

**Abstract.**—The tautog (*Tautoga onitis*) is one of two temperate labrid species commonly inhabiting the coastal marine and estuarine waters of the mid-Atlantic coast of the United States. To delineate population structure throughout its primary range, we examined samples collected from three sites (Rhode Island, Delaware, Virginia). Five regions of the mitochondrial genome (COI, ATPase 6, cyt *b*, ND2 and control region) and one nuclear intron were amplified by PCR and screened for sequence variation with a battery of restriction enzymes (RFLP analysis), or by denaturing gradient gel electrophoresis (DGGE).

With RFLP analysis an average of 129 restriction sites per individual were revealed and 532 bases per individual were surveyed. Polymorphisms were observed in the ND2 and control region fragments, but not in the COI, ATPase 6, or cyt *b* fragments. Mean within-sample haplotype diversity was 0.6905 ( $\pm 0.00184$ ), within the range of values reported for other marine species. However, mean nucleotide diversity was 0.000782, one of the lowest values reported for a marine teleost. Corrected nucleotide divergence between samples was essentially zero, suggesting the absence of population structuring along the mid-Atlantic Coast. DGGE analyses of COI, cyt *b*, and a lactate dehydrogenase (LDH) intron revealed little additional variation; each product possessed a single common haplotype and occasional rare variants.

The low level of genetic diversity observed in the tautog may reflect a small effective population size resulting from historical population bottlenecks or large variance in reproductive success. The apparent absence of geographic differentiation suggests that tautog from Rhode Island to Virginia form a single genetic stock; data from additional genetic polymorphisms are needed to confirm or disprove this conclusion.

## Genetic structure of tautog (*Tautoga onitis*) populations assayed by RFLP and DGGE analysis of mitochondrial and nuclear genes

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The tautog (*Tautoga onitis*) is one of two temperate labrid species commonly inhabiting the coastal marine and estuarine waters of the mid-Atlantic coast of the United States. Although the species range extends from the outer coast of Nova Scotia to Georgia, tautog are most abundant from Cape Cod to Chesapeake Bay (Bigelow and Schroeder, 1953). They are generally found in high relief, reeflike habitats such as those associated with jetties, breakwaters, and wrecks (Auster, 1989).

In the northern part of their range, adult tautog generally overwinter in a state of torpor in sheltered areas in deep water offshore and move inshore during the spring to spawn in estuaries and near-shore waters (Cooper, 1966; Olla et al., 1974). Some tautog remain offshore throughout the year, particularly in the southern part of their range (Olla and Samet, 1977; Eklund and Targett, 1991). Those that migrate offshore do not always return to the same sites to overwinter (Olla et al., 1979). Juveniles and some adults have been found to overwinter at inshore sites off Virginia (Hostetter and Munroe, 1993) and Delaware (Eklund and Targett, 1991). In a tagging study, Cooper (1966) found that adult tautog tagged in Narragansett Bay tended to return to the same spawning site each year, and adult movement into and out of the area was negligible. In general, tautog do not

appear to undergo extensive along-coast migration (Cooper, 1966; Olla et al., 1974; Briggs, 1977).

Tautog spawn between mid-May and mid-August; spawning activity peaks in June (Colton et al., 1979). Eggs are buoyant and generally confined to coastal waters. Hatching occurs in 42–45 hours at 20–22°C, and pelagic larval duration is approximately 20–30 days (Victor, 1986). Although spawning occurs primarily in estuaries, offshore spawning has been reported (Eklund and Targett, 1990; Hostetter and Munroe, 1993).

Tautog support important recreational and small commercial fisheries throughout their range. After peaking in 1986, annual harvests have declined, and the species is believed to be overexploited, particularly in the northern part of its range, between New York and Massachusetts.<sup>1</sup>

Despite the economic importance of tautog, little information is available regarding stock structure of the species. Tagging studies suggest that there is little mixing of adult fish between geographical regions (Cooper, 1966; Briggs, 1977). Hostetter and Monroe (1993) reported latitudinal variation in size-at-age; fish from Virginia were found to grow

<sup>1</sup> Atlantic States Marine Fisheries Commission. 1996. Fishery management plan for tautog, April 1996. Fishery Management Report 25 of the Atlantic States Marine Fisheries Commission. 1444 Eye Street, N.W., 6<sup>th</sup> Floor, Washington D.C. 20005.

