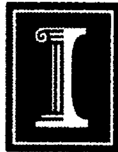


ILLINOIS

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

PRODUCTION NOTE

University of Illinois at
Urbana-Champaign Library
Large-scale Digitization Project, 2007.

597.73
P94
CAE
1984(7)

Natural History Survey
Library

THE PRODUCTION AND EVALUATION OF DIFFERENT GENETIC STOCKS OF LARGEMOUTH BASS, *MICROPTERUS SALMOIDES*, FOR DIFFERENT THERMAL ENVIRONMENTS

FINAL REPORT

Federal Aid Project F-35-R
U.S. Fish and Wildlife Service

1 April 1980 through 30 September 1983

Principal Investigator: Dr. David P. Philipp

Co-investigator: Dr. Gregory S. Whitt

Submitted to:

Illinois Department of Conservation

Submitted by:

Aquatic Biology Section
Illinois Natural History Survey

March 22, 1984

TABLE OF CONTENTS

		Pages
Chapter 1:	An Evaluation of Meristic Characters to Identify Populations of Northern, Florida, and F_1 Hybrid Largemouth Bass	
Authors:	Jeffrey L. Van Orman, Gregory S. Whitt and David P. Philipp	1-1 to 1-34
Chapter 2:	Survival and Growth of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass in Central Illinois	
Authors:	David P. Philipp, Christine Kaminski and Gregory S. Whitt	2-1 to 2-21
Chapter 3:	Reproductive Success of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass in Central Illinois	
Authors:	David P. Philipp, Jeffrey B. Koppelman, Christine Kaminski and Gregory S. Whitt . .	3-1 to 3-16
Chapter 4:	A Comparison of the Embryonic Development of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass in Different Thermal Environments	
Authors:	David P. Philipp, Christine Kaminski and Gregory S. Whitt	4-1 to 4-28
Chapter 5:	Biochemical Genetic Evaluation of Patterns of Gene Expression During the Embryonic Development of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass	
Authors:	Henry R. Parker, Gregory S. Whitt and David P. Philipp	5-1 to 5-38
Chapter 6:	Levels of Transcription and Translation During the Embryonic Development of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass	
Authors:	David P. Philipp, Henry R. Parker and Gregory S. Whitt	6-1 to 6-13

Chapter 7: Thermal Effects on the Metabolic Biochemistry of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass

Authors: David P. Philipp, Christine Kaminski, Henry R. Parker, Manijeh Pasdar and Gregory S. Whitt . . . . 7-1 to 7-39

Chapter 8: Thermal Preferenda of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass

Authors: Jeffrey B. Koppelman, Gregory S. Whitt and David P. Philipp . . . . 8-1 to 8-30

Chapter 9: Acute and Chronic Thermal Maxima of Northern, Florida, and Reciprocal F_1 and F_2 Hybrid Largemouth Bass

Authors: Robert Fields, Shirley S. Lowe, Christine Kaminski, Gregory S. Whitt and David P. Philipp . . . . 9-1 to 9-21

Chapter 10: Biochemical Genetic Analysis of Largemouth Bass Populations in Illinois

Authors: Jeffrey B. Koppelman, Christine Kaminski, Gregory S. Whitt and David P. Philipp . . . . 10-1 to 10-22

General Recommendations

STUDY 101
JOB 1

CHAPTER 1

An Evaluation of Meristic Characters to Identify Populations of Northern, Florida, and F_1 Hybrid Largemouth Bass

Jeffrey L. Van Orman[1,2], Gregory S. Whitt[3,1] and David P. Philipp[1,2]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Meristic and morphometric characters were first used by Bailey and Hubbs (1949) as a plausible method for separating the two subspecies of largemouth bass, the northern largemouth bass (NLMB), *Micropterus salmoides salmoides*, and the Florida largemouth bass (FLMB), *M. s. floridanus*. Previously established meristic counts used in this study as a potential means of subspecific separation consisted of the number of lateral line scales, scales above the lateral line, scales below the lateral line, caudal peduncle scales, pyloric cacea, and caecal tips. Characters observed in FLMB broodstock (Lake Dora, Florida) possessed higher meristic counts than NLMB (Bone Lake, Wisconsin), confirming previous reports. In addition, using these broodstocks, four genetically confirmed F_1 stocks, NLMB, FLMB, and reciprocal hybrids, NxF and FxN, were produced in ponds in Champaign, Illinois to evaluate the efficacy of these meristic characters in subspecies identification. No significant differences were observed between Illinois F_1 NLMB and Wisconsin NLMB broodstock, whereas Illinois F_1 FLMB differed significantly from Florida FLMB broodstock in several meristic counts, presumably as a result of latitudinal (temperature) differences. Identification of F_1 NLMB and FLMB using a combination of meristic characters in either a modified index or a discriminant function analysis revealed these methods were more discriminating for subspecies separation than any single character except pyloric caecal tips. Reciprocal F_1 hybrids were not significantly different from each other and were pooled for analysis. Meristic values of the pooled F_1 hybrid stock were intermediate between F_1 NLMB and FLMB in three meristic counts and abnormally

high in the remaining three meristic counts, paralleling or surpassing values of the FLMB. Production of F_2 NxF and F_2 FxN hybrids demonstrated an expansion of the range of meristic values over that observed for the two pure subspecies. Individual meristic characters measured in F_2 stocks also expressed means independent of one another, some high, some low, and some intermediate compared to the parental F_1 hybrids. The presence of F_1 and F_2 hybrids in intergrade populations contribute meristic counts which overlap both subspecies to the point that one cannot expect to classify individuals to either subspecific or hybrid categories with any reasonable accuracy on the basis of meristic counts.

INTRODUCTION

The currently accepted taxonomic classification of the species within the genus *Micropterus* has been summarized by McCrimmon and Robbins (1975). In largemouth bass, *Micropterus salmoides*, two subspecies are recognized. Bailey and Hubbs (1949) used morphometric and meristic techniques to distinguish the northern subspecies, *M. s. salmoides*, from the Florida subspecies, *M. s. floridanus*. These authors described the range of the pure northern subspecies as north and west of the Chocktawahatchee River and Apalachicola River drainages in Florida, Alabama, and Georgia, and north and east of the Savannah River drainage in South Carolina. They described the range of the pure Florida subspecies as peninsular Florida to the south and east of the Suwannee River drainage, including the St. John's River system. The areas between these two regions were stated to contain intergrade populations in which both subspecies existed and freely interbred. These original range designations have not been reevaluated for over 30 years, despite the numerous subsequent relocations of largemouth bass.

Fisheries researchers have relied on this possibly outdated range description and have routinely used a combination of meristic counts to separate populations of the two subspecies. Meristic indices have been devised based upon scale counts (Bailey and Hubbs 1949), numbers of pyloric caeca (Buchanan 1973), numbers of rib-bearing vertebrae (Bryan 1969), and a combination of these techniques (Thrasher 1974). These procedures have been assumed to be valid by fisheries workers in the absence of direct verification and even in the presence of contradictory data (Smith and Crumpton 1977).

The use of biochemical genetic characters eliminates the possible confusion caused by variable penetrance and expressivity associated with certain anatomical traits. The genetic structure of populations has often been studied using electrophoretic techniques (Lewontin 1974, Avise and Smith 1977) with good methodological (Nei 1972, 1978, Rogers 1972) and statistical support (Fitch and Margoliash 1967, Farris 1972, Sneath and Sokal 1973). Genetic differences among homologous proteins are not always associated with genetic differences in gross morphology (Turner 1974, King and Wilson 1975, Mickevich and Johnson 1976, Schnell et al. 1978). Electrophoretic analysis of one or a few mutational differences among proteins provides a better estimate of the "time" populations have been separated than do genetic differences in morphology which may result from the interactions of several or many genes (Langley and Fitch 1974, Wilson et al. 1977). Discrepancies between enzymatic and morphological variance reflects, in part, differences in mutations of "structural" versus "regulatory" genes (Wilson et al. 1977).

Philipp et al. (1981, 1983) reported the results of an electrophoretic survey of 28 enzyme loci among 90 populations of largemouth bass from areas throughout the range of this species. Allele frequencies at each locus, as well as the mean number of alleles at each locus, the average number of polymorphic loci and the mean level of heterozygosity were calculated for each population. Matrices of genetic identity and distance were used to assess interpopulational relationships. These analyses revealed substantial genetic differences among populations in the United States. The northern subspecies *M. s. salmoides* and the Florida subspecies *M. s. floridanus* were shown to have fixed allelic differences at two loci, isocitrate dehydrogenase-B and aspartate aminotransferase-B. The allele frequencies at these loci can then be used to

determine contributions of each subspecies to the gene pool of any population. In this manner, the intergrade zone was redescribed by these authors as consisting of northern Florida, Mississippi, Alabama, Georgia, South Carolina, North Carolina, Virginia, and Maryland, as well as Texas, California, and perhaps a few other states in which largemouth bass with at least some of the genes of the Florida subspecies have been purposely introduced. This newly described intergrade zone is larger than previously proposed, and casts considerable question on the validity of the morphological methods previously used.

This study used genetically confirmed pure stocks of _M. s. salmoides_ and _M. s. floridanus_ to produce four stocks of largemouth bass, pure northern largemouth bass (NLMB), pure Florida largemouth bass (FLMB), and both reciprocal F_1 hybrids, northern♀ x Florida♂ (NxF) and Florida♀ x northern♂ (FxN). These stocks were all produced in similar environments in central Illinois and used to assess the accuracy of meristic measurements for the purpose of identifying these various stocks. Finally, the amount of meristic variation among F_2 generation offspring resulting both from the reproduction of NxF and of FxN F_1 hybrid largemouth bass was also assessed. In this way the relative usefulness of meristic and electrophoretic techniques to determine the subspecific status of largemouth bass populations was assessed.

Production of Genetic Stocks:

Pure northern largemouth bass (NLMB), *M. s. salmoides*, were collected from Bone Lake, Wisconsin during October, 1978. Right pectoral fin clips were removed from each adult prior to stocking and utilized for electrophoretic analyses of each individual (Philipp et al. 1979, 1983). All individuals used contained only the Mdh-B^1, Idh-B^1, Sod-A^2 and Aat-B^1 or B^2 alleles, and represented the pure northern subspecies. These individuals were held outdoors in 0.08 hectare ponds until the onset of the project in April 1980. Pure Florida largemouth bass (FLMB), *M. s. floridanus*, were collected from Lake Dora, Florida during January, 1980 and again during February, 1981. These fish were air shipped to Champaign and held indoors at 8-12°C. Left pectoral fin clips were removed from each adult prior to stocking outdoors and utilized for electrophoretic analyses of each individual. All individuals contained only the Mdh-B^2, Idh-B^3, Sod-A^1 or Sod-A^2 and Aat-B^3 or Aat-B^4 alleles, and represented the pure Florida subspecies. In March of 1980 and 1981, the collected individuals were stocked outdoors in 0.08 hectare ponds.

During the spring of 1981, these brood stocks were used to produce NLMB, FLMB and both reciprocal F^1 hybrids, NLMB x FLMB (NxF) and FLMB x NLMB (FxN) by stocking 0.08 hectare ponds as follows:

Pond 1: 5 NLMB♀ and 5 NLMB♂
Pond 2: 5 NLMB♀ and 6 FLMB♂
Pond 3: 6 FLMB♀ and 5 NLMB♂
Pond 4: 8 FLMB♀ and 6 FLMB♂

Spawning was successful in all four production ponds. Ponds were drained on September 21, 22, 25 and 28, 1981, and approximately 1,200 50 mm fingerlings

were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

During the spring of 1982 the NxF F_1 stock was used to produce F_2 offspring. Similarly, during the spring of 1983 the FxN F_1 stock was used to produce F_2 offspring, as well. Specifically, equal numbers of male and female F_1 hybrids were placed in 0.08 hectare ponds and allowed to spawn naturally. Young-of-the-year F_2 hybrid offspring remained in ponds until late September. These offspring were randomly sampled for electrophoretic evaluation, with some being used for meristic evaluation as well.

Meristic Characterization. Meristic characters used to distinguish *M. s. salmoides* from *M. s. floridanus* consisted of four scale counts originally described by Bailey and Hubbs (1949) and two pyloric caecal counts. Characters noted include: scales along the lateral line (LLS); scales above the lateral line (SALL); scales below the lateral line (SBLL); scale rows around the caudal peduncle (CPS); the number of pyloric caeca (PC); and the number of pyloric caecal tips (CT). All scale counts were made according to methods outlined by Hubbs and Lagler (1958). Pyloric caeca, first evaluated by Buchanan (1973), are fingerlike outpocketings at the junction of the stomach and intestine. Pyloric caeca (basal caecal units) in largemouth bass are typically branched near the base in contrast to the simple development usually seen in other *Micropterus* (Hubbs and Bailey 1940) and are counted by the number of caecal tips whenever branching occurs.

Scale and caecal counts of FLMB broodstock populations were provided by the Florida Game and Freshwater Fish Commission. Scale counts from NLMB broodstock, as well as the F_1 and F_2 stocks, were taken from randomly selected frozen samples following electrophoretic analyses. Before examining the

pyloric caeca in these fish, a mid-ventral incision was made from the anal opening to a point anterior to the pectoral fins. Incisions were than made dorsally to form a flap which could be laid open to expose the stomach and attached pyloric caeca. Stomach, caeca, and intestine were removed intact and soaked for several days in a 10% formalin solution after which the caeca were cleaned and enumerated.

Data Analysis. Previous methods used in the categorization of largemouth bass subspecies include a Character Index (CI) described by Bailey and Hubbs (1949) and Discriminant Function Analysis (DFA) first used by Buchanan (1973). The Bailey and Hubbs character index is the summation of meristic values for five scale characters: CI = LLS + SALL + SBLL + CPS + CS (CS = scale rows on the cheek). This CI separates NLMB from FLMB on the basis of the typically higher meristic counts in M. s. floridanus. Functional ranges used by Bailey and Hubbs for subspecies identification consisted of 113-130 for M. s. salmoides and 131-145 for M. s. floridanus. In the present study a modification of this index was necessary since rows of scales on the cheek (CS) was not a character which was assessed. To modify the index, a value of 11.0, which is the high CS value for M. s. salmoides and the low CS value for M. s. floridanus, was subtracted from the index. The outcome was a range of values of 102-119 for M. s. salmoides and of 120-134 for M. s. floridanus. This modified index (MI) was then used to compare the various largemouth bass stocks by comparing values obtained from the equation: MI = LLS + SALL + SBLL + CPS. Neither the CI nor the MI has values which can identify an individual as a hybrid or a population as an intergrade.

Each fish was additionally categorized as either M. s. salmoides or M. s. floridanus using discriminant function analysis (DFA), as described by Fisher

(1936) and using the equations of Buchanan (1973). In each equation, for each individual, calculated coefficients are multiplied by the number of lateral line scales and caudal peduncle scales. A calculated constant is then subtracted from the sum of the products.

Discriminant Function 1 (*salmoides*):

$$10.91328 \text{ (no. lateral line scales)} + 12.12798 \text{ (no. caudal peduncle scales)} - 518.02170 = X_1$$

Discriminant Function 2 (*floridanus*):

$$11.97298 \text{ (no. lateral line scales)} + 12.82365 \text{ (no. caudal peduncle scales)} - 608.94987 = X_2$$

If the resulting number for function one is higher ($X_1 > X_2$), the fish is categorized as NLMB. If function two results in a higher value ($X_2 > X_1$), the fish is categorized as FLMB. Again, this analysis has no score in which an individual could be classified as a hybrid. The meristic characters selected for DFA were shown by Buchanan (1973) to be most divergent in one-way analysis of variance tests.

A technique used in separation of species or subspecies along with hybrids in intergrade populations is Hotelling's T^2 statistic, in which selected characters (LLS and PC) form the basis of two overlapping ellipses (Rao 1965). In this manner, individuals can be categorized as *M. s. salmoides*, *M. s. floridanus*, hybrids, or aberrant specimens (Buchanan 1973). The two ellipses represent the acceptance regions for the two populations constructed at the 0.01 level of significance. A zone of overlap contains the area of intermediacy between the two theoretical populations by which the hybrids are determined. This test was used in an attempt to categorize F_2 hybrid individuals.

RESULTS

Broodstock Meristic Analysis: Table 1 contains means, standard deviations, and ranges of meristic counts from NLMB and FLMB broodstock populations. Means were compared between subspecies and indicated significantly higher (x = 0.01) FLMB means in all scale counts. Similarly, a 9.6 CT (caecal tips) significant difference was observed between NLMB (28.3) and FLMB (37.9) means.

F_1 Stock Meristic Analysis: Scale counts of the F_1 generation of NLMB, FLMB, and reciprocal hybrid stocks, NxF and FxN, are summarized in Table 2. Comparing NLMB to FLMB, significantly higher mean FLMB scale counts were observed in LLS, SBLL, and CPS. Presented in Table 3 are values for two pyloric caecal counts along with comparative branching schemes among the four F_1 stocks. Mean CT comparisons indicated a significantly higher mean in FLMB. Based upon the meristic counts, it was possible by individual character evaluation to separate a portion of the NLMB and the FLMB from each other. Specifically, for each meristic character we calculated the percentage of NLMB which had meristic counts falling below the range of FLMB and the percentage of FLMB which had counts falling above the range of the NLMB. For the different counts these percentages were as follows: 1. pyloric caecal tips (CT): 100% of the F_1 NLMB were outside the range of the FLMB and 100% of the FLMB were outside the range of the NLMB: 2. lateral line scales (LLS): 70% NLMB and 80% FLMB; 3. scales below the lateral line (SBLL): 50% NLMB and 70% FLMB; 4. caudal peduncle scales (CPS): 30% NLMB and 90% FLMB; and pyloric caeca (PC): 0% NLMB and 20% FLMB. SALL proved totally ineffective in subspecies

identification of F_1 stocks since NLMB and FLMB possessed the same range (7-9) and essentially the same mean, 8.0 and 8.1, respectively.

Individuals from F_1 NLMB and F_1 FLMB stocks were analyzed by established methods using multiple meristic characters to determine the percent of individuals that could be correctly categorized. The first method used was the modified character index (MI) based upon values of Bailey and Hubbs (1949) and described in Materials and Methods (Table 6). The F_1 NLMB and F_1 FLMB stock values introduced into the modified index produced these results: 100% of the F_1 NLMB were correctly categorized as *M. s. salmoides*, but only 70% of F_1 FLMB were correctly categorized as *M. s. floridanus*. Low mean index values were observed for both F_1 NLMB and F_1 FLMB in comparison to those of Bailey and Hubbs (1949) as modified by us, and to those of Smith and Crumpton (1974) using the same modified index. If a modified index cutoff value of 112 had been used, 100% of both F_1 stocks would have been categorized correctly. Similar results were obtained from the discriminant function analysis: 100% of the F_1 NLMB were categorized as *M. s. salmoides*, but only 70% of the F_1 FLMB were categorized as *M. s. floridanus*.

Means and ranges of meristic characters of NLMB and FLMB presented in this study were compared to counts reported from previous studies (Table 4). Comparisons between F_1 NLMB and the Bone Lake NLMB broodstock population showed no significant differences in mean scale counts. However, the mean CT value (25.0) observed in F_1 NLMB was significantly lower than that (28.3) observed in the Bone Lake NLMB broodstock. Interestingly, significant differences in the LLS, SBLL, and CPS were observed for the F_1 FLMB and Lake Dora FLMB broodstock population. LLS (69.9) and CPS (32.7) means in the Lake Dora FLMB were higher

than those in the F_1 FLMB (LLS = 66.7 and CPS =27.9), whereas the SBLL (17.3) was higher in the F_1 FLMB stock than in the Lake Dora broodstock (SBLL =16.4).

NLMB broodstock (58.5) and F_1 NLMB (57.7) LLS means were approximately 5-7 scales lower than previously reported means (63.3-64.3) for NLMB. Other than the Bone Lake CT mean (28.3) the remainder of the NLMB counts fell within reasonable limits of previous descriptions (Table 4). FLMB population comparisons (Table 4) indicated that the F_1 FLMB LLS mean (66.7) was significantly lower than the FLMB broodstock (69.9) and lower than previous accounts (69.8-70.6). Conversely, high CT means in both F_1 (38.2) and broodstock (37.9) FLMB populations were higher than earlier studies (35.8-36.8). Although the CPS mean (27.9) observed for the F_1 FLMB stock was lower than previously reported values (29.0-30.0), the Lake Dora broodstock mean (32.7) was on the high end of previously reported values. The SBLL mean was the only meristic value for which the Illinois F_1 FLMB stock produced a significantly higher mean value (17.3) than the Lake Dora broodstock FLMB values (16.4).

Meristic counts of the two reciprocal F_1 hybrid stocks, NxF and FxN, presented in Tables 2 and 3 were significantly different between PC means only. Due to the inability to separate reciprocal hybrid stocks on the basis of meristic counts, NxF and FxN stock data were pooled into an F_1 hybrid largemouth bass category (Table 5). Mean meristic values of the F_1 hybrid stocks expressed conditions intermediate of broodstock means in all counts. Mean CT (29.6) and CPS (28.5) counts, while intermediate, were reasonably close to the NLMB broodstock means of 28.3 and 27.8, respectively. A similar condition exists between the F_1 hybrid LLS mean (67.5) and the FLMB broodstock mean (69.9). Although the F_1 hybrid meristic count means exceeded the F_1 FLMB

means for LLS, SALL, and CPS. The remainder of the F_1 hybrid mean counts were intermediate to the two F_1 subspecific stocks. The ranges of values for F_1 hybrid LMB showed, for the most part, a good deal of overlap with F_1 NLMB and F_1 FLMB values.

F_2 Meristic Analysis: Observed meristic values for F_2 hybrid stocks summarized in Table 7 indicated that a low F_2 FxN LLS mean (57.8) paralleled those of the F_1 NLMB (57.7) and NLMB broodstock (58.5), whereas the F_2 NxF LLS (63.9) mean was more intermediate to those of the NLMB and the F_1 FLMB (66.7) or FLMB broodstock (69.9) values. Both hybrid LMB F_2 stock LLS averages were considerably lower than the means observed for F_1 hybrids. Conversely, F_2 stock CT means increased 8-9 caecal tips from F_1 means, F_1 NxF (28.6) to F_2 NxF (37.8) and F_1 FxN (30.5) to F_2 FxN (38.4). Similarly, both F_2 hybrid LMB stocks possessed greater SALL means than all other stocks studied. The F_2 NxF SBLL mean (17.7) greatly exceeded the FLMB broodstock means (16.4) but resembled that of the F_1 FLMB (17.3). The F_2 NxF SBLL mean (17.7) increased in value from the F_1 NxF mean (16.3), whereas the F_2 FxN mean (15.9) decreased in value from the F_1 FxN mean.

Hotellings T^2 statistic was used to categorize nine F_2 NxF and eight F_2 FxN into pure NLMB, FLMB, or hybrid largemouth bass categories (Fig. 1). Analysis of F_2 hybrids revealed these results: one individual (5.9%) was classified as NLMB; two (11.8%) classified as FLMB; five (29.4%) classified as hybrid; and nine (52.9%) were outside the ellipses constructed by Buchanan (1973) and were thereby considered to be aberrant specimens. Only eight points are present outside the ellipses in Figure 1 as one FxN specimen, LLS:53 CT:34, did not correspond to values provided by Buchanan (1973). Two (22.2%) of the

aberrant specimens were NxF, the remaining seven (77.8%) were FxN.

DISCUSSION

Bailey and Hubbs (1949) reported that mean scale count comparisons among largemouth bass populations were typically higher in the Florida subspecies, *M. s. floridanus*, than in the northern subspecies, *M. s. salmoides*. Subsequent studies using additional meristic characters (Bryan 1969, Buchanan 1973) and populations (Addison and Spencer 1972, Thrasher 1974, Inman 1974) agreed with these findings. Unfortunately, none of these studies used genetically confirmed stocks for their analyses. However, comparatively higher meristic counts obtained for FLMB relative to NLMB in the present study substantiates these previous reports by using genetically confirmed broodstock populations. An earlier attempt by Pelzman (1980) to compare meristic and electrophoretic analyses must be considered somewhat suspect since the Mdh-B and Sod-A loci which were used as diagnostic markers for subspecies identification were not truly diagnostic. The two subspecies are not fixed for alternative alleles at these loci (Philipp et al. 1981, 1983).

The scale count providing the best single character differentiation between F_1 NLMB and FLMB stocks proved to be LLS. The difference in means of nine scales was the greatest observed among the different scale characters. In addition, the LLS had the lowest range overlap, 11.0%. Thrasher (1974) reported LLS as the most useful feature for subspecies separation. However, in our study, comparing F_1 NLMB and F_1 FLMB, the CT provided the most discriminating character overall with a difference in means of 13.9 CT. The absence of overlapping counts between the ranges of these two F_1 stocks enabled 100% separation.

Previous studies, however, have shown high CT range overlap as evidenced by the 25% overlap observed by Buchanan (1973) and 53% by Thrasher (1974).

Since the number of individuals used in the present study to determine CT means was low, additional CT counts may expand the ranges enough to produce some overlap, but most likely not to the extent observed in previous accounts. Sources of NLMB used by Thrasher (1974) include many populations which, although considered pure NLMB at that time, presently fall within a larger, redescribed intergrade zone (Philipp et al. 1981, 1983). These populations would most likely contain some of the FLMB genome and, therefore, contribute higher than normal counts, extending the NLMB range and increasing the perceived percent overlap between subspecies. In addition, in a comparison of mean CT values among 40 populations of largemouth bass in Florida, Smith and Crumpton (1977) demonstrated that the Lake Dora, Florida population had one of the highest mean CT values. Using this stock as our FLMB broodstock resulted in fairly high CT values in the F_1 FLMB.

Differences between F_1 NLMB and FLMB CT means seem to stem from differences in the degree of PC branching. Johnson (1907) reported largemouth bass PC were typically bifid, separating this species from the rest of the centrarchids. Ramsey (1975), using largemouth bass data provided by S. J. Zolczynski from experimental ponds at Auburn University, reported the presence of bifurcate (single branch) and quadrifurcate (double branched) PC conditions in _M. salmoides_. He noted quadrifurcate PC prevailed in individuals with higher CT counts and bifurcate PC in individuals with lower CT counts. This author further postulated that the higher CT counts of _M. s. floridanus_ stemmed from the increased division of individual pyloric caeca. The PC means between F_1 NLMB (11.0) and FLMB (12.0) were determined to be significantly different, but by only a single count. Since little PC variation exists between NLMB and FLMB stocks, the difference between CT means seems to be a consequence of

variation in branching conditions. Observations in the present study showed pyloric caeca were present at higher frequencies and in more extensively branched conditions in the F_1 FLMB compared to the F_1 NLMB. In agreement with Ramsey (1975), we observed that bifurcate and quadifurcate conditions similarly varied with CT frequencies.

Latitudinal differences in the spawning sites for the Bone Lake broodstock obtained from northern Wisconsin and the F_1 NLMB produced at the INHS Aquatic Research Field Laboratory, Champaign, Illinois, had no apparent effect on meristic counts. However, significant differences in some meristic counts did occur between the Lake Dora FLMB broodstock and F_1 FLMB. The broodstock FLMB means were higher than the F_1 FLMB means for LLS and CPS, but lower for SBLL. Since climatic conditions in central Illinois more closely resemble those in Wisconsin than in Florida, variation in values between FLMB populations may be a result of latitude. NLMB exist throughout the range of Wisconsin and Illinois, principally in pure form, whereas pure FLMB are limited to peninsular Florida (Bailey and Hubbs 1949, McCrimmon and Robbins 1975, Philipp et al. 1981, 1983). From this information it is evident that the Illinois spawning conditions during F_1 NLMB and F_1 FLMB production may have been less typical of Florida than of Wisconsin.

The plasticity of meristic characters as a consequence of environmental influence has been well documented (Hubbs 1922, Vladykov 1934, Taning 1952, Lindsey 1954, 1958, 1962a, Orska 1957, 1962, Seymour 1959, Barlow 1961, Bryan 1969, McCrimmon and Kwain 1969, Wallace 1973, Kwain 1975). The most overwhelming evidence has been the observance of meristic elements progressively increasing within a species from south to north (Hubbs 1926, Vladykov 1934, Taning 1952). Apparently the number of serial elements is

determined by developmental rate (Hubbs 1926, Gabriel 1944, Garside 1966). Longer developmental periods usually produce higher counts in meristic structures as a result of low temperatures. Other agents which retard development, such as high salinity and low oxygen tension, have effects parallel to those of low temperatures (Hubbs 1926, Taning 1952, Seymour 1856). Studies contradicting this relationship report the reverse is also true. Some characters increase as a result of high temperatures during development (Hubbs 1921, 1926, Schultz 1927, Vladykov 1934, Barlow 1961, Bryan 1969). Other studies have described the thermal plasticity of meristic values with V-shaped relationships in which the number of meristic elements is lowest or highest at some intermediate temperature (Schmidt 1921, Taning 1952, Seymour 1956, Molander and Molander-Swedmark 1957, Ali and Lindsey 1974). Wallace (1973) described an S-shaped relationship in which the meristic counts fluctuated in no specific manner. These studies reflect the inability to predict the directional change of a specific meristic character following a latitudinal change. Work by Taning (1952), Lindsey (1954), and Molander and Molander-Swedmark (1957), stressed consistent temporal effects of developmental temperature change on the counts of meristic elements. On the other hand, Orska (1962) showed that several phenocritical periods exist during development in which the average number of vertebrae would respond positively or negatively to a higher incubation temperature. Not only can the meristic counts of a particular character be influenced at different developmental stages, but different characters seem to be affected independently during the developmentally sensitive periods in which they can be influenced (Barlow 1961). All of these factors taken into consideration may well account for the inconsistency in the deviation of the F_1 FLMB mean meristic counts from those

of the FLMB broodstock: a decrease in LLS and CPS; an increase in SBLL; and no change in SALL or CT.

Interpopulational deviations may reflect alterations more complex than simple latitudinal variations in temperature, dissolved oxygen or light duration and intensity. Genetic histories have been postulated to combine with thermal environmental parameters to play a potential role in the control of the meristic makeup in certain species (Ricker 1972, Lindsey and Harrington 1972, Ali and Lindsey 1974, Lindsey 1975, MacGregor and McCrimmon 1977). These studies and others reviewed by Barlow (1961) have used individuals of the same parentage or lineage and comparisons between populations or races to indicate that variation exists within a particular stock irrespective of environmental control. Since most morphological characteristics are considered to be polygenic in nature (Svardson 1945, Fowler 1970), it is difficult to quantify the degree of genetic control involved.

Although the NxF and FxN F_1 reciprocal hybrids compared closely to the maternal subspecies in a number of physiological aspects of development (Philipp et al. 1984), they were intermediate to the two subspecies in morphology. This relationship between development and morphology parallels work by Heuts (1956) with sticklebacks. Comparison of pooled F_1 hybrids (NxF and FxN) to the NLMB and FLMB broodstocks indicated all of the characters except SBLL were intermediate of NLMB and FLMB but not necessarily median in their average. This agreed well with previous reports (Baily and Hubbs 1949, Buchanan 1973, Inman 1974, Thrasher 1974). Since the F_1 hybrids were produced under different climatic conditions from both broodstock (Illinois versus Wisconsin and Florida), comparisons have also been made with F_1 NLMB and FLMB. Under these similar circumstances of development, the F_1 hybrids were

abnormally high in three meristic counts, surpassing the F_1 FLMB means in LLS, SALL and CPS. Abnormally high counts in meristic characters is a condition common among interspecific and intergeneric hybrids (Hubbs and Strawn 1957, Smitherman and Hester 1962, West and Hester 1964, Simon and Noble 1968, Berry and Low 1970, Ross and Lavender 1981, Leary et al. 1983). Hybridization among taxonomic groups below the species level has been assumed to be intermediate. This in fact may only be the norm, not the rule. The existence of intergrade populations that have meristic counts more closely resembling the FLMB than the NLMB has been reported (Bailey and Hubbs 1949, Thrasher 1974, Pelzman 1980). It is apparent from these overlapping ranges of NLMB, FLMB, and F_1 hybrids that one cannot expect to classify individual fish from mixed populations as to subspecific or hybrid categories with reasonable accuracy on the basis of these meristic counts. In fact, it is apparent that it is also not possible to accurately classify a population as to pure NLMB, pure FLMB or intergrade using these meristic characters.

Through the use of a combination of meristic characters methods have been previously described which attempt to increase the probability of correctly identifying subspecies using meristic counts. The use of a modification of the Bailey and Hubbs (1949) character index (CI), which they described as more discriminant than any single character, was indeed more effective in the present study than any single meristic character with the exception of CT. Both the modified index (MI) and discriminant function analysis (DFA) reported 100% accuracy in NLMB identification and 70% accuracy for FLMB. However, both methods have severe limitations because neither method can categorize individuals as hybrids.

F_2 hybrids were produced from both NxF and FxN F_1 stocks in an effort to simulate further introgression in intergrade populations and to evaluate the efficacy of meristic analyses for these individuals. Although intermediate, there were no F_2 hybrid mean meristic counts which were median in average between NLMB and FLMB. Most meristic characters gave fairly high mean counts similar to those found for the F_1 FLMB. In addition, the ranges of some of the F_2 meristic characters were extended beyond the normal ranges of both NLMB and FLMB. This extension occurred bidirectionally, but in most cases the extension was beyond the upper range.

Hotelling's T^2 test statistic previously described by Rao (1965) was used by Buchanan (1973) in inverted form to classify NLMB, FLMB and hybrid individuals from mixed populations. This test was used in the present study to determine the percentage of F_2 hybrid individuals which would be identified as NLMB, FLMB, hybrid LMB or aberrant specimens. From a total of 17 individuals only five (29.4%) were classified as hybrids and all of these were F_2 individuals produced from the NxF F_1 stock. The majority of the FxN F_2 hybrid counts were low, falling outside the combination of ellipses thereby classifying these individuals as aberrant specimens. It should be noted that these ellipses were centered about the means obtained from the populations used by Buchanan (1973). The usefulness of this test, therefore may not have been adequately assessed. However, low LLS counts in combination with high CT counts were sufficient to cause the classification of seven out of eight FxN F_2 hybrid individuals as aberrant simply because the assumed pattern of intermediacy among hybrids does not apply.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collection and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. We also wish to acknowledge the assistance of the Wisconsin Department of Natural Resources in obtaining northern largemouth bass broodstock. We particularly want to thank Rick Cornelius and Jerry Perkins for the assistance they gave in field collections. Finally, we wish to thank Dr. Bruce Taubert, Dr. Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Christine Kaminski and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Addison, J. H. and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass, Micropterus salmoides (Lacepede), stocked in ponds in south Alabama. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 25:366-374.

Ali, M. Y. and C. C. Lindsey. 1974. Heritable and temperature induced meristic variation in the medaka, Oryzias latipes. Canadian Journal of Zoology 52:959-976.

Avise, J. C. and M. H. Smith. 1977. Gene frequency comparisons between sunfish (Centrarchidae) populations at various stages of evolutionary divergence. Systematic Zoology 26:319-335.

Bailey, R. M. and C. L. Hubbs. 1949. The black basses (Micropterus) of Florida, with description of a new species. University of Michigan Museum of Zoology Occasional Papers 516:1-40.

Barlow, G. W. 1961. Causes and significances of morphological variation in fishes. Systematic Zoology 10:1-105-117.

Berry, P. Y. and M. P. Low. 1970. Comparative studies on some aspects of the morphology and histology of Ctenopharyngodon idellus, Aristicthys nobilis, and their hybrid (Cyprinidae). Copeia 1970:708-726.

Bottroff, L. J. 1967. Intergradation of Florida bass in San Diego County. Masters Thesis. San Diego State College, San Diego, California, USA.

Bryan, C. F. 1969. Variation in selected meristic characters of some basses, Micropterus. Copeia 1969:370-373.

Buchanan, J. P. 1973. Separation of the subspecies of largemouth bass, Micropterus salmoides salmoides and M. s. floridanus and intergrades by use of meristic characters. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 27:608-619.

Davies, W. D. 1973. Florida largemouth bass in Alabama waters as a management tool. Auburn University Agricultural Station, Department of Fisheries and Allied Aquaculture, Annual Report, Volume 14, Auburn University, Alabama, USA.

Farris, J. S. 1972. Estimating phylogenetic trees from distance matrices. American Naturalist 106:645-668.

Fisher, A. 1936. The use of multiple measurements in taxonomic problems. Annals of Eugenics 7:179-188.

Fitch, W. M. and E. Margoliash. 1967. Construction of phylogenetic trees. Science (Washington, District of Columbia) 155:279-284.

Fowler, J. A. 1970. Control of vertebral number in teleosts-an embryological problem. Quarterly Review of Biology 45:148-167.

Gabriel, M. L. 1944. Factors affecting the number and form of vertebrae in Fundulus heteroclitus. Journal of Experimental Zoology 95:105-114.

Garside, E. T. 1966. Developmental rate and vertebral number in salmonids. Journal of the Fisheries Research Board of Canada 23:1537-1551.

Hubbs, C. L. 1921. Geographic variation of Notemigonus crysoleucas - an American minnow. Transactions of the Illinois Academy of Science 11:147-151.

Hubbs, C. L. 1922. Variations in the number of vertebrae and other meristic characters of fishes correlated with temperature of water during development. American Naturalist 56:;360-372.

Hubbs, C. L. 1926. The structural consequences of modifications of the developmental rate in fishes, considered in reference to certain problems of evolution. American Naturalist 60:57-81.

Hubbs, C. L. and R. M. Bailey. 1940. A revision of the black basses (Micropterus and Huro) with descriptions of four new forms. Miscellaneous Publications Museum of Zoology. No. 48, University of Michigan, Ann Arbor. 51 pp.

Hubbs, C. L. and K. F. Lagler. 1958. Fishes of the Great Lakes Region. Cranbrook Institute of Science, Bulletin No. 26, Revised Ed. 213 pp.

Hubbs, C. L. and K. Strawn. 1957. Relative variability between the darters, Etheostoma spectabile and Percina caprodies. Evolution 11:1-10.

Heuts, M. J. 1956. Temperature adaptation in Gasterosteus aculeatus L. Publ. Staz. Zool. Napol. 28:44-61.

Inman, C. R. 1974. Annual (unpublished) job report for State Fisheries Investigation, Region 3, Job 72-2. Florida Bass Study. Texas Parks and Wildlife Department, Austin. 7 p.

Johnson, R. H. 1907. The individuality and variations of the pyloric caeca of the Centrarchidae. Transactions of the Wisconsin Academy of Science 15:713-733.

King, M.-C. and A. C. Wilson. 1975. Evolution at two levels in humans and chimpanzees. Science (Washington, District of Columbia) 188:107-116.

Kwain, W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (Salmo gairdneri) exposed to combinations of light intensity and temperature. Journal of the Fisheries Research Board of Canada 32(3):397-402.

Langley, C. H. and W. M. Fitch. 1974. An examination of the constancy of the rate of evolution. Journal of Molecular Evolution 3:161-177.

Leary, R. F., F. W. Allendorf and K. L. Knudsen. 1983. Consistently high meristic counts in natural hybrids between brook trout and bull trout. Systematic Zoology 32(4):369-376.

Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia University Press, London, England.

Lindsey, C. C. 1954. Temperature controlled meristic variation in the paradise fish, Macropodus opercularis (L.). Canadian Journal of Zoology 30:87-98.

Lindsey, C. C. 1958. Modification of meristic characters by light duration in kokanee, Oncorhynchus nerka. Copeia 1958(2):134-136.

Lindsey, C. C. 1962a. Experimental study of meristic variation in a population of three-spine sticklebacks, Gasterosteus aculeatus. Canadian Journal of Zoology 40:271-312.

Lindsey, C. C. 1975. Pleiomerism, the wide spread tendency among related fish species for vertebral number to be correlated with maximum body length. Journal of the Fisheries Research Board of Canada 32:2453-2469.

Lindsey, C. C. and R. W. Harrington, Jr. 1972. Extreme vertebral variation induced by temperature in a homozygous clone of self-fertilizing cyprinodontid fish (Rivulus marmoratus). Canadian Journal of Zoology 50:733-744.

MacCrimmon, H. R. and W. Kwain. 1969. Influence of light on early development and meristic characters in rainbow trout, Salmo gairdneri Richardson. Canadian Journal of Zoology 47:631-637.

MacCrimmon, H. R. and W. H. Robbins. 1976. Distribution of the black basses in North America. Pages 56-66 In R. H. Stroud and H. Clepper (Eds.) Black bass biology and management. Sport Fishing Institute, Washington, DC, USA.

MacGregor, R. B. and H. R. MacCrimmon. 1977. Evidence of genetic and environmental influences on meristic variation in the rainbow trout, Salmo gairdneri Richardson. Environmental Biology of Fish 2(1):25-33.

Mickevich, M. F. and M. S. Johnson. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Systematic Zoology 25:260-270.

Molander, A. R. and M. Molander-Swedmark. 1957. Experimental investigations on variation in plaice (Pleuronenctes platessa [L.]). Institute of Marine Research, Lysekil, Ser. Biol., Fisheries Research Board of Sweden, Report No. 7:1-45.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Orska, V. 1957. The influence of temperature on the development of the skeleton in teleosts. Zool. Poloniae. 7(3):271-329.

Orska, V. 1962. The influence of temperature on the development of meristic characters of the skeleton in Salmonidae. Part 1. Temperature controlled variations of the number of vertebrae in Salmo irideus Gibbon. Zool. Poloniae. 12(3):309-340.

Pelzman, R. J. 1980. Impact of Florida largemouth bass, Micropterus salmoides floridanus, introduction at selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. California Fish and Game 66:133-162.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, Micropterus salmoides salmoides, and smallmouth bass, Micropterus dolomieui). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (Micropterus salmoides) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Philipp, D. P., C. Kaminski and G. S. Whitt. 1984. A comparison of the embryonic development of northern, Florida, and reciprocal F_1 hybrid largemouth bass in different thermal environments. Pages 4-1 to 4-28 In D. P. Philipp and G. S. Whitt (Eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Ramsey, J. S. 1975. Taxonomic history and systematic relationships among species of Micropterus. Pages 67-75 In R. H. Stroud and H. Clepper (Eds.) Black bass biology and management. Sport Fishing Institute, Washington, DC, USA.

Rao, C. R. 1965. Linear statistical inference and its applications. John Wiley and Sons, Inc., New York. 513 p.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. University of British Columbia, H. R. MacMillan Lectures in Fisheries, The stock concept in pacific salmon, 1972:27-160.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. University of Texas Studies in Genetics 7:145-153.

Ross, M. R. and T. M. Cavender. 1981. Morphological analyses of four experimental intergeneric cyprinid hybrid crosses. Copeia 1981:377-387.

Seymour, A. H. 1956. Effects of temperature upon young chinook salmon. Dissertation Abstracts 16:2249.

Seymour, A. H. 1959. Effects of temperature upon the formation of vertebrae and fin rays in young chinook salmon. Transactions of the American Fisheries Society 88:58-59.

Schmidt, J. 1921. Racial investigations. VII. Annual fluctuations of racial characters in Zoarces viviparus (L.). C. R. Trav. Lab. Carlsberg 14:1-24.

Schnell, G. D., T. L. Best and M. L. Kennedy. 1978. Interspecific morphological variation in kangaroo rats (Dipodomys): degree of concordance with genic variation. Systematic Zoology 27:34-48.

Schultz, L. P. 1927. Temperature controlled variation in the golden shiner, Notemigonus crysoleucas. Papers of the Michigan Academy of Science 7:417-432.

Simon, R. C. and R. E. Noble. 1968. Hybridization in Oncorhynchus (Salmonidae). I. Viability and inheritance in artificial crosses of chum and pink salmon. Transactions of the American Fisheries Society 97:109-118.

Smith, S. L. and J. E. Crumpton. 1974. Evaluation of largemouth bass meristic characters. Florida Game and Freshwater Fish Commission, Federal Aid in Fish Restoration, F-22-11, Study V, Job 6, Progress Report, Tallahassee, Florida, USA.

Smith, S. L. and J. E. Crumpton. 1977. Evaluation of largemouth bass meristic characters. Florida Game and Freshwater Fish Commission, Federal Aid in Fish Restoration F-22-11, Study V, Job 6, Annual Report, Tallahassee, Florida, USA.

Smitherman, R. O. and F. E. Hester. 1962. Artificial propagation of sunfishes, with meristic comparisons of three species of Lepomis and five of their hybrids. Transactions of the American Fisheries Society 91:333-341.

Sneath, P. H. A. and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman, San Francisco, California, USA.

Svardson, G. 1945. Polygenic inheritance in *Lebistes*. Arkiv. Zoologi 36A(6):1-9.

Taning, A. V. 1952. Experimental study of meristic characters in fishes. Biological Review of the Cambridge Philosophical Society 27:169-193.

Thrasher, D. R. 1974. A comparison of northern largemouth bass, *Micropterus salmoides salmoides* (Lacepede), the Florida subspecies, *Micropterus salmoides floridanus* (Le Seur), and their intergrades. M.S. Thesis. Auburn University, Auburn, Alabama, USA. 76 p.

Turner, B. J. 1974. Genetic divergence of Death Valley pupfish populations. Species-specific esterases. Comparative Biochemistry and Physiology B, Comparative Biochemistry 46:57-70.

Vladykov, V. D. 1934. Environmental and taxonomic characters of fishes. Transactions of the Royal Canadian Institute 20:99-140.

Wallace, C. R. 1973. Effects of temperature on developing meristic structures of smallmouth bass, *Micropterus dolomieui* (Lacepede). Transactions of the American Fisheries Society 102:142-144.

West, J. L. and F. E. Hester. 1964. Intergeneric hybridization of centrarchids. Transactions of the American Fisheries Society 93:280-288.

Wilson, A. C., S. S. Carlson and T. J. White. 1977. Biochemical evolution. Annual Review of Biochemistry 46:573-649.

Table 1. Meristic counts of NLMB (Bone Lake, Wisconsin) and FLMB (Lake Dora, Florida) broodstock populations.

Stock	Number	LLS	SALL	SBLL	CPS	CT
NLMB	15	58.5±2.2[a] (54-62)[b]	8.0±4.2 (7-9)	15.3±0.56 (14-16)	27.8±2.0 (25-31)	28.3±2.4 (23-31)
FLMB	14	69.9±2.3 (66-74)	8.4±0.45 (8-9)	16.4±0.31 (16-17)	32.7±2.1 (30-36)	37.9±5.6 (28-50)

[a]Mean ± Std. Dev.
[b]Range

Table 2. Scale counts of four F_1 young-of-the-year largemouth bass stocks.

Stock	Number	LLS	SALL	SBLL	CPS
NLMB	10	57.7±2.4 (54-61)	8.0±0.50 (7-9)	15.4±0.70 (14-16)	26.6±0.70 (25-27)
N x F	9	68.8±3.4 (62-71)	8.3±0.71 (7-9)	16.3±0.90 (15-18)	28.4±0.50 (28-29)
F x N	5	65.4±2.1 (62-67)	8.4±0.60 (8-9)	16.4±0.60 (16-17)	28.9±0.80 (28-30)
FLMB	10	66.7±3.5 (60-71)	8.1±0.60 (7-9)	17.3±1.3 (16-20)	27.9±0.30 (27-28)

Table 3. Pyloric caecal counts of four F_1 young-of-the-year largemouth bass stocks.

Stock	Number	PC	Caecal Branching[a] 0	1	2	3	4	5	6	CT
NLMB	8	11.0±1.1 (10-13)	1.6[b] (6)[c]	5.8 (8)	3.1 (8)	0.50 (4)	0.13 (1)	-	-	25.0±2.2 (22-29)
N x F	5	10.8±0.84 (10-12)	0.40 (2)	5.4 (5)	2.8 (5)	1.2 (4)	0.80 (3)	0.20 (1)	-	28.6±2.0 (26-31)
F x N	6	12.3±0.82 (11-13)	0.50 (2)	7.0 (6)	2.8 (6)	1.8 (6)	-	-	-	30.5±1.6 (28-33)
FLMB	7	12.0±2.1 (9-15)	0.43 (3)	3.3 (7)	4.6 (7)	1.7 (6)	0.86 (5)	1.0 (4)	0.14 (1)	38.9±5.8 (30-47)

[a] Caecal branching represents the comparative branching scheme between stocks, 0 = No branching (single tip) to 6 = 6 forks (7 tips)

[b] Mean

[c] Number of fish possessing a particular branched caecal types from n individuals possible for a fish to possess several to all types of branching.

Table 4. Meristic comparisons of NLMB and FLMB populations to previous studies.

Investigator	Number	Source	LLS	SALL	SBLL	CPS	CT
			NLMB				
Bailey and Hubbs (1949)	37-78	Great Lakes and Miss. R.	63.3±2.1 (59-69)	7.9±0.48 (7-9)	15.2±0.90 (14-17)	27.2±1.3 (24-30)	NA
Buchanan (1968)	117	Arkansas	63.9	7.6	15.8	27.8	23.2
Thrasher (1974)	560	Southeastern United States	64.3 (55-73)	7.3 (6-9)	14.7 (14-17)	27.6 (24-30)	25.7 (10-45)
Present study	15	Wisconsin (Bone Lake)	58.5±2.2 (54-62)	8.0±0.43 (7-9)	15.3±0.56 (14-16)	27.8±2.0 (25-31)	28.3±2.4 (23-31)
Present study	10	Illinois F_1 (INHS)	57.7±2.4 (54-61)	8.0±0.50 (7-9)	15.4±0.70 (14-16)	26.6±0.70 (25-27)	25.0±2.2 (22-29)
			FLMB				
Bailey and Hubbs (1949)	72-74	Florida Peninsula	70.4±2.1 (65-75)	8.6±0.60 (7-10)	17.0±0.67 (16-18)	29.7±1.3 (27-32)	
Buchanan (1968)	121	Florida	69.8	8.1	16.8	29.0	36.8
Thrasher (1974)	96	Florida	70.6 (66-77)	8.3 (7-11)	16.6 (15-18)	30.0 (27-34)	35.8 (22-49)
Present study	14	Florida[1] (Lake Dora)	69.9±2.3 (66-74)	8.4±0.45 (8-9)	16.4±0.31 (16-17)	32.7±2.1 (30-36)	38.7±5.8 (28-50)
Present study	10	Illinois F_1 (INHS)	66.7±3.5 (60-71)	8.1±0.60 (7-9)	17.3±1.3 (16-20)	27.9±0.30 (27-28)	38.2±5.6 (30-47)

[1] Values provided by the Florida GFWFC, Smith and Crumpton 1974.

Table 5. Meristic counts of the two pooled F_1 reciprocal hybrid LMB stocks, NxF and FxN.

Number	LLS	SALL	SBLL	CPS	PC	CT
11-14	67.5±3.1 (62-74)	8.3±0.17 (7-9)	16.3±0.75 (15-18)	28.5±0.66 (28-30)	11.6±1.1 (10-13)	29.6±2.1 (26-33)

Table 6. Subspecies determinations and index comparisons using Modified Index values of F_1 NLMB and FLMB stocks.

F1 NLMB Stock MI values	F1 FLMB Stock MI values
107	121
109	113
103	122
111	121
111	113
107	125
105	120
110	118
107	121
	126
$\bar{X}$ = 107.7	
range = 103 - 111	$\bar{X}$ = 120.0
	range = 113 - 126

Modification of Bailey and Hubbs (1949) Character Index
CI - CS(11.0) = MI
M. s. salmoides = 102-119 $\bar{X}$ = 114.0
M. s. floridanus = 120-134 $\bar{X}$ = 126.9

Illinois F_1 NLMB categorized as NLMB = 100%
(MI $\leq$ 119)

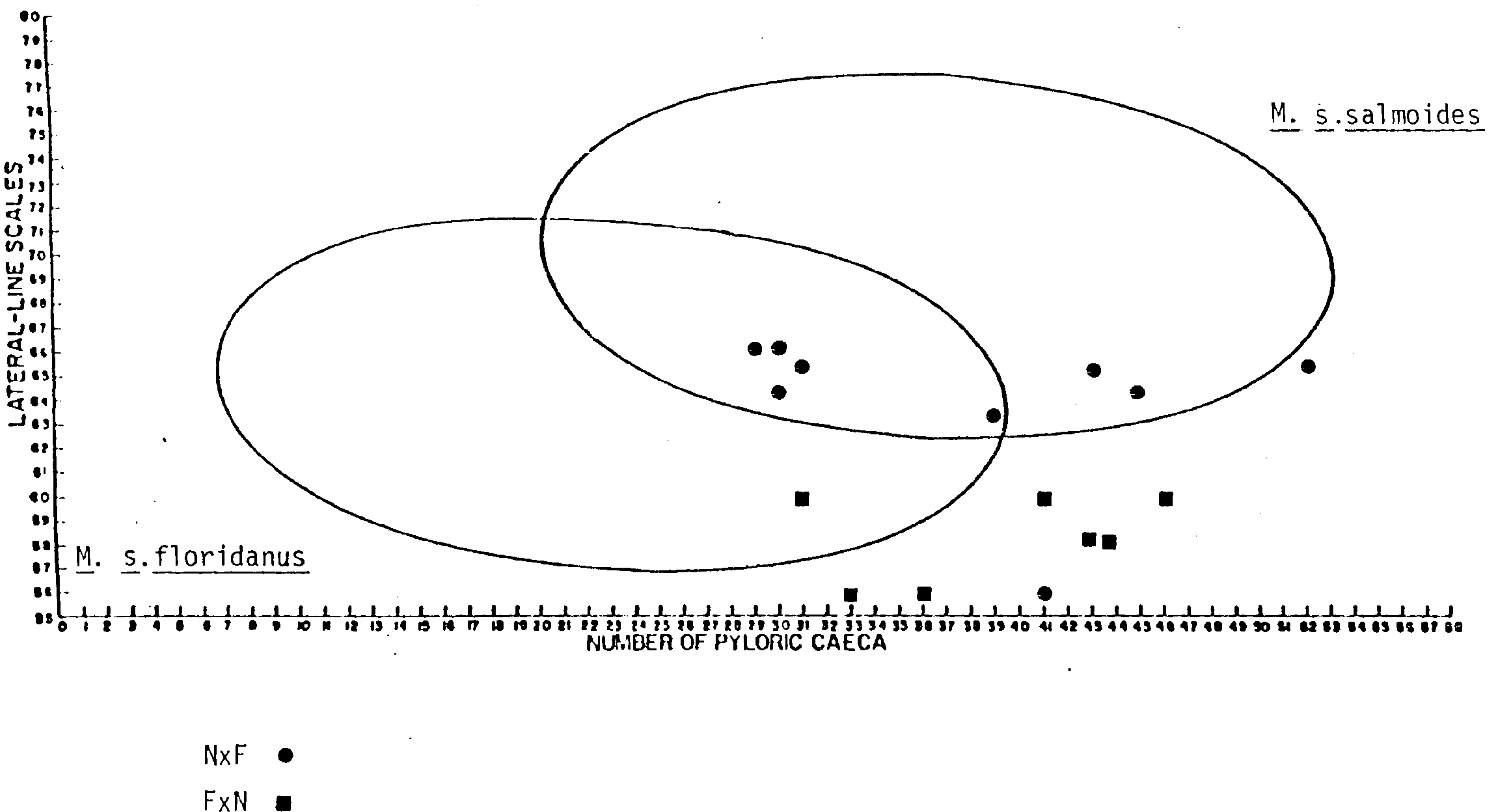
Illinois F_1 FLMB categorized as FLMB = 70%
(MI $\geq$ 120)

Smith and Crumpton (1974): Modified Index
LLS + SALL + SBLL + CPS = MI
M. s. salmoides $\bar{X}$ = 116.9
M. s. floridanus $\bar{X}$ = 125.6

Table 7. Meristic counts of F_2 hybrid stocks produced from NxF F_1 and FxN F_1 parent stocks.

Parent Stock	Number	LLS	SALL	SBLL	CPS	PC	CT
NxF	9-10	63.9±2.9 (56-66)	8.7±0.50 (8-9)	17.7±1.6 (15-21)	27.9±1.1 (26-30)	11.9±1.8 (9-15)	37.8±8.2 (29-52)
FxN	8-10	57.8±2.4 (53-60)	8.6±0.50 (8-9)	15.9±0.9 (15-18)	28.0±0.70 (27-29)	12.4±1.9 (10-15)	38.4±5.6 (31-46)

Figure.1-Subspecies separation of F_2 hybrid individuals using overlapping elipses constructed from Hotelling's T^2 test statistic.



STUDY 102
JOBS 1, 2

CHAPTER 2

Survival and Growth of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass in Central Illinois

David P. Philipp[1,2], Christine Kaminski[1] and Gregory S. Whitt[3,1]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Genetically confirmed stocks of the northern largemouth bass (*Micropterus salmoides salmoides*), Florida largemouth bass (*M. s. floridanus*) and both reciprocal F_1 hybrids were produced in Champaign, Illinois through natural spawning in 0.08 hectare earthen ponds. These stocks were used to assess the age 0, age 1 and age 2 overwinter survival and the second and third year growth of individuals in each of these four stocks of largemouth bass. Results indicated that the northern largemouth bass has a significantly greater overwintering capability in central Illinois than does the Florida largemouth bass. The hybrids were somewhat intermediate in their overwintering abilities. Results also indicated that the northern largemouth bass exhibits much greater second and third year growth in central Illinois than the Florida largemouth bass. One hybrid, *M. s. floridanus* ♀ x *M. s. salmoides* ♂, exhibited intermediate growth, while the reciprocal hybrid, *M. s. salmoides* ♀ x *M. s. floridanus* ♂, exhibited growth comparable to the northern largemouth bass in many instances.

INTRODUCTION

The original range of the largemouth bass, *Micropterus salmoides*, consisted of much of the midwestern, eastern and southeastern areas of the country, however, this has been expanded considerably through extensive stocking programs (MacCrimmon and Robbins 1975). Almost any body of water that could conceivably support a largemouth bass population has been stocked, and in many cases stocked repeatedly with largemouth bass. Unfortunately, little consideration has been given to the genetic consequences of these stockings. Fingerlings have been transported great distances to be stocked into lakes which have environments quite different from that which was native to those fingerlings. In some cases they are being stocked into lakes containing largemouth bass populations with a quite different genetic background. Of particular concern are the management thrusts currently implemented or being considered by various states which involve the propagation of the Florida subspecies, *M. s. floridanus*, for introduction into native populations of the northern largemouth bass, *M. s. salmoides*. The effects of such stocking practices on the genetic structure of the native or introduced populations are at present unknown, but this has been disregarded by many of the fishery biologists who determine largemouth bass management programs. We feel that this attitude should be changed so that sound genetic conservation principles will be incorporated into current and future state and national largemouth bass management programs.

Since the description of the two subspecies of largemouth bass, a few laboratory studies have been performed to analyze their thermophysiological differences (Hart 1952, Cichra et al. 1981). Observations are generally consistent with the assumption that the Florida subspecies is less tolerant to

colder environments than the northern subspecies, but comprehensive, quantitative analyses do not exist for genetically verified and identically tested individuals of each subspecies. A number of studies have compared survival and growth of the two subspecies in controlled field situations (Clugston 1964, Addison and Spencer 1972, Davies 1973, Graham 1973, Inman et al. 1976, Zolczynski and Davies 1976, Latta 1977, Smith and Wilson 1981, Wright and Wigtil 1981). However, the findings of these studies were often contradictory due to a consistent flaw in all of these experiments, that is, they lack persuasive data on the genetic structure of the populations studied. Without verification of the subspecific status of the largemouth bass utilized, comparisons between the subspecies are not valid. Meristic counts have been routinely used to "separate" the two subspecies. However, the number of scales along, above and below the lateral line, the number of scales around the caudal peduncle and/or the number of pyloric caeca simply cannot provide unambiguous resolution of the taxonomic status of different largemouth bass populations and therefore are not effective in distinguishing pure subspecies or identifying intergrade populations (Smith and Crumpton 1977; Van Orman et al. 1984). A number of other studies designed to assess the impact of the introduction of the Florida subspecies into existing populations of the northern subspecies in California (von Geldern and Mitchell 1975; Bottroff and Lembeck 1978; Moyle and Holzhauser 1978; Pelzman 1980) and in Texas (Inman et al. 1976) also lack subspecific genetic confirmation. Without appropriate broodstock analysis, the genetic integrity of the "Florida" largemouth bass transplanted to and subsequently propagated in these states is unconfirmed and questionable.

