Basin-scale patterns of mtDNA differentiation and gene flow in the bay scallop *Argopecten irradians concentricus*

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ABSTRACT: The bay scallop *Argopecten irradians concentricus* Say is a commercially and recreationally important species along the East Coast of the US that consistently shows evidence of recruitment limitation. To determine if limited planktonic larval exchange between populations inhabiting lagoonal basins or sounds contributes to recruitment limitation in central North Carolina, a hypervariable portion of mtDNA (885 bp) was sequenced from 219 adults from 4 sounds (Topsail, Bogue, Back, and Pamlico), 3 of which (Bogue, Back, and Pamlico) are connected by inshore waterways. Although the largest sound (Pamlico) harbored the greatest genetic diversity, diversity was high in all 4 sounds, with no evidence of recent bottlenecks. An analysis of molecular variance indicates significant genetic structure among sounds (ϕ_{CT} = 0.0069, p = 0.023); all pairwise comparisons among sounds were significant, with the exception of those involving Pamlico Sound. Despite being connected by a short (3 km) inshore waterway, adjacent Bogue and Back Sounds were highly significantly differentiated (ϕ_{CT} = 0.0145, p = 0.000). Coalescent-based estimates of gene flow indicated that most sounds exchange ≤4 migrants each generation. Bogue and Back Sound populations, which were heavily impacted by a localized red tide outbreak in 1987 and which subsequently experienced a recruitment failure, each receive <4 migrants each generation from all other sounds combined. Dynamic physical processes in the vicinity of Beaufort Inlet can explain such limited larval exchange between Bogue and Back Sounds, but if dispersal is primarily controlled by tidally dominated surface flow, net dispersal distances may be limited throughout the region. Therefore, patterns of gene flow and genetic differentiation are consistent with the hypothesis that individual sounds are demographically independent and are closed with respect to fishery management issues.

KEY WORDS: Connectivity · Dispersal · Fisheries management · Marine protected areas · MIGRATE · Recruitment limitation · Tidal currents

INTRODUCTION

Although density variation in natural populations can occur as a consequence of a variety of post-settlement processes, many studies have demonstrated that larval supply can be an important factor influencing local variation in the abundance of benthic marine organisms (e.g. Doherty 1983, Warner & Chesson 1985, Sutherland 1987, Roughgarden et al. 1988). Under conditions known as recruitment limitation, larval supply limits population density rather than post-settlement processes such as predation, competition, disturbance, and resource limitation. Recruitment limitation has often been studied in demographically open populations, a situation in which colonists generally originate from exogenous sources (reviewed by Caley et al. 1996), as is the case for many invertebrates with planktonic larvae, including barnacles (Gaines & Roughgarden 1985), corals (Sammarco & Andrews 1989), and sea urchins (Karlson & Levitan 1990). However, a

handful of recent studies indicate that local retention of planktonic larvae and the subsequent demographic isolation of populations have the potential to generate localized patterns of recruitment limitation (e.g. Jones et al. 1999, Swearer et al. 1999). Species of commercial and recreational value that are subject to overexploitation, but whose populations rely on local larval production for persistence, may be particularly vulnerable to this kind of recruitment limitation.

The bay scallop, *Argopecten irradians concentricus* Say, is a model species for understanding patterns and consequences of recruitment limitation in marine populations. Limited to seagrass beds along the East Coast of the US (Thayer & Stuart 1974), hermaphroditic bay scallops broadcast spawn (both sperm and eggs) into the water column. Planktotrophic larvae feed and swim for 5 to 19 d, depending on food supply and temperature (Castanga & Duggin 1971, Castanga 1975). Seagrass habitat is patchily distributed and found predominantly in semi-enclosed lagoonal basins where salinities are relatively high and turbidity is low (Duggan 1973, Tettelbach et al. 1985). In the southeast USA, bay scallop habitat exists largely within a network of estuaries, basins, and waterways connected to continental shelf waters by a series of inlets or gaps between barrier islands. Adjacent basins or sounds are often connected by channels, some of which are maintained by the Army Corps of Engineers as part of the Intracoastal Waterway.

Results from field surveys and experimental manipulation suggest that bay scallop populations within individual basins are effectively closed with respect to recruitment. Three lines of evidence support this idea. (1) Although there is typically high year-to-year variation in the abundance of adults (e.g. Wolff 1988), recruitment failures and generally slow recoveries of bay scallop populations previously decimated by mass mortality events such as toxic algal blooms (Tettelbach et al. 1985, Bologna et al. 2001) suggest that small population size limits local recruitment due to spawning stock limitation (Summerson & Peterson 1990). (2) Field surveys have shown that adult density and recruitment are positively correlated among basins (Peterson & Summerson 1992). (3) Relocation of mature adults into areas affected by mass mortalities can have a positive effect on subsequent local recruitment (Tettelbach & Wenczel 1991, Peterson et al. 1996).

Although the population dynamics of the bay scallop have been studied extensively, particularly in North Carolina, no parallel assessment of genetic connectivity on similarly small spatial scales has been conducted. For bay scallops, as well as other harvested marine organisms, management stands to benefit from complimentary genetic studies that seek to characterize patterns of genetic differentiation in recruitment-limited systems. In theory, genetics offer both an independent tool for assessing connectivity among populations and more detailed information about specific dispersal pathways between populations than do ecological studies. Whether genetic methods can adequately describe patterns of larval exchange in an ecologically meaningful context remains an open question, given that the exchange of only a few tens of migrants each generation can drive levels of differentiation down to undetectable levels (Taylor & Dizon 1996, Waples 1998). Although coalescent-based methods provide powerful means of analysis, all genetic methods, including those based on the coalescent, generate estimates of gene flow that are time-averaged across generations and that assume an evolutionary equilibrium between gene flow and genetic drift. Therefore, only consistent isolation across many generations is likely to be detected with any genetic method.