**Table 1**

List of primer sequences used in polymerase chain reaction amplifications for RFLP and DGGE-heteroduplex analyses. All sequences are in 5'→3' orientation. Approximate size of product includes primers.

Primer	Sequence	Region	Reference
CO1a-H	AGTATAAGCGTCTGGGTAGTC	cytochrome oxidase subunit I (≈680 bp)	Palumbi et al., 1991
CO1f-L	CCTGCAGGAGGAGGAGAYCC		
CB2-H	CCCTCAGAATGATATTTGTCCTCA	cytochrome <i>b</i> (≈380 bp)	Palumbi et al., 1991
CB1-L	CCATCCAACATCTCAGCATGATGAAA		
t-Met c-Trp	AAGCTATCGGCCCATACCC CTGAGGGCTTTGAAGGCC	NADH dehydrogenase subunit 2 (≈1270 bp)	Park et al., 1993
12SAR-H	ATAGTGGGGTATCTAATCCCAGTT	control region (≈1250 bp)	Palumbi et al., 1991 Meyer et al., 1994
L15995	AACTCTCACCCCTAGCTCCCAAAG		
ATPase6-F	ATAAATAGGCTAATGTTCG	ATP synthase 6 (≈680 bp)	Quattro <sup>1</sup>
ATPase6-R	AAGCACTACGGTTTAAAGC		
LDHA6F1	TACACTTCTGGGCSATYGGBATG	LDH A intron 6 (≈240 bp)	Quattro and Jones, 1999
LDHA6R	CGTSAGGAASACCTCRTCTTCAC		

<sup>1</sup> Courtesy of J. Quattro, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208.

more than twice as fast as fish from Rhode Island during their first year of life. However, in a common-garden experiment with fish from Rhode Island, Delaware, and Virginia, Martin (1993) found no genetic basis for the difference in growth rates. To date, there have been no published genetic analyses of population structure in this species.

The purpose of this study was to evaluate patterns of genetic variation in tautog across the primary range of the species, by examining DNA sequence variation in several mitochondrial and nuclear genes. We used restriction fragment length polymorphism (RFLP) analysis to examine several regions of the mitochondrial genome amplified by the polymerase chain reaction (PCR). These included portions of four protein-coding genes (cytochrome *b*, cytochrome *c* oxidase subunit I, ATP synthetase subunit 6, and NADH dehydrogenase subunit 2) and a segment including the entire control region, tRNA<sup>Phe</sup>, and part of the 12S rRNA gene. In addition, we used denaturing gradient gel electrophoresis (DGGE) heteroduplex analysis to examine a nuclear gene intron and portions of the mitochondrial cytochrome *b* and cytochrome *c* oxidase subunit I genes.

## Materials and methods

### Sample collection and DNA extraction

Juvenile tautog under age two were collected between 1991–1993 from three sites in the northern, middle,

and southern portions of the tautog's primary range: Narragansett Bay, Rhode Island; Delaware Bay, Delaware; and Chesapeake Bay, Virginia (for details regarding collection techniques see Martin, 1993). Fish were stored at –20°C. In addition, fin clips were taken from adult fish collected from Delaware Bay in 1996.

Total DNA extracts were prepared from white muscle tissue and fin clip samples from 24 fish from each of the three sites by using a Puregene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN) following the protocol specified for animal tissue.

### Polymerase chain reaction amplification of DNA

The polymerase chain reaction (PCR) was used to amplify parts of five regions of the mitochondrial genome: cytochrome *c* oxidase subunit I (COI), ATP synthetase 6 (ATPase 6), cytochrome *b* (*cyt b*), NADH dehydrogenase subunit 2 (ND2), and the control region (D-loop). In addition, intron 6 of the LDH-A gene was amplified. Amplifications were performed in a Perkin-Elmer 480 thermocycler under conditions optimized for each primer pair. Primer sequences are listed in Table 1. To create PCR products suitable for use in DGGE, primers CO1f-L, CB1-L, and LDH849R were modified through the addition of a 15 base pair (bp) GC clamp (5'-CCCGCCGCCGCC-3') to the 5' ends.

