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Management Data Series
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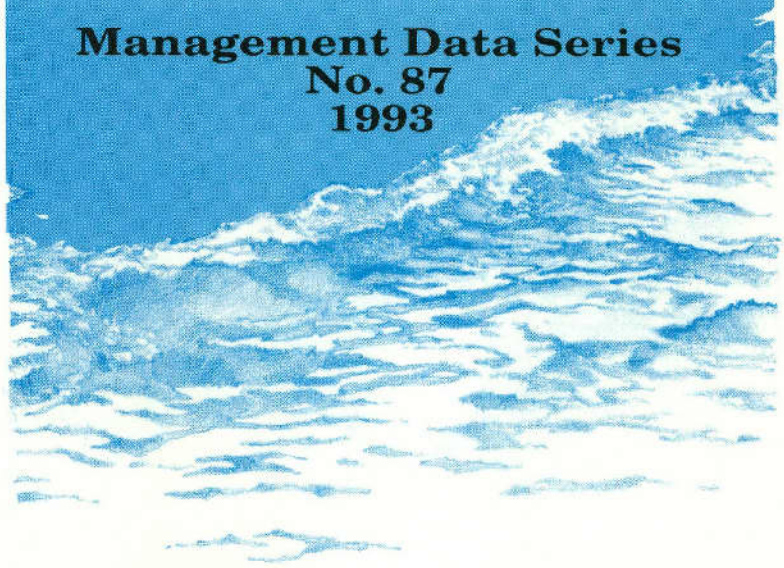

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GENETIC ANALYSIS OF PADDLEFISH: A COMPARISON OF INDIVIDUALS FROM MISSOURI, SOUTH DAKOTA AND TEXAS

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance provided by Catherine J. Dryden, Fish and Wildlife Technician II, who performed the electrophoretic analyses.

ABSTRACT

Protein electrophoresis was conducted on 53 presumptive loci from two stocks of cultured paddlefish (N=20) and from wild-caught paddlefish (N=2). General proteins from muscle and eye were also examined. EST-2*, PGM-1*, and parvalbumin demonstrated polymorphisms, while all other loci were monomorphic. Small sample size of the wild-caught fish complicated data interpretation, however the low level of variation seen in paddlefish probably renders protein electrophoresis an inadequate means to study stock dynamics.

INTRODUCTION

Paddlefish Polyodon spathula (Walbaum) are listed as a species of special concern by the American Fisheries Society (Deacon et al. 1979) and since 1977 have been considered endangered in Texas by the Texas Parks and Wildlife Department (TPWD) (Pitman 1992). As part of a statewide plan to re-establish paddlefish in Texas, the species is currently cultured at the A.E. Wood Fish Hatchery in San Marcos, Texas. Because paddlefish are rare in Texas, however, obtaining native broodfish for use in stocking efforts is unfeasible. Instead, paddlefish eggs are obtained from other states. The eggs are then incubated and the young are reared to stocking size at the A.E. Wood facility. To date, TPWD has reared paddlefish obtained from two sources: the Missouri River, South Dakota and Table Rock Reservoir, Missouri. The hatchery also maintains adults collected from the Trinity River (N=1) and the Red River (N=4) and tissue samples from two fish collected in the Neches River.

Currently, there is some debate regarding stocking practices. Some biologists believe that indigenous populations warrant protection and that the introduction of non-native fish is potentially destructive to endemic fisheries (e.g., Rutledge and McCarty 1989, Allendorf 1991, Phillip 1991, Krueger and May 1991). In spite of these concerns, many state and Federal agencies continue to augment natural populations using exogenous stocks. While some data are available on paddlefish genetics, information on stock dynamics is limited. Carlson et al. (1982) reported low levels of genetic variation in six paddlefish populations. Epifanio et al. (1989) followed with an expanded study that included 15 populations, additional enzyme loci, and restriction endonuclease fragment analysis (REFA) of mitochondrial DNA (mtDNA): this study also revealed low levels of genetic variation.