The purpose of this study was to produce genetically confirmed stocks of each subspecies of largemouth bass and both of their reciprocal F_1 hybrids.

These stocks were then evaluated to determine their relative growth performance and overwinter survival in ponds in central Illinois. In this manner, we assessed the impacts that the introductions of FLMB or one of the F_1 hybrids would have upon largemouth bass populations in Illinois.

MATERIALS AND METHODS

Production of Genetic Stocks:

Pure northern largemouth bass (NLMB), *M. s. salmoides*, were collected from Bone Lake, Wisconsin during October, 1978. Right pectoral fin clips were removed from each adult prior to stocking and utilized for electrophoretic analyses of each individual (Philipp et al. 1979, 1983). All individuals retained contained only the Mdh-B^1, Idh-B^1, Sod-A^2 and Aat-B^1 or B^2 alleles, indicating they represented the pure northern subspecies. These individuals were held outdoors in 0.08 hectare ponds until the onset of the project in April 1980. Pure Florida largemouth bass (FLMB), *M. s. floridanus*, were collected from Lake Dora, Florida during January, 1980 and again during February, 1981. These fish were air shipped to Champaign and held indoors at 8-12°C. Left pectoral fin clips were removed from each adult prior to stocking outdoors and utilized for electrophoretic analyses of each individual. All individuals contained only the Mdh-B^2, Idh-B^3, Sod-A^1 or Sod-A^2 and Aat-B^3 or Aat-B^4 alleles, indicating they represented the pure Florida subspecies. In March of 1980 and 1981, the collected individuals were stocked outdoors in 0.08 hectare ponds.

During the spring of 1980, adults were stocked in 0.08 hectare ponds as follows:

Pond 1: 6 NLMB ♀ and 6 NLMB♂
Pond 2: 5 FLMB ♀ and 6 FLMB♂

and allowed to spawn naturally. Spawning was successful in both production ponds. Ponds were drained on September 11, 1980, and approximately 2,000 50 cm fingerling largemouth bass were recovered from each. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

During the spring of 1981, these brood stocks were used to produce NLMB, FLMB and both reciprocal F_1 hybrids, NLMB♀ x FLMB♂ (NxF) and FLMB♀ x NLMB♂ (FxN) by stocking 0.08 hectare ponds as follows:

Pond 1: 5 NLMB♀ and 5 NLMB♂
Pond 2: 5 NLMB♀ and 6 FLMB♂
Pond 3: 6 FLMB♀ and 5 NLMB♂
Pond 4: 8 FLMB♀ and 6 FLMB♂

Spawning was successful in all four production ponds. Ponds were drained on September 21, 22, 25 and 28, 1981, and approximately 1,200 50 cm fingerlings were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

Evaluation of Growth and Survival:

1980 Year Class:

Two 0.08 hectare ponds (80A and 80B) were each stocked on September 17, 1980 with 62 individuals of the NLMB (right pectoral clip) and FLMB (left pectoral clip) stocks. Survival was monitored over the 1980-81 winter, ponds 80A and 80B being drained on April 4 and 8, respectively. These two competition ponds were each restocked on May 2 with 25 individuals of each of the two stocks. In addition, a 1.0 hectare pond (80C) was stocked with the remaining individuals of each of the two stocks. Survival and growth of these ponds were monitored over the 1981 growing season, ponds 80A and 80B being drained on October 13 and 15, respectively, and pond 80C being drained on November 15. The two 0.08 hectare ponds were again each stocked on October 13 and 15, respectively, with 23-25 individuals each of the NLMB and FLMB stocks. Survival was monitored over the 1981-82 winter, with the ponds being drained March 23 and 25, 1982, respectively. These two competition ponds were each

restocked with 20 individuals each of the two stocks on March 23 and 25, 1982. Survival and growth were monitored in these ponds during the 1982 growing season, with the ponds being drained on October 28, 1982. Two competition ponds (80A and 80B) were again stocked with 18-20 individuals each of the NLMB and FLMB stocks on October 28, 1982. Survival was monitored over the 1982-83 winter, with the ponds being drained March 10 and 11, 1983, respectively.

<u>1981 Year Class</u>:

Three 0.08 hectare ponds were each stocked with equal numbers of each of the four stocks, NLMB (right pectoral clip), FLMB (left pectoral clip) and both of the two reciprocal F_1 hybrids, NxF (right pelvic clip) and FxN (left pelvic clip). Pond 81A was stocked on October 12 with 15 individuals of each stock. Ponds 81B and 81C were both stocked on November 4 with 50 individuals of each stock. In addition to these competition ponds, during the period September 21-28, a 0.08 hectare holding pond was established for each of the four stocks into which 200 individuals (50-80 mm in length) of one specific stock were introduced. Finally, during this same period, a 0.04 hectare holding pond was established for each of the four stocks into which 1,000 individuals (50-80 mm in length) of one specific stock were introduced. Survival was monitored over the 1981-82 winter. Competition ponds 81A, 81B and 81C were drained and censused on April 21, May 12 and May 5, 1982, respectively. The four 0.2 acre holding ponds were drained and censused June 21-29, 1982, and the four 0.1 acre holding ponds were drained and censused April 5-13, 1982. Three 0.08 hectare ponds, 81A, 81B and 81C, were reestablished with 20 individuals of each of the four stocks on May 8, 12 and 6, respectively. Survival and growth in these ponds were monitored over the 1982 growing season, ponds 81A, 81B and 81C being

drained and censused on October 26-27, 1982. Three new competition ponds were each restocked on October 26-27, 1982 with 11-20 individuals of each of the four stocks. Survival was monitored over the 1982-83 winter, with the ponds being drained on March 10 and 17. Only two competition ponds were stocked with 20 individuals of each of the four stocks on March 15, 1983. Survival and growth during the 1983 growing season were monitored in these ponds, with both ponds being drained on October 13, 1983.

RESULTS

Overwinter Survival:

Overwinter survival data for the NLMB and FLMB stocks produced in 1980 were obtained for the winters of 1980-1981 (age 0), 1981-1982 (age 1) and 1982-1983 (age 2). In addition, overwinter survival data for the NLMB, NxF, FxN and FLMB stocks produced in 1981 were obtained for the winters of 1981-1982 (age 0), 1982-1983 (age 1) and 1983-1984 (age 2).

Results for age 0 largemouth bass stocked in competition ponds are summarized in Table 1. Additional results obtained for age 0 largemouth bass stocked not in competition, but in individual ponds are shown in Table 2. The NLMB demonstrated substantially greater overwinter survival than did the FLMB. The two reciprocal F_1 hybrids demonstrated intermediate values, with the NxF approaching the survival of NLMB in most instances. Tables 3 and 4 summarize the results obtained for age 1 and age 2 largemouth bass stocks, respectively. The NLMB again demonstrated substantially greater overwinter survival than did the FLMB, with the two F_1 hybrids, NxF and FxN, having intermediate results. The overwinter survival values for age 1 and age 2 fish, however, were higher than those for age 0. In addition, the 1982-82 winter, which was the most severe (5709 heating degree days), also resulted in the much lower overwinter survival figures for the age 0 FLMB than did the less severe 1980-81 winter (5124 heating degree days): 24.4% versus 69.8%.

Growth:

Since each of the different stocks of largemouth bass were produced through natural spawning in separate ponds, no valid comparisons of first year

growth could be made. However, second and third year growth data for the NLMB and FLMB stocks produced in 1980 were obtained for the 1981 and 1982 growing seasons, respectively. In addition, second and third year growth data for the NLMB, NxF, FxN and FLMB stocks produced in 1981 were obtained for the 1982 and 1983 growing seasons, respectively.

Results for second year growth of the largemouth bass stocks are summarized in Table 5. In both 1981 and 1982, the NLMB showed substantially greater growth in both length and weight than did the FLMB. Although the two reciprocal F_1 hybrids, NxF and FxN, showed intermediate growth characteristics during their second year, the NxF hybrid very closely approached the NLMB both in length and weight gain. Results for third year growth of the largemouth bass stocks are summarized in Table 6. These results were similar to those for the second year growth with two possible alterations. First, the NLMB appeared to outperform the FLMB even to a greater extent than in year 2. Second, the NxF F_1 hybrid actually nosed out the NLMB in gains in length (101% versus 100%) and in weight (101% versus 100%).

DISCUSSION

Comparing the four stocks of largemouth bass for potential use in natural environments in Illinois, the NLMB is clearly superior than the FLMB or either reciprocal F_1 hybrid. The NLMB had the greatest overwinter survival at all ages tested. In addition, although the NxF F_1 hybrid showed similar growth in its third year using fairly small fish, increased growth of NLMB during the second year would result in the largest fish in a pond after three years being NLMB. No other stock demonstrated any advantage over the NLMB for Illinois waters.

Most of the previous studies comparing survival and growth of NLMB and FLMB have been performed in much more southern climates (Clugston 1964; Addison and Spencer 1972; Davies 1973; Inman et al. 1976; Zolczynski and Davies 1976). Some studies have been performed in more northerly climates (Latta 1977; Smith and Wilson 1981; Wright and Wigtil 1981). However, all of these studies have one major flaw in that the stocks used were not genetically confirmed as being pure NLMB or pure FLMB. In addition, for many of these studies, the source location for one or both stocks used makes the purity of their subspecific status highly questionable. As a result, we feel that comparison of our results with those of these other studies may not be meaningful.

Much of the recent interest in propagating FLMB for introduction into waters outside of peninsular Florida stems from "trophy" catches of largemouth bass in California and Texas following the introduction of FLMB into waters of these states (von Geldern and Mitchell 1975; Inman et al. 1976; Bottroff and Lembeck 1978; Moyle and Holzhauser 1978; Pelzman 1980). It must be stressed that the largemouth bass populations in California prior to the introduction of Florida largemouth bass were themselves only the result of previous

introductions, not immigration and natural selection. The fish used in these initial introductions were obtained from Illinois and, therefore, represented the pure northern subspecies. These fish had been genetically tailored through natural selection for Illinois environments, not those of southern California. Comparing the climates of Illinois and Florida to that of southern California, it is not surprising that the newly introduced Florida largemouth bass and the F_1 hybrids created from the crossing of the two subspecies thrived. However, the long term effects of mixing the two gene pools can at this time only be surmised.

In a specific environment, the relative performance of one stock of largemouth bass compared to another depends upon the relative fitness of that stock for that specific environment. In addition, the fitness of each individual is determined by its genetic composition, and that of a stock by the combination of genotypes present among the individuals comprising the stock. Through natural selection, stocks of largemouth bass which existed in the native range of this species prior to man's intervention, were, most likely, genetically quite finely tailored to the environments they inhabited. As such, at both structural and regulatory loci, specific allele combinations and frequencies which proved advantageous, and thereby which maximized fitness, were generated in these populations. The introduction of a stock of largemouth bass obtained from one environment into a population of largemouth bass existing in a different environment could very well disrupt the advantageous allele combinations and frequencies present in the recipient population. This would, most likely, result in a reduction in the fitness of the pre-introduction population. Although this scenario would hold for most populations of largemouth bass in and to the east of the Mississippi River

drainage, it may not hold for recipient populations of largemouth bass not native to the lakes which they inhabit, as was the case for the California populations. Utmost care needs to be taken to protect the integrity of the various genetic stocks of largemouth bass existing today and current and future fisheries management programs need to protect, not exploit, the genetic resources of the largemouth bass, as well as all other managed species of fish.

Fishermen of today certainly deserve the best fishing possible. Fisheries managers, administrators and researchers should strive to provide optimal fisheries for them. However, we must be exceptionally careful that the consequences of our actions do not compromise the natural resources which we are supposed to be protecting and upon which future generation will depend for future enjoyment. Some management programs involve taking steps that are essentially irreversible, e.g., introducing genes from one population into another. There should be no time limit set upon the research required to determine the future effects of these types of programs. These programs should be postponed until all questions are answered, or they should be abandoned. Premature actions on this scale would only be gambling.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collections of Florida largemouth bass and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. We also wish to thank the Wisconsin Department of Natural Resources for permitting field collections of northern largemouth bass. We particularly want to thank Rick Cornelius and Jerry Perkins for their assistance in these collections. Finally, we wish to thank Dr. William F. Childers for advice and Dr. Paul R. Beaty, Dr. Bruce Taubert, Dr. Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Shirley Lowe, Jeffrey Van Orman, Jeffrey Koppelman, and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Addison, J. H. and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass, _Micropterus salmoides_ (Lacepede), stocked in ponds in south Alabama. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 25:366-374.

Bottroff, L. J. and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass, _Micropterus salmodies floridanus_. California Fish and Game 64:4-23.

Cichra, C. E., W. E. Neill and R. L. Noble. 1981. Differential resistance of northern and Florida largemouth bass to cold shock. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 34:19-24.

Clugston, J. P. 1964. Growth of the Florida largemouth bass, _Micropterus salmoides floridanus_ (Le Sueur), and the northern largemouth bass, _M. s. salmoides_ (Lacepede), in subtropical Florida. Transactions of the American Fisheries Society 93:146-154.

Davies, W. D. 1973. Florida largemouth bass in Alabama waters as a management tool. Annual Report of the Fisheries and Allied Aquacultures Department, Auburn University Agricultural Experiment Station, vol. 14.

Graham, L. K. 1973. The introduction of Florida largemouth bass in Missouri ponds. Dingell-Johnson Project F-1-R-22. Study I-14, Job No. 2. Missouri Department of Conservation.

Hart, J. S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. University of Toronto Biological Series Publication 60, Ontario Fish Research Laboratory 72:1-79, Toronto, Canada.

Inman, C. R., R. C. Dewey and P. P. Durocher. 1976. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the 30th Annual Conference of the Southeastern Association of Game and Fish Commissions 30:1-17.

Latta, W. C. 1977. Survival and growth of Florida largemouth bass in ponds and lakes in Michigan. Michigan Department of Natural Resources, Dingell-Johnson Project F-35-R-3, Study XXVII:101-114.

MacCrimmon, H. R. and W. H. Robbins. 1975. Distribution of the black basses in North America. Pages 56-66 _In_ R. H. Stroud and H. Clepper, editors. Black Bass Biology and Management. Sport Fishing Institute, Washington, DC.

Moyle, P. B. and N. J. Holzhauser. 1978. Effects of the introduction of Mississippi silverside (Menidia audens) and Florida largemouth bass (Micropterus salmoides floridanus) on the feeding habits of young-of-the-year largemouth bass in Clear Lake, California. Transactions of the American Fisheries Society 107:574-582.

Pelzman, R. J. 1980. Impact of Florida largemouth bass, Micropterus salmoides floridanus, introductions at selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. California Fish and Game 66:133-162.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, Micropterus salmoides salmoides, and smallmouth bass, Micropterus dolomieui). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Smith, S. L. and J. E. Crumpton. 1977. Evaluation of largemouth bass meristic characters. Dingell-Johnson Project F-22-11, Study V, Job 6. Florida Game and Freshwater Fish Commission Annual Report.

Smith, R. P. and J. L. Wilson. 1981. A growth comparison of two subspecies of largemouth bass in Tennessee ponds. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissions 34:25-30.

Van Orman, J. L., G. S. Whitt and D. P. Philipp. 1984. An evaluation of meristic characters to identify populations of northern, Florida, and F_1 hybrid largemouth bass. Pages 1-31 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

von Geldern, C. E., Jr. and D. F. Mitchell. 1975. Largemouth bass and threadfin shad in California. Pages 426-449 In R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

Wright, G. L. and G. W. Wigtil. 1981. Comparison of growth, survival, and catchability of Florida, northern, and hybrid largemouth bass in a new Oklahoma reservoir. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissions 34:31-38.

Table 1.-Overwinter survival of age 0 largemouth bass stocks in competition ponds.

Season	Heating Degree Days	Stock/Year/Pond	Fall Stocking			Spring Draining	
			Number	Length (mm)	Weight (g)	Number	% Recovery
1980-1981	5124	NLMB/80/A	62	108±4.2	12.6±3.5	53	85.5
		NLMB/80/B	62	110±4.7	12.9±4.1	61	98.4
							$\bar{X}$ = 92.0
		FLMB/80/A	62	114±9.8	13.9±3.6	42	67.7
		FLMB/80/B	62	116±9.7	14.2±4.1	47	71.8
							$\bar{X}$ = 69.8
1981-1982	5709	NLMB/81/A	15	115±16.8	18.6±11.2	15	100
		NLMB/81/B	50	65.2±3.1	2.5±0.4	46	92.0
		NLMB/81/C	50	65.4±3.8	2.4±0.4	49	98.0
							$\bar{X}$ = 96.7
		NxF/81/A	15	120±21.1	22.5±13.2	14	93.3
		NxF/81/B	50	58.3±5.1	1.8±0.5	44	88.0
		NxF/81/C	50	57.7±4.7	1.8±0.5	48	96.0
							$\bar{X}$ = 92.4
		FxN/81/A	15	100±28.5	15.0±6.8	10	67.7
		FxN/81/B	50	51.1±7.3	1.5±0.6	42	84.0
		FxN/81/C	50	51.5±6.0	1.3±0.6	42	84.0
							$\bar{X}$ = 78.6
		FLMB/81/A	15	123±21.0	23.6±13.4	2	13.3
		FLMB/81/B	50	60.1±7.4	2.1±0.6	19	38.0
		FLMB/81/C	50	58.8±7.2	1.9±0.6	11	22.0
							$\bar{X}$ = 24.4

Table 2. Overwinter (1981-82: 5709 Heating Degree Days) survival of age 0 largemouth bass stocks in individual ponds.

Stock/Year/Pond	Initial Number	Final Number	% Survival
A. 0.08 hectare ponds			
NLMB/81/1	200	159	79.5
NxF/81/2	200	135	67.5
FxN/81/3	200	118	59.0
FLMB/81/4	200	11	5.5
B. 0.04 hectare ponds			
NLMB/81/5	1000	421	42.1
NxF/81/6	1000	157	15.7
FxN/81/7	1000	68	6.8
FLMB/81/8	1000	5	0.5

Table 3.-Overwinter survival of age 1 largemouth bass stocks in competition ponds.

Season	Heating Degree Days	Stock/Year/Pond	Fall Stocking			Spring Draining	
			Number	Length (mm)	Weight (g)	Number	% Recovery
1981-1982	5709	NLMB/80/A	25	196±8.1	94.6±12.6	25	100
		NLMB/80/B	25	235±3.9	180±12.5	25	100
							$\bar{X}$ = 100
		FLMB/80/A	23	190±8.5	91.1±18.9	20	86.9
		FLMB/80/B	25	227±6.4	147±15.8	23	92.0
							$\bar{X}$ = 89.4
1982-1983	4595	NLMB/81/A	20	220±16.8	145±17.0	20	100
		NLMB/81/B	18	208±6.3	112±12.6	18	100
		NLMB/81/C	19	185±11.8	82.1±15.0	19	100
							$\bar{X}$ = 100
		NxF/81/A	20	205±9.2	117±10.5	20	100
		NxF/81/B	15	208±8.3	116±13.9	15	100
		NxF/81/C	19	188±5.9	83.3±8.7	18	94.7
							$\bar{X}$ = 98.2
		FxN/81/A	19	192±6.4	92.0±9.9	18	94.7
		FxN/81/B	15	198±6.1	99.0±11.6	15	100
		FxN/81/C	18	177±7.2	73.7±9.7	16	88.9
							$\bar{X}$ = 94.5
		FLMB/81/A	20	180±6.7	70.7±7.1	18	90.0
		FLMB/81/B	11	182±4.8	73.9±6.9	11	100
		FLMB/81/C	12	174±4.8	64.0±7.1	9	75.0
							$\bar{X}$ = 88.3

Table 4.-Overwinter survival of age 2 largemouth bass stocks in competition ponds.

Season	Heating Degree Days	Stock/Year/Pond	Fall Stocking			Spring Draining	
			Number	Length (mm)	Weight (g)	Number	% Recovery
1982-1983	4595	NLMB/80/A	19	245±10.1	211±17.2	19	100
		NLMB/80/B	20	261±8.9	265±16.9	19	95
							$\bar{X}$ = 97.5
		FLMB/80/A	18	227±8.8	155±12.7	16	88.9
		FLMB/80/B	20	241±6.9	199±15.2	17	85.0
							$\bar{X}$ = 87.0
1983-1984	5387	NLMB/81/A	17	202±6.7	101±8.6		
		NLMB/81/B	18	210±6.8	115±12.4		
		NxF/81/A	20	200±5.5	100±8.4		
		NxF/81/B	19	210±7.0	120±12.9		
		FxN/81/A	12	194±4.2	94.3±7.4		
		FxN/81/B	12	207±5.3	115±8.4		
		FLMB/81/A	15	194±6.2	85.0±11.7		
		FLMB/81/B	17	203±4.7	103±8.3		

Table 5.-Second year growth of largemouth bass stocks in competition ponds.

Season	Cooling Degree Days	Stock/Year/Pond	Spring Stocking			Fall Draining			Absolute Percent Increase		Normalized Percent Increase	
			Number	Length (mm)	Weight (g)	Number	Length	Weight	Length	Weight	Length	Weight
1981	790	NLMB/80/A	25	123±5.2	24.6±4.2	25	196±8.1	94.6±12.6	59.3	285	100	100
		NLMB/80/B	25	119±5.1	20.1±3.1	25	235±3.9	180±12.5	97.5	795	100	100
		NLMB/80/C	40	115±6.2	16.8±2.8	38	290±23.4	497±92.8	153	2854	100	100
		FLMB/80/A	25	133±2.7	29.7±9.4	24	189±8.7	74.3±12.0	42.1	150	71.0	52.6
		FLMB/80/B	25	123±8.9	21.0±5.6	25	224±6.4	145±24.0	82.1	590	84.2	74.2
		FLMB/80/C	20	106±12.8	12.0±7.4	19	252±15.1	276±67.8	138	2191	90.2	76.7
											$\bar{X}$ = 81.8	$\bar{X}$ = 67.8
1982	866	NLMB/81/A	20	72.4±3.7	3.6±0.23	20	220±16.8	145±17.0	204	3928	100	100
		NLMB/81/B	20	70.4±3.4	3.0±0.31	18	208±6.3	112±12.6	195	3633	100	100
		NLMB/81/C	20	70.1±2.8	3.0±0.21	19	185±11.8	82.1±15.0	164	2637	100	100
		NxF/81/A	20	71.8±7.2	3.3±0.34	20	205±9.2	117±10.5	186	3445	91.2	87.7
		NxF/81/B	20	70.3±6.3	3.0±0.29	17	208±8.3	116±13.9	196	3767	101	104
		NxF/81/C	20	69.4±4.3	2.8±0.31	19	188±5.9	83.3±8.7	171	2857	104	108
											$\bar{X}$ = 98.7	$\bar{X}$ = 99.9
		FxN/81/A	20	68.3±7.0	3.0±0.37	19	192±6.4	92.0±9.9	181	2967	88.7	75.5
		FxN/81/B	20	67.8±10.8	2.7±0.30	17	198±6.1	99.0±11.6	192	3556	98.5	97.9
		FxN/81/C	20	68.1±7.8	2.9±0.38	18	177±7.2	73.7±9.7	160	2417	97.6	91.7
											$\bar{X}$ = 94.9	$\bar{X}$ = 88.4
		FLMB/81/A	20	73.1±6.2	3.0±0.40	20	180±6.7	70.7±7.1	146	2257	71.6	57.5
		FLMB/81/B	12	73.1±10.1	3.3±0.42	11	182±4.8	73.9±6.9	149	2139	76.4	58.9
		FLMB/81/C	12	75.2±8.7	3.6±0.44	12	174±4.8	64.0±7.1	132	1678	80.5	63.6
											$\bar{X}$ = 76.2	$\bar{X}$ = 60.0

Table 6.-Third year growth of largemouth bass stocks in competition ponds.

Season	Cooling Degree Days	Stock/Year/Pond	Spring Stocking			Fall Draining			Absolute Percent Increase		Normalized Percent Increase	
			Number	Length (mm)	Weight (g)	Number	Length	Weight	Length	Weight	Length	Weight
1982	866	NLMB/80/A	20	192±7.8	104±14.1	19	245±10.1	211±17.2	27.6	103	100	100
		NLMB/80/B	20	236±4.5	180±12.3	20	261±8.9	265±16.9	10.6	47.2	100	100
		FLMB/80/A	20	190±8.5	91.1±18.9	18	227±8.8	155±12.7	19.5	70.1	70.7	68.1
		FLMB/80/B	20	230±6.3	154±14.9	20	241±6.9	199±15.2	4.8	29.2	45.3	61.9
											$\bar{X}$ = 58.0	$\bar{X}$ = 65.0
1983	1313	NLMB/81/A	20	161±3.3	51.2±7.9	17	202±6.7	101±8.6	25.4	97.3	100	100
		NLMB/81/B	20	150±2.8	43.2±4.9	18	210±6.8	115±12.4	40.0	166	100	100
		NxF/81/A	20	160±6.5	51.5±9.2	20	200±5.5	100±8.4	25.0	94.2	98.4	96.8
		NxF/81/B	20	148±3.3	43.8±5.1	19	210±7.0	120±12.9	41.9	174	104	105
											$\bar{X}$ = 101	$\bar{X}$ = 101
		FxN/81/A	20	159±6.6	48.5±8.1	12	194±4.2	94.3±7.4	22.0	94.4	86.6	97.0
		FxN/81/B	20	146±2.6	43.0±5.5	12	207±5.3	115±8.4	41.8	167	104	100
											$\bar{X}$ = 95.3	$\bar{X}$ = 98.5
		FLMB/81/A	20	165±6.7	51.0±8.9	15	194±6.2	85.0±11.7	17.8	66.7	70.1	68.6
		FLMB/81/B	20	146±3.2	41.3±3.9	17	203±4.7	103±8.3	39.0	149	97.5	89.8
											$\bar{X}$ = 83.8	$\bar{X}$ = 79.2

STUDY 102
JOB 3

CHAPTER 3

Reproductive Success of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass in Central Illinois

David P. Philipp[1,2], Jeffrey B. Koppelman[1], Christine Kaminski[1] and Gregory S. Whitt[3,1]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Genetically confirmed stocks of the northern largemouth bass, *Micropterus salmoides salmoides* (NLMB), Florida largemouth bass, *M. s. floridanus* (FLMB), and both reciprocal F_1 hybrids (NxF and FxN) were produced in Champaign, Illinois through natural spawning in 0.08 hectare earthen ponds. These stocks were assessed as to the relative amounts of NLMB, FLMB and F_1 hybrid reproduction in a pond stocked with equal numbers of adult NLMB and FLMB. Results indicated that although the greatest percentage of resulting offspring were pure FLMB, the NLMB were by far the largest offspring produced. The F_1 hybrid offspring were intermediate in size. The stocks were also used to assess the relative amounts of NLMB, FLMB, F_1 hybrid, and non-F_1 hybrid production in a pond stocked with equal numbers of NLMB, NxF, FxN, and FLMB adult largemouth bass. Results indicated that the greatest number of offspring produced were again pure FLMB and again, these offspring were the smallest, NLMB offspring being significantly larger throughout the growing season. The hybrids were intermediate in size with F_1 hybrids being somewhat greater in size than the non-F_1 hybrids.

INTRODUCTION

The largemouth bass, *Micropterus salmoides*, is one of the most sought after and highly managed sport fish in the United States. Fishery biologists have long suspected morphological and physiological differences between the two subspecies of largemouth bass and their hybrids. However, management practices have sometimes disregarded the possible deleterious effects of stocking individuals which are genetically inappropriate for a given environment or genetically incompatible with the existing population. Recently, various state fishery programs have implemented or are currently considering, management programs which involve the propagation of the Florida subspecies, *M. s. floridanus*, for introduction into native populations of largemouth bass. We feel this practice should be halted, at least until the appropriate research has been completed to determine the effects that such a program would have on the native populations. In this way, sound genetic conservation principles will be incorporated into current and future state and national largemouth bass management programs.

A number of studies have compared survival and growth of the two subspecies in controlled field situations (Clugston 1964, Addison and Spencer 1972, Davies 1973, Graham 1973, Inman et al. 1976, Zolczynski and Davies 1976, Latta 1977, Smith and Wilson 1981, Wright and Wigtil 1981). However, the findings of these studies were often different, which may be explained by the one consistent flaw in all of these experiments, the absence of persuasive data on the genetic structure of the study populations. This absence prevents the verification of the subspecific status of the largemouth bass utilized. Routinely employed meristic counts (most often a combination of the number of scales along, above and below the lateral line, the number of scales around the

caudal peduncle and/or the number of pyloric caeca) cannot provide unambiguous resolution of the taxonomic status of different largemouth bass populations and thus, are not effective in distinguishing the pure subspecies, as well as identifying intergrade populations (Smith and Crumpton 1977, Van Orman et al. 1984). A number of other studies designed to assess the impact of the introduction of the Florida subspecies into existing populations of the northern subspecies in California (von Geldern and Mitchell 1975, Bottroff and Lembeck 1978, Moyle and Holzhauser 1978, Pelzman 1980) and in Texas (Inman et al. 1976) also lack a firm genetic basis. Without the appropriate genetic data the integrity of the Florida largemouth bass originally planted and subsequently propagated in these states is unconfirmed and questionable.

The purpose of this study was to compare the relative reproductive success of northern, Florida and their reciprocal F_1 hybrid largemouth bass in central Illinois. The numerical percentage composition of year class production was determined and also the relative growth of each of these types of offspring. In this manner, the quality of the relative recruitment produced by each of the two subspecies, as well as the degree of resulting hybridization was assessed.

MATERIALS AND METHODS

Production of Genetic Stocks:

Pure northern largemouth bass (NLMB), *M. s. salmoides*, were collected from Bone Lake, Wisconsin during October, 1978. Right pectoral fin clips were removed from each adult prior to stocking and utilized for electrophoretic analyses of each individual (Philipp et al. 1979, 1983). All individuals retained contained only the $Mdh\text{-}B^1$, $Idh\text{-}B^1$, $Sod\text{-}A^2$ and $Aat\text{-}B^1$ or B^2 alleles, indicating they represented the pure northern subspecies. These individuals were held outdoors in 0.08 hectare ponds until the onset of the project in April 1980. Pure Florida largemouth bass (FLMB), *M. s. floridanus*, were collected from Lake Dora, Florida during January, 1980 and again during February, 1981. These fish were air shipped to Champaign and held indoors at 8-12°C. Left pectoral fin clips were removed from each adult prior to stocking outdoors and utilized for electrophoretic analyses of each individual. All individuals contained only the $Mdh\text{-}B^2$, $Idh\text{-}B^3$, $Sod\text{-}A^1$ or $Sod\text{-}A^2$ and $Aat\text{-}B^3$ or $Aat\text{-}B^4$ alleles, indicating they represented the pure Florida subspecies. In March of 1980 and 1981, the collected individuals were stocked outdoors in 0.08 hectare ponds.

During the spring of 1980, adults were stocked in 0.08 hectare ponds as follows:

Pond 1: 6 NLMB♀ and 6 NLMB♂
Pond 2: 5 FLMB♀ and 6 FLMB♂

and allowed to spawn naturally. Spawning was successful in both production ponds. Ponds were drained on September 11, 1980, and approximately 2,000 50 cm fingerling largemouth bass were recovered from each. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

During the spring of 1981, these brood stocks were used to produce NLMB, FLMB and both reciprocal F_1 hybrids, NLMB♀ x FLMB♂ (NxF) and FLMB♀ x NLMB♂ (FxN) by stocking 0.08 hectare ponds as follows:

Pond 1: 5 NLMB♀ and 5 NLMB♂
Pond 2: 5 NLMB♀ and 6 FLMB♂
Pond 3: 6 FLMB♀ and 5 NLMB♂
Pond 4: 8 FLMB♀ and 6 FLMB♂

Spawning was successful in all four production ponds. Ponds were drained on September 21, 22, 25 and 28, 1981, and approximately 1,200 50 cm fingerlings were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

Experimental Reproduction Ponds:

Pond 1: Comparison of the two subspecies (NLMB and FLMB):

From March 17-25, 1982, a 1.0 hectare pond devoid of fish was stocked with: 15 of the adult breeder NLMB collected from Bone Lake, WI (8 and 7 , 1-2 kg each); 15 of the adult breeder FLMB collected from Lake Dora, FL (8 and 7 , 1-2 kg each); 43 of the 1980 year class of NLMB produced in Champaign, IL (20 with TL = 236 ± 4.0 mm and Wt = 181 ± 11.7 g; 23 with TL = 204 ± 4.9 mm and Wt = 101 ± 8.2 g); and 43 of the 1980 year class of FLMB produced in Champaign, IL (20 with TL = 232 ± 6.5 mm and Wt = 153 ± 17.1 g; 23 with TL = 199 ± 5.9 mm and Wt = 97.5 ± 9.3 g). Adult lake chubsuckers, fathead minnows and redear sunfish were stocked simultaneously to provide forage.

These fish were allowed to spawn and the 1982 year class of largemouth bass was sampled by a combination of seining and electrofishing techniques on August 11, 1982, October 2, 1982, May 28, 1983, and October 7, 1983. Due to the extremely mild winter of 1982-83, mortalities of NLMB and FLMB were both negligible in our research ponds. As a result, approximately equal numbers of

NLMB and FLMB breeders were again present during the spring of 1983. These fish were allowed to spawn, and the 1983 year class of largemouth bass was sampled by a combination of seining and electrofishing techniques on August 14, 1983 and September 22-October 7, 1983.

Pond 2: Comparison of all four stocks (NLMB, NxF, FxN, FLMB):

On March 15 and 16, 1983, a 1.8 hectare pond devoid of fish was stocked with 10 larger individuals each of the following stocks of the 1981 year class: NLMB (TL = 205 ± 7.3 mm, Wt = 113 ± 15.5 g); NxF (TL = 204± 5.6 mm, Wt = 8.4 g); FxN (TL = 196 ± 4.8 mm, Wt = 104 ± 7.3 g); FLMB (TL = 187 ± 3.6 mm, Wt = 81 ± 5.5 g) and with 50 smaller individuals each of the following stocks of the 1981 year class: NLMB (TL = 158 ± 6.5 mm, Wt = 50.7 ± 7.9 g); NxF (TL = 153 ± 6.6 mm, Wt = 47.6 ± 7.2 g); FxN (TL = 147 ± 7.5 mm, Wt = 40.3 ± 8.1 g); FLMB (TL = 150 ± 18 mm, Wt = 42.9 ± 14 g). Adult lake chubsuckers, fathead minnows and bluegills were simultaneously stocked to provide forage.

These fish were allowed to spawn and the 1983 year class of largemouth bass was sampled by a combination of seining and electrofishing techniques on August 10, 1983 and October 22, 1983.

Analysis of Offspring Production:

When sampled, individual largemouth bass offspring collected were weighed and measured, wrapped and numbered individually in aluminum foil and frozen at -20°C until further analysis. To determine the parentage of each offspring, samples of muscle and liver were subjected to vertical starch gel electrophoretic analysis to determine the genotype of each individual at the Mdh-B, Idh-B, Aat-B and Sod-A locus as described in Philipp et al. (1979,

1983). This genotypic determination was used to classify each individual as a NLMB, FLMB, F_1 hybrid or a non-F_1 hybrid (either an F_2 or a backcrossed hybrid).

RESULTS

Comparison of the two subspecies (NLMB and FLMB):

Reproduction in this pond was successful in both 1982 and 1983. The percentage production of each of the three distinguishable types of offspring (pure NLMB, pure FLMB, F_1 hybrids) and their relative abundance during the 1982 and 1983 growing seasons are shown in Tables 1 and 2. In addition, the relative sizes of each of these types in the samples collected during the 1982 and 1983 growing seasons are also shown in Tables 1 and 2. Both in 1982 and, especially in 1983, the bulk of the offspring produced in this pond were pure FLMB (63% and 88%, respectively). However, even though the numbers of pure NLMB were low (20% and 4%) in these two years, the NLMB were clearly the longest in length and weight at all sampling dates. Relative to the NLMB, the growth in length and weight, respectively, of the 1982 year class of FLMB and F_1 hybrid (a combination of both possible reciprocals, NxF and FxN) after the first growing season were: FLMB = 80.2% and 43.7%; F_1 = 82.5% and 48.1%, and after the second growing season were: FLMB = 82.8% and 40.0%; F_1 = 83.7% and 46.5%. This same relationship was seen in the 1983 year class, as well. However, much of this class was eliminated by the dominant class of one year olds produced in 1982.

Comparison of the four stocks (NLMB, NxF, FxN, FLMB):

Reproduction in this pond was successful in 1983. The percentage production of each of the four distinguishable types of offspring (pure NLMB, pure FLMB, F_1 hybrids, non-F_1 hybrids [backcrosses and F_2 generations]) and their relative abundance during the 1983 growing season are shown in Table 3.

In addition, the relative sizes of each of these types in the samples collected during the 1983 growing season are also shown in Table 3. In this pond in 1983, there was a great deal more F_1 production compared to Pond 1. This may have been due to the presence of the F_1 hybrids modifying the behavior of the two parental subspecies. There was also a significant production of non-F_1 hybrids indicating that, indeed, the NxF and FxN readily partake in spawning. Again, however, there was a fairly low degree of pure NLMB offspring production. However, in this pond the sizes of the NLMB at both sampling dates were overwhelmingly larger than FLMB, F_1, and non F_1 hybrids. In fact, the substantial increase in percentage of NLMB in the pond from August 10, 1983 to October 22, 1983, may reflect some cannibalism of the smaller largemouth bass by these NLMB.

DISCUSSION

It has been shown previously that the NLMB has greater growth properties and demonstrates greater overwinter survival in central Illinois than FLMB, NxF, or FxN stocks (Philipp et al. 1984a). The results presented here demonstrate the potentially dangerous impact that the introduction of FLMB can have on native NLMB populations: the production of groups of offspring, making up large portions of a year class, which are apparently much less fit for the northern environments than are the native NLMB stocks already present.

The climatic conditions in central Illinois during the spring spawning seasons of 1982 and 1983 were similar and perhaps a bit atypical. In both years there was a very slow and gradual warming with a relatively late arrival of sustained warm weather. As a result, largemouth bass spawning throughout central Illinois was relatively late. These types of spring conditions have been postulated to favor the production of the earlier spawning FLMB (Philipp et al. 1984b). This differential in spawning times is currently being evaluated.

It is interesting to note that the presence of F_1 hybrids with NLMB and FLMB (as in Pond 2) contributes greatly to the production of non-NLMB offspring. The following scenario appears likely to unfold when FLMB or F_1 hybrid largemouth bass are stocked into NLMB populations. A substantial portion of the year classes produced would be made up of non-NLMB. These would be slower growing fish, but eventually, these fish would enter the breeding pool. Thus, in succeeding generations the number of pure NLMB spawnings that would occur would become less and less. Eventually, it would appear that a totally hybrid or intergrade population would result. These fish would be slower growing than the original, resident NLMB stock, which was present prior

to its "augmentation" with FLMB. In all fairness, the conclusions reached with these data may only be valid for regions with climatic conditions similar to those of central Illinois. However, the advantages of NLMB over non-NLMB in our study certainly introduce valid questions for any FLMB stocking program outside of peninsular Florida. Research evaluating the impact of FLMB on native populations needs to be performed before programs for FLMB stocking are instituted. The genetic integrity of native stocks of largemouth bass from all regions of the country needs to be protected.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collections of Florida largemouth bass and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. We also wish to thank the Wisconsin Department of Natural Resources for permitting field collections of northern largemouth bass. We particularly want to thank Rick Cornelius and Jerry Perkins for their assistance in these collections. Finally, we wish to thank Dr. William F. Childers for advice and Dr. Paul R. Beaty, Dr. Bruce Taubert, Dr. Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Shirley Lowe, Jeffrey Van Orman, and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Addison, J. H. and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass, Micropterus salmoides (Lacepede), stocked in ponds in south Alabama. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 25:366-374.

Bottroff, L. J. and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass, Micropterus salmodies floridanus. California Fish and Game 64:4-23.

Clugston, J. P. 1964. Growth of the Florida largemouth bass, Micropterus salmoides floridanus (Le Sueur), and the northern largemouth bass, M. s. salmoides (Lacepede), in subtropical Florida. Transactions of the American Fisheries Society 93:146-154.

Davies, W. D. 1973. Florida largemouth bass in Alabama waters as a management tool. Annual Report of the Fisheries and Allied Aquacultures Department, Auburn University Agricultural Experiment Station, vol. 14.

Graham, L. K. 1973. The introduction of Florida largemouth bass in Missouri ponds. Dingell-Johnson Project F-1-R-22. Study I-14, Job No. 2. Missouri Department of Conservation.

Inman, C. R., R. C. Dewey and P. P. Durocher. 1976. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the 30th Annual Conference of the Southeastern Association of Game and Fish Commissions 30:1-17.

Latta, W. C. 1977. Survival and growth of Florida largemouth bass in ponds and lakes in Michigan. Michigan Department of Natural Resources, Dingell-Johnson Project F-35-R-3, Study XXVII:101-114.

Moyle, P. B. and N. J. Holzhauser. 1978. Effects of the introduction of Mississippi silverside (Menidia audens) and Florida largemouth bass (Micropterus salmoides floridanus) on the feeding habits of young-of-the-year largemouth bass in Clear Lake, California. Transactions of the American Fisheries Society 107:574-582.

Pelzman, R. J. 1980. Impact of Florida largemouth bass, Micropterus salmoides floridanus, introductions at selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. California Fish and Game 66:133-162.

Phillipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, Micropterus salmoides salmoides, and smallmouth bass, Micropterus dolomieui). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Philipp, D. P., C. Kaminski and G. S. Whitt. 1984a. Survival and growth of northern, Florida, and reciprocal F_1 hybrid largemouth bass in central Illinois. Pages 2-1 to 2-21 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Philipp, D. P., C. Kaminski and G. S. Whitt. 1984b. A comparison of the embryonic development of northern, Florida and reciprocal F_1 hybrid largemouth bass in different thermal environments. Pages 4-1 to 4-28 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Smith, S. L. and J. E. Crumpton. 1977. Evaluation of largemouth bass meristic characters. Dingell-Johnson Project F-22-11, Study V, Job 6. Florida Game and Freshwater Fish Commission Annual Report.

Smith, R. P. and J. L. Wilson. 1981. A growth comparison of two subspecies of largemouth bass in Tennessee ponds. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissions 34:25-30.

Van Orman, J. L., G. S. Whitt and D. P. Philipp. 1984. An evaluation of meristic characters to identify populations of northern, Florida, and F_1 hybrid largemouth bass. Pages 1-1 to 1-31 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

von Geldern, C. E., Jr. and D. F. Mitchell. 1975. Largemouth bass and threadfin shad in California. Pages 426-449 In R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

Wright, G. L. and G. W. Wigtil. 1981. Comparison of growth, survival, and catchability of Florida, northern, and hybrid largemouth bass in a new Oklahoma reservoir. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissions 34:31-38.

Zolczynski, S. J. and W. D. Davies. 1976. Growth characteristics of the northern and Florida subspecies of largemouth bass and their hybrid, and a comparison of catchability between the subspecies. Transactions of the American Fisheries Society 105:249-243.

Table 1. Percentage of sample and average size (total length and weight) of NLMB, F_1 hybrid and FLMB offspring produced in Pond 1 in 1982.

		Sample Dates			
		8/11/82 (n = 100)	10/2/82 (n = 100)	5/28/83 (n = 100)	10/7/83 (n = 100)
NLMB	% of total	20	31	15	25
	Ave TL (mm) (SD)	102 (26.9)	126 (25.5)	139 (20.9)	227 (46.6)
	Ave wt (g) (SD)	17.6 (7.6)	29.5 (24.3)	39.3 (16.6)	197 (141)
F_1 hybrid	% of total	17	19	28	37
	Ave TL (mm) (SD)	80 (13.1)	104 (11.3)	118 (12.6)	190 (21.1)
	Ave wt (g) (SD)	7.5 (4.1)	14.2 (4.4)	23.1 (7.9)	91.6 (34.9)
FLMB	% of total	63	50	57	38
	Ave TL (mm) (SD)	82 (14.6)	101 (17.4)	110 (17.9)	188 (14.9)
	Ave wt (g) (SD)	7.0 (4.1)	12.9 (7.2)	18.8 (10.3)	78.8 (24.1)

Table 2. Percentage of sample and average size (total length and weight) of NLMB, F_1 hybrid and FLMB offspring produced in Pond 1 in 1983.

		Sample Dates	
		8/14/83 (n = 100)	10/7/83 (n = 17)
NLMB	% of total	4	6
	Ave TL (mm)	73.5	163
	(SD)	(9.4)	-
	Ave wt (g)	4.9	51.7
	(SD)	(2.0)	-
F_1 hybrid	% of total	8	12
	Ave TL (mm)	65.5	142
	(SD)	(16.2)	(46.0)
	Ave wt (g)	3.76	35.1
	(SD)	(3.3)	(32.0)
FLMB	% of total	88	82
	Ave TL (mm)	52.6	144
	(SD)	(9.0)	(32.0)
	Ave wt (g)	1.62	37.0
	(SD)	(.8)	(17.1)

Table 3. Percentage of sample and average size (total length and weight) of NLMB, F_1 hybrid, non-F_1 hybrid and FLMB offspring produced in Pond 2 in 1982.

		Sample Dates	
		8/10/83 (n = 100)	10/22/83 (n = 100)
NLMB	% of total	9	21
	Ave TL (mm)	120	184
	(SD)	(8.7)	(7.9)
	Ave wt (g)	24.9	84.6
	(SD)	(5.9)	(14.5)
F_1 hybrid	% of total	35	16
	Ave TL (mm)	56	125
	(SD)	(13.5)	(43.0)
	Ave wt (g)	2.7	34.6
	(SD)	(3.6)	(26.4)
non-F_1 hybrid	% of total	24	27
	Ave TL (mm)	52	89
	(SD)	(15.5)	(22.1)
	Ave wt (g)	2.3	9.8
	(SD)	(2.7)	(12.4)
FLMB	% of total	32	36
	Ave TL (mm)	40	70
	(SD)	(8.9)	(15.7)
	Ave wt (g)	.95	4.2
	(SD)	(.36)	(2.4)

STUDY 103
JOB 1

CHAPTER 4

A Comparison of the Embryonic Development of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass in Different Thermal Environments

David P. Philipp[1,2], Christine Kaminski[1], and G. S. Whitt[3,1]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
328 Mumford Hall
University of Illinois
Urbana, Illinois 61801

[3]Department of Genetics and Development
515 Morrill Hall
University of Illinois
Urbana, Illinois 61801

ABSTRACT

The two subspecies of largemouth bass, *Micropterus salmoides salmoides* and *M. s. floridanus*, naturally occur in different geographic and climatic regions of the United States, with hybrids occurring in a zone of intergradation between these regions. Genetic differences between these stocks are reflected by differing physiological responses to the thermal environment. To determine the extent to which these differences contribute to natural geographic separation of the subspecies we produced the embryos of the four genetic stocks of largemouth bass (*M. s. salmoides*, *M. s. floridanus*, *M. s. salmoides* ♀ x *M. s. floridanus* ♂, and *M. s. floridanus* ♀ x *M. s. salmoides* ♂ *in vitro* using artificial fertilization techniques. The developmental success of the embryos and the schedule of embryogenesis for each stock were compared at each of a series of different temperatures. The developmental success of each stock at each incubation temperature was determined by total hatching percentage. In addition, the thermal requirements for embryonic development of each of these four stocks were compared by determining the α-threshold temperatures of development, as well as the number of thermal developmental units required to reach each of 22 key embryonic stages. Significant differences in the thermal requirements for embryogenesis exist among these different stocks of largemouth bass. The implications of these findings on current and future largemouth bass management programs are discussed.

INTRODUCTION

Two subspecies of largemouth bass, the northern (Micropterus salmoides salmoides) and the Florida (M. s. floridanus), were originally described by Bailey and Hubbs (1949). Since that time a number of studies designed to assess the differences between the subspecies have been conducted (Hart 1952, Clugston 1964, Addison and Spencer 1972, Zolczynski and Davies 1976, Inman et al. 1977, Cichra et al. 1981, Smith and Wilson 1981 and Wright and Wigtil 1981). These studies have shown that a variety of biological differences exist between these two subspecies.

Our recent electrophoretic survey of the genetic structure of largemouth bass populations in the United States (Philipp et al. 1981, 1982, 1983) has quantified the degree of genetic divergence between these two subspecies. We have described a fast and reliable means of identifying pure northern, pure Florida or intergrade populations of largemouth bass, that of the electrophoretic determination of the allele frequencies at the Idh-B and Aat-B loci. Meristic and morphometric counts routinely used in the past by fisheries biologists to distinguish these types of populations (Bryan 1969, Addison and Spencer 1972, Buchanan 1973, Inman et al. 1977, Moyle and Holzhauser 1978 and Bottroff and Lembeck 1978) are ambiguous and, hence, unreliable. In at least one study (Pelzman 1980) electrophoretic techniques also proved unreliable since analyses were inappropriate and data misinterpreted.

Our biochemical genetic analyses of largemouth bass populations (Philipp et al. 1981, 1982, 1983) also demonstrate that the intergrade zone between the ranges of the two pure subspecies, as it exists today (northern Florida, Mississippi, Alabama, Georgia, South Carolina, North Carolina, Virginia and Maryland), is much more extensive than that originally described (Bailey and

Hubbs 1949). As a result, the previously assigned genetic status of the stocks of largemouth bass used as representatives of the two pure subspecies in many previous studies appears questionable. In the absence of genetic confirmation of the stocks used, the data generated by these earlier studies and their resulting conclusions must be considered very cautiously.

In 1959, Florida largemouth bass, or at least largemouth bass containing some portion of the genome of _M. s. floridanus_, were introduced into certain waters in California (Sasaki 1961). The subsequent establishment of largemouth bass populations with a substantial proportion of the gene pool contributed by the Florida subspecies has been well documented (Smith 1971, von Geldern and Mitchell 1975, Bottroff and Lembeck 1978, Moyle and Holzhauser 1978). The populations of largemouth bass which existed in California prior to 1959 were the result of introductions of northern largemouth bass imported from Illinois in 1891 (Shebley 1917) and were not the result of immigration and natural selection since the state of California is well outside the native range of largemouth bass (MacCrimmon and Robbins 1975). It is not surprising that the introductions of largemouth bass from Florida were successful in southern California considering the climatic conditions which more closely resemble those of Florida than of Illinois.

The apparent success of the recent introductions of _M. s. floridanus_ in southern California waters and the demand from fishermen for more and larger largemouth bass has apparently been the impetus for a number of states to initiate Florida largemouth bass programs. These programs range from controlled research with limited introductions to large scale propagation and widespread stockings. Unlike California, many of these states already contained populations of naturally established largemouth bass. Due to the lack of reliable, quantitative data concerning the genetic differences of the

native and introduced stocks and the relative fitnesses of these stocks in different environments, the long term effects of these introductions upon the existing largemouth bass fisheries cannot be accurately predicted at this time. However, the impact of these stocking programs on the genetic integrity of the native largemouth bass populations in these states could potentially be catastrophic. We have postulated (Philipp et al. 1981, 1982, 1983) that when alleles present in the Florida subspecies are introduced into a population of the northern subspecies the unique genic combinations initially present may become irreversibly altered. In addition, some of these new Florida alleles are likely to be less fit for these new environments. Although the resulting populations may be genetically sufficient in terms of short term survival, the long term effect of this genetic mixture would be a lowering of fitness in the recipient population. For these reasons, we have recommended that programs designed to introduce the Florida subspecies into states outside of peninsular Florida but within the native range of the largemouth bass be halted, until appropriate research has determined the effects on the recipient populations.

Factors which affect year-class strength among largemouth bass populations are complex. It has been suggested that spawns hatched early in a given year may suffer substantially less mortality than those hatched later in the season (Aggus and Elliot 1975). Therefore, in mixed populations, differential thermal effects upon the reproductive behavior and the rate and success of development between northern and Florida largemouth bass embryos may play a crucial role in determining the relative contribution of each stock to the total year-class production. The current study was designed to assess thermal effects upon the rate and success of development of embryos of genetically defined stocks of both pure subspecies, *M. s. salmoides* and *M. s. floridanus*, and of both reciprocal F_1 hybrids.

MATERIALS AND METHODS

Parental Stocks. Northern largemouth bass were collected from Clinton Lake, Illinois. Electrophoretic analyses of individuals from this population showed the frequency of the northern Idh-B allele (Idh-B^1) and of the sum of the northern Aat-B alleles (Aat-B^1 and Aat-B^2) both to be 1.000, confirming that this population consisted of pure *M. s. salmoides* (Philipp et al. 1981, 1982, 1983). Florida largemouth bass were collected from Lake Dora, Florida. Electrophoretic analyses of individuals from this population showed the frequency of the Florida Idh-B allele (Idh-B^3) and of the sum of the Florida Aat-B alleles (Aat-B^3 and Aat-B^4) both to be 1.000, confirming that this population consisted of pure *M. s. floridanus* (Philipp et al. 1981, 1982, 1983). Adult males and females from only these two genetically confirmed populations were used as brood stock throughout this study.

Production of Embryos. Florida largemouth bass (FLMB) and Florida ♀ x northern ♂ F_1 hybrid largemouth bass (F x N) embryos were produced at the Florida Game and Freshwater Fish Commission, Eustis Fisheries Research Laboratory, as follows. Northern largemouth bass (NLMB) males were collected in December, 1980 from Clinton Lake, Illinois and held indoors at 10°C at the Illinois Natural History Survey. On February 2, 1981, these fish were marked with a right pectoral clip, transported to Florida, and allowed to reach reproductive readiness in outdoor earthen ponds at the Richloam State Fish Hatchery. During February, 1981 ripe male NLMB, retrieved from the hatchery and ripe male FLMB, collected from Lake Dora by electrofishing, were brought to the Eustis laboratory where they were held in indoor flow-through raceways. On February 21 and 26, 1981, mature, ripe female FLMB were collected from Lake Dora, Florida by electrofishing and brought to the Eustis laboratory. The

eggs from individual FLMB females were manually stripped into a bowl, mixed, and split into two equivalent aliquots in separate petri dishes. One aliquot was fertilized with sperm from a single FLMB male and the other with sperm from a single NLMB male using methods described in Childers (1967) and Philipp et al. (1979).

Northern largemouth bass (NLMB) and northern ♀ x Florida ♂ F_1 hybrid largemouth bass (N x F) embryos were produced in essentially the same manner with the following exceptions. FLMB males, collected from Lake Dora in February, 1981, were marked with a left pectoral clip and air shipped to the Illinois Natural History Survey to be held indoors at 10°C until they were stocked outdoors in INHS earthen ponds on March 10, 1982 and allowed to reach reproductive readiness. During May, 1981, FLMB males retrieved from INHS ponds and NLMB males collected from Clinton Lake by electrofishing were brought to the INHS laboratory and held indoors. On May 13, 22, and 28, mature, ripe NLMB females were collected from Clinton Lake by electrofishing and brought to the INHS laboratory. NLMB and N x F embryos were produced in the laboratory using the procedures described previously for producing FLMB and F x N embryos.

Rearing the Embryos. Procedures and equipment used for rearing the FLMB and F x N embryos were identical to those used for rearing the NLMB and N x F embryos and are as follows. The very dense egg-sperm mixtures produced as described above were allowed to stand in minimal water for 5 minutes to allow for completion of the fertilization process. These newly fertilized embryos were transferred to plastic containers holding about 2-3 cm of water. The eggs were thinly spread within these containers and incubated for one hour at 24°C until initial cleavage. For each cross, samples of 100 normally cleaving

eggs (2-4 cell stage) were then removed, transferred to each of a number of glass finger bowls containing 24°C water, and allowed to adhere to the glass. The embryos in each finger bowl were acclimated over a one hour period to a specified test temperature, covered with nylon netting to allow water circulation but prevent loss of embryos, and immersed in an aerated, filtered 75 liter constant temperature bath at the test temperature. The temperature of each incubation bath was monitored continuously using dual water temperature probes and a calibrated 12 channel recorder (Chino Works, Ltd., Tokyo, Japan, Model EW 1200).

Morphological development of each set of embryos reared at each temperature was visually monitored using a dissecting microscope. Prior to retinal pigmentation, each set of embryos was monitored every 2-4 hours. After retinal pigmentation had progressed, the frequency of visual observation was reduced to every 6-8 hours. The times required to reach each of 22 key morphological stages was recorded for the embryos at each test temperature. Dead eggs or embryos were counted, removed, and recorded at each visual inspection. A photographic record of the morphological development of these embryos was made using an Olympus JM dissecting microscope with an Olympus photographic attachment and an Olympus OM-2 camera.

Data Analysis. The success of development was determined by calculating for each set of embryos the percentage of eggs which hatched, and of these the proportion which appeared normal.

The α-threshold temperature of development, a theoretical thermal value below which embryonic development ceases (Childers 1967) was determined as follows. For each set of embryos raised at each temperature, the time to reach each of 11 readily identifiable morphological stages was determined and

the cumulative average temperature of development was calculated from the thermal record for each of these stages. For each of the four genetic stocks (NLMB, N x F, F x N and FLMB) at each developmental stage, average cumulative temperature was plotted versus the inverse of developmental time in hours. The regression equation describing the linear relationship for each of the four stocks was determined from these values (10-12 data points per stage for the FLMB and F x N embryos and 16-18 data points per stage for the NLMB and N x F embryos). The x-intercept determined from each equation is the α-threshold temperature determined for that stock using the values for that developmental stage. The final α-threshold temperature for each genetic stock was determined as the average of the values for these eleven stages.

The number of thermal developmental units (TDU) required for an embryo to reach a given stage of development is defined as the number of degree-hours above the α-threshold temperature which needs to be accumulated. TDU values were calculated using the final value for the α-threshold temperature together with the times of development and average cumulative temperatures for each set of embryos of each genetic stock at each of 22 morphological stages of development.

RESULTS

Patterns of Development. No qualitative differences in morphogenetic events could be detected among the four genetic stocks of largemouth bass studied (NLMB, N x F, F x N and FLMB). Therefore, the following morphological features and their developmental sequence hold for the embryogenesis of all four stocks.

The cortical reaction occurs immediately upon fertilization with the cortical layer becoming raised by one minute post-fertilization. There follows a confluence of cytoplasm around the zygote nucleus and the resultant formation of the germinal disc. Cleavage is telolecithal, with the initial formation of the two cell stage occurring within one hour at 24°C. Rates of development during the rest of this study depended upon temperature and will be discussed in detail later. Figures 1A through 1P illustrate the periods of morphogenesis of one set of embryos, FLMB embryos raised at 24.2° ± 0.4°C. These serve to illustrate the patterns of development for each of the four stocks of largemouth bass embryos studied (NLMB, N x F, F x N and FLMB). The developmental progression paralleled that described for other centrarchid species (Morgan 1951, Balon 1959, Champion and Whitt 1976, Taubert 1977).

Early cleavage (Fig. 1A) continues, eventually resulting in blastula formation (Fig. 1B). Epiboly commences (Fig. 1C) and progresses through the yolk plug stage (Fig. 1D) prior to formation of a body axis (Fig. 1E). Embryonic development continues with the formation of increasing numbers of somite pairs (Fig. 1F). The optic cup and pericardial cavity continue to develop. The embryonic heart begins beating and true circulation with colorless blood starts shortly afterward (Fig. 1G). The tail increases in length and body contractions increase in number and severity. Red blood cells

containing hemoglobin appear just prior to hatching (Fig. 1H). Hatching occurs fairly synchronously for most eggs at normal temperatures (90% of eggs hatched in a 3-4 hour period at 24°C). The newly hatched embryo is still quite underdeveloped (Fig. 1I), having only limited and unguided movement. After hatching, the heartbeat becomes more vigorous and the first pigment granules become visible in the retina (Fig. 1J). Pectoral and pelvic fins form as the retina becomes quite darkly pigmented (Fig. 1K). The tapetum lucida develops to give the eye first a silvery then a golden appearance (Fig. 1L). During this period the rudiments of many of the internal organs, such as the liver, urinary bladder, intestine and swim bladder, are developing. Following this stage the jaw begins to form (Fig. 1M) and eventually starts to open and close regularly (Fig. 1N), as body pigmentation commences. At this point, the embryos begin to swim off the substrate with increasing regularity (Fig. 1O), eventually reaching a free swimming stage and active feeding as yolk sac absorption is completed (Fig. 1P). Our monitoring of the development of largemouth bass ceased at this point and did not include an investigation of the subsequent fry and fingerling stages.

