A Precocious Population of Channel Catfish with Potential as a Research Model

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Abstract: A population of catfish presumptively identified as channel catfish *(Ictalurus punctatus)* found in Lake Maurepas in southeast Louisiana matures at a small size and early age (<170 mm TL and <2 years) compared to other populations in southern Louisiana (>500 mm TL and 3 to 4 years). In addition, these catfish have a protracted spawning period. The peak spawning time coincides with cultured channel catfish, but a percentage of the population spawns throughout the summer months. These fish are harvested at a small size and support an important but contentious commercial fishery. The small size and early age at sexual maturity have raised several questions: are these channel catfish, a distinct subspecies, or a hybrid between channel catfish and another ictalurid? These reproductive characteristics would prove useful for research, especially if the taxonomic uncertainty could be resolved. In this study, we differentiated these catfish by fin shape and external coloration from all other ictalurids present in Lake Maurepas except for channel catfish and blue catfish *(Ictalurus furcatus)*. Blue catfish were differentiated by the absence of spots, anal fin shape and anal fin ray count. Genome size (cellular DNA content) determined for Lake Maurepas catfish in this study (2.11 ± 0.01 pg; $N = 36$) agreed with values for the Kansas strain of channel catfish (2.11 ± 0.01 pg; $N = 15$). Staining of chromosomes to determine the location (chromosome pair) of the nucleolar organizing region (NOR) revealed that the NOR for Lake Maurepas catfish did not differ from that of channel catfish. In addition, NOR for hybrids of Lake Maurepas catfish x blue catfish were located on heteromorphic chromosomes. A segment of an immunoglobin gene was sequenced from channel catfish, Lake Maurepas catfish, blue catfish, and black bullhead *(Ictalurus melas)*. The nucleotide sequences for channel catfish and Lake Maurepas catfish were identical, while differences

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were found among the other catfishes. These data indicate the existence of a distinct population of channel catfish in Lake Maurepas that possesses traits useful for research.

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Genetic improvement of fish stocks benefits greatly from controlled spawning of individuals to produce progeny for evaluation. Long generation times and large sizes have hampered application of these techniques to commercially important species such as channel catfish *(Ictalurus punctatus)* which typically require 3 to 4 years to reach sexual maturity at a weight of 1.5 to 2 kg. There is a unique catfish population in southeastern Louisiana that may avoid these problems and serve as a research model for channel catfish. Lake Maurepas (northwest of New Orleans), contains a population of catfish that reaches sexual maturity as early as age 1 (second summer of growth) and at sizes as small as 170 mm total length (TL) (McElroy et al. 1990). This population has been presumptively classified as channel catfish, although they can be differentiated from channel catfish of other Louisiana waters by discriminate analysis of morphological measurements (Lutz et al. 1987). The unique reproductive characteristics of these catfish, combined with the measurable morphological differences have spawned debate as to whether they are a distinct subspecies or a hybrid between channel catfish and another ictalurid.

Hypotheses regarding the genetic constitution of this population can be separated into taxonomic or genetic groups. Taxonomic hypotheses are based on the possibility that the Lake Maurepas catfish are not channel catfish, or are inter-specific hybrids, and therefore would have a: 1) chromosome number; 2) genome size (amount of DNA per cell), or 3) meristic profile different from that expected for channel catfish (e.g., Lake Maurepas catfish could be hybrids between channel catfish and another ictalurid, such as the black bullhead *(Ameiurus melas)*). Genetic hypotheses include divergence from normal channel catfish such that the Lake Maurepas catfish are: 4) aneuploid, or 5) polyploid. The objectives of this study were to: 1) compare meristics and external morphology for ictalurid catfishes common to Lake Maurepas; 2) compare genome size of Lake Maurepas catfish to a cultured strain of channel catfish and to a population of channel catfish from a nearby lake; 3) compare karyotypes from crosses of channel catfish (female) x Lake Maurepas catfish (male) to the standardized channel catfish karyotype, and 4) compare DNA sequence data from Lake Maurepas catfish and other ictalurids. This study was supported in part by the Louisiana Catfish Promotion and Research Board and USDA special grants. We thank D. Arnoldi, B. Prima and W. Wolters for help in obtaining fish and samples, and J. Buchanan, C. Figiel, T. Foshee, D. Glenn, and W. Wayman for assistance during spawning season. We thank J. Avault, R. Cooper, G. Lutz, M. Fitzsimons, and C. Short for critical review. This manuscript was approved by the Director of the Louisiana Agricultural Experiment Station as number 97-22-0370.

