0.001$), but this difference was minor and likely had limited biological importance. Diploid fish ranged 48-65 mm total length with a mean of 54.0 (SE = 0.48), and triploids ranged 42-86 mm total length with a mean of 52.3 (SE = 1.04). Flow cytometry revealed that only 63% of individuals from a sub-sample of the Group 2 treated group were triploid, and some triploids (9%) were found in the control group indicating that accidental mixing of the two treatment types had occurred. Therefore I used individual field verification of ploidy to differentiate diploid and triploid fish upon recapture.

No difference could be detected in growth between juvenile diploid and triploid largemouth bass (Figure 4.1). For Group 1, growth of diploid largemouth bass was 0.81 mm/day ($n = 16$, SE = 0.07) through the end of age 0 (240 days at large). Triploid fish grew slightly faster, increasing in length at a rate of 0.91 mm/day ($n = 13$, SE = 0.09). This difference was not significant ($t_{27} = 0.89$, $P = 0.38$), but the power of this test (0.05) was considerably lower than the widely accepted desired power level (0.80), indicating that sample size may be too low to detect a difference if present. The use of a t-test to detect differences in mean daily growth rates for juvenile bass should be valid over all juvenile size ranges despite initial size differences because growth rates are linear up to maturity (Neal et al. 2002; Neal and Noble 2002). Age-0 growth rates of Group 2 appeared similar to Group 1, but numbers of recaptures during age 0 (diploid = 4, triploid = 1) were insufficient to compare diploid and triploid groups.
Figure 4.1: Growth in length of diploid (solid circles) and triploid (open circles) largemouth bass stocked into Lucchetti Reservoir on 5 May 2000 (Group 1, Top), and on 30 June 2000 (Group 2, Bottom). Solid and open triangles indicate initial mean total length of diploid and triploid fish, respectively. The horizontal line at 275 mm represents the approximate size at maturity for Puerto Rico largemouth bass (Gran 1995), and the dashed vertical line indicates recruitment to age 1.
Both ploidy groups of Group 1 reached maturity size (at about 275 mm total length) during late-winter to early-spring 2001 (age 1, Figure 4.1 Top). Group 2, which was stocked later, approached maturity size in early summer 2001 (Figure 4.1 Bottom). Comparison of diploid and triploid growth after 275 mm was limited by low sample size. Visual interpretation suggested that the similarities in growth continued, although the largest fish recovered for each group during age 1 were triploids, and individual triploids were often substantially larger than other tagged conspecifics (see Figure 4.1). The largest diploid recaptured during the study was 334 mm total length (581 g) and the largest triploid recapture was 357 mm total length (636 g).

High gonadosomatic index (GSI) values indicative of maturity were seen in diploid fish beginning March 2001 at sizes as small as 228 mm for males and 265 mm for females (Figure 4.2). Female diploids had significantly higher GSI values than triploid females at age 1 (All fish > 250 mm TL, both stockings combined, \( t_7 = 3.65, P = 0.008 \)), and no triploid females displayed maturing ovaries. No differences in GSI were apparent for male ploidy groups for fish larger than 250 mm TL (\( t_{12} = 0.133, P = 0.896 \)), and several triploid males displayed GSI values marginally indicative of maturation. The highest GSI values for both females and males were seen in diploid individuals.

Condition of both groups of largemouth bass as measured by relative weight (\( W_r \)) increased with total length (Figure 4.3). Linear regression analyses found significant relationships for total length and diploid (\( F_{1, 30} = 21.05, P < 0.001 \)) and triploid (\( F_{1, 24} = 18.275, P < 0.001 \)) relative weights. Diploids displayed higher mean relative weight than triploids at age-0 (87.9 versus 83.4, respectively) and age-1 (99.6 and 93.5, respectively).
Figure 4.2: Reproductive development of diploid and triploid largemouth bass females (Top) and males (Bottom) as determined using the gonadosomatic index (GSI). Values of GSI exceeding 2.0 for females and 0.2 for males are considered mature (Gran 1995). Both Group 1 and Group 2 stockings are included in these figures; only fish collected during the spawning season (January-June) are shown.
Figure 4.3: Relative weight ($W_r$) of diploid and triploid largemouth bass stocked in Lucchetti Reservoir. Linear regressions indicate ontogenetic changes in mean relative weight.
but analysis of covariance (ANCOVA) did not detect a significant difference in condition between the two ploidy groups ($F_{2, 54} = 1.20, P > 0.25$).

