Journal of the Arkansas Academy of Science

Volume 55 Article 12

2001

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Abstract

The Florida subspecies of the largemouth bass (LMB) has often been introduced into waters outside of its range, with escape of individuals into associated waterways common. Sustaining pure lines within controlled hatchery settings is also difficult. The present study investigated LMB populations by way of allozyme analysis of three diagnostic loci of 115 LMB in three public and seven private aquaculture ponds within Arkansas. The goal was to determine the success of hatcheries in maintaining pure subspecies. None of the pond populations studied were fixed for all alleles. Private ponds had northern LMB allele frequencies of up to 0.40 in putative Florida LMB ponds. State fish hatcheries had higher proportions of predicted alleles. Most bass surveyed were intergrades (63%).

Introduction

The Florida largemouth bass (*Micropterus salmoides floridanus*; FLMB) is one of two subspecies of largemouth bass and was considered to possess superior growth characteristics to the northern largemouth bass (*M. s. salmoides*, NLMB) {Addison and Spencer, 1971}. Its range has been greatly enhanced due to stocking, including northern U.S. waters. However, several studies have suggested countergradient variation for growth rates versus latitude (Inman et al., 1976; Cichra et al., 1980; Fields et al., 1987; Philipp and Whitt, 1991), prompting fisheries managers to predominantly stock FLMB in southern waters. As a result of these stockings, introgression of FLMB with native stocks of northern largemouth bass (*M. s. salmoides*; NLMB) has been prevalent (Philipp et al., 1983). The Arkansas Game and Fish Commission has been stocking FLMB in reservoirs since the mid-1970's (D. Brader, Manager, Andrew Hulsey State Fish Hatchery, pers. comm.). Consequently, many Arkansas reservoir bass populations contain FLMB alleles (Philipp et al., 1983; Dunham et al., 1993; Fulton, 1998). Most of these reservoirs are no longer stocked with FLMB (D. Brader, pers. comm.).

Maintenance of pure stock of largemouth bass (LMB) in controlled hatchery settings has proven difficult. Intraspecific contamination of broodstock in state hatcheries has been a common phenomenon (Philipp et al., 1983; Gilliland and Whittaker, 1989). Indeed, 70% of the FLMB at the Andrew Hulsey Fish Hatchery (Hot Springs, Arkansas) in 1988 were intergrades (D. Brader, pers. comm.). These findings resulted in ongoing genetic testing within the Hulsey State Fish Hatchery FLMB broodstock and the separation of subspecies between hatcheries. LMB stocked from other Arkansas state fish hatcheries were expectedly NLMB; however the genetic identity of the broodstock has never been verified (B. Beavers, Manager, Joe Hogan State Fish Hatchery, pers. comm.).

The objective of the present study was to evaluate the genetic purity of putative fixed LMB populations of public and commercial farm ponds in Arkansas using three diagnostic loci. Levels of introgression were determined from allelic combinations.

Study Sites.--LMB were obtained from three of the four state fish hatcheries maintained by the Arkansas Game and Fish Commission: the William H. Donham State Fish Hatchery (Corning, AR); Joe Hogan State Fish Hatchery (Lonoke, AR); and Andrew Hulsey State Fish Hatchery (Hot Springs, AR). The Hulsey Fish Hatchery is the only public hatchery in the state rearing FLMB, whereas the other two hatcheries rear and provided NLMB. The Hulsey Fish Hatchery stocked almost 500,000 fingerling FLMB into seven southern Arkansas reservoirs during the past year (B. Beavers, pers. comm.). The Hogan Fish Hatchery stocked almost 250,000 NLMB fingerlings into 24 Arkansas reservoirs, as compared to less that 30,000 NLMB fingerlings stocked into seven Arkansas reservoirs from the Donham Fish Hatchery. The fourth state fish hatchery (Centerton State Fish Hatchery) is located in northwest Arkansas (Centerton, AR). This hatchery stocked less than 30,000 NLMB fingerlings into three reservoirs, and was not included in the present study. Additionally, a commercial fish farm in east-central Arkansas provided LMB from seven ponds. Six of the seven ponds contained putative FLMB, and the seventh contained putative NLMB.