Methods

Meristics and Morphology

A table of meristic traits and external morphology was prepared for ictalurid catfishes common to southern Louisiana to compare Lake Maurepas catfish with other species or hybrids that might exist in the lake. The variables were maximum total length, maximum weight, body coloration, anal fin ray count, adipose fin shape, presence or absence of spots, and caudal fin shape. Values for the table were taken from published sources (Williams 1992, Douglas 1974).

Estimation of Genome Size by Flow Cytometry

Blood samples were collected in acid-citrate-dextrose (ACD) solution (Becton-Dickinson vacutainer 4606) from 15 Kansas strain channel catfish (obtained from the USDA-ARS, Catfish Genetics Res. Lab., Stoneville, Miss.), 36 catfish collected from Lake Maurepas, and 10 catfish collected from Lac Des Allemands (40 km south of Lake Maurepas) and were refrigerated until analysis. Blood cells of the catfish under study were suspended with those of domestic chicken *(Gallus gallus)* as a mixture in 0.5 mL of buffer containing 25 μg of buffered RNase, 0.1% sodium citrate, 0.1% Triton X100, and 25 μg of propidium iodide (Tiersch et al. 1990). DNA content of the cells was estimated with a PROFILE flow cytometer (Coulter Electronics, Hialeah, Fla.) with an argon-ion laser at a wavelength of 488 nm. Fluorescence values of at least 40,000 propidium-iodide-stained nuclei were digitized individually and used to calculate DNA content in relation to a value of 7.0 picograms (pg) DNA assigned for fresh human (male) leukocytes. In each test, the value of the internal reference was cancelled during the calculation of DNA content, according to the formula:

$$\text{Nuclear DNA (pg)} = 7.0 \times C/R \times R/H$$

where C is the fluorescence value for the nuclei of catfish, R is the fluorescence value for the nuclei of the chicken internal reference, and H is the fluorescence value for the nuclei of human blood cells. Genome size values were compared by 1-way ANOVA (Data Desk, version 4.2, Data Description, Inc., Ithaca, N.Y.) with $P \leq 0.05$ chosen as the level of statistical significance.

Karyotyping and NOR Staining

Karyotypes were prepared from cultured leukocytes (Zhang and Tiersch 1995, 1998) of Lake Maurepas catfish, crosses of channel catfish (a research population maintained at La. State Univ. [LSU]) x Lake Maurepas catfish, and hybrids of channel catfish x blue catfish *(Ictalurus furcatus)*. The leukocytes were isolated from whole blood by gradient centrifugation on ficoll hypaque, and were cultured in RPMI-1640 medium (Sigma Chemical Corp., St. Louis, Mo.) with the addition of concanavalin A (10 μg/ml) to stimulate mitosis. The RPMI-1640 medium was diluted to 270 mOsmol/kg and supplemented specifically for culture of channel catfish leukocytes as described in Miller and Clem (1988). Chromosomes were arrested at

metaphase by addition of colchicine (0.5 µg/ml). Procedures for hypotonic treatment and cold fixation were based on the methods of LeGrande (1981).

The karyotyping process was aided by use of the Optimas (Bioscan, Inc., Edmonds, Wash.) and Kary (Pro Data, Inc., Oslo, Norway) computer software packages. The chromosomes were sorted by relative size (percent of total complement length, %TCL) and centromeric index (*CI*), and divided into groups. Size determination was based on the formula:

$$TCL\ (\%) = (\text{length of chromosome pair/total complement length}) \times 100$$

where *TCL* was the total length of all chromosomes in the spread.

The *CI* was determined by using the equation:

$$CI\ (\%) = (\text{short arm length/total chromosome length}) \times 100.$$

Chromosomes were classified as telocentric (CI = 0% to 12%), subtelocentric (CI = 13% to 25%), submetacentric (CI = 25% to 37%), or metacentric (CI = 38% to 50%) (Levan et al. 1964).

Staining of the nucleolar organizing region (NOR), an area of active ribosomal RNA synthesis, was based on the method of Howell and Black (1980). Slides were covered with a solution of 30% silver nitrate and 1.5% gelatin and incubated at 50 C for 8 to 10 min. The NOR-bearing chromosomes from 5 channel catfish x Lake Maurepas catfish, and 5 channel catfish x blue catfish hybrids were measured, and the *CI* was determined as previously described. The Student's *t*-test was used to determine differences between *CI* values with $P \leq 0.05$ chosen as the level of significance.