An approximately 680-bp portion of the COI gene was amplified with universal primers CO1a-H and CO1f-L (Palumbi et al., 1991) by using an initial

denaturation step of 2 min at 94°C followed by 35 cycles of 1 min at 95°C, 1 min at 50°C, and 1 min at 72°C, with a final step of 5 min at 72°C. Reaction volumes of 100 µL contained 1 µL of DNA extract, 2 mM MgCl<sub>2</sub>, 200 µM each dNTP, 2.5 units *Taq* polymerase (Promega), and 0.17 µM of each primer. An approximately 380-bp fragment of the *cyt b* gene was amplified with universal primers CB2-H and CB1-L (Palumbi et al., 1991) by using the same protocol, except that the final primer concentrations were 0.2 µM.

An approximately 680-bp fragment of the ATPase 6 gene was amplified with primers ATPase6-F and ATPase6-R by using an initial denaturation step of 2 min at 94°C followed by 30 cycles of 45 sec at 94°C, 1 min at 52°C, and 2 min at 72°C, with a final step of 5 min at 72°C. Reagent and template concentrations were identical to those used to amplify the *cyt b* fragment. This PCR reaction protocol was also used to amplify an approximately 1270-bp fragment of ND2 with universal primers t-Met and c-Trp (Park et al., 1993) and an approximately 1455-bp fragment containing the entire control region, tRNA<sup>Phe</sup>, as well as part of the 12S rRNA gene with universal primers L15995 (L-Pro; Meyer et al., 1994) and 12SAR-H (Palumbi et al., 1991).

An approximately 240-bp fragment of the LDH intron 6 was amplified with primers LDHA6F1 and LDHA6R (Quattro and Jones, 1999) by using an initial denaturation step of 2 min at 94°C followed by 35 cycles of 1 min at 95°C, 1 min at 52°C, and 1 min at 72°C, and a final step of 2 min at 72°C. Reaction volumes of 100 µL contained 3 µL of DNA extract, 2 mM MgCl<sub>2</sub>, 200 µM each dNTP, 2.5 units *Taq* polymerase, and 0.2 µM of each primer.

### Restriction endonuclease digestion of PCR products

Restriction enzyme digestions were performed as specified by the manufacturer (New England Biolabs, Inc., Beverly, MA) in 20 µl reactions containing 5 units of enzyme per reaction. Digestions were incubated for a minimum of 5 hours before being stopped with loading dye (20% Ficoll 400, 0.1 M Na<sub>2</sub>EDTA pH 8, 1% SDS, 0.25% bromophenol blue, 0.25% orange G). The digests were run for electrophoresis on 2% agarose gels for at least 2 hours at 100 volts. Gels were stained with ethidium bromide and photographed under UV light. Fragment sizes were determined from migration distances in relation to known standards [*Bst*I digest of pBR322 (New England Biolabs) and *Hae* III digest of pUC18 (Sigma, St. Louis, MO)] with the computer package Anagel (Mrázek and Spanova, 1992).

A subset of 24 fish, eight from each geographical region, was screened for polymorphisms in the five

amplified mitochondrial DNA segments with the following 16 restriction enzymes: *Alu* I, *Aci* I, *Bsm*A I, *Bst*U I, *Dde* I, *Dpn* II, *Hae* III, *Hha* I, *Hinf* I, *Mnl* I, *Mse* I, *Msp* I, *Nla* III, *Rsa* I, *αTaq* I, and *Tsp*509 I. Restriction enzyme and mtDNA region combinations that revealed variation in the initial screening, ND2-*Hinf* I and control region-*Hae* III, were repeated for the entire sample of 72 fish, 24 from each geographic region.