Effect of Temperature on Developmental Success. The percentage of hatched embryos (both normal and abnormal) was calculated for each set of embryos reared. These results are given in Table 1. The results for the NLMB and FLMB embryos are also compared graphically in Figure 2. Comparing the two pure subspecies, the FLMB embryos exhibited optimal hatching rates at temperatures (21-24°C) substantially higher than those exhibited by the NLMB embryos (17-22°C) (Table 1, Fig. 2). These temperature ranges are somewhat lower than the values reported by McCormick and Wegner (1981). In addition, the lower thermal limits for successful hatching occurred at temperatures

higher for FLMB embryos than for NLMB embryos. Conversely, the upper thermal limits for successful hatching occurred at temperatures lower for NLMB embryos than for FLMB embryos. The percentage of hatched embryos which appeared morphologically abnormal was less than 15% at each test temperature except for the NLMB and N x F at 30.5°C (100% abnormal in each case); the NLMB and N x F at 30.3° (68.6% and 75.0% abnormal, respectively); and the FLMB and F x N at 18.5° (16.3% and 18.5% abnormal, respectively).

The tendency for both hybrids to exhibit developmental patterns more similar to the maternal than to the paternal subspecies was notable (Table 1). Although the relationship between temperature and hatching percentage for the N x F embryos paralleled that for the NLMB embryos, the absolute hatching percentage for the N x F embryos was less than that for the corresponding NLMB embryos at most temperatures. The relationship between temperature and hatching percentage for the F x N embryos paralleled that for the FLMB embryos as well. However, the absolute hatching percentage for the F x N embryos was greater than that for the corresponding FLMB embryos at most temperatures.

Thermal Limits of Embryogenesis. The α-threshold temperatures of development were calculated for each of the four stocks of largemouth bass (NLMB, N x F, F x N and FLMB) using data from each of 11 distinct developmental stages (body axis, 10, 15, 20 and 25 somites, heartbeat, 50% hatching, onset of light and even retinal pigmentation, and jaw movement). We chose to monitor these eleven stages because they were easily identifiable visually. Because the rates of development for the NLMB and N x F embryos were similar at all of the temperatures studied, the data for these two stocks were combined for α-threshold temperature calculations. Data for the FLMB and F x N embryos were similarly combined (Table 2).

The overall α-threshold value for each stock was calculated as the average of the values calculated individually from the data for each of the eleven individual developmental stages used (Table 2). The overall α-threshold temperature determined for the NLMB and N x F embryos, 12.62 ± 0.27, was significantly higher ($P<0.01$, Wilcoxon's signed-ranks test, Sokal and Rohlf 1973) than that determined for the FLMB and F x N embryos, 11.52 ± 0.77. In addition, the stage-specific α-threshold temperatures calculated for the NLMB and N x F embryos were higher than those calculated for the FLMB and F x N embryos at each of the developmental stages used (Table 2).

The upper thermal limits of development can be estimated by extrapolating from the decrease in hatching percentage for a few of the trials at elevated temperatures, >30°C (Table 1). NLMB and N x F embryos did not hatch at temperatures above 30.5°C and even at temperatures between 30° and 30.5°C there was an extremely high percentage of morphological deformities. These results agree closely with those reported by McCormick and Wegner (1981) for northern largemouth bass embryos. FLMB and F x N embryos, however, had successful hatching at 30.8°C, although at 31.7°C no successful hatching was observed. It appears, therefore, that the upper thermal limit for successful embryonic development of FLMB and F x N embryos is 0.5°-1.0°C higher than that for the NLMB and N x F embryos.

Thermal Requirements of Embryogenesis. Using the overall α-threshold temperatures calculated for the NLMB and N x F embryos, as well as for the FLMB and F x N embryos, 12.62 ± 0.27 and 11.51 ± 0.77, respectively, the numbers of thermal developmental units (TDU) required to reach each of 22 development stages were calculated and are shown in Table 3. For the first twelve stages (mid-blastula through end of hatching), embryos from all stocks required comparable thermal input (TDU). However, for the remainder of

development, the FLMB and F x N embryos required substantially greater thermal input (TDU) than the NLMB and N x F embryos, ranging from 11.3% more for the onset of retinal pigmentation to 30.1% more for the final yolk absorption stage.

DISCUSSION

The environmental thermal regimes which exist among the aquatic communities throughout the United States are extremely varied. As such, temperature potentially plays a major role in the processes of natural selection serving to genetically tailor stocks of largemouth bass to specific environments (Childers 1975). Genetic differences between the two subspecies of largemouth bass, M. s. salmoides and M. s. floridanus, have been assessed and documented (Philipp et al. 1981, 1982, 1983). Indeed, a variety of physiological and behavioral differences most likely result from these genetic differences, each stock reacting somewhat differently to variable thermal conditions. Because these physiological/genetic differences among the two subspecies and their hybrids determine relative fitness in a given environment, a quantitative assessment of thermal response differences among these stocks is critically needed for effectively formulating present and future management programs for largemouth bass.

Although Swingle (1956) reported that major largemouth bass spawning periods occur between 68-75°F (20-24°C), Chew (1975) documented spawning of FLMB in Lake Weir, Florida at 59°F (15°C). Fluctuations in water temperature during the spring spawning season are usually not as severe in lakes in peninsular Florida as in lakes in more northerly regions of the country. Largemouth bass spawns in Florida lakes during the very initial periods of the seasonal spawning period may be less likely to result in the exposure of eggs or embryos to very cold temperatures (<12°C) than would correspondingly early spawns in northern regions. It has been suggested that, in mixed populations the Florida subspecies tends to spawn earlier in the season, at lower temperatures, than the northern subspecies of largemouth bass (Hunsacker and

Crawford 1964, Bottroff and Lembeck 1978, Moyle and Holzhauser 1978). Observations of spawning in ponds at the Illinois Natural History Survey during 1981 agree with this suggestion (unpublished results); further experimentation is currently underway to verify this.

Interestingly, the α-threshold temperature of development of the FLMB (11.52 ± 0.77) is significantly lower than that of the NLMB (12.62 ± 0.27). This relationship suggests a survival strategy of delayed spawning in the northern subspecies which presumably reflects a more variable, as well as a lower mean water temperature during spawning periods in the north. In addition, the northern subspecies apparently can use environmental incubation temperatures more efficiently. This is evidenced by the significantly lower number of thermal developmental units required by the NLMB embryos to reach the free swimming/actively feeding stage ($2,031 \pm 229$) than that required by the FLMB ($2,620 \pm 203$). Only at temperatures below about 16.2°C would FLMB eggs require less incubation time than NLMB eggs to reach the free swimming/actively feeding stage. At "normal" incubation temperatures NLMB embryos reach this stage sooner than FLMB embryos. For example, at 20°C NLMB embryos would reach the free swimming/actively feeding stage at 272 hrs, whereas the FLMB would require 309 hrs to reach this stage. This more rapid development gives NLMB embryos a clear advantage over FLMB embryos at temperatures normally encountered during the incubation periods in the wild (17°-25°C).

Interestingly, comparing embryological development of the two subspecies, major differences in numbers of thermal developmental units required, occurs at retinal pigmentation and later. These periods of embryonic development are associated with organogenesis and are periods during embryogenesis in which many of the genes encoding metabolic enzymes become activated (Philipp et al.

1979). This observation is consistent with the hypothesis that many of the metabolic processes up to the retinal pigmentation period may be under the control of maternal enzymes or m-RNA molecules synthesized during oogenesis. This observation also suggests that the two subspecies may have diverged in certain of their gene regulatory processes and now respond differently to temperature. The differences in the initial timing and levels of enzyme expression in developing embryos of these four stocks (NLMB, N x F, F x N and FLMB) strongly support this suggestion (Philipp et al. 1983, Parker, Philipp and Whitt, unpublished results).

The two subspecies also differ in hatching success at various incubation temperatures. The peak of successful hatching for the NLMB occurs at lower temperatures (17-22°C) than for the FLMB (21-24°C). In addition, the FLMB embryos apparently survive higher incubation temperatures than the NLMB (Fig. 2). These relationships most probably provide the NLMB and FLMB embryos an advantage in their respective environments.

It is interesting that the N x F embryos have consistently lower hatching percentages than their thermal counterpart NLMB embryos, but that the opposite holds true for the F x N and FLMB embryos (Table 1). This set of relationships is an example of the inherent differences between reciprocal hybrids. We have postulated that these non-additive asymmetrical responses by reciprocal hybrids result from the differences in the interaction of the paternal genes with the maternal gene effector molecules (Whitt et al. 1977, Philipp et al. 1983).

Our analyses reveal that the developing embryos of NLMB and FLMB react differently to differing thermal conditions. We feel that these differences have resulted from these two subspecific genomes evolving independently in response to different thermal selective pressures. We postulate that to better survive colder climates, NLMB have evolved a reproductive strategy of

delayed spawning until higher threshold temperatures are encountered. This helps prevent premature spawns which could be destroyed by severe cold fronts. In addition, NLMB have evolved schedules of embryonic gene expression, and thus mechanisms of controlling embryonic metabolism and morphogenesis, that operate more efficiently and rapidly at lower temperatures. These patterns of gene expression also allow NLMB embryos to reach maximal hatching rates at lower temperatures than FLMB embryos. However, during the protracted spawning seasons which occur in peninsular Florida, early spawns produce individuals as much as 3 months earlier than late spawns. FLMB appear to have evolved a reproductive strategy which allows them to spawn at lower temperatures than NLMB. These early spawned fish have distinct competitive advantages over ones spawned later. FLMB embryos have also evolved an increased tolerance to higher incubation temperatures than NLMB embryos. Thus, FLMB embryos are better able to tolerate the warmer water temperatures found in peninsular Florida during periods of the largemouth bass spawning season.

Divergence of developmental response to temperature in the two subspecies of largemouth bass has significant implications for the management programs which concern this species. The extent of differential fitness probably varies greatly depending upon the geographic location of the population, the physical characteristics of the body of water being considered, and the specific weather conditions during a given spawning season. However, it is not unreasonable to assume that in the long run, stocks of largemouth bass which are introduced into inappropriate thermal environments will not perform as well as stocks which are introduced into thermal environments for which they have been genetically tailored. We feel that our findings support a recommendation that management programs which result in mixing the two subspecies be discontinued. Precautions must be taken to protect the genetic

integrity of the two subspecies. Specifically, we recommend that no largemouth bass containing any portion of the genome of the Florida subspecies be propagated for introduction into waters north of the intergrade zone as described by Philipp et al. (1981, 1982, 1983). We also recommend, of course, that the introduction of largemouth bass containing any portion of the genome of the northern subspecies into waters of peninsular Florida similarly be prohibited.

For maximum effectiveness, it is imperative that sound genetic principles be incorporated into current and future fisheries management programs (Smith 1981, Philipp et al. 1981, 1982, 1983). Individual genetic stocks which comprise a species must be identified, characterized, and recognized as distinct units justly requiring their individual consideration in management efforts. The genetic integrity of discrete stocks must be preserved, because as Ryman (1981) so aptly stated, "Genetic variation in natural populations constitutes a biological resource that must be properly managed so as not to reduce future opportunities for use of the resource."

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collection and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. Finally, we wish to thank Dr. Bruce Taubert, Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Addison, J. H., and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass, Micropterus salmoides (Lacepede), stocked in ponds in south Alabama. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 25:366-374.

Aggus, L. R., and G. V. Elliot. 1975. Effect of cover and food on year-class strength of largemouth bass. Pages 317-322 in R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

Bailey, R. M., and C. L. Hubbs. 1949. The black basses (Micropterus) of Florida, with description of a new species. University of Michigan Museum of Zoology Occasional Papers 516:1-40.

Balon, E. 1959. Spawning of Lepomis gibbosus (Linne 1758) acclimatized in the backwaters of the Danube and its development during the embryonic period. Zeitschrift fur Fisheries Deren Hilfswissen 8:1-27.

Bottroff, L. J., and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass, Micropterus salmodies floridanus. California Fish and Game 64:4-23.

Bryan, C. F. 1969. Variation in selected meristic characters of some basses, Micropterus. Copeia 1969:370-373.

Buchanan, J. P. 1973. Separation of the subspecies of largemouth bass, Micropterus salmoides salmoides and M. s. floridanus and intergrades by use of meristic characters. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 27:608-619.

Champion, M. J., and G. S. Whitt. 1976. Synchronous allelic expression at the glucosephosphate isomerase A and B loci in interspecific sunfish hybrids. Biochemical Genetics 14:723-737.

Chew, R. L. 1974. Early life history of the Florida largemouth bass. Florida Game and Fresh Water Fish Commission Fishery Bulletin 7, 76 pp.

Childers, W. F. 1967. Hybridization of four species of sunfishes (Centrarchidae). Illinois Natural History Survey Bulletin 29:159-214.

Childers, W. F. 1975. Bass genetics as applied to culture and management. Pages 362-372 in R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

Cichra, C. E., W. E. Neill, and R. L. Noble. 1981. Differential resistance of northern and Florida largemouth bass to cold shock. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 34:19-24.

Clugston, J. P. 1964. Growth of the Florida largemouth bass, *Micropterus salmoides floridanus* (Le Sueur), and the northern largemouth bass, *M. s. salmoides* (Lacepede), in subtropical Florida. Transactions of the American Fisheries Society 93:146-154.

Hart, J. S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. University of Toronto Biological Series Publication 60, Ontario Fish Research Laboratory 72:1-79, Toronto, Canada.

Hunsacker, D., and R. W. Crawford. 1964. Preferential spawning behavior of the largemouth bass. Copiea 64:240-241.

Inman, C. R., R. C. Dewey, and P. P. Durocher. 1977. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissions 30:1-17.

MacCrimmon, H. R., and W. H. Robbins. 1976. Distribution of the black basses in North America. Pages 56-66 *In* R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

McCormick, J. H. and J. A. Wegner. 1981. Responses of largemouth bass from different latitudes to elevated water temperatures. Transactions of the American Fisheries Society 110:417-429.

Morgan, G. D. 1951. Life history of the bluegill sunfish, *Lepomis macrochirus*, of Buckeye Lake, Ohio. Journal of the Science Laboratory-Dennison University 41:21-49.

Moyle, P. B., and N. J. Holzhauser. 1978. Effects of the introduction of Mississippi silverside (*Menidia audens*) and Florida largemouth bass (*Micropterus salmoides floridanus*) on the feeding habits of young-of-the-year largemouth bass in Clear Lake, California. Transactions of the American Fisheries Society 107:574-582.

Pelzman, R. J. 1980. Impact of Florida largemouth bass, *Micropterus salmoides floridanus*, introductions at selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. California Fish and Game 66:133-162.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, *Micropterus salmoides salmoides*, and smallmouth bass, *Micropterus dolomieui*). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (*Micropterus salmoides*) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1982. Biochemical genetics of largemouth bass. Electric Power Research Institute, Palo Alto, California, 262 pp.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Ryman, N. 1981. Conservation of genetic resources: experiences from the brown trout (*Salmo trutta*). *In* N. Ryman, editor, Fish Gene Pools. Preservation of Genetic Resources in Relation to Wild Fish Stocks. Ecological Bulletins (Stockholm) 34:61-74.

Sasaki, S. 1961. The introduction of Florida largemouth bass into San Diego County. California Fish and Game, Inland Fisheries Administration, Report 61-11. Sacramento, California, USA.

Shebley, W. H. 1917. History of the introduction of food and game fishes into the waters of California. California Fish and Game 3(1):3-12.

Smith, G. 1971. Florida largemouth bass in southern California. Florida Wildlife 25(4):30-33.

Smith, M. H., and R. K. Chesser. 1981. Rationale for conserving genetic variation of fish gene pools. *In*: N. Ryman, editor, Fish Gene Pools. Preservation of Genetic Resources in Relation to Wild Fish Stocks. Ecological Bulletins (Stockholm) 34:13-20.

Smith, R. P., and J. L. Wilson. 1981. Growth comparison of two subspecies of largemouth bass in Tennessee ponds. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 34:35-30.

Sokal, R. R., and F. J. Rohlf. 1973. Introduction to Biostatistics. W. H. Freeman and Co., San Francisco, California, 368 pp.

Swingle, H. S. 1956. Appraisal of methods of fish population study--Part IV determination of balance in farm fish ponds. Transactions of the 21st North American Wildlife Conference 21:289-322.

Taubert, B. D. 1977. Early morphological development of the green sunfish, *Lepomis cyanellus*, and its separation from other larval *Lepomis* species. Transactions of the American Fisheries Society 106:445-448.

Whitt, G. S., D. P. Philipp, and W. F. Childers. 1977. Aberrant gene expression during the development of hybrid sunfishes (*Perciformes, Teleostei*). Differentiation 9:97-109.

von Geldern, C. E., Jr., and D. F. Mitchell. 1975. Largemouth bass and threadfin shad in California. Pages 426-449 in R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

Wright, G. L., and G. W. Wigtil. 1981. Comparison of growth, survival, and catchability of Florida, northern, and hybrid largemouth bass in a new Oklahoma reservoir. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 34:31-38.

Zolczynski, S. J., and W. D. Davies. 1976. Growth characteristics of the northern and Florida subspecies of largemouth bass and their hybrid, and a comparison of catchability between the subspecies. Transactions of the American Fisheries Society 105:240-243.

Table 1. Hatching percentages of largemouth bass eggs incubated at various temperatures.

Largemouth Bass Stocks							
NLMB		N x F		F x N		FLMB	
Temp	% Hatch	Temp	% Hatch	Temp	% Hatch	Temp	% Hatch
34.0	0	34.0	0	36.0	0	36.0	0
30.9	0	30.9	0	31.7	0	31.7	0
30.5	31	30.5	30	30.8	47	30.8	41
30.3	35	30.3	24				
28.8	35	28.8	28				
28.4	45	28.4	37	28.4	52	28.4	54
28.0	56	28.0	53				
27.5	59	27.5	57	27.7	63	27.7	52
27.4	49	27.4	55	27.6	54	27.6	46
26.6	58	26.6	53				
26.1	54	26.1	49				
25.3	62	25.3	55	24.5	66	24.5	61
24.5	68	24.5	61	24.2	65	24.2	56
22.6	70	22.6	66	23.0	61	23.0	57
22.5	67	22.5	64	22.8	71	22.8	62
17.8	72	17.8	60	19.8	54	19.8	55
17.2	65	17.2	61	18.5	54	19.8	55
17.0	67	17.0	62				

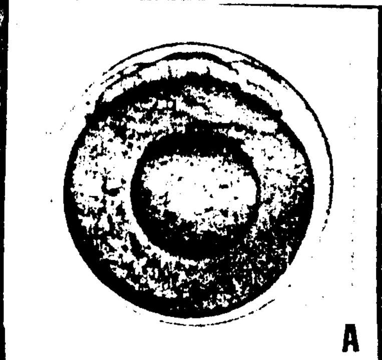
Table 2. Alpha threshold temperatures of development for the four stocks.

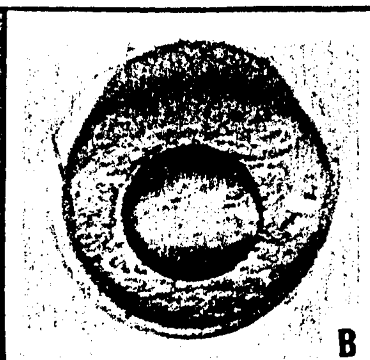
Developmental Stages	Embryos of NLMB and F_1 hybrid (NxF)	Regression Equation (Correlation Coefficient)	Embryos of FLMB and F_1 hybrid (FxN)	Regression Equation (Correlation Coefficient)
Body axis formation	12.17	$y = 0.00492x - 0.0599$ (0.944)	12.07	$y = 0.00550x - 0.0664$ (0.980)
10 somites	12.49	y $0.00400x - 0.0499$ (0.891)	10.67	$y = 0.00394x - 0.0420$ (0.975)
15 somites	12.58	$y = 0.00358x - 0.0451$ (0.905)	11.10	$y = 0.00354x - 0.0393$ (0.984)
20 somites	12.79	$y = 0.00334x - 0.0427$ (0.928)	11.41	$y = 0.00322x - 0.0367$ (0.990)
25 somites	12.69	$y = 0.00300x - 0.0380$ (0.914)	12.23	$y = 0.00314x - 0.0385$ (0.990)
Initial heartbeat	12.72	$y = 0.00272x - 0.0346$ (0.926)	12.22	$y = 0.00277x - 0.0339$ (0.990)
50% hatch	12.76	$y = 0.00184x - 0.0235$ (0.972)	12.18	$y = 0.00184x - 0.0224$ (0.994)
Onset of retinal pigmentation	12.89	$y = 0.00162x - 0.0209$ (0.900)	12.52	$y = 0.00147x - 0.0184$ (0.974)
Light retinal pigmentation	12.60	$y = 0.00131x - 0.0164$ (0.983)	11.14	$y = 0.00113x - 0.0126$ (0.970)
Even retinal pigmentation	12.15	$y = 0.00110x - 0.0134$ (0.986)	10.14	$y = 0.000904x - 0.00917$ (0.957)
Initial jaw movement	13.03	$y = 0.000877x - 0.0115$ (0.987)	11.01	$y = 0.000670x - 0.00738$ (0.958)
Mean	12.62		11.52	
Standard Deviation	0.27		0.77	

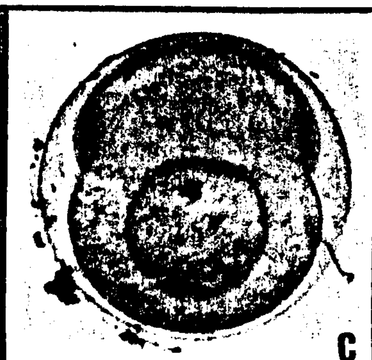
Table 3. Developmental unit requirements of the four stocks.

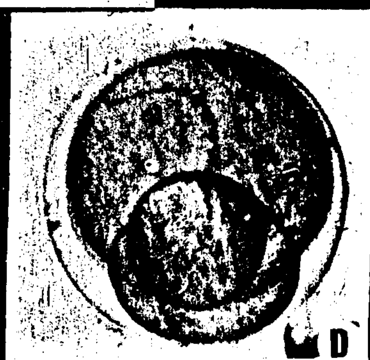
Developmental Stages	Embryos of NLMB and NxF F_1 hybrid (SD)	Embryos of FLMB and FxN F_1 hybrid (SD)
Mid-blastula	69 (14)	53 (11)
1/2 Epiboly	123 (29	122 (24)
Yolk plug	166 (23)	158 (23)
Initial body axis	197 (23)	193 (22)
10 somites	249 (36)	240 (19)
15 somites	278 (33)	275 (20)
20 somites	312 (37)	310 (19)
25 somites	345 (30)	340 (18)
Heartbeat	379 (41)	385 (18)
Begin hatch	503 (48)	508 (17)
50% hatch	556 (48)	576 (17)
End hatch	619 (53)	660 (13)
Onset of retinal pigmentation	670 (44)	746 (53)
Light retinal pigmentation	769 (44)	851 (58)
Even retinal pigmentation	865 (54)	993 (75)
Initial jaw movement	1,199 (77)	1,452 (122)
Onset of body pigmentation	1,318 (89)	1,574 (136)
Red spleen formation	1,372 (110)	1,674 (135)
Begin swim-up	1,501 (132)	1,833 (111)
Begin free swimming	1,715 (185)	2,136 (159)
Active free swimming	1,873 (208)	2,417 (177)
Yolk absorbed	2,031 (229)	2,620 (203)

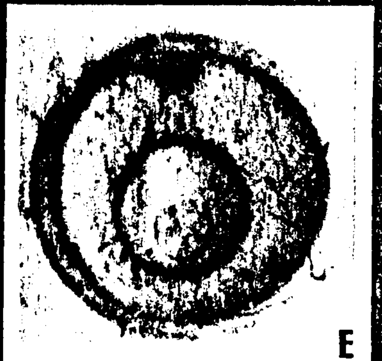
'gu . mor en of gem b mbr A -ly ava
B. Blastula; C. Epiboly-50%; D. Yolk plug; E. Body Axis; F. Somite development; G. Heartbeat; H. Prehatch; I. Posthatch; J. Onset of retinal pigmentation; K. Dark retinal pigmentation; L. Golden eyes; M. Jaw formation; N. Jaw movement; O. Swim-up; P. Free swimming/ actively feeding.

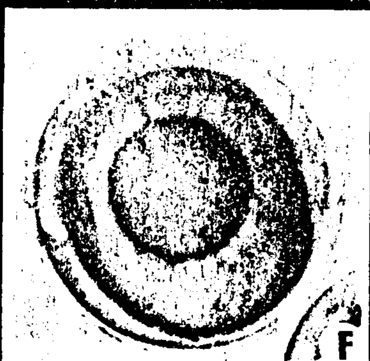


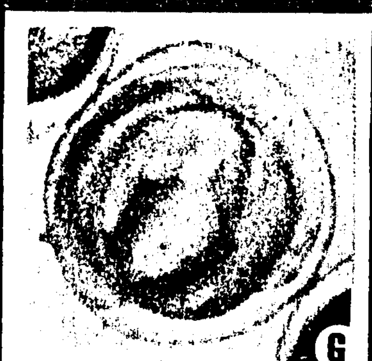


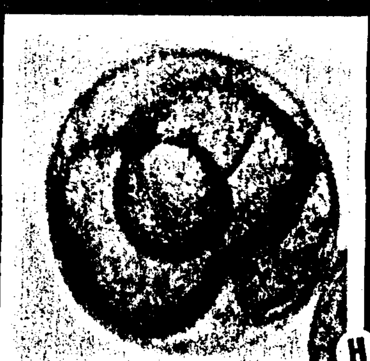


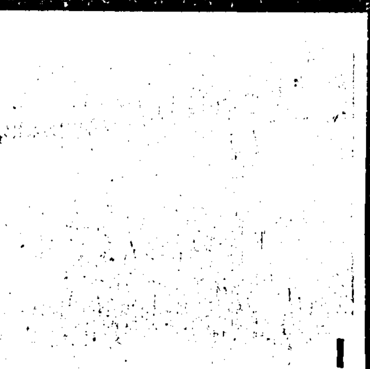


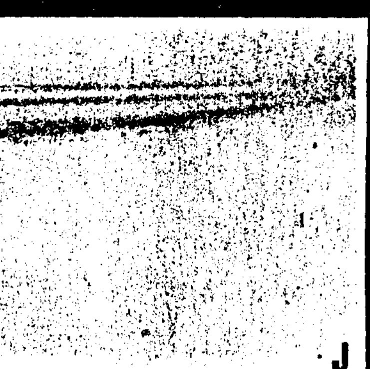




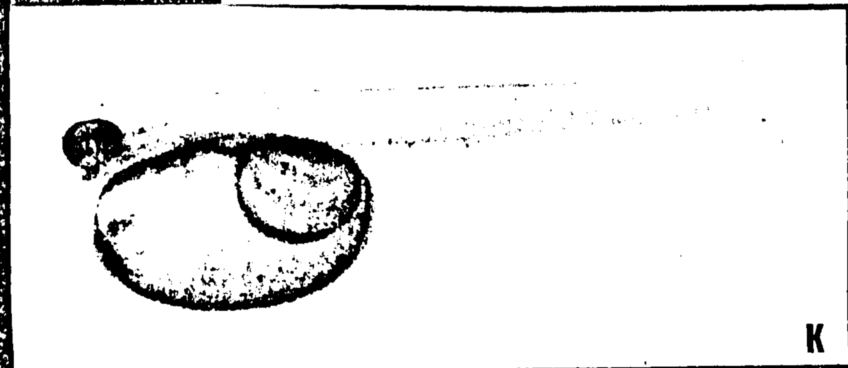


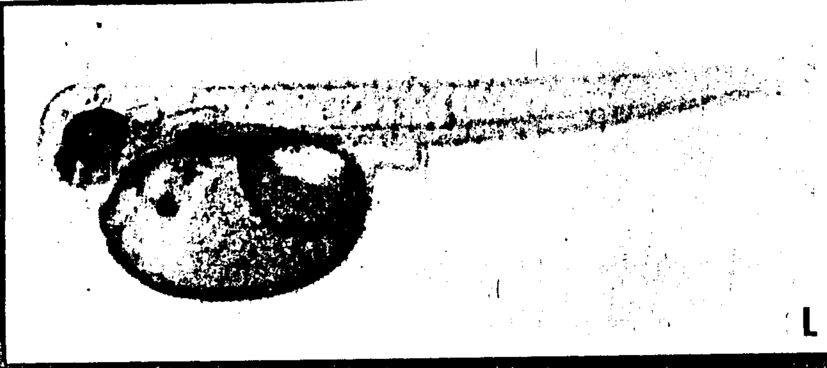


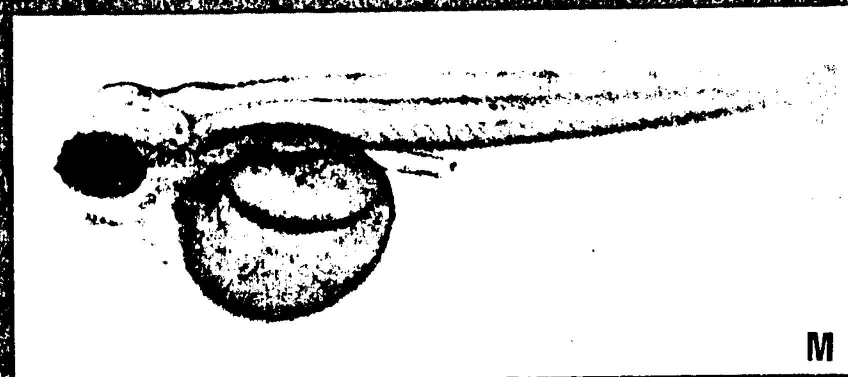


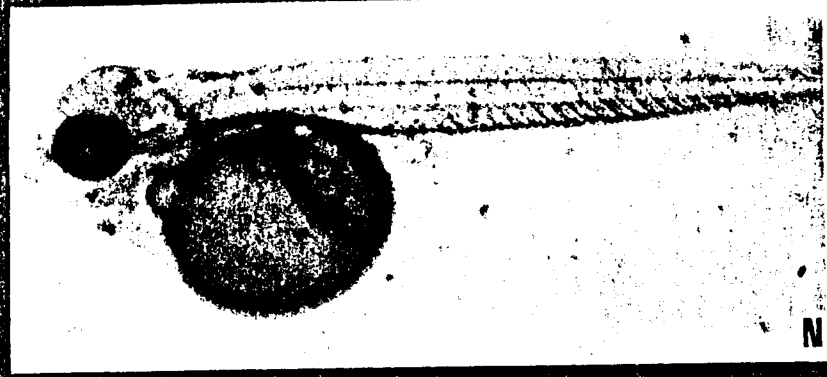


Fig[illegible]. [illegible] mo[illegible]ger[illegible] of [illegible]ger[illegible] [illegible] Body Axis; F. Somite [illegible]
B. Blastula; C. Epiboly-50%; D. Yolk plug; E. Body Axis; F. Somite development; G. Heartbeat; H. Prehatch; I. Posthatch; J. Onset of retinal pigmentation; K. Dark retinal pigmentation; L. Golden eyes; M. Jaw formation; N. Jaw movement; O. Swim-up; P. Free swimming/ actively feeding.









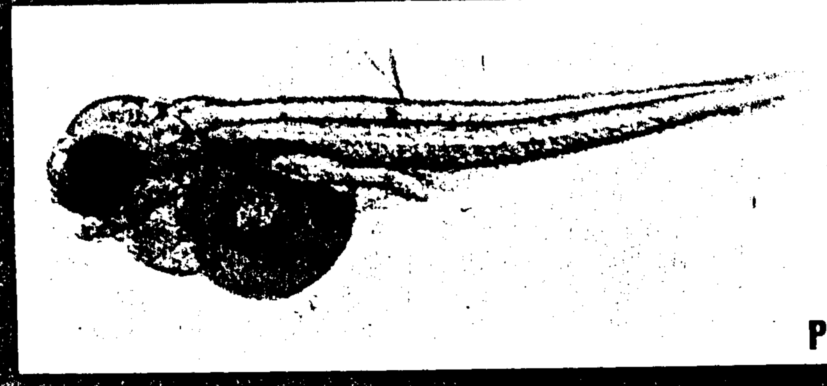
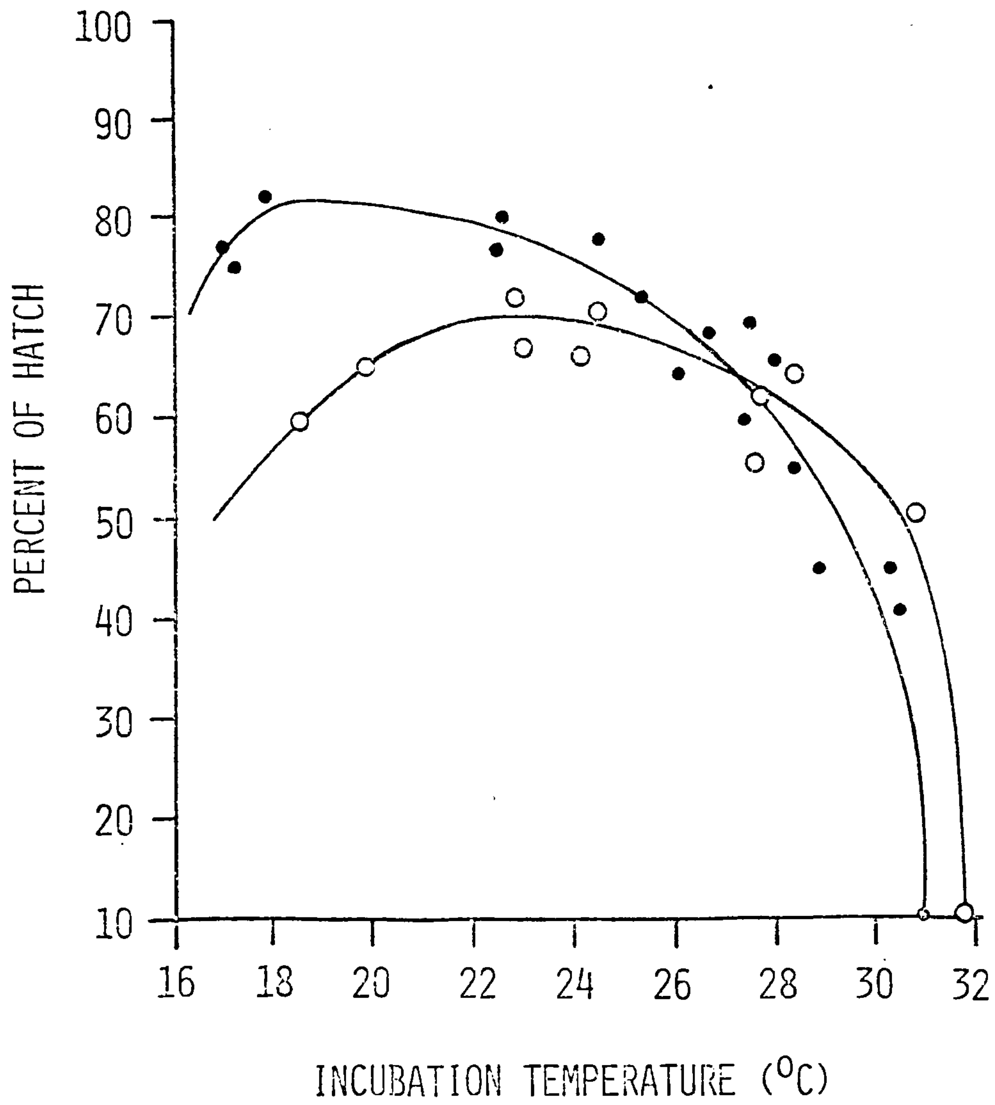


Figure 2. The effect of temperature on hatching success of northern largemouth bass (—●—) and Florida largemouth bass (—○—).



STUDY 103
JOB 2

CHAPTER 5

Biochemical Genetic Evaluation of Patterns of Gene Expression During the Embryonic Development of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass

Henry R. Parker[1], Gregory S. Whitt[1,2], and David P. Philipp[2,3]

[1] Department of Genetics and Development
University of Illinois
515 Morrill Hall
505 S. Goodwin Avenue
Urbana, Illinois 61801

[2] Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody
Champaign, Illinois 61820

[3] Department of Animal Science
328 Mumford Hall
University of Illinois
Urbana, Illinois 61801

ABSTRACT

Differences in patterns of gene expression among embryos derived from crossing two subspecies of largemouth bass, northern largemouth bass, NLMB, (*Micropterus salmoides salmoides*), and Florida largemouth bass, FLMB (*M. s. floridanus*) were used to investigate the extent of divergence of developmental gene regulation. These different subspecies have diverged at a number of their structural genes as indicated by their genetic distance (D = 0.133, Nei, 1978). The relative morphological and molecular developmental success was determined for embryos formed by crosses within each subspecies (FLMB x FLMB, NLMB x NLMB) and between each subspecies (FLMB x NLMB, NLMB x FLMB). The morphological facets of development; fertilization percentages, hatching percentages, and schedules of anatomical development were very similar among the progeny of the four crosses. In contrast, the developmental patterns of expression of each of 16 enzyme loci often showed substantial differences among the four types of embryos.

Slight differences in the schedules of morphogenesis coupled with the considerable differences in the schedules and levels of enzyme locus expression indicate that the two subspecies have diverged at genes controlling developmental processes. However, the extent of gene divergence between these two subspecies has not progressed far enough to cause serious regulatory incompatibilities in their F_1 hybrids. Although some gene regulatory divergence has occurred, additional mutations influencing developmental regulation will have to be incorporated into the genomes of these subspecies before sufficient post-mating incompatibilities bring about reproductive isolation.

INTRODUCTION

Two subspecies of largemouth bass, the northern largemouth bass, *Micropterus salmoides salmoides*, and the Florida largemouth bass, *M. s. floridanus*, are currently recognized (MacCrimmon and Robbins, 1975). These subspecies were originally described by Bailey and Hubbs (1949) on the basis of morphometric and meristic characteristics. Although today largemouth bass populations exist in a continuum from the southern tip of Florida to southern Ontario, populations in peninsular Florida and those further north have gone through periods of allopatry during the Pleistocene (Remmington, 1968). An analysis of allele frequencies at 28 enzyme loci in largemouth bass populations throughout the United States defined the genetic differences among these populations (Philipp et al., 1981, 1983b). In addition, this study described the range of each subspecies, defined the extent of intergradation between them and quantified the genetic differences between the subspecies (D = 0.133, Nei, 1978). Populations at the extremes of the range of each subspecies have fixed allelic differences at three enzyme loci, with each of these loci exhibiting latitudinal clines in allele frequencies. Kinetic analyses of the clinally distributed MDH-B_2 allelic isozymes revealed K_m differences at certain temperatures (Hines et al., 1984). These associations of specific alleles with specific environments, as well as the detection of thermal kinetic differences for allelic isozymes are consistent with the hypothesis that each subspecies is best adapted to quite different thermal environments.

The degree of developmental success of F_1 hybrid embryos provides a means of assessing the compatibility of the gene regulatory mechanisms of two different genomes. The extent that developmental processes are disrupted in

these hybrid embryos has been used to estimate the amount of gene regulatory divergence both within species and between species. A direct relationship between increasing taxonomic distance between parental species and decreasing developmental success plus increasingly abnormal patterns of gene expression in developing hybrid embryos has been found in many taxa (Whitt et al., 1973, 1977; Wilson et al., 1974; Prager and Wilson, 1975; Woodruff, 1979; Oliver, 1979; Whitt, 1981; Frost, 1982; Philipp et al., 1984).

The present report assesses the amount of gene regulatory divergence between the two subspecies of largemouth bass by analyzing embryogenesis in the progeny of intrasubspecific and of reciprocal intersubspecific F_1 hybrid crosses. We present evidence for significant developmental regulatory divergence which may be a key factor in the ongoing process of speciation among these fishes.

MATERIALS AND METHODS

Production and Rearing of Embryos

In February, 1981, male northern largemouth bass (*Micropterus salmoides salmoides*) (NLMB) collected previously from Clinton Lake, Illinois were transported in aerated water to the Florida Game and Freshwater Fish Commission Fisheries Research Laboratory at Eustis, Florida. Ripe male and female Florida largemouth bass (*M. s. floridanus*) (FLMB) were collected by electrofishing techniques during the natural spawning season from Lake Dora, Florida. The eggs of the FLMB were manually stripped, divided into two equal aliquots, and each aliquot separately fertilized *in vitro* by sperm from FLMB and NLMB males, respectively, thus producing the FLMB x FLMB and FLMB x NLMB cross progeny.

The other two crosses (NLMB x NLMB, NLMB x FLMB), were produced at the Illinois Natural History Survey (INHS) laboratory in Champaign, Illinois in May, 1981. In preparation for these crosses, FLMB males collected from Lake Dora, Florida were transported in aerated water to Champaign, Illinois. Ripe male and female NLMB were collected from Clinton Lake, Illinois by electrofishing techniques during the natural spawning season. The eggs of the NLMB were manually stripped, divided into two equal aliquots and each aliquot separately fertilized *in vitro* by sperm from NLMB and FLMB males, respectively.

Once fertilized, eggs were maintained at 22°C in 200-mm x 10-mm plastic petri dishes containing a minimal amount of natural pond water for approximately 20 minutes to allow for water hardening. The fertilized eggs were then washed and transferred to glass baking dishes containing 22°C pond

water. Fresh aerated water was replenished periodically to insure that the embryos had sufficient oxygen and that toxic levels of waste products did not accumulate. Dead embryos were removed at regular intervals. Once hatching began, newly hatched embryos were placed into a separate dish of freshly aerated water.

Symbols

The following symbols will be used to indicate the different types of crosses:

Female x Male

FLMB = the FLMB x FLMB ♀ cross ♂

F x N = the FLMB x NLMB ♀ cross ♂

N x F = the NLMB x FLMB ♀ cross ♂

NLMB = the NLMB x NLMB ♀ cross ♂

Fertilization success was assessed 30-60 minutes post fertilization by determining the percentage of embryos with fertilization membranes. The fertilization percentages of the reciprocal hybrids were normalized to those of the embryos of the subspecies of the maternal parent used for the hybrid cross. Hatching success was calculated in a similar manner by determining the percentage of fully hatched individuals (normal and abnormal) in each cross. The hatching percentages of the reciprocal hybrids were normalized to those of embryos of the subspecies of the maternal parent used for the hybrid cross.

Sampling of Embryos

Morphological development of the embryos of each cross was monitored with a dissecting microscope. The thermal requirements for development are

expressed in thermal developmental units, TDU (Childers, 1967) which are defined as the effective degree-hours above the alpha-threshold temperature. The alpha-threshold temperatures used for these calculations were 12.62°C for the NLMB and 11.52°C for the FLMB as determined by Philipp et al. (1984). In addition, for each cross, two duplicate series of separately pooled samples of 50 embryos each were removed at each of the following 17 developmental periods; 8-16 cells; blastula; 50% epiboly; yolk plug; body axis; somite formation; heartbeat; pre-hatch; hatching; post-hatch; appearance of hemoglobin; retinal pigmentation; jaw formation; jaw movement; body pigmentation; swim-up; and free-swimming. The embryos were placed into 0.25 ml of 0.1 M Tris-HCl, pH 7.0 and frozen at -20°C for later analysis.

Spectrophotometric Analysis

In preparation for analyses of enzyme ontogeny, the samples of embryos were thawed and homogenized at 4°C in a motorized Potter-Elvehjem homogenizer. These homogenates were centrifuged at 4°C for 20 minutes at 23,500xg and following decantation, the supernatants were recentrifuged under the same conditions.

The supernatants of the first developmental series were analyzed spectrophotometrically at 25°C for the following enzymes: (1) creatine kinase, CK (EC 2.7.3.2), (2) adenylate kinase, AK (EC 2.7.4.3), (3) phosphoglucomutase, PGM (EC 2.7.5.1), and (4) glucosephosphate isomerase, GPI (EC 5.3.1.9). The reaction mixtures used for each assay were, with slight changes, based on Shaklee et al. (1977) with the exception of that used for PGM, which was based on Dawson and Mitchell (1969).

(1) AK - 20 mM Tris-HCl pH 7.5, 4.0 mM $MgCl_2$, 3.3 mM glucose, 0.5 mM ADP, 0.15 mM NADP, 1.0 unit/ml hexokinase, 1.0 unit/ml glucose-6-phosphate dehydrogenase.

(2) CK - identical to the AK reaction mixture except that 10.0 mM phosphocreatine was added.

(3) PGM - 4 mM Imidazole-HCl pH 7.5, 3.0 mM $MgCl_2$, 1.5 mM EDTA, 0.17 mM NADP, 1.7 mM glucose-1-phosphate, 3.3 mM glucose-1, 6-diphosphate, 1.0 unit/ml glucose-6-phosphate dehydrogenase (Dawson and Mitchell, 1969).

(4) GPI - 20 mM Tris-HCl pH 7.5, 3 mM $MgCl_2$, 0.13 mM NADP, 2.0 mM fructose-6-phosphate, 1.0 unit/ml glucose-6-phosphate dehydrogenase.

The supernatants of the second developmental series were analyzed spectrophotometrically at 25°C for the following enzymes: (5) malate dehydrogenase, MDH (EC 1.1.1.37), (6) lactate dehydrogenase, LDH (EC 1.1.1.27), (7) isocitrate dehydrogenase (NADP), IDH (EC 1.1.1.41), (8) 6-phosphogluconate dehydrogenase, 6-PGDH (EC 1.1.1.44), and (9) aspartate aminotransferase, AAT (EC 2.6.1.1). The reaction mixtures used, with slight modifications, were based on those of Shaklee et al. (1977).

(5) LDH - 0.1 M phosphate buffer pH 7.0, 1.0 mM pyruvate, 0.14 mM NADH.

(6) MDH - 0.1 M phosphate buffer pH 7.0, 0.33 mM oxaloacetate, 0.14 mM NADH

(7) IDH - 50 mM Tris-HCl pH 7.5, 2.0 mM MgCl2, 0.15 mM NADP, 1.0 mM isocitrate.

(8) 6-PGDH - 50 mM Tris-HCl pH 7.5, 2.0 mM $MgCl_2$, 0.15 mM NADP, 2.0 mM 6-phosphogluconate.

(9) AAT - 50 mM Tris-HCl pH 7.5, 0.7 mM ketoglutarate, 10.0 mM aspartate, 0.08 mM pyridoxal-5-phosphate, 0.1 mM NADH, 1.0 unit/ml malate dehydrogenase.

Enzyme activities were determined by monitoring the changes in absorbance at 340 nm using a Beckman Kintrac VII spectrophotometer and are expressed as International Units. The final volume of the reaction mixture was 1.0 ml. Reactions were initiated with the addition of enzyme extract (1-50 ul of the appropriate supernatant, depending upon the enzyme being assayed). Protein concentrations of each supernatant were determined using the procedure of Lowry et al. (1951) as modified by Mason et al. (1973).

Electrophoresis

Vertical starch gel electrophoresis and histochemical staining analyses of parental tissue and embryonic extracts were performed essentially as described in Philipp et al. (1979). To determine the relative contribution of each isozyme to the total enzyme activity,two-fold serial dilutions were also made of each embryo extract and then electrophoresed on starch gels according to the method of Klebe (1975).

Statistical Analysis

Principal component analysis of the ontogeny of enzyme specific activities for each of the crosses was performed. One principal component analysis was carried out for the developmental timing of gene expression of 16 loci, i.e., normalized times of first expression of enzyme activity or the first stable developmental increase in activity for each of the loci being analyzed. The second factor analysis was of the enzyme specific activity levels at selected stages throughout the entire developmental period. In the latter analysis, we determined enzyme specific activities at each of six

different developmental periods: onset of hatching, onset of retinal pigmentation, mid-retinal pigmentation, jaw movement, body pigmentation, and swim-up. The analyses determined the two major factors that contributed to the variation observed among the four crosses.

RESULTS

Morphological Development

All four sets of embryos exhibited fertilization percentages in excess of 95%, and hatching percentages in excess of 80%. The fertilization and hatching percentages of the hybrids were essentially indistinguishable from those of the pure crosses serving as controls.

There were slight differences in the thermal requirements for morphogenesis between the two subspecies (Table 1). Because of these differences and slight differences in the incubation temperatures used for various individual crosses, the developmental schedule of each cross was normalized to the jaw movement stage. This stage occurred at 1632 TDU post-fertilization in the crosses in which the maternal parent was the Florida largemouth bass and at 1183 TDU post-fertilization in the crosses in which the northern largemouth bass was the maternal parent. In addition, hybrid embryos exhibited the same morphogenetic schedules as the embryos from the intrasubspecific cross of the maternal parent. There is a greater number of developmental units required by the FLMB and F x N embryos compared to the NLMB and N x F embryos to reach a specific morphological stage as embryogenesis proceeded (Table 1). These results are in agreement with those obtained in a more detailed determination of the thermal requirements for embryonic development of NLMB, FLMB and their reciprocal hybrids (Philipp et al., 1984).

Molecular Ontogeny

Soluble Protein

The soluble protein levels in all of the four different stocks of embryos steadily decreased from fertilization to the time of swim-up. The

embryos produced from FLMB females had slightly higher concentrations of soluble proteins than did the embryos produced from female NLMB. This higher concentration of soluble protein may be a reflection of the larger egg size produced by the FLMB females used. This larger egg size resulted from the larger sizes of FLMB collected relative to NLMB.

Enzyme Ontogeny

Electrophoretic analyses of the ontogeny of enzyme locus expression in developing embryos of NLMB have been reported previously (Philipp et al., 1979, 1983a). The isozyme patterns obtained during the present study were quite similar, as expected. As a result, instead of presenting these figures in detail, the times of first detected expression during development, for each locus, for each cross, has been summarized in Table 2.

Spectrophotometric analyses of total enzyme specific activity coupled to the serial dilution electrophoretic analyses (Klebe, 1975) permitted the quantitative determination of the activity contributed by each isozyme at each developmental stage. These data were used to construct the developmental profiles of enzyme specific activity (Figs. 1-7) as well as the relative contributions of each isozyme locus at each developmental stage (Table 3).

Creatine Kinase Activity

In largemouth bass, CK activity is encoded in four loci. The Ck-A locus is expressed predominantly in white skeletal muscle, the Ck-B locus in eye and brain and the Ck-C locus in many tissues, but predominantly in stomach. The Ck-D locus is expressed in mature ripe testes only, and so was not observed during embryogenesis. The developmental patterns of total CK specific

activity was not greatly dissimilar in all four sets of embryos (Fig. 1). The total CK activity increased by the onset of retinal pigmentation in the FLMB and F x N embryos but not until mid-retinal pigmentation and jaw movement in the N x F and NLMB embryos. These increases in total CK activity were due to increases in the expression of all three isozymic loci (Fig. 2-4). However, each set of embryos showed a specific but different pattern of relative contribution for each of the CK isozymes (Table 3). These differences are presumably reflections of differences in patterns of gene regulation between the subspecies.

Glucosephosphate Isomerase Activity

Two loci, located on different chromosomes, encode GPI activity (Whitt et al., 1976) in the largemouth bass. The Gpi-A locus is expressed in many tissues but predominantly in the liver, whereas the Gpi-B locus is primarily expressed in white skeletal muscle. Over the entire developmental period, the FLMB and F x N embryos possessed relatively higher GPI activities than those of the NLMB and N x F embryos (Fig. 5). GPI-A activity was present throughout development in all embryos (Fig. 6), with varying times in initial increases observed among the embryo types (Fig. 6). Initial expression of the Gpi-B locus was also variable among the embryo stocks, occurring from post-hatch through retinal pigmentation (Fig. 7).

Lactate Dehydrogenase Activity

Isozymes of LDH are encoded in three different loci in centrarchid fishes (Whitt et al., 1971). Ldh-A is expressed to some degree in all adult tissues but it predominates in the white skeletal muscle. Ldh-B is expressed in all tissues examined and predominates in the heart. Ldh-C is predominantly

expressed in the eye and brain. The developmental profiles of total LDH activity were very similar in all four types of embryos up to retinal pigmentation at which point the activity patterns diverged. The LDH-A_4 isozyme was expressed throughout development in all four types of embryos. In the FLMB and F x N embryos, the first increase in LDH-A activity was slightly earlier in development than in the NLMB and N x F embryos (Table 2). The Ldh-B locus was expressed at quite variable times among the embryos of the four stocks (Table 2). Ldh-C expression was detected only late in development in all embryos (Table 2).

Malate Dehydrogenase Activity

Three MDH loci are expressed in largemouth bass. The MDH-M_2 isozyme is restricted to the mitochondria and is found in all tissues. MDH-M_2 activity is present in unfertilized eggs and throughout embryogenesis at very low levels. The cytosolic malate dehydrogenase isozymes are encoded in two loci, Mdh-A and Mdh-B, which are located on separate chromosomes (Wheat et al., 1972). In adult fish, Mdh-A expression is observed in all tissues and is particularly high in liver. The Mdh-B locus is expressed predominantly in white skeletal muscle. The ontogenetic patterns of total MDH activity during the development were very similar for four sets of embryos. In the embryos of all four stocks, the Mdh-A locus is expressed throughout development, whereas the Mdh-B is not expressed until late retinal pigmentation. The relative contributions during development of the Mdh-A and Mdh-B loci are very similar among three out of the four sets of embryos (Table 3).

Adenylate Kinase Activity

AK activity is encoded in two loci in largemouth bass, only one of which, AK-A, is expressed in embryos and it is expressed throughout development. The total AK activity profile of the hybrid paralleled that for normal embryos from the subspecies serving as the maternal parent with the FLMB and F x N embryos having higher AK activity levels than the NLMB and N x F embryos (Table 2).

Phosphoglucomutase Activity

There is at least one PGM locus present in sunfish, Pgm-A, and it is expressed predominantly in white skeletal muscle and liver of adult fishes. PGM-A activity was present throughout development in all of the embryos, however, the time of increase in activity differed among the four embryo stocks (Table 2). However, the overall developmental trend of PGM activity in each of the hybrid embryos is similar to that for the subspecies of the maternal parent.

Isocitrate Dehydrogenase Activity

The IDH isozymes appear to be encoded in two loci in the largemouth bass. In adult fish, the Idh-A locus is predominantly expressed in white skeletal muscle and the Idh-B locus primarily in the liver. Developmental profiles of total activity for IDH appeared similar for all four embryo stocks. The NLMB and N x F embryos exhibited an earlier initial increase in the IDH-A isozyme activity than the FLMB and F x N embryos (Table 2). The onset of the Idh-B locus expression was undetectable in the N embryos because the IDH-A and B isozyme possessed the same electrophoretic mobility. The IDH-B activity in

the N x F embryos first appeared when they were well into retinal pigmentation. However, in the embryos derived from a female FLMB, the Idh-B locus expression was initially detected just prior to hatching (Table 2).

6-Phosphogluconate Dehydrogenase Activity

In the largemouth bass, 6-PGDH is encoded in a single locus, 6-Pgdh-A. The activity of 6-PGDH was present throughout development in the embryos of all four crosses. The initial increase in 6-PGDH activity occurred at various developmental times in the embryos of the four stocks (Table 2). The absolute activity levels were also quite variable.

Aspartate Aminotransferase Activity

In largemouth bass, AAT is encoded in three loci. The Aat-A locus encodes a cytosolic form of the enzyme expressed predominantly in the white skeletal muscle. The Aat-B locus encodes a cytosolic form of the enzyme expressed predominantly in the liver. The Aat-M locus encodes a mitochondrial form of the enzyme expressed to some extent in all tissues. Throughout development, very low levels of AAT-M_2 activity were observed in all crosses. No detectable Aat-B expression was observed, but a low initial expression of Aat-A was observed at quite different times among the four stocks (Table 2).

Temporal Patterns of Gene Expression in the Two Subspecies and Their Reciprocal Hybrids

A comparison of the enzyme activity of the two intrasubspecific and the two reciprocal F_1 hybrid intersubspecific embryos revealed quite a variety of different temporal patterns (Fig. 8) depending upon the enzyme locus involved. In one observed pattern, as exemplified by Ck-A, the times of initial

expression (or initial increase in activity) were different for the embryos of the two different subspecies, and the timing of both of the reciprocal hybrids was the same and intermediate to those of the two subspecies. However, for the Pgm-A locus the embryos of the hybrids (F x N and N x F) both exhibited a earlier expression than the embryos of either pure subspecies.

A principle component analysis was performed to determine whether a general pattern of enzyme activity differences throughout development could be detected among the four classes of embryos. The enzyme activity levels of the hybrids tended to resemble those of the embryos of the subspecies serving as the female parent (Figure 9). The horizontal axis shows the factor weighting which has developmental time as a predominant component. The verticle axis is the factor weighting with the time component removed, of the enzymes that contributed the most to the variation among the four crosses. The enzymes were in two groups, the first consisted of Ck-B, Ck-C, Gpi-A and Ldh-C, and the second consisted of Ldh-B, Idh-A, Idh-B, Mdh-A, Mdh-B, Aat-A. When the activities were high in the first group, they were low in the second and vice versa. These two factors account for 85% of the variance among the crosses.

DISCUSSION

The two *Micropterus* subspecies, *M. s. floridanus* and *M. s. salmoides*, now overlap in a large intergrade zone which extends throughout the southeastern United States (Philipp et al., 1981, 1983). These subspecies, however, have been periodically separated during their evolutionary history, the latest of which occurred during the Pleistocene (Cooke, 1945). Templeton (1982) has stated that when a species occupies a broad range encompassing a wide variety of environments, it will tend to organize into demes based on common environments. Therefore, when a widely distributed species such as the largemouth bass is separated into two such demes (the northern and Florida subspecies), adaptive divergence may lead to pre-mating and/or post-mating reproductive isolation. The completion of reproductive isolation may be rapid or slow depending upon the kinds of mutational differences present between the populations, and the nature of the selective and stochastic forces acting upon the populations involved.

Different thermal environments exist in the ranges of the two different subspecies. Recent laboratory and field studies on genetic stocks of both subspecies and both reciprocal F_1 hybrids have demonstrated that the two subspecies have quite different thermal physiological properties (Philipp and Whitt, this report). Therefore, environmental temperature appears to be a major selective force governing the microdifferentiation of the various stocks of largemouth bass.

Morphogenesis

As observed in this study, as well as in a separate study (Philipp et al., 1984), the number of developmental units required to reach any given

morphological stage are similar, but not identical, for the two subspecies of largemouth bass (Table 1). Despite these slight differences in rates of development betweem the different progeny classes, the gene regulatory mechanisms of the different subspecies appear to be reasonably compatible. Although no obvious pre-mating or post-mating barriers between these subspecies have been detected, we cannot exclude the possibility that the observed subtle developmental differences between the subspecies may be leading to reduced fitness in certain intergrade populations over longer periods of time.

The relatively high level of compatibility between the genomes of the two subspecies is further evidenced by the high percentages of hatching and fertilization observed for embryos from all four of the crosses. Indeed, the FN embryos consistently demonstrated a higher hatching percentage than control values, 109% versus 100%. This increased percentage of hatching relative to controls has been observed for certain other _Micropterus_ hybrids (Beaty, 1980; Philipp et al., unpublished results). Although the mechanism(s) behind this effect are not known, it is unlikely that this increase is due to a release from inbreeding depression. If this were the case, this effect would be expected in both reciprocal F_1 hybrids.

Molecular Ontogeny

The differences in the developmental regulation of enzyme locus expression were assessed by measuring three things: (1) the times of enzyme activity appearance; (2) the times of the first stabilized increase in enzyme activity; and (3) the absolute enzyme activity levels at specific stages throughout embryogenesis. The first assessment was used only for those enzymes not present in the egg initially. The second assessment was used only

for those enzymes carried over from oogenesis and present throughout embryogenesis. The third assessment was used for all enzymes.

The time of first enzyme appearance was determined for seven of the 16 loci studied: Ck-A, Ck-B, Gpi-B, Mdh-B, Ldh-B, Ldh-C, and Idh-B (Table 2). These loci exhibited quite different temporal patterns of expression. More importantly, with the exception of Mdh-B, the time of initial expression of each locus was different for each of the two subspecies. The temporal patterns of gene expression in the hybrids varied from locus to locus. However, for most loci the time of first enzyme appearance in the hybrids was similar to that of the subspecies serving as the maternal parent.