Of the stocks maintained at A.E. Wood, only the Missouri River fish have been studied through protein electrophoresis (Epifanio et al. 1989). Allozyme analysis of paddlefish stocks could provide TPWD with baseline information necessary to enhance stock management. Such information is potentially useful even if few native fish are available for comparisons. Since stocking with non-native fish is in progress, an investigation of paddlefish stock genetics was initiated.

MATERIALS AND METHODS

Samples of eye, muscle and liver were obtained from 10 advanced fingerlings of each stock of paddlefish reared at A.E. Wood and from the two Neches River fish. Samples were stored at -40 C until analyzed by agarose gel electrophoresis or polyacrylamide gel isoelectric focusing. Electrophoretic methods generally followed the methods of Epifanio et al. (1989) except that agarose was substituted for starch as the electrophoretic medium (Table 1). Additionally, general proteins and parvalbumin were examined using isoelectric focusing which provided better resolution than conventional electrophoretic methods. Electrofocusing methods followed Fries and Harvey (1989) but pH 3-10 ampholytes were used for the general protein analyses.

RESULTS AND DISCUSSION

Of the 53 presumptive enzyme-coding loci analyzed in this study only two, EST-2* and PGM-1*, were polymorphic (Table 2). This low level of variation is consistent with the findings of Carlson et al. (1982) and Epifanio et al. (1989). Additionally, the Texas fish exhibited phenotypic differences in general proteins (presumably parvalbumins) from muscle tissue.

Interpretation of the data is difficult due to factors related to

sampling bias. The hatchery reared stocks were produced from only a few broodfish and provided very limited genetic information. Because of this founder effect, it was thought that additional sampling of the same fish would have provided little added data. The small sample size of the native Texas fish (N=2) renders statistical methods inappropriate, therefore, the relevance of the apparent difference a the EST-2* locus is unknown. Similarly, the implications of the apparent polymorphism in parvalbumin, while consistent and repeatable, are also unknown. Perhaps general protein patterns are reflective of the ontogeny of the animals and are undeveloped in very immature fish. Departure from expected protein profiles has been observed in very young palmetto bass by one of the authors (LTF). Another possible explanation is that the juvenile paddlefish were fed a commercially prepared feed that is different from naturally occurring food sources. The disparate diets could also be a factor contributing to different electromorphs.

The most notable conclusion from this study is that, due to low levels of variation, protein electrophoresis is an inadequate means to measure paddlefish stock dynamics. The low level of variation observed in allozymes, however, is most likely indicative of low diversity throughout the paddlefish genome. Carlson et al. (1982) hypothesized several explanations for the low level of detectable genetic variation, including fixation for a highly adaptive genotype. While Epifanio et al. (1989) admitted that genetic data did not necessarily support the existence of drainage-specific stocks, the report still recommended that caution be used in reintroduction efforts. Among the reasons was evidence that growth and age of maturity varied between drainages. Such performance traits are as likely to be controlled by environment as genetic influences.

The consequences of introducing non-native paddlefish into Texas waters are largely unknown. However, restocking efforts are concentrated in areas where paddlefish are in danger of becoming extirpated. In such situations, non-native paddlefish may be preferable to no paddlefish.

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Table 1.--Enzyme loci, tissue, and electrophoresis buffers used to examine paddlefish isozymes (after Epifanio et al., 1989).

