Comparison of Diploid and Triploid Largemouth Bass Growth and Maturation in Puerto Rico

J. Wesley Neal, *Department of Wildlife, Fisheries & Aquaculture, Box 9690, Mississippi State, MS 39759*

Abstract: Triploid largemouth bass may have potential in sport fish management and in food fish production as a means to eliminate reproduction, which would, in turn, potentially increase somatic growth. To examine this potential, four cohorts of diploid and triploid largemouth bass were produced over a 10-yr period and tagged intramuscularly with coded wire tags. Bass were stocked into Lucchetti Reservoir, Puerto Rico, and recaptured during subsequent sampling events. Growth rates, condition (relative weight, W_r), and reproductive investment (gonadosomatic index, GSI) were compared for diploid and triploid fish. Mean daily growth rate (MDG) did not differ ($P \geq 0.050$) between diploids and triploids overall (diploid MDG ± SE = 0.75 ± 0.02 mm/d; triploid MDG ± SE = 0.74 ± 0.03), or by age class through age 2. von Bertalanffy growth parameters were similar between ploidy groups (diploid: $L_\infty = 384.5$ mm, $K = 1.244$, $t_0 = -0.237$; triploid: $L_\infty = 387.0$ mm, $K = 1.231$, $t_0 = -0.31$). Unlike triploid largemouth bass, diploid fish exhibited advanced reproductive development following maturation at age 1; thus, mean GSI was greater for diploids versus triploids for both males and females ($t \geq 2.52$, $P \leq 0.010$). Relative weight was consistently greater for diploid largemouth bass ($P < 0.008$), likely due to greater GSI. The lack of significant growth advantage in tropical environments precludes using triploid largemouth bass to enhance trophy bass potential in Puerto Rico reservoirs. However, triploid largemouth bass may have utility in systems where largemouth bass reproduction is unwanted.

Key words: Micropterus salmoides, sterile, reproductive development, somatic growth

Journal of the Southeastern Association of Fish and Wildlife Agencies 1:1–6

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 for control applications (Parsons 1993). Particularly applicable to fish management and husbandry is the potential for faster growth, which theoretically results from the reduction in reproductive investment often observed with triploid individuals (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).

The concept of using sterile or same-sex fish to eliminate reproduction and thereby to increase somatic growth is a growing research area (e.g., Wolters et al. 1982, Parsons and Meals 1997, Neal and Noble 2008). One method to produce sterile fish is to manipulate chromosome number (Stanley 1979, Gervai et al. 1980, Thorgaard et al. 1981, Wolters et al. 1982). Polyploidy can be readily induced in many fish species by shocking eggs early in development with sharp temperature changes, increases in hydrostatic pressure, or chemical treatments (Thorgaard and Allen 1987, Ihssen et al. 1990). For triploidy production, the shock must be administered shortly after fertilization, which causes the egg to retain the second polar body that is normally shed, increasing the number of chromatids to three, and presumably resulting in sterilization of the offspring (Thorgaard 1983).

In Puerto Rico, growth of largemouth bass (*Micropterus salmoides*) is rapid, with fish usually reaching maturity by age 1 (Neal and Noble 2002). However, growth slows considerably after maturation, and is nearly negligible by age 2. Furthermore, longevity of largemouth bass in Puerto Rico is greatly reduced, with few fish surviving to age 3 (Lilyestrom et al. 1994, Waters 1999). Neal and Noble (2006) suggested that multiple spawning events per spawning season and extended reproductive period result in reduced growth rates of largemouth bass because of energy reallocation from growth to reproduction. Thus, they hypothesized that sterile triploid largemouth bass could exhibit faster growth than diploid conspecifics. Garrett et al. (1992) earlier showed that pressure shock has been effective at inducing sterility in largemouth bass by creating triploid eggs. This pressure shock technique has been effectively replicated in tropical environments (Neal et al. 2004).