For 9 out of 16 loci (Ck-C, Ak-A, Pgm-A, Gpi-A, Ldh-A, Idh-A, Mdh-A, Aat-A and 6-Pgdh-A), the enzymes encoded at these loci are present in the embryos throughout embryogenesis, and the presence of these maternal enzymes masked the appearance of enzymes synthesized by the embryonic genes. Thus, only the time of the first increase in enzyme activity was determined for 9 of the 16 enzyme loci (Table 2). This increase in enzyme activity has been asAumed to approximate the time of initial gene expression for these loci. The two subspecies differed in their times of first increase of enzyme activity for many of the enzyme loci. The different kinds of departures from the expected temporal pattern in the hybrid presumably reflect different kinds and/or magnitudes of difference between regulatory elements of the two subspecies.

The data from both types of assessments of the timing of enzyme locus expression in the embryos were pooled and analyzed by a principle component procedure. The FLMB and F x N embryos were closely grouped, whereas the N x F hybrid embryos and the NLMB embryos belonged to separate groups. The FLMB egg cytoplasm appears to be exerting a strong dominant effect over NLMB genome

expression in the F x N embryos, resulting in a strong maternal influence on the developmental schedules of gene expression. In the reciprocal hybrid, the presence of the FLMB paternal genome in the female NLMB cytoplasm appears to result in a more disruptive developmental schedule. The NLMB egg cytoplasm does not appear to exert the overwhelming dominant maternal effect observed in the F x N embryos. These results suggest that the cytoplasmic compositions of the eggs of the two subspecies have diverged.

The absolute levels of specific enzyme activity over the entire developmental period must certainly be partially dependent upon the time of the first appearance of embryonic expression of specific genes. However, the differences in the rates of accumulation of enzyme activity, presumably due to differences in the rates of synthesis, appear to be an even more important determinant in the differences in enzyme levels at later developmental periods. Therefore, comparison of differences in enzyme activity profiles throughout embryogenesis among the four crosses has provided another means of monitoring divergence of developmental regulation. The developmental patterns vary from locus to locus (Table 2). However, in general, the embryos produced from eggs of a FLMB exhibited higher enzyme activities over the course of development than did the embryos from NLMB eggs. In addition, the hybrid embryos of both reciprocal crosses tended to have slightly higher enzyme activity levels than those of the embryos of their maternal subspecies.

The results of the principle component analyses which clustered the sets of embryos at each of six different developmental times according to the levels of activity of several enzyme loci, underscore the obvious fact that enzyme activity increases with developmental time (Fig. 9). More important is the observation that enzyme activity levels differed between the two

subspecies at 11 of the 16 loci. These developmental differences indicate that the differences in the patterns of embryonic gene expression between the subspecies and the alterations in the normal patterns of expression of some loci in the hybrids are due to slight incompatibilities between the gene regulatory mechanisms in these subspecies. The existence within species of naturally occurring regulatory polymorphisms that alter the rates of protein synthesis provides a basis for the hypothesis that the enzyme activity differences observed during the development of different subspecies and their hybrids are the consequence of the divergence of gene regulatory factors affecting the timing of expression of the many enzymes in these subspecies (Abraham and Doane, 1978; McDonald and Ayala, 1978; Ayala and McDonald, 1980; Hickey, 1981; Klose, 1982; Anderson and McDonald, 1983).

The differences in the patterns of gene expression observed for the loci studied, together with the high degree of success and the relative similarity in the rates of morphological development between the subspecies imply that the mutational differences accumulated in the regulatory genes of the two subspecies are relatively small. If this interpretation is correct, it would imply that there has been a rather gradual rate of divergence between the northern and Florida subspecies of largemouth bass during their evolution. Thus, there appears to be no major post-mating isolation mechanisms. Mixture of these two stocks in the wild will result in the production of viable F_1 hybrids with no apparent loss in production compared to intrasubspecific matings. However, it must be stressed that the developmental success of the F_1 intersubspecific hybrid embryos does not indicate a concomitant success of F_2 or backcrossed hybrid embryos. The "balanced" genomes found in the F_1 may result in a short term stability or luxuriance. However, following

recombination and independent assortment during gamete production, it is our prediction that the resulting embryos produced would show a decreased fitness by exhibiting decreases in the success of morphological development accompanied by increases in the abnormalities of developmental patterns of gene expression.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collection and providing laboratory space at the Eustis Fisheries Research Laboratory. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment, and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf, and Paul Shafland. We would also like to thank Dr. Bruce Taubert, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Todd Powless, and Christine Kaminski of the Illinois Natural History Survey. We thank Manijeh Pasdar for her assistance with the electrophoresis. We thank David L. Swofford for his helpful advice on the statistical analyses of the data. Finally, we wish to thank Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration frunds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Abraham, I. and W. W. Doane. 1978. Genetic regulation of tissue-specific expression of amylase structural genes in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. USA 75(9):4446-4450.

Anderson, S. M. and J. F. McDonald. 1983. Biochemical and molecular analysis of naturally occurring *Adh* variants in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 80:4798-4802.

Ayala, F. J. and J. F. McDonald. 1980. Continuous variation: Possible role of regulatory genes. Genetics 52/53:1-15.

Bailey, R. M. and C. L. Hubbs. 1949. The black basses (*Micropterus*) of Florida, with description of a new species. University of Michigan Museum of Zoology Occasional Paper 516:1-40.

Beaty, P. R. and W. F. Childers. 1980. Hybridization of northern largemouth bass (*Micropterus salmoides salmoides*) and northern smallmouth bass (*Micropterus dolomieui dolomieui*). Pp. 1-66. Bass Research Foundation, Starkville, Miss.

Childers, W. F. 1967. Hybridization of four species of sunfish (Centrarchidae). Ill. Nat. Hist. Surv. Bull. 29:159-214.

Cooke, C. W. 1945. Geology of Florida. Florida Geol. Surv. Geol. Bull. 29:339.

Dawson, D. M. and A. Mitchell. 1969. The isoenzymes of phosphoglucomutase. Biochemistry 8(2):609-614.

Frost, J. S. 1982. Functional genetic similarity between geographically separated populations of Mexican leopard frogs (*Rana pipiens* complex). Syst. Zool. 31:57-67.

Hickey, D. A. 1981. Regulation of amylase activity in *Drosophila melanogaster*: Variation in the number of enzyme molecules produced by different amylase genotypes. Biochem. Genet. 19:783-796.

Hines, S. A., D. P. Philipp, W. F. Childers, and G. S. Whitt. 1984. Thermal kinetic differences between allelic isozymes of malate dehydrogenase (Mdh-B locus) of largemouth bass, *Micropterus salmoides*. Bioch. Gen. (In press).

Klebe, R. J. 1975. A simple method for the quantitation of isozyme patterns. Biochem. Gen. 13(11/12):805-812.

Klose, J. 1982. Genetic variability of soluble proteins studied by two-dimensional electrophoresis on different inbred mouse strains and on different mouse organs. J. Mol. Evol. 18:315-328.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mason, T. L., R. O. Poyton, D. C. Wharton, and G. Schatz. 1973. Cytochrome c oxidase from baker's yeast: I. Isolation and properties. J. Biol. Chem. 248:1346-1354.

MacCrimmon, H. R. and W. H. Robbins. 1976. Distribution of the black basses in North America. Pages 56-66 _In_ R. H. Stroud and H. Clepper, eds. Black bass biology and management. Sport Fishing Institute, Washington, District of Columbia, USA.

McDonald, J. F. and F. J. Ayala. 1978. Gene regulation in adaptive evolution. Can. J. Genet. and Cytol. 20:159-175.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Newman, H. H. 1914. Modes of inheritance in teleost hybrids. J. Ex. Zool. 16:447-500.

Oliver, C. G. 1979. Genetic differentiation and hybrid viability within and between some _Lepidoptera_ species. Am. Nat. 114:681-694.

Parker, H. R., D. P. Philipp, and G. S. Whitt. 1984. Gene regulatory divergence among species estimated by altered developmental patterns in interspecific hybrids. (In preparation).

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1979. Evolution of patterns of differential gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, _Micropterus salmoides salmoides_, and smallmouth bass, _Micropterus dolomieui_). J. Exp. Zool. 210:473-488.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (_Micropterus salmoides_) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Trans. Am. Fish. Soc. 112:1-20.

Philipp, D. P., C. Kaminski, and G. S. Whitt. 1984. A comparison of the embryonic development of northern, Florida, and reciprocal F_1 hybrid largemouth bass in different thermal environments. Ill. Nat. Hist. Surv. Bull. (In press).

Philipp, D. P., H. R. Parker, and G. S. Whitt. 1983a. Evolution of gene regulation: Isozymic analysis of patterns of gene expression during hybrid fish development. Pp. 193-238 *In* M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt, eds. Isozymes: Current Topics in Biological and Medical Research. Vol. 10. Alan R. Liss, New York, New York.

Prager, E. M. and A. C. Wilson. 1975. Slow evolutionary loss of the potential for interspecific hybridization in birds: A manifestation of slow regulatory evolution. Proc. Natl. Acad. Sci. USA 72:200-204.

Remington, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Pp. 321-428 *In* T. Dobzhansky, M. K. Hecht, and W. C. Steere, eds. Evolutionary Biology. Vol. 2. Appleton-Century-Crofts, New York, New York.

Shaklee, J. B., J. A. Christiansen, B. D. Sidell, C. L. Prosser, and G. S. Whitt. 1977. Molecular aspects of temperature acclimation in fish: Contributions of changes in enzyme activities and isozyme patterns to metabolic reorganization in the green sunfish. J. Exp. Zool. 201:1-20.

Templeton, A. R. 1982. Genetic architectures of speciation. Pp. 105- 121 *In* Mechanisms of Speciation. Alan R. Liss, Inc., New York, New York.

Wheat, T. E., G. S. Whitt, and W. F. Childers. 1972. Linkage relationships between the homologous malate dehydrogenase loci in teleosts. Genetics 70:337-340.

Whitt, G. S. 1981. Developmental genetics of fishes: Isozymic analyses of differential gene expression. Amer. Zool. 21:549-572.

Whitt, G. S., W. F. Childers, and P. L. Cho. 1973. Allelic expression at enzyme loci in an intertribal hybrid sunfish. J. Hered. 64:55-61.

Whitt, G. S., W. F. Childers, J. B. Shaklee, and J. Matsumoto. 1976. Linkage analysis of the multilocus glucosephosphate isomerase isozyme system in sunfish (Centrarchidae, Teleostii). Genetics 82:35-42.

Whitt, G. S., W. F. Childers, and T. E. Wheat. 1971. The inheritance of tissue-specific lactate dehydrogenase isozymes in interspecific bass (*Micropterus*) hybrids. Biochem. Gen. 5:257-273.

Whitt, G. S., D. P. Philipp, and W. F. Childers. 1977. Aberrant gene expression during the development of hybrid sunfishes (Perciformes, Teleostei). Differentiation 9:97-109.

Wilson, A. C., L. R. Maxson, and V. M. Sarich. 1974. Two types of molecular evolution: Evidence from studies of interspecific hybridization. Proc. Natl. Acad. Sci. USA 71:2843-2847.

Woodruff, D. S. 1979. Postmating reproductive isolation in Pseudophryne and the evolutionary significance of hybrid zones. Science 203:561-563.

Table 1

The Time in Developmental Units (Normalized Developmental Time) Necessary to Reach Specific Developmental Stages

stages / embryos	8-16 cells	blastula	body axis	20 somites	heartbeat	50% hatch	onset of retinal pigmentation	jaw movement	swim-up
M. s. floridanus	22 (.01)	65 (.04)	218 (.13)	359 (.22)	446 (.27)	544 (.33)	707 (.43)	1632 (1.00)	2252 (1.38)
M. s. salmoides	21 (.02)	67 (.06)	185 (.16)	298 (.25)	389 (.33)	454 (.38)	670 (.57)	1183 (1.00)	1665 (1.41)
floridanus / salmoides	1.05	0.97	1.18	1.20	1.15	1.20	1.06	1.38	1.35

Table 2

The Normalized Developmental Time of Initial Expression or Increase of Enzyme Activity

locus \ embryos	F	FN	NF	N
[1]Ck-A	0.55	0.75	0.77	1.00
[1]Ck-B	0.55	0.55	0.77	1.00
[2]Ck-C	0.55	0.55	0.77	1.00
[2]Ak-A	0.66	0.45	0.60	0.60
[2]Pgm-A	0.55	0.36	0.47	0.60
[2]Gpi-A	0.83	1.00	1.27	1.00
[1]Gpi-B	0.55	0.55	0.47	0.60
[2]Ldh-A	0.17	0.17	0.23	0.23
[1]Ldh-B	1.12	1.00	1.17	0.77
[1]Ldh-C	1.12	1.13	1.41	1.41
[2]Idh-A	0.55	0.55	0.47	0.47
[1]Idh-B	0.55	0.55	0.60	0.47
[2]Mdh-A	0.67	0.67	0.60	0.47
[1]Mdh-B	0.76	0.76	0.60	0.77
[2]Aat-A	0.76	0.66	0.60	0.47
[2]6Pgdh-A	0.56	0.56	0.77	0.34

1) Enzyme loci that are first expressed during embryogenesis.
2) Enzyme loci that encode for activities that are present throughout embryogenesis.

Table 3

The Percentage of Enzyme Activity Contributed

by Each Isozyme Locus

Embryos	Enzyme	Locus	Normalized Developmental Time				
			0.40	0.65	0.85	1.00	1.30
F x F	CK	A	0	43	39	38	30
		B	0	14	41	21	45
		C	100	43	20	21	25
	LDH	A	100	100	100	100	85
		B	0	0	0	0	14
		C	0	0	0	0	1
	MDH	A	100	93	90	91	83
		B	0	7	10	9	17
	GPI	A	100	77	89	89	86
		B	0	23	11	11	14
F x N	CK	A	0	0	26	32	30
		B	0	19	37	34	35
		C	100	81	37	34	35
	LDH	A	100	100	100	90	85
		B	0	0	0	10	14
		C	0	0	0	0	1
	MDH	A	100	100	79	92	83
		B	0	0	21	8	17
	GPI	A	100	89	73	73	73
		B	0	11	27	27	27

Table 3, continued

Embryos	Enzyme	Locus	Normalized Developmental Time 0.40	0.65	0.85	1.00	1.30
N x F	CK	A	0	0	17	18	30
		B	0	0	33	41	35
		C	100	100	50	41	35
	LDH	A	100	100	100	100	70
		B	0	0	0	0	30
		C	0	0	0	0	0
	MDH	A	100	98	90	92	84
		B	0	2	10	8	16
	GPI	A	100	92	76	75	59
		B	0	8	24	25	41
N x N	CK	A	0	0	0	0	30
		B	0	0	0	0	39
		C	100	100	100	100	31
	LDH	A	100	100	84	79	67
		B	0	0	16	21	33
		C	0	0	0	0	0
	MDH	A	100	100	98	96	83
		B	0	0	2	4	17
	GPI	A	100	87	74	73	74
		B	0	13	26	27	26

Fig. 1. The ontogenetic profiles of total creatine kinase specific activity for the four types of embryos. The horizontal axis indicates normalized developmental time from the 8-16 cell stage to the active free swimming stage. The vertical axis indicates the specific activity expressed in Internation Units, U, per mg soluble protein. The types of embryos, FLMB (-□-), FxN (-○-), NxF (-●-) and NLMB (-■-), are produced from the crosses described in Materials and Methods.

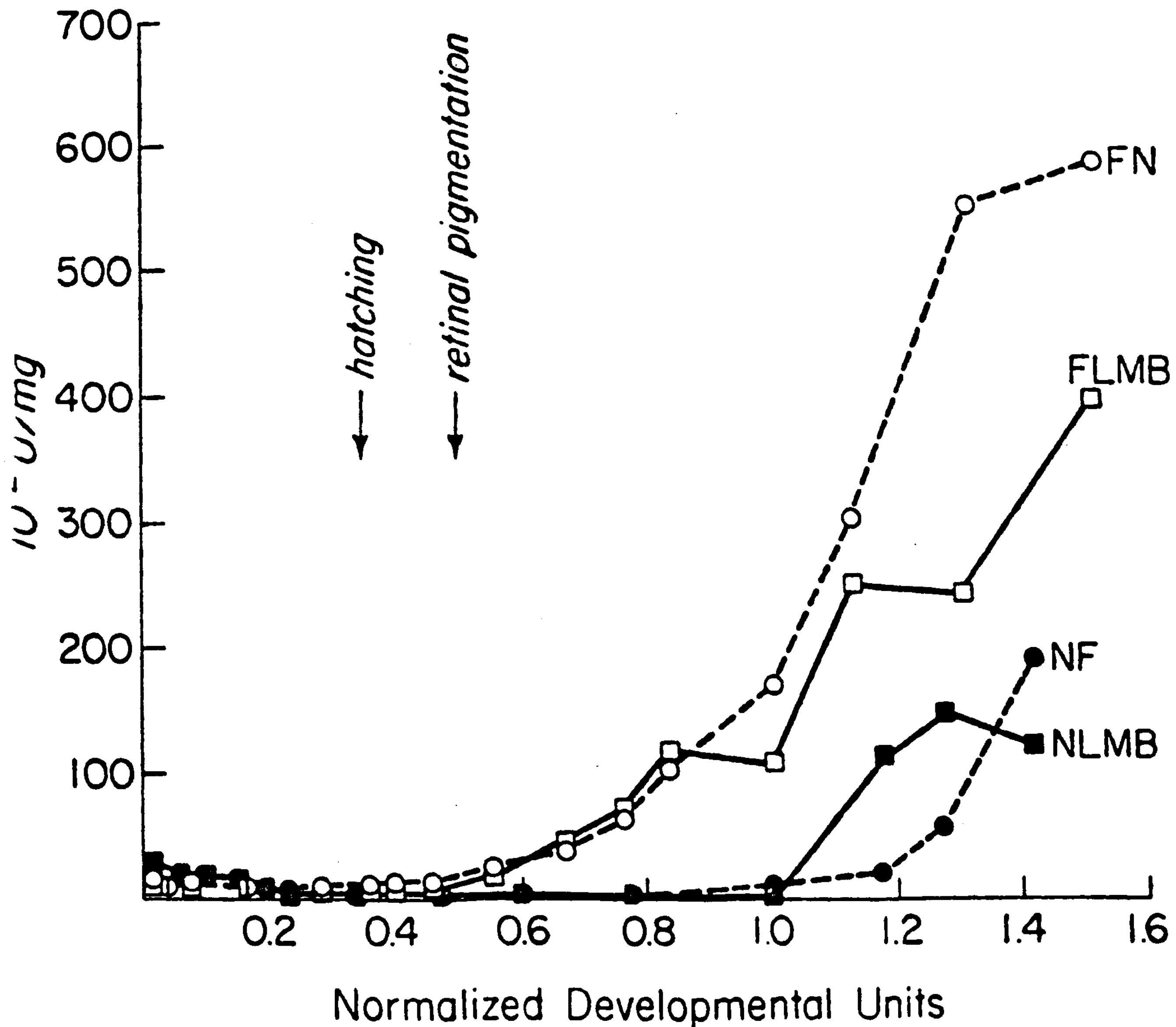


Fig. 2. The ontogenetic profiles of creatine kinase A subunit activity for each of the four kinds of embryos. The horizontal axis indicates normalized developmental time from the onset of retinal pigmentation stage to the active free swimming stage. See Figure 1 legend for details.

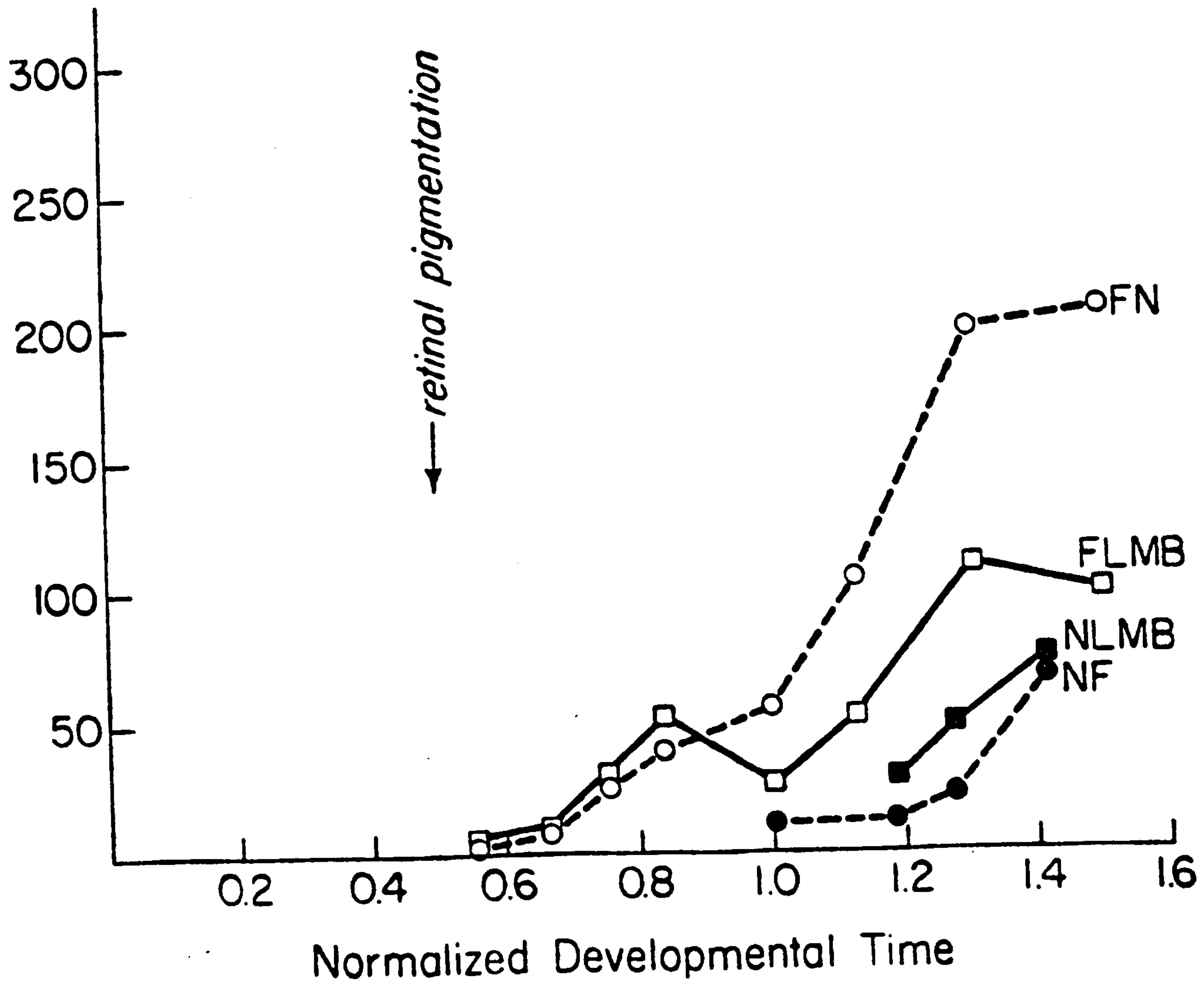


Fig. 3. The ontogenetic profiles of creatine kinase B subunit activity for each of the four types of embryos. The horizontal axis indicates normalized developmental time from the onset of retinal pigmentation stage to the active free swimming stage. See Figure 1 legend for details.

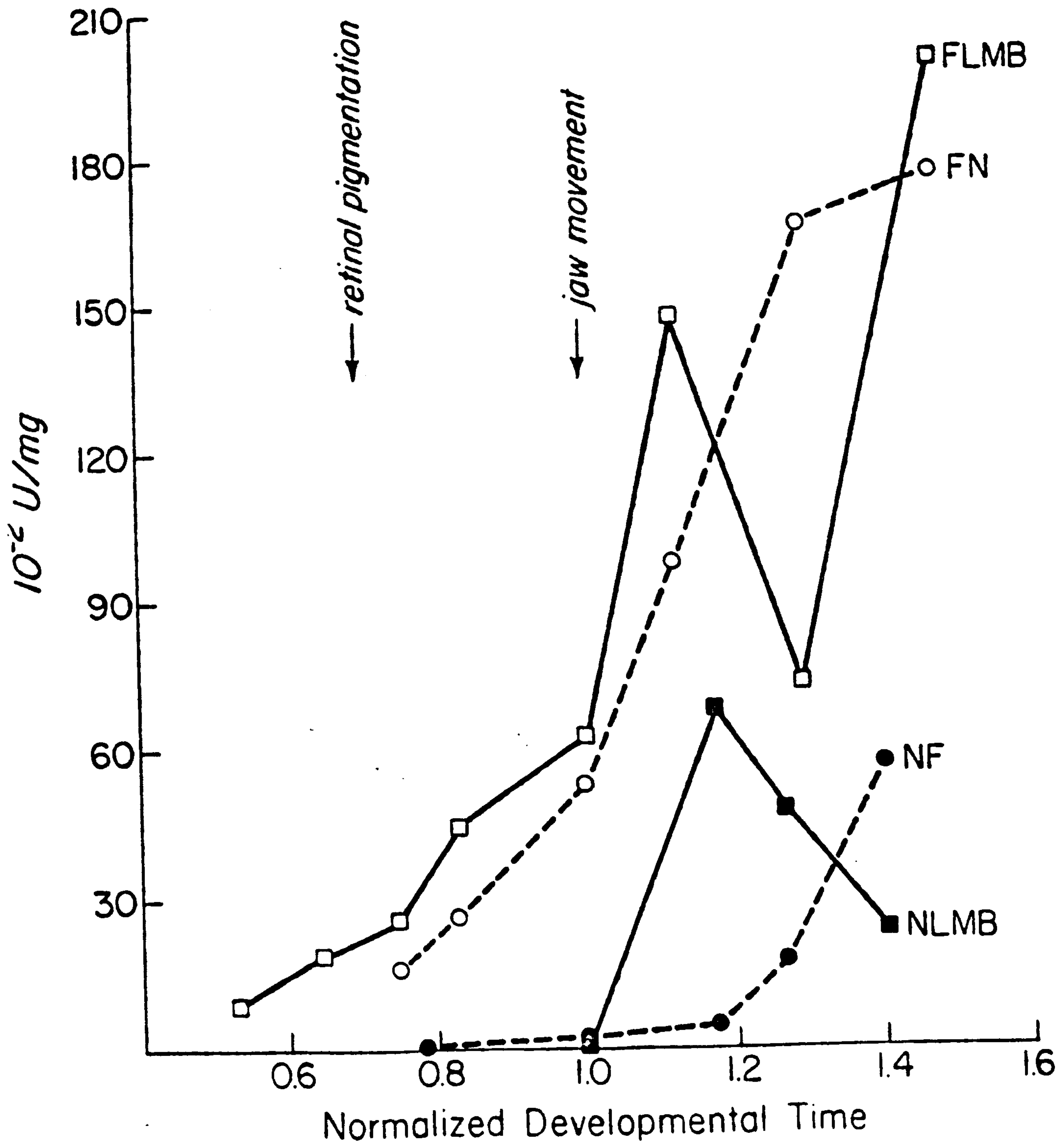


Fig. 4. The ontogenetic profiles of creatine kinase C subunit activity for each of the four kinds of embryos. See Figure 1 legend for details.

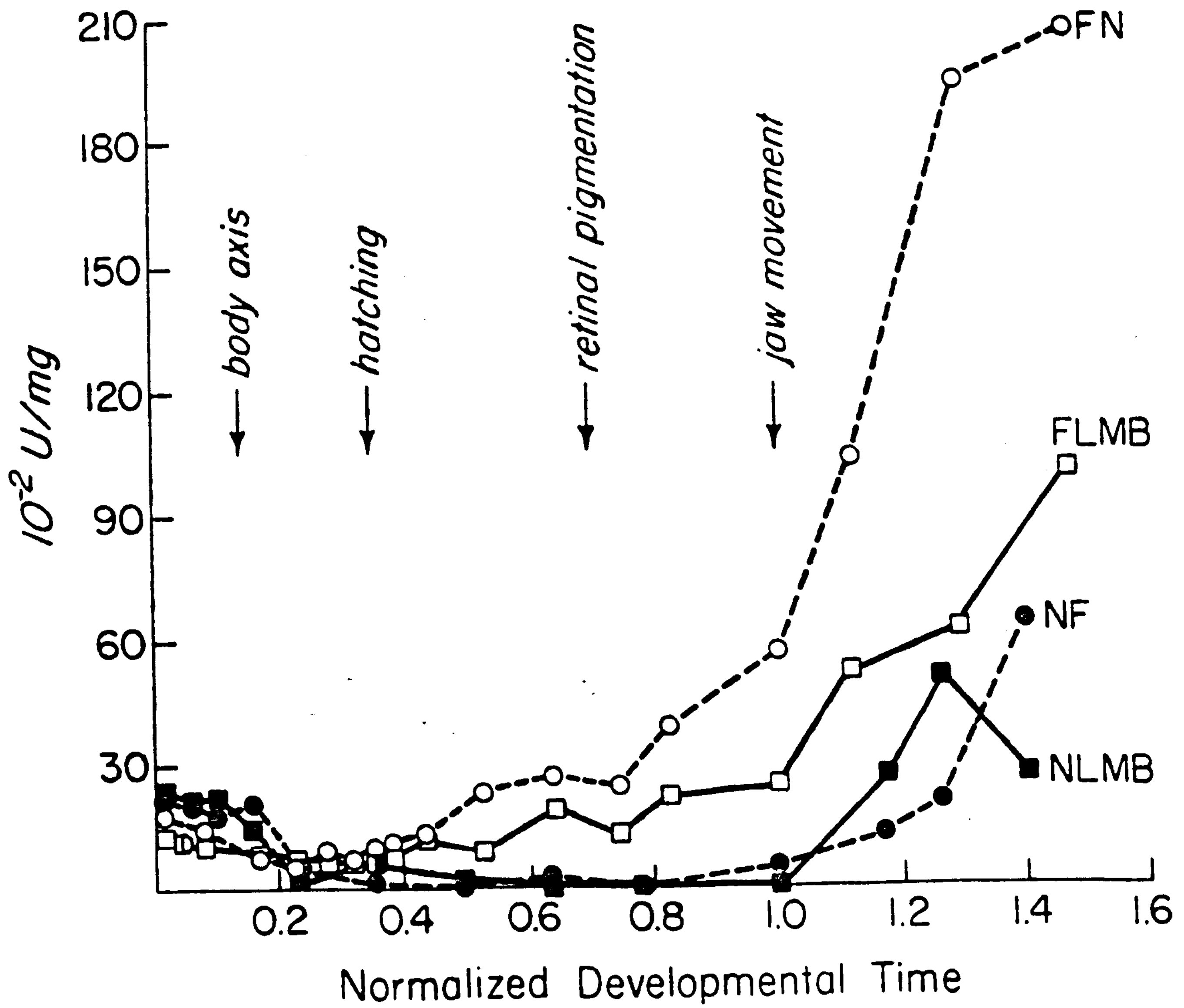


Fig. 5. The ontogenetic profiles of total glucosephosphate isomerase subunit activity for each of the four kinds of embryos. See Figure 1 legend for details.

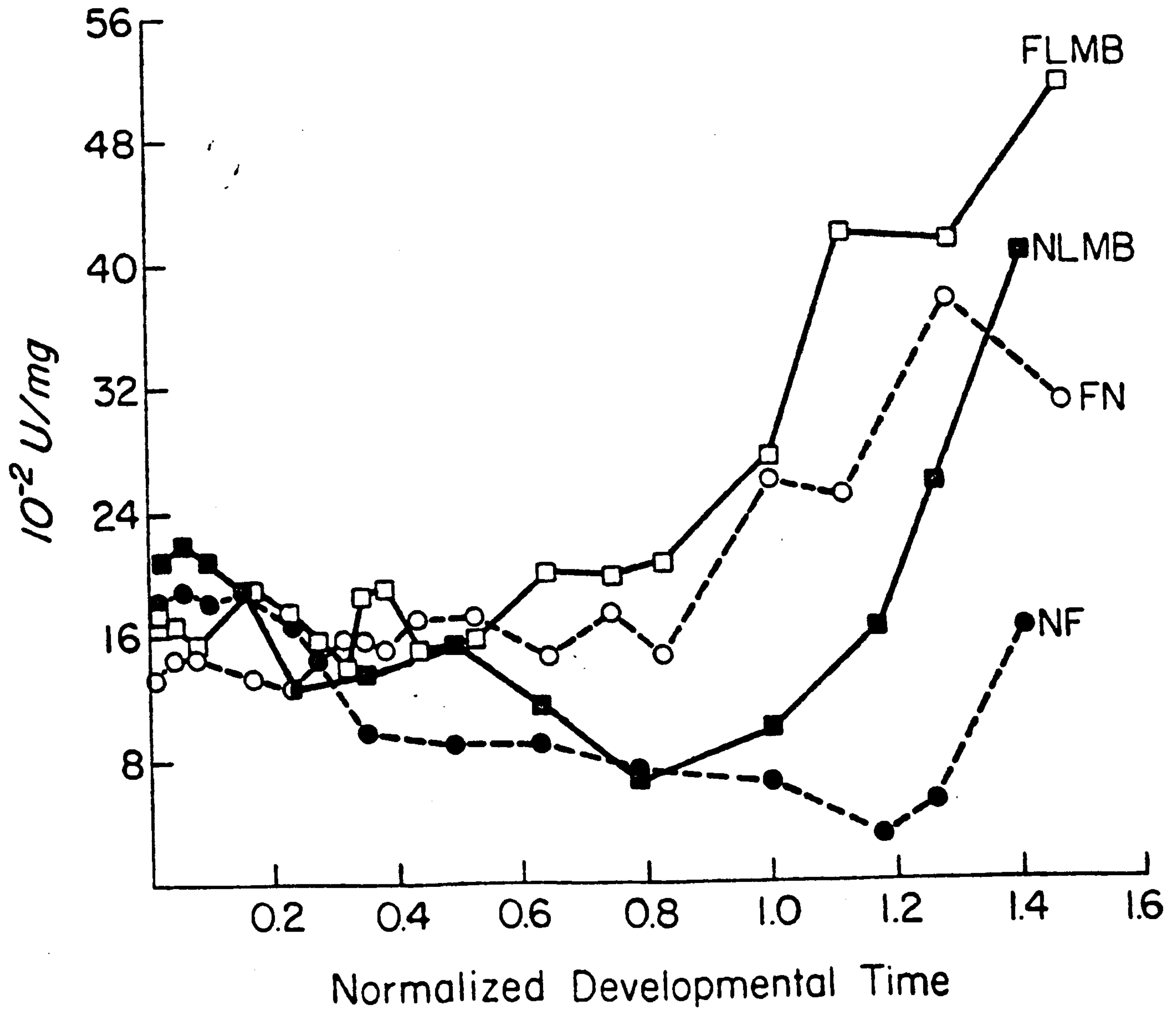


Fig. 6. The ontogenetic profiles of glucosephosphate isomerase A subunit activity for each of the four kinds of embryos. See Figure 1 legend for details.

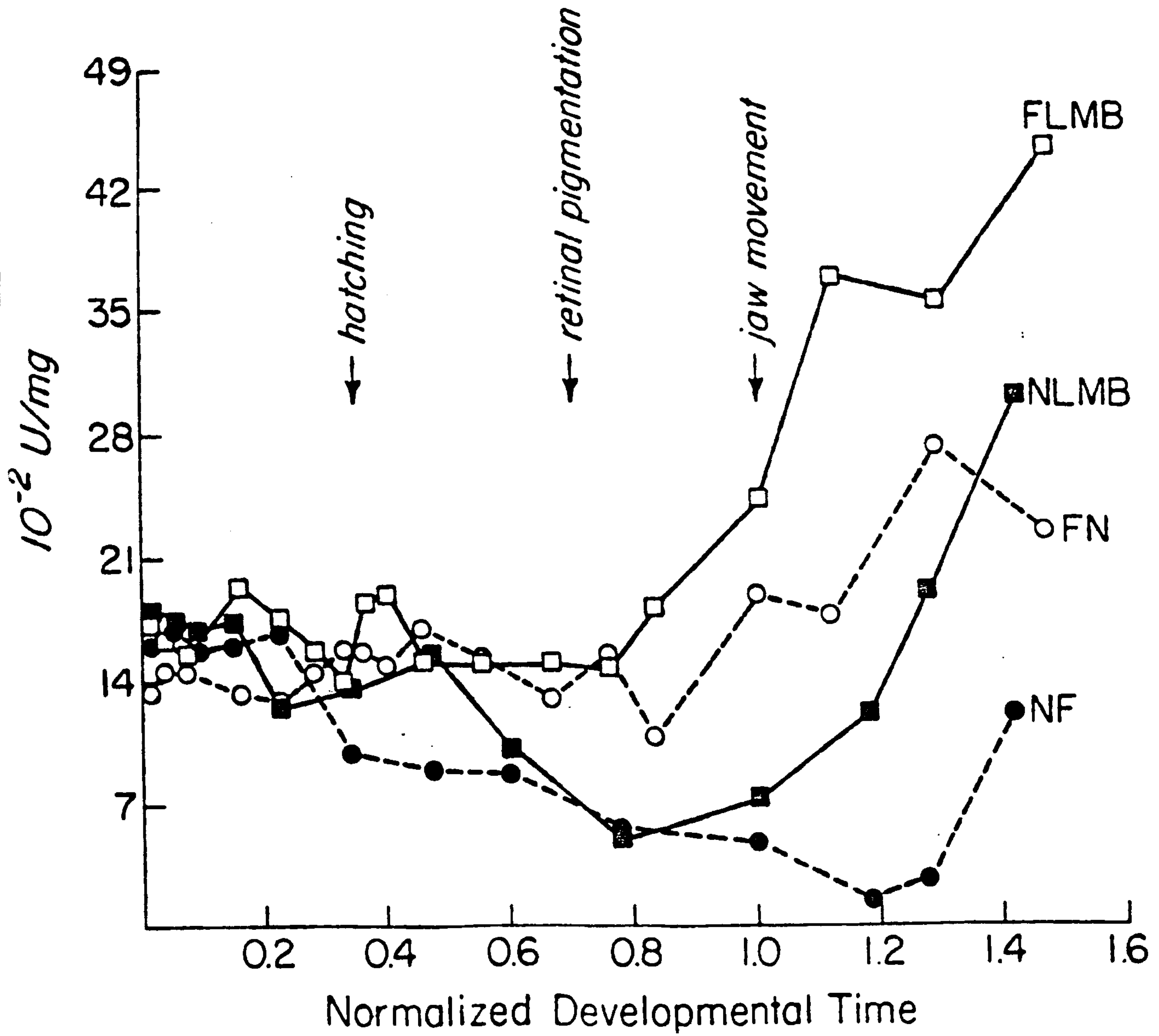


Fig. 7. The ontogenetic profiles of glucosephosphate isomerase B subunit activity for each of the four kinds of embryos. The horizontal axis indicates normalized developmental time from the posthatch stage to the active free swimming stage. See Figure 1 legend for details.

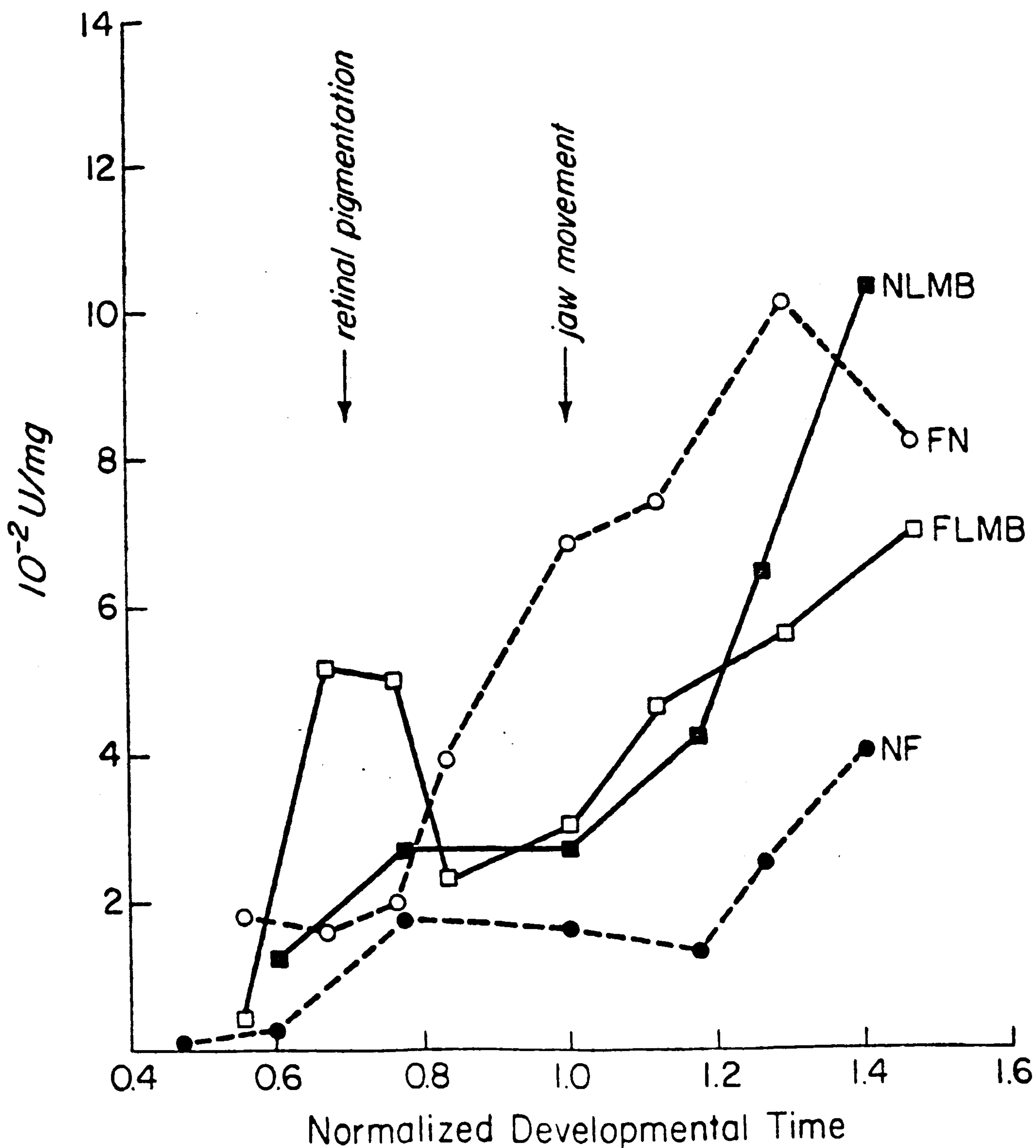


Fig. 8. The developmental schedules of initial gene expression of 16 loci for each of the four types of embryos. A separate set of histograms is given for each enzyme locus. The horizontal axis of each histogram indicates normalized developmental time with the vertical line marking the time of the jaw movement stage (1.00 ndt). The horizontal stipled bars indicate the times of first appearance of an enzyme or its first sustained increase in activity. The horizontal stripes mark the presence of enzyme activity throughout embryogenesis.

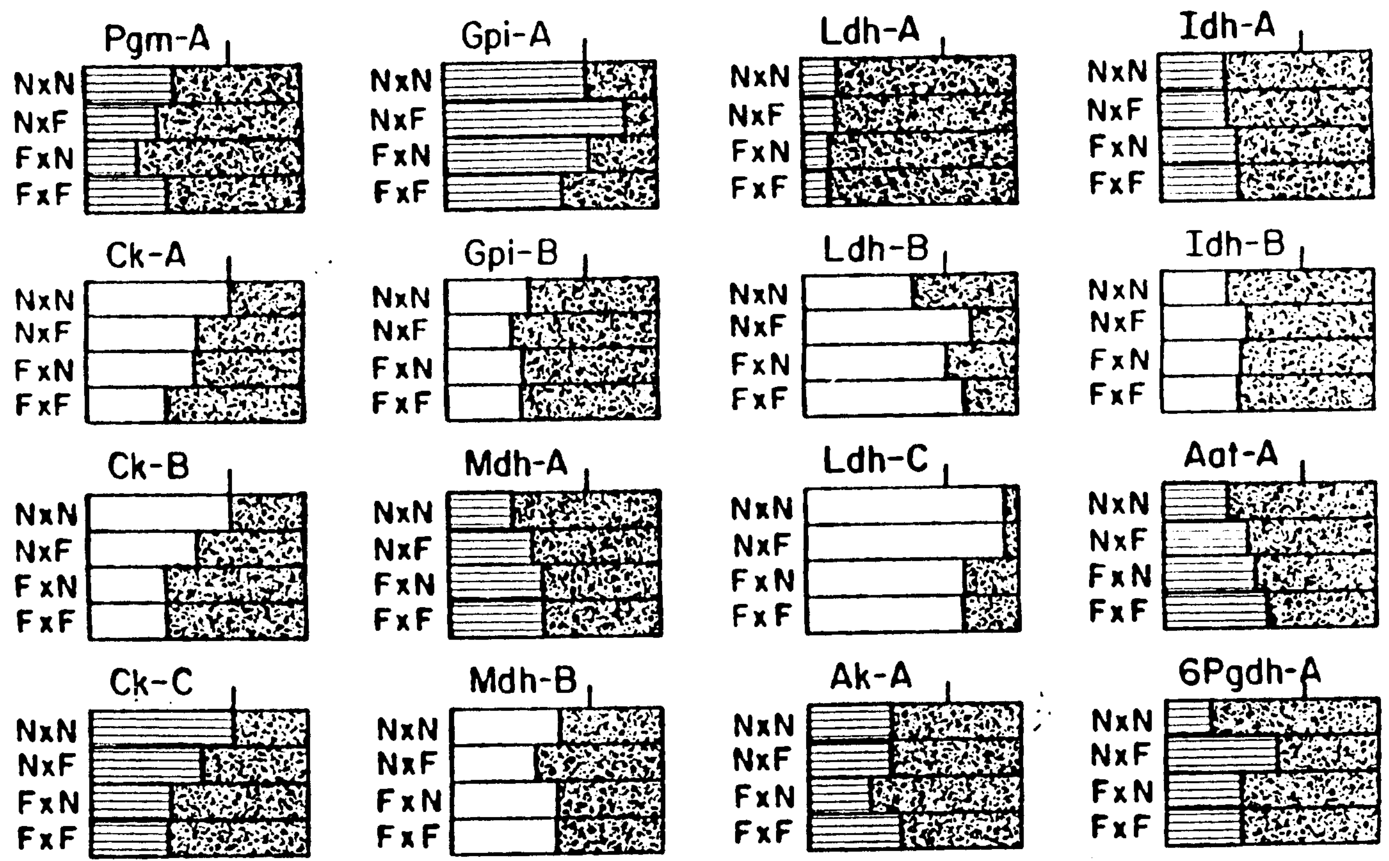
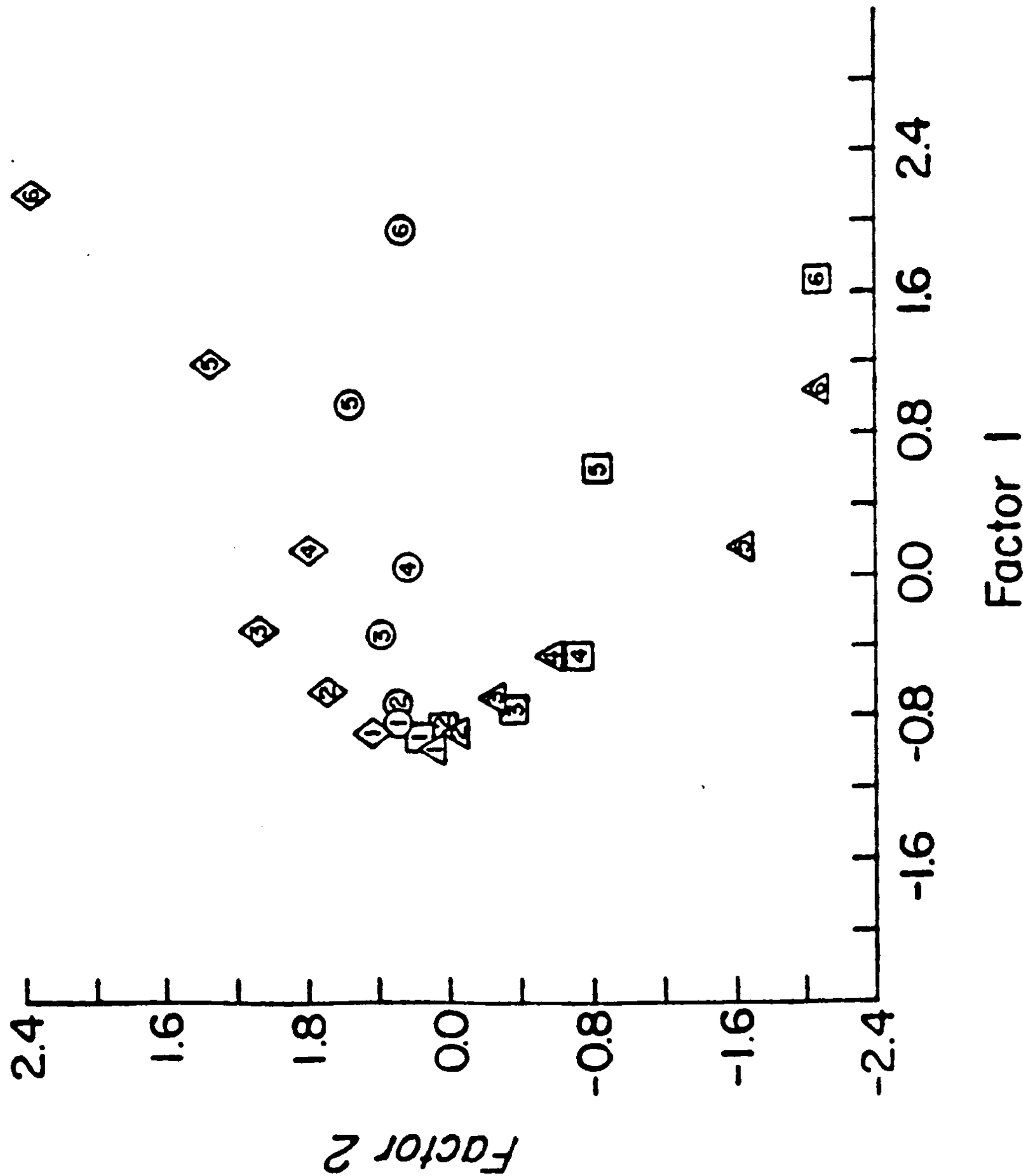


Fig. 9. The graphical representation of the principal component analysis. The horizontal axis indicates the weighted scores of the first factor of the analysis. The first factor has a strong developmental time component and accounts for 76% of the variance. The vertical axis indicates the weighted scores of the second factor of the analysis. The second factor ignores the time component and focuses on the enzyme loci that contributes the most to the between-cross variation with regard to the levels of enzyme activity. The symbols are as follows: ◇ , FxN; ○ , FLMB; △ , NxF; and □ , NLMB. The numbers within each symbol designates the developmental times, 1-6 (see Materials and Methods), during which the enzyme activity levels were assessed.



STUDY 103
JOB 2

CHAPTER 6

Levels of Transcription and Translation During the Embryonic Development of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass

David P. Philipp[1,2], Henry R. Parker[3]
and Gregory S. Whitt[3,1]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Genetically confirmed stocks of the northern largemouth bass (*Micropterus salmoides salmoides*), Florida largemouth bass (*M. s. floridanus*), and both reciprocal F_1 hybrids were produced in the laboratory using manual stripping/artificial fertilization techniques. Eggs from each stock were raised through hatching at 20±1°C. Samples of eggs at each of six key morphological stages of development were removed and incubated with ^{3}H-leucine or ^{3}H-uridine to measure amounts of protein or RNA synthesis, respectively. Although radioactively labelled precursors were incorporated at low levels, which increased through embryogenesis, no significant differences in the pattern of protein or of RNA synthesis could be detected among the four stocks.

INTRODUCTION

Compared to amphibians and echinoderms, rather limited data are available concerning the processes of transcription and translation in fishes. Available reports indicate that the synthesis of RNA during teleost oogenesis (Barmeister 1973, Neyfakh and Abramova 1974, Mazabraud et al. 1975) is similar to the pattern of transcription in amphibian oogenesis (Davidson 1976). During early teleost oogenesis the bulk of the transcribed RNA is tRNA and 5S RNA stored as 42S ribonucleoproteins within the cytoplasm (Mazabraud et al 1975). The bulk of the 5S RNA in teleost oocytes differs from 5S RNA found in somatic cells and, therefore, is presumably transcribed from genes normally repressed in these cells (Mazabraud et al. 1975). The genes encoding the 18S and 28S rRNA molecules apparently become amplified during early oogenesis (Vincent et al. 1968) with the corresponding 18S and 28S rRNA molecules not becoming transcribed until late oogenesis (Mazabraud 1975). Only after the synthesis of these rRNA molecules are the teleost ribosomes assembled and stored in the yolk (Kafiani and Timofeeva 1962). Throughout teleost oogenesis variable amounts of high molecular weight heterogeneous RNA accumulates. This heterogeneous RNA presumably represents the maternal pre-mRNA and mRNA species required to support protein synthesis during early embryogenesis. As in many other developing organisms, when the teleost oocyte reaches maturation, transcription and translation abruptly halt and the egg remains synthetically quiescent until after fertilization (Kafiani 1971).

From fertilization to organogenesis, there is relatively little change in the total amount of RNA within a developing fish egg. There is, however, a significant influx of RNA from the yolk into the cells of the developing

embryo. This RNA is most likely rRNA primarily (Kafiani and Timofeeva 1962). Despite the relatively constant level of total RNA in developing fish eggs, the level of transcription of specific classes of genes during this period changes dramatically. Prior to the formation of the blastula only a low level of transcription is detectable, much of which has been assigned to the mitochondria (Baltus et al 1965, Kafiani et al. 1969). Recent evidence, however, indicates that at least a portion of the synthesis of precursor tRNA's during cleavage may be nuclear in origin. However, these tRNA species are apparently processed only after the mid-blastula stage (Timofeeva and Solovjeva 1973).

In most teleosts, at the mid-blastula stage transcription increases dramatically (Neyfakh and Abramova 1974), however, the bulk of this newly synthesized RNA is heterogeneous nuclear RNA (HnRNA) (Kafiani and Timofeeva 1964, Kafiani et al. 1969). As in other developing eukaryotes, this HnRNA is characterized by having a high molecular weight, a DNA-like base composition and a fast turnover rate within the nucleus (Rachkus et al. 1969a). It has been postulated that this HnRNA contains precursor mRNA which requires one or more processing steps and transport from the nucleus to the cytoplasm before maturation of the mRNA molecules is complete (Neyfakh et al. 1972). These authors have demonstrated a slight but significant temporal separation between the initial burst of transcription (at mid-blastula) and the appearance of this newly synthesized RNA in the cytoplasm. Furthermore, it has been demonstrated that the majority of the RNA synthesized in the nucleus is never transported to the cytoplasm, much of the RNA transported to the cytoplasm being found in large ribonucleoprotein particles of unknown function (Spirin 1966, 1969). The results obtained from DNA-RNA annealing experiments demonstrated that the

cytoplasmic mRNA molecules synthesized during blastula formation and epiboly are more homogeneous than the HnRNA synthesized during this same period (Rachkus et al. 1969a). As development proceeds from epiboly through organogenesis, however, the cytoplasmic mRNA molecules become more heterogeneous in their molecular weight as well as their hybridization characteristics. These results suggest that as development proceeds, mRNA tends to become increasingly transcribed from new unique sequences. Competition hybridization experiments, however, have revealed that there is a significant overlap of RNA species produced at all stages of development (Rachkus et al. 1969b).

In teleosts there is a low level of protein synthesis in the unfertilized egg which changes little after fertilization and during cleavage (Krigsgaber and Neyfakh 1968, 1972, Neyfakh and Abramova 1974). This low level of synthesis, however, appears necessary for normal morphogenetic development (Crawford et al. 1973). Protein synthetic activity starts to rise at mid-blastula, presumably corresponding to the time at which mRNA molecules synthesized after fertilization first become translated. Translation continues to increase at least through epiboly (Krigsgaber and Neyfakh 1968, 1972, Neyfakh and Abramova 1974). This pattern of activation of protein synthesis more closely resembles that found in the amphibians than in the sea urchins.

Embryonic protein synthesis occurs almost exclusively in the cells of the blastoderm, although translation in the mitochondria probably occurs at low levels in the yolk (Krigsgaber and Neyfakh 1968, Neyfakh and Abramova 1974). Prior to epiboly, however, translation occurs on preformed ribosomes, some of which have migrated into the blastoderm cells from the yolk (Kafiani and Timofeeva 1962, Ajtkhoghin et al. 1964, Krigsgaber and Neyfakh 1968). The

majority of the proteins synthesized during these periods of early embryogenesis become associated with the nucleus, while almost none of the proteins become associated with mitochondria. However, very little information is available concerning the synthesis of specific protein molecules during teleost development, and the factors governing their regulation have not yet been elucidated.

MATERIALS AND METHODS

Embryos of each of four stocks of largemouth bass, NLMB, NxF, FxN, and FLMB, were formed in the laboratory by manually stripping ripe eggs from NLMB (collected from Clinton Lake, Illinois) or FLMB (collected from Lake Dora, Florida) females, splitting the expressed eggs into two batches in petri dishes, and fertilizing one-half of the eggs with sperm from NLMB and one-half with sperm from FLMB. These sperm-egg mixtures were allowed to sit for five minutes to allow for fertilization. They were then spread thinly into glass dishes containing approximately 1.0 cm water and allowed to water harden for about 30 minutes. These eggs were incubated at 20°± 1°C until they hatched. Dead embryos were removed and water changed three to four times daily. Morphological development was monitored visually using a dissecting microscope. At each of six key morphological stages during prehatch embryogenesis, samples of 200 eggs were removed, washed with sterilized water, and placed in 2.00 ml sterilized water containing 400 uCi ^{3}H-leucine or 40 uCi ^{3}H-uridine. These eggs were incubated for 20 minutes at 20°C with gentle agitation. Following incubation, the ^{3}H-incubation mixture was removed and the eggs were washed with 5.0 ml sterilized water and then frozen in 1.0 ml sterilized water at -20°C awaiting analysis.

To determine levels of protein synthesis, the samples incubated with ^{3}H-leucine were thawed, homogenized in a ground-glass Potter-Elvejehm homogenizer, and centrifuged for 10 minutes at 10,000xg at 4°C to remove structural debris. The resulting supernatants were brought to 70% saturation with ammonium sulfate to precipitate proteins. The precipitated protein mixture was collected by centrifugation at 20,000xg for 10 minutes at 4°C and resuspended in 2.0 ml of 100 mM Tris-HCl, pH 7.0. This precipitation and

resuspension procedure was repeated twice to remove unincorporated ^{3}H-leucine. A 0.10 ml sample of the radioactively labelled protein was precipitated using cold 10% trichloroacetic acid and collected on millipore filters. The radioactivity was then measured in a Packard Tri-carb scintillation counter as described in Philipp and Parsons (1979).

To determine levels of RNA synthesis, the samples incubated with ^{3}H-uridine were thawed, homogenized in a ground-glass Potter-Elvejehm homogenizer, and centrifuged at 10,000xg for 10 minutes at 4°C to remove debris. Following addition of unlabelled carrier RNA, labelled RNA was isolated from the supernatants using a Phenol-SDS extraction procedure (Phenol-saturated with 10 mM Tris-HCl, pH 9.0 and 0.25% sodium dodecylsulfate), followed by cold ethanol precipitation of the RNA. Following precipitation, RNA was collected by centrifugation at 20,000xg for 20 minutes at 4°C, and the resulting precipitated RNA was resuspended in 1.0 ml of 10 mM Tris-HCl, pH 8.1. A 0.10 ml sample of the radioactively labelled RNA was precipitated using cold 10% trichloroacetic acid and collected on millipore filters. The radioactivity was then measured in a Packard Tri-carb scintillation counter as described in Philipp and Parsons (1979).

RESULTS

Morphological development of the NLMB, NxF, FxN and FLMB embryos followed the pattern described by Phillipp et al. (1984). The percentage of fertilized eggs in each cross tested was greater than 98%. Hatching percentage for each cross was greater than 85%. No increased level of morphological abnormalities were observed in any one of the four crosses. No mortality was observed uring the ^{3}H-leucine or ^{3}H-uridine incubation phases.