Methods

All specimens (n = 115) from the fish hatcheries were shipped live to Arkansas State University where they were stored at −70°C. Specimens ranged from approximately 3-31 cm TL. Liver and muscle homogenates were electrophoresed on cellulose acetate plates (Helena Laboratories). The plates were stained for allozymes using the recipes of Hebert and Beaton (1989). Loci used to distinguish the LMB phenotypes (Gilliland, 1992) were isocitrate dehydrogenase (sIDHP-B, Enzyme Number 1.1.1.42) in Tris-Glycine (TG) buffer, aspartate aminotransferase (sAAT-B, 2.6.1.1) in TG buffer, and malate dehydrogenase (sMDH-B, 1.1.1.37) in Tris-Citrate buffer. Alleles are differentially fixed for the loci sIDHP-B and sAAT-B for both FLMB and NLMB. The two alleles of *sAAT-B**, which occur solely in FLMB, *sAAT-B*3* and *sAAT-B*4*, were combined, as were the two alleles solely occurring in NLMB, *sAAT-B*1* and *sAAT-B*2*, to clarify reporting within the goal of the study. Two alleles for *sMDH-B* (**1* and **2*) occur in the NLMB, one of which (*sMDH-B*2*) is fixed for the FLMB.

Allele frequencies were determined for each locus. Individual LMB were designated as FLMB or NLMB if they were homozygous for all three loci specific for that subspecies, F_1 intergrades if they were heterozygous for each locus, and F_x if they had other allelic combinations. It should be noted that these designations are for communication and labeling purposes, since bass fixed for each of the three loci may be an intergrade (Johnson and Fulton, 1999).

Results

Ten (10) specimens from each pond, but Hulsey Hatchery (n = 25), were screened for products of the three diagnostic loci. None of the public or private LMB populations sampled were fixed for all alleles (Table 1). Allele frequencies from the seven private pond populations differed greatly from what would be expected of pure strains of LMB. The six ponds containing putative FLMB had NLMB allele frequencies of up to 0.65, with ranges typically from 0.30 to 0.40. Alleles of the sMDH-B locus were closest to the expected, although a confounding factor is that the NLMB also can possess the *sMDH*2* allele. The LMB of the Hulsey State Fish Hatchery were closest to being fixed for all alleles, with single individuals being heterozygous for the sIDH-B and sMDH-B loci. Allele frequencies of the bass for the Donham and Hogan State Fish Hatcheries were more variable than for those bass of Hulsey Hatchery, but less so than that found in the private pond populations.

Phenotypic results showed that most populations had a large number of F_x individuals. This was particularly true for the commercial pond populations which were predominated by F_x individuals (Table 2). Very few individuals were fixed for all alleles of these three diagnostic loci (x 16%; range of 0-40%). The private ponds 1-6 were putative FLMB, but fixation only ranged from 0-40%, and pond 7, putative NLMB, had 10% of individuals fixed for NLMB alleles. Among state fish hatcheries, the Donham and Hulsey Hatcheries had less than 50% of bass having a phenotype of F_x, whereas Hogan State Fish Hatchery bass had an F_x phenotype frequency of 90%. The Donham and

Table 1. Allele frequencies of sAAT-B, sIDH-B, s-MDH-B for largemouth bass populations found in seven commercial ponds, William H. Donham, Joe Hogan, and Andrew Hulsey State Fish Hatcheries.

Locus / Allele	Site									
	Commercial Pond							State Fish Hatchery		
	1	2	3	4	5	6	7*	Donham*	Hogan*	Hulsey
sAAT-B										
sAAT-B*1&2	0.25	0.40	0.40	0.30	0.40	0.10	0.75	0.80	0.85	0.00
sAAT-B*3&4	0.75	0.60	0.60	0.70	0.60	0.91	0.25	0.20	0.15	1.00
sIDH-B										
sIDH-B*1	0.35	0.50	0.35	0.30	0.65	0.15	0.85	1.00	0.90	0.02
sIDH-B*3	0.65	0.50	0.65	0.70	0.35	0.85	0.15	0.00	0.10	0.98
sMDH-B										
sMDH-B*1	0.00	0.15	0.20	0.00	0.00	0.08	0.75	0.90	0.75	0.02
sMDH-B*2	1.00	0.85	0.80	1.00	1.00	0.92	0.25	0.10	0.25	0.98

*Putative NLMB populations

Table 2. Phenotypic frequencies for FLMB, NLMB, F_1 and F_x individuals at seven private hatchery ponds, Donham, Hogan, and Hulsey State Fish Hatcheries.

