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Mitochondrial DNA Variation in Natural and Cultured Populations of the Bay Scallop, Argopecten irradians (Lamarck) and the Calico Scallop, Argopecten gibbus (Dall)

Sandra Grace Blake
College of William and Mary - Virginia Institute of Marine Science

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MITOCHONDRIAL DNA VARIATION IN NATURAL AND CULTURED POPULATIONS OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK), AND THE CALICO SCALLOP, *ARGOPECTEN GIBBUS* (DALL)

A thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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Sandra G. Blake

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This thesis is submitted in partial fulfillment of

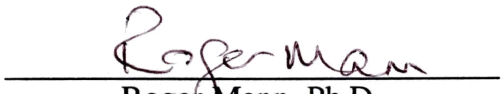
the requirements for the degree of

Master of Arts

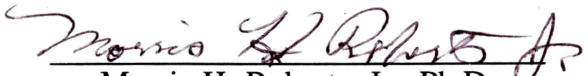
Sandra G. Blake

Approved, May 1994

John E. Graves, Ph.D
Committee Co-chair/Co-advisor

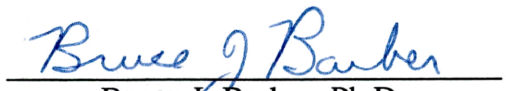


Roger Mann, Ph.D.
Committee Co-chair/Co-advisor



Morris H. Roberts, Jr., Ph.D.

Bruce Grant, Ph.D.



Bruce J. Barber, Ph.D.
University of Maine
Orono, Maine

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ABSTRACT

The genus *Argopecten* includes two species found on the Atlantic and Gulf coasts of the United States. *Argopecten irradians* Lamarck, the bay scallop, is a short-lived hermaphroditic species which grows rapidly and reaches reproductive maturity at a relatively young age. The three commonly recognized subspecies of *A. irradians* have been distinguished on the basis of shell morphology, with diagnostic features including rib count, valve color and valve inflation. Overlap in these features complicates identification, and no clear geographic boundaries exist to delineate the ranges of the subspecies.

The calico scallop, *A. gibbus* Dall, exhibits a life history very similar to that of *A. irradians*, but dwells in a sandy-bottom offshore habitat rather than the shallow grass-bed regime of the bay scallop. While their different habitats may effectively isolate the two species, the range of *A. gibbus* overlaps considerably with that of the southern bay scallop subspecies, *A. i. concentricus* Say. *A. gibbus* has been shown to differ morphologically from *A. i. concentricus*, but the high degree of variation and overlap in the distinguishing characters complicates identification.

The primary objective of this study was to assess the variation in the mitochondrial DNA of the subspecies of bay scallop and of the calico scallop, in order to supplement the morphological data used to distinguish them taxonomically. Secondly, variation in cultured populations was compared to that in samples approximating natural set, in order to assess the effects of hatchery breeding, and to give clues to the origin of broodstock where this was not known.

Restriction site variation in the mitochondrial DNA of *Argopecten irradians* ssp. was surveyed for four populations sampled from the U.S. Atlantic coast and the Gulf of Mexico. A population from North Carolina was resampled one year after the first collection, to provide a measure of temporal variation within a population. Samples from two cultured populations were also examined. Haplotype diversity was high, with 51 haplotypes revealed among a total of 198 bay scallops screened with 8 endonucleases. Nucleotide sequence divergences among populations ranged from 0.00 percent, for the temporal samples, to 0.33 percent, when corrected for within-sample variation. Tests for heterogeneity indicated that no two of the geographically separated populations shared a common gene pool, and neither of the cultured populations shared a common gene pool with any other population.

An UPGMA cluster analysis based on nucleotide sequence divergence suggests that bay scallops in North Carolina are more closely related to those from the Florida Gulf than to those in New England, though only slightly. This is in accordance with morphological features, which have traditionally been used to place North Carolina and Florida populations in one subspecies, and New England populations in another. The same UPGMA analysis placed the two cultured samples in a cluster with that from New England, suggesting that bay scallops of the northern subspecies formed a large component of the broodstock for these cultured populations.

Two populations of calico scallop, from the Atlantic and Gulf coasts of Florida, were sampled and analyzed similarly. In these, haplotype diversity was high, with 6 restriction endonucleases revealing 19 different haplotypes in 51 individuals. Nucleotide sequence divergence between the populations was low, however, when corrected for within-sample variation, and a test for heterogeneity indicated that the two populations do share a common gene pool. No restriction fragments or patterns were common to the bay and calico scallop, reinforcing their classification as separate species.

MITOCHONDRIAL DNA VARIATION IN NATURAL AND CULTURED POPULATIONS OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK), AND THE CALICO SCALLOP, *ARGOPECTEN GIBBUS* (DALL)

INTRODUCTION

Paleohistory and Classification.

In a definitive publication, Waller (1969) detailed the evolution of the "*Argopecten gibbus* stock", which includes both living and extinct members of the genus *Argopecten*. Among the extant members of the stock are the bay and calico scallops, *A. irradians* Lamarck and *A. gibbus* Dall, common in the Western Atlantic and Gulf of Mexico; the less common *A. nucleus* Born, of the Caribbean and southern Florida; and two scallop species common in the eastern Pacific, *A. purpuratis* Lamarck and *A. circularis* Sowerby (Waller, 1969).

Early classification efforts by Dall (1898, as cited by Waller, 1969) considered *A. irradians* ssp. and *A. nucleus* as intergradational forms of *A. gibbus*, with valve color, convexity and rib number as distinguishing features. Dall later separated the taxa into three specific groups (Dall, 1925), but his earlier system was followed by many researchers through the 1960's (Waller, 1969). This has led to some uncertainty in the interpretation of older faunal lists. *A. irradians* ssp. and *A. gibbus* were not commonly distinguished from each other until Dodge (1952) summarized the morphological differences between the three extant subspecies of *A. irradians*, and enumerated the morphological and ecological characteristics that distinguish *A. irradians* from *A. gibbus*. Sastry (1962) expanded upon this by providing quantitative measurements for morphological features that distinguish the two species, and further emphasizing the differences in their habitats. Clarke (1965) performed a similar quantitative analysis for the three recognized subspecies of *A. irradians*.

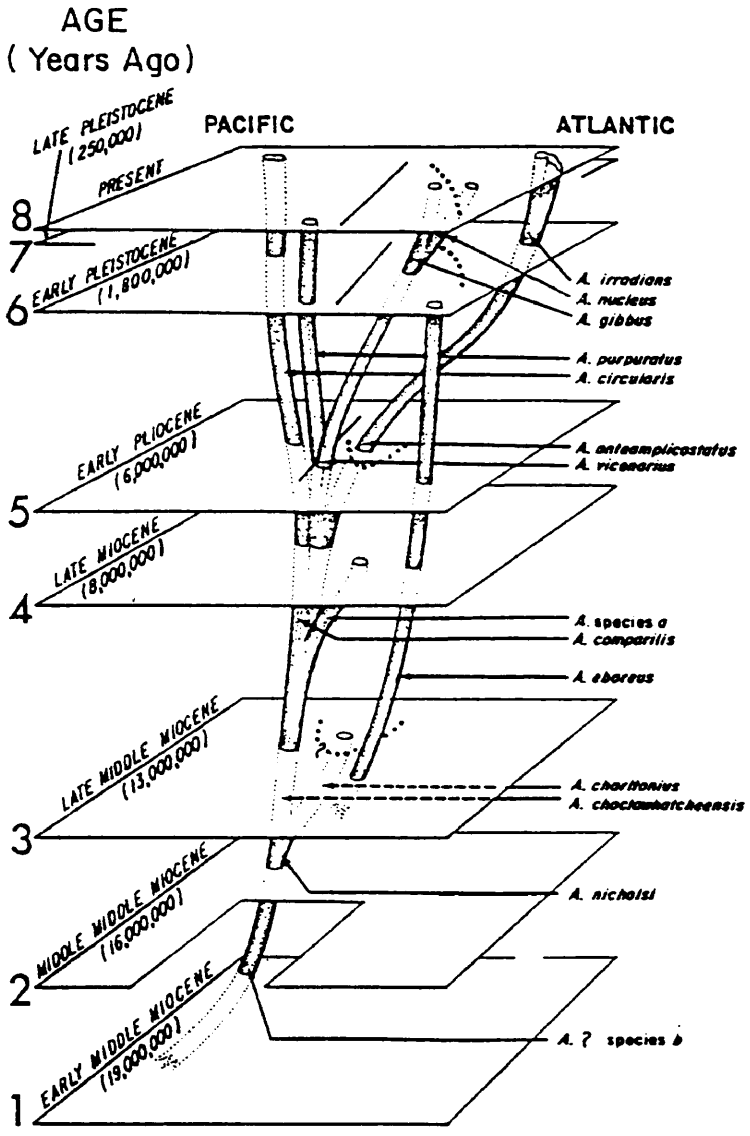
In literature published prior to 1970 it is common to see members of the *Argopecten* species group referred to with the generic synonyms, *Aequipecten,*

Plagioctenium and *Pecten*. This confusion persisted until Waller (1969) standardized the nomenclature, declaring the generic name of American species related to *A. gibbus* to be *Argopecten*.

Working through the fossil record, and using the morphological and ecological relationships of the living species as a model, Waller (1969) sought to deduce the evolutionary relationships of the ancestral members of the *A. gibbus* stock. He measured 70 morphological features in fossilized individuals collected from the upper Cenozoic strata of the U.S. Atlantic and Gulf Coastal Plains. The postulated phylogeny showing the Cenozoic evolution of the stock, provides a useful graphic representation of the relationships among the extant species and subspecies of the genus *Argopecten* (Fig. 1). These are thought to have arisen from a hypothetical *Argopecten* species b, which evolved phyletically through a series of now-extinct species. *A. compalaris*, the presumed ancestor of the Pacific lineage, was apparently "broadly adapted and widely distributed, living in bays, sounds and open marine waters in the western Atlantic, Gulf of Mexico and Caribbean and probably extending through seaway passages to the Pacific..." The primitive bay scallop, *A. anteamplicostatus*, was probably restricted to bays and sounds like its living descendents, and was present in the eastern Americas by the late Miocene. *A. vicenarius* is the presumed ancestor of the calico scallop, and was likewise probably limited to open marine waters. Unlike the ancestral bay scallop, this species appears to have radiated into the Pacific region, to give rise to *A. purpuratis*. The extant tropical species, *A. nucleus*, is inferred to have split from *A. gibbus* in the mid-Pleistocene, then converged morphologically on the bay scallop form. The separate *A. eboreus* lineage was common in the Miocene and Pliocene, but in this scheme, gave rise to no other species and was extinct by the early Pleistocene.

It has been asserted (Waller, 1969) that the evolution of *Argopecten*,

Fig. 1 Phylogeny of the *Argopecten gibbus* stock (from Waller, 1969).



including speciation and phyletic change, has been faster in the Atlantic than in the Pacific. The living Pacific species appear to be quite primitive, resembling the Atlantic species of the late Miocene and early Pliocene. This may have resulted from coastal tectonic activity in the Pacific, which destroyed the island barriers so important to the development of genetic isolation and speciation in the Atlantic stock.

Intraspecific variation in *A. irradians*, specifically in terms of geographic trends in morphological features, have been observed to vary from north to south (Waller, 1969). These trends may be indicative of local adaptation to latitudinally varying environmental conditions, such as temperature. The plasticity of these phenotypic features, that is, that they may be environmentally rather than genetically controlled, has not been rigorously tested. These geographic trends in morphological data were not observed in *A. gibbus* over its latitudinal range (Waller, 1969).

Waller (1969) noted that almost all Pleistocene bay scallop samples appear to resemble the southern subspecies, *A. i. concentricus*, on the basis of shell dimensions and rib count. Neither *A. i. irradians*- nor *A. i. amplicostatus*-type individuals are apparent until the early Holocene, suggesting that *A. i. concentricus* may be ancestral among the subspecies.

Life Histories.

Argopecten irradians and *A. gibbus* are highly prolific annual spawners, producing pelagic larvae which remain in the water column for 10 to 19 days (Allen and Costello, 1972; Fay et al., 1983). With an average longevity of 12 to 16 months, most bay scallops spawn only once before dying (Fay et al., 1983). Calico scallops live somewhat longer – 18 to 20 months on average – and may spawn twice (Allen and Costello, 1972). In both species, the young of a given year mature to form the bulk of the broodstock in the succeeding spawning season (Marshall, 1960).

A. irradians and *A. gibbus* are hermaphroditic, and an individual may release both eggs and sperm in a single spawning period (Peters, 1978). Gametogenesis is controlled primarily by water temperature and food availability (Sastry, 1963), while spawning is induced by rapid fluctuations in the environment. *A. irradians* and *A. gibbus* are both broadcast spawners, releasing eggs and sperm into the water column for fertilization (Fay et al., 1983).

In the bay scallop, top-shaped trochophore larvae develop from the swimming gastrulae and are transformed into feeding, shelled veligers by 48 h. post-fertilization (Sastry, 1965). The veliger stage lasts about 10 days, then the onset of metamorphosis is indicated by the appearance of the foot. This is followed by loss of the velum, movement of the mouth to an anterior, dorsal position, and the development of gills (Sastry, 1965). After metamorphosis, larvae settle and attach to the substratum by byssus threads. Here they grow rapidly to achieve the ribbed adult form by 30-40 days post-fertilization (Sastry, 1965). Once the valves are formed, the number of ribs they contain remains constant (Sastry, 1965).

The range of movement by larvae that may occur during the two-week pelagic stage is not precisely known. For the calico scallop, it has been proposed that this range may be quite extensive. Kirby-Smith (1970) suggested that juvenile calico scallops found off the North Carolina coast may be recruited from populations spawning on the Atlantic coast of Florida. This would explain the highly transient nature of North Carolina calico scallop populations, which may be very large in one season only to disappear completely in the next (Peters, 1978). The basis for this suggestion was the observation that calico scallop juvenile abundances off North Carolina are not correlated with the spawning of adults in the same populations. Larvae generated in the earlier, Florida spawnings, then, may be entrained in the Gulf Stream and carried northward, to settle on beds off North Carolina (Kirby-Smith, 1970). The

hydrographic conditions that permit this may be sporadic, resulting in the apparent declines in population size.