The results of the translation experiments using ^{3}H-leucine as a labelled precursor to measure protein synthesis in the embryos of each of the four stocks is shown in Table 1. No significant differences in translation patterns were observed among the four stocks. There was a gradual increase in the rate of ^{3}H-leucine incorporation as embryogenesis proceeded from the ealy cleavage stages until just prior to hatching. The greatest increase appeared to be during formation of blastula and epiboly.

The results of the transcription experiments using ^{3}H-uridine as a labelled precursor to measure RNA synthesis in the embryos of each of the four stocks is shown in Table 2. No significant differences in the patterns of transcription were observed among the four stocks. There was a gradual increase in the rate of ^{3}H-uridine incorporated as embryo development proceeded from early cleavage through hatching. There was no obvious period of major increase in transcriptional rates.

DISCUSSION

In previous studies it has been shown that translation levels in developing fish embryos are initially low, but increase during formation of the blastula and epiboly (Krigsgaber and Neyfakh 1968, 1972, Crawford et al. 1973, Neyfakh and Abramova 1974). The results obtained in the present study agree with these other studies. The fact that the overall quantitative patterns of protein synthesis among the four stocks did not significantly differ may not be surprising, since the morphological patterns of development were so similar. Based upon the results obtained by Parker et al. (1984), qualitative differences in the specific proteins synthesized would be expected. However, since the amount of ^{3}H-leucine which was incorporated into newly synthesized protein was so low, no further fractionation of these labelled proteins was performed in an attempt to determine qualitative differences among the four stocks.

Previous studies revealed that levels of transcription during embryogenesis remain fairly constant (Kafiani and Timofeeva 1962, 1964, Baltus et al. 1965, Kafiani et al. 1969, Rachkus et al. 1969, Timofeeva and Solovjeva 1973). This was observed in the present study. A gradual rise in transcription rates from early cleavage through hatching did occur, but there were no major increases at any one stage. Due to the low level of ^{3}H-uridine incorporated during the incubations, no further fractionation of the ^{3}H-labelled RNA molecules was attempted. The use of cell-free _in vitro_ translation systems may in fact reveal greater qualitative differences in gene expression at this level than this type of _in vivo_ labelling procedure. These _in vitro_ procedures will be tested in the future.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collections and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. Finally, we wish to thank Dr. Bruce Taubert, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Christine Kaminski and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Baltus, E., J. Quertier, A. Ficq and J. Brachet. 1965. Biochemical studies of nucleate and anucleate fragments isolated from sea-urchin eggs. A comparison between fertilization and parthenogenetic activation. Biochim. Biophys. Acta 94:408-417.

Baumeister, H. G. 1973. Lampbrush chromosomes and RNA-synthesis during early oogenesis of _Brachydanio rerio_ (Cyprinidae, Teleostei). Z. Zellforsch. 145:145-150.

Crawford, R. B., C. E. Wilde, M. H. Heinemann and F. J. Hendler. 1973. Morphogenetic disturbances from timed inhibitions of protein synthesis in _Fundulus_. J. Embryol. Exp. Morph. 29:363-382.

Davidson, E. H. 1976. Gene activity in early development. 2nd Ed., Academic Press, New York, NY.

Kafiani, C. 1970. Genome transcription in fish development. Pages 209-283 _In_ Adv. Morph. Academic Press, New York, NY.

Kafiani, C. and M. J. Timofeeva. 1962. Inorganic orthophosphate and phosphoproteins in the embryonic development of _Misgurnis fossilis_, L. Biokhimiya 27:938-943.

Kafiani, C. and M. J. Timofeeva. 1964. Synthesis of nuclear RNA in early embryonic development. Dokl. Akad. Nauk. SSSR 164:721-724.

Kafiani, C., M. J. Timofeeva, A. A. Neyfakh, N. L. Melnikova and J. A. Rachkus. 1969. RNA synthesis in the early embryogenesis of a fish (_Misgurnis fossilis_). J. Embryol. Exptl. Morphol. 21:295-308.

Krigsgaber, M. R. and A. A. Neyfakh. 1968. Protein synthesis in the loach embryo blastoderm. Dokl. Akad. Nauk. SSSR 180:1259-1263.

Krigsgaber, M. R. and A. A. Neyfakh. 1972. Investigation of the mode of nuclear control over protein synthesis in early development of loach and sea urchins. J. Embryol. Exp. Morphol. 28:491-502.

Mazabraud, A., M. Wegnez and H. Denis. 1975. Biochemical research on oogenesis. RNA accumulation in the oocytes of teleosts. Dev. Biol. 44:326-332.

Neyfakh, A. A. and N. B. Abramova. 1974. Biochemical embryology of fishes. Pages 261-285 _In_ Chemical Zoology, Vol. VIII. Academic Press, New York.

Neyfakh, A. A., A. A. Kostomarova and T. A. Bourakova. 1972. Transfer of RNA from nucleus to ctyoplasm in early development of fish. An autoradiographical study. Exp. Cell. Res. 72:223.

Parker, H. R., D. P. Philipp and G. S. Whitt. 1984. Biochemical Genetic Evaluation of Patterns of Gene Expression During the Embryonic Development of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass. Pages 5-1 to 5-38 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, *Micropterus salmoides*, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Philipp, D. P. and P. Parsons. 1979. Isolation and purification of long chain fatty acyl-Coenzyme A ligase from rat liver mitochondria. J. Biol. Chem. 254:10776-10784.

Philipp, D. P., C. Kaminski and G. S. Whitt. 1984. A Comparison of the Embryonic Development of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass in Different Thermal Environments. Pages 4-1 to 4-28 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, *Micropterus salmoides*, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Rachkus, J. A., M. J. Timofeeva and C. Kafiani. 1969a. Transcription of repetitive DNA sequences in embryogenesis. Mol. Biol. 3:428-440.

Rachkus, J. A., H. C. Kuprianova, M. J. Timofeeva and C. Kafiani. 1969b. Homologies in RNA populations at different stages of embryogenesis. Mol. Biol. 3:617-626.

Spirin, A. S. 1966. On "masked" forms of messenger RNA in early embryogenesis and in other differentiating systems. Curr. Top. Develop. Biol. 1:1-38.

Spirin, A. S. 1969. Informosomes. Eur. J. Biochem. 10:20-35.

Timofeeva, M. J. and I. A. Solovjeva. 1973. Transfer RNA synthesis in early embryogenesis. FEBS Lett. 33:327-330.

Table 1. Incorportion of ^{3}H-leucine into protein during the development of NLMB, NxF, FxN, and FLMB embryos.

Morphological stage	Incorporated ^{3}H-leucine (cpm/.10 ml aliquot) (mean of two incubations)			
	NLMB	NxF	FxN	FLMB
8-16 cell	294	174	192	243
Blastula	386	341	361	392
1/2 Epiboly	478	407	393	441
Body Axis	481	480	461	504
14-16 Somites	546	502	553	510
Prehatch	533	553	608	579

Table 2. Incorportion of ^{3}H-uridine into RNA during the development of NLMB, NxF, FxN, and FLMB embryos.

Morphological stage	Incorporated ^{3}H-uridine (cpm/.10 ml aliquot) (mean of two incubations)			
	NLMB	NxF	FxN	FLMB
8-16 cell	132	164	153	158
Blastula	146	159	168	170
1/2 Epiboly	198	206	200	189
Body Axis	242	251	228	233
14-16 Somites	291	303	254	286
Prehatch	306	315	298	329

STUDY 104
JOB 1

CHAPTER 7

Thermal Effects on the Metabolic Biochemistry of Northern, Florida, and Reciprocal F_1 Hybrid Largemouth Bass

David P. Philipp[1,2], Christine Kaminski[1], Henry R. Parker[3], Manijeh Pasdar[3], and Gregory S. Whitt[3,1]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Genetically confirmed stocks of the northern largemouth bass (*Micropterus salmoides salmoides*), Florida largemouth bass (*M. s. floridanus*), and both reciprocal F_1 hybrids were produced in Champaign, Illinois through natural spawning in 0.08 hectare earthen ponds. Individuals of each of these stocks were removed during autumn and placed in aerated tanks at collection temperatures. Temperatures were adjusted at a rate of 1°C per day until final acclimation temperatures of 8°, 16°, 24° or 32°C were reached. Individuals of each of the four stocks were acclimated for 30 days at each of these four temperatures. Muscle and liver tissue extracts were assayed spectrophotometrically to determine specific activity levels for six enzyme systems (lactate dehydrogenase, creatine kinase, glucosephosphate isomerase, malate dehydrogenase, isocitrate dehydrogenase and aspartate aminotransferase). Serial dilution electrophoretic analysis of these extracts permitted further partitioning of total enzyme activities into individual locus contributions. Several of the enzyme loci expressed in specific tissues, Ldh-A (muscle), Ck-A (muscle), Gpi-B (muscle), Mdh-B (muscle) and Aat-B (liver) exhibited a commmon pattern in which the FLMB and FxN stocks had much lower activity levels at 8°C than the NLMB and NxF stocks, but much higher activity levels at 32°C. These results indicated that there is a differential regulation of these important metabolic enzyme loci in these stocks in response to temperature. In addition, differential thermal regulation of the northern (B^1) and Florida (B^3) alleles at the Aat-B locus among the NxF and FxN F_1 hybrids indicated the existence of different regulatory alleles associated with these different structural alleles.

INTRODUCTION

The temperature of the aquatic environment plays a vital role in the life history of fishes. Most fish usually deal with abrupt shifts in water temperature, such as those caused by sudden climatic changes or by sudden influx or cessation of a thermal discharge, through behavioral thermoregulation. Largemouth bass, *Micropterus salmoides*, have a distinct preferred temperature range, as well as upper and lower avoidance and lethal temperatures (Fry 1950, Hart 1952, Ferguson 1958, Coutant 1975a, b, Reynolds and Casterlin 1976, Reynolds et al. 1976, Magnuson and Beitinger 1978, Venables et al. 1977, Cichra et al. 1981, Fields et al. 1984, Koppelman et al. 1984). When abrupt localized temperature shifts occur, largemouth bass are usually able to migrate to more preferred temperatures and thus physically avoid undesirable or adverse thermal conditions.

However, when normal seasonal changes occur, thermal fluctuations are usually gradual, and areas of preferred temperatures often do not exist. Most fish species exhibit at least a partial capacity for physiological thermoregulation during seasonal temperature changes (Carey and Teal 1969, Stevens and Fry 1970, 1974). Thermal acclimation involves metabolic alterations at a number of different molecular levels (Hart 1952, Brett 1956, Fry 1967, Fry and Hochachka 1970, Prosser 1973, 1975, Hochachka and Somero 1973, Coutant et al. 1974, Hazel and Prosser 1974, 1979, Somero 1975, Moon 1975, Wilson et al. 1975, Shaklee et al. 1977). The ability or inability of one individual in a population to acclimate its metabolism to seasonal thermal regimes is a key factor in governing individual fitness. This acclimation ability has a strong genetic component which probably encompasses differences at both structural and regulatory genes (Shaklee et al. 1977).

Since different populations of a single species often occupy quite different geographic regions, there may be no one genotype within that species to allow for optimal thermal acclimation under all environmental conditions encountered within its range. Selection for enzymatic attributes suitable for populations in specific thermal regimes becomes increasingly important for species such as the largemouth bass which inhabit geographic areas encompassing a variety of thermal environments. The metabolic requirements for largemouth bass inhabiting waters in the northern extreme of the range (e.g., Minnesota, Wisconsin) must certainly be different from those for largemouth bass inhabiting waters in the southern extreme of the range (e.g., Florida). Indeed, significant differences in the thermal properties and requirements at the organismic level have been reported for the two subspecies of largemouth bass, the northern largemouth bass, *M. s. salmoides*, and the Florida largemouth bass, *M. s. floridanus* (Hart 1952, Clugston 1964, Addison and Spencer 1972, Chew 1975, Inman et al. 1976, Zolczynski and Davies 1976, Latta 1977, Bottroff and Lembeck 1978, Chichra et al. 1981, Smith and Wilson 1981, Wright and Wigtil 1981). The fact that allelic differences exist at loci encoding metabolically significant enzymes of largemouth bass (Philipp et al. 1981, 1983a) and that different allelic isozymes have different thermal kinetic properties (Hines et al. 1983) suggests that some of these differences may be contributing to differential organismic fitness.

The purpose of the present study was to monitor quantitative changes in key metabolic enzymes among four stocks of largemouth bass (NLMB, NxF, FxN, FLMB) in response to changes in acclimation temperature. In this manner, we have assessed the variability among these different genetic stocks in the acclimation responses of different enzyme loci among different tissues. This

assessment of differences in acclimation responses among the four genetic stocks serves also to assess the differences in the ability of each of these stocks to adapt to different thermal ranges.

MATERIALS AND METHODS

Production of Genetic Stocks:

Pure northern largemouth bass (NLMB), *M. s. salmoides*, were collected from Bone Lake, Wisconsin during October, 1978. Right pectoral fin clips were removed from each adult prior to stocking and utilized for electrophoretic analyses of each individual (Philipp et al. 1979, 1983a). All individuals retained contained only the Mdh-B^1, Idh-B^1, Sod-A^2 and Aat-B^1 or B^2 alleles, indicating they represented the pure northern subspecies. These individuals were held outdoors in 0.08 hectare ponds until the onset of the project in April 1980. Pure Florida largemouth bass (FLMB), *M. s. floridanus*, were collected from Lake Dora, Florida during January, 1980 and again during February, 1981. These fish were air shipped to Champaign and held indoors at 8-12°C. Left pectoral fin clips were removed from each adult prior to stocking outdoors and utilized for electrophoretic analyses of each individual. All individuals contained only the Mdh-B^2, Idh-B^3, Sod-A^1 or Sod-A^2 and Aat-B^3 or Aat-B^4 alleles, indicating they represented the pure Florida subspecies. In March of 1980 and 1981, the collected individuals were stocked outdoors in 0.08 hectare ponds.

During the spring of 1981, these brood stocks were used to produce NLMB, FLMB and both reciprocal F^1 hybrids, NLMB ♀ x FLMB ♂ (NxF) and FLMB ♀ x NLMB ♂ (FxN) by stocking 0.08 hectare ponds as follows:

Pond 1: 5 NLMB ♀ and 5 NLMB ♂
Pond 2: 5 NLMB ♀ and 6 FLMB ♂
Pond 3: 6 FLMB ♀ and 5 NLMB ♂
Pond 4: 8 FLMB ♀ and 6 FLMB ♂

Spawning was successful in all four production ponds. Ponds were drained on September 21, 22, 25 and 28, 1981, and approximately 1,200 50 mm fingerlings

were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

Acclimation Procedure:

Fingerlings of each of the four stocks of bass produced in 1981 were collected and held in thermally regulated aquaria under a 12L-12D 24 hour light schedule. The temperatures of the aquaria were adjusted at the rate of 1°C per day until the desired acclimation temperatures were reached. The acclimation temperatures were 8°, 16°, 14°, and 32°C. These largemouth bass were then held for a 30 day period to allow for total thermal acclimation. During this period these fingerlings were fed live *Daphnia magna* and frozen brine shrimp three times daily. Following total thermal acclimation, these largemouth bass were frozen awaiting enzyme analysis.

Enzymatic Determinations:

For enzyme activity determinations, liver and muscle were dissected and homogenized in 100 mM Tris-HC1, pH 7.0. After centrifugation, the resulting supernatants were split into two aliquots. The first aliquot was used to spectrophotometrically determine total enzyme activity (specific activity: International Enzyme Units/mg soluble protein) and protein concentrations. The specific enzymatic assays were performed spectrophotometrically at 25°C for the following enzymes: (1) creatine kinase, CK (EC 2.7.3.2), (2) adenylate kinase, AK (EC 2.7.4.3), (3) phosphoglucomutase, PGM (EC 2.7.5.1), (4) glucosephosphate isomerase, GPI (EC 5.3.1.9), (5) malate dehydrogenase, MDH (EC 1.1.1.37), (6) lactate dehydrogenase, LDH (EC 1.1.1.27), (7) isocitrate dehydrogenase (NADP), IDH (EC 1.1.1..41), (8) 6-phosphogluconate dehydrogenase, 6-PGDH (EC 1.1.1.44),

and (9) aspartate aminotransferase, AAT (EC 2.6.1.11). The reaction mixtures used for each assay were, with slight changes, based on Shaklee et al. (1977) with the exception of that used for PGM, which was based on Dawson and Mitchell (1969).

(1) AK - 20 mM Tris-HCl pH 7.5, 4.0 mM $MgCl_2$, 3.3 mM glucose, 0.5 mM ADP, 0.15 mM NADP, 1.0 unit/ml hexokinase, 1.0 unit/ml glucose-6-phosphate dehydrogenanse.

(2) CK - Identical to the AK reaction mixture except that 10.0 mM phosphocreatine was added.

(3) PGM - 4 mM Imidazole-HCl pH 7.5, 3.0 mM $MgCl_2$, 1.5 mM EDTA, 0.17 mM NADP, 1.7 mM glucose-1-phosphate, 3.3 mM glucose-1, 6-diphosphate, 1.0 unit/ml glucose-6-phosphate dehydrogenase (Dawson and Mitchell 1969).

(4) GPI - 20 mM Tris-HCl pH 7.5, 3 mM $MgCl_2$, 0.13 mM NADP, 2.0 mM fructose-6-phosphate, 1.0 unit/ml glucose-6-phosphate deydrogenase.

(5) LDH - 0.1 M phosphate buffer pH 7.0, 1.0 mM pyruvate, 0.14 mM NADH.

(6) MDH - 0.1 M phosphate buffer pH 7.0, 0.22 mM oxaloacetate, 0.14 mM NADH.

(7) IDH - 50 mM Tris-HCl pH 7.5, 2.0 mM $MgCl_2$, 0.15 mM NADP, 1.0 mM isocitrate.

(8) 6-PGDH - 50 mM Tris-HCl pH 7.5, 2.0 mM $MgCl_2$, 0.15 mM NADP, 2.0 mM 6-phosphogluconate.

(9) AAT - 50 mM Tris-HCl pH 7.5, 0.7 mM ketoglutarate, 10.0 mM aspartate, 0.08 mM pyridoxal-5-phosphate, 0.1 mM NADH, 1.0 unit/ml malate dehydrogenase.

Enzyme activities were determined by monitoring the changes in absorbance at 340 nm using a Beckman Kintrac VII spectrophotometer and are expressed as International Units. The final volume of the reaction mixture was 1.0 ml. Reactions were initiated with the addition of enzyme extract (1-50 ul of the appropriate supernatant, depending upon the enzyme being assayed). Protein concentrations of each supernatant were determined using the procedure of Lowry et al. (1951) as modified by Mason et al. (1973).

The second aliquot was subjected to a serial dilution-starch gel electrophoretic analysis to partition out total enzyme activity to the individual isozyme components present in each tissue (Klebe 1975, Magee and Philipp 1982). In this way, the relative contribution of the 16 individual genes was determined for both tissues of each fish incubated at all four temperatures. The vertical starch gel electrophoresis and histochemical staining procedures used in these analyses were performed essentially as described in Philipp et al. (1979).

The specific activity determinations for the level of total lactate dehydrogenase activity, as well as the individual contributions of the Ldh-A and Ldh-B loci for both muscle and liver tissue in all four stocks are given in Table 1 and diagramatically represented in Figures 1 and 2. In muscle, the predominant LDH activity is contributed by the Ldh-A locus. The different stocks demonstrated an interesting thermal activity pattern, and one which was seen for several enzyme loci (Fig. 1). At 8°C, the FLMB and FxN had relatively low LDH-A activity levels. However, as acclimation temperature increased, their LDH-A activity levels sharply increased as well, to levels much higher than the NLMB and NxF stocks at 32°C. Very minimal and constant LDH activity patterns were observed in liver samples from all stocks (Fig. 2).

The specific activity determinations for the level of total creatine kinase activity, as well as the individual contributions of the Ck-A, Ck-B, and Ck-C loci for both muscle and liver tissue in all four stocks are given in Table 2 and diagramatically represented in Figures 3 and 4. In muscle, the predominant CK activity is contributed by the Ck-A locus. The stocks exhibited a thermal activity pattern similar to that for the Ldh-A locus in muscle. The FLMB and FxN stocks had the lowest activity levels at 8°C, but the highest ones at 32°C (Fig. 3). Again, only minimal and relatively constant thermal activity patterns for CK were observed for all stocks in liver tissue (Fig. 4).

The specific activity determinations for the level of total glucosephosphate isomerase activity, as well as the individual contributions of the Gpi-A and Gpi-B loci for both muscle and liver tissue in all four stocks are given in Table 3 and diagramatically represented in Figures 5 and 6. In muscle, the predominant GPI activity is contributed by the Gpi-B locus. The

four stocks again exhibited a thermal activity pattern similar to those of the Ldh-A locus and the Ck-A locus in muscle. The FLMB and FxN stocks had the lowest activity levels at 8°C, but the highest ones at 32°C (Fig. 5). In liver, the predominant GPI activity is contributed by the Gpi-A locus. In this case, the FLMB had the lowest level of activity at 8°C and the NLMB the highest level of GPI-A activity. At 32°C, these positions remained unchanged, but they did grow much closer (Fig. 6).

The specific activity determinations for the level of total malate dehydrogenase activity, as well as the individual contributions of the Mdh-A, Mdh-B, and Mdh-M loci for both muscle and liver in all four stocks are given in Table 4 and diagramatically represented in Figures 7 and 8. In muscle, the predominant MDH activity is contributed by the Mdh-B locus, although measureable Mdh-A locus contribution is also present. Although no definitive thermal activity pattern was observed among the stocks of largemouth bass for the Mdh-A locus, the pattern seen before for other loci in muscle (Ldh-A, Ck-A, Mdh-B) was observed for the Mdh-B locus in this tissue (Fig. 7). The FLMB and FxN had the lowest Mdh-B contributions at 8°C, but the highest at 32°C. The NLMB and FLMB used in this study had fixed allelic differences at this locus. As a result, the genotype of the four stocks are as follows: NLMB = B^1/B^1; NxF = B^1/B^2; FxN = B^1/B^2; FLMB = B^2/B^2. The differences in thermal activity patterns among the stocks do not simply reflect differences in the allelic isozymes because the MDH activity patterns of the two reciprocal hybrids are quite different, even though their genotypes at this locus are identical. In addition, no differences in allelic contributions between the B^1 and B^2 alleles were noted in these heterozygous individuals. In all cases, serial dilution electrophoretic analyses revealed a 1:1 ratio of MDH-B^1:MDH-B^2 subunits. In

the liver, the predominant MDH activity is contributed by the Mdh-A locus. For all stocks, the thermal activity pattern was similar, peak activity occurring at 24°C (Fig. 8). In this case, the NLMB had the highest levels of MDH activity and the FLMB the lowest.

The specific activity determinations for the level of total isocitrate dehydrogenase activity, as well as the individual contributions of the Idh-A and Idh-B loci for both muscle and liver in all four stocks are given in Table 5 and diagramatically represented in Figure 9. In muscle the predominant IDH activity is contributed by the Idh-A locus, whereas in liver it is contributed by the Idh-B locus. Relatively constant and similar thermal activity patterns for all four stocks were observed for both the Idh-A locus in muscle and the Idh-B locus in liver (Fig. 9). Interestingly, the NLMB and FLMB used in this study had fixed allelic differences at the Idh-B locus. Similar to the Mdh-B locus, no differential allelic contributions were noted for the Idh-B^1 (NLMB) and the Idh-B^3 (FLMB) alleles at any of the acclimation temperatures studied.

The specific activity determinations for the level of total aspartate aminotransferase activity, as well as the individual contributions of the Aat-A, Aat-B, and Aat-M loci for both muscle and liver in all four stocks are given in Table 6 and diagramatically represented in Figures 10 and 11. In addition, the individual contributions of the Aat-B^1 (M. s. salmoides) and Aat-B^3 (M. s. floridanus) alleles in the liver of the NxF and FxN F_1 hybrids are represented in Figure 12. In muscle the predominant AAT activity is contributed by both the Aat-A and Aat-M loci. Relatively constant and similar thermal activity patterns for all four stocks were observed for both AAT loci in this tissue (Fig. 10). In liver the predominant AAT activity is contributed by the Aat-B locus. Here again, the four stocks exhibited a thermal activity

pattern similar to Ldh-A (muscle), Ck-A (muscle), Gpi-B (muscle) and Mdh-B (muscle). The FLMB and FxN stocks had the lowest AAT-B subunit activity levels at 8°C, but the highest at 32°C (Fig. 11). The NLMB and FLMB used in this study were fixed for alternative alleles at this locus too. NLMB had a B^1/B^1 genotype, FLMB a B^3/B^3 genotype and both hybrids a B^1/B^3 genotype. The thermal activity patterns for the AAT-B subunits in liver of the two hybrids were quite different (Fig. 11). However, unlike the Mdh-B and Idh-B loci, which also had fixed allelic differences between the subunits, the two alleles showed differential thermal activity patterns in the two F_1 hybrid stocks, both with B^1/B^3 genotypes (Fig. 12). In both the NxF and FxN hybrid, the activity contributions of the B^1 and B^3 alleles were approximately equal at 8°C. However, as the acclimation temperature increased, the relative amount of the B^3 allele compared to the B^1 allele increased dramatically. At 32°C, the ratio of B^3:B^1 contribution was 1.35 in the NxF and 1.40 in the FxN hybrid. This divergence from true codominant allelic expression indicates the existence of some differential regulation of allele expression at the molecular level.

DISCUSSION

It has been fairly well accepted that most fish species have at least some capacity to thermoregulate their physiological responses during seasonal changes (Carey and Teal 1969, Stevens and Fry 1970, 1974). In fact, thermoregulatory acclimation involves metabolic alterations at a variety of molecular levels (Hart 1952, Brett 1956, Fry 1967, Fry and Hochachka 19770, Prosser 1973, 1975, Hochachka and Somero 1973, Coutant et al. 1974, Hazel and Prosser 1974, 1979, Somero 1975, Moon 1975, Wilson et al. 1975, Shaklee et al. 1977).

It has also been shown that largemouth bass have distinct upper/lower avoidance and lethal temperatures (Fry 1950, Hart 1952, Ferguson 1958, Venables 1977, Cichra et al. 1981, Fields et al. 1984), as well as distinct thermal preference ranges (Coutant 1975a,b, Reynolds and Casterlin 1976, Reynolds et al. 1976, Magnuson and Beitinger 1978, Koppelman et al. 1984). In fact, Fields et al. (1984) have shown that different genetic stocks of largemouth bass have significantly different upper thermal tolerance limits, and that these limits greatly depend upon acclimation temperature. Similarly, Koppelman et al. (1984) have shown that different stocks of largemouth bass have somewhat different preferred temperatures, and that these values greatly depend upon acclimation temperature, as well. These studies suggest that the different stocks of largemouth bass thermally acclimate their metabolisms differently. The present study was designed to test this hypothesis.

Although some of the loci studied were expressed at only very low levels in one or both of the tissues studied, others, which were expressed extensively, showed quite interesting thermoregulatory patterns. Most interesting was a common pattern exhibited by five enzyme loci, Ldh-A, Ck-A,

Gpi-A, Mdh-B, all predominantly expressed in the white skeletal muscle, and Aat-B, predominantly expressed in the liver (Figs. 1, 3, 5, 7 and 11, respectively). For these five loci, the levels of expression in the FLMB and FxN stocks are lower than those expressed in the NLMB and NxF stocks at 8°C, but higher at 32°C. A somewhat analogous pattern of activity was observed for the Gpi-B locus predominantly expressed in the liver (Fig. 6). At 8°C, the FLMB and FxN stocks had much lower activities than the NLMB and NxF stocks. However, at 32°C the levels of all four stocks were comparable.

This common pattern indicated that these different stocks were regulating their levels of expression of certain specific genes differentially in response to temperature. These changes in levels of key metabolic enzymes must certainly have significant effects upon the biochemical metabolic states of these fish stocks. The suggestion is that the two subspecies, the *M. s. salmoides* and the *M. s. floridanus*, have different complements of regulatory loci which are responding differentially to varying thermal conditions and thereby regulating the expression of orthologous genes differentially in the different stocks.

The patterns of thermal activity demonstrated by the Aat-B locus in the liver tissue among the two F_1 hybrids, NxF and FxN, further illustrate this differential regulation. The two subspecific broodstocks of largemouth bass used to produce the F_1 stocks in this study had fixed allelic differences at three loci monitored, Mdh-B, Idh-B and Aat-B. As a result, all individuals of both F_1 hybrid stocks were heterozygous at these loci, Mdh-B^1/B^2, Idh-B^1/B^3 and Aat-B^1/B^3. The expression of the two alleles at the Mdh-B and the Idh-B loci was exactly codominant at all temperatures tested. That is, the ratio of MDH-B^1:MDH-B^2 and IDH-B^1:IDH-B^3 subunit activity was always 1.0. This was not

the case for the expression of the two alleles at the Aat-B locus (Fig. 12). At 8°C the expression of the B^1 and B^3 alleles was relatively codominant in both F_1 hybrids. However, as temperature increased, the disparity of allele expressions increased. At 32°C, the level of the FLMB allele (B^3) was substantially higher than the level of the NLMB allele (B^1), reaching B^3:B^1 ratios of 1.35 in the NxF hybrid and 1.40 in the reciprocal FxN hybrid. There is a strong suggestion that the Aat-B loci in the two subspecies may have different regulatory elements that are cis-located to the structural gene itself.

The results presented in this study clearly suggest that the two subspecies of largemouth bass and their reciprocal F_1 hybrids regulate their biochemical metabolisms differentially in response to different thermal regimes. In addition, we postulate that this regulatory divergence stems from an evolutionary divergence between these subspecies in the regulatory genes controlling the expression of specific enzyme loci. We feel that the evolutionary divergence among stocks is most dramatically affected by divergence at these regulatory loci and less by divergence at structural loci (Whitt and Philipp 1977, Philipp et al. 1979, 1981, 1983b, Parker et al. 1984). We also feel that this divergence among stocks at specific regulatory loci plays a major role in the genetic tailoring of specific stocks for specific environments through natural selection, and thus, in determining the fitness of one stock relative to another.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collection and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. Finally, we wish to thank Dr. Bruce Taubert, Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Shirley Lowe, Todd Powless, and especially, Dale Burkett for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Addison, J. H. and S. L. Spencer. 1972. Preliminary evaluation of three strains of largemouth bass, Micropterus salmoides (Lacepede), stocked in ponds in south Alabama. Proceedings of the 25th Annual Conference of the Southeastern Association of Game and Fish Commissioners 25:366-374.

Bottroff, L. J. and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass, Micropterus salmoides floridanus. California Fish and Game 64:4-23.

Brett, J. R. 1956. Some principles in the thermal requirements of fish. Quarterly Reviews of Biology 31:75-87.

Carey, F. G. and J. M. Teal. 1969. Regulation of body temperature by the bluefin tuna. Comparative Biochemistry and Physiology 28:205-213.

Chew, R. L. 1975. The Florida largemouth bass. Pages 450-458 In R. H. Stroud and H. Clepper (Eds.) Black bass biology and management. Sport Fishing Institute, Washington, D.C.

Cichra, C. E., W. E. Neill and R. L. Noble. 1981. Differential resistance of northern and Florida largemouth bass to cold shock. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissioners 34:19-24.

Clugston, J. P. 1964. Growth of the Florida largemouth bass, Micropterus salmoides floridanus (Le Sueur), and the northern largemouth bass, M. s. salmoides (Lacepede), in subtropical Florida. Transactions of the American Fisheries Society 93:146-154.

Coutant, C. C. 1975a. Responses of bass to natural and artificial temperature regimes. Pages 272-285 In R. H. Stroud and H. Clepper (Eds.) Black bass biology and management. Sport Fishing Institute, Washington, D.C.

Coutant, C. C. 1975b. Temperature selection by fish - a factor in power plant impact assessments. Pages 575-595 In Environmental effects of cooling systems at nuclear power plants. International Atomic Energy Agency, Vienna.

Coutant, C. C., H. M. Ducharme, Jr. and J. R. Fisher. 1974. Effects of cold shock on vulnerability of juvenile channel catfish (Ictalurus punctatus) and largemouth bass (Micropterus salmoides) to predation. Journal of the Fisheries Research Board of Canada 31:351-354.

Dawson, D. M. and A. Mitchell. 1969. The isoenzymes of phosphoglucomutase. Biochemistry 8(2):609-614.

Ferguson, R. G. 1958. The preferred temperature of fish and their mid-summer distribution in temperate lakes and streams. Journal of the Fisheries Research Board of Canada 15:607-624.

Fields, R., S. S. Lowe, C. Kaminski, G. S. Whitt and D. P. Philipp. 1984. Acute and chronic thermal maxima of northern, Florida, and reciprocal F_1 and F_2 hybrid largemouth bass. Pages 9-1 to 9-19 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, *Micropterus salmoides*, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Fry, F. E. J. 1950. Effects of the environment on animal activity. University of Toronto Studies in Biology Series 55. 62 p.

Fry, F. E. J. 1967. Responses of vertebrate poikilotherms to temperature. Pages 375-409 In A. H. Rose (Ed.) Thermal biology. Academic Press, New York.

Fry, F. E. J. and P. W. Hochachka. 1970. Fish. Pages 79-134 In G. C. Whittow (Ed.) Comparative physiology of thermoregulation. Academic Press, New York.

Hart, J. S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. University of Toronto Biology Series 60. Publication of the Ontario Fisheries Research Laboratory No. 72 60:1-79.

Hazel, J. R. and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiological Reviews 54:620-677.

Hazel, J. R. and C. L. Prosser. 1979. Incorporation of $1-^{14}$ C-acetate into fatty acids and sterols by isolated hepatocytes of thermally acclimated rainbow trout (*Salmo gairdneri*). Journal of Comparative Physiology 134:321-329.

Hochachka, P. W. and G. N. Somero. 1973. Strategies of biochemical adaptation. W. B. Saunders Co., Philadelphia. 358 p.

Inman, C. R., R. C. Dewey and P. P. Durochere. 1976. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the 30th Annual Conference of the Southeastern Association of Game and Fish Commissioners 30:1-17.

Klebe, R. J. 1975. A simple method for the quantitation of isozyme patterns. Biochemical Genetics 13:805-812.

Koppelman, J. B., G. S. Whitt and D. P. Philipp. 1984. Thermal preferenda of northern, Florida, and reciprocal F_1 hybrid largemouth bass. Pages 8-1 to 8-30 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, *Micropterus salmoides*, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Latta, W. C. 1977. Survival and growth of Florida largemouth bass in ponds and lakes in Michigan. Michigan Department of Natural Resources, Dingell-Johnson Project F-35-R-3, Study XXVII:101-114.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Magee, S. M. and D. P. Philipp. 1982. Biochemical genetic analyses of the grass carp ♀ x bighead carp ♂ F_1 hybrid and the parental species. Transactions of the American Fisheries Society 111:593-602.

Magnuson, J. J. and T. L. Beitinger. 1978. Stability of temperature preferred by centrarchid fishes and terrestrial reptiles. Pages 181-216 In E. S. Reese and F. J. Lighter (Eds.) Contrasts in behavior. John Wiley and Sons, Inc., New York.

Mason, T. L., R. O. Poyton, D. C. Wharton and G. Schatz. 1973. Journal of Biological Chemistry 248:1346-1354.

Moon, T. W. 1975. Temperature adaptation: isozymic function and the maintainence of heterogeneity. Pages 207-220 In C. L. Markert (Ed.) Isozymes II: Physiological function. Academic Press, New York.

Parker, H. R., G. S. Whitt and D. P. Philipp. 1984. Biochemical genetic evaluation of patterns of gene expression during the embryonic development of northern, Florida, and reciprocal F_1 hybrid largemouth bass. Pages 5-1 to 5-38 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, *Micropterus salmoides*, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, *Micropterus salmoides salmoides*, and smallmouth bass, *Micropterus dolomieui*). Journal of Experimental Zoology 210:473-488.

Phillipp, D. P., W. F. Childers and G. S. Whitt. 1981. Management implications for different stocks of largemouth bass (Micropterus salmoides) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Phillipp, D. P., W. F. Childers and G. S. Whitt. 1983a. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Phillipp, D. P., H. R. Parker and G. S. Whitt. 1983b. Evolution of gene regulation: Isozymic analysis of patterns of gene expression during hybrid fish development. In: Isozymes: Current Topics in Biological and Medical Research 10:193-237.

Prosser, C. L. 1973. Comparative animal physiology. W. B. Saunders Co., Philadelphia. 966 p.

Prosser, C. L. 1975. Physiological adaptations in animals. Pages 3-18 In J. J. Vernberg (Ed.) Physiological adaptations to the environment. Intext, New York, NY.

Reynolds, W. W. and M. E. Casterlin. 1976. Thermal preferenda and behavioral thermoregulation in three centrarchid fishes. Pages 185-190 In G. W. Esch and R. W. McFarland (Eds.) Thermal ecology II: Proceedings of a symposium held at Augusta, Georgia, April 2-5, 1975. U.S. Atomic Energy Commission Symposium Series, CONF-750425.

Reynolds, W. W., R. W. McCauley, M. E. Casterlin and L. I. Crawshaw. 1976. Body temperatures of behaviorally thermoregulating largemouth black bass (Micropterus salmoides). Comparative Biochemistry and Physiology 54A:461-463.

Shaklee, J. B., J. A. Christiansen, B. D. Sidell, C. L. Prosser and G. S. Whitt. 1977. Molecular aspects of temperature acclimation in fish: Contributions of changes in enzyme activities and isozyme patterns to metabolic reorganization in the green sunfish. Journal of Experimental Zoology 201:1-20.

Smith, R. P. and J. L. Wilson. 1981. A growth comparison of two subspecies of largemouth bass in Tennessee ponds. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissioners 34:25-30.

Somero, G. N. 1975. The roles of isozymes in adaptation to varying temperatures. Pages 221-234 In C. L. Markert (Ed.) Isozymes II: Physiological function. Academic Press, New York.

Stevens, E. D. and F. E. J. Fry. 1970. The rate of thermal exchange in a teleost, Tilapia mossambica. Canadian Journal of Zoology 48:221-226.

Stevens, E. D. and F. E. J. Fry. 1974. Heat transfer and body temperatures in non-thermoregulatory teleosts. Canadian Journal of Zoology 52:1137-1143.

Venables, B. J., W. D. Pearson and L. C. Fitzpatrick. 1977. Thermal and metabolic relations of largemouth bass, Micropterus salmoides, from a heated reservoir and a hatchery in north central Texas. Comparative Biochemistry and Physiology 57(A):93-98.

Whitt, G. S., D. P. Philipp and W. F. Childers. 1977. Aberrant gene expression during the development of hybrid sunfishes (Perciformes, Teleostei). Differentiation 9:97-109.

Wilson, F. R., M. J. Champion, G. S. Whitt and C. L. Prosser. 1975. Isozyme patterns in tissues of temperature-acclimated fish. Pages 193-206 In C. L. Markert (Ed.) Isozymes II: Physiological function. Academic Press, New York, NY.

Wright, G. L. and G. W. Wigtil. 1981. Comparison of growth, survival and catchability of Florida, northern and hybrid largemouth bass in a new Oklahoma reservoir. Proceedings of the 34th Annual Conference of the Southeastern Association of Game and Fish Commissioners 34:31-38.

Zolczynski, S. J. and W. D. Davies. 1976. Growth characteristics of the northern and Florida subspecies of largemouth bass and their hybrid, and a comparison of catchability between the subspecies. Transactions of the American Fisheries Society 105:240-243.

Table XIII.-Lactate dehydrogenase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

Specific Activity (Units/min/mg)

		8°C			16°C			24°C			32°C		
		TOT	LDH-A	LDH-B	TOT	LDH-A	LDH-B	TOT	LDH-A	LDH-B	TOT	LDH-A	LDH-B
							MUSCLE						
NLMB	$\bar{X}$	149	136	16.4	153	135	14.3	175	158	14.7	261	249	13.8
	SD	15.2	9.5	1.68	8.01	7.5	2.51	23.9	20.5	2.29	91.0	95.0	5.15
NxF	$\bar{X}$	254	230	18.3	193	170	21.3	225	213	12.7	266	259	10.3
	SD	30.5	23.2	7.70	35.3	25.9	4.12	13.4	12.2	1.75	59.4	53.0	3.11
FxN	$\bar{X}$	114	100	19.9	147	137	18.4	264	246	15.7	398	384	13.1
	SD	13.5	24.6	3.92	6.7	13.0	3.43	79.7	71.0	7.00	54.5	58.5	6.30
FLMB	$\bar{X}$	154	140	9.70	170	157	9.9	284	270	12.5	358	348	11.1
	SD	15.7	19.5	0.92	20.4	22.9	1.41	79.0	80.5	2.10	84.6	84.5	2.51
							LIVER						
NLMB	$\bar{X}$	6.95	5.10	2.56	11.9	6.70	3.05	9.60	6.45	1.44	8.60	5.05	3.20
	SD	2.95	1.86	0.59	5.66	4.06	0.47	4.54	3.15	0.19	1.19	1.50	0.96
NxF	$\bar{X}$	3.97	2.65	1.08	7.31	5.45	1.92	3.60	2.96	.630	5.80	4.19	2.01
	SD	1.99	1.04	0.64	2.31	1.58	0.87	0.48	0.36	0.18	3.06	2.42	0.56
FxN	$\bar{X}$	5.62	3.92	1.58	5.21	3.82	1.39	3.00	2.24	0.72	5.20	3.81	1.62
	SD	1.49	1.22	0.52	1.74	1.38	0.36	1.54	0.98	0.52	.399	1.71	0.13
FLMB	$\bar{X}$	5.87	4.28	1.69	10.2	7.60	2.84	5.20	4.35	1.39	7.10	5.25	1.76
	SD	0.61	0.55	0.26	1.05	1.60	0.67	1.17	1.98	0.18	2.79	2.23	0.56

Table XIV.-Malate dehydrogenase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

Specific Activity (Units/min/mg)

		8°C				16°C				24°C				32°C			
		TOT	MDH-A	MDH-B	MDH-M	TOT	MDH-A	MDH-B	MDH-M	TOT	MDH-A	MDH-B	MDH-M	TOT	MDH-A	MDH-B	MDH-M
MUSCLE																	
NLMB	$\bar{X}$	73.3	23.7	52.0	1.25	83.1	20.6	59.9	1.79	70.4	19.9	48.7	1.55	80.3	28.2	51.0	2.65
	SD	12.4	3.30	11.9	0.55	9.80	2.20	8.35	0.24	9.55	4.09	6.90	0.69	8.31	7.15	7.85	0.98
NxF	$\bar{X}$	92.1	23.2	65.5	0.68	99.0	31.5	64.5	2.04	61.2	20.5	48.1	1.34	91.4	26.6	53.5	2.99
	SD	6.0	8.80	8.20	0.59	7.45	3.92	3.65	0.48	5.20	4.36	4.78	0.45	5.52	7.55	15.2	0.71
FxN	$\bar{X}$	67.2	22.5	43.4	1.18	82.5	23.6	60.0	1.77	74.7	20.1	54.0	1.10	95.2	26.7	63.5	3.09
	SD	10.5	1.98	7.85	0.79	7.02	2.69	14.6	0.65	10.8	5.15	7.85	0.27	10.3	4.58	7.90	0.73
FLMB	$\bar{X}$	65.0	26.5	34.2	2.17	80.3	24.8	53.5	2.13	78.2	22.2	54.5	1.76	81.1	20.1	60.5	2.76
	SD	11.6	6.45	2.95	1.40	8.23	2.36	4.04	0.59	0.59	2.67	19.4	0.66	12.9	5.00	8.40	0.85
LIVER																	
NLMB	$\bar{X}$	195	191	ND	4.03	402	393	ND	8.00	479	468	ND	10.8	327	317	ND	8.45
	SD	67.6	67.5		0.82	54.9	55.0		2.10	119	114		1.53	54.6	49.2		4.99
NxF	$\bar{X}$	210	208	ND	2.01	351	338	ND	10.5	427	419	ND	8.60	319	294	ND	6.75
	SD	59.3	60.5		0.75	37.5	34.3		1.08	117	116		1.60	30.1	24.9		2.16
FxN	$\bar{X}$	187	175	ND	1.89	299	292	ND	7.40	430	423	ND	8.80	361	323	ND	6.95
	SD	16.2	24.9		0.41	36.8	34.1		2.91	76.0	67.5		1.35	27.4	20.4		1.68
FLMB	$\bar{X}$	157	144	ND	3.13	276	272	ND	5.75	323	317	ND	6.20	241	228	ND	6.65
	SD	21.9	22.9		0.58	41.5	40.6		2.36	67.0	61.5		2.62	27.7	23.5		1.11

Table XV.-Isocitrate dehydrogenase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

Specific Activity (Units/min/mg)

		8°C			16°C			24°C			32°C		
		TOT	IDH-A	IDH-B	TOT	IDH-A	IDH-B	TOT	IDH-A	IDH-B	TOT	IDH-A	IDH-B
MUSCLE													
NLMB	$\bar{X}$	11.7	11.7	ND	10.0	10.0	ND	6.40	6.40	ND	9.10	9.10	ND
	SD	2.40	2.40		2.56	2.56		1.02	1.02		3.24	3.24	
NxF	$\bar{X}$	12.9	12.9	ND	11.4	11.4	ND	6.10	6.10	ND	9.60	9.60	ND
	SD	4.16	4.16		0.79	0.79		0.94	0.94		1.61	1.61	
FxN	$\bar{X}$	9.61	9.61	ND	7.50	7.50	ND	8.20	8.20	ND	8.80	8.80	ND
	SD	2.74	2.74		2.25	2.25		0.55	0.55		1.85	1.85	
FLMB	$\bar{X}$	4.02	4.02	ND	9.00	9.00	ND	3.70	3.70	ND	7.40	7.40	ND
	SD	1.72	1.72		0.63	0.63		1.41	1.41		1.61	1.61	
LIVER													
NLMB	$\bar{X}$	8.98	ND	8.98	12.9	ND	12.9	9.20	ND	9.20	10.1	ND	10.1
	SD	2.95		2.95	0.94		0.94	0.74		0.74	3.57		3.57
NxF	$\bar{X}$	8.97	ND	8.97	10.8	ND	10.8	9.40	ND	9.40	7.90	ND	7.90
	SD	0.88		0.88	2.04		2.04	1.04		1.04	1.20		1.20
FxN	$\bar{X}$	6.77	ND	6.77	7.90	ND	7.90	11.4	ND	11.4	19.4	ND	19.4
	SD	1.46		1.46	0.94		0.94	1.61		1.61	3.40		3.40
FLMB	$\bar{X}$	7.72	ND	7.72	6.40	ND	6.40	6.80	ND	6.80	8.40	ND	8.40
	SD	1.84		1.84	2.37		2.37	1.82		1.82	1.20		1.20

Table XVI.-Aspartate aminotransferase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

		Specific Activity (Units/min/mg)															
		8°C				16°C				24°C				32°C			
		TOT	AAT-A	AAT-B	AAT-M	TOT	AAT-A	AAT-B	AAT-M	TOT	AAT-A	AAT-B	AAT-M	TOT	AAT-A	AAT-B	AAT-M
MUSCLE																	
NLMB	$\bar{X}$	42.1	17.9	ND	20.3	42.4	21.7	ND	21.7	33.9	12.3	ND	20.7	34.7	23.6	ND	19.7
	SD	6.35	2.58		3.65	6.35	4.30		4.30	2.79	1.01		4.38	9.49	4.09		8.05
NxF	$\bar{X}$	36.7	18.4	ND	18.4	37.5	15.3	ND	23.4	33.3	17.2	ND	14.3	29.8	20.6	ND	15.1
	SD	4.67	2.34		2.34	2.28	1.40		3.88	2.37	2.74		2.00	4.12	4.01		5.25
FxN	$\bar{X}$	34.6	17.3	ND	14.8	38.9	13.8	ND	25.1	37.9	20.8	ND	16.5	33.1	20.2	ND	16.9
	SD	3.95	1.98		2.53	3.20	1.03		2.65	1.44	3.14		2.69	3.03	7.95		6.90
FLMB	$\bar{X}$	24.6	11.1	ND	13.0	27.3	12.7	ND	12.7	29.8	12.9	ND	18.8	26.2	18.3	ND	12.2
	SD	6.55	4.47		2.98	1.59	1.51		1.51	5.62	3.08		3.76	1.88	4.81		4.22
LIVER																	
NLMB	$\bar{X}$	132	ND	120	11.4	167	ND	141	23.0	131	ND	120	11.5	151	ND	123	23.9
	SD	11.7		13.8	3.23	27.7		24.5	6.95	10.5		14.2	5.50	34.7		25.1	12.0
NxF	$\bar{X}$	132	ND	123	13.1	162	ND	146	16.0	157	ND	140	12.4	117	ND	107	9.65
	SD	8.52		8.15	1.53	17.0		13.3	2.72	25.7		26.6	7.30	23.2		23.9	2.47
FxN	$\bar{X}$	100	ND	90.5	10.9	136	ND	117	15.6	153	ND	140	9.15	170	ND	152	13.1
	SD	16.3		18.8	2.72	8.58		10.4	3.36	14.4		14.0	0.59	9.1		10.9	5.95
FLMB	$\bar{X}$	122	ND	106	15.9	138	ND	124	18.1	142	ND	127	12.3	156	ND	139	14.3
	SD	30.0		30.2	2.65	10.6		15.8	8.65	17.0		14.9	1.62	13.0		3.9	4.71

Table XVII.-Creatine kinase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

		Specific Activity (Units/min/mg)																			
		8°C					16°C					24°C					32°C				
		TOT	CK-A	CK-B	CK-C	CK-D	TOT	CK-A	CK-B	CK-C	CK-D	TOT	CK-A	CK-B	CK-C	CK-D	TOT	CK-A	CK-B	CK-C	CK-D
MUSCLE																					
NLMB	$\bar{X}$	5468	4593	ND	122	ND	4300	3590	ND	96.0	ND	4700	3895	ND	145	ND	6680	5600	ND	174	ND
	SD	219	192		30.6		2560	2150		56.0		671	540		75.5		3740	3135		96.5	
NxF	$\bar{X}$	6200	5200	ND	113	ND	5490	4552	ND	94.5	ND	4500	3820	ND	138	ND	5920	5100	ND	123	ND
	SD	445	496		30.4		2104	2090		57.0		571	510		62.5		1530	1160		52.0	
FxN	$\bar{X}$	4880	4095	ND	111	ND	4100	3415	ND	75.5	ND	4750	3850	ND	130	ND	6830	6000	ND	103	ND
	SD	566	595		16.8		2520	2125		18.6		339	162		15.8		3330	2605		52.0	
FLMB	$\bar{X}$	3780	3075	ND	90.5	ND	4200	2875	ND	81.5	ND	3330	3110	ND	89.0	ND	4460	5937	ND	98.0	ND
	SD	467	362		7.85		1710	920		20.4		847	703		39.1		724	1680		26.1	
LIVER																					
NLMB	$\bar{X}$	35.2	5.75	ND	15.8	ND	43.4	9.75	ND	17.1	ND	22.8	3.01	ND	12.4	ND	34.8	2.16	ND	13.2	ND
	SD	5.24	1.02		1.96		3.20	2.74		1.86		8.82	1.50		5.60		13.1	2.13		3.65	
NxF	$\bar{X}$	35.6	6.20	ND	15.5	ND	38.2	7.10	ND	15.0	ND	15.4	2.44	ND	7.40	ND	30.0	2.91	ND	14.7	ND
	SD	6.97	1.41		3.03		6.97	1.45		4.06		1.28	0.23		2.00		14.4	2.57		8.25	
FxN	$\bar{X}$	38.0	7.50	ND	15.2	ND	63.4	8.45	ND	17.0	ND	22.0	3.14	ND	12.6	ND	31.4	1.94	ND	16.5	ND
	SD	7.12	1.01		1.64		21.7	0.70		1.28		5.02	0.69		3.25		3.42	1.86		0.91	
FLMB	$\bar{X}$	15.3	4.91	ND	13.7	ND	42.7	7.20	ND	15.9	ND	16.6	2.47	ND	11.7	ND	33.4	2.35	ND	16.0	ND
	SD	2.49	1.54		3.15		10.8	0.61		3.08		7.56	0.46		6.05		6.60	2.24		1.96	

Table XVIII.-Glucosephosphate isomerase activity in tissues of the four stocks acclimated to 8, 16, 24, and 32°C.

		Specific Activity (Units/min/mg)											
		8°C			16°C			24°C			32°C		
		TOT	GPI-A	GPI-B	TOT	GPI-A	GPI-B	TOT	GPI-A	GPI-B	TOT	GPI-A	GPI-B
MUSCLE													
NLMB	$\bar{X}$	89.1	10.6	77.5	104	9.70	95.0	144	10.1	134	189	10.2	163
	SD	14.4	0.96	15.5	9.67	1.43	8.95	15.4	2.86	17.3	56.5	4.57	50.5
NxF	$\bar{X}$	136	13.7	121	131	11.8	121	151	9.35	145	149	11.1	137
	SD	30.7	1.85	28.4	14.1	2.42	13.3	19.3	2.31	19.7	27.2	3.49	30.1
FxN	$\bar{X}$	59.4	6.00	53.0	101	15.1	105	199	13.2	186	238	9.35	225
	SD	6.7	0.54	5.9	5.66	1.06	30.7	35.4	5.40	30.9	47.6	1.55	44.8
FLMB	$\bar{X}$	70.3	8.95	58	75.2	10.9	67.0	159	9.10	145	196	9.60	194
	SD	15.3	1.17	10.7	4.92	2.22	4.17	46.5	0.53	45.9	67.9	2.40	71.0
LIVER													
NLMB	$\bar{X}$	155	155	ND	155	155	ND	167	167	ND	141	141	ND
	SD	26.0	26.0		20.4	20.4		16.3	16.3		33.6	33.6	
NxF	$\bar{X}$	147	147	ND	152	152	ND	153	153	ND	110	110	ND
	SD	17.8	17.8		7.13	7.13		21.1	21.1		14.3	14.3	
FxN	$\bar{X}$	108	108	ND	120	120	ND	157	157	ND	127	127	ND
	SD	13.3	13.3		15.4	15.4		18.5	18.5		8.58	8.58	
FLMB	$\bar{X}$	96.1	96.1	ND	112	112	ND	130	130	ND	105	105	ND
	SD	23.3	23.3		11.3	11.3		26.7	26.7		11.9	11.9	

Figure 1. The effect of acclimation temperature on the LDH activity levels in muscle tissue of four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(----), FLMB(-•-).

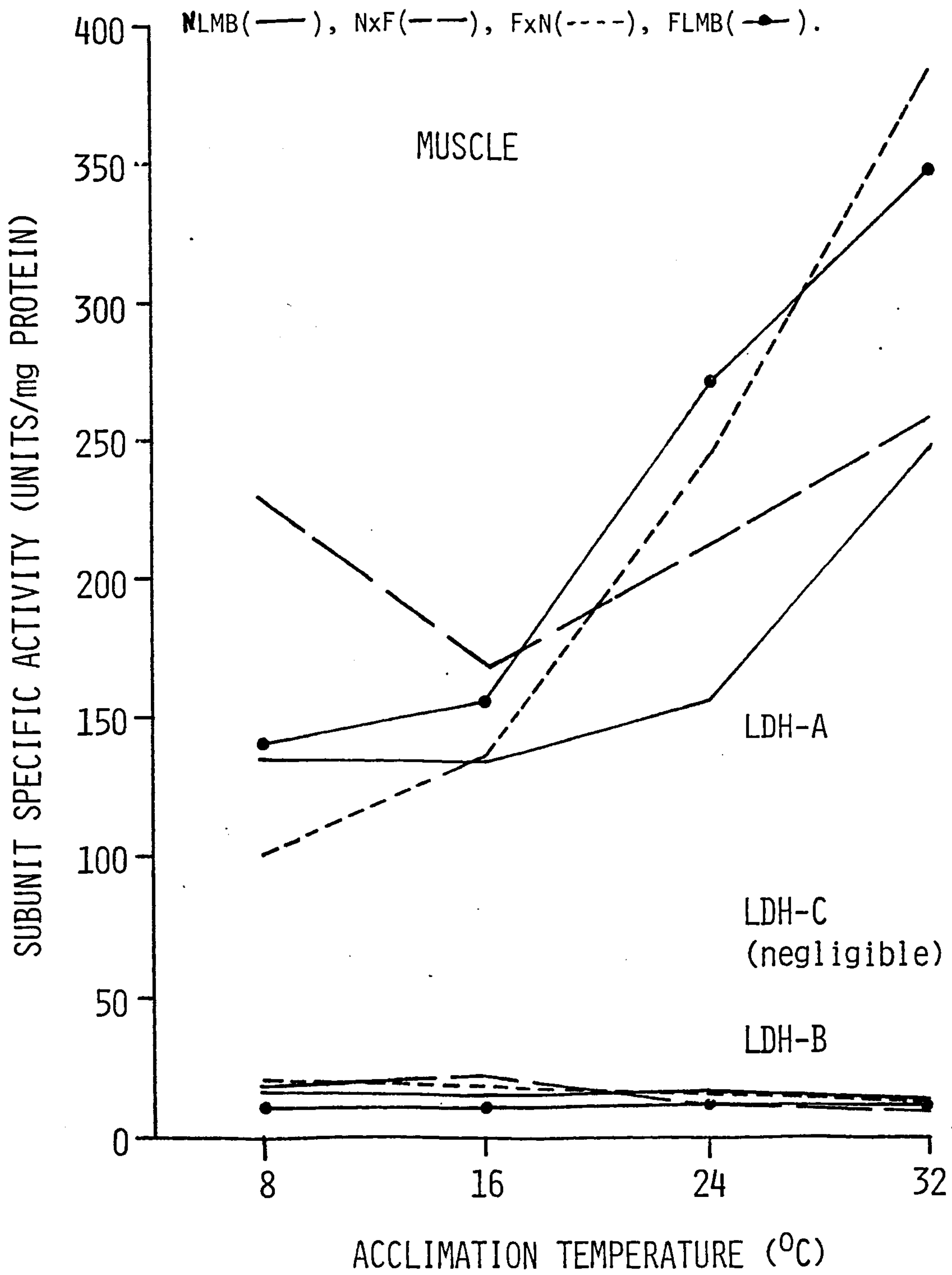


Figure 2. The effect of acclimation temperature on the LDH activity levels in liver tissue for four stocks of largemouth bass, NLMB(——), NxF(— — —), FxN(- - - -), FLMB(—●—).