**DISCUSSION**

I found no difference in growth between diploid and triploid largemouth bass during age 0 (juveniles). This finding is consistent with results from other fish species, for which no significant increase in growth from triploidy was found in juveniles (Swarup 1959; Purdom 1976; Gervai et al. 1980; Wolters et al. 1982). However, sample sizes were limited due to low initial stocking densities and low return rates from the Group 2 stocking event. Hence, the comparison of growth rates had low power and thus higher than acceptable probability of a Type II error ($\beta$), which is a failure to detect a significant difference when a difference exists. For this reason, negative findings (no difference in growth) must be interpreted cautiously. However, it is unlikely that growth rates of triploid largemouth bass differ from those of diploid fish as juveniles because the theoretical mechanism behind improved growth in triploids is the lack of reproductive development after reaching the size of normal maturity.

Water level at the time of and immediately following stocking appeared to have an impact on initial survival of stocked diploid and triploid largemouth bass in this study. Water level was 170.1 m above sea level at the time of the Group 1 stocking, and increased to full pool one week later (Figure 4.4) providing large amounts of inundated vegetation and increased littoral area. The low water level of 167.1 m above sea level that was present during the Group 2 stocking event reduced available cover substantially. Neal et al. (2001) found that vegetative cover in Lucchetti Reservoir completely
Figure 4.4: Water level dynamics of Lucchetti Reservoir in 2000. Arrows and group designations indicate the water levels at which experimental stockings occurred. Dotted lines indicate missing data.
disappears when water levels drop below 170 m above sea level, and determined a positive correlation between habitat availability and recruitment of largemouth bass to age 1. This change in available habitat appeared to impact survival and hence recapture rates of the two stocking events, with Group 1 exhibiting more returns than Group 2, despite a higher initial stocking density of Group 2.

No immediate differences in adult growth rates were apparent in this study. Differences between control and experimental fish would not become apparent until reaching sizes characteristic of mature largemouth bass, which was reached around the end of the spawning season in 2001. Most fish exceeded 275 mm total length (the approximate size of maturity) after April 2001, and the first mature female was observed 27 March 2001. Whereas primary spawning occurs from January to April, many of the stocked largemouth bass may have matured after the spawning season. Hence, timing of maturity may have prevented significant spawning of control bass during their first spawning season, limiting energetic diversion to reproduction and resulting in growth rates similar to triploid bass.

Both ploidy groups reached the size of maturity during age 1, and diploid largemouth bass underwent greater reproductive development. Male triploids demonstrated some reproductive maturation, but gonadosomatic index values were reduced when compared to fully developed diploid largemouth bass. Similarly, reduced reproductive development was reported for male triploid rainbow trout (Simon et al. 1993), suggesting that male triploids invest less in reproduction than normal diploids. My female triploids showed no apparent increase in ovarian size following maturity while similar-size diploids often produced well-developed ovaries. These results are
consistent with results for other fish species in which triploid females did not undergo sexual maturation (e.g., Parsons 1993; Simon et al. 1993), and suggest that the triploid females do not invest significant energy into reproductive development. Whereas reproductive energy requirements are high for diploid largemouth bass in Puerto Rico (Chapter 2), the lack of reproductive energy investment by triploids may prevent the slow grow seen for reproductive age-2 diploids.

Growth rates of largemouth bass in Lucchetti Reservoir typically are moderate during age-1, and then slow to near 0 mm/day at age 2 (the first full spawning season). Hence it is likely that energetic superiority of triploid largemouth bass will not become obvious until they reach age 2. Interestingly, mean total length (mm) of triploid largemouth bass during the last successful recapture period was 332.3 (SE = 12.8) compared to 308.3 (SE = 10.9) for diploids, although sample sizes were only 2 and 3 fish, respectively. A long-term evaluation is needed to determine if age-2+ growth rates differ between the ploidy groups. Further evaluation could also address longevity, another potential advantage of triploids that could not be studied in the time frame of this project.