Neal and Noble (2008) compared the first 2 yrs at large of a single stocking of diploid and triploid largemouth bass in Lucchetti Reservoir, Puerto Rico. That study did not find initial differences in growth between triploid and control largemouth bass, but postulated that more time post-maturation was needed. In addition, sample sizes were low due to inherent difficulties of producing triploid largemouth bass and subsequent low recapture rates during later sampling events. In the current study, some of these limitations were overcome by combining data from Neal and Noble (2008) with three additional cohort stockings

to compare growth, reproductive development, and condition of diploid and triploid largemouth bass in tropical reservoirs. Statistical comparisons were possible through age 2 and qualitative comparisons were possible up to age 5.

Methods

Study Reservoir

Located in the mountain region of southwestern Puerto Rico, Lucchetti Reservoir is a 108-ha impoundment with a maximum depth of 22.2 m. The area was originally tropical forest, although much of the landscape is now used for agriculture. Rainfall in the region of the reservoir averages 198 cm annually. Lucchetti Reservoir has been categorized from mesotrophic to eutrophic on the basis of nutrients, physical limnology, chlorophyll *a* concentrations, and phytoplankton biomass data (Churchill et al. 1995). The primary function of the reservoir is water storage for irrigation, but the creation of the Lucchetti Field Station and associated facilities has improved recreational access and increased reservoir popularity among boating anglers. This reservoir was chosen for this study because there is a historical database on largemouth bass population dynamics for this system.

Triploid Production

Two cohorts of triploid largemouth bass were produced in 2000, and data from one cohort through age 1 were reported in Neal and Noble (2008). Methods for production of their two cohorts were similar to those we describe below and were presented in Neal and Noble (2008). Two additional cohorts were produced in 2008 and 2009. Broodstock used to produce experimental fish were collected from Lucchetti Reservoir using boom-mounted boat electrofishing at 7–8 amps and 60 pps DC. Only fish with free-flowing gametes or advanced gonadal development were transported to hatchery facilities for spawning.

Fish with naturally free-flowing gametes were immediately spawned; others were induced to release gametes using hormone injections. Both males and females recieved Ovaprim injections of 0.1 mL kg^{-1} initial and 0.5 mL kg^{-1} resolving dose (8 h post-initial dose). Once gametes were free flowing, individual females were placed in an anesthetic bath of buffered MS-222 solution. A male largemouth bass was euthanized by overdose with MS-222; the testes were removed and macerated, with approximately 10 mL of 0.3% NaCl irrigation solution added to increase volume. Females were stripped into a mixing bowl with no water, and the macerated testes were mixed with water and poured through a fine mesh over the eggs to remove testicular tissue. Fertilization was assumed to be instantaneous. Eggs were stirred for 1 minute with a feather before being divided into diploid and triploid treatments.

Table 1. Stocking history of diploid and triploid largemouth bass in Lucchetti Reservoir, Puerto Rico. Stocking date, numbers stocked, mean total length, standard error, and percent triploid of each treatment group are presented.

Stocking date	Diploid			Triploid			
	n	TL (mm)	SE (mm)	*n*	TL (mm)	SE (mm)	% Trip (*n*)
May 2000	487	46.7	1.30	477	63.3	1.41	100% (23)
Jun 2000	535	54.0	0.48	537	52.3	1.04	82% (50)
Jun 2008	973	66.6	0.29	896	56.9	1.04	100% (100)
May 2009	548	70.7	0.39	532	74.5	1.43	100% (100)
Total	2,543			2,442			
Mean		59.5			61.8		96%

Eggs from the triploid treatment were placed into a mesh basket and the basket was inserted into a water-filled pressure chamber. At 5 min post-fertilization, eggs were subjected to 563 kg cm^{-2} (8,000 PSI) for 1 min (Garrett et al. 1992). Control eggs were not subjected to pressure shock. Eggs from both treatments were placed on incubation mats within aerated 37.8-L hatching aquaria. Ambient water temperatures were about 23° C during spawning and hatching.