DNA Sequencing

Genomic DNA was extracted from whole blood of 2 Lake Maurepas catfish, 2 blue catfish, and 1 black bullhead with the QIAmp blood and tissue kit (Qiagen Inc., Chatsworth, Calif.). Primers designed to target the gene encoding a portion of the heavy chain of channel catfish immunoglobulin (Wilson et al. 1990) were synthesized at the LSU Gene Probe and Expression Laboratory (LSU School of Veterinary Medicine, Baton Rouge). The primer DNA sequences were (5' to 3'): TCCCCAAG-GTTTACTTGCTCGCTCC (designated CH4-1) and CGATGGATCTGGATAT-GTGGCGCAC (CH4-2). These primers, designed to yield a 303 base pair (bp) fragment, were used for polymerase chain reaction (PCR) analysis of genomic DNA. Each PCR reaction contained 0.2 µM of CH4-1 and CH4-2, 10 µM of each deoxynucleotriphosphate (dNTP) (G, A, T, or C), 1.5 mM $MgCl_2$, 1% DMSO, 2.5 units of Ampli*Taq* DNA polymerase (Roche Molecular Systems, Inc., Branchburg, N.J.), 1-x Ampli*Taq* buffer (supplied as a 10-x concentrate with Ampli*Taq* DNA polymerase), ~ 0.25 µg of sample DNA (template), and sufficient sterile, distilled water to bring the reaction volume to 100 µL. The reaction conditions were 95 C for 5 minutes to denature the template DNA, followed by 30 cycles of 95 C for 30 seconds (DNA denaturation step), 52 C for 30 seconds (primer annealing), and 72 C for 1 minute (primer extension). After PCR, samples were electrophoresed at 8.0 V/cm in a 2%

agarose gel for 1.5 hours to determine relative size and number of bands amplified by the CH4 primers for each sample.

For DNA sequencing, purity and concentration of DNA in PCR products were estimated using the GeneQuant RNA/DNA calculator (Model 80-2104-98, Pharmacia Biotech, Cambridge, England). The CH4-1 primer was used with the Ready Reaction Kit (Perkin Elmer, Foster City, Calif.) to prepare the PCR products for sequencing in an ABI Prism 310 Genetic Analyzer (Perkin Elmer, Foster City, Calif.). A 245-bp channel catfish sequence corresponding to base pairs 33 to 278 of the 303 bp sequence amplified by the CH4 primers from genomic DNA of channel catfish (LSU research population) was available to use as a reference sequence. The reference sequence was verified by alignment to the complete gene sequence of the channel catfish immunoglobulin heavy chain reported by Wilson et al. (1990). Sequences were aligned and analyzed with the Sequence Navigator software package (ABI Inc., San Diego, Calif.) on a Power Macintosh 6100 computer (Apple Computer, Cupertino, Calif.). A list of restriction enzyme sites was generated for each sequence with the PC Gene software package (Intelligenetics Inc., Mountainview, Calif.).

Results

Meristics and Morphology

The meristic and morphometric analysis (Table 1) differentiated all species by coloration and caudal fin shape except the blue catfish, channel catfish, and Lake Maurepas catfish. Blue catfish usually can be differentiated from channel catfish by 3 characteristics: 1) absence of spots in blue catfish (although some mature channel catfish do not have spots); 2) presence of a straight anal fin margin in blue catfish (that of channel catfish is rounded), and 3) presence of 30 to 36 anal fin rays in blue catfish (24 to 31 rays in channel catfish). The Lake Maurepas catfish could be distinguished from blue catfish, but not from channel catfish by these criteria.

Estimation of Genome Size by Flow Cytometry

The grand mean of all values of genome size in this study ($N = 61$) was 2.11 ± 0.01 pg DNA (mean ± SD). Genome size was not significantly different ($F_{60} = 3.26$, $P = 0.05$) among the populations tested (Table 2). The range between the lowest (2.09 pg) and highest (2.14 pg) values was 0.05 pg, or 2.37% of the mean. Therefore, the genome size of the Lake Maurepas catfish is not different from that published for channel catfish.