Sample sizes of recaptured largemouth bass after reaching maturity were low, limiting the application of statistical tests to the data. Whereas I developed the technology for artificial propagation and triploidy induction during the production of my experimental fish, the number of fingerlings that I could produce was limited. Now that methods for triploid production and verification have been established (Chapter 3), an intensive evaluation using greater numbers of diploid and triploid largemouth bass can be performed. I recommend a follow-up study involving intensive production, stocking, and
evaluation of triploid largemouth bass over a greater time period, which would determine the efficacy of triploidy as a management tool.
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CHAPTER 5

SYNTHESIS OF RESEARCH ON REPRODUCTIVE ENERGETICS, GROWTH, AND MORTALITY OF TROPICAL LARGEMOUTH BASS

Discussion of Findings

The results presented in this study further the understanding of largemouth bass reproduction, growth, and survival in tropical environments. Largemouth bass in Puerto Rico reach sexual maturity in one year, and unlike largemouth bass in temperate regions, spawning occurs during a six-month period beginning in mid-winter with multiple spawning by individuals each season. In this dissertation, I sought to determine if excessive energy drain from extended reproduction is responsible for or contributes to the slow growth observed in Lucchetti Reservoir. The study design was threefold – to identify seasonal trends in mortality and relate them to reproduction; to model the energetic demand of multiple reproduction and assess the potential for reduction in growth; and to evaluate the growth of mature non-reproductive largemouth bass in comparison to normal reproducing fish.

Seasonal trends in mortality were detected using length-frequency analysis and biotelemetry data from Waters (1999), and these trends indicated that most mortality (natural and total) closely coincided with largemouth bass spawning. Although the mechanisms of natural mortality were not directly determined, excessive reproduction could result in increased risk for adult largemouth bass to disease, parasites, predation, or other means of natural mortality. The investment in reproduction by some fish species may be at the expense of bodily maintenance (Calow 1978). This could increase risk of
mortality through a reduction in body size, deterioration of body tissues, or a reduction in immune capacity (Shevchenko 1972, Roff 1983). It could also lead to a reduction in swimming ability (Koch and Wieser 1983) and consequently affect their ability to escape predators or capture prey.

Spawning effort is difficult to quantify in natural systems, and no reliable information on frequency or sex product deposition of individual spawning events has been determined for Puerto Rico. Gran (1995) was successful at demonstrating that largemouth bass in Lucchetti Reservoir exhibit multiple-clutch, group-synchronous ovarian development similar to that exhibited by other members of the family Centrarchidae (Merriner 1971). However, her estimates of annual fecundities ranging 8,000-84,000 eggs per kilogram body weight used methods that likely significantly underestimated annual fecundities. Lack of information on spawning effort necessitates a more robust method for evaluating the energetic investment of reproduction.

I used bioenergetic modeling to estimate reproductive energy requirements for a range of spawning frequencies and fecundities possible in Lucchetti Reservoir. This method provided a basic model for interpreting the effects of multiple partial spawning. The daily energy requirements presented in Chapter 2 can be converted to growth potential, providing an estimate of somatic growth that could have occurred in the absence of energy expenditures for gametes (Figure 5.1). This model suggests that when largemouth bass spawn at high frequency and fecundity, large quantities of energy are reallocated to reproduction from other potential uses such as growth. This reallocation provided a reasonable explanation for the discrepancy between observed and predicted growth as discussed in Chapter 2. Whereas the female bioenergetics model did not
**Figure 5.1**: Estimated growth in weight lost due to spawning for a 500-g largemouth bass female over a six-month spawning period. Simulation included up to 20 spawning events with 1-10% body weight lost in the form of gametes during each spawning event. This figure is based on daily maintenance and spawning energy requirements presented in Figure 2.6, Chapter 2.
account for behavioral changes that affect energy use and acquisition (e.g., pre-spawn
searching behavior and courtship), it is likely that the actual costs of reproduction are
slightly higher, and the model estimates are conservative. Spawning costs of male
largemouth bass are almost completely due to behavior, and the behavioral energy model
presented in Chapter 2 reasonably explained the growth discrepancy as well.