Hatching began within 48 h, and swim-up and first feeding followed about 3 days post-hatching. 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 at least 40 mm total length (TL), they were tagged with decimal coded wire tags that differentiated between triploid and diploid fish, and then transported to and released in Lucchetti Reservoir. Four separate stocking events were conducted (Table 1). Ploidy of the two cohorts in 2000 was assayed using flow cytometry with methods previously described (Kerby and Harrell 1990, Neal and Noble 2008). Ploidy of the 2008 and 2009 cohorts was determined using the erythrocyte size technique described in Neal et al. (2004).

Field Comparison of Ploidy Groups

Following each stocking event, largemouth bass were recaptured periodically beginning 1 month post-stocking and continuing for up to 5 yr post-stocking. Fish were collected using the same techniques described previously for broodstock collection. Sampling was conducted in conjunction with other periodic sampling activities, so sampling times and target numbers varied through time.

Field verification of ploidy status of recaptured largemouth bass was performed using measurement of erythrocyte length (Neal et al. 2004). Blood samples were taken from all tagged bass in the field using a large-bore syringe inserted into the blood sinuses located behind the gill arches. A small (<0.5 cm^3) sample of blood was diluted using one drop of 0.5% NaCl solution, and analyzed within 6 hours of collection. All microtagged fish collected were euthanized, placed on ice, and returned to the laboratory for

analysis. Total length, weight with stomach contents removed, and gonad weight (g) were recorded. Blood samples were analyzed using a compound microscope following the methods of Neal et al. (2004).

Periodic sampling of largemouth bass in Lucchetti Reservoir provided estimates of growth rate, condition, and reproductive development. Mean daily growth (MDG) 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. Body condition of individuals ≥150 mm TL was assessed using relative weight (W_r) (Anderson and Neumann 1996). Gonadosomatic index (GSI), which is the gonad weight expressed as a percentage of body weight (excluding stomach contents), was used as an index of reproductive development for fish collected during the spawning season (January–June, Gran 1995). Threshold limits for GSI of 0.2 for males and 2.0 for females were used to identify sexually mature individuals (Gran 1995), with fish exceeding these gender-specific limits considered reproductively mature.

Comparison of mean daily growth rates of diploid and triploid largemouth bass across stockings, age class, and ploidy group was performed using a general linear model approach through age 2 (PROC GLM, SAS Institute 2008). Older ages were not included in statistical analyses due to low sample size, but were included in growth modeling using Von Bertalanffy growth equations fit using Fishery Analysis and Simulation Tools (FAST) Version 3.0 (Slipke and Maceina 2006). Differences in GSI between largemouth bass ploidy groups were assessed by gender using a *t*-test, after testing confirmed that normality assumptions were met. A general linear model (PROC GLM, SAS Institute 2008) was used to compare W_r for diploid and triploid groups at each age, as ontogenetic changes in condition needed to be considered. All statistical tests were considered significant at $P \geq 0.05$.

Results

Diploid ($n = 2{,}543$) and triploid ($n = 2{,}442$) largemouth bass were successfully produced, tagged, and stocked into Lucchetti Reservoir during four separate stocking events from 2000 to 2009 (Table 1). Flow cytometry or erythrocyte length analysis confirmed the ploidy status of sub-samples of both diploid and triploid largemouth bass, with an overall triploid induction success of 96%. Due to natural growth variability in the fingerling production ponds, individual cohorts varied on average by 1.7 to 16.6 mm TL, although the average sizes during all stocking events combined were nearly identical (2.3 mm difference).

A total of 101 diploid and 48 triploid largemouth bass were recaptured over the course of the study (Table 2). All recaptures were individually verified for ploidy and correctly matched their initial stocking treatment in all instances (i.e., no diploid fish from the triploid treatment were recaptured). During the first two stockings, recapture rates were high from age 0 to age 1, but recaptures diminished at the end of age 1, and no further sampling occurred due to project completion. In the two more recent stockings, sampling did not target age-0 fish in hopes that greater recruitment to age 1 might occur. Recapture rates declined sharply from age 1 to age 2, with only 4 diploids and 1 triploid recaptured at ages 3–5.