Location	Phenotype			
	NLMB	F_1	F_x	FLMB
Pond 1	0 (0%)	0 (0%)	7 (70%)	3 (30%)
Pond 2	0 (0%)	1 (10%)	9 (90%)	0 (0%)
Pond 3	0 (0%)	1 (10%)	7 (70%)	2 (20%)
Pond 4	0 (0%)	0 (0%)	9 (90%)	1 (10%)
Pond 5	0 (0%)	0 (0%)	10 (100%)	0 (0%)
Pond 6	0 (0%)	0 (0%)	6 (60%)	4 (40%)
Pond 7	1 (10%)	0 (0%)	9 (90%)	0 (0%)
Donham	7 (70%)	0 (0%)	3 (30%)	0 (0%)
Hogan	1 (10%)	0 (0%)	9 (90%)	0 (0%)
Hulsey	0 (0%)	0 (0%)	2 (8%)	23 (92%)

Hogan State Fish Hatcheries were putative NLMB. The Donham Hatchery bass did possess a 70% fixed NLMB population, but the Hogan Hatchery possessed only a single individual designated as NLMB. Conversely, the bass originating from the Hulsey State Fish Hatchery were mostly fixed for FLMB alleles (92%).

Discussion

It was not the intent of this study to provide an in-depth assessment of the allelic and phenotypic frequencies of these bass populations. Rather, the goal of this study was to determine if genetic contamination of breeding stocks had occurred. Despite periodic genetic screening, the Hulsey Hatchery bass possessed NLMB alleles. The two state fish hatcheries without a genetic screening program for bass (Hogan and Donham) had greater allelic contamination. Florida bass alleles were common in both hatchery populations, resulting in a high degree of introgression. FLMB from Hulsey Hatchery are stocked in selected southern Arkansas reservoirs, whereas bass from Hogan and Donham State Fish Hatcheries are shipped throughout the state. Bass from the private fish farm are represented and sold commercially as FLMB. Introgression was particularly high within these populations (84%). These bass can legally be stocked in private ponds throughout the state. Although illegal, bass are often transported to other waterways by anglers (S. Barkley, Arkansas Game and Fish Commission, pers. comm.). Thus, alleles from these sources can potentially enter public water systems.

There is great concern about the long-term effects of d uting native coadapted gene complexes of NLMB with g nes from Florida stocks, and about the ability of FLMB a d intergrades between FLMB and NLMB to survive and t ive in northern waters (Philipp, 1992). Many studies have f used on differential growth parameters and thermal tol- e nce of the two subspecies and their intergrades (e.g., I nan et al., 1976; Cichra et al., 1980; Fields et al., 1987). A though differential growth and survival have been identi- f d for NLMB and FLMB when they co-exist in temperate w ters (Fields et al., 1987; Philipp and Whitt, 1991), no sig- n ficant differences were identified in a northern Arkansas b ss population containing alleles of both subspecies (Johnson and Fulton, 1999). Nonetheless, once exogenous alleles enter a system they may have long term effects (Philipp, 1992).

The cause for concern may be lessened for a non-native species introduced into an artificial system such as a reservoir. However, of perhaps greater concern is the potential effect of escaped individuals into surrounding lotic waters. Annual stockings of up to 750,000 LMB annually within 41 reservoirs (B. Beavers, pers. comm.) provides great opportunity for the spread of exogenous alleles. Water levels of many of these reservoirs are maintained by pumping water out of and in to adjacent streams, which can lead to genetic contamination of native riverine stocks. Exogenous alleles have been detected in native LMB populations of Oklahoma (Gelwick et al., 1995) and Alabama (Dunham et al., 1992). This potential of escape behooves fisheries managers to develop not only long-term management plans but also containment strategies for the stocking of FLMB and other introduced species.

ACKNOWLEDGMENTS.—This research was partially funded by the Arkansas Game and Fish Commission. We are thankful to the fisheries managers of the Arkansas Game and Fish Commission state fish hatcheries, D. Caldwell, B. Beavers, and D. Brader, for providing fish for analysis. Our thanks are also extended to the anonymous donor of the commercial fish farm bass. Helpful insight was provided by M. Huss and other reviewers, to whom we are most grateful.

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