The extended spawning season of largemouth bass in tropical reservoirs increases
the potential for multiple spawning by individuals. Although the number of spawns per
individual is not known, a synthesis of data on various life history aspects suggests that
largemouth bass may spawn many times within the primary spawning season. For
instance, hatching periodicities suggest that spawning generally occurs over a period of at
least 3 months and as long as 6 months (Ozen 2002). Gran (1995) determined that during
the peak of spawning (February-April), about 80% of female largemouth bass had
oocytes in final maturation at any given time. In response to hormone injections
discussed in Chapter 3, 50% of female largemouth bass that demonstrated advanced
gonadal development could be induced to ovulate within 24 hours (this excludes fish with
free-flowing gametes at the time of collection). Hence, 40% of female bass are capable
of spawning within 24 hours at any given time. If the other 60% are on similar but offset
reproductive cycles, it would appear that each female is capable of spawning at least
every 3 days, or about 30 times during a 3 month season. Longer spawning seasons
would increase the total number of individual spawning events accordingly. Many other
factors determine spawning frequency, and females would be unlikely to reproduce at
their physiological maximum. Male largemouth bass must guard nests and broods for
longer intervals, reducing potential spawning opportunities, but increasing parental investment.

If reproduction limits growth of adult largemouth bass in Puerto Rico, non-reproductive individuals should experience higher growth rates and attain larger maximum size. Interestingly, many reservoirs in Puerto Rico occasionally produce unusually large individuals that appear to have avoided the slow growth experienced by most other adults. For example, marking efforts during a population estimate in Carite Reservoir in 2000 (Neal et al. 2001) collected 11.3% of the adult largemouth bass population in the reservoir, including a small mode that was about 200 mm longer than the presumed age-3 length class (Figure 5.2). Similarly, largemouth bass 3.5-5.1 kilograms were occasionally collected in Lucchetti Reservoir (e.g., Figure 1.6), with relatively few intermediates. The absence of significant numbers of intermediate-size individuals and the slow adult mean growth rates reported in these reservoirs suggest that this large size-class was not simply older fish. Instead, it appears that this group experienced much faster growth than the rest of the population for reasons that are not known. Unfortunately, none of these large fish were sacrificed for examination of sex characteristics.

It is possible that these few fish that reach larger size invest less energy into reproduction. One scenario could be that their individual genetic combination is conducive to less reproductive effort, such as spawning only once per season. Another possibility is that these few fish in the population are sexually aberrant, which has been shown to increase growth rates in some species (Wolters et al. 1982). To address this possibility, I developed technologies for producing sterile triploid largemouth bass
Figure 5.2: Length distribution of largemouth bass in Carite Reservoir during the marking period of a Petersen-type population estimate. Figure adapted from Neal et al. (2001).
(Chapter 3), and I compared the performance of sterile and reproductive largemouth bass (Chapter 4).

The propagation, triploid induction, and verification methods developed during this study lay the groundwork for intensive evaluation of the reproductive energetics hypothesis. Although my small-scale assessment of diploid and triploid largemouth bass did not find significant growth differences between ploidy groups, low sample sizes and limited assessment time severely hampered analyses. Gonadosomatic values indicated reproductive investment in diploids, while triploid males showed reduced investment and triploid females did not experience reproductive development. Consequently, the mechanism exists for differential growth. However, because the diploid group did not reach maturity until late in the age-1 spawning season, it is unlikely that significant energy was diverted to reproduction during the study period. Without reproductive activity, no differences would be apparent between the ploidy groups.

Differences in energetic allocation would become much more pronounced during the spring of age 2, when all diploid largemouth bass are capable of spawning during the entire season. Unfortunately, the low initial stocking densities combined with low water levels and related high mortalities limited the number of study fish surviving in the reservoir, and sampling efforts by the end of age 1 collected no tagged fish despite electrofishing about 200% of the shoreline. Thus, evaluation of growth differences between ploidy groups was not possible during the second spawning season when these differences should have become apparent.

A large-scale evaluation is needed to determine if triploid largemouth bass will demonstrate superior growth due to reduced reproductive development. Additionally, a
long-term study with high stocking densities could provide information on longevity, which was not assessed during my short-term study. Technology for large-scale production of triploids is now available, and my ploidy discrimination technique greatly simplifies field determination of ploidy. A large-scale assessment could provide definitive evidence to support or refute the reproductive energetics hypothesis, and would determine if triploid largemouth bass are a viable management option for growing trophy largemouth bass in Puerto Rico.

**Research Implications**

*A Temperate Fish in the Tropics*

Largemouth bass, which are native to temperate and subtropical habitats in North America, have been widely introduced around the world. In tropical environments such as Puerto Rico, the environmental conditions experienced by largemouth bass are quite different from those in native environments for which they have evolved. Consequently, largemouth bass experience marked differences in life history strategies, including fast juvenile growth, multiple spawning and increased spawning period duration, slow adult growth, and high adult mortality. The altered ecology and population dynamics of this species in tropical systems necessitates a customized management approach.