To ascertain the nature of gene flow between populations of bay scallops in adjacent basins in central North Carolina, we have conducted a population genetic survey with a highly polymorphic, non-coding mitochondrial DNA (mtDNA) fragment. We focused on populations in neighboring Bogue, Back, and Pamlico Sounds on the central North Carolina coast (Fig. 1) that have each differentially experienced recent mortality events. Bogue and Back Sounds are 2 relatively small, adjacent basins that are connected by a short, deep-water channel (Morehead Channel), but which include the region's most productive scallop harvesting grounds. Back Sound and neighboring Core Sound to the north are contiguous and are connected to Pamlico Sound, a much larger basin to the north.

In 1987, a red tide (*Ptychodiscus brevis*) outbreak devastated local populations in both Bogue and Back Sounds and moderately affected populations in southern Core Sound, but did not extend to Pamlico Sound (Tester et al. 1991). In both 1987 and 1988, recruitment in Bogue and Back Sounds averaged only 2% of the mean of the 3 previous years (Summerson & Peterson 1990). From 1988 to 1991, bay scallop populations showed no signs of recovery in Bogue Sound despite a slow recovery of populations in Back/Core Sound and persistence of populations in Pamlico Sound (Peterson & Summerson 1992). A similar catastrophic decline (of unknown cause) in 1970 was also followed by an extremely slow recovery, taking 5 to 12 yr for populations to return to levels recorded in the late 1960s (Peterson & Summerson 1992). Following the mass mortality of 1987, transplantation of mature adults into Bogue Sound resulted in a significant increase in localized recruitment, but with no measurable effect on recruitment in neighboring basins (Peterson et al. 1996). Peterson et al. (1996) concluded that a moder-
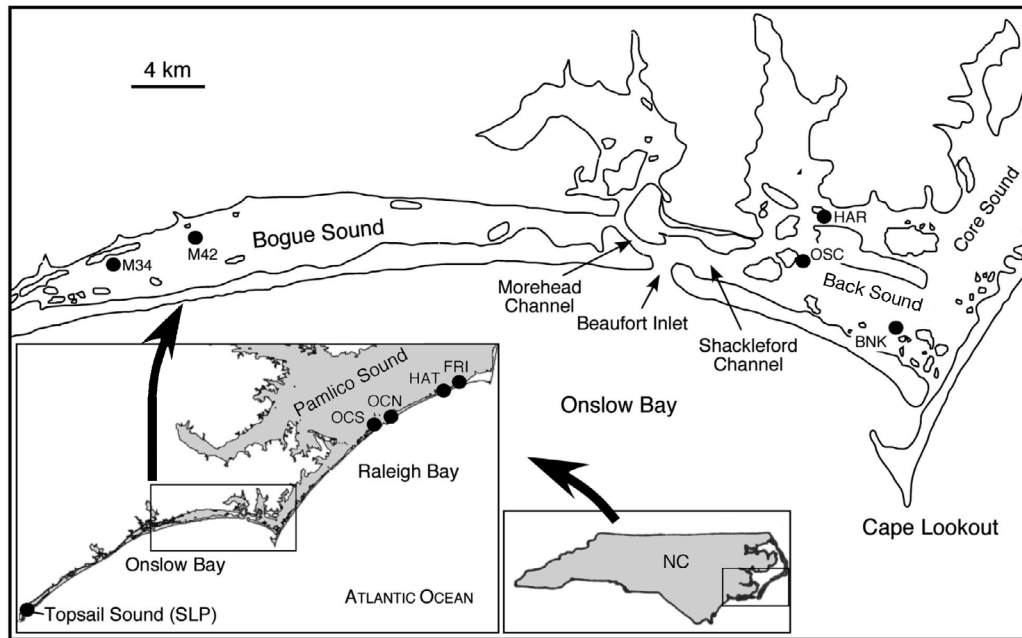
Fig. 1. Cape Lookout region of North Carolina, USA, showing sample sites for *Argopecten irradians concentricus*. Site abbreviations (from west to east)—SLP: Sloop Point; M: channel marker (Nos. 34 and 42 on map); OSC: Oscar Shoals; HAR: Harkers Island; BNK: Banks Bay; OCS: Ocracoke Inlet south; OCN: Ocracoke Inlet north; HAT: Hatteras Inlet; FRI: Frisco

ately short larval period, limited adult mobility, and tidally dominated water circulation (Luettich et al. 1998, 1999) combine to effectively isolate individual basins, rendering them demographically independent.

Our study focuses on 2 questions. (1) Do genetic analyses of bay scallops detect a basin-scale pattern of limited larval exchange that is consistent with recent patterns of recruitment and recovery in North Carolina? (2) Are patterns of genetic diversity within sounds consistent with population recovery driven by endogenous or exogenous larval production?