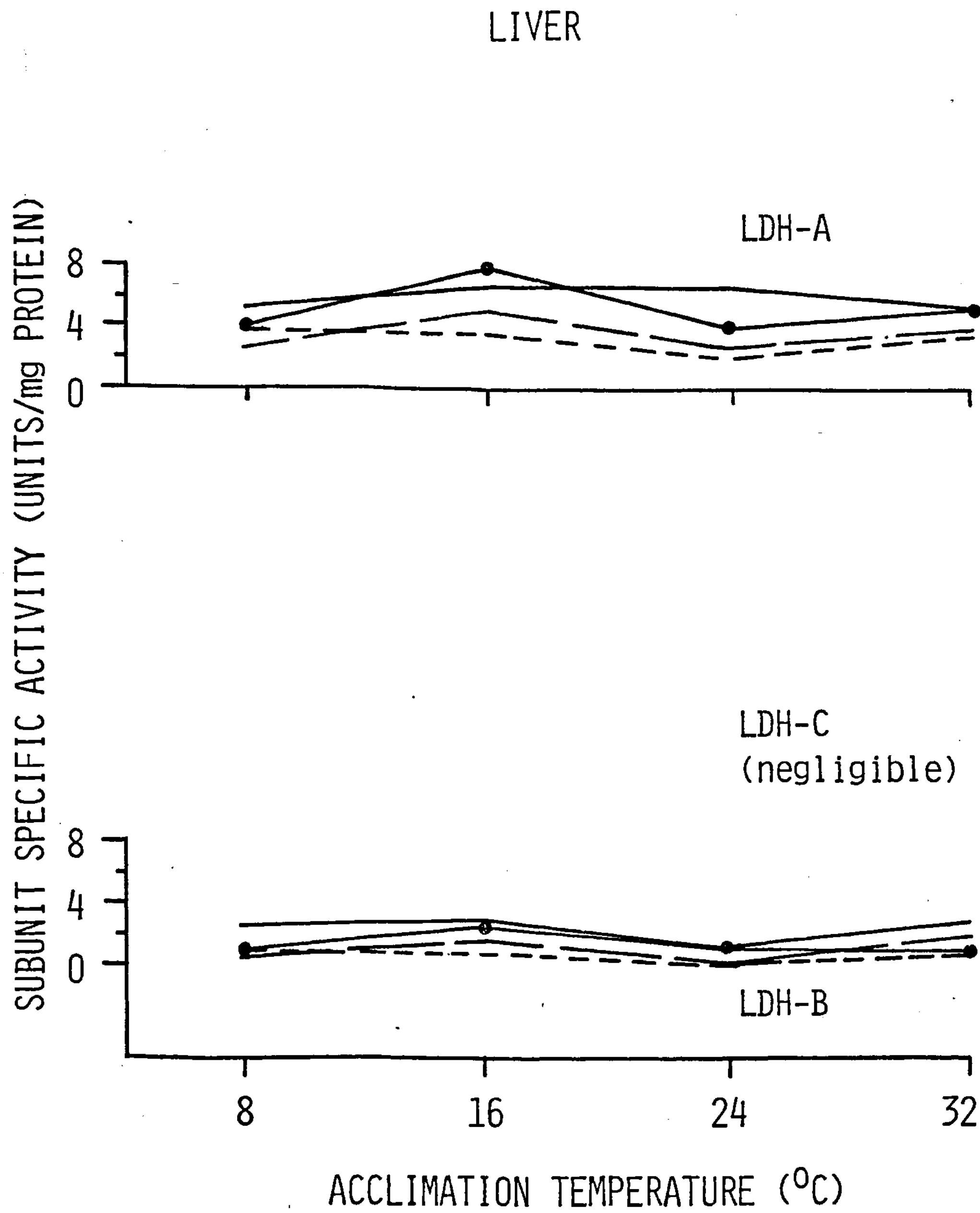


Figure 3. The effect of acclimation temperature on the CK activity levels in muscle tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(- - - -)FLMB(—●—).

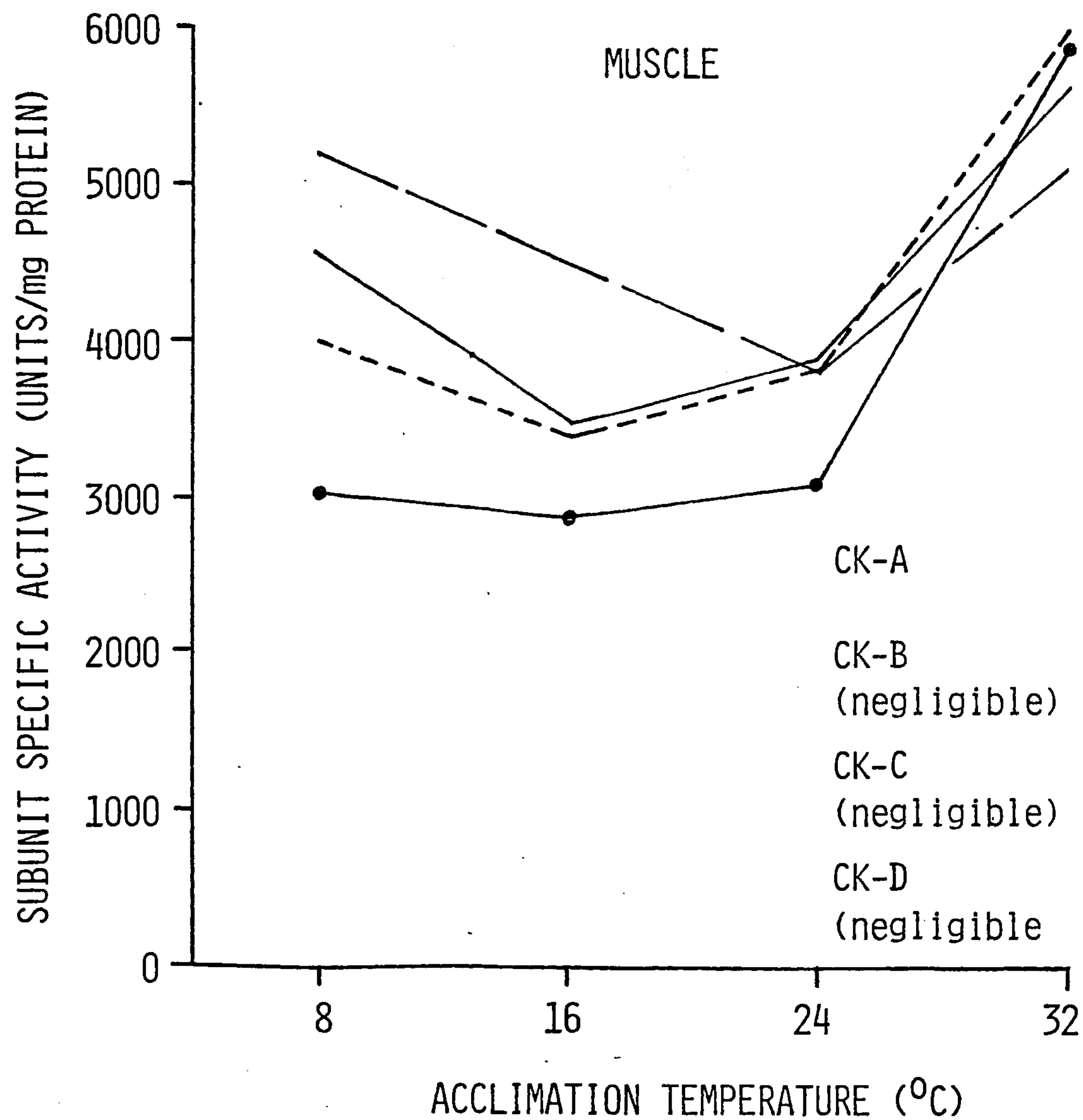


Figure 4. The effect of acclimation temperature on the activity lavels in liver tissue for four stocks of largemouth bass, NLMB(———), NxF(— —), FxN(----), FLMB(–●–).

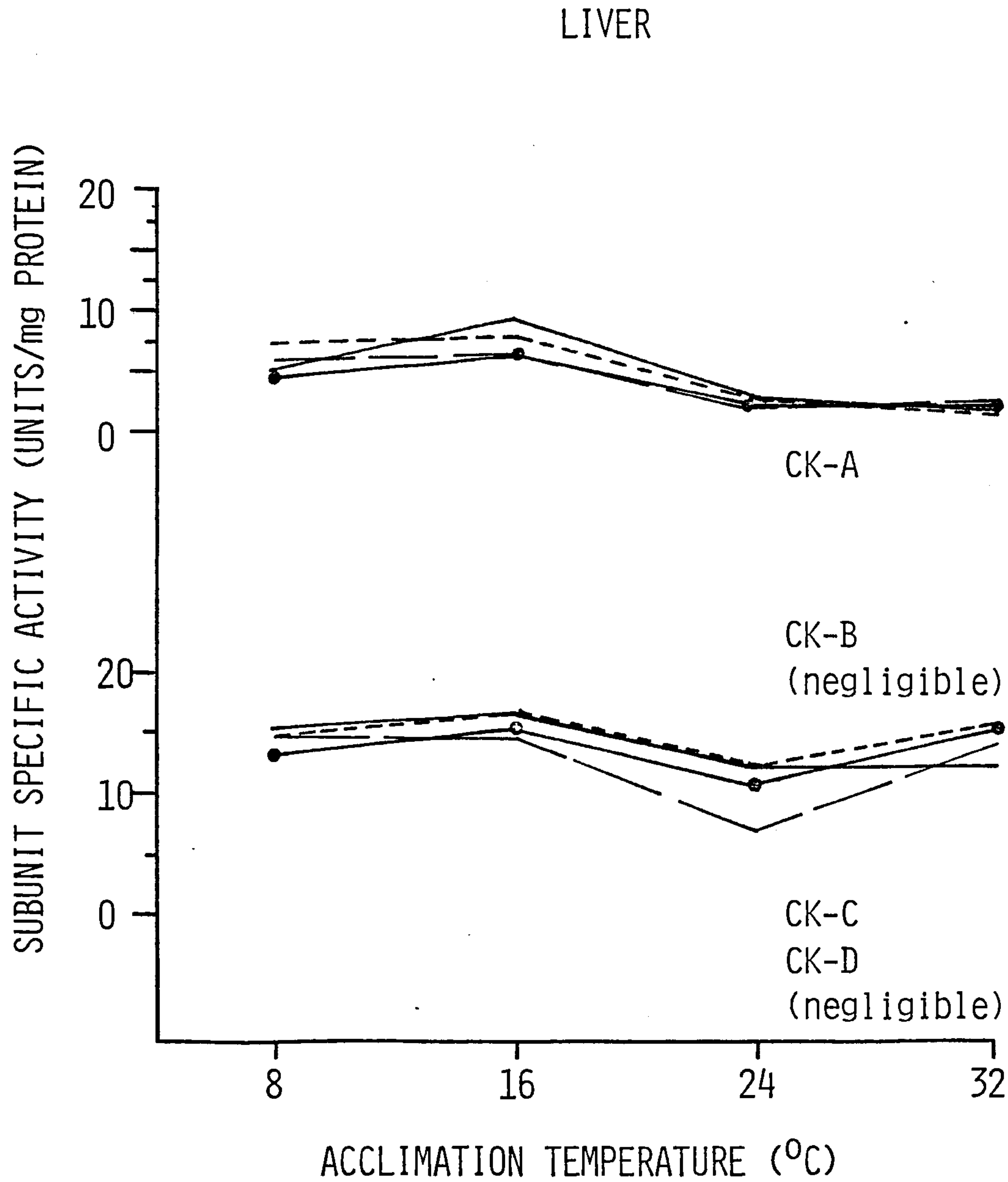


Figure 5. The effect of acclimation temperature on the GPI activity levels in muscle tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(----), FLMB(—●—).

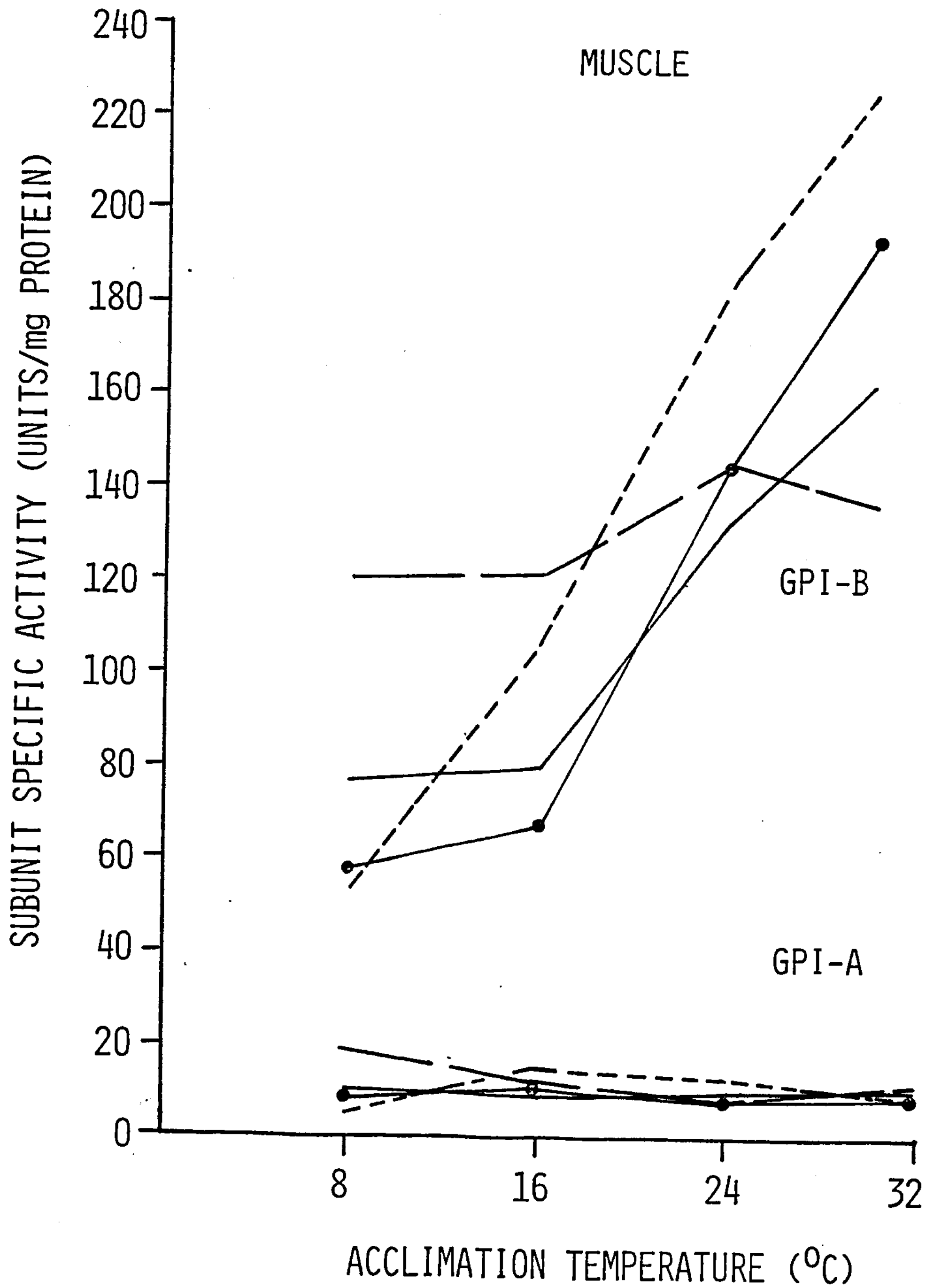


Figure 6. The effect of acclimation temperature on the GPI activity levels in liver tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(- - - -), FLMB(—●—).

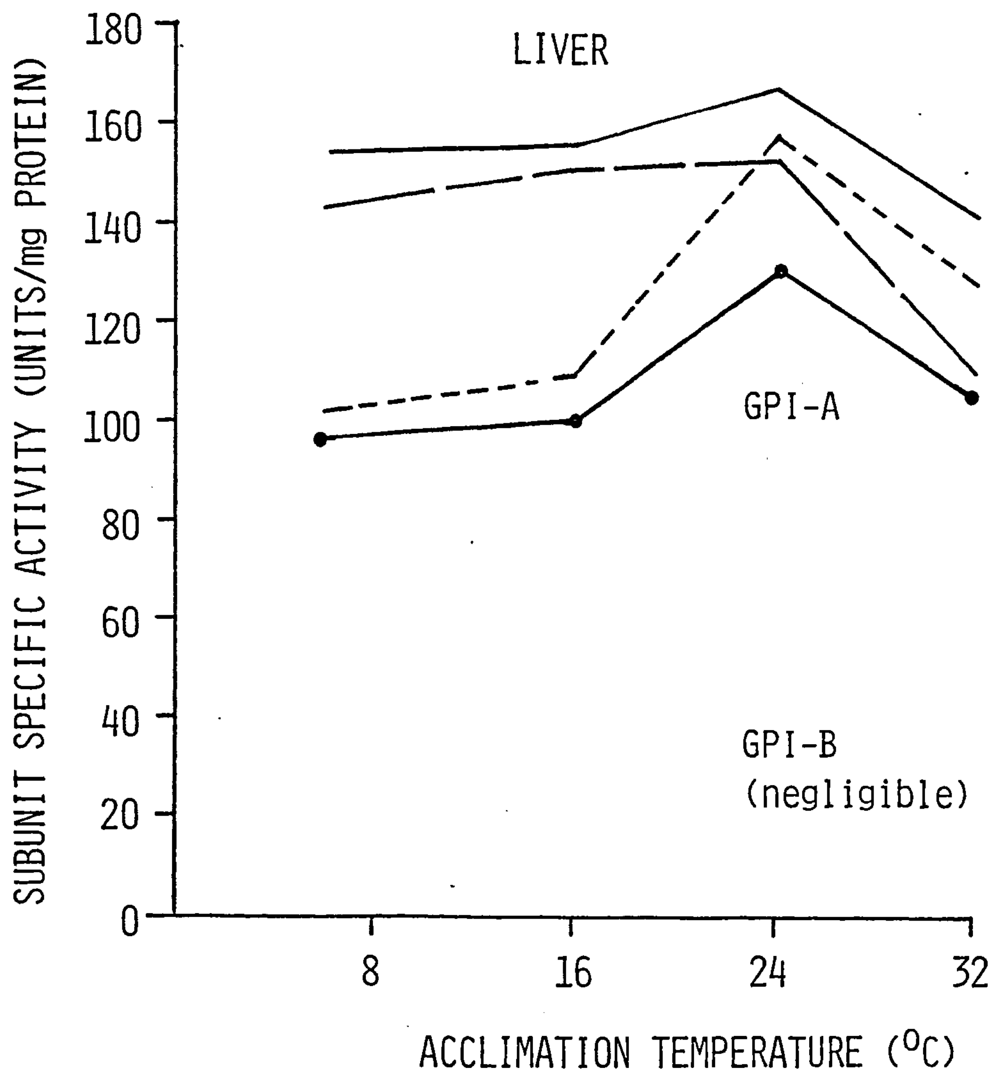


Figure 7. The effect of acclimation temperature on the MDH activity levels in muscle tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(----), FLMB(-●-).

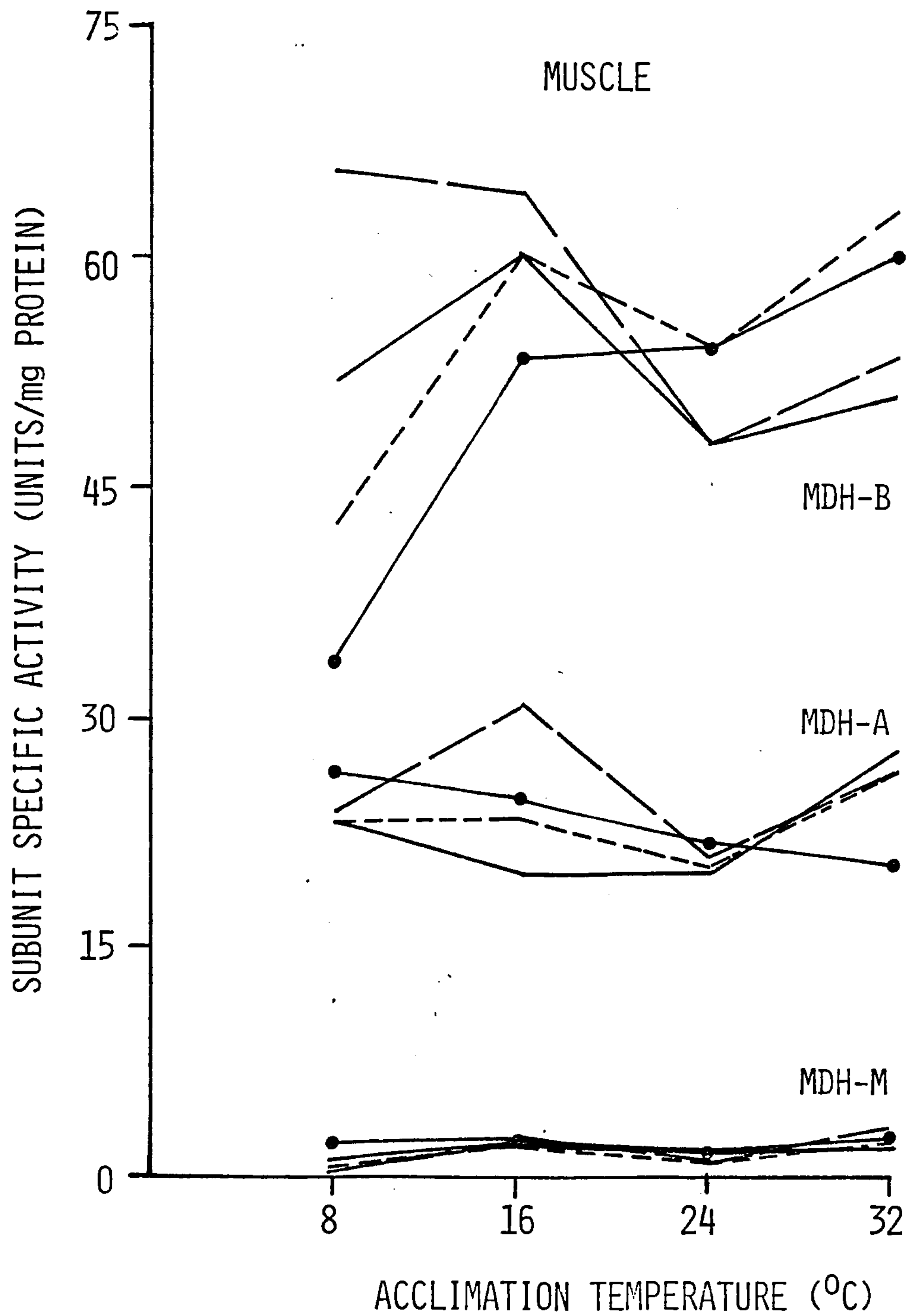


Figure 8. The effect of acclimation temperature on the MDH activity levels in liver tissue for four stocks of largemouth bass, NLMB(——), NxF(— —),FxN(----), FLMB(—●—).

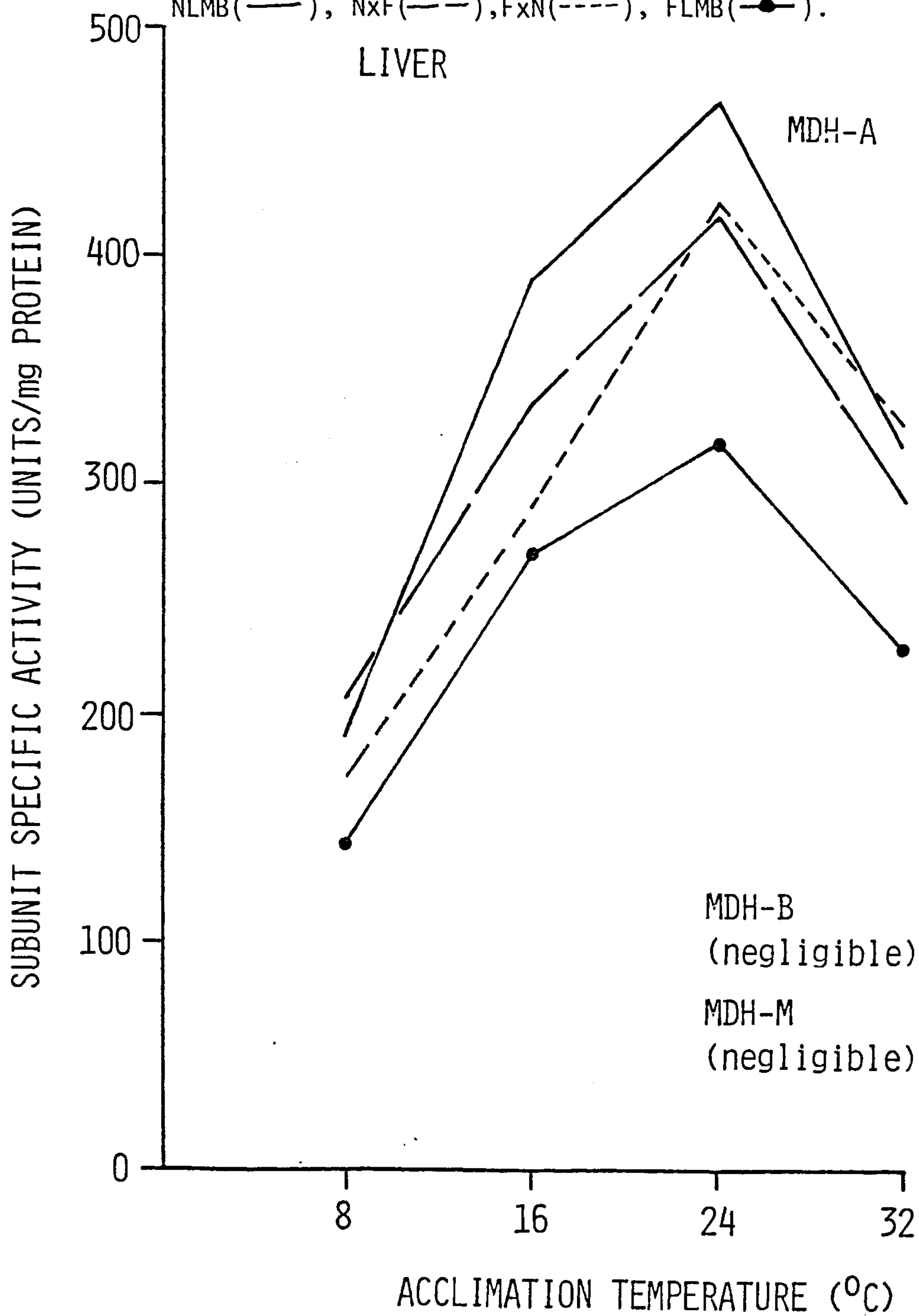


Figure 9. The effect of acclimation temperature on the activity levels of IDH in muscle and liver tissues for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(----), FLMB(—●—).

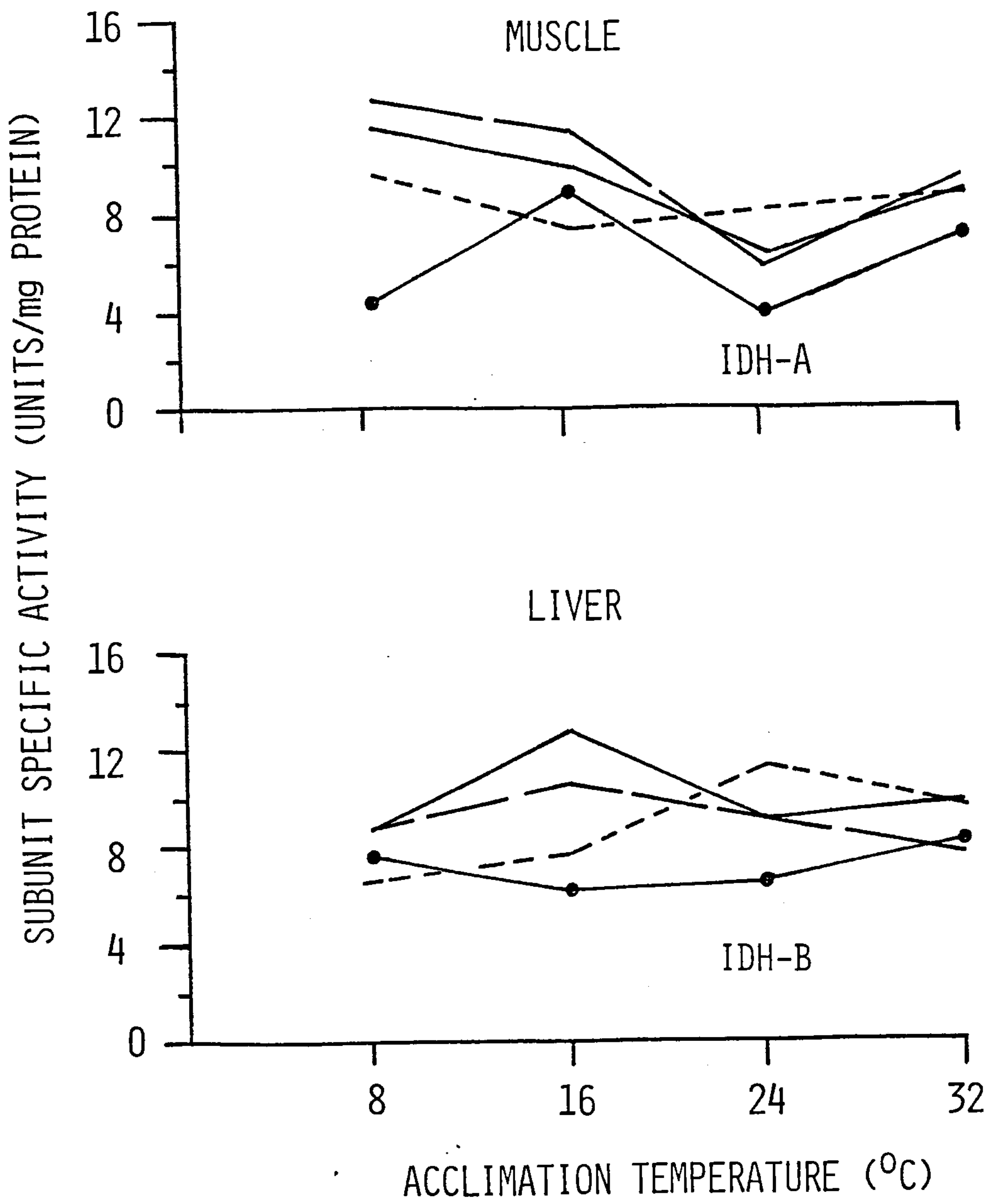


Figure 10. The effect of acclimation temperature on the AAT activity levels in muscle tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), FxN(----), FLMB(—●—).

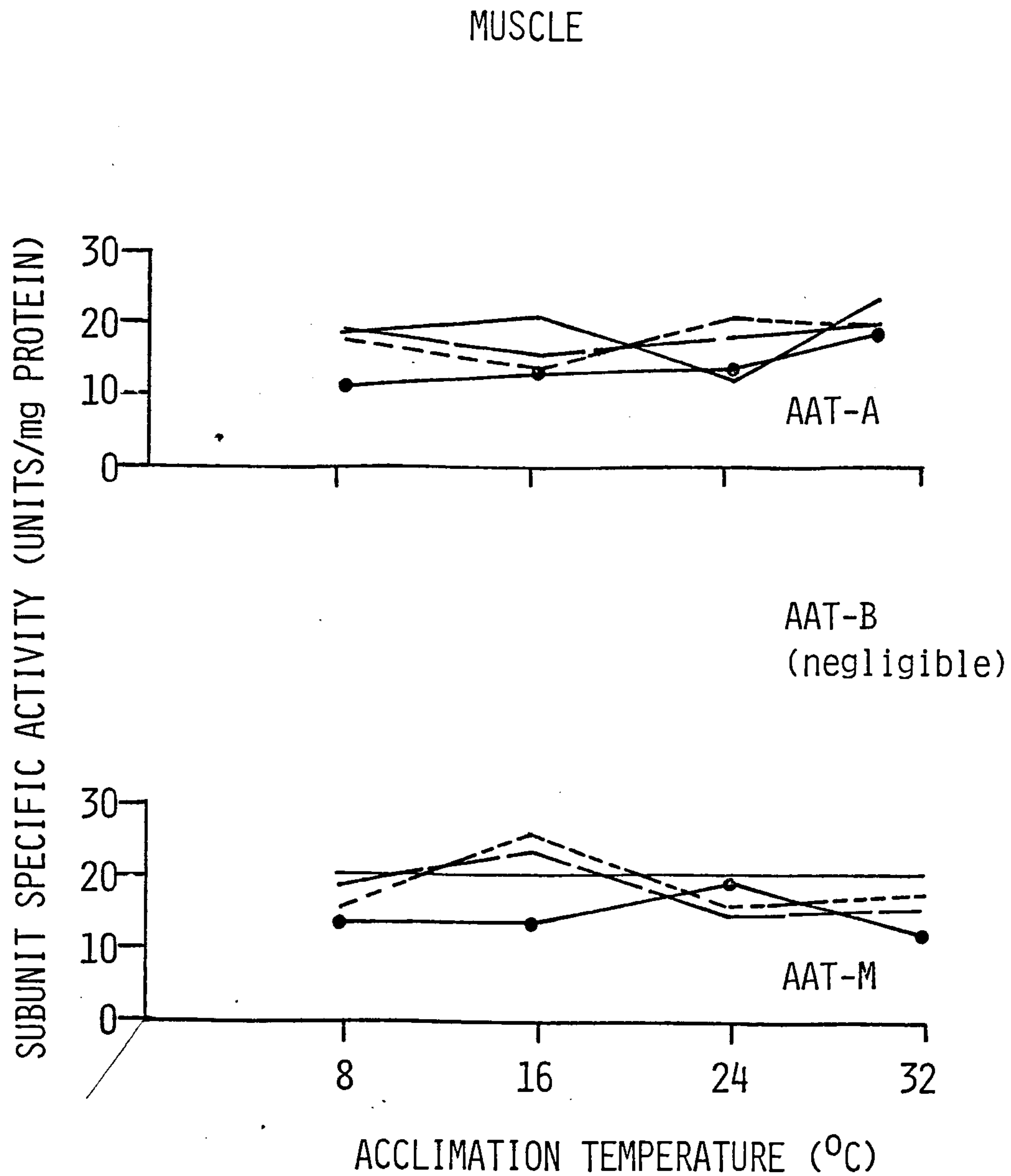


Figure 11. The effect of acclimation temperature on the AAT activity levels in liver tissue for four stocks of largemouth bass, NLMB(——), NxF(— —), LIVER FxN(----), FLMB(-●-).

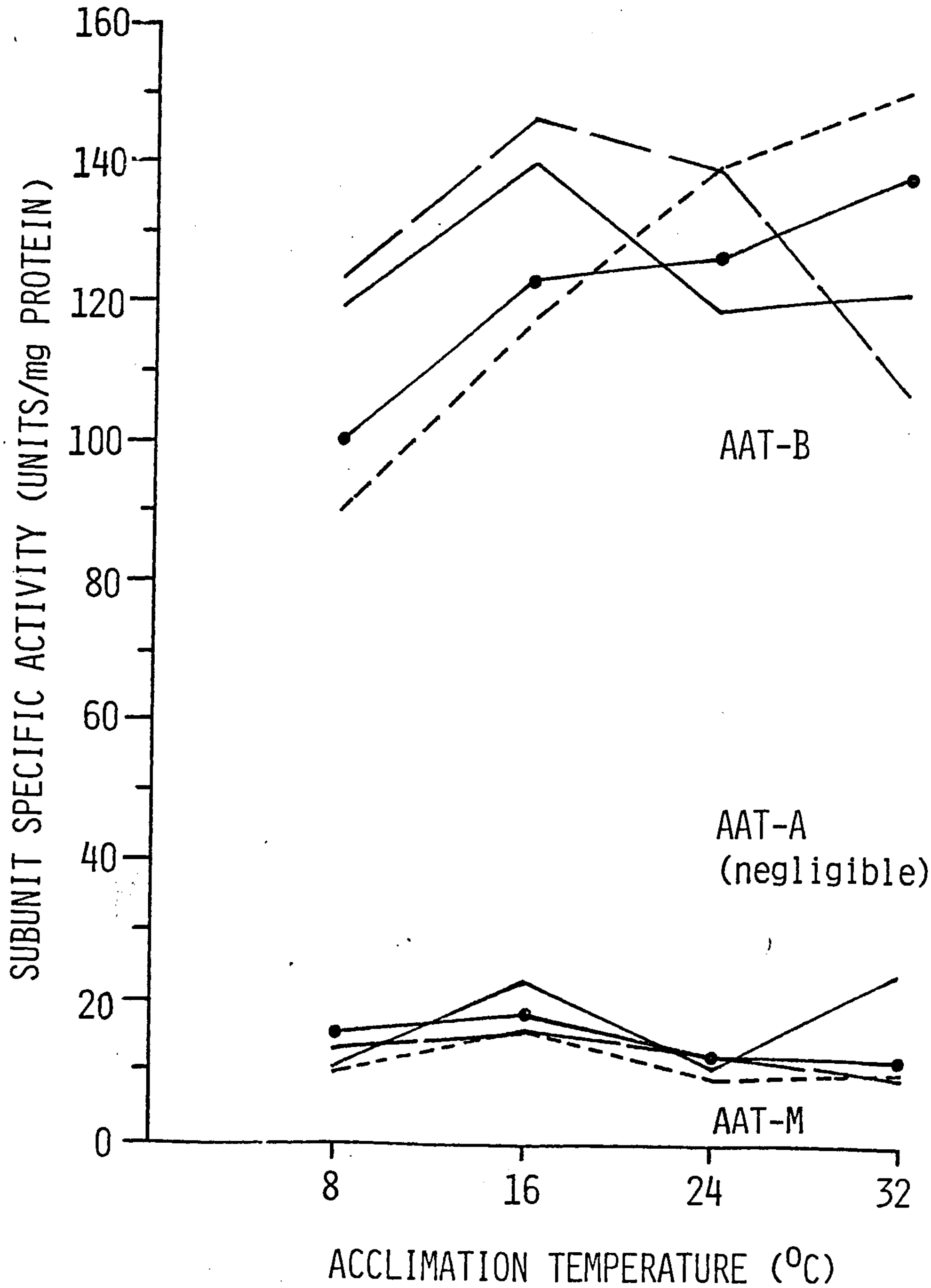
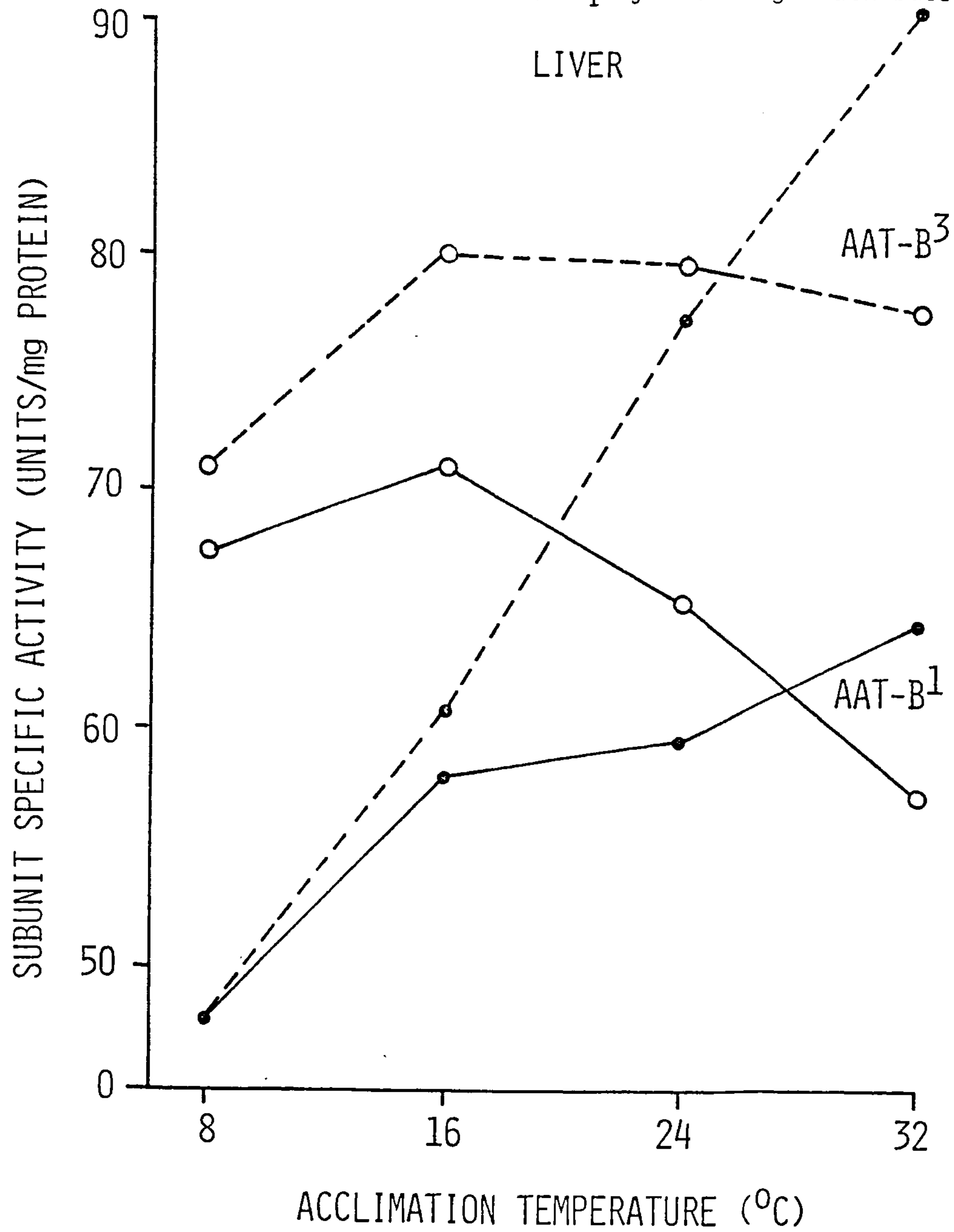


Figure 12. The effect of acclimation temperature on the allelic expression patterns for the Aat-B^1 and Aat-B^3 alleles in the liver of NxF and FxN F_1 hybrid largemouth bass.



Study 104
Job 2

CHAPTER 8

Thermal Preferenda of Northern, Florida and Reciprocal F_1 Hybrid Largemouth Bass

Jeffrey B. Koppelman[1], Gregory S. Whitt[3,1] and David P. Philipp[1,2]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Four genetically confirmed stocks of largemouth bass (northern, Florida, and both reciprocal F_1 hybrids) were evaluated to establish whether thermoregulatory behavioral differences exist among them. Acute testing methods were used in the laboratory to determine thermal preferenda in relation to seven acclimation temperatures ranging from 8° to 32°C. Cubic regression analyses of the results of the testing demonstrated that both pure subspecies and both reciprocal F_1 hybrids have similar final preferred temperatures. However, each stock responded differently to the various acclimation temperatures, indicating that the thermoregulatory behavior of these different stocks differ.

INTRODUCTION

The impact of temperature on fish populations has been proposed to be greater than any other abiotic factor (Beitinger and Fitzpatrick 1979). The various thermal regimes associated with environments located in different regions of the country must certainly be a strong selective force driving the divergence of different stocks of fishes. As a result, it is important to evaluate the various effects of environmental temperature on all life stages of fish (Beitinger and Magnuson 1979).

Thermal preference studies have been proposed to be precisely measurable analyses of thermal adaptation among fish species (Magnuson et al. 1979). These types of analyses have been conducted for various fish species of different size and geographic locations to assess the effects of environmental parameters such as season, photoperiod, salinity, and dissolved oxygen (Meldrim and Gift 1971, Barans and Tubb 1973, Cherry et al. 1975, Goddard and Tait 1976, Hall et al. 1978,,Richards and Ibara 1978, Peterson et al. 1979, Tranquilli et al. 1979, Mathur et al. 1981). Neill (1971) and Reynolds et al (1976), using largemouth bass (Micropterus salmoides) in laboratory testing during daylight hours, determined final thermal preferenda of 29 and 30.1°C, respectively. Coutant (1975), when monitoring largemouth bass in small Tennessee lakes, observed a final thermal preference of 27°C. Other centrarchid analyses have yielded varied results according to the species tested. Smallmouth bass (Micropterus dolomieui) have been observed to prefer temperatures somewhat lower than the largemouth bass. Barans and Tubb (1973) reported a thermal preference range of 18-30°C. Ferguson (1958) reported a 28°C preferenda for smallmouth bass, a value close to that for largemouth bass reported by both Coutant (1975) and Reynolds et al. (1976). Different studies on bluegills

(*Lepomis macrochirus*) have reported values ranging from 27.2°C observed during night testing in the field (Neill 1971) to laboratory values of 32.3°C (Ferguson 1958, Reynolds and Casterlin 1976). During laboratory testing, green sunfish (*Lepomis cyanellus*) expressed final preferred temperatures of 27.3, 28.2, and 30.6°C (Jones and Irwin 1965, Beitinger et al. 1975, Cherry et al. 1975).

Although testing procedures differed among the various investigators with regard to fish size and age, season, etc., it is apparent that two imperative conditions must be met when testing behavioral responses to a temperature regime. First, all test fish should be genetically analyzed to account for potential physiological or behavioral response differences that may influence movement toward a preferendum (Reynolds and Casterlin 1979). Secondly, all fish used for testing purposes need to be fully (metabolically and physiologically) acclimated to test temperatures. Reynolds and Casterlin (1979) further emphasized the importance of using confirmed genetic strains of fish in these studies.

Philipp et al. (1981, 1983) recently completed a biochemical genetic survey of 90 largemouth bass populations in the U.S. and determined that substantial genetic differences exist among these populations. In addition, latitudinal clines in the allele distributions at several loci were observed (Philipp et al. 1982, 1983). In trying to understand the evolutionary basis and thus the mode of selection for existing genetic variation, researchers have attempted correlations between enzyme phenotypes and certain environmental variables (Lewontin 1974). Since fish are poikilothermic, temperature is a major environmental factor in controlling many aspects of their life cycle. Detecting clinal distributions of allele frequencies does not necessarily mean

that these allele frequency differences arose as a result of these environmental influences (Kimura and Ohta 1971, Ohta and Kimura 1971, Aspinwall 1974). However, clinal distributions of allele frequencies correlated with environmental factors such as temperature can be the basis for further investigations into genetic differentiation. Observed gene frequencies can be used to assess stock structures and subsequent analyses of each stock may reveal functional differences.

Unfortunately, little consideration has been given to protecting the integrity of these different stocks. Significant genetic differences exist between Florida largemouth bass (*M. s. floridanus*) and northern largemouth bass (*M. s. salmoides*) (Philipp et al. 1983). Stocking efforts to achieve bigger largemouth bass have involved the transportation of the Florida subspecies to areas outside of their natural range (peninsular Florida). Florida largemouth bass grow to much greater sizes than northern largemouth bass when each are in their respective native ranges. However, this is mainly the result of a combination of the longer growing season in the south and the unique genetic attributes of the native largemouth bass which have been selected for this specific environment.

In any given environment, performance of a stock determines the fitness and thus the success of the stock in that environment. Selection pressures have altered the genetic composition of the species, resulting in "tailored" populations for specific environments. Thus, in a given environment, specific genes in specific combinations which were advantageous, or at least not deleterious, persisted, resulting in stocks tailored by thousands of generations of selection in that environment. The introduction of a stock of largemouth bass obtained from one environment into a population of largemouth

bass existing in a different environment could very well disrupt these advantageous allele combinations and any co-adapted gene complexes present in the recipient population. This could result in a substantial reduction in the fitness of the recipient population. Current and future fisheries management programs need to protect the diversity of genetic stocks of largemouth bass (Ryman 1981, Philipp et al. 1983). In addition, information regarding the performance of the genetic stocks in different environments and their physiological and behavioral qualities as determined by their genetic structure must be fully evaluated.

The purpose of this study was to assess the thermoregulatory behavioral differences among four genetically confirmed stocks of largemouth bass. Specifically, it was to determine thermal preference values for each of the two pure subspecies, the northern and Florida largemouth bass, *M. s. salmoides* and *M. s. floridanus*, respectively, and both reciprocal F_1 hybrids obtained by crossing NLMB♀ with FLMB♂ (NxF) and FLMB♀ with NLMB♂ (FxN).

MATERIALS AND METHODS

Production of Genetic Stocks:

Pure northern largemouth bass (NLMB), *M. s. salmoides*, were collected from Bone Lake, Wisconsin during October, 1978. Right pectoral fin clips were removed from each adult prior to stocking and utilized for electrophoretic analyses of each individual (Philipp et al. 1979, 1983). All individuals retained contained only the $Mdh\text{-}B^1$, $Idh\text{-}B^1$, $Sod\text{-}A^2$ and $Aat\text{-}B^1$ or B^2 alleles, indicating they represented the pure northern subspecies. These individuals were held outdoors in 0.08 hectare ponds until the onset of the project in April 1980. Pure Florida largemouth bass (FLMB), *M. s. floridanus*, were collected from Lake Dora, Florida during January, 1980 and again during February, 1981. These fish were air shipped to Champaign and held indoors at 8-12°C. Left pectoral fin clips were removed from each adult prior to stocking outdoors and utilized for electrophoretic analyses of each individual. All individuals contained only the $Mdh\text{-}B^2$, $Idh\text{-}B^3$, $Sod\text{-}A^1$ or $Sod\text{-}A^2$ and $Aat\text{-}B^3$ or $Aat\text{-}B^4$ alleles, indicating they represented the pure Florida subspecies. In March of 1980 and 1981, the collected individuals were stocked outdoors in 0.08 hectare ponds.

During the spring of 1981, these brood stocks were used to produce NLMB, FLMB and both reciprocal F_1 hybrids, NLMB♀ x FLMB♂ (NxF) and FLMB♀ x NLMB♂ (FxN) by stocking 0.08 hectare ponds as follows:

Pond 1: 5 NLMB♀ and 5 NLMB♂
Pond 2: 5 NLMB♀ and 6 FLMB♂
Pond 3: 6 FLMB♀ and 5 NLMB♂
Pond 4: 8 FLMB♀ and 6 FLMB♂

Spawning was successful in all four production ponds. Ponds were drained on September 21, 22, 25 and 28, 1981, and approximately 1,200 50 mm fingerlings were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

Acclimation Procedure:

During fall of 1981, young-of-the-year largemouth bass (40-60 mm total length) were removed from production ponds and placed in holding tanks at ambient temperatures in Conviron controlled environmental chambers. Acclimation to a succession of temperatures from 8 to 32°C was achieved by first altering water temperatures 1°C per day until reaching the desired testing temperature and then holding the fish at that temperature for a minimum of three weeks. Fish were fed frozen brine shrimp and live *Daphnia* daily. A 12D/12L natural photoperiod was maintained during the acclimation period.

Temperature Preference Determinations:

Four stainless steel test chambers (3.0 x 0.51 x 0.25 m) patterned after Meldrim and Gift (1971), with the addition of 11 plywood baffles (35.6 x 1.9 x 25.4 cm) positioned tranversely to create a shifting flow pattern, were used for all preference testing (Fig. 1). Water 5 cm in depth was heated as it flowed the length of a test chamber by three series of incandescent lamps which established a horizontal thermal gradient of approximately 20°C in range. Water depth was maintained by pumping cooled water from a Min-O-Cool circulating water bath into one end of a test chamber and draining the excess through a standing pipe at the heated end. Perforated 5 mm Tygon tubing attached to the inside bottom of each test chamber supplied the aeration which

successfully eliminated any vertical temperature stratification and prevented any super-saturation of gases caused by heating the water. Calibrated thermometers were suspended in the center of each compartment created by the baffles and readings were taken every 15 minutes during testing.

For a given run, five fish of a single genetic stock were placed in each test chamber. Individuals of one each of the four stocks were placed in one of four test chambers, so that all stocks were tested simultaneously. When introduced into the test chamber, the fish were placed at a point in the gradient at which the temperature was the same as the acclimation temperature. A 30 minute period was allowed for adjustment to the new environment. The temperatures at which each of the fish was positioned were recorded every 10 minutes for 2.5 hours, for a total of 75 observations per test chamber run. Five runs per stock (for a total of 25 individuals) were conducted at each of seven acclimation temperatures. Test chambers used by each stock were rotated each successive run to eliminate variability due to possible test chamber differences. All testing occurred between 1000 and 1500 hours.

One of three methods of analysis was used to determine the thermal preferendum at each acclimation temperature for each stock: (1) a mean of all observations for all runs at a particular acclimation temperature; (2) an asymptotic regression to determine when movement toward a preferendum had stabilized; and (3) a segmented regression, which divided the curve of movement by time into two "segments", a curvelinear portion (movement) and an asymptotic portion. The method having the lowest standard error was selected. Once preferred temperatures had been determined for each stock at each acclimation temperature, cubic regressions were used to plot a line for preferred versus acclimation temperature for each stock. The point at which the regression line

intersected a 45° line (preferred temperature = acclimation temperature) was used to determine the final preferred temperature for each stock.

RESULTS

Temporal patterns of thermal behavior after release into the test chambers revealed gradual movement toward the preferred temperature. Figures 2-5 show examples of this temporal behavior for one stock, FxN, acclimated to each of four temperatures, 8°, 16°, 24°, and 32°C. However, all stocks exhibited very similar patterns when acclimated to the same temperature level. The closer the acclimation temperature was to the final preferred temperature, the smaller the amount of gravitation was needed to reach the final preferendum. Table 1 shows the preferred temperature values for each stock acclimated to each of the seven different acclimation temperatures. For all acclimation temperatures except 32°C, all four stocks preferred higher or equal temperatures than those at which they were acclimated. When acclimated to 32°C all stocks preferred areas of the test chambers below 32°C.

Fry (1947) defined final preferred temperature as that temperature to which a fish will eventually gravitate, regardless of its previous thermal history, and the temperature at which acclimation and preferred temperatures are equal. Although cubic regression analysis resulted in the two F_1 hybrid stocks, NxF and FxN, having slightly higher final preferred temperatures than the two pure subspecies, NLMB and FLMB (Figs. 6-9), the final preferred temperatures for all four stocks statistically were not different from each other (Table 2). However, the thermoregulatory behavior at temperatures above and below that value did vary among the stocks (Figure 10). For example, the pure Florida stock displayed the lowest preferred temperatures at the lowest and highest acclimation temperatures, but intermediate to high preferred temperatures at intermediate acclimation temperatures (12-28°C).

DISCUSSION

For a species as a whole, the greater the developmental flexibility of the phenotype, the better that species can cope with selection pressure. This flexibility is clearly advantageous for those organisms exposed to highly unpredictable environmental conditions (Mayr 1983). Different thermal surroundings exert a great influence on developing and adult largemouth bass by affecting growth, reproductive success, swimming speed, and metabolic rates (Coutant 1975). A population of largemouth bass must have within its constituents the ability to meet the physiological demands for growth and development before reproduction efforts can determine the success of a population. Growth in fish will occur only after temperature dependent metabolic maintenance requirements are met (Cox and Coutant 1981). Temperature also can have an effect on food availability, habitat conditions, and predator/prey interactions, all of which can directly influence growth and success of largemouth bass.

Philipp et al. (1981, 1983) determined through electrophoretic analysis that Florida and northern largemouth bass have fixed allelic differences at two loci (Idh-B, Aat-B) and show significant differences in allelic frequencies at two other loci (Mdh-B, Sod-A). These authors showed that the geographic distributions of the alleles at these four loci described specific latitudinal clines, suggesting geographically induced genetic differences among these populations. A large area of intergradation exists between the ranges of the pure subspecies (Philipp et al. 1983). The distribution of a species over a large geographic area presumably leads to individual populations within that area becoming genetically tailored through time to the thermal environments which they inhabit. Thus, not only are there differences between the two

subspecies, but there also exists an area between the subspecific ranges that contains populations of largemouth bass that potentially may show quite variable thermoregulatory behavior.

Temperature of optimum growth values of 25° and 30°C were obtained for largemouth bass in Ontario and Texas, respectively (Strawn 1961, Niimi and Beamish 1974). These values are consistent with the hypothesis that populations of largemouth bass become genetically tailored to their thermal environments (Philipp et al. 1981, 1983). However, the results of this study indicate that the FLMB bass prefers water temperatures of 28°C and lower. These results seem to indicate that this FLMB stock may prefer a cooler than optimal metabolic thermal environment. This may be a result of learned food availability or learned upper thermal avoidance behavior.

The similarities in the determined final preferred temperatures for pure northern and Florida largemouth bass and for both reciprocal intersubspecific F_1 hybrids do not suggest that major thermoregulatory behavioral differences exist among the four stocks. However, the thermoregulatory behavior of each stock after acclimation to temperatures above and below the final preferred temperature did differ. Mathur (1981) observed the same phenomenon upon testing a variety of fish species other than largemouth bass. Hall (1978), in testing white perch (Morone americana), determined there are significantly different thermal preference responses in fish from different geographic areas. These responses support the hypothesis that fish undergo physiological and biochemical adaptations to environmental temperature (Hochachka and Somero 1971). A population becomes genetically suited to its specific environment through selection for a particular phenotype (Mayr 1970, Holland et al. 1974).

Tranquilli et al. (1979) found through radiotracking studies of largemouth bass in Lake Sangchris, Illinois, a power plant cooling lake, that final preferred temperatures ranged from 28.2 to 30.5°C. These values are somewhat higher than the results obtained in this study for NLMB (Table 2). In Lake Shelbyville, Illinois, an unheated lake, the investigators found final preferred temperatures of 26.5°C, a value only slightly lower than our results for NLMB. An explanation of these results may be found in the fact that acclimation to high water temperatures did not occur in unheated Lake Shelbyville as occurred in the heated Lake Sangchris. Similarly, largemouth bass, when fully acclimated in the laboratory to temperatures of 28° and 32°, had a thermal preferenda of approximately 28°C. The period required for full metabolic thermal acclimation has been determined to be from 3 to 4 weeks (2-4 weeks for smaller fish) (Sidell et al. 1973, Sidell 1977). Thus, seasonal differences in natural environmental temperature (field acclimation temperatures) would have substantial effects upon the preferred temperature values determined, unless fish were removed from the field and sufficiently acclimated to a common temperature.

Two methods have been used for determining thermal preferenda in the laboratory, acute or gravitational (chronic). Acute experiments commonly have been 2-3 hours in length and gravitational 2-3 days in length. Both have had criticisms as to their ability to reveal true results (Reynolds and Casterlin 1979). For example, the phenomenon of overshoot in gravitational methods could lead to higher results, as would linearly extrapolating the preferenda data to determine final preferred temperatures in acute testing. Richards et al. (1977) concluded that other factors involved in testing, i.e., age, size, and season may have more effect on the outcome of experiments than the type of test used. Meldrim and Gift (1971) and McCauley and Huggins (1979) showed no

significant differences between fish of different ages or size. Hence, for this study, first year largemouth bass offspring were used for all tests.

A number of different statistical methods have been used to assess the relationship between preferred temperatures and acclimation temperatures when determining final thermal preferences. The problem of using linear equations to describe non-linear relationships has been overcome in this treatment of the data by computing cubic regressions, which more accurately define the behavior observed. Intersection with a line of equality between acclimation and preferred temperatures (Fry 1947) remains the determinant method of calculating the final thermal preferenda.

In conclusion, northern and Florida largemouth bass were shown to have similar final thermal preferenda but different thermoregulatory behavior at acclimation temperatures above and below that temperature. This most likely results from selective adaptations to the thermal conditions present in the range of each subspecies. Hochachka and Somero (1971, 1973) stated that adapations have occurred which can enable lower vertebrates to succeed or at least survive fluctuating environmental conditions. Thus, current and future stocking programs need to consider a variety of issues pertaining to the genetic background of the fish being propagated for introduction into different geographic regions. It must be determined whether the genetic composition of these fish will enable them to thrive in the new environment. If the introduced fish are not well suited for the recipient environment, deleterious effects to these native populations would be expected to occur. Before largemouth bass management and stocking practices continue to unknowingly manipulate different genetic stocks of largemouth bass, more information must be obtained on the physiological and genetic qualities of this species and of its constituent genetic stocks.

ACKNOWLEDGMENTS

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collections of Florida largemouth bass and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. We also wish to thank the Wisconsin Department of Natural Resources for permitting field collections of northern largemouth bass. We particularly want to thank Rick Cornelius and Jerry Perkins for their assistance in these collections. We especially want to thank David Swofford for his advice and assistance throughout the statistical analyses period. Finally, we wish to thank Dr. Paul R. Beaty, Dr. Bruce Taubert, Dr. Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Shirley Lowe, Jeffrey Van Orman, Christine Kaminski, and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Aspinwall, N. 1974. Genetic analysis of North American populations of the pink salmon, *Onchorhynchus garbuscha*, possible evidence for the neutral mutation - random drift hypothesis. Evolution 28:295-305.

Barans, C. A. and R. A. Tubb. 1973. Temperatures selected seasonly by four fishes from western Lake Erie. Journal of the Fisheries Research Board of Canada 30:1697-1703.

Beitinger, T. L. and L. C. Fitzpatrick. 1979. Physiological and ecological correlates of preferred temperature in fish. American Zoologist 19:319-329.

Beitinger, T. L. and J. J. Magnuson. 1979. Growth rates and temperature selection of bluegill, *Lepomis macrochirus*. Transactions of the American Fisheries Society 108:378-382.

Beitinger, T. L., J. J. Magnuson, W. H. Neill and W. R. Shaffer. 1975. Behavioral thermoregulation and activity patterns in the green sunfish, *Lepomis cyanellus*. Animal Behavior 23:222-229.

Cherry, D. S., K. L. Dickson, and J. Cairns, Jr. 1975. Temperatures selected and avoided by fish at various acclimation temperatures. Journal of the Fisheries Research Board of Canada 32:485-491.