Size structure of the adult largemouth bass stock in Lucchetti Reservoir appears to be comprised primarily of young bass of only a few year-classes. Accordingly, diminished recruitment in any single year can have profound effects on stock size. Some buffering of recruitment variation may be provided by the extended spawning season; however, factors such as water level decline from preferred spawning grounds interrupt
spawning and reduce year-class size (Waters 1999, Ozen 2002). Conversely, because the stock is comprised of few year-classes, stocking of hatchery-produced fish can significantly impact stock size. Previous research has suggested that wild year classes can be highly variable, and that supplemental stocking during low recruitment years can be used effectively to stabilize stock size (Neal et al. 2002).

The rapid growth to maturity followed by high incidence of adult mortality suggests that liberal size and creel limits are warranted. Growth to reproductive size is rapid (less than one year) and slows considerably following maturity. Hence, there is no biological need for a minimum size limit in Puerto Rico reservoirs. A negative public perception generally is associated with anglers retaining very small largemouth bass, so in most instances anglers will not keep very small fish because of issues associated with practicality and perception. The high natural mortality of adult bass indicates that creel limits should be liberal in order to maximize harvest and minimize natural losses (e.g., Ozen 2002). Furthermore, catch-and-release mortality may be a factor during summer months when water temperatures exceed 25° C (Neal and Lopez-Clayton 2001), so it may be advantageous to encourage harvest during summer months.

Research presented in this dissertation supported the hypothesis that reproductive costs limit the growth of largemouth bass in Puerto Rico. Hence, manipulation of reproductive effort could lead to improvements in growth rates, maximum size, and possibly survival. Since spawning is highly dependent on water level (Ozen 2002), water level manipulation might be used to restrict largemouth bass populations in Puerto Rico reservoirs to a short spawning period each year if the costs are primarily for gamete production rather than gamete maintenance and associated behavior. In turn, reduced
spawning costs result in increased energy for alternative uses such as growth and maintenance. However, management of water level is unlikely due to conflicts with other uses.

Reproductive sterility is likely a more realistic option than water level management. In this dissertation, I refined the techniques to produce and verify sterile (triploid) largemouth bass for research and management purposes in Puerto Rico. The next step is a thorough evaluation of triploidy performance, which would establish the efficacy of triploidy as a management option. If further research determines that triploid largemouth bass show superiority in adult growth rates, then these fish could be mass-produced and stocked as part of the supplemental stocking program already established. Whereas the objective of a triploid stocking program would be to create trophy fisheries, large stocking densities or protected size ranges would be required to ensure adequate survival to large size. An alternative would be to designate specific reservoirs as trophy fisheries, implement conservative size and creel limits, and stock only triploid largemouth bass in these systems.

I assessed the production and evaluation of intergrade triploid largemouth bass because this strain has been intensively studied in Puerto Rico. However, Florida subspecies largemouth bass live significantly longer than intergrade largemouth bass in Puerto Rico (Neal and Noble 2002), and is currently the only strain produced and stocked on the island. Further evaluation of triploid largemouth bass should use the Florida subspecies to exploit the increased longevity and the growth potential it represents. The combination of increased maximum age of Florida largemouth bass combined with
reduced reproductive investment of triploids may produce a longer-lived faster-growing fish that is capable of reaching trophy sizes.

Global Implications

The issue of growth and mortality of largemouth bass in Puerto Rico may have importance beyond that of island fisheries management. The shortened life expectancy and slow growth not only limits the trophy potential of largemouth bass in local reservoirs; it forecasts the possibility of similar responses of animal life history strategies to our changing global climate.

Global climatic change has received considerable attention in recent years from scientists and policy makers alike (e.g., McGinn 2002). It has become obvious that our environment is changing in substantial ways that we often cannot predict (Mooney 1991, Firth and Fisher 1992, Neilson 1993). The complexity of biological systems greatly confounds attempts to isolate and understand the mechanisms of ecological change, as does the lack of appropriate tools to measure biotic processes (Mooney 1991). Also, the brevity of most research programs results in a trade-off between accurate evaluation of long-term changes and production of short-term results. Global climate change is a continuum, however, and impacts on organisms often cannot be observed or predicted within the time frame necessary to fulfill most grant requirements. Thus, most of our understanding is based on models and theories instead of direct field observations.