Karyotyping and NOR Staining

The Lake Maurepas catfish x channel catfish karyotype was not different from the standardized karyotype for channel catfish detailed in Zhang and Tiersch (1998). The karyotype of the Lake Maurepas catfish x channel catfish cross was chosen for this report because it offers an intrinsic control for differential chromosome preparation (e.g., varied exposure to reagents) not possible with separate karyotypes. As

Table 1. Meristic and external morphology for ictalurids common to Lake Maurepas, La.

Species	Max. Length (cm)	Max weight (kg)	Barbels	Coloration dorsal/ lateral/ventral	Anal fin rays	Anal fin shape	Adipose fin morph-ology[a]	Spots	Caudal fin morph-ology
Channel catfish *Ictalurus punctatus*	120	26.3	4 pairs	blue gray/ lt. blue- silver white	24–31	rounded	free	yes	deeply forked
Blue catfish *Ictalurus furcatus*	110	45.4	4 pairs	blue-slate/ lighter/ white	30–36	straight	free	none	deeply forked
Flathead catfish *Pylodictis olivaris*	140	41.4	4 pairs	olive-yellow to light brown/yellow/ yellow	14–17	rounded	free (elon-gated)	none	weakly notched
Black bullhead *Ameirus melas*	43	1.2	4 pairs	olive-black/ yellow to black/yellow	17–21	rounded	free	none	slightly notched
Yellow bullhead *Ameirus natalis*	46	1.4	4 pairs	olive brown/ yellow- brown/ yellow	24–27	rounded	free	none	slightly notched
Black madtom *Noturus funebris*	9	NA	4 pairs	dark gray- black/dark gray-black/ whitish	NA	rounded	adnate	none	trun-cate

a. Adipose fin morphology: free = adipose fin free and flap-like at its posterior edge, completely separated from the caudal fin; adnate = adipose fin continuous with caudal fin.

Table 2. Comparison of genome sizes (diploid DNA content) for populations of channel catfish.

Strain/population	*N*	minimum-maximum (range)	Mean ± SD	% variation (range/ mean) x 100
Kansas	15	2.10–2.12 (0.02)	2.11 ± 0.01	1.14
Lake Maurepas	36	2.09–2.13 (0.04)	2.11 ± 0.01	1.99
Lac Des Allemands	10	2.10–2.14 (0.04)	2.12 ± 0.01	1.98

with the standardized channel catfish karyotype, the Lake Maurepas catfish x channel catfish chromosomes formed pairs that could be divided into 8 groups: A, 2 large metacentrics; B, 3 large subtelocentrics; C, 3 medium metacentrics; D, 5 medium submetacentrics; E, 5 medium subtelocentrics; F, 2 telocentrics; G, 5 small metacentrics; and H, 4 small submetacentrics for a total of 29 pairs (Fig. 1). Karyotypes of pure Lake Maurepas catfish were not different from those of Lake Maurepas catfish x channel catfish or pure channel catfish, and in every case the NOR-bearing chromo-

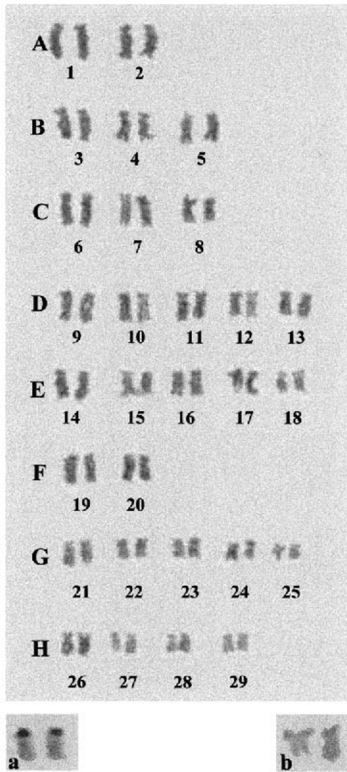

Figure 1. Karyotype of channel catfish x Lake Maurepas catfish. The chromosomes were grouped as: A, large metacentric; B, large subtelocentric; C, medium metacentric; D, medium submetacentric; E, medium subtelocentric; F, telocentric; G, small metacentric; and H, small submetacentric. Chromosomes staining positive for nucleolar organizer regions (NOR) from channel catfish x Lake Maurepas catfish (a) and channel catfish x blue catfish hybrid (b). It can be inferred for the hybrid NOR-bearing chromosomes that the metacentric (left) was derived from the blue catfish and the submetacentric (right) was from the channel catfish.

some pair (designated as D-11 for the channel catfish) (Zhang and Tiersch 1998) was the same (data not shown).