Table 2. Number of recaptured fish at age presented by stocking date and group. Sampling was not conducted 2002–2008 and age-0 fish were not targeted following the 2008 and 2009 stockings. Three dashes (---) indicate that targeted sampling was not conducted for the corresponding cohort in that year. Zeroes indicate that targeted sampling was conducted for the corresponding cohort in that year, but no fish were collected.

	Recaptures at age (*n*)					
Cohort	**0**	**1**	**2**	**3**	**4**	**5**
May 2000						
Diploid	16	17	---	---	---	---
Triploid	13	15	---	---	---	---
June 2000						
Diploid	4	10	---	---	---	---
Triploid	1	4	---	---	---	---
June 2008						
Diploid	---	31	17	2	0	1
Triploid	---	6	3	0	0	0
May 2009						
Diploid	---	1	1	0	1	0
Triploid	---	5	0	0	1	0
Total						
Diploid	20	59	18	2	1	1
Triploid	14	30	3	0	1	0

Growth was simulated using the von Bertalanffy growth model fit to treatment groups, and parameter estimates were similar between groups (Figure 1; diploid: $L_\infty = 384.5$ mm, $K = 1.244$, $t_0 = -0.237$; triploid: $L_\infty = 387.0$ mm, $K = 1.231$, $t_0 = -0.31$). Mean daily growth rates ($\pm SE$) for age 0 fish were 0.85 ± 0.06 mm day^{-1} for diploids and 0.86 ± 0.09 mm day^{-1} for triploids, and declined for older fish in both ploidy groups. Mean daily growth was similar between diploid and triploid largemouth bass overall ($F = 1.20$, $P = 0.275$) and by age class through age 2 ($F = 0.19$; $P \geq 0.662$). Statistical comparisons beyond age 2 were not possible due to low sample sizes.

Overall condition differed substantially between diploid and triploid largemouth bass (both sexes combined, $F = 7.33$, $P = 0.008$), with diploid largemouth bass having overall greater condition estimates. Relatively low sample sizes, particularly for larger size classes, precluded more refined statistical analysis by size and gender. However, mean relative weight was greater for diploids in all size-gender comparisons (Table 3).