One way to observe the immediate impacts of climate change on an organism is to immediately change the climate in which that organism is located. These “ecologically displaced” organisms could provide valuable insight into the changes in life history characteristics that can be expected with the impending changes in our global climate.
This type of ecological displacement has been done with many species through exotic introductions all over the world, and is especially prominent with freshwater fish. Fish are appealing study organisms for climate research because of their reliance on climate as it affects water temperature. As poikilothermic animals, fish rely heavily on water temperature for biological processes, behavioral cues, and activity levels. All three are manifested in the growth and survival of an individual.

For these reasons, largemouth bass in Puerto Rico may provide valuable insight into the possible effects of global warming on largemouth bass and similar species within their native range. As mean global temperatures rise, can we expect the high mortality and slow growth experienced by Puerto Rico largemouth bass populations to become characteristic of largemouth bass populations further north? Results from this and other subtropical and tropical studies suggest that alterations in temperature regimes will impact the species throughout their distribution, although the exact nature and magnitude of the impact is not clear. Global climate change will involve an increase in mean temperature within the native range of largemouth bass, but seasonal temperature cycles will continue. Hence, it is unlikely that temperature largemouth bass will experience the magnitude of change observed in growth and reproductive characteristics of largemouth bass stocked in Puerto Rico.

Most research on the impacts of global climate change has focused on cool and cold freshwater fisheries and marine environments (see McGinn 2002), but it is likely that warm freshwater systems will be significantly impacted as well. Abiotic factors play a crucial role in year-class formation and recruitment of largemouth bass, and global warming is anticipated to produce substantial weather changes over large areas of the
largemouth bass range (Ridgeway and Philipp 2002). Hence, we need the ability to predict the changes that we will face, and we need to anticipate management approaches that can be used in light of these changes.

The best evidence of impending changes to largemouth bass biology and behavior comes from latitudinal variations in their life history strategies. For instance, there is an increase in spawning season duration and frequency of multiple spawning with decrease in latitude. In Ontario, largemouth bass spawning begins in late-May and lasts only a few weeks (Stocek and MacCrimmon 1965). In North Carolina, spawning typically begins in late-April and lasts about 6 weeks (Phillips 1994), while spawning in Florida begins in early to mid-April and lasts about 8 weeks (Clugston 1966). Variation in the spawning season of largemouth bass is even more pronounced in lower latitudes where this species is non-native, with spawning generally commencing when water temperatures reach minimum annual values (19-24 ºC), and the duration of spawning activity is extended for up to 8 months (Caldwell et al. 1957; Clugston 1966; Guerra et al. 1980; Dadzie and Aloo 1990).

Modde and Scalet (1986) addressed latitudinal influences on largemouth bass maximum size using record weights recognized by state fish and wildlife agencies of the 48 contiguous states (as of October 1984). They found a strong negative linear relationship between maximum body size and latitude for record largemouth bass caught between 27 and 48 degrees north latitude, suggesting that the largest absolute size is obtained at the lowest latitudes. But this analysis did not include largemouth bass stocked at tropical latitudes outside of the native range.
I repeated the comparison of record weight and latitude by updating with current records and including available records from subtropical and tropical reservoirs in the northern hemisphere (Figure 5.3). The resulting relationship was not linear, and was best fit using the 4-parameter log-normal equation:

\[
\text{Record weight} = 4.5466 + 4.3395 \times \exp\left(-0.5\left[\frac{\ln\left(\frac{\text{latitude}}{28.9147}\right)}{0.1601}\right]^2\right)
\]

with \( R^2 = 0.71 \) (\( F_{3,47} = 38.56, P < 0.0001 \)). This relationship suggests that optimum latitudes for largemouth bass maximum size are around 28.9 degrees north latitude, and environmental conditions are sub-optimal to the north and south. Largemouth bass to the north are limited by cool water temperature and short growing period (e.g., Kramer and Smith 1960; Coutant 1975), while populations to the south are likely limited by year-round warm water temperature and extended reproductive drain.