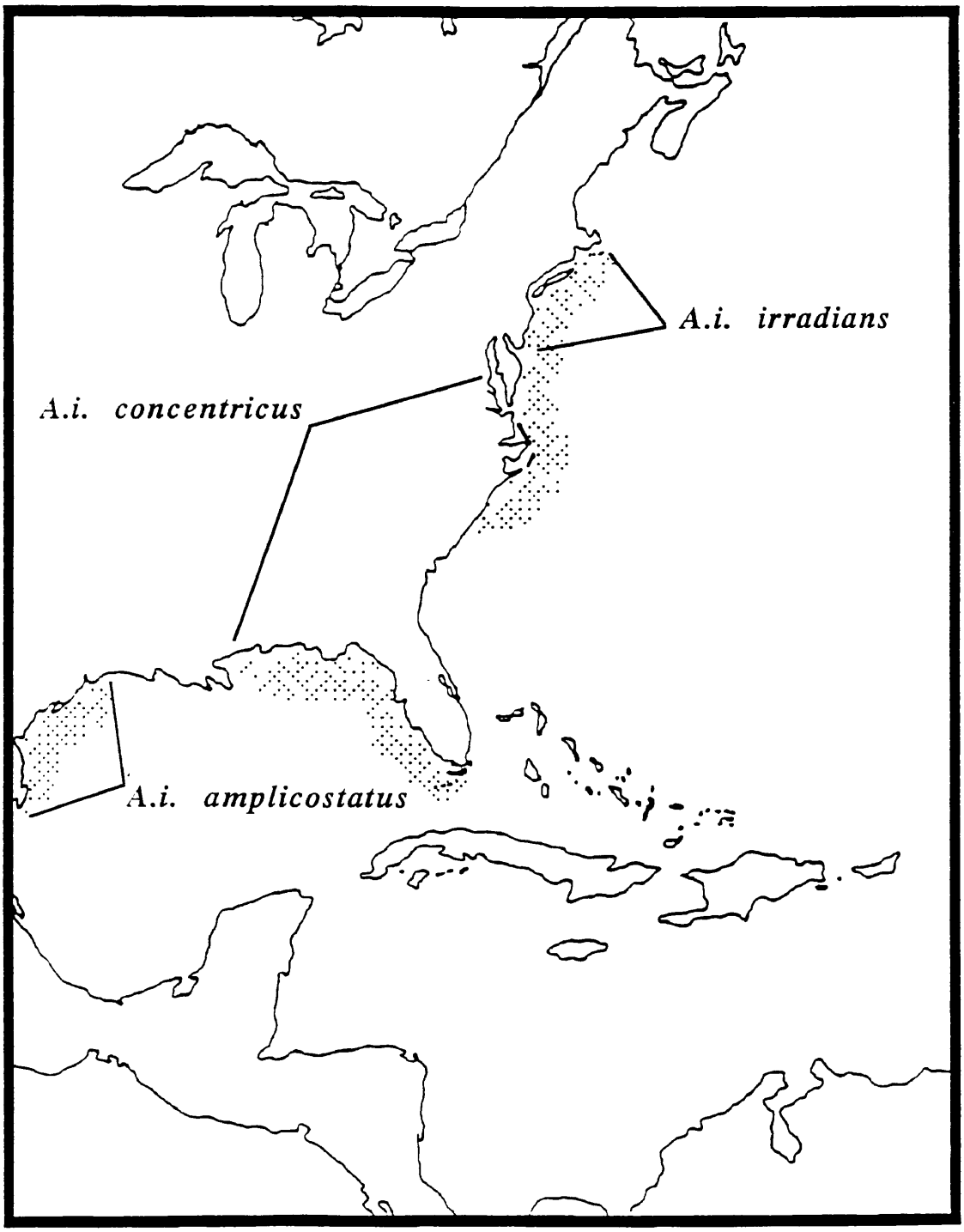
This type of long distance dispersal is not likely to occur for the bay scallop, whose habitat is restricted to near-coastal grass beds and the shelter of barrier islands. A unidirectional current with a surface velocity of one knot could feasibly transport a planktonic larva 240 miles in a 10 day period, possibly sweeping it from one bay or estuary into another with suitable habitat (Waller, 1969). However, it is thought that most bay scallop larval recruitment depends on tidal circulation which acts to retain the planktonic larvae in a habitable bay or estuary (Marshall, 1960). The open waters of the Gulf Stream do not commonly provide transport to suitable habitat. These features may in part explain the isolated character of bay scallop populations, and the more continuous pattern of distribution exhibited by the calico scallop.

Atlantic and Gulf Populations of Argopecten irradians.

The bay scallop is distributed along the east coast of the United States from Cape Cod, Massachusetts to Laguna Madre, Texas. It has been reported from the coast of Mexico and as far south as Cartagena, Colombia (Fig. 2) (Clarke, 1965). The distribution of bay scallops is not continuous in this range, and there is a large gap on the Atlantic coast south of North Carolina, in which no natural populations are present. Only one unequivocal record of the species exists for South Carolina (Clarke, 1965), and the species is assumed to be completely absent from the coasts of Georgia and eastern Florida.

The bay scallop distribution is limited to estuarine and near-coast environments, with protected bays forming the primary habitat (Fay et al., 1983; Heffernan et al., 1988).

Fig. 2 Geographic distributions of the subspecies of the bay scallop, *Argopecten irradians.*



Marshall (1960) defined certain hydrographic conditions found to be common to estuaries containing bay scallops: the ratio of tidal volume to river flow is high, so that higher salinities are maintained; the basin is shallow; and the circulation in the basin is such that planktonic larvae are retained for reseeding and adequate food supplies are drawn from offshore waters. It would appear, however, that these conditions exist in many areas where bay scallops are not commonly found, including sites in the Chesapeake Bay and along the coasts of Virginia, Maryland and Delaware (Marshall, 1960). Some of the Georgia sounds would also appear to possess the required conditions (Marshall, 1960), and indeed, *A. i. irradians*, the northern bay scallop, has been successfully cultivated in the coastal waters of Georgia (Heffernan et al., 1988). It has been suggested that the absence of *A. irradians* from these regions may be due to the lack of submerged aquatic vegetation necessary for recruitment and growth (Heffernan et al., 1988). The once-abundant populations of Virginia's Eastern Shore are now virtually non-existent, their disappearance attributed to the loss of *Zostera* beds in the bays along that coast (Marshall, 1960; Castagna and Duggan, 1971). Other potentially-suitable regions on the Atlantic coast, such as the lagoonal estuaries of Florida's Indian River, may have salinity regimes that periodically range below the 14 ppt tolerance level (Belding, 1910) of the bay scallop (Chew, 1956).

The characters used to distinguish the three subspecies of bay scallop are summarized in Table 1, and a diagnostic key is provided in Appendix 1. Of the three subspecies, the northern *A. i. irradians* is characterized by the most compressed shell morphology (Fig. 3). It possesses a rib count of 17 to 18, and is found from Cape Cod south to an area between New Jersey and Maryland, where it may intergrade with *A. i. concentricus* (Clarke, 1965).

A. i. concentricus has 18 or more ribs, and a more inflated shell shape than *A. i. irradians* (Fig. 4). Its range along the Atlantic coast spans the region between New

Table 1. Characters differentiating the subspecies of *Argopecten irradians* and *Argopecten gibbus* (adapted from Clarke, 1965 and Sastry, 1962)

Feature	*A. i. irradians*	*A. i. concentricus*	*A. i. amplicostatus*	*A. gibbus*
# of ribs	17-18	18-24	12-17	18-23
shape of ribs	low, roundish	elevated, roundish to squarish	high squarish to slightly roundish	flat, squarish
color of lower valve	slightly lighter than upper	lightest, commonly white	commonly all white	few reddish mottlings
color of upper valve	gray-brown with indistinct darker markings	dull bluish gray to brown	similar to concentricus	reddish mottlings on cream background
relative inflation of valves	most compressed	upper valve flatter than lower, more gibbous than irradians	more gibbous than concentricus	both valves deep, gibbous

Fig. 3 Representative valves of *A. irradians irradians*.

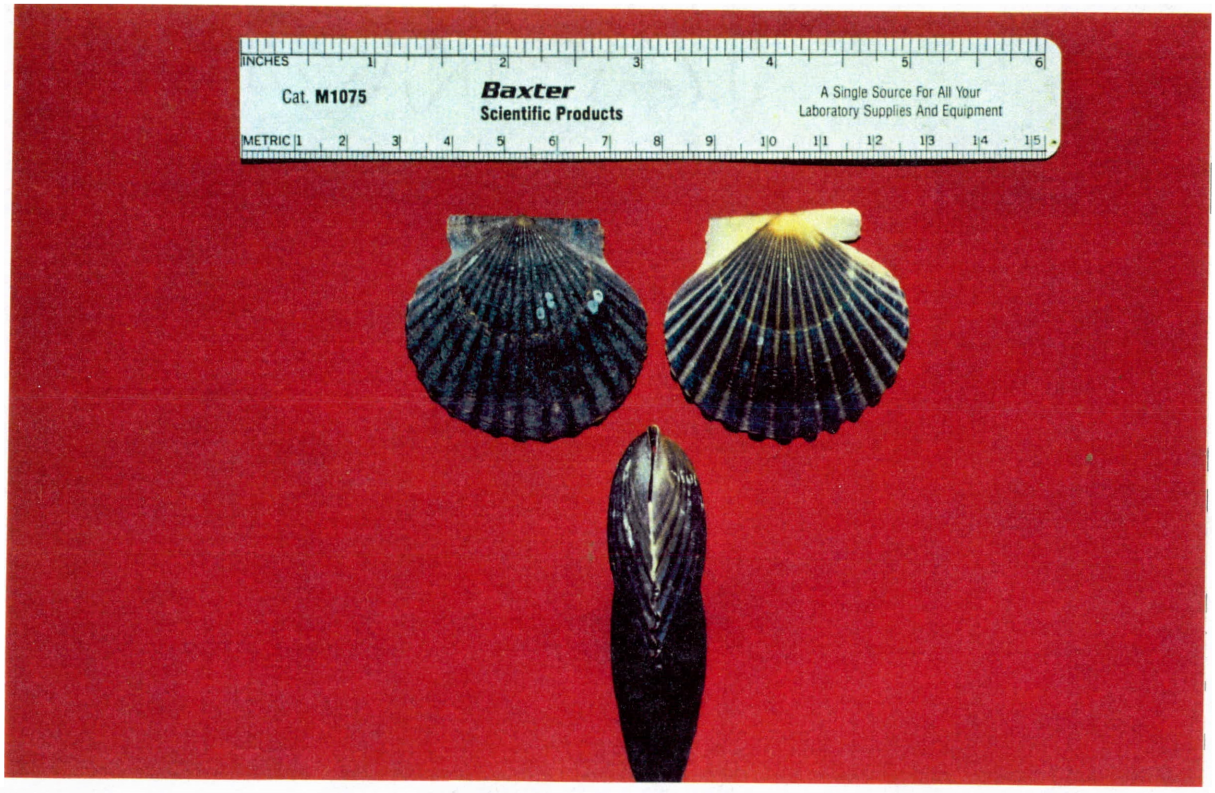
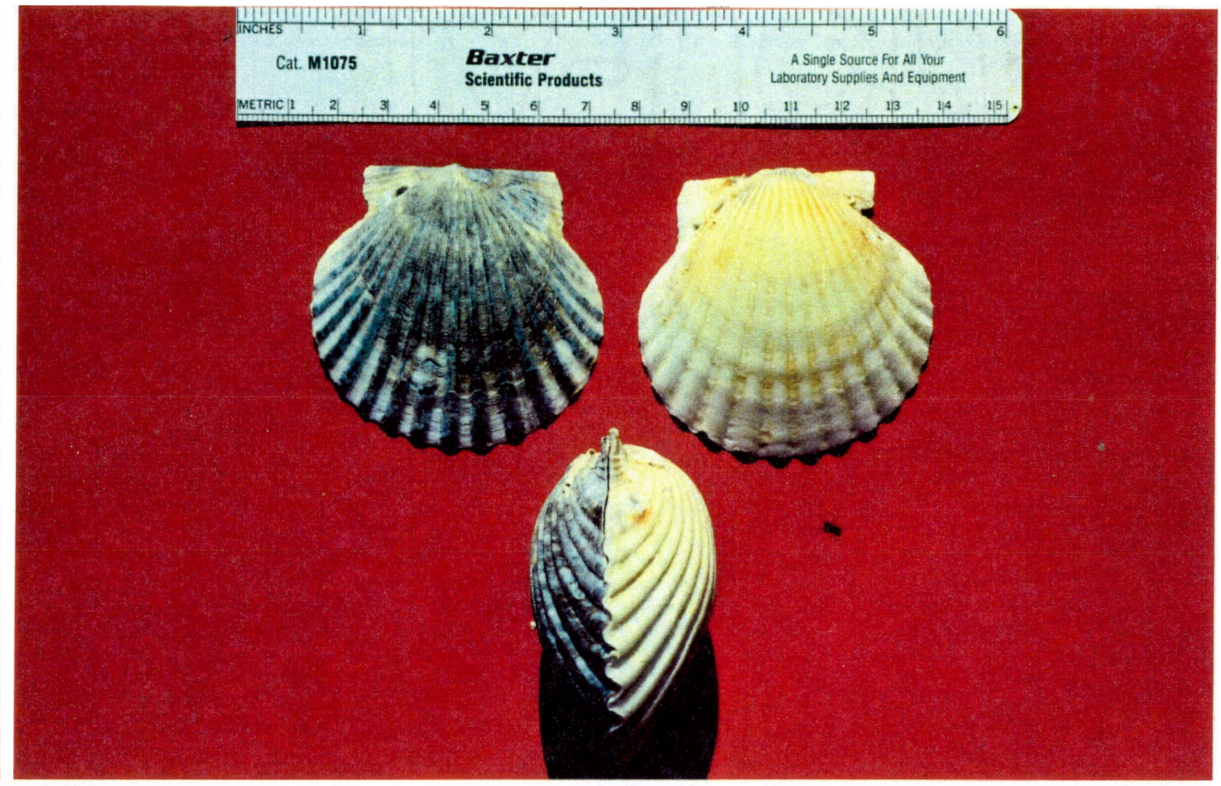


Fig. 4 Representative valves of *A. irradians concentricus*.



Jersey and North Carolina, though remaining healthy populations appear to be limited to coastal North Carolina. *A. i. concentricus* are found in abundance, however, on the Gulf coast, extending from Florida Bay to a region possibly as far west as eastern Texas (Clarke, 1965; Marelli et al., in press).