### Denaturing gradient gel electrophoresis

Perpendicular gradient denaturing gels were run to determine the approximate denaturing points of the COI, *cyt b*, and LDH intron PCR products. For each fragment, PCR product was mixed with an equal volume of neutral loading dye (20% sucrose; 10 mM Tris-HCl, pH 7.8; 1 mM ethylenediaminetetraacetic acid [EDTA]; 0.1% bromophenol blue) and run on 6.5% acrylamide gels (14 cm × 19 cm, 0.75 mm thick) containing a perpendicular gradient of 0 to 80% denaturant [100% denaturant was defined as 7 M urea/ 40% (v/v) formamide]. Gels were electrophoresed at 150 volts for a minimum of 5 hours in a recirculating 1 × TAE buffer bath at 60°C (CBS Scientific, Inc., Del Mar, CA). Gels were visualized with ethidium bromide staining and photographed under UV light.

The entire sample of 72 fish was screened for polymorphisms in COI, *cyt b*, and the LDH intron by means of parallel DGGE. The parallel gradient gels spanned a range of 10% denaturant on either side of the experimentally determined melting point of each region: 40% to 60% gels were used to screen COI and *cyt b*, and 20% to 40% gels to screen the LDH intron. Parallel denaturing gels were run at 150 volts under conditions identical to those used for the initial perpendicular DGGE; the running times were optimized for resolution of each region: 4 hours for the LDH intron, 5 hours for *cyt b*, and 6 hours for COI.

In addition, heteroduplex analysis was performed on each individual from each of the three regions. Heteroduplexes were formed by heating samples containing equal volumes of PCR product from two individuals to 95°C for 5 min and then incubating them at -20°C for at least 30 min. Each heteroduplex sample was allowed to thaw slowly at room temperature and was run on the appropriate gradient. To create a chain of comparison linking each individual to all of the others, the samples were sequentially combined: the first was mixed with the second, the second with the third, etc., including the last sample which was combined with the first. Pairs of samples that exhibited a single homoduplex band and no het-

**Table 2**

Frequencies of composite haplotypes from restriction digestion of the COI, *cyt b*, ATPase 6, ND2, and the control region amplified fragments from *T. onitis*. Restriction enzymes used for each PCR product are listed. RI = Rhode Island, DE = Delaware, VA = Virginia. All individuals had identical haplotypes for COI, *cyt b*, and ATPase 6 for the enzymes surveyed.

COI: *Hae* III, *Hinf* I, *Nla* III, *Dde* I, *Mnl* I, *Aci* I and *Tsp509* I

*cyt b*: *Hae* III, *Hha* I,  $\alpha$ *Taq* I, *Nla* III, *Bst*U I, *Mse* I, *Dde* I, *Mnl* I, *Aci* I, and *Tsp509* I

ATPase 6: *Alu* I, *Rsa* I, *Hae* III, *Hha* I,  $\alpha$ *Taq* I, *Hinf* I, *Nla* III, *Bst*U I, *Mse* I, *Dde* I, *Mnl* I, *Aci* I, and *Tsp509* I

ND 2:*Alu* I, *Hae* III, *Rsa* I, *Hha* I, *Msp* I, *Hinf* I, *Dpn* II, *Nla* III, *Bst*U I, *Mse* I, *Dde* I, *Mnl* I, *Aci* I and *Tsp509* I

control region: *Alu* I, *Rsa* I, *Hae* III, *Hha* I,  $\alpha$ *Taq* I, *Msp* I, *Hinf* I, *Dpn* II, *Nla* III, *Bst*U I, *Mse* I, *Dde* I, *Mnl* I, *Aci* I and *Tsp509* I

Haplotype		RI	DE	VA
ND 2	Control region			
AAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAA	4	5	4
AAAAA <b>B</b> AAAAAAAA	AAAAAAAAAAAAAAAA	2	2	2
AAAAAAAAAAAAAAAA	AA <b>B</b> AAAAAAAAAAAA	2	1	1
AAAAAAAAAAAAAAAA	AACA <b>A</b> AAAAAAAAAAAA	0	0	1

eroduplex bands were considered to possess identical haplotypes.

### DNA sequencing

Haplotypes identified by DGGE-heteroduplex screening of the cytochrome *b* fragment were sequenced by using an ABI 373 automated DNA sequencer with dye terminator chemistry. Sequencing was done in both directions with the original PCR primers.