In the channel catfish x blue catfish hybrid, the NOR-bearing chromosomes stained differentially with one always darker than the other. The *CI* of the NOR-bearing chromosomes from hybrids of channel catfish x blue catfish (38.3 ± 8.4 %, mean ± SD; $N = 10$) were not different ($P = 0.42$) from those of Lake Maurepas catfish x channel catfish (35.7 ± 5.2%; $N = 10$). The NOR-bearing chromosomes of channel catfish x blue catfish hybrids, however, were heteromorphic: all of the darker-staining chromosomes were metacentric and all of the lighter chromosomes were submetacentric. The darker-staining chromosomes had a *CI* of 45.2 ± 4.5% ($N = 5$) while the lighter chromosomes had a *CI* of 31.4 ± 4.2% ($N = 5$). There were no detectable morphological differences between the NOR-bearing chromosomes from the crosses of Lake Maurepas catfish x channel catfish.

DNA Sequencing

A single band of the expected size (303 bp) resulting from PCR with the CH4 primers was observed for each species. Because in each case the CH4 primers ampli-

Sequence	10	20	30	40	50	60
1) Channel catfish	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG
2) LM[a] catfish 1	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG
3) LM[a] catfish 2	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG
4) Blue catfish 1	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG
5) Blue catfish 2	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG
6) Black bullhead	GCCCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	ATTCTACCCT	AAGGAGGTGG
7) Ambiguity	--*-------	----------	----------	----------	*---------	----------
8) Consensus	GCTCTGGTGA	ATCAGTGACC	CTGACTTGCT	ATGTTAAAGA	CTTCTACCCT	AAGGAGGTGG

	70	80	90	100	110	120
1) Channel catfish	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGTCGGCTAT	GAGCAGAACA
2) LM[a] catfish 1	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGTCGGCTAT	GAGCAGAACA
3) LM[a] catfish 2	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGTCGGCTAT	GAGCAGAACA
4) Blue catfish 1	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGGCGGCTAT	GAGCAGAACA
5) Blue catfish 2	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGGCGGCTAT	GAGCAGAACA
6) Black bullhead	CTGTGTCTTG	GCTTGTTGAC	GATAAACAAG	TGGAAGAAGT	GGGCGGCTAT	GAGCAGCACA
7) Ambiguity	----------	-------*--	----------	----------	--*-------	------*---
8) Consensus	CTGTGTCTTG	GCTTGTTAAC	GATAAACAAG	TGGAAGAAGT	GGkCGGCTAT	GAGCAGAACA

	130	140	150	160	170	180
1) Channel catfish	CCACTGCAGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA
2) LM[a] catfish 1	CCACTGCAGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA
3) LM[a] catfish 2	CCACTGCAGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA
4) Blue catfish 1	CCACTGCAGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA
5) Blue catfish 2	CCACTGCAGT	TATCGACACA	AACAACCTCT	TTTCAGTGTA	CAGCCACCTG	ATTATCAAAA
6) Black bullhead	CCACTGCGGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA
7) Ambiguity	-------*--	----------	----------	----------	----------	----------
8) Consensus	CCACTGCAGT	TATCGACAGA	AACAACCTCT	TTTCAGTGTA	CAGCCAGCTG	ATTATCAAAA

	190	200	210	220	230	240
1) Channel catfish	CTGCAGACTG	GAACAGTGGC	AGTGTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
2) LM[a] catfish 1	CTGCAGACTG	GAACAGTGGC	AGTCTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
3) LM[a] catfish 2	CTGCAGACTG	GAACAGTGGC	AGTGTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
4) Blue catfish 1	CTGCAGACTG	GAACAGTGGC	AGTTTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
5) Blue catfish 2	CTGCAGACTG	GAACAGTGGC	AGTTTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
6) Black bullhead	CTACACAATG	GAACAATGGC	AGTGTGTTCA	GCTGCCTGGT	TTATCATGAG	TCCATCAAGG
7) Ambiguity	--*--*-*--	-----*----	---*------	----------	----------	----------
8) Consensus	CTGCAGACTG	GAACAGTGGC	AGTGTGTTCA	GCTGCCTGGT	TTATCATGAC	TCCATCAAGG