It has been suggested that populations designated *A. i. concentricus* in North Carolina may in fact be more closely related to those of the northern subspecies than to the Gulf populations with the same taxonomic designation (Marelli et al., in press). Allozyme data place individuals of a North Carolina population of *A. i. concentricus* intermediate to the well-clustered *A. i. irradians* and Florida *A. i. concentricus* (Marelli et al., in press) (Fig. 5). Genetic-distance values (Nei's D) (Nei, 1972) among the subspecies of *A. irradians* were found to range between 0.047 and 0.188, with increased divergences between populations separated by greater geographic distance (Table 2, Fig. 6). The genetic distance between bay scallops from western Florida and North Carolina was no smaller than that between the North Carolina population and a sample of *A. i. irradians* from Massachusetts (Marelli et al., in press). This is consistent with observed clinal variation in the species, and it suggests that no clear geographic boundaries exist to delineate the ranges of *A. i. irradians* and *A. i. concentricus* .

The third bay scallop subspecies, *A. i. amplicostatus* Dall is found in the Gulf of Mexico between Matagorda, Texas and Cape Rojo, Mexico (Clarke, 1965). Disjunct populations have also been reported from the coasts of Cartagena, Colombia and Miami, Florida (Clarke, 1965) This subspecies is characterized by the fewest ribs, 12 to 18, and the most gibbous shell morphology (Fig. 7). The ecology of *A. i. amplicostatus* has been little studied, probably because it does not have the commercial importance of the other two subspecies. The degree of intergradation between this and the other subspecies, and the extent to which they are genetically distinct is not known.

Fig. 5 Dendrogram from UPGMA cluster analysis of populations of *A. irradians* ssp. (from Marelli et al., in press). Scale is Nei's genetic distance (Nei, 1972). MA: Martha's Vineyard, Massachusetts; CO: Niantic River, Connecticut; LI: Northwest Harbor, New York; NC: Core Banks, North Carolina; SJ: St. Joseph Bay, Florida; HS: Homosassa Bay, Florida; RK: Rabbit Key Basin, Florida.

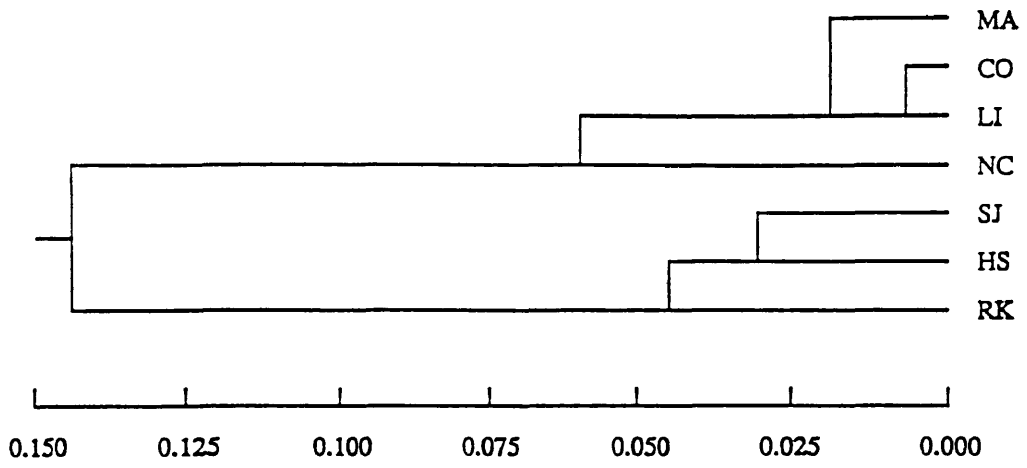


Table 2. Nei's genetic distances (Nei, 1972) between sampled populations of *A. irradians* ssp. (from Marelli et al., in press). MA: Martha's Vineyard, Massachusetts; CO: Niantic River, Connecticut; LI: Northwest Harbor, New York; NC: Core Banks, North Carolina; SJ: St. Joseph Bay, Florida; HS: Homosassa Bay, Florida; RK: Rabbit Key Basin, Florida.

	MA	CO	LI	NC	SJ	HS	RK
MA	0						
CO	0.0124	0					
LI	0.0231	0.0064	0				
NC	0.0814	0.0490	0.0470	0			
SJ	0.1530	0.1400	0.1524	0.0992	0		
HS	0.1663	0.1439	0.1655	0.0935	0.0298	0	
RK	0.1712	0.1504	0.1883	0.0875	0.0536	0.0347	0

Fig. 6 Florida sampling sites for *A. irradians* ssp. (from Marelli et al., in press).

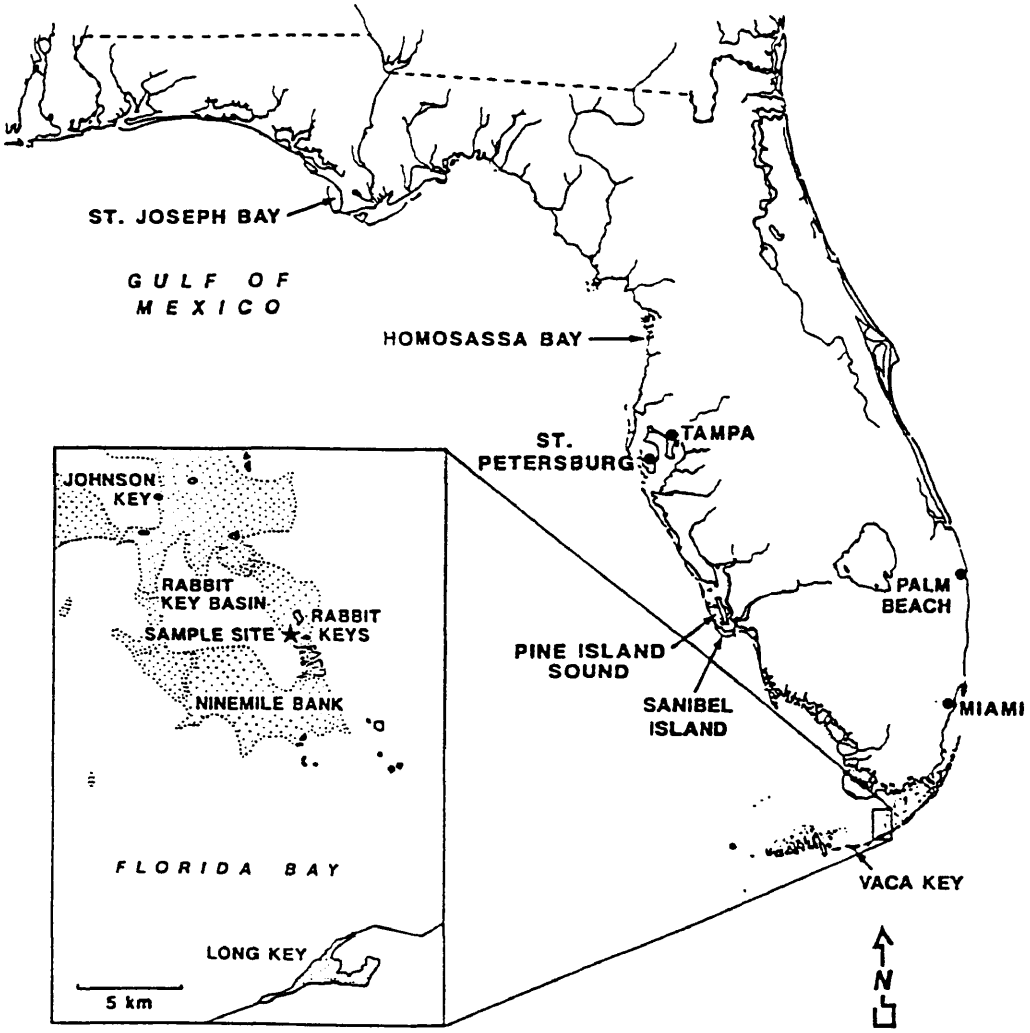
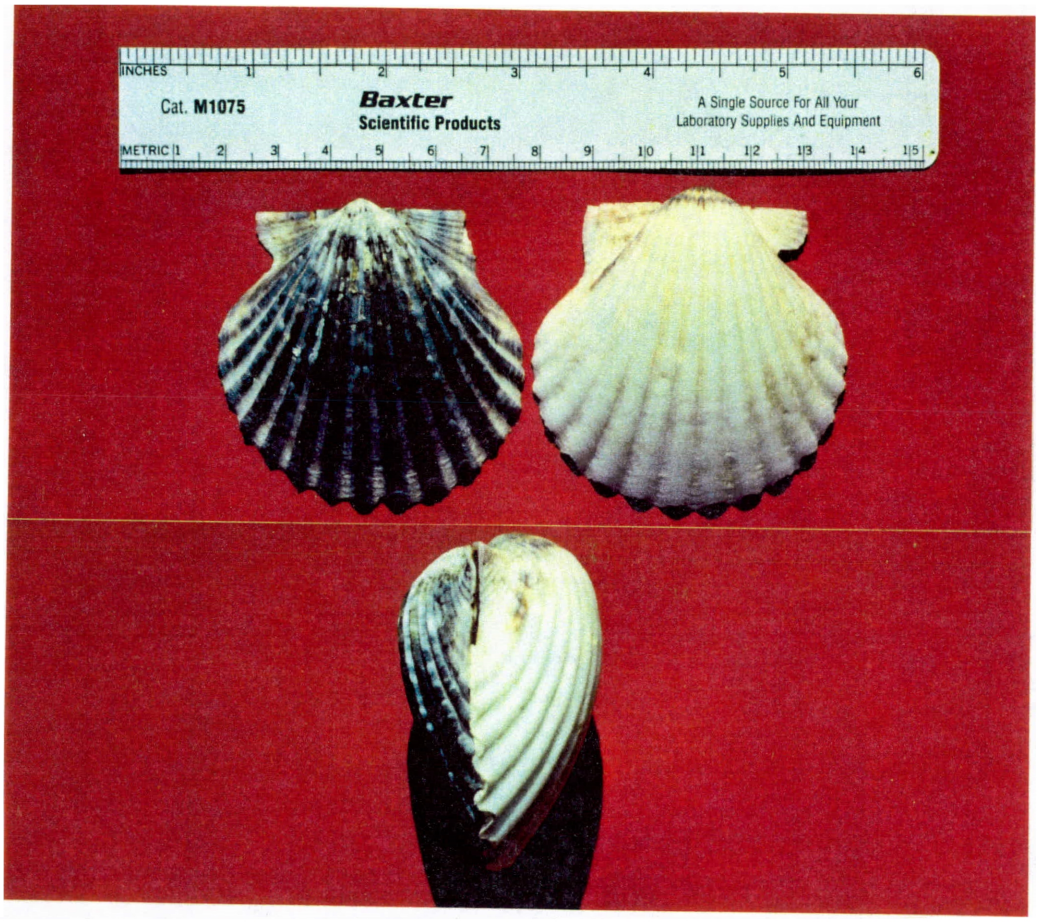


Fig. 7 Representative valves of *A. irradians amplicostatus*.



In 1987, the existence of a fourth *A. irradians* subspecies, *A. i. taylorae*, was proposed by Petuch (1987). The holotype for the subspecies was collected in 10 ft. of water, on a solid *Thalassia* bottom, in the Rabbit Key Basin of Florida Bay in 1983 (Petuch, 1987). It is described as "thin, fragile, moderately inflated, with the lower valve slightly more convex than the upper valve;...rib count ranging from 23-25; color of upper valve composed of mottled streaks and patches of dark brown, pink yellow and white on khaki green and gray background;...bottom valve yellow or yellow-white, copiously covered with brown and white mottlings...." (Fig. 8) Petuch (1987) submitted that the range of *A. i. taylorae* Petuch is restricted to the dense, well developed *Thalassia* beds of Florida Bay and the waters west of the middle and upper Keys. Comparing it to its closest relative, *A. i. concentricus*, he described it as much smaller and more fragile, with more and flatter ribs. It is also distinguished by its more colorful upper valve, and yellow rather than white lower valve (Petuch, 1987).

The unique subspecific designation for the Rabbit Key scallops has been debated by some, and indeed an independent morphological and genetic examination by Marelli et al. (in press) suggested that *A. i. taylorae* was described from juvenile *A. i. concentricus*. A study of allozyme variation in a range of *A. irradians* populations revealed "small but significant" allele frequency differences at only two of six polymorphic loci between individuals from Rabbit Key and *A. i. concentricus* from Homasassa Bay (Marelli et al., in press). This was taken as evidence for differential selection acting among genotypes between the sites, or as an indication that some restriction of gene flow exists between the populations. Given the distance separating the populations, neither possibility seems unlikely, but the genetic similarity is still too great to suggest partitioning them into two distinct subspecies. Furthermore, several of the morphological distinctions enumerated by Petuch (1987) were refuted by Marelli et al. (in press). The latter researchers found larger valves from recently dead scallops in

Fig. 8 Representative valves of *A. irradians taylorae*.

Rabbit Key basin, more similar in size to those of *A. i. concentricus*. Variations in coloration were attributed to different fouling conditions in the sampled regions, and mean rib number for Rabbit Key scallops was not significantly different from that in an *A. i. concentricus* sample (Marelli et al., in press).

Atlantic and Gulf Populations of Argopecten gibbus.

The range of the calico scallop extends on the Atlantic coast from Cape Hatteras, North Carolina to Cape Canaveral, Florida (Sastry, 1962) (Fig. 9). It is also present in harvestable numbers on the northern Gulf coast of Florida, and has been reported to occur off the West Indies (Sastry, 1962). It has been noted that the highest concentrations of calico scallops, and therefore the best fishing grounds, are found near coastal prominences such as Cape San Blas, Florida, Cape Canaveral, Florida, and Cape Hatteras, North Carolina. This is thought to be due to an interrupting, eddying effect created by these land masses, that causes a concentration of larvae and higher settlement in these regions (Allen and Costello, 1972).

The calico scallop is generally smaller in size than the southern bay scallop subspecies, *A. i concentricus*. It is characterized by an upper valve with 17 to 23 ribs and a brightly mottled, more rounded morphology (Sastry, 1962; Allen and Costello, 1972) (Table 1, Fig. 10).

Bay Scallop Culture.