MATERIALS AND METHODS

Adult bay scallops (n = 219) were collected from sea grass beds from 9 sites in Bogue, Back, and Pamlico Sounds along the central North Carolina coast (Fig. 1, Table 1) in the summer of 2002. For comparison, we also obtained an additional sample from Topsail Sound, which is not physically connected to any of the other 3 focal basins. Genomic DNA was extracted by incubation in CTAB (Cetyl Trimethyl Ammonium Bromide) in the presence of Proteinase K and β-mercaptoethanol followed by 2 chloroform:isoamyl alcohol extractions (24:1) and precipitation with ethanol. A 1025 base pair fragment of mtDNA (Seyoum et al. 2003, GenBank™ accession no. AF261938) was amplified with the primers ANONF1 (5′-AGTCAGC-

CACTGGTTTATAG-3′) and ANONR1025 (5′-GC-CACCCACTAAATTAGATC-3′) and Taq Extenders™ (Stratagene). Although originally isolated from an anonymous mtDNA library, subsequent work has shown that the fragment includes a portion of the mitochondrial ATP-synthase-6 coding region (Wilbur et al. 2005), is maternally inherited, and shows no evidence of heteroplasmy or heterozygosity (Seyoum et al. 2003). Amplification products were gel purified (Qiagen) and sequenced in both directions on an automated sequencer (Applied Biosystems) using long (42 cm) sequencing plates. No electropherograms showed any 'double-peaking,' an indication of the am-

Table 1. Sample localities for the bay scallop *Argopecten irradians concentricus*, from west to east in North Carolina, USA. Abbreviations as in Fig. 1

Site	Sound	Lat. (°W)	Long (°N)
SLP	Topsail	35° 00.000′	78° 45.012′
M42	Bogue	34° 40.443′	77° 43.327′
M34	Bogue	35° 12.314′	77° 02.448′
OSC	Back	34° 41.594′	76° 59.360′
HAR	Back	34° 43.141′	76° 34.494′
BNK	Back	34° 49.210′	76° 21.512′
OCS	Pamlico	35° 04.718′	76° 05.091′
OCN	Pamlico	35° 05.940′	76° 00.547′
FRS	Pamlico	35° 17.656′	75° 57.685′
HAT	Pamlico	35° 12.314′	75° 43.327′

plification and sequencing of paralogous loci, such as pseudogenes.

Sequences were aligned easily by eye due to the rarity of insertions and deletions. We then calculated Tajima's D for each sample using ARLEQUIN v. 2.001 (Schneider et al. 1997). The significance of the D statistic was determined by comparison to a table of the β-distribution. We next used PAUP* version 4.0 (Swofford 2001) to build a haplotype tree with the optimality criterion of parsimony. Population genetic structure was then characterized with an ANOVA method (Excoffier et al. 1992) implemented in ARLEQUIN. We calculated both 'conventional' F-statistics based on the variance in haplotype frequencies among samples (i.e. F_{ST}) and φ-statistics derived from comparisons of sequence divergences within and between samples (i.e. Analysis of Molecular Variance or AMOVA). AMOVAs employed Kimura's 2-parameter substitution model (Kimura 1980) with rate heterogeneity among sites; model parameters were estimated separately with PAUP*. Given that mtDNA is haploid but bay scallops are hermaphroditic, we calculated gene flow (Nm, the number of migrants exchanged between populations each generation) from φ-statistics whenever statistically significant, using the equation $\phi_{ST} = 1/(1 + 2Nm)$ from Hudson et al. (1992).

To assess the impacts of recent population crashes and subsequent recoveries, we used the Metropolis-Hastings sampling approach implemented in the program Fluctuate (Kuhner et al. 1998) to obtain maximum-likelihood estimates of both current and past population sizes within each basin. This method uses a sampling of gene trees to jointly estimate genetic diversity (Θ) and an exponential growth parameter (g). Estimates of these parameters were then used to infer Θ 15 generations ago (i.e. in approximately 1987) with the expression $\Theta_t = \Theta e^{(-g\mu t)}$, where Θ_t corresponds to Θ at any time t (in generations) in the past and μ is the mutation rate, per site per generation. Nucleotide substitution parameters were first estimated with PAUP*. Because we have no estimate of the mutation rate for the particular fragment we analyzed, we conducted this analysis across a wide range of mutation rates. Because Fluctuate can only detect recent exponential growth, we also used Arlequin to conduct an analysis of pairwise distributions of differences between haplotypes, more commonly known as a 'mismatch' analysis (Rogers & Harpending 1992).

Although Nm can be calculated from F-statistics (or φ-statisitcs), these estimates of gene flow do not use genealogical information that DNA-sequence data provide; they do assume symmetrical migration between populations and equal population sizes. Therefore, migration rates between sounds were inferred using MIGRATE version 2.1.3 (Beerli & Felsenstein 2001), which employs a maximum-likelihood method that jointly estimates Nm and the coalescent parameter Θ (genetic diversity) for n populations in a genealogical framework (for mtDNA in hermaphroditic species, MIGRATE generates values of 2 Nm and Θ = $2N\mu$, where N = the effective population size and μ = the substitution rate per generation per nucleotide site). We ran 10 replicate analyses using default search settings (10 short chains of 10 000 sampled, 500 recorded, followed by 3 long chains of 100 000 sampled, 5000 recorded). One of these initial 10 runs, which all used F_{ST}-based estimates for starting values for Θ and Nm, resulted in unrealistically large final values of Θ and Nm (i.e. 1×10^{20}) for one basin (Topsail Sound); 95th percentiles could also not be calculated for this population, indicating that the search had reached a portion of parameter space with very flat likelihood surfaces. Because the F_{ST}-based starting values for Θ were extremely small for some sounds (contrary to coalescent-based estimates), we instead used FLUCTUATE (Kuhner et al. 1998) to estimate starting Θ values. This method yielded results from MIGRATE that were consistent across 50 replicate runs, from which the averages are reported. We also attempted to conduct the MIGRATE analysis using the 10 sites as populations, but in this case the 95th percentiles were often extremely wide, indicating extremely flat likelihood surfaces and therefore little discrimination among a wide range of values.