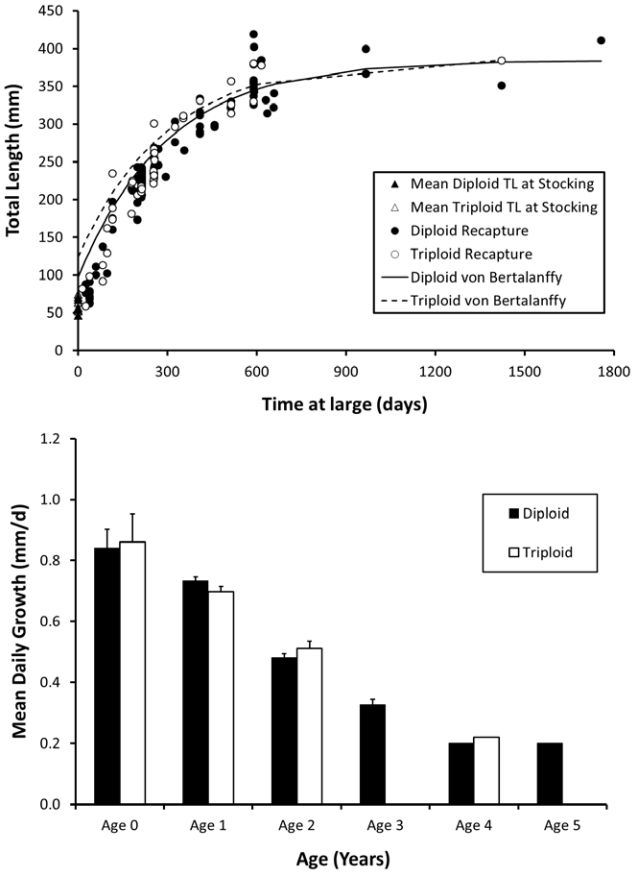

Figure 1. Top: Growth in total length of diploid and triploid largemouth bass stocked in Lucchetti Reservoir. Overall mean length at stocking for each group is displayed. Bottom: Mean daily growth rate by age for diploid and triploid largemouth bass calculated by dividing change in total length by total time at large. Error bars are one *SE*.

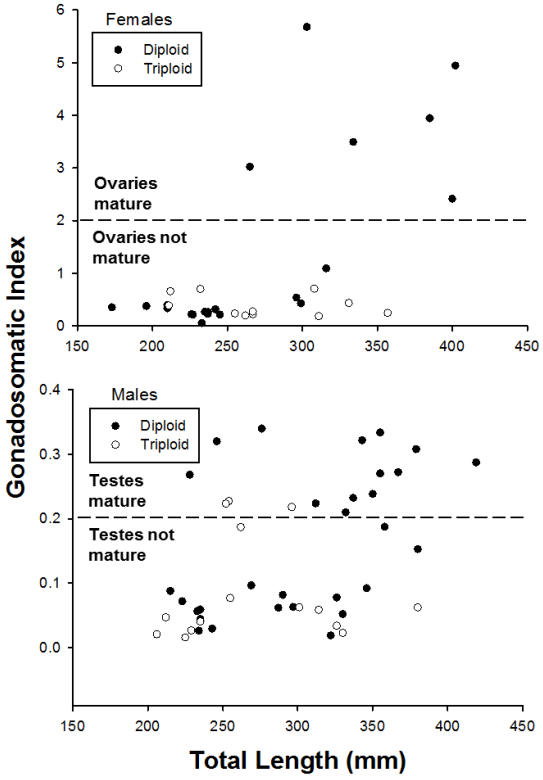

Figure 2. Reproductive development of diploid and triploid largemouth bass females (top) and males (bottom) as determined using the gonadosomatic index (GSI). Values of GSI exceeding 2.0 for females and 0.2 for males are considered mature (Gran 1995). Only fish collected during the spawning season (January–June) are presented.

Table 3. Comparison of gonadosomatic index (GSI) and relative weight (W_r) between diploid and triploid largemouth bass by size class and gender.

Size range and gender	Treatment group	GSI (%)			W_r		
		n	Mean	SE	*n*	Mean	SE
150–249							
Male	Diploid	13	0.127	0.053	22	89.7	1.6
	Triploid	6	0.032	0.005	12	83.9	1.9
Female	Diploid	17	0.271	0.027	27	89.7	2.1
	Triploid	9	0.312	0.091	10	80.5	1.7
250–349							
Male	Diploid	9	0.144	0.03	12	95.3	2.7
	Triploid	9	0.123	0.029	12	91.7	3.6
Female	Diploid	7	2.375	0.844	9	99.5	3.2
	Triploid	8	0.494	0.126	10	90.6	3.2
350+							
Male	Diploid	8	0.256	0.021	10	90.1	1.8
	Triploid	2	0.063	0.005	2	87.3	0.9
Female	Diploid	4	3.767	0.007	4	97.1	4.3
	Triploid	2	0.246	0.008	2	94.2	0.8

Both ploidy groups reached approximate size at maturity (275 mm TL) during late-winter to early-spring (age 1), approximately 9 mo following stocking. Elevated gonadosomatic index (GSI) values indicative of maturity were observed in diploid fish beginning in March at lengths as small as 228 mm TL for males and 265 mm TL for females. Female diploids had significantly greater GSI values than female triploids at age 1 ($t = 2.52$, df = 21, $P = 0.010$; Table 3; Figure 2), and no triploid females displayed maturing ovaries. Similar differences were observed between males of each ploidy group, with diploid males having greater GSI values ($t = 2.76$, df = 43, $P = 0.004$). Although several triploid males displayed GSI values marginally indicative of maturation, the greatest GSI values for females and males were consistently observed in diploid individuals.