The first significant bay scallop culturing effort was undertaken by Castagna and Duggan at the Virginia Institute of Marine Science (VIMS) (1971) in the late 1960's, using methods described by Loosanoff and Davis (1963). These researchers felt that the high market value of scallop meats was sufficient to support a

Fig. 9 Geographic distribution of the calico scallop, *Argopecten gibbus.*

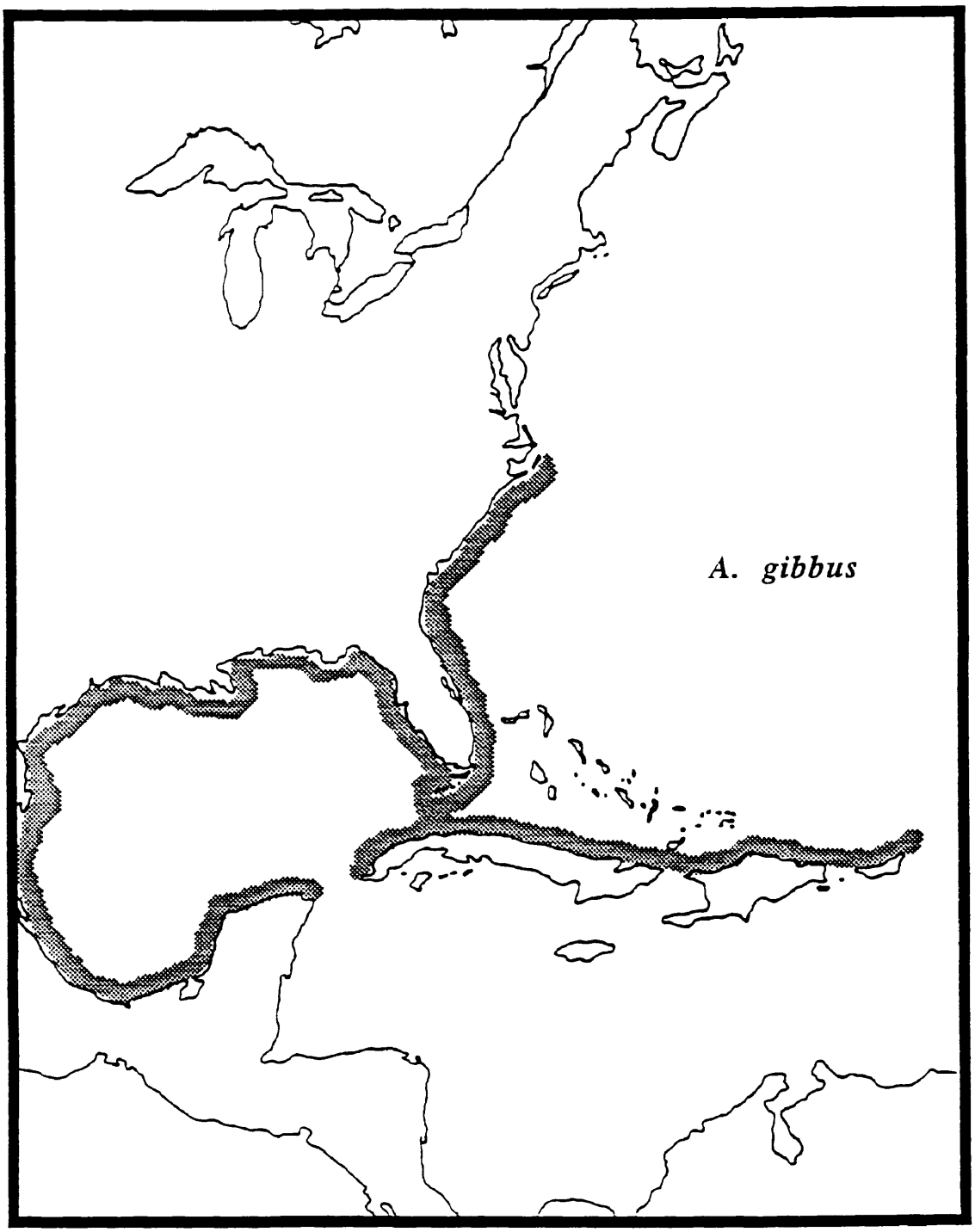


Fig. 10 Representative valves of the calico scallop, *Argopecten gibbus*.

mariculture effort, and that cultured scallops could stabilize a market supply that was subject to wide fluctuations in natural population densities.

In the Castagna and Duggan (1971) study, adult bay scallops were conditioned to produce ripe gonads, and induced to spawn with raised water temperatures. Periodic self-fertilization was observed, but more often only one gamete product was released from an individual. Larvae were reared to metamorphosis in polyethylene garbage cans, then were transferred to plastic photographic trays, outdoor tanks and finally to rectangular, wooden floats for growth to market size (50-65 mm). The time from fertilization to harvest was 6 months.

The waters off Virginia's Eastern Shore supported a significant bay scallop fishery until the early 1930's, when the disappearance of the eelgrass beds and a change in the salinity regime with the Hurricane of 1933 essentially eliminated the populations in this area. Since this time, ephemeral bay scallop populations appear periodically, settled presumably with larvae transported in from the bay scallop populations off North Carolina. It was with individuals from one of these periodic settlement events that the VIMS broodstock was established in the late 1960's. In addition to this original "Virginia" broodstock, periodic introductions have been made with individuals from Massachusetts, North Carolina, and Texas (presumed *A. i. amplicostatus*) (M. Castagna, Virginia Institute of Marine Science, 1993, pers. comm.), so that the current subspecific composition of the population is unknown. This culturing effort has continued at the Eastern Shore Laboratory of the Virginia Institute of Marine Science, and scallops reared by these methods have been specially marketed in recent years, for consumption as a whole product (M. Oesterling, Virginia Institute of Marine Science, 1993, pers. comm.).

Culture of the bay scallop has been accomplished on a much grander scale by the Chinese, an undertaking that was initiated by Dr. Fusui Zhang of the Institute of

Oceanology, Academia Sinica in Qingdao. Professor Zhang arranged for the import of U.S. bay scallops into China for use as broodstock in the early 1980's. The first shipment, harvested from the Niantic River, in Connecticut (E. Rhodes, Cultivos Marinos Internationales, 1994, pers. comm.), was sent by the U.S. National Marine Fisheries Service Labs, and consisted of 45 scallops. These reportedly arrived in Beijing either dead or in poor enough condition that they were not able to spawn. A second attempt to ship 200 animals from Connecticut was also unsuccessful, but in late 1982, Professor Zhang was able to hand-carry 128 scallops, provided by Michael Castagna of the Virginia Institute of Marine Science, to his laboratories in Qingdao. Twenty-six of these survived the journey, and were induced to spawn in January, 1983 (Chew, 1990).

By 1987, Chinese scallop production originating with the 26 founder individuals exceeded 20,000 tonnes in-shell live weight (Chew, 1990). By 1989 this figure exceeded 50,000 tonnes (Chew, 1990), and by 1993, 200,000 tonnes (Dr. Xue Qinzhao, Institute of Oceanology, Academia Sinica, 1994, pers. comm.). For comparison, total annual landings of bay scallops in the U.S. in 1992 totalled approximately 356,000 pounds meat weight (Holliday and O'Bannon, 1992). Assuming that the adductor (meat) weight is 10% of the total in-shell live weight (William DuPaul, Virginia Institute of Marine Science, 1994, pers. comm.), this U.S. harvest was equivalent to less than 1620 tonnes.

Chinese spawning and larval culture methods are much like those used by Castagna and Duggan (1971), but growout occurs in lantern nets attached to floating longlines which now cover several thousand hectares along the coast of China (Chew, 1993). The most recent scallop-spawning effort by the Chinese has given their worst yield to date, with up to 50% mortality in juvenile scallops (Dr. Xue Qinzhao, Institute of Oceanology, Academia Sinica, 1993, pers. comm.) A parasite is suspected, but the

possibility of deleterious inbreeding effects is also of concern to the Chinese researchers.

This brief overview has highlighted the historic problems with the specific and subspecific status of isolated populations of *Argopecten sp.* Morphological criteria have supplied the primary means of distinguishing the taxa, but the more recent techniques of molecular genetics may provide important supplemental information about the relationships among these groups.

Genetic Analysis.

Extensive genetic studies on scallops have been conducted using techniques of chromosomal analysis and manipulation, and protein electrophoresis (Beaumont and Zouros, 1990). Examinations of population structure and stock isolation have used allozyme data primarily, as summarized by Beaumont and Zouros (1990), and Beaumont (1991). Populations are said to have little gene flow between them when electrophoretic analysis reveals significant allele frequency differences at a locus. To assess the possibility that these differences are caused by natural selection acting in different environmental regimes rather than to restricted gene flow, the observed frequencies must be compared with those predicted by the Hardy-Weinberg model. This model states that, in the absence of selection, co-dominant alleles A and a, present at frequencies p and q, should be represented in genotypes AA, Aa and aa with frequencies predicted by the binomial distribution, $p^2+2pq+q^2$. Unfortunately, deviations from the Hardy-Weinberg may also be caused by non-random mating, migration, genetic drift in populations of finite size, and for allozyme studies, by the presence of non-staining protein products (Beaumont, 1991). Conclusions about genetic differentiation then, are difficult to draw when allozyme data do not fit the Hardy-Weinberg model.

While allozyme studies detect genetic variation at the level of the polypeptide product of the gene, analysis of the DNA itself can provide information about untranslated genetic variation. The mitochondrial genome, a covalently closed, circular molecule, has been found to possess several unique properties which make it useful for the quantification of genetic differences among populations. First, it is small, consisting of 15,000 to 18,000 base pairs in most metazoans (Ferris and Berg, 1987; Moritz et al., 1987), and it has been shown to evolve 5-10 times faster than nuclear DNA in primates, probably due to an accelerated rate of mutation (Brown et al., 1979). Because they accrue so rapidly, differences in mtDNA may be apparent between populations and closely related species, where variation in the nuclear DNA might not be detected. With the suspected exception of mussels of the genus *Mytilus* (Hoeh et al., 1991), mtDNA is thought to be strictly maternally inherited, and does not undergo recombination. This permits the tracing of lineages, much as paternally inherited surnames do in many human cultures.

Restriction fragment length polymorphism (RFLP) analysis of mtDNA has been widely applied in population genetics. The use of the polymerase chain reaction to amplify portions of the mtDNA molecule for direct sequencing has also gained attention recently (Kocher and White, 1989; Kocher et al., 1989). RFLP analysis utilizes enzymes to cut the mtDNA molecule at particular base pair recognition sequences. The sizes of the resulting fragments can then be compared to provide a measure of relatedness between mtDNA haplotypes. The technique has been described in detail by Lansman (1981).

Studies employing RFLP analysis to measure population- and species-level variation have been performed on a wide array of species. In the marine environment, fishes have enjoyed the most extensive application of these techniques, particularly in

the area of stock assessment as reviewed by Ovenden (1990). Invertebrates have been the focus of fewer studies, but several species have now been examined in some detail.

The published research most relevant to this study has examined geographic differentiation in mtDNA of species distributed continuously along the Atlantic coast of the U.S., and south into the Gulf of Mexico. The horseshoe crab, *Limulus polyphemus*, was the first to be examined in this manner (Saunders et al., 1986). RFLP analysis was performed on a total of 99 horseshoe crabs from 15 localities between the Florida panhandle and New Hampshire, in order to assess genetic diversity within populations and over the species range. The most striking find of the study was the detection of a "major genetic 'break'", which distinguished crabs collected north and south of a particular region on the Florida Atlantic coast (Saunders et al., 1986). This break was similar to those observed for terrestrial and freshwater organisms, and the point at which it occurred has been generally accepted as the zoogeographic boundary between temperate and tropical marine bios. To explain the pattern, Saunders et al. (1986) suggested that the genetic differences on either side of the break may be maintained by differential selection pressures or by the presence of gene flow barriers at the transition zone.

A similar observation to that made for horseshoe crabs was made for the American oyster in 1990 (Reeb and Avise, 1990). Restriction site variation in the mtDNA of *Crassostrea virginica* was examined for 212 individuals from 14 localities between the Gulf of St. Lawrence, Canada, and Brownsville, Texas. As in the Saunders (1986) study, the observed mtDNA haplotypes were found to group into two distinct genetic arrays, with the boundary dividing them detected on the Atlantic coast of Florida. The high degree of variability measured in oyster mtDNA, and the notable break in haplotype frequencies, were counter to the results of previous studies in which allozyme frequencies were compared (Buroker, 1983). Reeb (1990) presented several

hypotheses to account for this discrepancy, and in a later study, Karl and Avise (1992) suggested that it is due to balancing selection acting at the allozyme loci—in other words, allozyme markers in this case are non-neutral, and should not be assumed to be so.

The use of mtDNA RFLP analysis to distinguish species of bivalves has been used extensively on those of the genus *Mytilus* (Skibinski, 1985; Edwards and Skibinski, 1987; Blot et al., 1990). In general, it has been found that *Mytilus* species are distinguishable by these methods, and that populations expected to contain hybrids on the basis of allozyme and morphological data, indeed contain mtDNA haplotype frequencies intermediate to those of the supposed parent species (Skibinski, 1985).

A potential problem facing researchers who wish to continue this research on *Mytilus*, is the observation by Hoeh et al. (1991) that a large proportion of individuals of the species *Mytilus edulis* exhibit heteroplasmy. In these cases, more than one mtDNA type is present within the individual. This phenomenon is also known to occur at low levels in some scallop species (Gjetvaj et al., 1992).

The mitochondrial genomes of several members of the scallop family, Pectinidae, have been examined in some detail (Snyder et al., 1987; Gjetvaj et al., 1992). The family shows a broad range of mtDNA features, many very different from those of other metazoans. *Placopecten magellanicus*, the deep-sea scallop, exhibits the most dramatic divergence from the standard, with the largest known mtDNA molecule of any multicellular animal (Gjetvaj et al., 1992). It also shows a high degree of size polymorphism within the species, with a measured range of 31 to 44 kb. This variation in size has been attributed to varying copy numbers of a tandemly repeated sequence. No such sequences have been detected in the mtDNA of *Argopecten irradians*, which was found to possess a 16.2 kb molecule of invariant size (Gjetvaj et al., 1992). Five other pectinid species display mtDNA types larger than typical, with some degree of

intraspecific size polymorphism due to the presence of repeated sequences. The efficacy of most mtDNA population genetic techniques is limited in these species, as a consistency in the size of the whole mtDNA molecule is necessary for comparative analysis of RFLP's across individuals (Gjetvaj et al., 1992). The mtDNA of the calico scallop, *A. gibbus*, has not been characterized by these techniques.

An attempt was made by Boulding et al. (1993) to circumvent the problem of size variation in the mtDNA of *Patinopecten yessoensis*, by amplifying four smaller (0.6 to 1.5 kb) coding fragments, then subjecting them to RFLP analysis. In a comparative analysis of mtDNA from hatchery-propagated and wild *P. yessoensis*, they observed similar haplotype frequency distributions. This was taken as an indication that the hatchery stock was not severely inbred, and that gene flow between the wild populations was sufficient to prevent genetic divergence.