An increase in global temperatures may result in a northward shift of the curve presented in Figure 5.3. Maximum sizes will likely increase at latitudes just north of the peak in response to increased growing season and mean water temperatures. Conversely, maximum sizes will likely decline at the current peak latitudes and further south as summer water temperatures become more oppressive and spawning effort increases. Accompanying the changes in absolute size will be corresponding changes in reproductive strategies and maximum lifespan. An overall reduction in longevity and increase in duration of spawning season would be expected across the current range of the species. Other warm water species may respond similarly.
**Figure 5.3:** Record weights (as of December 2001) of maximum largemouth bass sizes recognized for the 48 contiguous states including Florida (FL), Georgia (GA), and California (CA); Hawaii (HI); Puerto Rico (PR); and Mexico (MX). Nonlinear regression using a 4 parameter log-normal equation provided the best fit and is presented on the figure.
Literature Cited


Shevchenko, V. V. 1972. Dynamics of the content of dry fat-free residue and of lipid content in the body and organs of the North Sea haddock (Melanogrammus aeglefinus) in the course of growth and gonad maturation 12:830-837.


**APPENDIX**

**TIMING OF PRESSURE TREATMENTS TO INDUCE TRIPLOIDY AND DEPENDENCE ON WATER TEMPERATURE**

Chromosome manipulation such as induction of triploidy is an important technique in aquaculture and fish management. Shocking eggs using sharp temperature changes, hydrostatic pressure, or chemical treatments can cause eggs to retain the second polar body that is normally lost during development, adding an additional set of chromosomes to the resulting embryo (see review in Chapter 3). Optimizing shock induction requires empirical determination of a shock’s magnitude, duration, and time of application following fertilization. The optimum time of application depends on temperature, which affects the rate of embryonic development in poikilothermic species. I evaluated triploidy induction success for largemouth bass at a range of temperatures and post-fertilization times to determine a time-temperature model for future production of triploids.

I used propagation and induction methods described in Chapters 3 and 4. I manipulated water temperatures using ice and a YSI 55 meter, maintaining temperatures to the desired temperature treatment in both the saline dilution fluid and within the pressure chamber. Temperature treatments evaluated were 18, 20, 23, 25, and 27 °C. Eggs were stripped into a dry container, and milt was diluted in treatment temperature saline solution. The solution was poured over the eggs and timing of post-fertilization period began. The fertilized eggs were transferred to a mesh basket and placed in the treatment temperature saline solution within the pressure chamber. Post-fertilization
times of pressure treatments were 210, 240, 270, and 300 seconds. One sample of each
time-temperature combination was conducted, hatched, and evaluated for induction
success. Verification was performed using the erythrocyte major axis length model
described in Chapter 3.

Induction success of 94% or better was achieved for at least one time interval for
each temperature (Table A.1). At 18 °C, a 300-s interval provided the highest induction
success (96%), and less than half of the 210-s interval sample fish were triploids.
Surprisingly, all time treatments produced 100% triploids at 20 °C, and the highest
induction success for warmer temperatures was achieved in the 270-s interval.

It appears that the time intervals used in this study were too large to produce an
accurate time-temperature model for predicting the post-fertilization interval at any given
temperature. However, this study offers two important results. First, it demonstrates that
an interval of 270-300 s will produce high percentages of triploids over a range of
temperatures, allowing for some error in timing to occur. Second, this research showed
how easy it is to manipulate temperature during fertilization and pressure treatment. If a
constant temperature is always maintained, there is no need to determine post-fertilization
interval during each induction event.
Table A.1: Triploidy induction success of 20 time-temperature combinations. Induction success is a percentage, and the number of individuals examined for ploidy status is given in parentheses. Bold values indicate the highest induction success for each temperature.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>210</th>
<th>240</th>
<th>270</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>48 (25)</td>
<td>92 (25)</td>
<td>89 (28)</td>
<td>96 (25)</td>
</tr>
<tr>
<td>20</td>
<td>100 (16)</td>
<td>100 (21)</td>
<td>100 (25)</td>
<td>100 (25)</td>
</tr>
<tr>
<td>23</td>
<td>27 (11)</td>
<td>39 (18)</td>
<td>100 (24)</td>
<td>88 (8)</td>
</tr>
<tr>
<td>25</td>
<td>89 (28)</td>
<td>76 (21)</td>
<td>94 (35)</td>
<td>79 (19)</td>
</tr>
<tr>
<td>27</td>
<td>83 (30)</td>
<td>80 (25)</td>
<td>100 (15)</td>
<td>96 (28)</td>
</tr>
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</table>