Discussion

The expectation that sterile triploid largemouth bass would experience growth advantages due to lack of reproductive investment was not supported in this study. No differences in growth rate or maximum size were observed, despite reduced energy allocation

to gonadal development by triploid largemouth bass. Reduced reproductive investment and increased growth rates have been demonstrated for some fish species, but the results are conflicting. For example, triploid and diploid channel catfish (*Ictalurus punctatus*) reared indoors differed in both GSI and weight by 8 mo of age, with triploids being significantly less sexually developed and heavier in weight (Wolters et al. 1982). Yet, when diploid and triploid channel catfish were raised outdoors at high densities, no differences in weight were detected, though significantly greater GSI values were observed for diploids (Wolters et al. 1991). Similarly, Parsons and Meals (1997) reported reduced gonadal development of triploid hybrid crappie (*Pomoxis annularis* × *P. nigromaculatus*) compared to diploid white crappie (*P. annularis*), but could not detect a consistent growth advantage of triploid fish.

Diploid largemouth bass underwent greater reproductive development than triploid largemouth bass. Male triploids demonstrated some reproductive maturation, but GSI values were reduced when compared to fully-developed diploid largemouth bass. Female largemouth bass triploids showed no apparent increase in ovarian size following maturity, while similar-sized diploids often contained well-developed ovaries. These results were generally consistent with those for other fish species in which triploids did not undergo sexual maturation (e.g., Parsons 1993, Simon et al. 1993). Therefore, these data suggested that triploid largemouth bass did not invest significant energy into reproductive development. This was supported by consistently greater relative weights in diploid fish as compared to triploid fish.

Survival also appeared to have been reduced for triploid largemouth bass. Although difficulty in producing triploid largemouth bass limited initial stocking densities, which reduced long term recapture probabilities, this alone does not explain differences between treatment groups. Despite similar stocking rates, less than half as many triploids as diploids were recaptured. It is likely that immediate post-stocking survival influenced recapture rates. In each stocking event, the two ploidy groups were transported and stocked separately. For the first two cohorts (both in 2000), ploidy treatments were obtained from different facilities (triploids were produced in earthen ponds in the warmer Lajas Valley; diploids were produced in lined ponds in the much cooler Maricao area). The last two stockings used treatment fish from the Maricao Fish Hatchery, but diploids and triploids were hauled and stocked separately.

Neal and Noble (2008) proposed that, although no differences were observed between ploidy groups through age 1, these differences might become apparent for older fish with greater reproductive opportunity. This study did not support that contention, as growth rates converged to a nearly identical maximum size for both ploidy groups. These data implied that triploid largemouth bass do not have utility for trophy management in tropical reservoirs because enhanced growth was not realized, production of triploids was difficult, and the reduced longevity reported for normal diploid fish in Puerto Rico affects triploid largemouth bass as well.

Triploid largemouth bass may have greater potential in temperate systems, where longer lifespans provide more time for differences to be realized. However, this is unlikely because temperate largemouth bass generally spawn once or a few times during the spring (Heidinger 1975), and must contend with water temperature extremes that reduce growth during winter and summer (e.g., Rice et al. 1983). Conversely, largemouth bass in Puerto Rico spawn multiple times for up to 6 mo (Gran 1995), and water temperatures do not approach winter or summer extremes that are common in temperate habitats (Neal and Noble 2006). Therefore, the theoretical energetic advantage of foregoing reproduction should be greater in tropical systems.