Testable Hypotheses.

The objective of this study was to test the following null hypotheses, using mitochondrial DNA variation as determined by RFLP analysis, in the bay and calico scallops:

H_{O1}: The sampled populations of *A. irradians* ssp. share a common gene pool.

H_{O2}: Morphologically similar populations of *A. irradians* ssp. share a common gene pool.

H_{O3}: Scallops sampled from the same population in two consecutive years share a common gene pool.

H_{O4}: Individuals of the purported *A. i. taylorae* subspecies share a common gene pool with those of a sampled population of *A. i. concentricus*.

H_{O5}: Cultured bay scallops from China share a common gene pool with a broodstock source population.

H_{O6}: *A. irradians* and *A. gibbus* share a common gene pool.

H_{O7}: The sampled populations of *A. gibbus* share a common gene pool.

MATERIALS AND METHODS

Sample Collection.

A summary of sample sites, sizes and dates of collection is provided in Table 3. The abbreviated codes for these, to be used in subsequent discussion, are also indicated.

Samples of *A.irradians* were obtained from Taylor Seafood, in Woods Hole, Massachusetts, Harker's Island, North Carolina, Rabbit Key, Florida, and Crystal River, Florida. These were assumed to approximate natural set in their sites of origin. The Massachusetts sample was hatchery-produced with broodstock from Martha's Vineyard, Buzzards Bay and Nantucket Sound. The broodstock had been maintained in the hatchery since 1988, and was used to produce seed scallops to augment the natural bay scallop set in the area. The sample was harvested from seeded beds in Nascatucket Bay, but may also contain scallops from natural set. Taylor Seafood has not introduced any scallops from outside New England into their broodstock (R. Taylor, Taylor Seafood, 1993, pers. comm.).

The Crystal River, Florida sample was also composed of laboratory-reared F_1 progeny (from approximately 2 dozen parent individuals), but the broodstock from Crystal River had not been combined with any other (N. Blake, University of South Florida, 1992, pers. comm.).

Scallops in the Harker's Island, North Carolina sample were natural set collected from the clam beds of Carolina Cultured Shellfish, Inc. A temporal replicate of this sample was collected one year after the first collection.

Cultured bay scallops were provided by the Virginia Institute of Marine Science labs at Wachapreague, and by Dr. Fusui Zhang, of the Institute of Oceanology,

Table 3. Summarized Sample Descriptions.

Population	Code	Approx. Date of Collection	Source	n
Bay Scallops				
Crystal River, FL	FL	9/92	Hatchery F_1	27
New England	MA	9/92	Hatchery $F_?$ + Field?	26
Harker's Island, NC	NC	10/12/92	Field	27
Harker's Island, NC	NCT	11/12/93	Field	21
Wachapreague, VA	VA	5/3/93	Hatchery $F_?$	27
Rabbit Key, FL	RK	9/22/93	Field	34
Qingdao, China	Q	10/6/93	VA + 10 gens.	36
Calico Scallops				
Cape Canaveral, FL	CA	2/17/93	Field	27
Apalachicola, FL	AP	4/14/93	Field	24

Qingdao, China. The Wachapreague sample is presumed to have come from a spawning of 100-200 animals (M. Oesterling, Virginia Institute of Marine Science, 1994, pers. comm.).

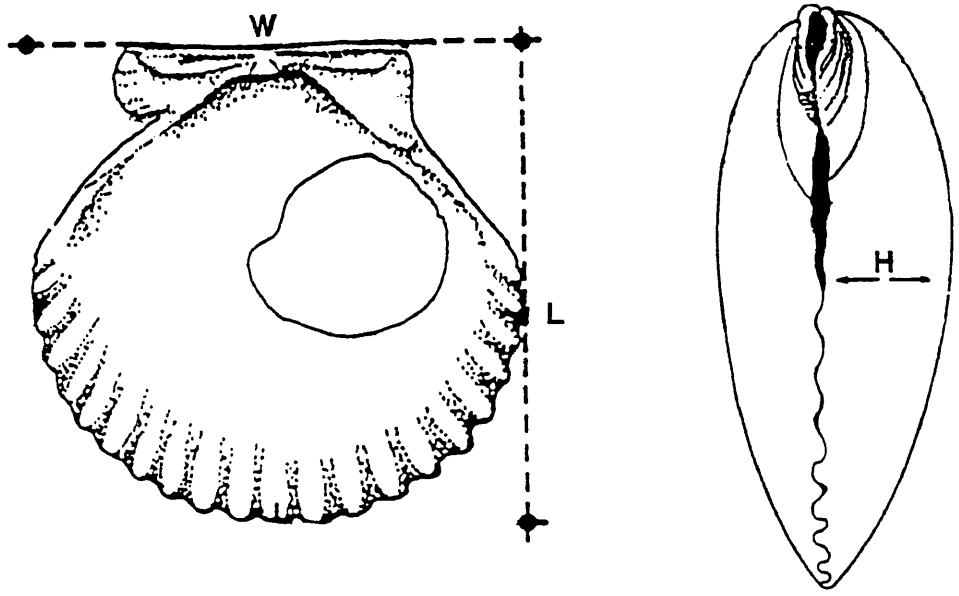
The initial bottleneck of 26 breeding individuals was not sustained in the Chinese culturing operation, as production of bay scallops in China grew extremely rapidly. The number of individuals spawned to produce the sampled population is not known, but is presumed to be many orders of magnitude higher than that used in Wachapreague.

Calico scallop samples were obtained from the waters off Cape Canaveral, Florida, and Apalachicola, Florida. They represent natural set for these regions.

Morphological Measurements.

To confirm that the sampled individuals fell within the ranges reported for their designated subspecies, the following measurements were performed and compared with Clarke's (1965) morphological criteria: average number of ribs, percent white or mostly white, average width to length ratio, and average height to length ratio (valve inflation) (Fig. 11). Measurements were conducted on right (lower) valves of individuals from all sampled populations except the Rabbit Key bay scallops and the Cape Canaveral calico scallops. Tissue from the latter two populations was provided by William Arnold of the Florida Department of Natural Resources, who performed an independent morphological analysis. Valves measured from the first North Carolina and Crystal River, Florida samples were from different individuals than the tissue used in the mtDNA analysis, but were from individuals of the same year-class and collection site.

Fig. 11 Morphological measurements performed on right valves of *Argopecten* sp.



Tissue Handling.

With the exception of the Chinese and Rabbit Key bay scallops, and the Apalachicola calico scallops, samples were delivered or hand-carried to the Virginia Institute of Marine Science (VIMS) alive, where gonad, and/or mantle tissue were dissected and frozen at -70°C. A limited number of individuals were processed fresh when time permitted. The Rabbit Key bay scallops and Apalachicola calico scallops were provided by William Arnold of the Florida Department of Natural Resources. In these cases, animals were shucked and frozen whole in liquid nitrogen, then stored at –70°C until transport to VIMS on dry ice.

The difficulties of transporting and processing tissue from the Chinese culturing facilities required a somewhat different preparative protocol. Thirty-six individuals, 18 from a northern facility (Laizhou) and 18 from a facility in Qingdao (Tiaonan), were processed fresh at the Institute of Oceanology in Qingdao. While these individuals had been reared at the different growout sites, the seed originated from the same broodstock (X. Qinzhao, Institute of Oceanology, Academia Sinica, 1993, pers. comm.). Following the addition of CsCl-saturated water (see below) to the tissue preparations, the samples were maintained on ice for transport to VIMS, where mtDNA isolation and analysis were completed.

Mitochondrial Isolation.

Mitochondrial DNA was purified according to a modified standard protocol (Lansman et al., 1981). Tissue (usually 2-3 g) was homogenized with a Tekmar Tissuemizer, in cold grinding buffer (50mM Tris, 25mM EDTA, 1.5% KCl, 200mM sucrose, pH 8.3). Homogenate was held on ice throughout subsequent processing. Nuclei and cellular debris were removed by centrifugation at 1200 g for 5 min, and the supernatant carefully decanted into a clean, prechilled centrifuge tube. The low speed

spin was repeated until little or no pellet remained. The sample was then spun at 18,000 g for 25 min to pellet the mitochondria, and the resulting supernatant discarded. The pellet was washed by resuspension in grinding buffer, followed by an additional high speed spin. All centrifugation was carried out at 4°C. The final mitochondrial pellet was resuspended in 2.5 ml Tris/EDTA (TE) buffer, and the mitochondria were lysed with the addition of 0.32 ml 20% SDS. The suspension was observed to clarify, and an additional 10 min room temperature incubation insured thorough lysis. CsCl-saturated water (0.47 ml) was added to the lysate, and the solution was incubated on ice for at least 30 min to precipitate the protein-CsCl-SDS complex. A final, 10 min spin at 12,000 g removed mitochondrial debris, leaving the desired nucleic acids in the supernatant. The sample was then prepared for ultracentrifugation.

MtDNA Purification.

To the total volume of the supernatant were added 1.0 volume of solid CsCl and 0.178 volume of ethidium bromide (2mg/ml). The density of the solution was adjusted to 1.55-1.57 g/ml with the appropriate addition of CsCl or deionized water. The sample was then placed in an ultracentrifuge tube, and topped with mineral oil. Following 18-24h of ultracentrifugation at 70,000 rpm, DNA bands were visualized under UV light. The bright upper nuclear band and the dimmer mitochondrial band visible about 0.5 cm below the nuclear band, were collected separately by dripping through the tube bottom. Ethidium bromide was removed from the DNA (usually 0.5 ml or less in volume) by repeated extraction with NaCl-saturated butanol. Serial dialysis against changes of TE buffer (twice at 1X and twice at 0.1X) removed remaining CsCl, excess EDTA, and other impurities. DNA was stored in sterile microfuge tubes at –20°C.

Quantification of mtDNA.

The quantity of closed circular mtDNA obtained by this method of isolation was estimated for several preparations to provide a basis for the determination of DNA volumes used in subsequent digestions. To do this, an aliquot of mtDNA was stained with ethidium bromide, and run on a 1% agarose minigel against λ phage DNA of known concentration.

Restriction Endonuclease Digestion.

Digestion of *A. irradians* mtDNA was carried out with the following restriction endonucleases used according to the manufacturer's recommendations: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, *Hin*dII. Each of these enzymes recognizes a 5.3 or 6-base pair restriction site. Approximate 10ng aliquots of DNA were digested with enzyme and the appropriate buffer for at least three hours at optimum temperature, as determined by the manufacturer. Overnight incubation and/or an additional spiking with enzyme were performed when necessary to achieve complete digestion.

Many of the individuals in the *A. gibbus* sample from Apalachicola yielded low quantities of closed-circular mtDNA, perhaps because of an extended storage time, or inadequate handling procedures. Limited DNA quantities required that only six of the above enzymes be included in the calico scallop analysis. These were *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, and *Hae*II. For comparison with *A. irradians*, Cape Canaveral calico scallop DNA was screened with all eight enzymes.

Since mtDNA quantities yielded by these methods are not sufficient for direct visualization, DNA fragments were visualized by endlabeling or Southern transfer and hybridization.

Endlabeling and Electrophoresis.

DNA from all *A. irradians* samples and the Cape Canaveral *A. gibbus* were visualized by endlabeling. To endlabel fragments, digests were treated with a "cocktail" containing the Klenow fragment of DNA polymerase I and ^{35}S-labeled nucleotides. 5µl of the labeling mixture were added to each digest and allowed to incubate 30-60 min at 37°C. Reactions were then stopped with the addition of 3µl of stop buffer (50% glycerol, 0.02% bromphenol blue, 5% SDS), and loaded into the wells of a prepared 1% agarose gel. Several lanes of ^{35}S-labeled 1 kb ladder DNA (BRL) were also loaded on each gel to provide a molecular weight size standard. Gels were run at 1 volt/cm overnight, fixed in an acetic acid fix solution for 30 min, soaked for 30 min in a commercially prepared scintillation enhancer, and dryed under heat and vacuum. Dried gels were exposed to X-ray film for 3-6 days at –70°C.

Southern Transfer and Hybridization.

The low yields of Apalachicola calico scallop mtDNA made it necessary to visualize some restriction patterns by Southern transfer and hybridization. Digests of mtDNA or aliquots of nuclear DNA were prepared as described previously, but were stopped without the addition of endlabeling cocktail. These were then loaded into the wells of prepared 1% agarose gels, with biotinylated l HindIII fragments as a size standard. Gels were run at 3 volts/cm for 3-4 hours, then subjected to depurinating (20 min in 0.25M HCl), denaturing (2x 15 min in 1.5MNaCl, 0.5M NaOH) and neutralizing (2x 15 min in 1M Sigma 7-9,1.5MNaCl pH 8) washes. The gels were blotted by overnight capillary transfer onto nylon membrane, and membranes were UV-crosslinked to immobilize the DNA.

For each blot, a 5ml prehybridization solution, consisting of 2.5ml formamide, 1.25ml 20X SSC (3.0M NaCl, 0.3M sodium citrate, pH 7.0), 0.5ml Denhardt's

solution, 0.25ml 5M $NaPO_4$, 0.3ml sterile water, and 0.2ml denatured calf thymus DNA, was prepared. Blots were prehybridized at 42°C for 2 hours, then an overnight (42°C) hybridization was initiated with the addition of 0.25-0.5µg biotinylated probe DNA. Probe was prepared from purified *A. irradians*, *A. gibbus* or *Placopecten magellanicus* mtDNA, by nick translation with biotin-7-dATP using the BRL Bionick Labeling System. Unincorporated nucleotides were removed from the biotin-labeled probe DNA by size-exclusion chromatography.

After hybridization, blots were subjected to a series of stringency washes and blocked with a 3% solution of bovine serum albumen. Restriction patterns were then visualized using the BluGene Nonradioactive Nucleic Acid Detection System.

Data Analyses.

Sizes of mtDNA fragments were estimated by fitting band migration distances to those of the standard by the local reciprocal method of Elder and Southern (1983). Fit calculations were performed by the program Gel Frag Sizer (Gilbert, 1989). Restriction sites were inferred from completely additive fragment patterns, and haplotype names were assigned to the different patterns. Composite haplotypes were constructed and analyzed separately for fragment and site data.