[a]LM = Lake Maurepas

Figure 2. Comparison of nucleotide sequence data from 3 ictalurid species corresponding to base pairs 33 through 278 of a 303 bp fragment of the channel catfish immunoglobulin M heavy chain gene amplified by PCR. The ambiguity sequence highlights base differences among the species with the symbol "*". Where a consensus (majority) could not be found among the sequences, a "k" was inserted in the consensus sequence. The final 5 nucleotides were identical for all species (ACTGT) and were omitted from the figure.

fied only 1 band, nucleotide sequencing could be carried out directly on the PCR products. We targeted a 270-bp fragment for each sample, allowing alignment with the 245-bp channel catfish reference sequence for all samples. The nucleotide sequences of the 2 Lake Maurepas catfish were in 100% consensus with each other, the 245-bp reference sequence for channel catfish, and the published sequence for this gene (Wilson et al. 1990). The sequences for the 2 blue catfish were in 100% consensus with each other, while the channel catfish and blue catfish sequences differed by a single base at 2 locations (base pairs 103 and 204, Fig. 2). The black bullhead sequence was also different from the channel catfish and blue catfish sequences. The sequence differences, while small, could be used to differentiate the 3 species and their hybrids.

Discussion

The early age and small size at sexual maturity of the Lake Maurepas catfish population has been studied previously. Catfish in Lake Maurepas, Lac Des Allemands, and Flat Lake (Atchafalaya Basin) were studied to determine if the small size at maturity of Lake Maurepas catfish was due to stunted growth which is characterized by individuals or populations that are well below the potential growth rate for a species (Burrough and Kennedy 1979). Stunted fish are sexually mature at normal age, but are short for their age (Woodhead 1978). Stunting can be caused by overcrowding or by competition for forage. Dietary analyses offered no evidence to indicate stunting or overcrowding in any of the 3 lakes (Zeringue et al. 1988).

A study of growth of channel catfish from 8 Louisiana lakes found that the Lake Maurepas catfish were substantially different from the other populations in terms of growth and length-weight relationships (Zeringue 1989). In a 3-year study, Lake Maurepas catfish and channel catfish from Lac Des Allemands were sampled to determine if age structure, growth rate, and length at maturity indicated stunting in these populations (McElroy et al. 1990). It was concluded that mean total length (TL) at age 2 and beyond compared favorably with commercially fished populations in other areas of the lower Mississippi River drainage and that Lake Maurepas catfish achieved higher percentages of sexual maturity at small size classes than did fish in nearby Lac Des Allemands. The 100% level of sexual maturity was reached by age 2+ in Lake Maurepas catfish (280–290 mm TL), 1 year earlier than in channel catfish from Lac Des Allemands (360–379 mm TL).

Given these observations, what is the taxonomic classification of the Lake Maurepas catfish? First, channel catfish were easily distinguishable from all other ictalurids in Lake Maurepas by use of external morphological characteristics and meristic data. Meristics may be useful in identifying some ictalurid hybrids such as the intergeneric hybrid channel catfish x black bullhead, which has characteristics intermediate to those of the parents (Goudie et al. 1993). However, meristics can fail to identify some ictalurid hybrids such as the interspecific hybrid of channel catfish x blue catfish, and the reciprocal cross because paternal dominance for some characteristics (external appearance, anal fin shape, and anal-fin rays) has been documented in these hybrids (Dunham et al. 1982). Thus, the Lake Maurepas catfish can be morphologically and meristically classified as channel catfish, but the possibility of hybridization could not be excluded by these observations.

Second, the genome size determined for Lake Maurepas catfish in this study agrees closely with that reported previously for populations of channel catfish (Tiersch et al. 1990) and was identical to the genome size of channel catfish from Lac Des Allemands and a commercial strain of channel catfish. Genome size has been reported for blue catfish, flathead catfish *(Pylodictus olivaris)*, and black bullhead, and for hybrid crosses of channel catfish with blue catfish, black bullhead, and flathead catfish (Tiersch and Goudie 1993). In that study, the genome sizes for all hybrids were exactly intermediate to those of the parental species, and could be predicted by dividing the sum of the genome values for the parental species by 2. In the

present study, the Lake Maurepas catfish can not be differentiated from channel catfish by genome size which suggests that they do not possess an aneuploid (abnormal) DNA content and are not the result of hybridization. These data also show that the Lake Maurepas catfish are not polyploid (e.g., triploid or tetraploid).