### Statistical analysis of RFLP data

Restriction patterns were analyzed with the Restriction Enzyme Analysis Package (REAP, version 4.0) (McElroy et al., 1992). For each of the three geographical samples, haplotype diversities were calculated following Nei (1987), and nucleotide diversities were calculated following Nei and Miller (1990). Haplotype diversity ranges from zero (all individuals share a common haplotype) to one (every individual has a unique haplotype) and estimates the probability that two randomly selected individuals in a sample will have different haplotypes. Nucleotide diversity estimates the average number of nucleotide substitutions for a pair of haplotypes randomly drawn from a sample. Nucleotide divergences among samples were estimated and corrected for within-sample variation according to Nei (1987). Heterogeneity of haplotype frequencies across samples was tested with exact  $R \times C$  tests of independence using the software program StatXact (Cytel, 1992). Additional analyses of population differentiation were

conducted using Arlequin 1.1 (Schneider et al.<sup>2</sup>). Power analysis of  $R \times C$  tests was performed with Power and Precision 1.0 (Borenstein et al., 1997).

## Results

### Restriction site variation

Digestion with 16 restriction enzymes revealed polymorphisms in the ND2 and control region fragments, but not in the COI, ATPase 6, or *cyt b* fragments. Restriction enzyme digestion revealed an average of 129 restriction sites per individual and surveyed an average of 532 bases in each fish.

ND2 gene sequence variation was revealed by digestion with *Hinf* I, and sequence variation in the control region fragment was revealed by digestion with *Hae* III. The remaining enzymes produced invariant patterns. The 16 enzymes revealed four composite haplotypes attributable to restriction site gains or losses in the 24 individuals surveyed (Table 2). No additional haplotypes were uncovered by increasing the sample size to 24 individuals from each geographic region for the ND2-*Hinf* I and control region-*Hae* III combinations (Table 3). No evidence of mitochondrial genome size variation or heteroplasmy was observed in the regions analyzed.

<sup>2</sup> Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin: a software for population genetic data analysis, vers. 1.1. Genetics and Biometry Lab., Department of Anthropology, Univ. Geneva, Geneva.

One of the composite haplotypes was found only in a single individual from Virginia, whereas the remaining three were found in all three geographical regions. Mean within-sample haplotype diversity was 0.6905 ( $\pm 0.00184$ ), and mean nucleotide diversity was 0.000782 (Table 4).

Corrected nucleotide divergence between samples varied between  $-0.0077$  and  $-0.0081\%$ , suggesting the absence of population structuring along the mid-Atlantic Coast. This inference is further supported by exact log-likelihood analysis of the distribution of the four composite haplotypes across the three sites (likelihood ratio statistic=2.82, 6 df, exact  $P=1.00$ ). A similar analysis of the larger data set ( $n=72$ , only polymorphic sites examined) indicated no significant heterogeneity (likelihood ratio statistic=6.05, 6 df, exact  $P=0.469$ ). Lack of population differentiation was further supported by a mean  $F_{ST}$  value of  $-0.005$

(i.e. zero), and the analysis of molecular variance (AMOVA), which found 100% of total haplotype variation to occur within populations.

### DGGE-heteroduplex analysis

For the entire sample of 72 individuals, analysis of the *cyt b* fragment revealed a total of three haplotypes, two of which were unique and found only in single individuals from Delaware and Virginia (Fig. 1). Sequence analysis of the three *cyt b* haplotypes revealed that the two unique haplotypes differed from the dominant haplotype by synonymous single base substitutions. The unique Virginia haplotype differed from the common haplotype by an A to G transition at base 276, whereas the unique Delaware haplotype resulted from the same substitution at base 291 (Fig. 2).

All 72 tautog appeared to possess a single COI haplotype, a result consistent with the extensive restriction enzyme screening of this region performed on the original subset of 24 individuals. The LDH intron was also relatively invariant. Of the 72 fish screened, DGGE-heteroduplex analysis revealed only a single heterozygous individual, collected in the Delaware region.