Statistical analyses were performed using the Restriction Enzyme Analysis Package (REAP) (McElroy et al., 1991). *A. irradians* and *A. gibbus* were analyzed separately, as they showed no similarity in restriction fragment patterns. For each sample, haplotype and nucleotide diversities were calculated following the methods of Nei (Nei, 1987) and Nei and Miller (1990), respectively. Mean nucleotide sequence divergence among samples was calculated as per Nei and Miller (1990), and was corrected for within-population polymorphism by subtracting the average of the within-sample diversities. As many of the haplotypes observed were rare, a Monte Carlo

simulation (Roff and Bentzen, 1989) was performed to estimate heterogeneity and assess the likelihood that the sampled populations share a common gene pool. Selected pair-wise analyses were also performed on samples that appeared more likely to show congruence.

RESULTS

Morphological Measurements.

Average rib number in the *A. irradians* samples ranged between 18.2 and 22.0 and was 22.4 for the *A. gibbus* sample (Table 4). There was considerable overlap in this character among the populations of bay scallop, even within subspecies. The three samples designated *A. i. concentricus* had mean rib counts between 19.3 and 22.0, while valves in the New England, Virginia and Qingdao samples had 18.2 to 18.7 ribs. The standard deviations of these counts, however, which ranged between 0.7 and 1.3, prevented clear distinction between populations and subspecies based on this character.

Valve inflation, measured as the mean height to length ratio for the valves in a sample, also showed considerable overlap among populations. New England *A. i. irradians* were the most compressed of all the measured bay scallops, as evidenced by the lower average height to length ratio of 0.22. The cultured samples from Virginia and China were somewhat more inflated than these, with average ratios of 0.28 and 0.25, respectively. *A. i. concentricus* samples were generally more gibbous than others, with average height to length ratios between 0.27 and 0.30. Again, standard deviations on these measures, 0.02 in all cases, created overlap in the variable that made clear distinctions between populations or subspecies impossible. The calico scallop sample showed a slightly higher degree of valve inflation than the *A. i. concentricus*, with an average height to length ratio of 0.31.

Fairly consistent differences in valve coloration were observed among bay scallop populations, with an overall range of 15 to 97 percent of right valves in a sample having a predominantly white hue. In the Crystal River and Harker's Island

Table 4. Morphometric features of right valves of representative *Argopecten irradians* ssp. and *Argopecten gibbus* individuals, (± s.d). MA = New England; NC = Harker's Island, North Carolina; NCT = NC + 1yr.; FL = Crystal River, Florida; VA = Wachapreague, Virginia; Q = Qingdao, China; CA = Cape Canaveral, Florida.

Population	Current Taxonomic Designation	n	Avg. # Ribs	% white or mostly white	Avg. width:length ratio	Avg. height:length ratio
MA	*A. i. irradians*	23	18.7 ± 0.7	17	1.09 ± 0.02	0.22 ± 0.02
NC	*A. i. concentricus*	30	19.3 ± 0.8	97	1.06 ± 0.04	0.30 ± 0.02
NCT	*A. i. concentricus*	21	19.4 ± 1.0	76	1.03 ± 0.03	0.29 ± 0.02
FL	*A. i. concentricus*	27	22.0 ± 1.3	96	1.02 ± 0.02	0.27 ± 0.02
VA	?	30	18.4 ± 1.0	15	1.09 ± 0.04	0.28 ± 0.02
Q	?	36	18.2 ± 1.0	25	1.06 ± 0.03	0.25 ± 0.02
CA	*A. gibbus*	30	22.4 ± 0.6	73	1.04 ± 0.02	0.31 ± 0.01

Length = dimension from umbo to opposite end of shell
Width = maximum dimension at right angle to length
Height = maximum vertical height

samples, 76 to 97 percent of the right valves were white or mostly white. The New England sample contained fewer white valves, with only 17 percent white or mostly white. These were present in the Virginia and Qingdao samples at frequencies of 15 and 25 percent, respectively. Seventy-three percent of the *A. gibbus* were white or mostly white when valves of uniform yellow hues were counted as white.

Quantification of mtDNA.

Yields of closed-circular mtDNA were estimated for 4 individuals, three of which had been frozen prior to mtDNA purification. Concentrations of the dialyzed volumes for the frozen samples were estimated at 1ng/µl. With total dialyzed volumes of approximately 0.5ml, mtDNA yields for these individuals were around 500ng, or for a 2g tissue sample, 250ng mtDNA/g tissue. MtDNA yield from the individual processed fresh was about 10 times higher, at 2.5µg mtDNA/g tissue. Based on these quantifications, digestions were performed with approximately 10-50ng mtDNA.

MtDNA yield was also observed to vary with tissue used, with ripe gonad providing better yield than mantle and gill, or immature or spent gonad. Imprecise estimates of the mtDNA yields, deduced from the intensity of the mtDNA band after ultracentrifugation, were usually sufficient to provide a basis for the determination of DNA volumes used in digestions.

Genetic Variation in Argopecten irradians *ssp.*

Analysis of 198 bay scallops with a battery of 8 restriction endonucleases revealed 51 distinct mtDNA haplotypes (Table 5). Enzymes produced between 4 and 10 restriction fragment patterns. None was invariant. A total of 133 fragments was visualized, and restriction site gains and losses were inferred from additive changes in fragment patterns (Appendix 2). The different fragment patterns were accounted for

Table 5. *Argopecten irradians*. Composite haplotypes from seven populations: MA = New England; NC = Harker's Island, North Carolina; NCT = NC + 1yr.; FL = Crystal River, Florida; RK = Rabbit Key, Florida; VA = Wachapreague, Virginia; Q = Qingdao, China. Restriction enzymes used: *Aval*, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, and *Hin*dII. Values in parentheses are totals from the Laizhou, China growout facility.

HAPLOTYPE	MA	NC	NCT	FL	RK	VA	Q	Total
AAAAAAAE	2							2
AABAAAAE	5					27		32
AABAAAAH	2							2
AACAAAAA	7	13	13	2			4 (4)	39
AACAAAAG	2							2
AACBAAAA	1							1
AADAAAEE	4							4
AAEAAAAE	2							2
AAFAAAEE	1							1
AACAAAAC		1						1
AACAAAAD		1						1
AACAAAGA		1						1
AACAABDD		1						1
AACABAAA		1						1
AACCAAAA		1						1
AAGAAAAE		1						1
ABCAAABA		1						1
ACCADAAA		1						1
ADCAAACA		1						1
AFCAAAAA		1						1
BACAAAAB		3		10				13
AEBAAAAA				2	3			5
AEBBAAAA				10				10
CABACAAA				2				2
AACAAAAI				1				1
AACAAAAE			1					1
AACAADAA			1					1
AACAAEAA			1					1
AACDAAAA			1					1
ACEAAAAE			1					1
AICAAAAA			1					1
DAIAAAAA			1					1
EACAAAAA			1					1
AABAAAAA					9			9
AABAAAAD					1			1
AABABAAJ					1			1
AABAEAAA					1			1
ACBAAAAA					1			1
ADBAAACA					1			1
AEBAAAAE					1			1
AEBAAAAH					1			1
AEBAAAEA					4			4
AEBAAAEK					1			1
AEBDAAAA					3			3
AGCAAAAA					1			1
AHHABAHA					1			1
DABABCAA					1			1
DCBABAAK					3			3
EEBDAAAA					1			1
AABAAAEE							22 (9)	22
ACCAAAAA							10 (5)	10
TOTAL n	26	27	21	27	34	27	36	198

with 6 to 16 sites, and eighty-three sites were scored in total. The most common haplotype, represented by 39 individuals, contained a total of 52 sites, accounting for approximately 1.7% of the mtDNA genome.

The size of the whole molecule was estimated at 16.7kb from *Ava*I digests, which provided the most consistent and easily-scored restriction patterns. All other enzymes produced either large fragments, which were sized with less accuracy, or large numbers of small fragments, which compounded the error on the overall size estimate.

Haplotype diversity, or the probability of encountering different haplotypes when two individuals are sampled from a population, was quite large (0.55-0.91) for all of the populations, with the exception of the monotypic cultured sample from Virginia (VA) (Table 6). Excepting the Virginia sample, that from the cultured Chinese population (Q), which contained 3 haplotypes, possessed the lowest haplotype diversity. The two less common haplotypes in the Qingdao sample, ACCAAAAA and AACAAAAA, differed by only one site change, while the third and most common, AABAAAEE, differed from these by several site changes. The latter, however, only differed from the single Virginia haplotype (AABAAAAE) by one site change. The higher haplotype diversities for the other populations (0.63 to 0.90) were due to the large numbers of rare variant haplotypes in these samples. These rare haplotypes differed from their more common counterparts in the populations by one to numerous site changes.

Mean nucleotide sequence diversities ranged from 0%, for the monotypic Virginia sample, to a high of 0.53% for the Crystal River sample (FL). The high value for the latter population was not correlated with haplotype diversity, but was caused by the predominance of two haplotypes in the sample, AEBBAAAA and BACAAAAB, that differed from each other by 5 sites. The North Carolina (NC) sample contained 11 rare haplotypes, each represented by a single individual, but 9 of these differed from

Table 6. *Argopecten irradians*. Summary statistics. MA = New England; NC = Harker's Island, North Carolina; NCT = NC + 1yr.;NC_{pool} = NC+NCT; FL = Crystal River, Florida; RK = Rabbit Key, Florida; VA = Wachapreague, Virginia; Q = Qingdao, China.

	MA	NC	NCT	NC_{pool}	FL	RK	VA	Q	Average
# individuals	26	27	21	48	27	34	27	36	
# haplotypes	9	13	9	21	6	17	1	3	
haplotype diversity *(h)*	0.87	0.74	0.63	0.69	0.74	0.91	0.00	0.55	0.63
% mean nucleotide sequence diversity *(p)*	0.48	0.28	0.22	0.25	0.53	0.50	0.00	0.33	0.33

the common haplotype by only one or two site changes. The nucleotide sequence diversity within this population was comparatively low at 0.28%.

Table 7 is a matrix of mean nucleotide divergences among the populations. Values below the diagonal have been corrected for within-sample variation. Most notable among these is the corrected divergence between the two North Carolina samples (NC and NCT) collected at the same site in different years. This value is negligible, indicating that the likelihood of sampling similar individuals from both populations is comparable to that of sampling them from within either population. At least at this site then, there was little year-to-year variation that might complicate among-sample comparison of geographically separate populations. The temporal samples were pooled for the determination of mean nucleotide sequence divergences between North Carolina and the other populations.

A test for heterogeneity was performed (Roff and Bentzen, 1989) on the pooled haplotype distributions from all of the bay scallop samples. One thousand Monte Carlo randomizations yielded no χ^2 values exceeding the observed, indicating that the populations do not share a common gene pool. A separate test was performed on the two subpopulations of bay scallops from China, to determine whether these shared a common gene pool (originated from a common broodstock), and could be treated in subsequent analyses as one population. This simulation yielded 126 out of 1000 χ^2 values that equalled or exceeded the value from the original data, indicating that at p=0.126, the populations are not significantly heterogeneous. The Chinese sample is discussed as a single "Qingdao" population.

The two North Carolina populations were also tested for heterogeneity using this method, with 987 out of 1000 simulations producing χ^2 values equal to or greater than the observed. This is consistent with the null hypothesis that these two populations share a common gene pool, and show little temporal partitioning of

Table 7. *Argopecten irradians*. Matrix of nucleotide sequence divergences among populations, in percent. Uncorrected above diagonal; below diagonal corrected for within-sample variation. MA = New England; NC = Harker's Island, North Carolina; NCT = NC + 1yr.;NC_{pool} = NC+NCT; FL = Crystal River, Florida; RK = Rabbit Key, Florida; VA = Wachapreague, Virginia; Q = Qingdao, China.

	MA	NC	NCT	NC_{pool}	FL	RK	VA	Q
MA		0.51	0.46	0.49	0.68	0.61	0.30	0.45
NC	0.13		0.25		0.53	0.58	0.47	0.49
NCT	0.11	-0.00*			0.51	0.54	0.41	0.45
NC_{pool}	0.12				0.52	0.56	0.44	0.47
FL	0.18	0.13	0.14	0.14		0.62	0.58	0.67
RK	0.12	0.19	0.18	0.19	0.11		0.45	0.57
VA	0.06	0.33	0.30	0.32	0.31	0.21		0.29
Q	0.04	0.18	0.17	0.18	0.24	0.15	0.13	

* This value is not actually zero, but was computed by REAP to be -0.0017. This indicates that the probability of drawing two similar individuals from NC and NCT was greater than or equal to the probability of drawing two similar individuals from one of those populations.

variation. Tests for heterogeneity were likewise performed on five pairs of populations that shared a haplotype: MA and NC, MA and NCT, MA and VA, MA and Q, and FL and RK. In all of these, the 1000 randomizations produced no χ^2 values higher than the observed, indicating that significant heterogeneity exists between the tested pairs.

Figure 12 is a dendrogram produced by the unweighted pair group method (UPGMA) of cluster analysis from average nucleotide sequence divergences among samples of *A. irradians*. The North Carolina temporal samples are clustered together, to graphically illustrate the lack of differentiation between them. These are also grouped more closely with the individuals from Rabbit Key (RK) and Crystal River (FL), than they are with the New England population (MA) or either of the cultured samples (Q and VA), which form a separate cluster.

Genetic Variation in Argopecten gibbus.

In all, 51 calico scallops were screened with 6 restriction endonucleases, to produce 19 distinct mtDNA haplotypes (Table 8). Enzymes produced between 3 and 12 restriction sites. None were invariant. A total of 60 fragments was visualized, and restriction site gains and losses were inferred from additive changes in fragment patterns (Appendix 3). The different fragment patterns were accounted for with 3 to 12 sites, and 42 sites were scored in total. The size of the whole mtDNA molecule was estimated at 16.5kb from the *Ava*I digests, and no indications of size polymorphism or heteroplasmy were apparent.

Five of the 19 haplotypes were shared between the two populations. The most common haplotype, aaaaaa, was present in both samples, in a total of 17 individuals. It contained 36 sites, accounting for an estimated 1.2% of the genome.

The haplotype diversities of the Cape Canaveral and Apalachicola populations were both large at 0.79 and 0.92, respectively. Mean nucleotide sequence diversities

Fig. 12. Dendrogram from UPGMA cluster analysis of seven populations of *A. irradians*. Scale is percent nucleotide sequence divergence. NC = Harker's Island, North Carolina; NCT = NC + 1 yr.; FL = Crystal River, Florida; RK = Rabbit Key, Florida; Q = China (cultured); VA = Virginia (cultured).