Conversely, the moderated water temperature in Puerto Rico could plausibly lead to the slow growth and accelerated mortality of largemouth bass in the tropics. The preferred water temperature for maximum prey consumption by largemouth bass is 27.5°C (Rice et al. 1993); however, preferred water temperature tends to decline with fish size and associated declines in metabolic rate (e.g., Clark and Johnston 1999). Thus, as largemouth bass in Puerto Rico grow, they require cooler temperatures, and are genetically driven to consuming proportionally less prey by volume. However, tropical fish in general tend to operate at higher maintenance feeding levels than temperate species in accordance with higher year-round environmental temperatures (Brett and Groves 1979). This paradox may lead to accelerated mortality in largemouth bass, which are a temperate species that is not genetically designed for a tropical existence.

Although this study concluded that triploid largemouth bass do not have efficacy for trophy management in tropical systems, they may be useful for creating non-reproductive populations where reproduction is not desired, such as trophy bass management in ponds and small impoundments (Neal and Willis 2012). Another application, although more remote, could be the creation of largemouth bass fisheries in areas where establishment of this species could otherwise threaten sensitive native species. However, ploidy verification of all fish stocked will be required to ensure that no reproductive fish are introduced, and this added cost may limit application of this management tool.

Acknowledgments

The author would like to express gratitude to the personnel of the Puerto Rico Department of Natural and Environmental

Resources, Division of Marine Resources and Division of Reserves and Refuges. None of this research could have been completed without the aid of D. Lopez-Clayton, C. Lilyestrom, and their staffs. The author thanks many North Carolina State University employees including R. Noble, G. Rodriguez-Ferrer, F. Del Toro, D. Neal, and N. Bacheler, and many Mississippi State University employees, including C. Mace, M. Muñoz-Hincapié, and several students and technicians. M. McGee of Caribe Fisheries, Inc., provided hatchery facilities and technical expertise. Funding was provided through Federal Aid in Sport Fish Restoration under Puerto Rico DNER Projects F-41-2 and F-53R, and through North Carolina Agricultural Research Service Project 06270.

Literature Cited

Allen, S. K., Jr., and J. G. Stanley. 1978. Reproductive sterility in polyploid brook trout, *Salvelinus fontinalis*. Transactions of the American Fisheries Society 107:473–478.

Anderson, R. O. and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447–482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Brett, J. R. and T. D. D. Groves. 1979. Physiological energetics. Pages 279–352 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, Volume VIII. Academic Press, New York.

Churchill, T. N., R. L. Noble, J. E. Gran, and A. R. Alicea. 1995. Largemouth bass recruitment in Lucchetti Reservoir. Puerto Rico Department of Natural and Environmental Resources, Federal Aid in Sport Fish Restoration, Final Report, Project F-16, Study 2. San Juan.

Clark, A. and N. M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology 68:893–905.

Garrett, G. P., M. C. F. Birkner, and J. R. Gold. 1992. Triploidy induction in largemouth bass, *Micropterus salmoides*. Journal of Applied Aquaculture 1:27–34.

Gervai, J., S. Peter, A. Nagy, L. Horvath, and V. Csanyi. 1980. Induced triploidy in carp, *Cyprinus carpio*. Journal of Fish Biology 17:667–671.

Gran, J. E. 1995. Gonad development and spawning of largemouth bass in a tropical reservoir. Master's Thesis, North Carolina State University, Raleigh.

Heidinger, R. C. 1975. Life history and biology of the largemouth bass. Pages 11–20 *in* R. Stroud and H. Clepper, editors. Black Bass Biology and Management, Sport Fishing Institute, Washington D.C.

Ihssen, P. E., L. R. McKay, I. McMillan, and R. B. Phillips. 1990. Ploidy manipulation and gynogenesis in fishes: cytogenetic and fisheries applications. Transactions of the American Fisheries Society 119:698–717.

Kerby, J. H. and R. M. Harrell. 1990. Hybridization, genetic manipulation, and gene pool conservation of striped bass. Pages 159–190 *in* R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. American Fisheries Society, Southern Division, Striped Bass Committee, Bethesda, Maryland.