**Table 3**

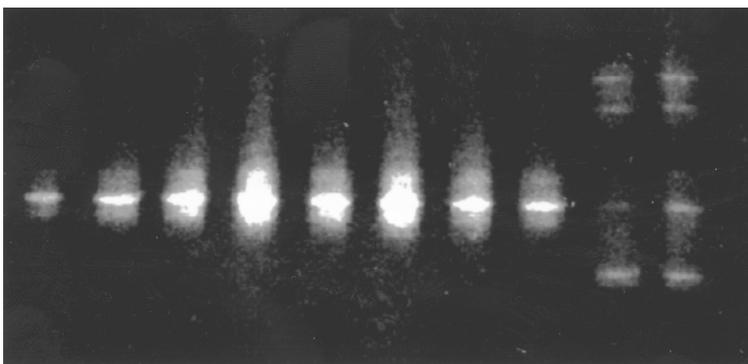
Composite haplotypes from restricted gene and restriction enzyme combinations (ND2-*Hinf* I and control region-*Hae* III) for entire sample ( $n=72$ ).

Haplotype	Rhode Island	Delaware	Virginia
AA	13	14	14
BA	6	9	7
AB	5	1	2
AC	0	0	1
Total	24	24	24

### Discussion

RFLP analysis revealed a mean mtDNA haplotype diversity value ( $0.6905 \pm 0.00184$ ) for tautog that is intermediate within the range of values reported for other marine fishes. However, the overall nucleotide diversity value (0.078%) for the tautog is one of the lowest reported for a marine fish. It should be noted that values for haplotype diversity are affected by the number of restriction enzymes used and should be compared across studies with caution. In addition, values for haplotype and nucleotide diversity may be biased upwards in analyses that include only polymorphic enzymes.

Low levels of intraspecific sequence diversity in mitochondrial genes are generally attributed to low effective population size ( $N_e$ ) or to reduced rates of mutation (Ovenden, 1990). Effective population sizes inferred from mtDNA data are often orders of magnitude smaller than present-day census estimates; this disparity may reflect the



**Figure 1**

Denaturing gradient gel of tautog *cyt b* PCR products. Each lane represents the pooling of PCR products from two individuals, treated to induce heteroduplex formation when two different DNA sequences were present. The single homoduplex bands in lanes 1–8 (from left to right) indicate sequence identity for the nine tautog examined in pairwise combinations. Lanes 9–10 (the two lanes farthest to the right) illustrate the combination of the common haplotype and one rare haplotype. The two lower (faster-migrating) bands represent alternative homoduplex molecules; the upper two bands are the heteroduplex molecules.

**Table 4**

Summary statistics from the analysis of variation in the mitochondrial COI, *cyt b*, ATPase 6, ND2, and control region genes. Samples are pooled according to geographic region.

Population	Sample size	Haplotype diversity	Nucleotide diversity
Rhode Island	8	0.7143 ±0.12275	0.000816
Delaware	8	0.6071 ±0.16398	0.000648
Virginia	8	0.7500 ±0.13913	0.000883
Overall	24	0.6905 ±0.00184	0.000782

action of historical fluctuations in population size, such as bottlenecks caused by disease epidemics or environmental change, as well as a large variance in reproduction among females (Bowen and Avise, 1990). Low levels of nucleotide sequence diversity observed in some teleost fish have also been attributed to rates of sequence evolution that are lower than those estimated for birds and mammals (Bernatchez and Danzmann, 1993).

The Pleistocene glaciation is believed to have decreased the genetic diversity of many species through bottleneck effects (Bernatchez et al., 1989; Avise, 1992). During the Pleistocene era, ten or more glacial advances and retreats that caused changes in sea level and climate are believed to have had significant impacts on the distributions of coastal and marine fauna (Avise, 1992). *Tautog* population size may have been reduced through the reduction of suitable habitats for both themselves and their food resources. During the most recent glacial event, ice covered all waters north of Long Island and would have forced temperate populations southward into regions where high relief, hard substrate habitats are patchier and less abundant. Glacial advances also resulted in drops in sea level that would have reduced the availability of estuarine environments. In addition, post-glacial dispersal from southern refugia may have created population bottlenecks if current populations were founded by a limited number of propagules. The same considerations may apply to the black sea bass *Centropristis striata*, an estuarine-dependent species with low nucleotide diversity (0.03%), corresponding to an estimate of  $\approx 5,000$  for  $N_{f(e)}$ , the effective number of females (Bowen and Avise, 1990).