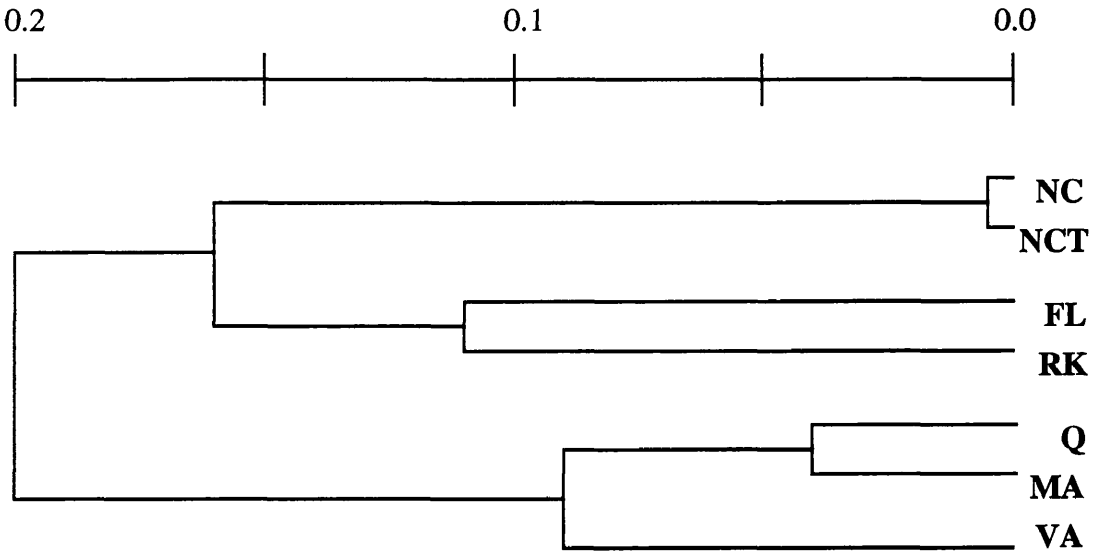


Table 8. *Argopecten gibbus*. Composite haplotypes from two populations: CA = Cape Canaveral, Florida; AP = Apalachicola, Florida. Restriction enzymes used: *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, and *Hae*II.

HAPLOTYPE	CA	AP
aaaaaa	12	5
aaaaac	1	
aadaaa	1	
aaeaaa	3	
adaaaa	1	3
babaaa	4	4
babaaf	1	
bacaaa	1	1
bafaaa	1	1
bbbbaa	1	
bcbabb	1	
aaaaad		1
aaaaag		1
aaaace		1
adeaaa		2
adeaac		1
aegaaa		1
babaab		1
bdbaaa		2
Total n	27	24

were 0.54% for the Cape Canaveral sample, and 0.69% for the Apalachicola individuals (Table 9). Corrected and uncorrected nucleotide sequence divergences between the populations were 0.007% and 0.621% respectively, indicating that most of the sequence divergence could be attributed to variation within the samples.

A test for heterogeneity (Roff and Bentzen, 1989) performed for the two calico scallop samples yielded 143 out of 1000 replicates with χ^2 values exceeding that calculated from the original data. This suggests that the populations are not significantly heterogeneous, at p=0.143.

A. irradians vs. *A. gibbus.*

The Cape Canaveral sample of calico scallops was screened with the full battery of eight restriction enzymes used in the bay scallop analysis. No similar banding patterns or shared fragments were observed between the two species.

Table 9. *Argopecten gibbus.* Summary statistics. CA = Cape Canaveral, Florida; AP = Apalachicola, Florida.

	CA	AP
# individuals	27	24
# haplotypes	11	13
haplotype diversity *(h)*	0.79	0.92
% mean nucleotide sequence diversity *(p)*	0.54	0.69

Nucleotide sequence divergence between CA and AP samples of *A. gibbus*, in percent:

Uncorrected: 0.621
Corrected for within-sample variation: 0.007

DISCUSSION

Morphological Features of A. irradians *ssp.*

Morphological measurements were used to confirm that samples of bay scallops fit the criteria for their presumed taxa in Clarke's (1965) key to subspecies. Marelli et al. (in press) have shown that, with few exceptions, variables related to valve size and shape vary allometrically, making them invalid for use in statistical comparisons of samples containing individuals of different sizes. They suggest that morphological examinations of the species utilize allometric transformation for these variables. Plical number (rib count) was not found to be significantly related to valve size, but the potential for error in the measurement of this character state is high. The "riblets" on the valve disk flanks are easily mistaken for plicae, and can lead to an inflated rib count.

The values presented in Table 4 are all consistent with Clarke's morphological criteria for the subspecies, although close examination reveals considerable overlap among samples. The New England sample, for example, possessed valves with an average rib number of 18.7 ± 0.7. Clarke's value for discriminating between *A. i. irradians* and *A. i. concentricus* is 19 (see Appendix 1), indicating that the New England sample is on the border between subspecies for this character. The sample may be considered *A. i. irradians* with some confidence, however, since several other characters fall within Clarke's ranges for this subspecies. The mean height to length ratio of 0.22 is within Clarke's range (0.19 to 0.29), and fewer than 50% (17%) of the right valves were counted as white.

The North Carolina samples and the sample from Crystal River, Florida all appear to have features consistent with those of the southern subspecies, *A. i.*

concentricus. Rib counts in these samples, 19.3 and 19.4 for the two from North Carolina, and 22.0 for the Crystal River sample, all exceeded Clarke's prescribed minimum of 19. Standard deviations on these counts, however, which ranged between 0.8 and 1.3, place the two North Carolina samples on the border between the subspecies for this character. Average height to length ratios were also borderline when standard deviations are considered. Clarke's range in this measure for *A. i. concentricus* is 0.28 to 0.35. The North Carolina samples possessed ratios of 0.29 and 0.30, with standard deviations of 0.02. The Crystal River sample had a height to length ratio of 0.27 (± 0.02 s.d.). The only feature that clearly places these populations within the bounds of *A. i. concentricus* is the proportion of white right valves. At 76% to 97% percent, all three samples exceeded Clarke's 50% cutoff value.

Morphological values for the cultured samples from Virginia and China indicate that both are slightly more similar to the northern *A. i. irradians* than to *A. i. concentricus*. Rib counts of 18.4 (± 1.0 s.d.) and 18.2 (± 1.0 s.d.) place them close to but not quite under the discriminating value of 19. Average height to length ratios for these populations were 0.28 (± 0.02) and 0.25 (± 0.02), also on the border of the range for the northern subspecies. The Virginia sample had 15% white right valves, however, and the China sample had 25%—both under the 50% mark that denotes *A. i. concentricus*.

Only one sample of calico scallop valves was available for morphological analysis, as the other included in the study was provided as tissue only. There is no published description comparable to Clarke's for this species. Sastry (1962) counted ribs on the left valve, and the sample on which he performed this count was from the Gulf of Mexico, complicating comparison with the sample measured in this study. The average rib count of 22.4 (± 0.06 s.d.) for the Cape Canaveral calico scallops falls within Sastry's range of 18-23 however, and other unquantified features such as shell

thickness, shape and coloration clearly distinguish the valves from those of the bay scallop.

Genetic Variation in A. irradians *and* A. gibbus.

The analysis of genetic variation within and among the sampled populations of bay and calico scallops allowed seven null hypotheses to be tested. These are addressed individually, and are followed by a more general discussion of the findings of this study.

H_{O1}: *The sampled populations of* A. irradians *ssp. share a common gene pool.* The pooled heterogeneity analysis indicated that the seven bay scallop populations do not share common gene pool. **Reject.**

This result is not surprising given the degree of morphological differentiation among the populations, and the fact that they were sampled over a wide geographic range. The precise dispersal capabilities of the bay scallop are not known. Adults are known to be effective swimmers, but the "clapping" behavior that propels them is used primarily as an escape mechanism (Fay et al., 1983). One study observed a maximum spatial displacement of 0.8m for an adult scallop over a six day period (Marshall, 1960). Any wider-range dispersal of bay scallops occurs most probably in the 2-week larval stage, and is governed entirely by hydrographic conditions.

No clear trends are apparent in the magnitudes of corrected nucleotide sequence divergences and geographic distribution. The New England sample is about equally divergent from the pooled North Carolina sample (0.12%) and the Rabbit Key sample (0.12%), but is more divergent from the Florida Gulf population (0.18%). The pooled North Carolina sample, however, is more divergent from the Rabbit Key scallops (19%) than from the Florida Gulf sample (0.14%). This raises the question of the

validity of suggesting subspecies designations on the basis of these data. Since within- and among-sample divergences are so high, it is difficult to determine trends among populations that might be indicative of relatedness at the subspecies level.

H_{O2}: *Morphologically similar populations of* A. irradians *ssp. share a common gene pool.* Pairwise testing of geographically separate populations with the same taxonomic designation revealed none that shared a common gene pool. **Reject.**

Again this is likely due to the isolated nature of bay scallop populations, and the geographic distance separating the populations included in this study. The effects of sampling on these results should be considered at this point, as considerable error may have been introduced when scallops were collected. Actual removal of scallops from the field was performed by other researchers or culturists in all cases, and little is known about collection procedures used (i.e. randomization techniques, if any). It is assumed that the populations were effectively sampled with the removal of 30 to 50 individuals, but the potential for encountering maternally-based patchy distributions within populations is fairly high. Hydrographic factors may act equally on a larval cohort, so that adults collected from a particular patch may be derived from the same maternal lineage. Diversity values within populations were high enough, 0.63 to 0.91 when the cultured Qingdao and Virginia populations are not considered, that bias introduced by non-random sampling might be considered negligible. Interpopulation comparisons of diversity are not valid, however, particularly because two of the "natural" samples were actually derived from cultured populations. The Massachusetts sample, as described earlier, had been subject to broodstock introductions from a number of New England sources. The degree to which this population had been inbred is not known. The Crystal River population is only one generation removed from the source, but the measured diversity within this sample cannot be said to approximate that

in Crystal River bay scallops. It is useful to note, however, that neither the New England nor the Crystal River samples show diversities below those of samples collected from naturally settled populations.

UPGMA analysis based on nucleotide sequence divergence places North Carolina and Crystal River *A. i. concentricus* in a cluster separate from the New England sample (Fig. 12). This is in contrast to the UPGMA dendrogram produced from an analysis of allozymes, which placed a North Carolina bay scallop population in a cluster with samples from Massachusetts, Connecticut and Long Island, while samples from the Florida Gulf and Rabbit Key clustered separately (Marelli et al., in press) (Fig. 5). Based on these dendrograms, the mitochondrial nucleotide sequence divergence data support classification of North Carolina bay scallops as *A. i. concentricus*, as did the morphological analysis, while the allozyme data suggest that these are more closely related to the northern subspecies. Despite this incongruity, the difference in the two studies' findings is not that great. Both dendrograms show North Carolina samples to be distantly related to the two major clusters, and place them intermediate to other samples clustered more closely along the lines of taxonomic designation. Nucleotide sequence divergence between North Carolina and New England (pooled for NC and NCT) is 0.12%, the same as that between North Carolina and Rabbit Key, which cluster together based on this measure.

The conclusion to be drawn from these studies is that bay scallops from North Carolina are separated from those of New England and the Florida Gulf by comparable genetic distances and divergences. It is clear that two subspecies exist, one present in New England and one found in the bays of Florida's west coast. What is not clear is to which, if either, of these classifications the bay scallops of North Carolina belong. Based purely on morphology, North Carolina bay scallops should continue to be classified as *A. i. concentricus*. The potential for gene flow between the three general

areas where bay scallops persist, New England, North Carolina and the Gulf, is not great. It has been shown for the Oyster Bay system of Long Island Sound, that bivalve larvae are retained within the bay, and develop to a stage where they may recruit to the same population as their parents (S. Siddall, Kenyon College, 1993, pers. comm.).

Clarke (1965) delineated the range of *A. i. concentricus* to include the region between New Jersey and North Carolina. Unfortunately, it has not been possible to apply the genetic techniques of RFLP analysis and allozyme variation to bay scallop populations from this region, as none remain to be sampled. It seems probable that the clinal variation that places North Carolina bay scallops intermediate to those of New England and the Gulf, would also be apparent in a New Jersey sample, with the latter possessing morphological and genetic characters intermediate to those of New England and North Carolina.

H_{O3}: *Scallops sampled from the same population in two consecutive years share a common gene pool.* Scallops sampled from the same North Carolina population in two consecutive years were found to share a common gene pool, indicating that within-population variation is reasonably consistent over time. **Do not reject.**

Of the haplotypes observed in the NC and NCT samples, only one (AACAAAA) was common to both, but this was found in 48% and 62% of the individuals, respectively. This finding permits more confidence in the interpretation of results from geographically rather than temporally separated populations. Since temporal variation is relatively small, the high levels of variation within the different samples may be taken as characteristic of their respective populations—that is, they are not just "noise" in the signal, that might prevent characterization and distinction of populations with these genetic data.

This finding also holds implications for the question of bay scallop larval dispersal ability. It is apparent from the lack of temporal variation in these samples, that the scallops recruiting to this population in the two years examined originated from the same or similar broodstock. The most probable manner in which this temporal homogeneity is maintained is by retention of larvae from a local spawning event, so that those recruiting to the population are offspring of the previous generation. For a species that is short-lived and essentially annual then, this may be used as a measure of recruitment success.

H_{O4}: *Individuals of the purported* A. i. taylorae *subspecies share a common gene pool with those of a sampled population of* A. i. concentricus. Heterogeneity analysis indicated that *A. i. taylorae* from Rabbit Key, Florida and the Crystal River population of *A. i. concentricus* do not share a common gene pool. **Reject.** The Rabbit Key scallops do cluster with the Crystal River sample in the UPGMA analysis, however, and the nucleotide sequence divergence of 0.12% between the two populations would not seem to justify a separate subspecies status for the Rabbit Key scallops. These are at least as related to the Crystal River sample as is the sample from North Carolina, and both are currently designated *A. i. concentricus*. The suggestion of Marelli et al. (in press), that *A. i. taylorae* was described from juvenile *A. i. concentricus*, seems probable. Morphological variation between the populations may be limited to phenotypically plastic characters that are expressed differently in the two regions.