RESULTS

An 885 bp sequence was obtained from 219 bay scallops. Polymorphisms were abundant, with 70% of individuals possessing unique haplotypes. Although 1 haplotype (#5) was common in each sound (Fig. 2), most haplotypes (>75%) were found in only 1 basin (i.e. 'private' haplotypes). Private haplotypes were not rare within sounds, however, with several shared exclusively between neighboring sites. For example, in each of Bogue Sound and Back Sound, an average of 4.0 haplotypes were shared between sites, compared to only 2.6 haplotypes shared between these 2 basins. Although Pamlico Sound shared several haplotypes with both Bogue and Back Sounds, no other sounds shared haplotypes other than haplotype 5 (Fig. 2). The midpoint-rooted haplotype tree has several distinct clusters, but no obvious phylogeographic pattern (Fig. 3). Nevertheless, many sister haplotypes tend to come from the same sound; several small clusters of 3 and 4 haplotypes also consist of those sampled from the same basin.

By nesting collection sites within sounds, calculation of conventional F-statistics (i.e. based on haplotype
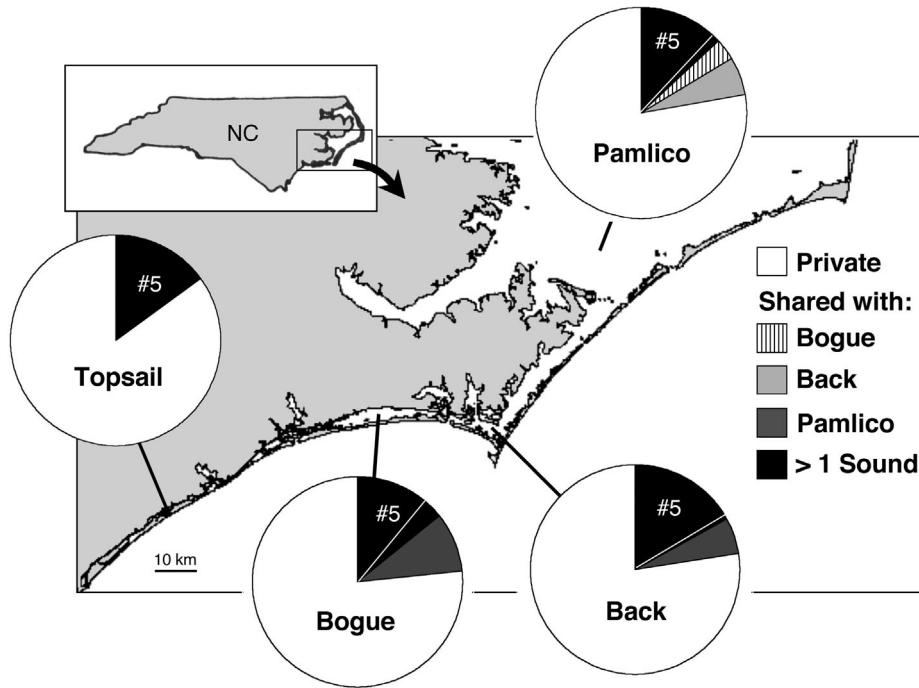
Fig. 2. *Argopecten irradians concentricus.* Frequencies of shared and private mtDNA haplotype frequencies in each of the 4 basins in North Carolina. The black sections of each pie chart indicate the haplotypes shared with more than one sound. The white lines subdivide the black sections into the sharing frequency of Haplotype 5 and of all other haplotypes, respectively, indicating the high frequency of Haplotype 5 in all 4 basins (i.e. very few other haplotypes were shared among >2 sounds)

frequencies) revealed no significant genetic structure between or within sounds (Table 2). However, an AMOVA (comparison of sequence divergences within and between sounds) with sites nested within sounds revealed significant differences among sounds at the 5% level (Table 2), indicating that haplotypes drawn from the same sound had significantly smaller sequence divergences than haplotypes drawn from different sounds. Separate pairwise AMOVAs showed a highly significant difference between adjacent Bogue and Back Sounds ($\phi_{CT} = 0.015$, p = 0.0001, $Nm = 33$) but no significant differences between Back and Pamlico ($\phi_{CT} = 0.004$, p = 0.135) and Pamlico and Bogue Sounds ($\phi_{CT} = 0.0008$, p = 0.407). The lone site in Topsail Sound (Sloop Point) was significantly differentiated from both Bogue ($\phi_{CT} = 0.028$, p = 0.0001, $Nm = 17$) and Back ($\phi_{CT} = 0.014$, p = 0.0001, $Nm = 35$) Sounds, but a pairwise AMOVA with Pamlico Sound ($\phi_{CT} = 0.006$, p = 0.210) revealed no significant differences. No analyses revealed any significant structure within sounds, and pooling of sites within sounds produced results similar to the nested designs.

Because no significant differences were detected within sounds, we elected to pool all individuals within sounds for joint estimation of Θ and Nm with MIGRATE (Table 3). This analysis showed that Θ was

greatest in Pamlico Sound, more than an order of magnitude larger than in either Bogue or Topsail Sounds, but only twice as large as in adjacent Back Sound (Table 3). Values of Nm among sounds were generally small but also generally within an order of magnitude of estimates of Nm from ϕ-statistics (see previous paragraph). Although MIGRATE indicated that point estimates of Nm ranged between 0 and 198 migrants each generation, the upper 95th percentiles for 8 of 12 com-

Table 2. *Argopecten irradians concentricus*. Population structure based on haplotype frequencies (i.e. conventional *F*-statistics; Hap. freq.) and DNA sequence divergences (i.e. ϕ-statistics; Seq. div.). AS: among sounds; AP: among populations; WP: within populations

Source of variation	df	SS	Variance components	% of variation	p-value
Hap freq					
AS	3	1.50	−0.0002	−0.04	0.469
AP	6	3.05	0.0009	0.17	0.170
WP	209	102.5	0.490	99.87	
Seq div					
AS	3	4.17	11.10	0.69	0.023
AP	6	3.71	15.28	−1.26	0.925
WP	209	26.78	724.62	100.12	

parisons were ≤2.3. Bogue and Back Sounds, which have experienced recruitment limitation, each received <4 migrants each generation from all other sounds combined.