TTT	GGC	TCC	CTA	CTA	GGC	CTC	TGC	TTA	GTC	GCA	CAA	ATT	CTT	ACA	45
F	G	S	L	L	G	L	C	L	V	A	Q	I	L	T	
GGG	CTT	TTC	CTA	GCC	ATA	CAC	TAC	ACC	TCG	GAT	ATC	GCC	ACA	GCC	90
G	L	F	L	A	M	H	Y	T	S	D	I	A	T	A	
TTT	TCA	TCA	GTC	GCC	CAC	ATC	TGC	CGC	GAC	GTT	AAT	TAC	GGC	TGA	135
F	S	S	V	A	H	I	C	R	D	V	N	Y	G	W	
CTA	ATT	CGA	AAC	ATA	CAT	GCA	AAC	GGC	GCC	TCC	TCC	TTC	TTC	ATC	180
L	I	R	N	M	H	A	N	G	A	S	S	F	F	I	
TGC	ATC	TAC	CTA	CAC	ATT	GCA	CGG	GGC	CTT	TAC	TAC	GGC	TCA	TAC	225
C	I	Y	L	H	I	A	R	G	L	Y	Y	G	S	Y	
CTC	CAC	AAA	GAA	ACG	TGA	AGC	ATC	GGC	GTT	GTT	CTC	CTC	CTC	CTT	270
L	H	K	E	T	W	S	I	G	V	V	L	L	L	L	
GTT	ATa	ATA	ACT	GCC	TTC	GTa	GGC	TAT	GTC	CTC	CCC				306
V	M	M	T	A	F	V	G	Y	V	L	P				

**Figure 2**

Nucleotide sequence and deduced amino acid sequence of a 306-bp portion of the mitochondrial *cyt b* gene in *T. onitis*. Reading frame was inferred from alignment with published teleost *cyt b* sequences. Two rare haplotypes possessed synonymous A→G transitions at nucleotides 276 and 291 (indicated by lower case letters).

In addition to the low levels of within-population nucleotide diversity observed, this analysis also failed to detect genetic heterogeneity among *tautog* samples from different geographical regions. The uncorrected mean nucleotide sequence divergences among *tautog* samples were of the same magnitude as mean nucleotide diversities within samples; the mean difference between haplotypes randomly drawn from a single sample was equivalent to the mean difference between haplotypes drawn from different samples.

Low levels of intraspecific genetic variation do not preclude the detection of genetic differentiation among stocks. Shulman and Bermingham (1995) detected significant population subdivision within the Caribbean reef fish *Stegastes leucostictus*, for which they reported a mean within-sample sequence diversity of 0.07%. Bowen and Avise (1990) reported a mean within-sample sequence diversity of 0.03%

for black sea bass but detected significant divergence between Gulf of Mexico and Atlantic populations.

The results of our study are consistent with the null hypothesis that the tautog form a single genetic stock within the species range. Although tagging studies in Rhode Island have suggested that adult movement into and out of the area is negligible (Cooper, 1966), tautog have a relatively long pelagic larval stage of approximately three weeks which could result in enough gene flow among geographic regions to prevent the genetic differentiation of subpopulations.

The current system off of the mid-Atlantic coast of the United States consists of three major features: the northeastward flow of the Gulf Stream, the southwestward flow of the along-shelf current, and the across-shelf-flowing warm-core ring streamers that split off from the Gulf Stream (Brooks, 1996). The direction of flow is also influenced by local river runoff, seasonal wind patterns and meteorological events, and by the onshore movement of Ekman currents. The flow patterns in the mid-Atlantic region suggest that a high degree of transport of larvae among regions is possible (Hare and Cowen, 1993), especially for species such as the tautog that spawn in nearshore waters and have pelagic eggs. Larval transport has been suspected as the cause of genetic homogeneity over wide geographic ranges for many marine species (Awise, 1994; Hedgecock, 1994).