Lilyestrom, C. G., P. Quinones, and G. Oliveras. 1994. Peacock and largemouth bass competition in La Plata Reservoir. Puerto Rico Department of Natural and Environmental Resources, Federal Aid in Sport Fish Restoration, Final Report, Project F-30, San Juan.

Neal, J. W., D. M. Neal, R. L. Noble, and M. V. McGee. 2004. Artificial propagation and induction of triploidy in largemouth bass, and ploidy discrimination using erythrocyte length. Journal of the World Aquaculture Society 35(1):46–54.

_____ and R. L. Noble. 2002. Growth, survival, and movement of Florida and intergrade largemouth bass in a tropical reservoir. North American Journal of Fisheries Management 22:528–536.

_____ and _____. 2006. A bioenergetics-based approach to explain largemouth bass size in tropical reservoirs. Transactions of the American Fisheries Society 135:1535–1545.

_____ and _____. 2008. Comparison of diploid and triploid largemouth bass growth and maturation through age 1 in Puerto Rico. North American Journal of Fisheries Management 28:688–693.

_____ and D. W. Willis, editors. 2012. Small impoundment management in North America. American Fisheries Society, Bethesda, Maryland.

Parsons, G. R. 1993. Comparison of triploid and diploid white crappies. Transactions of the American Fisheries Society 122:237–243.

_____ and K. Meals. 1997. Comparison of triploid hybrid crappie and diploid white crappie in experimental ponds. North American Journal of Fisheries Management 17:803–806.

Rice, J. A., J. E. Breck, S. M. Bartell, and J. F. Kitchell. 1983. Evaluating the constraints of temperature, activity and consumption on growth of largemouth bass. Environmental Biology of Fishes 9:263–275.

_____, L. B. Crowder, and K. A. Rose. 1993. Interactions between size-structured predator and prey populations—experimental test and model comparison. Transactions of the American Fisheries Society 122:481–491.

SAS Institute Inc. 2008. SAS/STAT 9.2 user's guide, second edition. SAS Institute, Cary, North Carolina.

Simon, D. C., C. G. Scalet, and J. C. Dillon. 1993. Field performance of triploid and diploid rainbow trout in South Dakota ponds. North American Journal of Fisheries Management 13:134–140.

Slipke, J. W. and M. J. Maceina. 2006. Fishery analyses and simulation tools (FAST 3.0). Auburn University, Auburn, Alabama.

Stanley, J. G. 1979. Control of sex in fishes with special reference to the grass carp. Pages 201–242 *in* J. V. Shireman, editor. Proceedings of the grass carp conference. Institute of Food and Agriculture Science, University of Florida, Gainesville.

Thorgaard, G. H. 1983. Chromosome set manipulation and sex control in fish. Pages 405–434 *in* W. S. Hoar, D. J. Randall, and E. M. Donaldson, editors. Fish physiology, volume 9, part B. Academic Press, New York.

_____ and S. K. Allen. 1987. Chromosome manipulation and markers in fisheries management. Pages 319–331 *in* N. Ryman and F. Utter, editors. Population genetics and fisheries management. University of Washington Press, Seattle.

_____, M. E. Jazwin, and A. R. Stier. 1981. Polyploidy induced by heat shock in rainbow trout. Transaction of the American Fisheries Society 110:546–550.

Waters, D. S. 1999. Spawning season and mortality of adult largemouth bass (*Micropterus salmoides*) in a tropical reservoir. Master's Thesis, North Carolina State University, Raleigh.

Wolters, W. R., G. S. Libey, and C. L. Chrisman. 1982. Effect of triploidy on growth and gonad development of channel catfish. Transactions of the American Fisheries Society 111:102–105.

_____, C. G. Lilyestrom, and R. J. Craig. 1991. Growth, yield, and dress-out percentage of diploid and triploid channel catfish in earthen ponds. The Progressive Fish-Culturist 53:33–36.