Significant gene flow asymmetries suggested that Pamlico is potentially a 'sink' for larval transport, a pattern that is consistent with pairwise values of ϕ_{CT}: despite significant values of ϕ_{CT} among Topsail,
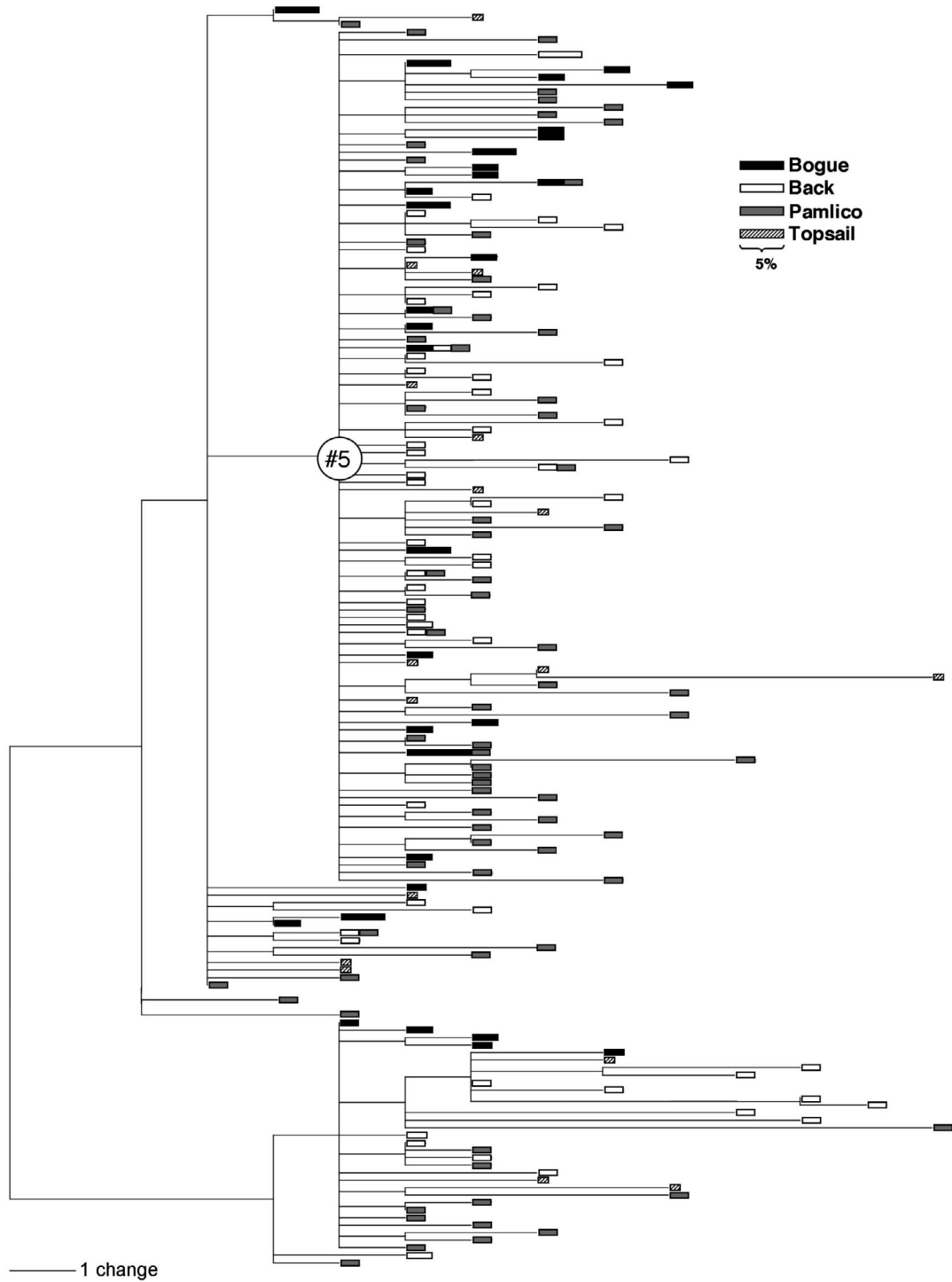

Fig. 3. *Argopecten irradians concentricus.* Midpoint-rooted parsimony network for bay scallop mtDNA haplotypes. Bars to the right of terminal branches: relative haplotype frequencies in each basin; circle: Haplotype 5 (the most common, present in all basins)

Table 3. *Argopecten irradians concentricus.* Point estimates and 95th percentiles (parentheses) for Markov chain Monte Carlo estimates of Θ and number of migrants (*N*m) among sounds

Population (Donor)	Θ	*N*m (Recipient)			
		Bogue	Back	Topsail	Pamlico
Bogue	0.048 (0.040–0.062)	–	2.0 (1.2–3.4)	0 (10^{-11}–0.3)	0.5 (0.1–1.2)
Back	0.132 (0.110–0.160)	2.3 (1.3–4.0)	–	0.6 (0.2–1.5)	0.6 (0.2–1.5)
Topsail	0.138 (0.121–1.091)	0 (10^{-13}–2.3)	28 (17–44)	–	0 (10^{-14}–2.3)
Pamlico	1.322 (0.586–4.983)	198 (134–295)	159 (133–204)	22 (8–47)	–

Bogue, and Back Sounds, Pamlico Sound was not significantly differentiated from any of these 3 basins. However, according to MIGRATE, the total number of migrants arriving in Pamlico Sound from all other sounds combined was only 379 ind. each generation. Although strongly asymmetrical, values of *N*m from MIGRATE were surprisingly similar in magnitude to estimates of *N*m from φ-statistics (see previous paragraph).