Other mtDNA analyses of stock structuring of coastal fish species in the mid-Atlantic region have likewise detected no population subdivision, e.g. weakfish (Graves et al., 1992b), bluefish (Graves et al., 1992a), summer flounder (Jones and Quattro, 1999), and Atlantic croaker (Lankford et al., 1999). None of these studies detected significant genetic heterogeneity among samples collected at different sites within the geographical region spanning from Rhode Island to Chesapeake Bay. For many fish species, the extensive mobility of juveniles and adults coupled with larval dispersal is likely to result in substantial gene flow.

We were unable to reject the null hypothesis of genetic homogeneity among three sites in the northern, middle, and southern portions of the tautog's range but we did not conclusively prove that tautog in the mid-Atlantic region constitute a single genetic stock. Population structure not resolved by our study might be detected by an examination of additional polymorphisms, with rapidly evolving markers better suited for detecting recent subpopulation divergence. Improved resolution would also be gained by increasing sample sizes; the current analysis with a sample size of 24 individuals per population had limited power (40%) to detect significant heterogeneity

among populations with the observed haplotype-frequency distributions. However, it is unlikely that by simply increasing sample sizes with the same set of molecular markers the picture of minimal geographic heterogeneity would alter substantially. If we had found the same haplotype proportions in a study with quadrupled sample sizes ( $n=96$  individuals per site), estimates of gene flow ( $Nm$ ) would still be substantial ( $Nm=8.6$  between RI and DE; 28 for VA-RI; 73 for DE-VA). We conclude that the sampled populations are probably genetically homogeneous, as the result of contemporary or recent gene flow.

In many genetic studies of population structure, PCR amplification of DNA is coupled with a restriction fragment length polymorphism (RFLP) analysis or direct sequencing. Although PCR is relatively simple, the subsequent analyses can become expensive and time-consuming when large numbers of individuals must be evaluated. These drawbacks can be alleviated through the use of mutation detection techniques such as DGGE and DNA heteroduplex mobility assays (Lessa 1992; Grompe, 1993; Lessa and Applebaum, 1993). These methods can be used to compare DNA fragments to determine rapidly which individuals have the same haplotype. Only one representative of each haplotype needs to be further characterized, and large numbers of individuals can be rapidly and efficiently screened. High-resolution DNA screening techniques such as DGGE and heteroduplex analysis provide the sensitivity of DNA sequencing and make it possible to screen greater numbers of individuals for less cost and effort than standard sequencing techniques.

Our study employed a DGGE-heteroduplex assay to screen regions of the mitochondrial and nuclear genomes of the tautog for polymorphisms useful for the analysis of population structure. Both the cytochrome *b* and cytochrome *c* oxidase products, as well as the LDH intron, appeared to be invariant in the tautog samples, raising the question of whether the DGGE-heteroduplex technique was providing the high degree of resolution anticipated.

In order to provide an independent assessment of the DGGE-heteroduplex technique, we examined the cytochrome *b* fragment from eight spot (*Leiostomus xanthurus*), a species that has been shown to have a high genetic diversity in a RFLP analysis of the ATPase 6 and control regions (Lankford et al., 1999), with the same protocol used to evaluate tautog samples. Four to six haplotypes were observed in the eight individuals. It is clear that DGGE-heteroduplex analysis is capable of revealing DNA sequence diversity in amplified mtDNA, as has been reported by other investigators (e.g. Campbell et al. 1995; Michikawa et al. 1997; Tek Kay et al. 1997).

Additionally, this study shows that DGGE-heteroduplex analysis is a powerful technique that makes it possible to screen large numbers of samples and to identify haplotypes that differ by as little as a single nucleotide. The unique tautog cytochrome *b* haplotypes identified by DGGE-heteroduplex analysis result from single base substitutions that would not have been revealed by a RFLP analysis because of the lack of restriction enzyme target sequences spanning the mutation sites.

The low level of genetic variation in the tautog mitochondrial genome suggests that further mtDNA analyses would likely prove to be unsuccessful in delineating tautog population structure. It might be more productive to focus instead on highly variable nuclear DNA sequences such as microsatellite loci, major histocompatibility complex genes, and introns. In addition, more thorough tagging studies and observations of tautog egg and larval transport should prove valuable in determining the amount of exchange among regions within the species range. However, until further studies are undertaken, the null hypothesis that tautog populations between Rhode Island and Virginia constitute a single genetic stock cannot be rejected.

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