IUNBC Number	Enzyme Name	Locus	Tissue	Electrophoretic Buffer[a]
1.1.1.1	Alcohol dehydrogenase	ADH-1*	Liver	EBT 8.5
		ADH-2*	Liver	EBT 8.5
1.1.1.8	Glycerol-3-phosphate dehydrogenase	GPDH-1*	Muscle	TC 8.0
1.1.1.27	Lactate dehydrogenase	LDH-A*	Muscle	TC 8.0
		LDH-B*	Muscle	TC 8.0
		LDH-C*	Liver	TC 8.0
1.1.1.37	Malate dehydrogenase	MDH-A*	Muscle	TC 8.0
		MDH-B*	Muscle	TC 8.0
		mMDH-1*	Muscle	TC 8.0
		mMDH-2*	Muscle	TC 8.0
1.1.1.40	Malic enzyme	ME-1*	Liver	EBT 8.5
		ME-2*	Liver	EBT 8.5
1.1.1.42	Isocitrate dehydrogenase ($NADP^+$)	IDHP-1,1*	Muscle	TC 8.0
		IDHP-1,2*	Muscle	TC 8.0
		mIDHP-1*	Muscle	TC 8.0
		mIDHP-2*	Muscle	TC 8.0
1.1.1.44	Phosphogluconate dehydrogenase	PGDH-1*	Muscle	TC 8.0
		PGDH-2*	Liver	TC 8.0
1.1.1.49	Glucose-6-phosphate dehydrogenase	G6PDH-1*	Muscle	EBT 8.5
		G6PDH-2*	Muscle	EBT 8.5
1.2.1.12	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-1*	Muscle	TC 8.0
		GAPDH-3*	Eye	TC 6.3
1.2.3.2	Xanthine dehydrogenase	XDH-1*	Liver	EBT 8.5
1.15.1.1	Superoxide dismutase	SOD-A,1*	Liver	EBT 8.5
		SOD-A-2*	Liver	EBT 8.5
2.6.1.1	Aspartate aminotransferase	mAAT-1*	Liver	TC 6.3
2.7.1.11	6-Phosphofructokinase	FDP-1*	Muscle	TC 8.0
		FDP-2*	Liver	TC 8.0
2.7.1.40	Pyruvate kinase	PK-1*	Muscle	TC 8.0
		PK-2*	Muscle	TC 8.0
2.7.2.3	Phosphoglycerate kinase	PGK-1*	Muscle	TC 8.0
		PGK-2*	Muscle	TC 8.0

IUNBC Number	Enzyme Name	Locus	Tissue	Electrophoretic Buffer[a]
2.7.3.2	Creatin kinase	CK-1.1*	Muscle	EBT 8.5
		CK-1.2*	Muscle	EBT 8.5
		CK-3*	Liver	EBT 8.5
2.7.4.3	Adenylate kinase	AK-1*	Muscle	EBT 8.5
3.1.1.-	Esterase	EST-1*	Liver	TC 8.0
		EST-2*	Liver	TC 8.0
4.1.2.13	Aldolase	ALD-1*	Muscle	TC 8.0
		ALD-3*	Eye	TC 8.0
4.2.1.2	Fumarate hydratase	FUM-1*	Muscle	TC 8.0
		FUM-2*	Muscle	TC 8.0
4.2.1.3	Aconitate hydratase	AH-1*	Muscle	TC 8.0
		AH-2*	Eye	TC 8.0
5.3.1.8	Mannose-6-phosphate isomerase	MPI-1*	Muscle	EBT 8.5
5.3.1.9	Glucose-6-phosphate isomerase	GPI-1*	Eye	TC 8.0
		GPI-2.1*	Muscle	TC 8.0
		GPI-2.2*	Muscle	TC 8.0
5.4.2.2	Phosphoglucomutase	PGM-1*	Muscle	TC 8.0
		PGM-2*	Muscle	TC 8.0
		PGM-3*	Muscle	TC 8.0
		PGM-4*	Muscle	TC 8.0
		PGM-5*	Muscle	TC 8.0
--	Parvalbumin		Muscle	IEF 3-5
--	General protein		Muscle	IEF 3-10
			Eye	IEF 3-10

[a]Electrophoretic buffers were as follows: EBT 8.5, 0.5 M tris/0.65 M borate/0.02 M EDTA; TC 8.0, 0.687 M tris/0.157 M citric acid; TC 6.3, 0.22 M tris/0.086 M citirc acid/0.031 M NaOH; IEF 3-5, isoelectric focusing, pH range 3-5; IEF 3-10, isoelectric focusing, pH range 3-10.

Table 2.--Allele frequencies at polymorphic loci in three putative populations of paddlefish.

Locus	Allele	Frequency		
		Missouri (N = 10)	South Dakota (N = 10)	Texas (N = 2)
EST-2*	EST-2*93	0.05	0.00	0.00
	EST-2*100	0.80	0.95	0.50
	EST-2*111	0.15	0.05	0.50
PGM-1*	PGM-1*82	0.50	0.40	0.50
	PGM-1*100	0.50	0.60	0.50

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