The FLUCTUATE analysis showed no evidence of recent exponential population growth in either Bogue Sound or Back Sound relative to populations in other sounds (Fig. 4). Although estimates of N_e in Bogue Sound 15 generations ago fell below 20% of the current population size at very high mutation rates ($\geq 10^{-4}$), Pamlico Sound, which was unaffected by the red tide, also showed a similar recent decline in population size when μ was assumed to be 10^{-4}. Tajima's *D* was marginally significant for 2 samples, one from Bogue Sound and one from Pamlico Sound; the *D*-statistic was marginally insignificant from a third sample

Table 4. *Argopecten irradians concentricus.* Mean pairwise differences and Tajima's *D* values. Abbreviations as in Fig. 1

Site	N	Mean pairwise differences	Tajima's *D*	p
SLP	20	14.46	0.366	0.356
M42	26	6.48	−1.613	0.046
M34	17	8.00	−0.403	0.366
OSC	35	11.05	−1.020	0.163
HAR	17	8.23	−0.533	0.320
BNK	19	6.53	−1.538	0.057
OCS	28	10.43	−1.149	0.131
OCN	18	8.76	−1.006	0.171
FRS	26	8.64	−1.250	0.109
HAT	13	7.51	−1.714	0.032

from Back Sound (Table 4). The mismatch analysis, however, indicated that the sudden expansion model could not be rejected for any of the 4 sounds (p > 0.59 for all), suggesting similar demographic history across sounds.
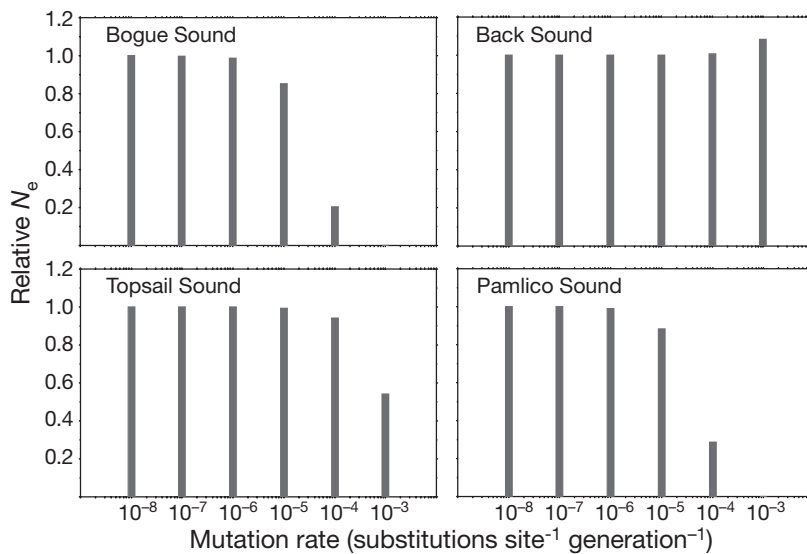

Fig. 4. *Argopecten irradians concentricus.* Estimates of effective population size (N_e, as a proportion of current population size) 15 generations prior to sample collection in 2002, across a range of possible mutation rates

DISCUSSION

Analysis of a hypervariable portion of mtDNA from central North Carolina bay scallop populations reveals an overall pattern of limited larval exchange among individual sounds, and that restrictions on gene flow are in fact sufficient to cause significant genetic differentiation between some of the basins. However, a small but significant amount of differentiation was detected at the basin scale when φ-statistics were calculated from sequence divergences among haplotypes. Most notably, ϕ_{CT} between Bogue Sound and adjacent Back Sound is highly significant despite the fact that these basins are directly connected by a relatively short waterway (Fig. 1).

With such high polymorphism at a single hypervariable locus, a Type I error could be committed when testing the significance of *F*-statistics if each basin were inadequately sampled and if the distribution of haplotype frequencies were non-normal. In our study, however, we found significance at the basin scale with ϕ-statistics, indicating that sequence divergences between haplotypes drawn from different basins were significantly larger than sequence divergences between haplotypes drawn from the same basin. Therefore, a scenario under which inadequate sampling of mtDNA diversity would lead us to gather more closely related haplotypes within basins simply due to chance seems unlikely. In fact, we found no significant differences in haplotype frequencies among basins, presumably because of the high diversity of private haplotypes within sounds (Fig. 2). Far more important sources of error in our migration estimates may instead be the high interlocus variation in the coalescent process and the fact that we only sampled a single genetic locus. We are therefore currently scoring multi-locus genotypes from microsatellite loci.

Surprisingly, the magnitude of mtDNA differentiation and inferred rates of gene flow between Bogue Sound and Back Sound were comparable to those observed between either Bogue or Back Sound and Topsail Sound, the latter being >60 km away from either Bogue Sound or Back Sound with no inshore connection (Fig. 1). Calculation of gene flow between Bogue and Back Sounds from ϕ_{CT} yielded $Nm = 33$. Although this estimate may rest on unrealistic assumptions (Whitlock & McCauley 1999, Neigel 2002), the coalescent-based estimates of Nm generated by Migrate are even smaller ($Nm \approx 2$, in either direction). Therefore, even though gene flow between Bogue Sound and Back Sound appears great enough to prevent the accumulation of large genetic differences (i.e. $Nm \geq 1$), both coalescent- and noncoalescent-based estimates of migration rates between these 2 basins appear to be ecologically insignificant, given that individual sounds historically support population sizes in the range of hundreds of thousands to several million ind. (Peterson & Summerson 1992; Peterson et al. 1996). Such low levels of migration between these 2 adjacent sounds should render them demographically independent, each unlikely to provide a significant 'rescue' effect following a mass mortality event such as was endured in 1987. Our genetic analysis is therefore consistent with the hypothesis that limited exogenous larval supply creates a potential for recruitment limitation at the spatial scale of individual basins in North Carolina (Peterson et al. 1996 and references within). Although our analysis only revealed the number of migrants that successfully recruit to, and reproduce in, any

given population, recruitment failures in 1988 in both Bogue and Back Sounds (followed by very sluggish recoveries) are not consistent with a scenario in which massive numbers of larvae are regularly transported between sounds, but experience extremely low reproductive success.