Coutant, C. C. 1975. Responses of bass to natural and artificial temperature regimes. Pages 272-285 *In* R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, DC.

Cox, D. K. and C. C. Coutant. 1981. Growth dynamics of juvenile striped bass as functions of temperature and ration. Transactions of the American Fisheries Society 110:226-238.

Ferguson, R. B. 1958. The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. Journal of the Fisheries Research Board of Canada 15:607-624.

Fry, F. E. J. 1947. Effects of the environment on animal activity. University of Toronto Studies, Biological Series Publication 55, Ontario Fisheries Research Laboratory 68, Toronto, Canada.

Goddard, C. I. and J. S. Tait. 1976. Preferred temperatures of F_3 to F_5 hybrids of *Salvelinus fontinalis* X *S. namaycush*. Journal of the Fisheries Research Board of Canada 33:197-202.

Hall, L. W., Jr., C. H. Hocutt, and J. R. Stauffer, Jr. 1978. Implication of geographical location on temperature preference of white perch, *Morone americana*. Journal of the Fisheries Research Board of Canada 35:1464-1468.

Hochachka, P. W. and G. N. Somero. 1971. Biochemical adaptation to the environment. Pages 99-156 In W. S. Hoar and D. J. Randall, editors. Fish Physiology. Vol. 6. Academic Press, New York.

Hochachka, P. W. and G. N. Somero. 1973. Strategies of biochemical adaptations. W. B. Saunders Company, Philadelphia, Pennsylvania. 358 p.

Holland, W. E., M. H. Smith, J. W. Gibbons, and D. H. Brown. 1974. Thermal tolerance of fish from a reservoir receiving heated effluent from a nuclear reactor. Physiological Zoologist 47:110-118.

Jones, T. C. and W. H. Irwin. 1965. Temperature preferences by two species of fish and the influence of temperature on fish distribution. Proceedings of the 26th Annual Conference of the Southeastern Association of Game and Fish Commissoners 26:323-333.

Kimura, M. and T. Ohta. 1971. Protein polymorphism as a phase of molecular evolution. Nature 229:467-470.

Lewontin, R. C. 1974. The genetic basis for evolutionary change. Columbia University Press, London.

Magnuson, J. J., L. B. Crowder, and P. A. Medvick. 1979. Temperature as an ecological resource. American Zoologist 19:331-343.

Mathur, D., R. M. Schutsky, E. J. Purdy, Jr., and C. A. Silver. 1981. Similarities in acute temperature preferences of freshwater fishes. Transactions of the American Fisheries Society 110:1-13.

Mayr, E. 1970. Populations, species, and evolution. Bellrap Press of Harvard University Press, Cambridge, Massachusetts.

Mayr, E. 1983. How to carry out the adaptationist program? The American Naturalist 121:324-334.

McCauley, R. W. and N. W. Huggins. 1979. Ontogenetic and non-thermal seasonal effects on thermal preferenda of fish. American Zoologist 19:267-271.

Meldrim, J. W. and J. J. Gift. 1971. Temperature preferences, avoidance and shock experiments with estaurine fishes. Icthyological Association Bulletin 7.

Neill, W. H. 1971. Distributional ecology and behavioral thermoregulation of fishes in relation to heated effluent from a steam-electric power plant (Lake Monona, Wisconsin). Ph.D. Thesis. University of Wisconsin, Wisconsin.

Niimi, A. J. and F. W. H. Beamish. 1974. Bioenergetics and growth of largemouth bass (Micropterus salmoides) in relation to body weight and temperature. Canadian Journal of Zoology 52:447-456.

Ohta, T. and M. Kimura. 1971. Behavior of neutral mutants influenced by associated overdomiant loci in finite populations. Genetics 69:247-260.

Peterson, R. H., A. M. Sutterlin, and J. L. Metcalfe. 1979. Temperature preference of several species of Salmo and Salvelinus and some of their hybrids. Journal of the Fisheries Research Board of Canada 36:1137-1140.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of patterns of differential gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, Micropterus salmoides salmoides, and smallmouth bass, Micropterus dolomieui). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (Micropterus salmoides) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1982. Biochemical genetics of largemouth bass. Electric Power Research Institute, Palo Alto, California, 262 p.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Reynolds, W. W. and M. E. Casterlin. 1976. Thermal preferenda and behavioral thermoregulation in three centrarchid fishes. Pages 185-190 in G. W. Esch and R. W. McFarlane (Eds.) Thermal Ecology II: Proceedings of the Symposium held at Augusta, GA, April 2-5, 1975. ERDA Symposium Series, CONF-750425, National Technological Information Services, Springfield, VA.

Reynolds, W. W. and M. E. Casterlin. 1979. Behavioral thermoregulation and the final preferendum paradigm. American Zoologist 19:211-224.

Reynolds, W. W., R. W. McCauley, M. E. Casterlin and L. I. Crawshaw. 1976. Body temperatures of behaviorally thermoregulating largemouth black bass, Micropterus salmoides. Comparative Biochemical Physiology 54:461-463.

Richards, F. P. and R. M. Ibara. 1978. The preferred temperatures of the brown bullhead, Ictalurus nebulosus, with reference to its orientation to the discharge canal of a nuclear power plant. Transactions of the American Fisheries Society 107:288-294.

Richards, F. P., W. W. Reynolds, and W. R. McCauley. 1977. Temperature preference studies in environmental impact assessments: an overview with procedural recommendations. Journal of the Fisheries Research Board of Canada 34:728-761.

Ryman, N. 1981. Conservation of genetic resources: Experiences from the brown trout (*Salmo trutta*). *In* N. Ryman (Ed.) Fish gene pools. Ecological Bulletin (Stockholm) 34:61-74.

Sidell, B. 1977. Turnover of cytochrome c in skeletal muscle of green sunfish (*Lepomis cyanellus* R.) during thermal acclimation. Journal of Experimental Zoology 199:233-250.

Sidell, B. D., F. R. Wilson, J. Hazel, and C. L. Prosser. 1973. Time course of thermal acclimation in goldfish. Journal of Comparative Physiology 84:119-127.

Strawn, K. 1961. Growth of largemouth bass fry at various temperatures. Transactions of the American Fisheries Society 90:334-335.

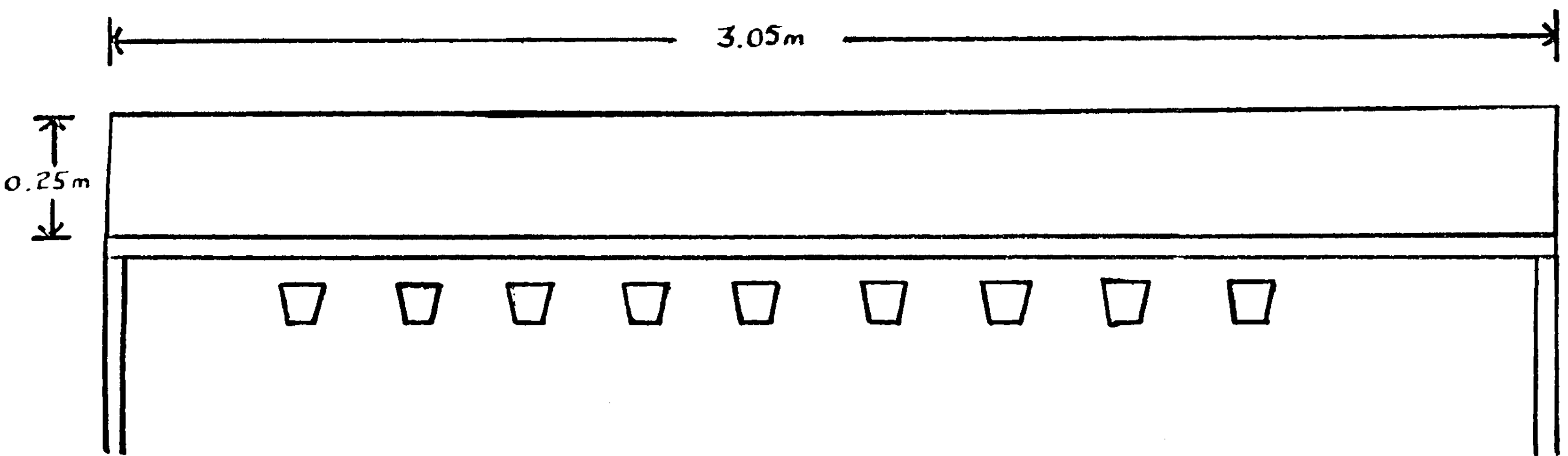
Tranquilli, J. A., D. W. Dufford, and R. W. Larimore. 1979. Activity and preferred temperature of radiotagged largemouth bass in Lake Sangchris and Lake Shelbyville. Pages 105-128 *in* Evaluation of a cooling lake fishery, Vol. III. Illinois Natural History Survey Final Report to Electric Power Research Institute, Palo Alto, CA.

Table 1. Preferred temperatures (°C) for four stocks of largemouth bass (NLMB, NxF, FxN, FLMB) at various acclimation temperatures.

Stock	Acclimation Temperature						
	8°	12°	16°	20°	24°	28°	32°
	Preferred Temperature						
NLMB	24.6	25.8	25.8	25.9	25.8	27.7	29.5
N x F	24.2	26.3	25.6	27.9	26.2	28.1	27.9
F x N	24.4	26.3	26.8	27.8	26.5	28.[illegible]	28.2
FLMB	22.0	25.8	27.9	26.0	26.3	28.1	27.3

Table 2. Final preferred temperatures (°C) for four stocks of largemouth bass (NLMB, NxF, FxN, FLMB).

Stock	Final Preferred Temperature
NLMB	26.7
N x F	28.4
F x N	27.8
FLMB	26.7



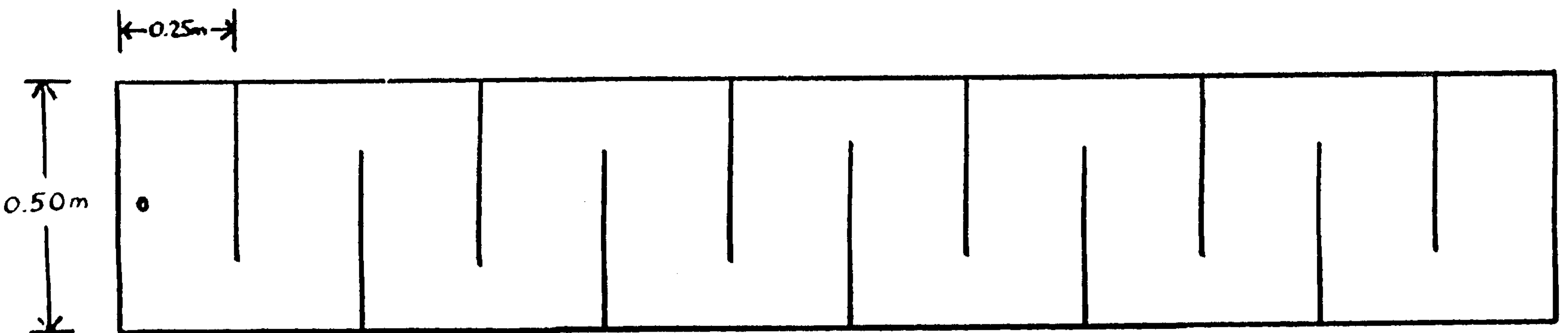


Figure 1. Diagram of preference testing apparatus.

FIGURE 2. THERMAL BEHAVIOR PATTERN OF *M.s.floridanus* x *M.s. salmoides* F_1 HYBRID ACCLIMATED TO 8°C

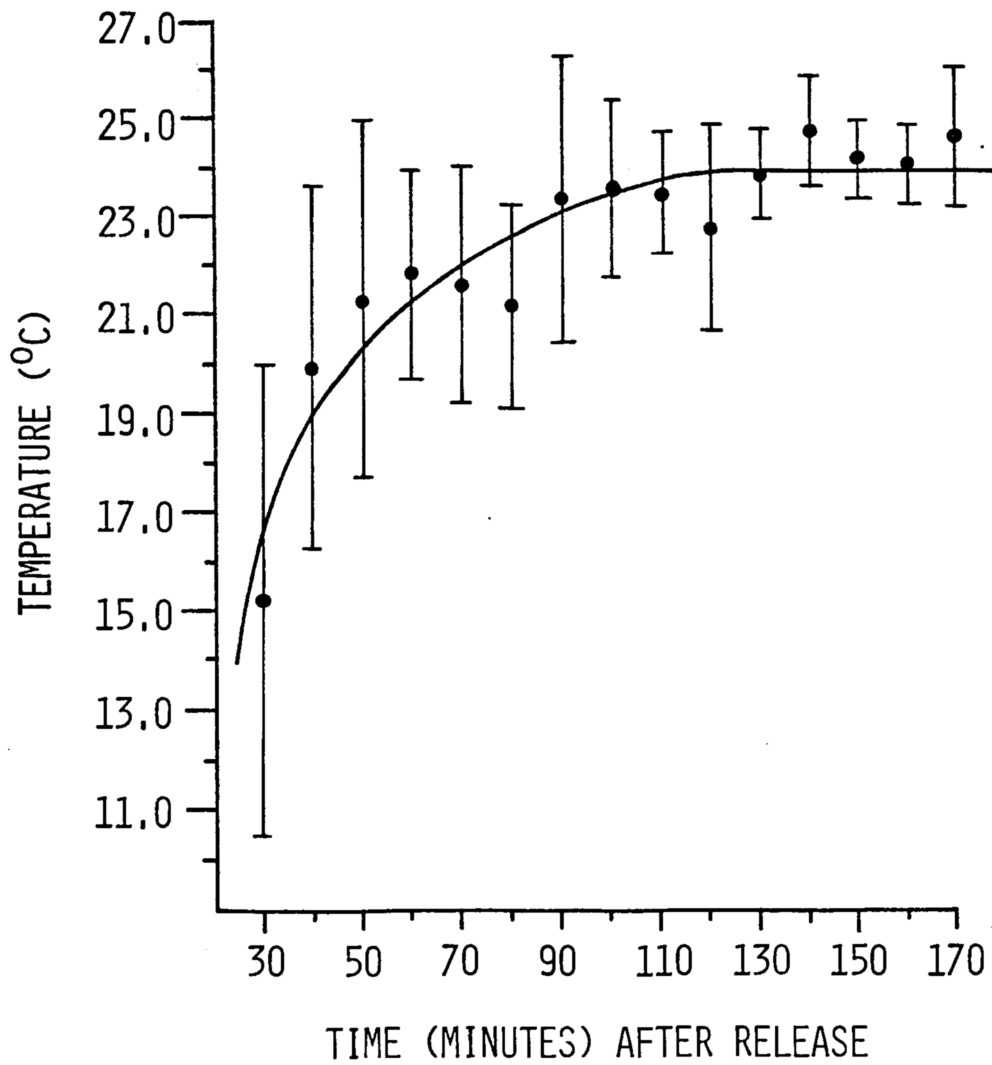


FIGURE 3. THERMAL BEHAVIOR PATTERN OF *M.s. floridanus* x *M.s. salmoides* F_1 HYBRID ACCLIMATED TO 16° C

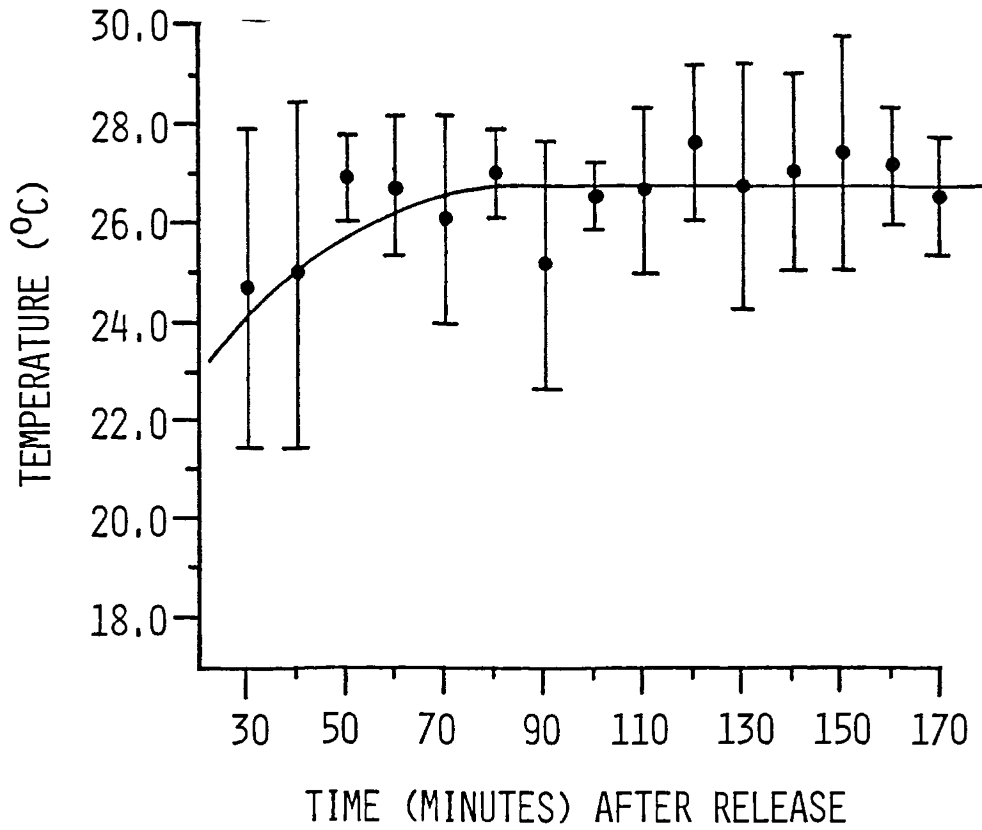


FIGURE 4. THERMAL BEHAVIOR PATTERN OF M.s. floridanus x M.s. salmoides F_1 HYBRID ACCLIMATED TO 24°C

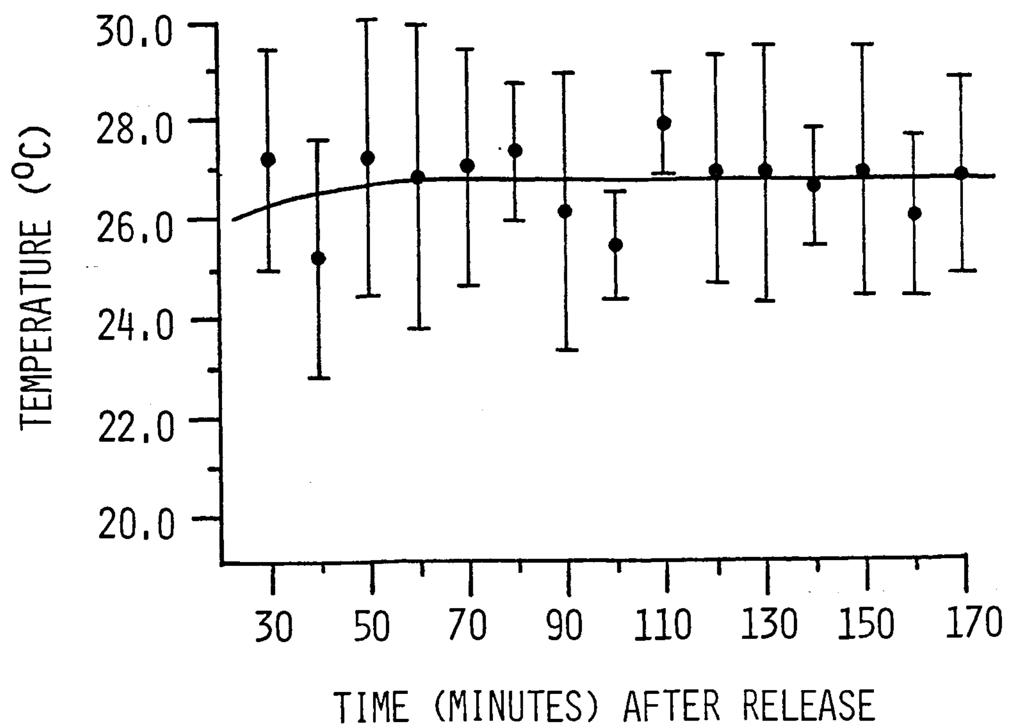


FIGURE 5. THERMAL BEHAVIOR PATTERN OF
M. s. floridanus x *M. s. salmoides*
F_1 HYBRID ACCLIMATED TO 32°C

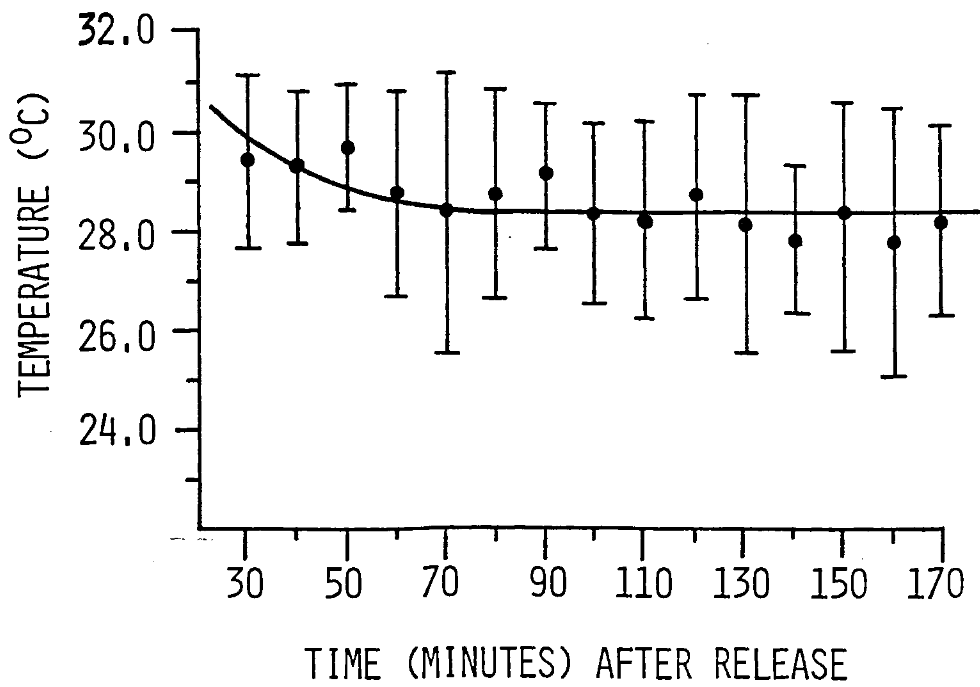


Figure 6. Final preferred temperature of northern largemouth bass as determined by cubic regression.

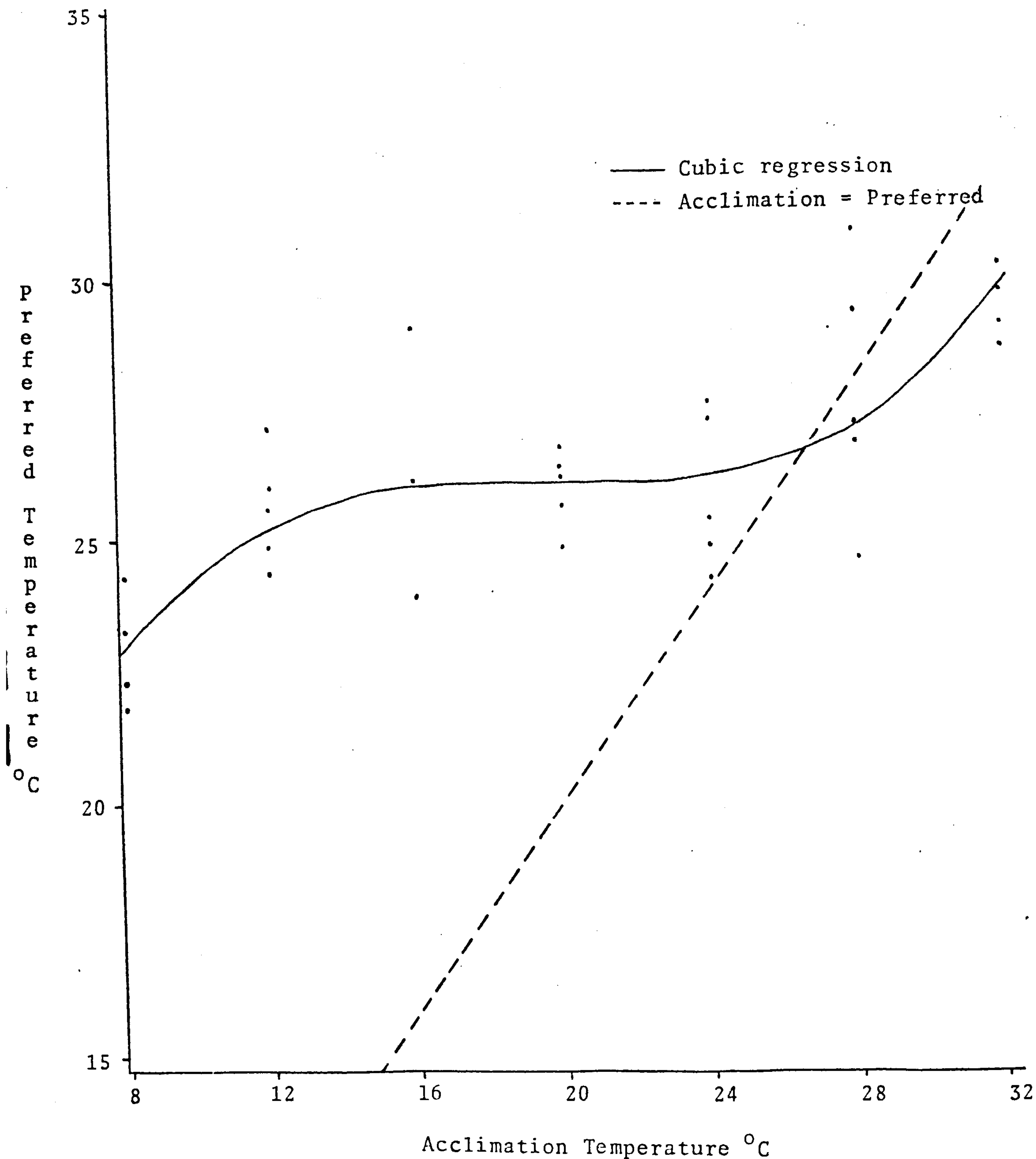


Figure 7. Final preferred temperature of northern x Florida largemouth bass as determined by cubic regression.

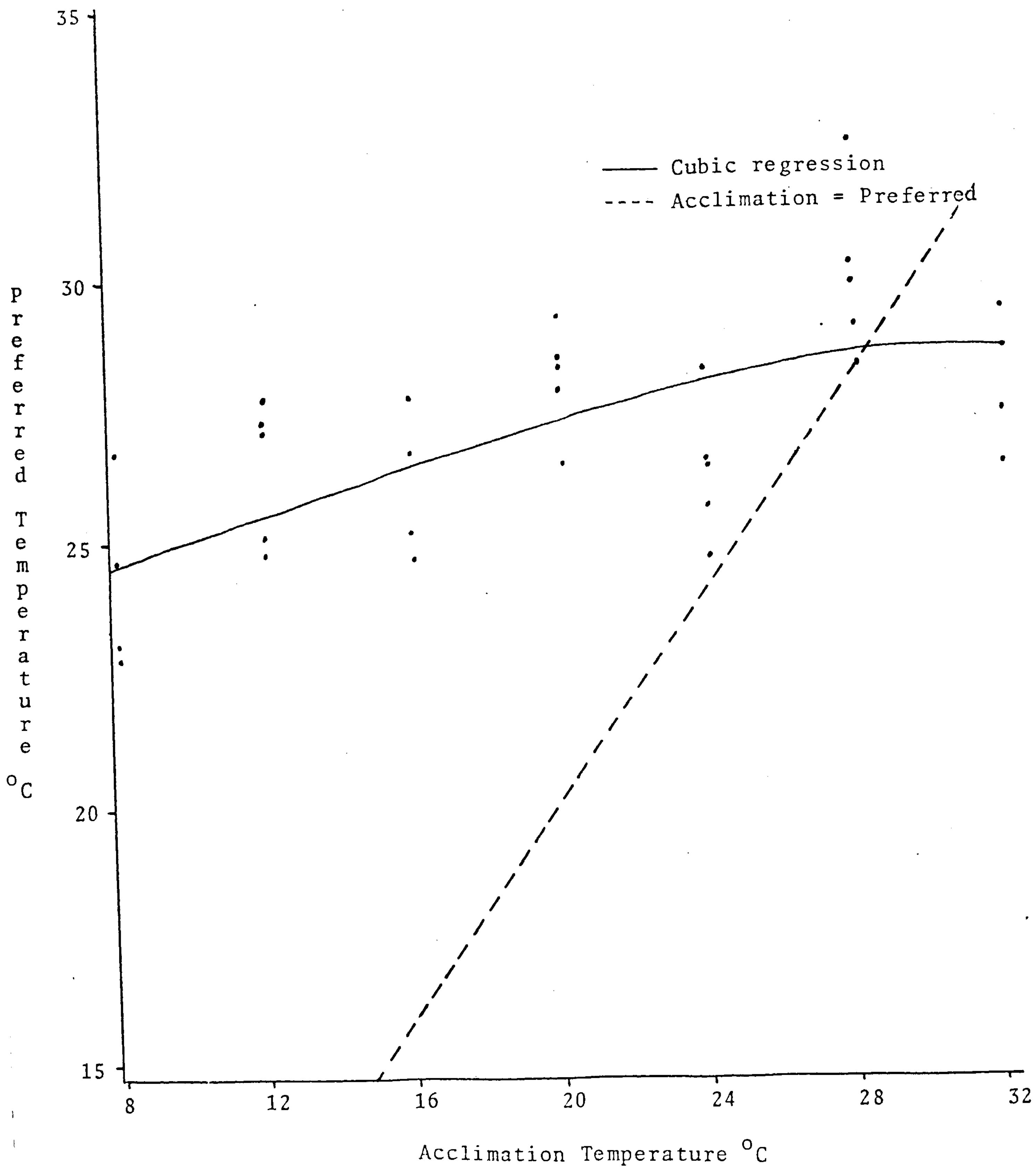


Figure 8. Final preferred temperature of Florida x northern largemouth bass as determined by cubic regression.

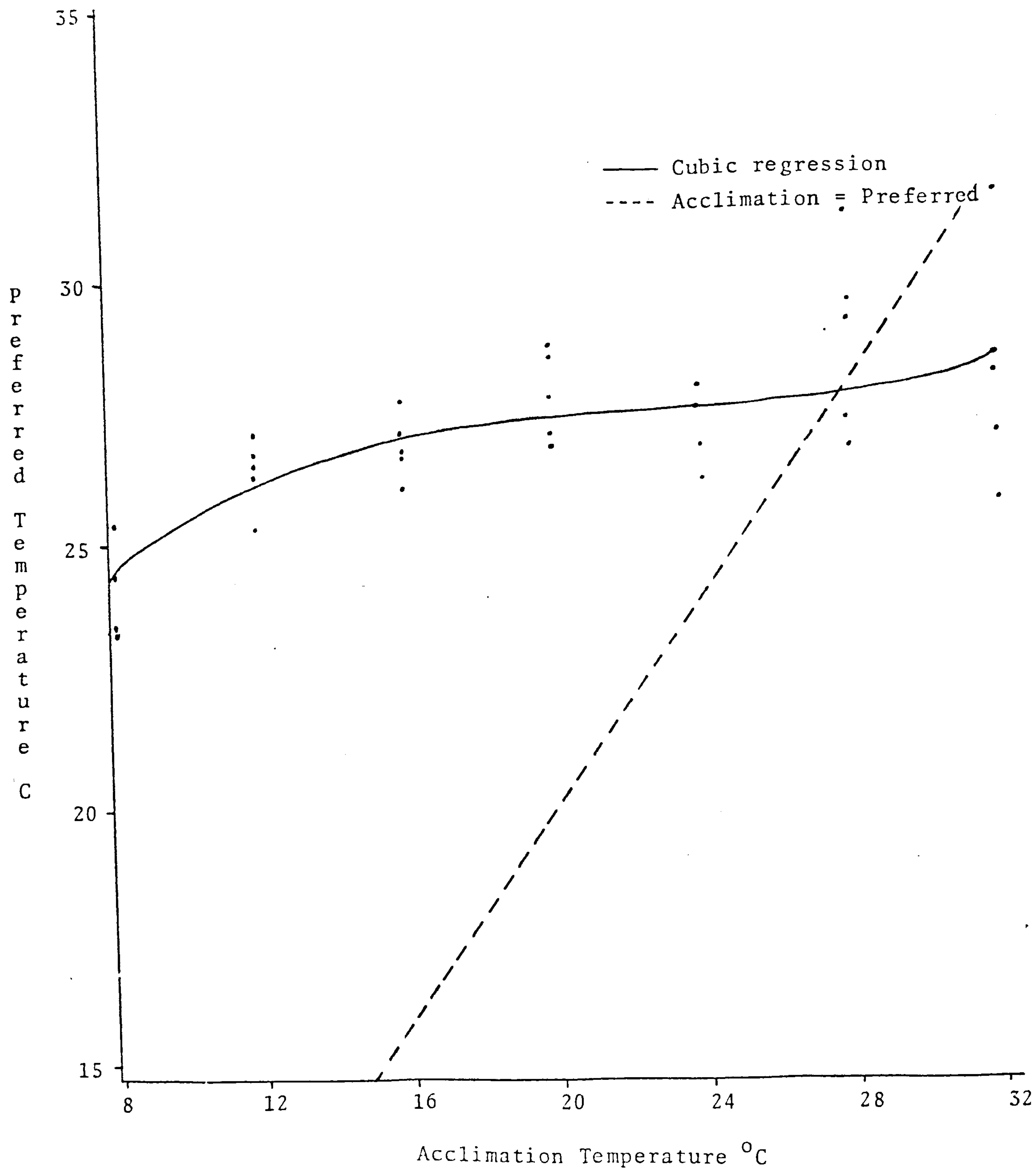


Figure 9. Final preferred temperature of Florida largemouth bass as determined by cubic regression.

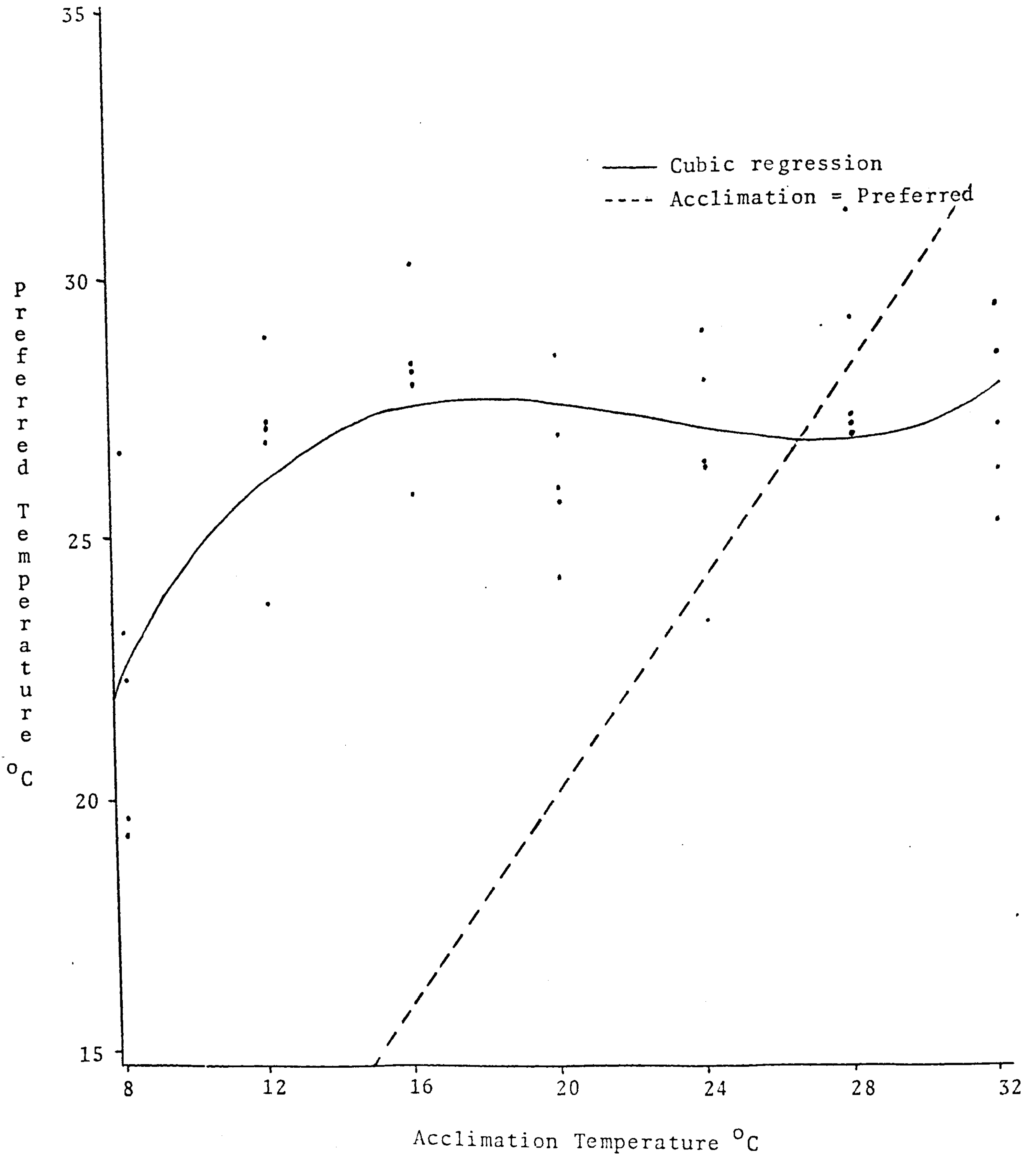
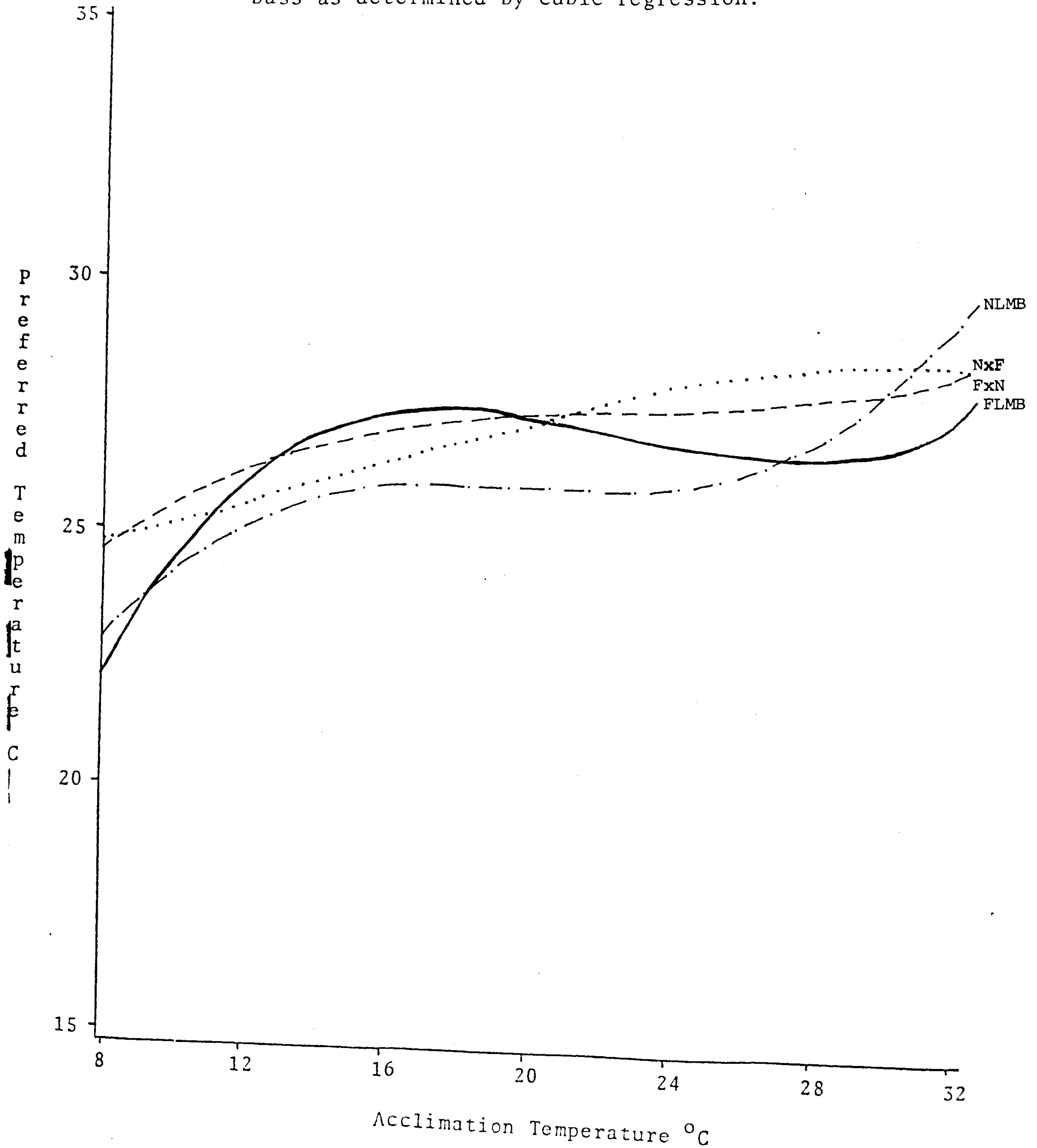


Figure 10. The relationships of acclimation temperature to preferred temperature for four stocks of largemouth bass as determined by cubic regression.



STUDY 104
JOB 3

CHAPTER 9

Acute and Chronic Thermal Maxima of Northern, Florida, and Reciprocal F_1 and F_2 Hybrid Largemouth Bass

Robert Fields[1], Shirley S. Lowe[1], Christine Kaminski[1], Gregory S. Whitt[3,1] and David P. Philipp[1,2]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

The relative effect of acclimation temperature on thermal tolerance was studied in four genetic stocks of juvenile largemouth bass: northern largemouth bass, NLMB (*Micropterus salmoides salmoides*), Florida largemouth bass, FLMB (*M. s. floridanus*) and both reciprocal F_1 hybrids, NLMB♀ x FLMB♂ (NxF) and FLMB♀ x NLMB♂ (FxN). Thermal tolerance for each of these stocks was measured by two methods. The first method determined Acute Thermal Maxima (ATM) at various acclimation temperatures. ATM is defined as the temperature at which death occurs during a rapid temperature increase (1°C per 5 minutes). Thermal tolerance was also studied in each of the four stocks by determining the Chronic Thermal Maxima (CTM) for fish acclimated to 32°C. CTM is defined as the temperature at which death occurs during a gradual temperature increase (1°C per day). The order of greatest ATM and CTM values were identical and were FxN > FLMB > NxF > NLMB. Results showed that increased acclimation temperature resulted in significant, proportional increases in ATM for all stocks. Second generation hybrids (NxF F_2 and FxN F_2) were acclimated at 24°C and examined for ATM. The NxF F_2 had a higher overall ATM (38.4) than did the FxN F_2 (37.8), however, these values were intermediate to those of the NLMB (37.3) and FLMB (39.2). Subsequent genetic analysis revealed no correlation between ATM and genotype at the four loci studied, showing that a large proportion of Florida alleles at these loci had no direct influence on thermal tolerance of hybrid offspring.

INTRODUCTION

Two recognized subspecies of largemouth bass, the northern (*Micropterus salmoides salmoides*) and the Florida (*M. s. floridanus*), were originally separated into subspecies by morphological differences (Bailey and Hubbs 1949). Despite inconsistencies in results of studies that attempted to compare growth qualities between the strains (Clugston 1964, Inman et al. 1976), some resource managers have assumed enhanced genetic and physiological traits in *M. s. floridanus* due to its superior growth in its native habitat. As a result, Florida largemouth bass stocking programs have been suggested as one method of enhancing trophy fish production in northern waters. The apparent success of the introductions of Florida largemouth bass in Texas and California have further provided impetus to the idea that artificially heated reservoirs in northern climates may be similar enough to the native habitat of *M. s. floridanus* that introduction of this subspecies may improve the fishery. However, the shortcoming of studies comparing the subspecies in Texas (Inman et al. 1976) and California (von Geldern and Mitchell 1975, Moyle and Holzhauser 1978) has been a lack of genetic verification of stocks used. This problem has been substantiated and partially redressed by Philipp et al. (1981, 1982, 1983a), but for the most part, no assessments have been made of the ultimate impact of the introduction of the Florida subspecies on the endemic stocks of largemouth bass in northern waters.

One potentially important physiological difference between the Florida and northern largemouth bass is thermal tolerance, or the ability to survive thermal stress. This may be measured via the Acute Thermal Maxima (ATM), the temperature at which disorientation, and ultimately, death occurs after rapid temperature increase (Cowles and Bogert 1944). This is a dynamic process dependent upon cumulative affects and prior conditioning, and recorded values

may depend somewhat upon subjective judgment by the experimenter. In a study of extended exposure to thermal stress, Hart (1952) found that northern largemouth bass were less susceptible to extended heat stress than Florica largemouth bass. As in other studies, increased acclimation temperature was found to significantly increase thermal tolerance. However, here too, the comparison between strains must be considered somewhat questionable due to a lack of genetic verification. Smith and Scott (1975) found that acclimation temperature also affects tolerance to acute thermal stress, as has been seen in numerous studies. In a South Carolina cooling pond, Yardley et al. (1974) found correlation between malate dehydrogenase allele frequencies and environmental thermal stress in largemouth bass, which implies a selective advantage for a single genotype. However, Smith and Scott (1975) found no relation between this environmental phenomenon and laboratory determined ATM values.

This study was undertaken to examine one potential parameter of thermal tolerance in pure northern and Florida largemouth bass and two generations of hybrid offspring. That is, fish of known genetic composition were assessed under identical conditions, so that the relative importance of genotype versus environmental conditioning, in this case acclimation temperature, was evaluated independently. Pure northern (NLMB), pure Florida (FLMB), and both reciprocal hybrid offspring (NLMB♀ x FLMB♂, NxF, and FLMB♀ x NLMB♂, FxN) were used to determine the Chronic Thermal Maxima (CTM) and the effect of acclimation temperature on Acute Thermal Maxima (ATM). In addition, the assertion that a large donation of Florida alleles at structural loci imposes genetic superiority for thermal tolerance was evaluated among NXF F_2 individuals for he single criterion of survivability. The enzyme loci selected for analysis were those used as markers to distinguish the two subspecies, Mdh-B, Idh-B, Aat-B.

Thus, through correlation of electrophoretic results with ATM results for each F_2 individual tested, we assessed the impact of NLMB versus FLMB alleles at each of those structural loci on thermal tolerance.

were recovered from each pond. Electrophoretic analysis of subsamples of 100 fingerlings from each stock confirmed their genetic purity.

During the spring of 1982 the NxF F_1 stock was used to produce F_2 offspring. Similarly, during the spring of 1983 the FxN F_1 stock was used to produce F_2 offspring, as well. Specifically, equal numbers of male and female F_1 hybrids were placed in 0.08 hectare ponds and allowed to spawn naturally. Young-of-the-year F_2 hybrid offspring remained in ponds until late September. The F_2 hybrid fingerlings were recovered by draining the pond which contained them or by seining and electrofishing methods.

Acclimation Procedure:

Fingerlings of each of the four F_1 stocks of largemouth bass produced in 1981 were collected and held in thermally regulated aquaria initially adjusted to the field temperature at collection time under a 12L-12D 24 hour light schedule. The temperatures of the aquaria were adjusted at the rate of 1°C per day until the desired acclimation temperatures were reached. These largemouth bass were then held for a 30 day period to allow for total thermal acclimation. During this period these fingerlings were fed live *Daphnia magna*, frozen brine shrimp or live fathead minnows (*Pimephales promelas*) three times daily. All Chronic Thermal Tolerance test (CTM) fish were acclimated to 32°C for 4 weeks prior to testing. All Acute Thermal Tolerance (ATM) test fish were acclimated to one of four temperatures, 8°, 16°, 24° or 32°C for at least three weeks prior to testing.

Determination of Chronic Thermal Tolerance (CTM):

Chronic (CTM) tests were conducted in aerated 20 gallon aquaria in a Conviron environmental chamber. Prior to testing, these fish had been acclimated to 32°C for 30 days. The temperatures in the tanks were controlled

by adjusting the temperature of the environmental chamber. Tests were conducted by raising the temperature 1°C per day. Survival of the fish was monitored three times daily and the temperature at which death occurred for each fish was recorded. All stocks were tested simultaneously with ten fish of each stock being tested. During testing, fish were fed live *Daphnia* three times daily.

Determination of Acute Thermal Tolerance (ATM):

Acute (ATM) tests were conducted in an aerated 20 gallon aquarium into which heated water was added at a constant rate. An outflow siphon maintained a constant water level. The water was mixed by vigorous aeration and a magnetic stirring bar isolated from the test chamber by a wire screen. Prior to the tests, fish were held in the chamber for 15 minutes to adjust to the new environment. The water was then heated at a rate of 1°C per minute or less. This rate was fast enough to minimize cumulative stress on the fish, yet allowed the observer sufficient time (5-10 seconds) to record fish behavior or time of death between 0.1°C intervals. For comparison of acclimation effects on thermal tolerance, times of initial loss of equilibrium, total loss of equilibrium and death were recorded. Death was presumed to occur when the gills flared and subsequent paralysis made respiration impossible. Only the time of death was recorded for the portion of the study assessing F_2 hybrids. All fish were frozen at -20°C immediately after death for subsequent genetic analysis.

Genetic Analyses:

The genotypes at each of three enzyme loci (Mdh-B, Idh-B and Aat-B) for each of the F_2 hybrids were determined by vertical starch gel electrophoresis. The procedures used for tissue preparation, electrophoresis and histochemical staining was essentially as described in Philipp et al. (1979).

RESULTS

The three data sets (Chronic Thermal Tolerance [CTM] of F_1 stocks, acclimation effects on Acute Thermal Tolerance [ATM] of F_1 stocks, and ATM determinations for individual F_2 fish) were evaluated independently using analysis of variance (ANOVA). The liberal Duncan's multiple range test and the more conservative Scheffe's test were used to determine the relatedness groupings of FLMB, NxF, FxN and FLMB stocks.

Chronic Thermal Maxima (CTM) of F_1 Stocks:

Ten individuals of each of the F_1 stocks, NLMB, NxF, FxN and FLMB, were tested simultaneously. The results are summarized in Table 2. The temperature at death was correlated with genetic origin ($F = 20.67$, $R^2 = .633$ and $P = .002$). The order of CTM was FxN > FLMB > NxF > NLMB. Pure *M. s. salmoides* had a CTM significantly lower than all other stocks according to both Duncan's and Scheffe's tests. Only the liberal Duncan's test separated the high CTM of the FxN F_1 from the other stocks.

Acute Thermal Tolerance (ATM) of F_1 Stocks:

Initial and total loss of equilibrium at all acclimation temperatures were consistent with temperatures of death. Mean death temperatures for each stock are summarized in Table 3. The effect of acclimation temperature on ATM values is shown in Figure 1. ANOVA results in Table 3 depict the genetic-dependent variation between groups. Genetic origin contributed significantly to the variation of ATM observed between stocks at all acclimation temperatures. It should be noted, however, that not all of the variation can be attributed to genetic origin alone (see R^2 values, Table 3). The order of

mean ATM for the test at each acclimation temperature (FxN > FLMB > NxF > NLMB) was the same as that for mean CTM values (Table 2). At low acclimation temperatures (8°C, 16°C), both Duncan and Scheffe's groupings isolated the ATM of NLMB from the FxN F_1. The FLMB did not differ significantly from either hybrid. Only at higher temperatures (24°C, 32°C) were the ATM values of the pure subspecies significantly different. Thus, as acclimation temperature is raised, the disparity between Florida and northern largemouth bass adaptation to thermal stress becomes more apparent.

<u>Acute Thermal Tolerance (ATM) of F_2 Stocks</u>:

The total average ATM values for both F_2 stocks are shown in Table 4. Interestingly, although at an acclimation temperature of 24°C, the ATM for the FxN F_1 is greater than that for the NxF F_1, the reverse was true for the F_2 generation. In addition, at an acclimation temperature of 24°C, the ATM of the NxF hybrids increased from the F_1 (37.0) to the F_2 (38.4) generation, whereas the reverse was true for the FxN hybrids. The ATM of the FxN F_1 (37.9) was higher than that of the FxN F_2 (37.8). However, NxF F_2 and FxN F_2 groups were not tested simultaneouslsy. Therefore, comparison between these two stocks and the other four stocks must be considered somewhat tentatively.

There was no correlation between ATM and genetic expression at any of the loci examined (Table 4). Variation within genetic groups was as large as the overall variation. ATM was not correlated with the number of Florida alleles, the number of homozygous Florida loci, the number of homozygous northern loci or the degree of heterozygosity.

DISCUSSION

Biochemical evolution in habitats under unique environmental pressures should result in quantifiable physiological differences between divergent stocks. Transient environmental conditions should affect the degree to which biochemical variations are expressed. In the present study, thermal acclimation was found to significantly affect subsequent response to thermal stress, within limits imposed by genetic origin. The correlation between CTM and allelic expression at three enzyme loci which had fixed allelic differences between the two subspecies, is consistent for only the pure subspecies (M. s. salmoides and M. s. floridanus). The relationship is not conclusive in first generation hybrids, and no correlation is apparent in second generation fish. M. s. salmoides exhibited significantly lower thermal tolerance than M. s. floridanus. It is important to note that the disparity between M. s. salmoides and M. s. floridanus becomes most apparent at high acclimation temperatures. The adaptive significance of genetically controlled thermal tolerance is thus directly related to the environment in which it evolved. This is contrary to the findings of Hart (1952), but the lack of genetic verification of stocks used in that study prevents a direct comparison. As can also be seen from the data (Tables 2 and 3), significant differences exist between the ATM values for reciprocal hybrids. The FxN F_1 ATM was consistently higher than that of the NxF F_1, and even higher than the FLMB stock. Clearly, this disparity cannot be explained by allelic variation at the tested enzyme loci, as all F_1 hybrids received equal genetic donation from each parent subspecies. The source of the observed disparity is unclear. However, non-reciprocity among reciprocal F_1 hybrids has been documented extensively (Whitt and Philipp 1977, Philipp et al. 1983b, Parker et al. 1984). Genetic expression does not account for all variation among test groups in the present study (see R^2 values, Table 3),

however, variation in size among juvenile (first-year) fish was not large, but there may have been some variation in nutritional state. It is also tempting to compare the data obtained for the two F_2 hybrids to those of the others. This must be done cautiously, since each of the F_2 tests was run separately and uncontrollable variables may have affected the absolute values obtained. For example, unnoticed transient deviations in acclimation temperature may significantly alter the final ATM.

The Chronic Thermal Tolerance (CTM) tests probably best simulate the intermittent exposure to sublethal temperatures in the environment. Thus, the data obtained may represent more realistic absolute lethal temperatures for each stock. Tolerance to short-term thermal stress may more closely reflect adaptations to rapidly rising temperatures in the environment. Although the stress is more acute than any likely to be encountered in unaltered environments, the consistency of these results and their relation to long term data indicate the usefulness of this measurement as an indirect parameter of adaptability.

The three enzyme loci examined (Mdh-B, Idh-B, Aat-B) exhibit allelic expressions (Table 1) that describe latitudinally generated geographic clines (Philipp et al. 1981, 1983a). If physiological response to thermal stress is partially dependent upon variable expression at a few important loci, then the above loci are likely candidates for investigation. Leigh-Brown (1977) described two criteria for testing the adaptive significance of phenotypic clinal distributions: the allelic isozymes under investigation must be shown to be differentially affected _in vitro_ by the presumed environmental selection pressure; and the expression of these isozymes must be correlated with _in vivo_ responses to the same selective pressure. The largemouth bass locus for which the most extensive data are available in this context is Mdh-B. Thermal

kinetic studies of the two allelic isozymes, MDH B^1B^1 and MDH B^2B^2 *in vitro* have shown a correlation with the latitude-dependent distribution of these two alleles among natural populations (Hines et al. 1983). The occurrence of specific alleles at another MDH locus in largemouth bass had been shown by Yardley et al. (1974) to be correlated with different thermal regimes within a heated body of water. However, the findings of Smith and Scott (1975) and the present study failed to relate this phenomenon for fish in a controlled thermal experiment. There are several possible explanations for this discrepancy. Perhaps the ATM is not a reliable indicator of adaptation to increased sublethal temperatures. This is not likely however, since the correlation of this parameter with that expected for the two pure subspecies is very good. Alternatively, the Mdh-B locus and other loci may not be as important to thermal tolerance as indicated. The enzyme systems studied represent only three among the many thousands expressed, and the occurrence of clines in allele distribution patterns may be the result of random mutation and genetic drift among ecologically isolated strains. However, this does not explain the differential occurrence of loci within a body of water such as that found by Yardley et al. (1974). It is most likely that polygenic complexes of regulatory as well as structural genes interact to provide adaptability to potential thermal variation. Hence, the F_2 generation would be expected to demonstrate highly variable ATM and CTM responses under controlled laboratory conditions. Also, the complex interactions of thermal preference and tolerance, as well as physiological efficiency, may serve to refine the genetic composition of fish for a specific set of environmental circumstances.

The implication of this study for manangement practices is that, although the pure Florida subspecies and its reciprocal hybrids with the northern subspecies appear to be genetically superior to NLMB for adaptation to high

temperature tolerance, high temperatures may not be the prevailing environmental condition which contributes most to the genetic tailoring in natural habitats. In fact, in most northern habitats the ability to tolerate cold temperatures during the winter appears to be more important to the survival of a stock. The difference among these stocks in their upper thermal tolerance does indicate the sometimes overlooked need of prohibiting the introduction of NLMB or any stock of largemouth bass containing a portion of NLMB alleles into waters in peninsular Florida. The genetic integrity of the FLMB needs to be protected as much as that of the NLMB. Before Florida largemouth bass or any non-native stock of largemouth bass are released into the waters of Illinois to remedy specific problems, extensive study of successive hybrid generations is needed to establish the relation of thermal tolerance and genetic origin. Further definition and refinement of the pertinent ecological factors involved in differential adaptability are also necessary for a complete understanding of how different genetic stocks perform in different environments.

The authors wish to thank the Florida Game and Freshwater Fish Commission for their support and assistance in this study from its inception. We especially wish to thank F. Gerry Banks, Dennis "Smokie" Holcomb, Forrest J. Ware, and Edward Zagar for permitting field collections of Florida largemouth bass and providing laboratory space at the Eustis Fisheries Research Laboratory, and Charles Starling and Harrell Revels for assistance at the Richloam hatchery. We also wish to thank a number of staff members of the FGFWFC who helped collect fish, assisted with experiments, lent equipment and offered valuable advice: Joseph Crumpton, William Coleman, William Johnson, A. Michael Wicker, Charles Mesing, Richard Krause, Levi J. Jenkins III, James Bitter, Robert Wattendorf and Paul Shafland. We also wish to thank the Wisconsin Department of Natural Resources for permitting field collections of northern largemouth bass. We particularly want to thank Rick Cornelius and Jerry Perkins for their assistance in these collections. We also especially wish to thank Dr. William Harvey for his advice and assistance in the statistical analyses. Finally, we wish to thank Dr. Paul R. Beaty, Dr. Bruce Taubert, Dr. Henry R. Parker, Nancy Frye, Lynn Dettman, Charles Stone, Sheila Magee, Jeffrey Koppelman, Jeffrey Van Orman, and Todd Powless for field and laboratory assistance and Suzanne Peratt for technically preparing the manuscript. This project was supported by funds from the Illinois Natural History Survey and by Federal Aid for Fish Restoration funds from the Illinois Department of Conservation, Project F-35-R.

REFERENCES

Bailey, R. M. and C. L. Hubbs. 1949. The black basses (Micropterus) of Florida, with description of a new species. Univ. Mich., Mus. of Zool., Occas. Papers 516:1-40.

Clugston, J. P. 1964. Growth of the Florida largemouth bass, Micropterus salmoides floridanus (LeSueur), and the northern largemouth bass, M. s. salmoides (Lacepede), in subtropical Florida. Trans. Am. Fish. Soc. 93:146-154.

Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Amer. Mus. Nat. Hist. 83:265-296.

Hart, J. S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. Publ. Ontario Fish Res. Lab. LXXII. 79 p.

Hines, S. A., D. P. Philipp, W. F. Childers and G. S. Whitt. 1983. Thermal kinetic properties of allelic isozymes at the MDH-B locus. In D. P. Philipp, W. F. Childers and G. S. Whitt (editors) Biochemical genetics of largemouth bass, Micropterus salmoides. Final Report to Electrical Power Research Institute, Palo Alto, CA. Illinois Natural History Survey, Champaign, Illinois.

Inman, C. R., R. C. Dewey and P. P. Durocher. 1976. Growth comparisons and catchability of three largemouth bass strains. Proceedings of the 30th Annual Conference of the SE Assoc. Game and Fish Comm. 30:1-17.

Leigh-Brown, A. J. 1977. Physiological correlates of an enzyme polymorphism. Nature 269:803-804.

Moyle, P. B. and N. J. Holzhauser. 1978. Effects of the introduction of the Mississippi silverside (Menidia audens) and Florida largemouth bass (Micropterus salmoides floridanus) on the feeding habits of young-of-the-year largemouth bass in Clear Lake, California. Trans. Am. Fish. Soc. 107:574-582.

Parker, H. R., G. S. Whitt and D. P. Philipp. 1984. Biochemical genetic evaluation of patterns of gene expression during the embryonic development of northern, Florida, and reciprocal F_1 hybrid largemouth bass. Pages 5-1 to 5-40 In D. P. Philipp and G. S. Whitt (eds.) The production and evaluation of different genetic stocks of largemouth bass, Micropterus salmoides, for different thermal environments. Final Report to Illinois Department of Conservation, Federal Aid Project F-35-R by the Illinois Natural History Survey, Champaign, Illinois, USA.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, *Micropterus salmoides salmoides*, and smallmouth bass, *Micropterus dolomieui*). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (*Micropterus salmoides*) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1982. Electrophoretic analyses of largemouth bass, *Micropterus salmoides*, populations. *In* D. P. Philipp, W. F. Childers and G. S. Whitt (editors) Biochemical genetics of largemouth bass *Micropterus salmoides*. Final Report to Electrical Power Research Institute, Palo Alto, CA. Illinois Natural History Survey, Champaign, IL. 262 p.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1983a. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Philipp, D. P., H. R. Parker and G. S. Whitt. 1983b. Evolution of gene regulation: Isozymic analysis of patterns of gene expression during hybrid fish development. Pages 193-237 *In* G. S. Whitt (Ed.) Isozymes: Current Topics in Biological and Medical Research.

Smith, M. H. and S. L. Scott. 1975. Thermal tolerance and biochemical polymorphism of immature largemouth bass *Micropterus salmoides* Lacepede. Bull. Ga. Acad. Sci. 34:180-184.

von Geldern, C. E. Jr. and D. F. Mitchell. 1975. Largemouth bass and threadfin shad in California. Pages 426-449 *In* R. H. Stroud and H. Clepper (editors) Black Bass Biology and Management. Sport Fishing Institute, Washington, DC.

Whitt, G. S. and D. P. Philipp. 1977. Differential lactate dehydrogenase gene expression during development of the medaka (*Oryzias latipes*). Isozyme Bulletin 10:36.

Yardley, D., J. C. Avise, J. W. Gibbons and M. H. Smith. 1974. Biochemical genetics of sunfish. III. Genetic subdivisions of fish populations inhabiting heated waters from nuclear reactors. Pages 255-263 *In* J. W. Gibbons and R. R. Sharitz (editors) AEC Symposium Series.

Table 1. Electrophoretic variation among NLMB and FLMB stocks.[a]

Enzyme	Source	Locus	Alleles found in northern	Alleles found in Florida
Malate Dehydrogenase	white skeletal muscle	B	1,2[b]	2
Isocitrate Dehydrogenase	liver	B	1	3
Aspartate Aminotransferase	liver	B	1,2	3,4

[a] Adapted from Philipp et al. 1982

[b] Although NLMB populations contain both the B^1 and B^2 alleles, the NLMB stock from Bone Lake used as NLMB broodstock in producing NLMB, NxF and FxN F_1 stocks was fixed for the B^1 allele.

Table 2. Mean long-term thermal maxima (LTM) values for the four F_1 stocks of largemouth bass, NLMB, NxF, FxN and FLMB.

	Long-Term Thermal Maxima (°C) (± one standard deviation from mean)
NLMB	37.3 ± 0.60
N x F	39.1 ± 0.97
F x N	40.1 ± 0.64
FLMB	39.2 ± 0.64

Table 3. Mean short-term thermal maxima (CTM) values for the four F_1 stocks of largemouth bass, NLMB, NxF, FxN and FLMB, and parameters of genetic-dependent analysis of variance.

Acclimation Temperature	NLMB	NxF F_1	FxN F_1	FLMB	F	P	R^2
32	40.9	41.6	41.9	41.8	8.13	0.0003	0.41
24	36.5	37.0	37.9	37.5	13.00	0.0001	0.52
16	33.6	34.0	34.4	34.1	3.17	0.0359	0.21
8	29.2	30.1	30.8	30.4	4.92	0.0058	0.29

Table 4. Mean short-term thermal maxima (CTM) values for NXF F_2 and FxN F_2 stocks and for individuals with different genotypes at the three diagnostic loci (Mdh-B, Idh-B and Aat-B).

	F x N F_2	N x F F_2
Overall mean ± S.D.	37.8 ± 0.62	38.4 ± 0.77
No. Florida alleles[a]	Mean temp.	Mean temp.
0	38.0	38.2
1	37.9	38.4
2	38.1	38.5
3	37.7	38.6
4	37.6	38.0
5	37.8	38.4
6	38.2	38.6
No. Heterozygous loci		
0	37.9	38.4
1	37.9	38.4
2	37.8	38.3
3	37.7	38.6
No. Homozygous loci with FLMB alleles		
0	37.9	38.4
1	37.7	38.2
2	37.8	38.3
3	37.7	38.6
No. Homozygous loci with NLMB alleles		
0	37.6	38.3
1	38.0	38.5
2	38.0	38.4
3	38.0	38.1

[a] At the Aat-B, Idh-B and Mdh-B loci.

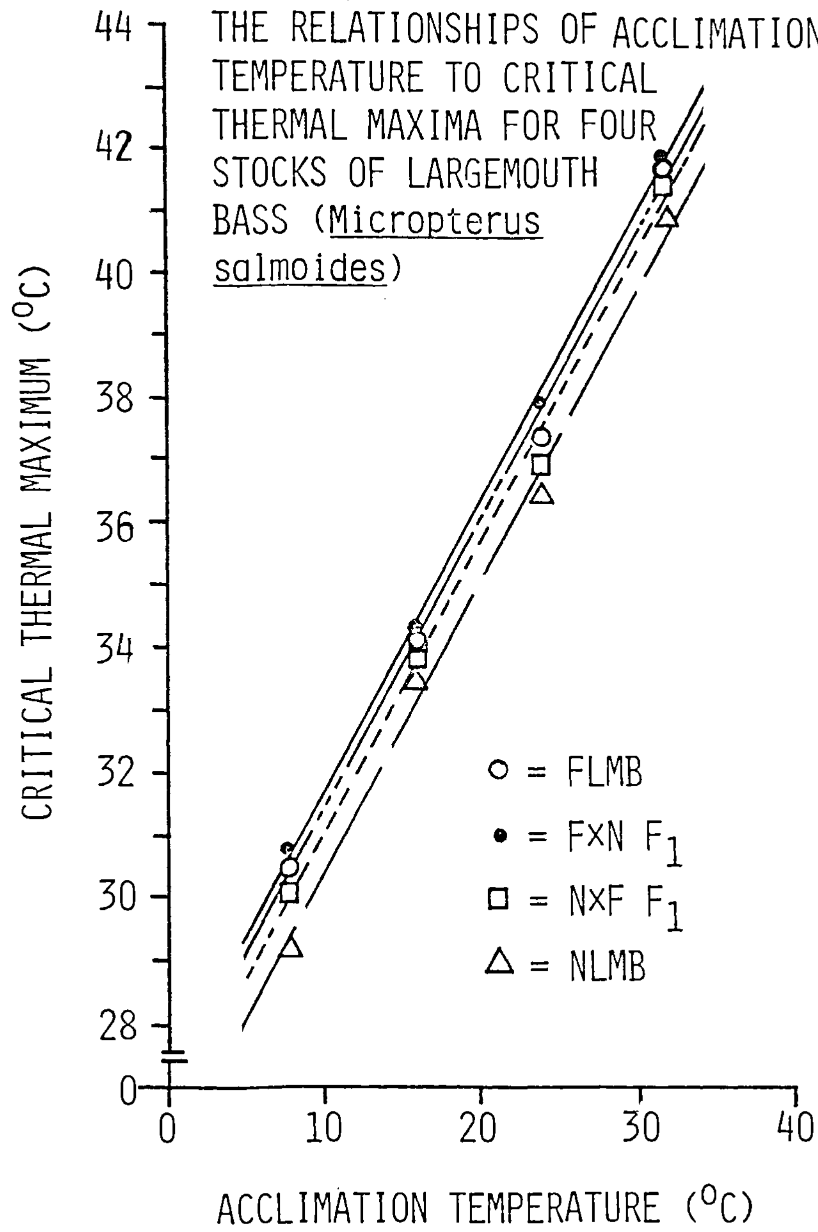


FIGURE 1.
THE RELATIONSHIPS OF ACCLIMATION TEMPERATURE TO CRITICAL THERMAL MAXIMA FOR FOUR STOCKS OF LARGEMOUTH BASS (Micropterus salmoides)
CRITICAL THERMAL MAXIMUM (°C)
44
42
40
38
36
34
32
30
28
0
0
10
20
30
40
ACCLIMATION TEMPERATURE (°C)
○ = FLMB
● = FxN F1
□ = NxF F1
△ = NLMB

STUDY 105
JOB 1, 2

CHAPTER 10

Biochemical Genetic Analysis of Largemouth Bass Populations in Illinois

Jeffrey B. Koppelman[1], Christine Kaminski[1], Gregory S. Whitt[3,1] and David P. Philipp[1,2]

[1]Aquatic Biology Section
Illinois Natural History Survey
607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Animal Science
University of Illinois
328 Mumford Hall
Urbana, Illinois 61801

[3]Department of Genetics and Development
University of Illinois
515 Morrill Hall
Urbana, Illinois 61801

ABSTRACT

Population genetics studies of largemouth bass, *Micropterus salmoides*, have definite applications in attempting to provide a successful fishery in the State of Illinois. Previous studies on largemouth bass populations existing in the United States have described clinal distributions of allele frequencies correlated with temperature. Electrophoretic methods were used to determine allele frequencies at each of six enzyme loci in 48 populations of largemouth bass throughout Illinois. Populations in areas throughout the United States have already been characterized with respect to the allele frequencies at these six loci. Fixed allelic differences between the two subspecies of largemouth bass, *M. s. salmoides* and *M. s. floridanus*, have been described at two loci, Idh-B and Aat-B. Although clinal distributions of alleles encoded at the Mdh-B locus exist in populations across the United States, no clinal distributions at this locus were observed in Illinois. This is most likly due to the transfer of different fish stocks from one body of water to another in Illinois throughout the years. If there is to be an increase or at least a maintainence of the present largemouth bass fishery, manangement programs must avoid mixing different genetic stocks of largemouth bass.

INTRODUCTION

The use of biochemical genetic markers in characterizing largemouth bass populations is a valuable and necessary tool in managing largemouth bass populations today (Philipp et al. 1981). Through time, native largemouth bass stocks have developed specific local attributes as a result of natural selection. Temperature is perhaps the greatest single environmental factor influencing poikilotherms. The thermal "tailoring" of a largemouth bass population results in the evolutionary development of a population that has maximized its fitness for the thermal situation which it inhabits. Previous genetic studies on other fish species have demonstrated the existence of geographic clines in the distribution of alleles encoded in a variety of enzyme loci (Nyman and Shaw 1971, Merritt 1972, Johnson 1974, 1977, Avise and Smith 1974, Utter et al. 1974, Place and Powers 1978, 1979, Powers and Place 1978). North-south clinal distributions, for example, suggest that differential selection pressures are resulting in the predominance of one allele in a geographic area and the predominance of the alternate allele in another area. Alternative explanations involving isolation and random drift have also been proposed (Kimura and Ohta 1971, Ohta and Kimura 1971, Aspinwall 1974).

Allelic differences in specific isozymes among intraspecific populations of largemouth bass from throughout the United States have been correlated with such physical factors as heating and cooling degree days, and latitude (Philipp et al. 1982). This has been postulated to result from the fact that largemouth bass are poikilothermic organisms, and therefore, that temperature plays such an important role in their life cycle. Philipp (1981) found that fixed allelic differences exist between Florida and northern largemouth bass at the Idh-B and Aat-B loci. In addition, *M. s. floridanus* was fixed for the Mdh-B^2 allele,

whereas the *M. s. salmoides* contained both the Mdh-B^1 and Mdh-B^2 alleles. Similarly, whereas the northern subspecies contained only the Sod-A^2 allele, the Florida subspecies contained both the Sod-A^1 and Sod-A^2 alleles.

Using these four gene loci and two others that were polymorphic within Illinois, Gpi-B and Ck-C, populations of Illinois largemouth bass were analyzed to reveal the genetic composition of 48 samples collected throughout the state. In addition, the level of correlation of the allele frequencies at the Mdh-B locus with environmental factors such as heating and cooling degree days were determined for the populations sampled. In this manner we assessed the degree of genetic variability which exists among largemouth bass populations within Illinois and if there were distinct genetic differences between populations in northern versus southern Illinois.

MATERIALS AND METHODS

Largemouth bass samples of 20 individuals each were collected from 48 sites located throughout the State of Illinois (Table 1, Figure 1). Two samples were taken from different areas in each of two lakes, Clinton Lake (population samples 3 and 4) and Baldwin Lake (population samples 5 and 6). Individual fish in all collections were individually wrapped in foil and frozen shortly after capture, and were kept frozen at -20°C until tissues were excised from each individual for genetic analysis.

White skeletal muscle and liver tissue samples were prepared as described in Philipp et al. (1979) and subjected to vertical starch gel electrophoresis in conjunction with specific histochemical staining procedures to determine the genotype for each individual at six enzyme loci (Philipp et al. 1979). The six enzyme loci monitored were Mdh-B, Idh-B, Aat-B, Sod-A, Gpi-B, and Ck-C. Allele frequencies at each locus for each population were calculated after the phenotypes were visualized following staining of the gel. The number of heating and cooling degree days (Table 1) were calculated according to Philipp et al. (1982). Regression equations were computed using $Mdh\text{-}B^1$ allele frequencies for each population in comparison with both heating and cooling degree days.

RESULTS

Allelic variation was observed among populations of largemouth bass studied in Illinois at all six enzyme loci examined, malate dehydrogenase-B (Mdh-B), isocitrate dehydrogenase-B (Idh-B), superoxide dismutase-A (Sod-A), aspartate aminotransferase-B (Aat-B), creatine kinase-C (Ck-C), and glucosephosphate isomerase-B (Gpi-B). However, no alleles were detected in these populations that had not been previously reported (Philipp et al. 1983.)

Two alleles were observed at the Mdh-B locus. Three populations were fixed for the Mdh-B^1 allele, whereas the remaining populations contained the alternate B^2 allele at frequencies of up to 0.375 (Table 2). The geographic distribution of the two alleles at this locus did not describe a latitudinal clinal distribution (Figure 2). In addition, no significant correlation was found between the frequencies of the Mdh-B^1 allele and the number of heating degree days (correlation coefficient of $r = -0.055$) and cooling degree days (correlation coefficient of $r = 0.047$) for each population sampled.

Two alleles were also observed at the Idh-B locus. Only three population samples were not fixed for the Idh-B^1 allele. These three populations, New City Lake, Jones Lake, and Little Grassy Lake, contained the alternate B^3 allele (representative of the Florida subspecies) at frequencies of 0.025, 0.025, and 0.050, respectively (Table 2, Figure 3).

Three alleles were observed at the Aat-B locus, two of which have been determined to be representative of the northern largemouth bass (Aat-B^1 and B^2). These alleles predominated in all of the populations sampled. However, Lake of Egypt, Jones Lake, and Little Grassy Lake had frequencies of an alternative B^3 allele, an allele representative of the Florida largemouth bass, of 0.050, 0.075, and 0.025, respectively (Table 2, Figure 4).

The Sod-A locus found in largemouth bass encodes two described alleles, A^1 and A^2. Northern largemouth bass are fixed for the A^2 allele, as were all of the Illinois populations examined except three populations. The Lake of Egypt, Newton Lake, and Little Grassy Lake largemouth bass population samples all exhibited a frequency for the A^1 allele (found only in the Florida subspecies) of 0.025 (Table 2, Figure 5).

Three Ck-C alleles have been observed in largemouth bass populations throughout the country. All three alleles were observed in the present study, as well. The C^2 allele was the predominant allele in all populations, being fixed or nearly fixed (frequency $\geq$ 0.900) in 38 of the 48 samples (Table 2, Figure 6). The alternate C^1 allele was present at frequencies of 0.025 to 0.475 in 27 populations, whereas Kincaid Lake was the only population to possess the C^3 allele, and at a frequency of only 0.05 (Table 2, Figure 6).

Three Gpi-B alleles have been observed in largemouth bass populations across the country. Again, all three alleles were observed among the populations examined in this study. Most populations were fixed for the Gpi-B^2 allele (39 of 48 populations) with a second allele, B^3, being present at frequencies from 0.025 to 0.300 in nine populations (Table 2, Figure 7). The third allele, Gpi-B^1, was found only in the Schuy-Rush Lake population and at a frequency of 0.025.

DISCUSSION

The existence of "Florida" alleles ($Aat\text{-}B^3$, $Idh\text{-}B^3$, $Sod\text{-}A^1$) in certain Illinois largemouth bass populations is most likely the result of "volunteer" stocking efforts that have undoubtedly succeeded to a certain degree due to the more southernly locality of these lakes (Little Grassy Lake in Wilmington County; New City Lake in Bond County; Jones Lake in Saline County; Lake of Egypt in Johnson County; and Newton Lake in Jasper County). In fact, Lake of Egypt and Newton Lake receive heated effluent. Bodies of water exposed to thermal effluents have been reported to favor genotypes heavily influenced by the Florida largemouth bass genome (Yardley et al. 1974). Obviously, these alleles have not arisen through mutation in these separate lakes. Personal conversations with fishing club members in southern Illinois confirmed the fact that largemouth bass from Georgia and Florida have repeatedly been introduced into Lake of Egypt. It is reasonable to assume that similar largemouth bass could have been introduced into surrounding lakes. Management efforts in Illinois need to guard against supplementing the spread of Florida alleles among other Illinois lakes.

Movement of largemouth bass throughout the state is strongly suggested by the great amount of heterogeneity in the frequency of the Mdh-B alleles among the populations studied. Distinct north-south clinal distributions of alleles at this locus throughout the United States would suggest that a similar clinal distribution would be expected in Illinois (Philipp et al. 1981, 1983). We would have expected populations in southern Illinois to have higher $Mdh\text{-}B^2$ frequencies than populations in northern Illinois. However, this was not the case. In fact, only older, well established populations, such as the Fox Chain of Lakes, Crab Orchard, and Devil's Kitchen Lakes, showed this trend. This

relatively random geographic distribution of Mdh-B alleles is indicative of introductions of hatchery stocks obtained from areas other than where they were stocked.

Mixing of different stocks in this manner becomes important when one considers that the different alleles at the Mdh-B locus may be related to or at least be an indication of the thermal characteristics possessed by the stock. These characteristics may directly bear on the success of that stock in a particular environment. Natural selection has taken many years to genetically tailor populations or stocks of largemouth bass to specific thermal environments. Undoubtedly, different thermal conditions exist which will influence the success of introducing different stocks of largemouth bass into different thermal environments. Tolerance to both high and low temperatures, both acute and chronic, is needed to succeed in many Illinois environments. Hatchery practices have, in the past, overlooked the genetic differences that exist among conspecific populations of largemouth bass. Today's management goals should include an evaluation of the different hatchery fish stocks both genetically and physiologically to assure that their performance in the environment is maximized and to protect the genetic integrity of largemouth bass populations in Illinois.

ACKNOWLEDGMENTS

The authors would like to express their thanks to the following persons who were instrumental in completing this report: Department of Conservation field personnel for collecting and delivering the required fish samples, Jeffrey Van Orman and Susan Malmer for their assistance in the laboratory analysis, Dr. William D. Harvey for his editorial advice, and most importantly, Shirley S. Lowe for her invaluable assistance in the laboratory and Suzanne Peratt for technically preparing the manuscript.

REFERENCES

Aspinwall, N. 1974. Genetic analysis of North American populations of the pink salmon, Onchorhynchus garbuscha, possible evidence for the neutral mutation-random drift hypothesis. Evolution 28:295-305.

Avise, J. C. and M. H. Smith. 1974. Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, Lepomis macrochirus. Evolution 28:42-56.

Johnson, M. S. 1974. Comparative geographic variation in Menidia. Evolution 28:607-618.

Johnson, M. S. 1977. Association of allozymes and temperature in the crested blennny, Anoplarchus purpurescens. Marine Biology 41:147-152.

Kimura, M. and T. Ohta. 1971. Protein polymorphism as a phase of molecular evolution. Nature 229:467-470.

Merritt, R. B. 1972. Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, Pimephales promelas. American Naturalist 106:172-184.

Nyman, L. and D. H. Shaw. 1971. Molecular weight heterogeneity of serum esterases in four species of salmonid fishes. Comparative Biochemical Physiology 40(B):563-566.

Ohta, T. and M. Kimura. 1971. Behavior of neutral mutants influenced by overdominant loci in finite populations. Genetics 69:247-260.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1979. Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, Micropterus salmoides salmoides, and smallmouth bass, Micropterus dolomieui). Journal of Experimental Zoology 210:473-488.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1981. Management implications for different genetic stocks of largemouth bass (Micropterus salmoides) in the United States. Canadian Journal of Fisheries and Aquatic Sciences 38:1715-1723.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1982. Correlation of allele frequencies of largemouth bass populations with physical/environmental parameters. Pages 15-209 In D. P. Philipp, W. F. Childers and G. S. Whitt, editors. Biochemical genetics of largemouth bass, Micropterus salmoides. Electric Power Research Institute, Palo Alto, CA, USA.

Philipp, D. P., W. F. Childers and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Transactions of the American Fisheries Society 112:1-20.

Place, A. R. and D. A. Powers. 1978. Genetic bases for protein polymorphism in *Fundulus heteroclitus* (L.). I. Lactate dehydrogenase (Ldh-B), malate dehydrogenanse (Mdh-A), glucosephosphate isomerase (Gpi-B), and phosphoglucomutase (Pgm-A). Biochemical Genetics 16:577-591.

Place, A. R. and D. A. Powers. 1979. Genetic variation and relative catalytic efficiencies: Lactate dehydrogenase-B allozymes of *Fundulus heteroclitus*. Proceedings of the National Academy of Science, USA 76:2354-2358.

Powers, D. A. and A. R. Place. 1978. Biochemical genetics of *Fundulus heteroclitus* (L.). I. Temporal and spatial variation in gene frequencies of Ldh-B, Mdh-A, Gpi-A, and Pgm-A. Biochemical Genetics 16:583-607.

Utter, F. M., H. O. Hodgins and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations. Page 213-228 *In* J. R. Sargent (ed.) Biochemical and Biophysical Perspective in Marine Biology 1. Academic Press, New York, USA.

Yardley, D., J. C. Avise, J. W. Gibbons and M. H. Smith. 1974. Biochemical genetics of sunfish. III. Genetic subdivision of fish populations inhabiting heated waters. Pages 255-263 *In* J. W. Gibbons and R. R. Sharitz (eds.) Thermal Ecology. U.S. Atomic Energy Commission.

Table 1. Populations of largemouth bass analyzed plus the corresponding heating and cooling degree day values.

Number	Lake Name (County)	HDD	CDD
1.	Lake Sangchris (Christian)	5046	1027
2.	Lake Shelbyville (Shelby)	5066	1084
3.	Clinton Lake SC (DeWitt)	5426	985
4.	Clinton Lake NF (DeWitt)	5426	985
5.	Baldwin Lake-cold (Randolph)	4080	1441
6.	Baldwin Lake-hot (Randolph)	4080	1441
7.	Coffeen Lake (Montgomery)	5066	1084
8.	Lake of Egypt (Johnson)	4057	1354
9.	Crab Orchard Lake (Williamson)	4057	1354
10.	Forbes Lake (Marion)	4452	1220
11.	Beaver Dam (Macoupin)	4835	1150
12.	Lake Springfield (Sangamon)	5197	1067
13.	Lake of the Woods (Champaign)	5379	929
14.	Dawson Lake (McLean)	6027	985
15.	Dale Lake (Wayne)	4266	1296
16.	Newton Lake (Jasper)	4956	1052
17.	Sam Parr Lake (Jasper)	4956	1052
18.	Red Hills Lake (Lawrence)	4414	1225
19.	Greenville Lake (Bond)	5118	1128
20.	New City Lake (Bond)	5118	1128
21.	Otter Lake (Macoupin)	4835	1150
22.	Lincoln Trail Lake (Clark)	5061	1048
23.	Collins Lake (Grundy)	5862	768
24.	Lake Vermilion (Vermilion)	5323	887
25.	Lake Marie (Lake)	6381	504
26.	Lake Catherine (Lake)	6381	504
27.	Grass Lake (Lake)	6381	504
28.	Argyle Lake (McDonough)	5539	952
29.	Gladstone Lake (Henderson)	5766	872
30.	Apple River Canyon Lake (Jo Daviess)	6672	582
31.	Johnson Sauk Lake (Henry)	5856	845
32.	Lake George (Roch Island)	6171	797
33.	Lake Le-Aqua-Na (Stephenson)	6672	582
34.	N. Spring Lake (Tazewell)	5800	872
35.	Pierce Lake (Winnebago)	6612	659
36.	Shabbona Lake (DeKalb)	6512	625
37.	Randolph Co. Lake (Randolph)	4080	1441
38.	Washington Co. Lake (Washington)	4080	1441
39.	Devil's Kitchen Lake (Williamson)	4057	1354
40.	Jones Lake (Saline)	3983	1369
41.	Cedar Lake (Jackson)	4057	1354
42.	Little Grassy Lake (Williamson)	4057	1354
43.	Kincaid Lake (Jackson)	4057	1354
44.	Dolan Lake (Hamilton)	4108	1382
45.	Schuy-Rush Lake (Schuyler)	5285	1052
46.	Weinberg-King Lake Pond #1 (Schuyler)	5285	1052
47.	Siloam Springs (Adams)	4957	1300
48.	Nauvoo State Lake (Hancock)	4957	1300

Table 2. Allele frequencies for largemouth bass populations in Illinois.

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12
Mdh-B	1	.700	.925	.900	.775	.975	.800	.750	.675	.750	.850	1.000	.975
	2	.300	.075	.100	.225	.025	.200	.250	.325	.250	.150	-	.025
Idh-B	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-
Aat-B	1	1.000	1.000	.825	.825	.875	.750	.825	.650	.875	.825	.650	.925
	2	-	-	.175	.175	.125	.250	.175	.300	.125	.175	.350	.075
	3	-	-	-	-	-	-	-	.050	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
Sod-A	1	-	-	-	-	-	-	-	.025	-	-	-	-
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000
Ck-C	1	.150	.050	.025	-	-	-	-	-	.025	.100	.100	-
	2	.850	.050	.975	1.000	1.000	1.000	1.000	1.000	.975	.900	.900	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-
Gpi-B	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	.700	.925	1.000	1.000	.800	1.000	1.000	.950	.950	1.000	1.000	1.000
	3	.300	.075	-	-	.200	-	-	.050	.050	-	-	-

Table 2. (continued).

Locus	Allele	13	14	15	16	17	18	19	20	21	22	23	24
Mdh-B	1	.900	.825	.900	.775	.875	.925	.950	.850	.850	.975	.950	.600
	2	.100	.175	.100	.225	.125	.075	.050	.150	.150	.025	.050	.400
Idh-B	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000
	3	-	-	-	-	-	-	-	.025	-	-	-	-
Aat-B	1	.925	.725	.925	.900	.875	.875	.825	.900	.800	.650	.850	.800
	2	.075	.275	.075	.100	.125	.125	.175	.100	.200	.350	.150	.200
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
Sod-A	1	-	-	-	.025	-	-	-	-	-	-	-	-
	2	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ck-C	1	.150	-	-	.025	.050	-	.150	.050	.125	.475	-	.250
	2	.850	1.000	1.000	.975	.950	1.000	.850	.950	.875	.525	1.000	.750
	3	-	-	-	-	-	-	-	-	-	-	-	-
Gpi-B	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. (continued).

Locus	Allele	25	26	27	28	29	30	31	32	33	34	35	36
Mdh-B	1	.850	.800	.825	.750	.850	.750	.725	.725	.900	.892	.625	.775
	2	.150	.200	1.75	.250	.150	.250	.275	.275	.100	.108	.375	.225
Idh-B	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-
Aat-B	1	.550	.600	.650	.775	.925	.825	.900	.850	1.000	.748	.850	.925
	2	.450	.400	.350	.225	.075	.175	.100	.150	-	.252	.150	.275
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
Sod-A	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ck-C	1	.075	.100	.125	.100	.025	-	-	.050	-	-	-	.025
	2	.925	.900	.875	.900	.975	.900	1.000	.950	1.000	1.000	1.000	.975
	3	-	-	-	-	-	-	-	-	-	-	-	-
Gpl-B	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	.975	1.000	1.000	1.000	.975	1.000	1.000	1.000	.964	1.000	.975	1.000
	3	.025	-	-	-	.025	-	-	-	.036	-	.025	-

Table 2. (continued).

Locus	Allele	37	38	39	40	41	42	43	44	45	46	47	48
Mdh-B	1	.775	.550	.625	.870	.775	.850	.925	1.000	.850	1.000	.700	.850
	2	.225	.450	.375	.130	.225	.150	.075	-	.150	-	.300	.150
Idh-B	1	1.000	1.000	1.000	.975	1.000	.950	1.000	1.000	1.000	1.000	1.000	1.000
	3	-	-	-	.025	-	.050	-	-	-	-	-	-
Aat-B	1	.900	.850	.625	.825	.875	.825	.825	.964	1.000	1.000	1.000	1.000
	2	.100	.150	.375	.100	.125	.150	.175	.036	-	-	-	-
	3	-	-	-	.075	-	.025	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
Sod-A	1	-	-	-	-	-	.025	-	-	-	-	-	-
	2	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000	1.000	1.000	1.000
Ck-C	1	-	.025	.325	-	.250	.025	.025	-	-	-	-	-
	2	1.000	.975	.675	1.000	.750	.975	.950	1.000	1.000	1.000	1.000	1.000
	3	-	-	-	-	-	-	.025	-	-	-	-	-
Gpi-B	1	-	-	-	-	-	-	-	-	.025	-	-	-
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.975	1.000	1.000	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-

Figure 1. Locations of the largemouth bass collections. Numbers correspond to those in Table 1.

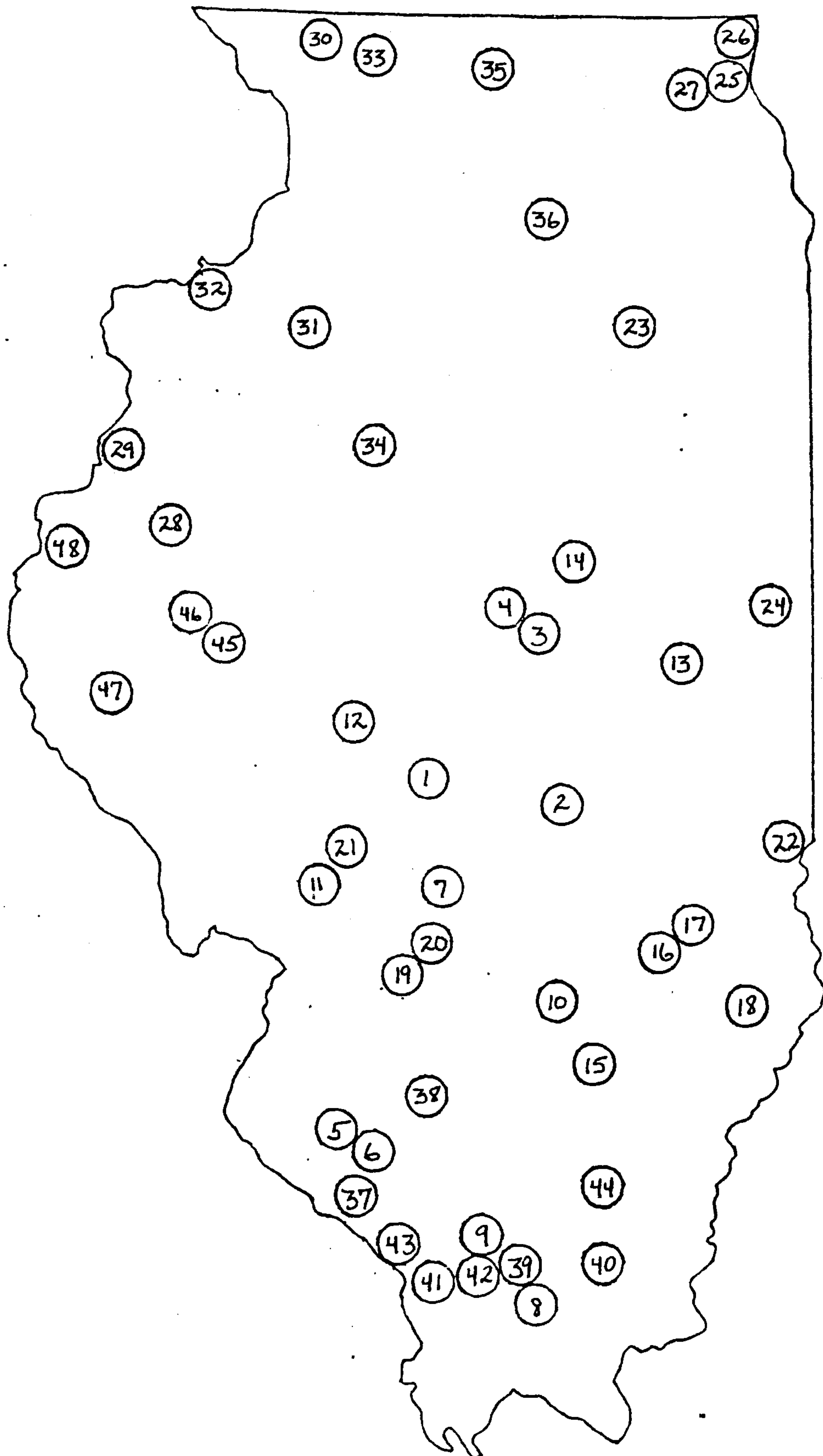


Figure 2. Distribution of the alleles at the Mdh-B locus. The frequency of the B^1 allele is shown in white, the B^2 allele in black.

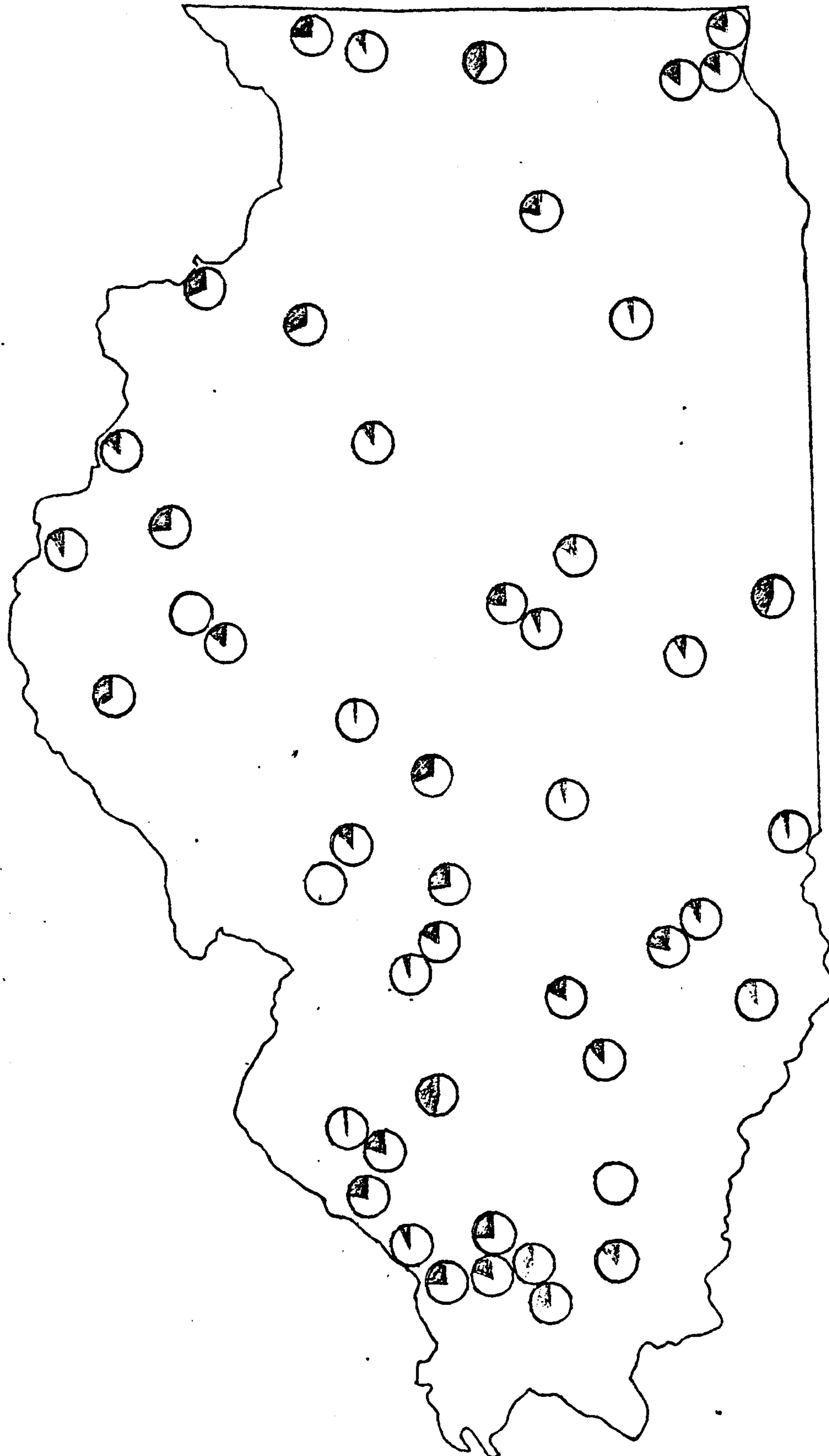


Figure 3. Distribution of the alleles at the Idh-B locus. The frequency of the B^1 allele is shown in white, the B^3 allele in black.

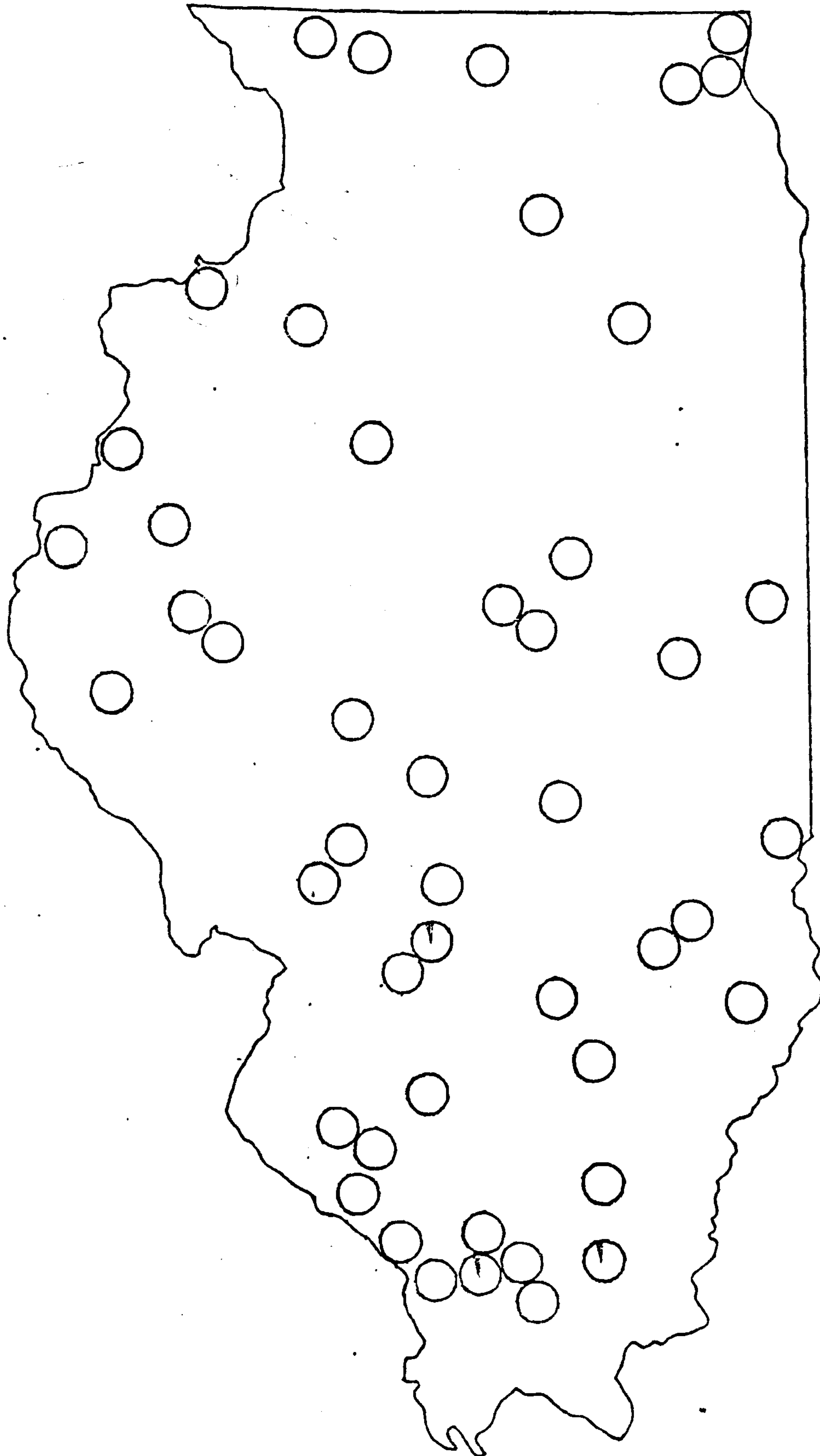


Figure 4. Distribution of the alleles at the Aat-B locus. The frequency of the B^1 allele is shown in white, the B^2 allele in black, the B^3 allele in spotted.

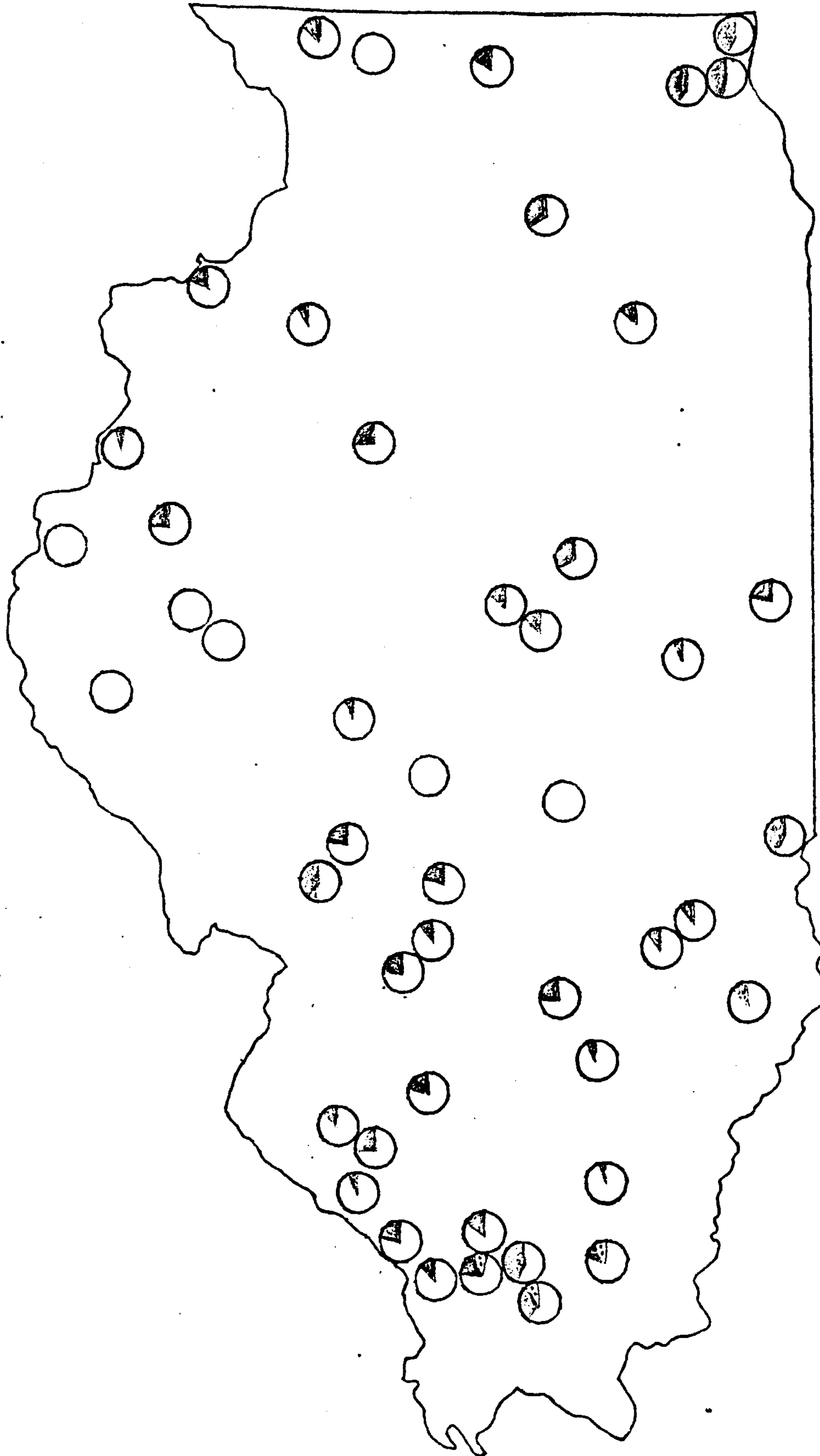


Figure 5. Distribution of the alleles at the Sod-A locus. The frequency of the A^1 allele is shown in black, the A^2 allele in white.

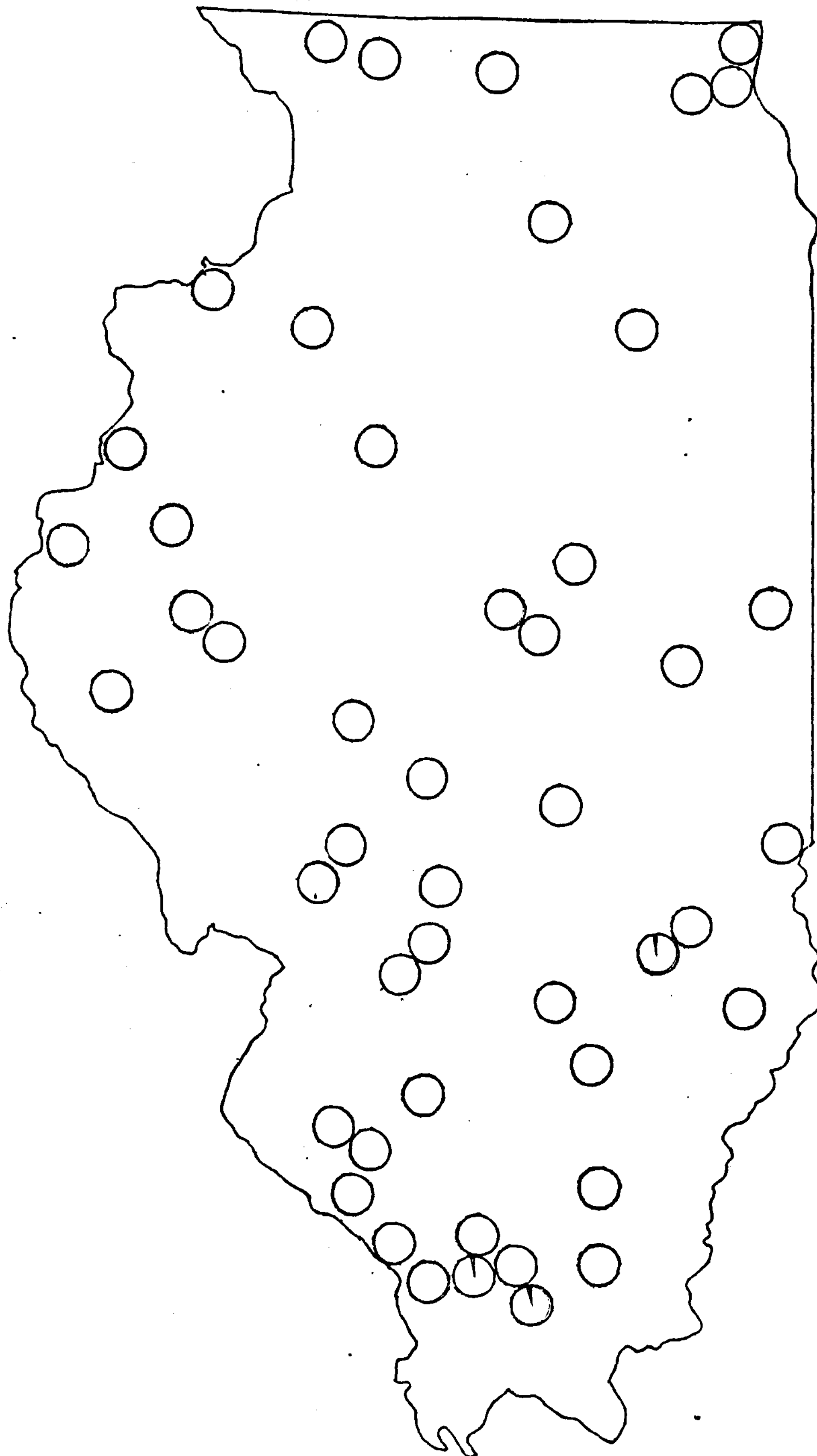


Figure 6. Distibution of the alleles at the Ck-C locus. The frequency of the C^1 allele is shown in black, the C^2 allele in white, the C^3 allele in spotted.

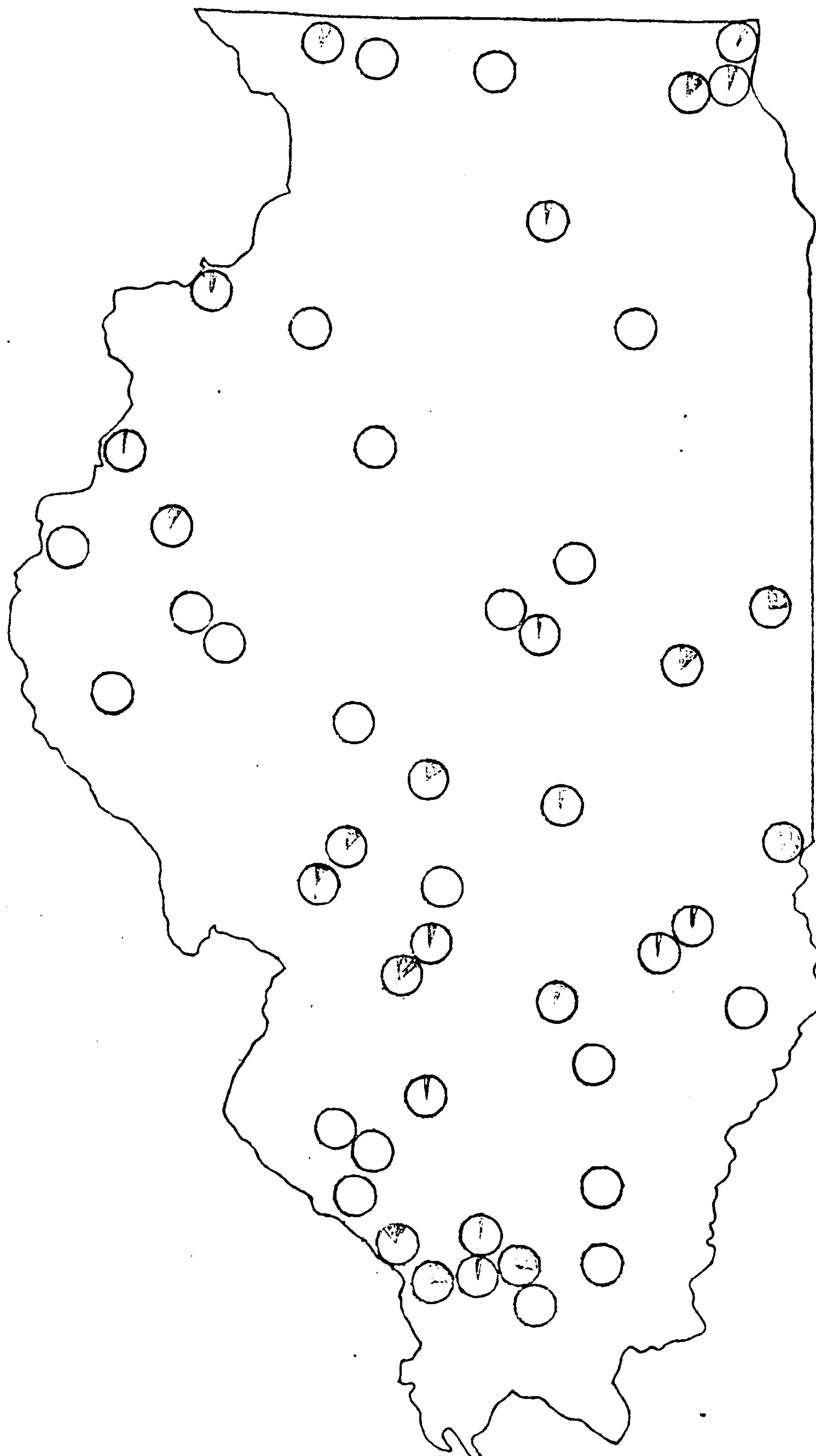
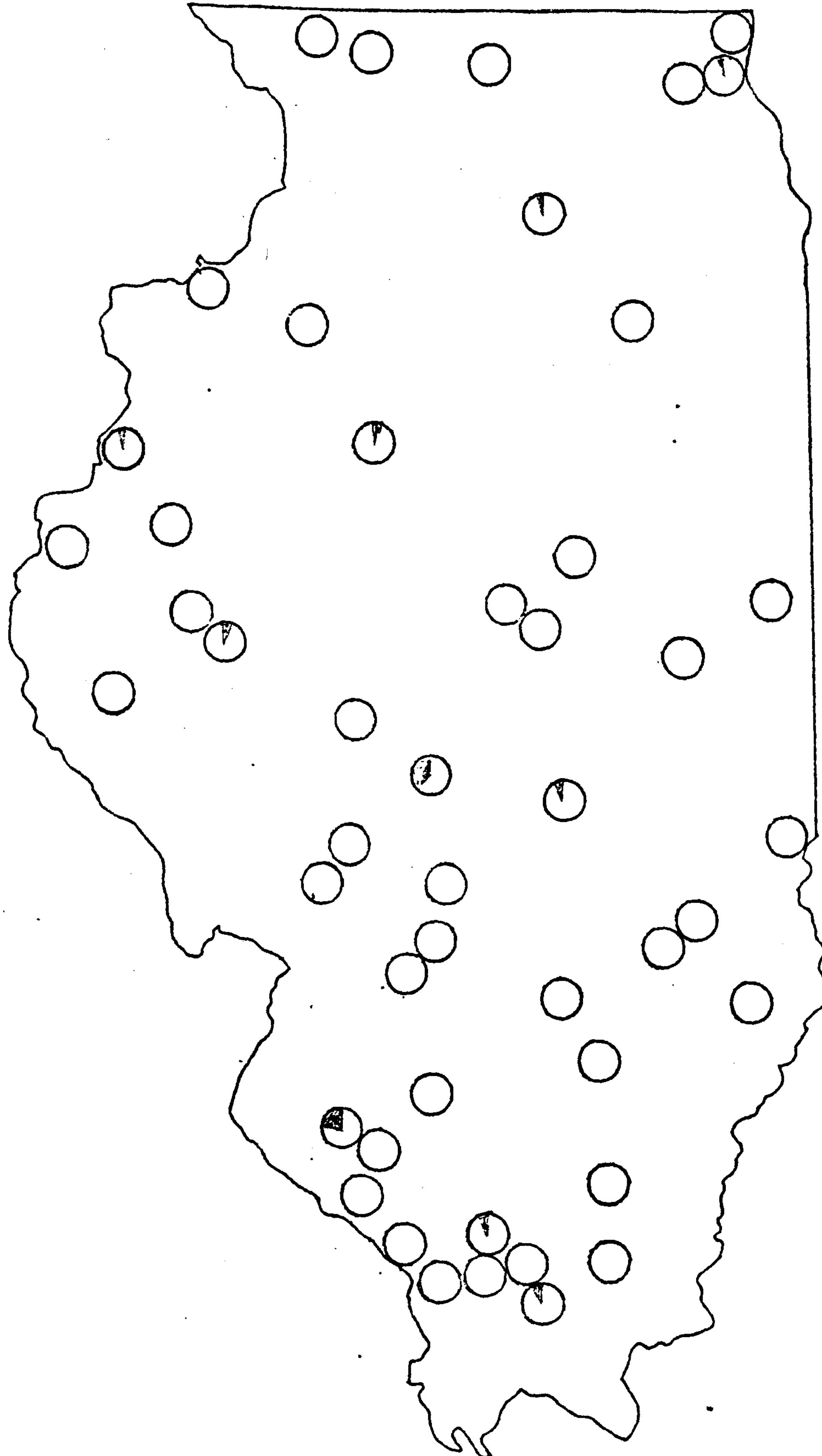


Figure 7. Distribution of the alleles at the Gpi-B locus. The frequency of the B^1 allele is shown in spotted, the B^2 allele in white, the B^3 allele in black.



GENERAL RECOMMENDATIONS

1. Since meristic counts routinely used in the past to identify the two subspecies of largemouth bass were shown to be invalid for this purpose, we recommend that only electrophoretic analyses (specifically, the determination of the genotypes at the Idh-B and Aat-B loci) be accepted as confirmation of the subspecific status of largemouth bass populations.

2. Since the northern largemouth bass outperforms all other genetic stocks of largemouth bass tested with respect to growth and survival in Illinois, we recommend that no largemouth bass containing any portion of the genome of the Florida subspecies be introduced into the waters of Illinois.

3. Since the reproductive potentials and embryonic thermal requirements of the four genetic stocks were shown to differ significantly, we recommend that studies assessing the long-term success of each stock in competition be performed to determine the potential impact that the introduction of the Florida subspecies may have upon native northern largemouth bass populations.

4. Since the four genetic stocks of largemouth bass were shown to have quite different gene regulatory mechanisms during embryogenesis and in response to different thermal regimes, we recommend that further studies be performed to determine at a molecular level how these genes are differentially regulated and to what extent this divergence in gene regulation has contributed to the evolution of the two subspecies.

5. Since the four genetic stocks of largemouth bass demonstrated differences in thermal tolerance and thermal preference we recommend that radiotelemetry studies be conducted to assess the differences in behavior which apparently exist among these stocks in response to different thermal conditions.

6. Since genetic differences were observed among different populations of largemouth bass from different regions of Illinois, we recommend that the potential of developing different hatchery stocks of largemouth bass for introduction into different regions of the state be investigated.

7. In general, we recommend that this investigation of different genetic stocks of largemouth bass be used as a model to illustrate how the stock concept can be incorporated into the management philosophy for all managed fish and wildlife species, so that the principles of genetic conservation are well served.

8. We also recommend that each state set up a State Fisheries Genetics Research Program to accomplish these goals, that the U.S. Fish and Wildlife Service sponsor a National Fisheries Genetics Research Program in a nationally coordinated effort, or that some combination of these two efforts be established.