Third, channel catfish, blue catfish, and hybrids of channel catfish x blue catfish all possess 58 chromosomes, and the karyotypes are indistinguishable from one another (LeGrande et al. 1984). The black bullhead has 60 chromosomes, and the hybrid of channel catfish x black bullhead has 59 chromosomes and would be identified by chromosome number alone (Zhang and Tiersch 1997). The location of NOR has been used to differentiate species (Amemiya and Gold 1988); however, the location of the NOR-bearing chromosome pair for blue catfish is unpublished. The data presented in this study showed that the NOR-bearing chromosomes of the channel catfish x blue catfish hybrid were of different types (submetacentric and metacentric). Because the NOR-bearing chromosomes of channel catfish are known to be submetacentric, it can be deduced that the NOR-bearing chromosomes from the blue catfish are metacentric. Therefore, NOR-staining could allow identification of parental species and offspring because the hybrid would inherit a different NOR-bearing chromosome from each parent. This shows that the Lake Maurepas catfish are not distinguishable from channel catfish, and provides further evidence that they are distinguishable from hybrids of channel catfish and blue catfish

Fourth, the DNA sequence data for the Lake Maurepas catfish and channel catfish were identical. A base pair difference (at bp 103) among channel catfish, blue catfish, and black bullhead would allow differentiation of all 3 species by restriction enzyme digest. A restriction enzyme recognizes and cuts DNA at a specific nucleotide sites. The restriction enzyme *Fnu*4HI, recognizes the 5 nucleotide sequence GCNGC (where N is any nucleotide: A, G, T, or C) and cuts after the first C. A site recognized and cut by *Fnu*4HI is present once in the channel catfish CH4 sequence (GCTGC, bp 211 to 215), while such a site occurs twice in the blue catfish sequence (GCGGC, bp 102 to 106; GCTGC, bp 211 to 215) and 3 times in the black bullhead catfish sequence (GCGGC, bp 102 to 106; GCAGC, bp 112 to 116, and GCTGC, bp 211 to 215). Successful digestion of the CH4 fragment with this enzyme would yield 2 fragments for the channel catfish, 3 fragments for the blue catfish, and 4 fragments for the black bullhead CH4 sequence. This method could also be useful for identification of hybrids because a restriction digest of PCR products amplified from genomic DNA extracted from an ictalurid hybrid would yield a mixture of fragments representative of both parents. These observations support once again that the Lake Maurepas catfish are not distinguishable from channel catfish, yet are clearly distinguishable from other species and hybrids.

Thus, this study presents a synthesis of morphologic, cytologic, cytogenetic, and molecular evidence that the catfish in Lake Maurepas do indeed belong to the species *Ictalurus punctatus*, and they appear to be normal in all features studied other than maturation at an early age and small size. The small size and age at sexual maturity of Lake Maurepas channel catfish is of use for genetic research because commercial strains of channel catfish have a generation time of 3 to 4 years and mature at

~1.5 kg and >500 mm TL (Busch 1985). Artificial spawning of large fish is made difficult and expensive by the size requirements for even a basic hatchery, whereas use of the smaller, early-maturing Lake Maurepas channel catfish would allow researchers to gather data more efficiently.

Furthermore, after typical populations of channel catfish have completed spawning for the year, around late June to early July in southern Louisiana, ripe Lake Maurepas broodstock can be collected and spawned (Bates 1997). At LSU, the use of channel catfish from Lake Maurepas has extended the research spawning season to more than 5 months as compared to the 5 to 6 weeks available otherwise with other strains of channel catfish. In addition, unlike genetic experiments with a model fish of another species, techniques developed using Lake Maurepas channel catfish would be directly applicable to commercial and research strains of channel catfish.

It must be noted that although small size and early maturity render the Lake Maurepas channel catfish useful as a genetic model, these traits are not commercially desirable in catfish aquaculture. Therefore, techniques developed with the Lake Maurepas channel catfish would likely have to be applied separately to commercial domesticated populations. One option would be to crossbreed between Lake Maurepas channel catfish and a fast-growing strain of channel catfish such as the Kansas strain (Dunham and Smitherman 1984). The product of such a cross could combine fast growth and early maturity.

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