Our data support the hypothesis that, from an ecological perspective, larval exchange among the 4 basins we sampled is low. Although these results are also consistent with the additional conclusion that each sound is effectively closed with respect to recruitment, the results from MIGRATE cannot be considered conclusive on this point. MIGRATE makes the important assumption that all significant source populations contributing recruits to sampled populations are represented in the data (Beerli & Felsenstein 2001). Therefore, the existence of several unsampled, genetically distinct 'ghost' populations (Beerli 2004) that supply recruits exclusively to populations that we sampled may cause MIGRATE to incorrectly characterize sampled populations as isolated from all others (Bilodeau et al. 2005). However, given that we sampled 4 adjacent basins, 3 of which are physically contiguous, it is unlikely that the differential supply of genetically-distinct larvae among all 4 of our sampled populations was responsible for generating a pattern of isolation among sampled populations. Although a situation in which a single source population supplies recruits to several or all sampled populations seems more likely in marine systems (Bilodeau et al. 2005), simulations have demonstrated that this scenario leads to overestimation of gene flow among sampled populations (Beerli 2004).

Circulation patterns in the vicinity of Beaufort Inlet can potentially explain low levels of larval transport between the Bogue and Back Sound populations (Fig. 5). Physical transport in the vicinity of barrier island inlets is strongly influenced by tides, local geometry, and bathymetry, which create a characteristic circulation pattern (e.g. Stommel & Farmer 1952,
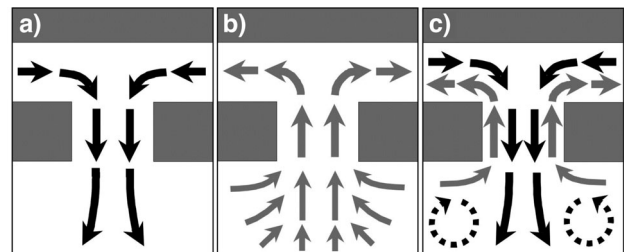

Fig. 5. Schematic of physical transport through an idealized barrier island inlet. (a) Ebb tide water flow from the landward to seaward side of the inlet. (b) Flood tide. (c) Residual tidal circulation (see 'Discussion'). Black and gray: outgoing and incoming currents, respectively; dotted: eddies

Zimmerman 1981, Luettich et al. 1999, Brown et al. 2000, Hench et al. 2002) that is expected to hydrographically isolate adjacent water bodies on the landward side of barrier islands (Luettich et al. 1999, Hench & Luettich 2003). On an ebb tide (Fig. 5a), a jet flows straight out of an inlet into offshore waters, but on the following flood tide, water converges towards an inlet from all directions, creating longshore flow towards the inlet (Fig. 5b). The physical dynamics of these flow patterns generate a dynamic 'wall' down the center of the inlet (perpendicular to the shoreline) that presents a significant barrier to cross-inlet exchange (Hench & Luettich 2003).

On the seaward side of the inlet, the differences between ebb and flood tide currents generate counter-rotating eddies on either side of the offshore flow (Fig. 5c) that entrain passive particles transported onto the shelf. The presence of these eddy currents tend to cause propagules expelled onto the shelf during an ebb tide to be returned to the same side of the inlet on a subsequent flood tide (Luettich et al. 1999). Therefore, if larvae are passive dispersers, cross-inlet dispersal should be low. Furthermore, if larvae transported into shelf waters on an ebb tide are not carried away in winddriven alongshore currents, they are expected to be predominantly returned to their basin of origin on the landward side of the inlet as a result of the retentive eddies formed on either side of an inlet. Particle-tracking models specifically developed for Beaufort Inlet in fact confirm both the relatively infrequent transport of passive propagules between Bogue and Back Sound and their consistent return to their basin of origin when exported onto the shelf (Luettich et al. 1999).

The results from our genetic study are therefore consistent with model expectations that inshore tidal currents likely play a major role in structuring bay scallop populations in this region, potentially isolating populations on opposite sides of Beaufort Inlet. The role of wind-driven currents on the continental shelf is less clear. In the fall, when bay scallops spawn, winds along the North Carolina coast blow toward the southwest, potentially carrying larvae south along the coast (Werner et al. 1999). Our analyses of genetic data, however, suggest a gene flow asymmetry in the opposite direction (Table 3), indicating that Pamlico Sound is a potential sink for larval transport. This surprising result may indicate that few larvae advected onto the continental shelf are successfully transported between basins in alongshelf currents, and that successful inter-basin transport is achieved via waterways that connect sounds. Although future modeling efforts can quantitatively assess the relative importance of inshore versus alongshelf transport, if our estimates of Nm are accurate, larval exchange between basins appears

demographically trivial, regardless of the particular dispersal pathways taken.

A recent decline in bay scallops, particularly within Bogue Sound, could in itself reduce connectivity by decreasing the number of larvae produced and increasing distances between populations. The most reliable sites to obtain scallops in Bogue Sound tend to be at the western end of the basin (i.e. our Marker 42 and Marker 34 sites) where grass beds are extensive; persistent populations at the eastern end of the basin, those that are most likely to receive larvae from adjacent Back Sound (and whose larvae are most likely to be transported to Back Sound), are rare, often containing few mature individuals during the fall spawning season despite high recruitment over the winter (Ambrose et al. 1992, P. B. Marko pers. comm.). Populations of bay scallops are also subject to intensive predation by schooling cownose rays that enter North Carolina basins during their south migration along the East Coast of the US, arriving in late summer and feeding on benthic invertebrates just prior to the spawning season (Peterson et al. 2001 and references within). The impacts of these predators tend to be greatest in the vicinity of barrier island inlets and could be an important natural factor influencing patterns of connectivity between basins.

Despite no significant genetic differentiation between the Back Sound population and the historically more stable population in Pamlico Sound, the MIGRATE analysis suggests that even though gene flow may be great enough to prevent genetic differentiation from rising to a level that can be detected by ϕ-statistics, migration rates are too small to cause any coupling of the population dynamics of these 2 basins. As with the Bogue Sound population, Back Sound was impacted by the 1987 red tide, experiencing a complete recruitment failure the following year. Some sites within Back Sound, however, showed pre-red tide levels of recruitment as few as 2 yr after the mortality, leading to an eventual, albeit slow, recovery in Back Sound (Peterson & Summerson 1992). The recovery of the Back Sound population may have been facilitated by larval supply from Core and Pamlico Sounds, given that Back Sound is essentially contiguous with these 2 basins, and that the Core and Pamlico bay scallop populations suffered no direct mortality during the 1987 red tide (Summerson & Peterson 1990). However, the results from MIGRATE are inconsistent with this hypothesis: if Back Sound recovery was fueled primarily by larval supply from Core and Pamlico Sounds, we would expect far greater average rates of gene flow between Back and Pamlico Sounds.

Because we did not analyze samples from Core Sound, we cannot rule out the possibility that there is high ecological connectivity between Back Sound and

southern Core Sound (explaining the recovery of Back Sound populations); southern Core Sound and Back Sound may be an isolated unit with respect to northern Core Sound and Pamlico Sound. A previous comparison of mtDNA found no genetic differentiation between Bogue Sound and Core Sound (Bologna et al. 2001), but these results were based on relatively smaller sample sizes and did not employ the hypervariable section of mtDNA used in this study. Although modeling efforts have not been extended beyond Beaufort Inlet, the presence of Drum Inlet, approximately halfway up Core Sound, may create a similar transport barrier. However, because the width of the sound behind Drum Inlet is much greater than the inlet's dynamic length, the dynamic wall effect described earlier could be minimal (Hench et al. 2002, J. Hench pers. comm.). Alternatively, a greater distance between Back Sound and Pamlico Sound may also be a factor explaining the absence of high connectivity between Back Sound and Pamlico Sound, especially if larval transport on the landward sides of barrier islands is governed primarily by tidal flow. Even in the absence of a transport barrier in the vicinity of Drum Inlet, bay scallop larvae retained on the landward sides of barrier islands may experience very little net movement.

Such low levels of gene flow between Bogue and Back Sounds are also surprising, given that restoration experiments in the early 1990s may have genetically 'polluted' Bogue Sound populations with scallops transplanted from Back Sound. The relocation of approximately 385,000 mature adults from Back Sound to western Bogue Sound between 1992 and 1994 (to the same localities that we sampled in 2002) was followed by apparent increases in recruitment of bay scallops to grass beds in Bogue Sound (Peterson et al. 1996). Similar restoration efforts in other regions involving genetically distinct bay scallops (Wilbur et al. 2005) detected no change in haplotype frequencies in resident populations following transplantation, suggesting that the genetic effects of transplantation may be difficult to distinguish, even in populations depleted by overfishing and natural catastrophes. The number of introduced individuals required to alter haplotype frequencies ultimately depends on the number introduced relative to the size of the resident population that is subject to restoration efforts. Although harvest data indicate that scallop populations in Bogue and Back Sounds were grossly depressed following the 1987 red tide, actual effective population size across the entire basin is probably difficult to ascertain. Genetic diversity in all 4 sounds is in fact high (Tables 2 & 4), and our estimates of past population growth show no clear evidence of severe population bottlenecks exclusive to either Bogue or Back Sounds.

Both the FLUCTUATE and mismatch analysis are likely invalid, because they implicitly assume monophyly of alleles or haplotypes within each sample, a condition clearly violated by our data set (Fig. 2). Alternatively, given that Bogue and Back Sound populations have undergone additional declines and recoveries since the red tide event of 1987, genetic drift may have since reestablished the small amount of genetic differentiation that we are able to detect with F_{ST}.

In summary, our mtDNA data indicate that although gene flow may be great enough to prevent the accumulation of large genetic differences between most basins, rates of larval transport among the basins we considered are likely too small to be ecologically significant, a result consistent with patterns of mortality and recruitment failure in North Carolina during the late 1980s. The greatest potential source of error in our study likely arises from the fact that we only surveyed mtDNA diversity; confirmation of these patterns awaits similar analyses of physically unlinked nuclear genetic markers, which are currently in progress. Use of multilocus markers will also allow the use of assignment tests to identify the population of origin of the recruits gathered from basins where we have previously sampled adults. From the perspective of fishery management, such low levels of gene flow likely explain the apparent decoupling of population dynamics of adjacent water bodies, supporting the idea that individual basins should be managed as independent units. Although we cannot yet address patterns of connectivity within Core Sound and between Core Sound and Back sound (2 historically important regions for commercial harvesting), our data provide no indication of any indirect connection between Pamlico and Back Sounds via the Core Sound population.

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