H_{O5}: *Cultured bay scallops from China share a common gene pool with a broodstock source population.* Cultured bay scallops from China were not found to share a common gene pool with the putative Virginia source population, or with the sample from New England. **Reject.**

Only one of the three haplotypes (AACAAAA) observed in the Qingdao sample was common to other populations, but this was found in the New England, both North Carolina, and Crystal River samples. Corrected mean nucleotide sequence divergences between the Chinese sample and these others ranged between 0.04% (Q vs. MA) and 0.24% (Q vs. FL). The Chinese sample was most closely related to the Virginia and New England populations in the UPGMA analysis, indicating that the three populations may have shared a common gene pool more recently than any has with the North Carolina or Florida populations. This potential relatedness is supported by morphological similarities measured in the valves from these samples.

The Chinese sample clustered more closely with the sample from New England than with the cultured Virginia population. One possible scenario to explain this is that, as reported, the Chinese culturing operation was founded exclusively with individuals from the Virginia population, but the latter has lost a greater proportion of the original variation in the intervening generations of hatchery breeding. At the time the scallops were sent to China, the Virginia population may have contained a greater genetic component resembling that found in New England. The introduction of New England bay scallops into the Virginia broodstock is known to have taken place, although it has been assumed that the majority of the stock originated from North Carolina-spawned animals that set naturally off Virginia's Eastern Shore.

H_{O6}: A.irradians *and* A. gibbus *share a common gene pool.* A rigorous test of this hypothesis was not necessary, as no similarity in mtDNA sequence was observed. **Reject.** This finding provides genetic evidence for the species-level distinction of the taxa. An estimate of the interspecific sequence divergence between *A. irradians* and *A. gibbus* would require the application of other molecular techniques, such as direct sequencing of more conserved regions of DNA.

H_{O7}: The sampled populations of A. gibbus *share a common gene pool.*

Heterogeneity analysis of the two samples of calico scallops gave results consistent with the null hypothesis, that the two populations share a common gene pool. **Do not reject.** This supports the suggestion (Kirby-Smith, 1970) that *A. gibbus* larvae are capable of wide dispersal. It would seem, however, that entrainment in the currents of the Gulf Stream would only account for a unidirectional, west to east, south to north transport of larvae. Larvae from individuals spawned in the northern Gulf of Mexico could feasibly recruit to populations on the Atlantic coast of Florida. Transport in the opposite direction, from the Atlantic into the Gulf, does not seem as likely. This one-way gene flow then, must be sufficient to prevent divergence of these geographically separate populations by the mechanisms of natural selection and genetic drift.

It would be very interesting to expand the study of genetic variation in *A. gibbus* to include samples from North Carolina and other points north of Cape Canaveral. The genetic discontinuity detected for the horseshoe crab (Saunders et al., 1986) and American oyster (Reeb and Avise, 1990) is more likely to be apparent in the continuously distributed calico scallop than in the spatially isolated and genetically dissimilar populations of bay scallop. The presence of a similar break in mtDNA haplotype frequencies for the calico scallop would require modification of the current theories of recruitment dynamics for this species.

General Discussion.

Trends in the genetic variation in *A. irradians* and *A. gibbus* can be compared with those observed in other bivalve species. Haplotype diversities *(h)* in these scallop species are high, up to 0.91 as measured for the Rabbit Key sample, but are comparable to diversities measured for the American oyster, *Crassostrea virginica.* (Reeb and Avise, 1990). Haplotype diversities in this species were found to range between 0.57, an average for populations sampled on the Atlantic coast, and 0.80, an average for populations from the Gulf of Mexico. A better comparative measure for populations, however, that is less dependent on the number of sites examined, is the percent mean nucleotide sequence diversity *(p).* This value for the bay scallop was measured at 0.22% to 0.53% (excluding the heavily cultured samples), and for the calico scallop was 0.54% (Cape Canaveral) and 0.69% (Apalachicola). For the Atlantic and Gulf assemblages of oysters, *p* values were 0.14% and 0.25%, respectively, somewhat lower than those for the scallop species.

A similar comparison between Atlantic and Gulf coast populations can be made for the calico scallop, with the Apalachicola (Gulf) population showing a higher haplotype diversity (0.92 vs. 0.79) and mean nucleotide sequence diversity (0.69 vs. 0.54) than the Cape Canaveral (Atlantic) sample. Broad conclusions cannot be drawn from this observation, however, as the Atlantic and Gulf "assemblages" in this case are comprised of a single population each. It would be necessary to sample at least several populations from each region, as was done with the oyster, in order to gain some certainty that the observed trend is real. The present study does not permit this type of Atlantic-Gulf comparative analysis for the bay scallop, as the only Gulf coast sample included (Crystal River) was derived from a population that had been subjected to a generation of hatchery rearing, and therefore did not yield a genetic diversity value representative of the natural population.

The effects of inbreeding on genetic diversity in bivalves have been studied using allozyme electrophoresis and mtDNA analysis. Allozyme frequencies compared for natural and cultured samples provide an indication of whether alleles at particular loci are lost, and whether mean heterozygosities or proportions of polymorphic loci are lowered in hatchery populations. In a study of this type performed on the Pacific oyster, *Crassostrea gigas*, three generations of hatchery propagation were observed to lead to a loss of rare alleles, but not decreased heterozygosity or loss of polymorphism. (Hedgecock and Sly, 1990).

RFLP analysis was performed on a related species, *Crassostrea virginica*, to determine whether native and selectively inbred populations could be distinguished with mtDNA (Brown and Paynter, 1991). This study found that a selectively inbred population, isolated from the wild for 8 to 10 generations, was characterized by a much different haplotype distribution than those observed for native populations. Six unique haplotypes were found in the inbred sample, when 28 individuals were screened with five restriction endonucleases. Only one haplotype, represented by a single individual in the inbred sample, was also observed in samples from three natural populations, and this was found in only 2 of 138 individuals examined. No real conclusions could be drawn from these data, except that processes have led to the divergence of the inbred population from the natural ones, without an accompanying, dramatic loss of diversity.

A similar study, comparing strains of *Crassostrea virginica* selected for disease resistance with their natural source populations, found marked genetic differences in these strains after only two generations of selective breeding (Graves and McDowell, 1993). Little genetic differentiation was measured among the 4 source populations, but the challenged strains were divergent both from each other, and from their respective sources. It is hypothesized that this differentiation was caused by the intense selection

for disease resistance in the two generations of hatchery rearing, or more probably, by genetic drift.

The specific effects of random genetic drift and selection on cultured bivalve populations has been examined in some detail by Gaffney et al. (1992). These researchers analyzed allozyme frequencies in three lines of *Crassostrea virginica* selected for rapid growth, and compared expected (allele frequencies in the parental source) with observed (those measured in the selected lines) haplotype distributions. The effective breeding number of individuals estimated for the lines was lower than the known number of adults mass-spawned to produce the lines. This discrepancy was reduced when lines were formed by pooling progeny from smaller group spawns, indicating that high levels of genetic drift observed for cultured bivalve strains (as in Brown and Paynter, 1991 and Graves and McDowell, 1993), may be due to small effective population sizes resulting from mass spawns in which few parental individuals are actually producing larvae.

A study of variation in mtDNA among natural and inbred populations has been conducted for the scallop species, *Patinopecten yessoensis*, using the polymerase chain reaction to amplify sections of the mtDNA genome prior to RFLP analysis (Boulding et al., 1993). Eleven haplotypes, determined by 10 variable restriction sites, were detected in 49 individuals. No significant differences in haplotype diversity or within-population nucleotide sequence diversity, were observed between the cultured sample and two natural populations. Nor was significant divergence measured among the samples, suggesting to these researchers that the hatchery stock had not been "substantially inbred" in three generations of culture.

The genetic aspects of hatchery rearing of bay scallops is of great interest to culturists in the U.S., where the bay scallop is native, and China, where culture of this scallop is being undertaken on a very large scale. The haplotype diversity values for

the cultured samples in this study indicate that mtDNA variation may be lost in the culturing process, but the factors influencing this loss are not clear. The Chinese population represented by the Qingdao sample presumably derived from 26 or fewer founder individuals, possessed a lower haplotype diversity (0.55) than the natural populations from Rabbit Key (0.91) and North Carolina (0.69, pooled). This value would not, however, seem to reflect 10 generations of potentially severe inbreeding. It may be that, since the bottleneck was not sustained—that is, in all generations after the first, far greater numbers of individuals were spawned—the variation present in the original broodstock was not appreciably decreased. The Chinese spawning protocol is not known, and it may be that instead of single mass spawnings, progeny from smaller spawning events are pooled, as recommended by Gaffney et al. (1992) to prevent loss of variation by genetic drift.

The Crystal River population, an F_1 product of approximately two dozen individuals, did not appear to have a lower haplotype diversity than the samples from natural populations, suggesting that this number of parents possessed sufficient diversity that interbreeding them did not produce a significant bottleneck, at least in one generation.

The cultured sample from the facility in Wachapreague, Virginia showed the only clear sign that a population had been inbred—a haplotype diversity of zero. This does not definitively indicate that all of the Virginia broodstock is of this type, since the sample was derived from one spawning event, reported to have taken place with 100-200 individuals. It is feasible that only one spawning female, or several of the same haplotype, produced viable gametes. If this single spawning is used to replace the broodstock for the subsequent generation, then a monotypic lineage would likely persist. If additional spawnings were undertaken with other individuals in the same season, greater diversity might be revealed and contributed to the next generation.

Likewise, if bay scallops from other locales are introduced to the broodstock, variant mtDNA haplotypes are also likely to be introduced. Spawnings should be conducted in small groups with the products pooled, to ensure the largest possible number of contributing parent individuals (Gaffney et al., 1992).

Genetic divergences among populations of bay and calico scallops reveal few clear trends or biogeographic patterns, as have been observed for the oyster, horseshoe crab, and other coastal marine species (Reeb and Avise, 1990). An UPGMA cluster analysis for the haplotypes observed in the calico scallop did not reveal two distinct phenetic groups characterizing the Atlantic and Gulf coast populations, as it did for *Crassostrea virginica.* If a break in the distribution of mtDNA haplotypes exists for the calico scallop, it may only be detectable at sites north of Cape Canaveral.

The non-continuous distribution and isolated nature of bay scallop populations has led to high mean nucleotide sequence divergences both within and among populations, such that a break in the haplotype distributions would not likely be detected. Furthermore, the location of the genetic break in the mtDNA of the oyster and horseshoe crab (Saunders et al., 1986) occurs in the middle of a large region devoid of bay scallops, so a similar break between Gulf and Atlantic bay scallop populations might reflect current geographic instead of (or in addition to) historic distributions .

Conclusions.

Restriction site variation in the mitochondrial DNA of *Argopecten irradians* revealed high haplotype diversities within populations, and high levels of genetic differentiation among populations. No two geographically separated populations were found to share a common gene pool, and neither of two cultured populations shared a common gene pool with any other population.

An UPGMA cluster analysis based on nucleotide sequence divergence suggests that bay scallops in North Carolina are more closely related to those from the Florida Gulf than to those in New England, though only slightly. This is in accordance with morphological features, which have traditionally been used to place North Carolina and Florida populations in one subspecies, and New England populations in another. The same UPGMA analysis placed the two cultured samples in a cluster with that from New England, suggesting that bay scallops of the northern subspecies formed a large component of the broodstock for these cultured populations.

Two samples of the calico scallop, *Argopecten gibbus* , also exhibited high haplotype diversity, but nucleotide sequence divergence between these was low. A test for heterogeneity was consistent with the null hypothesis that the two populations share a common gene pool. No restriction fragments or patterns were found in both the bay and calico scallop, indicating that the current species-level distinction between these taxa is valid.

Suggestions for Future Research.

Numerous questions were raised in the course of this study, that could be addressed by future research undertakings. Biogeographic patterns in the calico scallop should be easily detected, if present, by expanding the sampling regime to include more populations along the continuum of the species distribution. Such a study would also further elucidate questions addressed here, of gene flow and larval dispersal patterns for the calico scallop.

Sampling greater numbers of bay scallop populations, with every effort to obtain natural rather than hatchery-reared samples, would also provide greater insight into gene flow patterns and relationships among subspecies. Certainly, a sample of the Texas bay scallop, *Argopecten irradians amplicostatus,* should be included for

comparison, as little is known about this subspecies and how it fits phylogenetically with the others into the *Argopecten gibbus* stock (T. Waller, Smithsonian Institution, 1994, pers. comm.). Including samples of the extant *A. nucleus* and the Pacific species, *A. purpuratis* and *A. circularis*, might also be instructive, although it is likely that other molecular techniques would have to be employed in order to quantify differences among these.

Appendix 1. Key to the identification of the subspecies of *Argopecten irradians* (adapted from Clarke, 1965)

1. Mean rib count, 19 or more 2
   Mean rib count less than 19 3

2. More than 50% of the right valves are white.
   Mean H/L index of right valves 0.28 to 0.35.
   Known range: New Jersey south around coast
   of Florida to eastern Louisiana ..... *Argopecten irradians concentricus* Say

3. Less than 50% of the right valves are white.
   Mean H/L index of right valves 0.19 to 0.29.
   Width greater than length (mean W/L index
   usually 1.02-1.10). Known range: Massachusetts
   to New Jersey ..... *Argopecten irradians irradians* Lamarck

   More than 50% of the right valves are white.
   Mean H/L index of right valves 0.28 to 0.41.
   Width and length approximately equal (mean
   W/L index usually 0.96-1.04). Known range:
   New Jersey and south 4

4. Mean rib count 18-19. Known range: New Jersey
   south around coast of Florida to eastern Louisiana .....
   ..... *Argopecten irradians concentricus* Say

   Mean rib count 17 or less (usually 15 -16).
   Known range: eastern to southern Texas ..... *Argopecten irradians amplicostatus* Dall

Length (L) is measured from umbo to opposite end of shell.
Width (W) is the maximum dimension at right angle to length.
Height (H) is the greatest vertical height of the valve placed convex-side down on a flat surface.

Appendix 2. *Argopecten irradians*. MtDNA fragment length estimates, in kilobases, for eight restriction endonucleases: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, *Hin*dII. Fragment sizes can only be reliably estimated to the nearest 0.1 kb, but figures are provided to the next decimal place to indicate additivity and distinguish bands of similar sizes. Capital letters designate different haplotypes for each given enzyme.

*Ava*I	A	B	C	D	E
	-	-	-	-	-
	-	-	10.2	-	10.2
	6.5	6.5	-	6.5	-
	3.7	3.7	-	3.7	-
	3.4	-	-	3.4	3.4
	-	-	3.3	-	-
	3.1	3.1	3.1	-	3.1
	-	2.6	-	-	-
	-	-	-	2.3	-
	-	0.8	-	-	-
	-	-	-	0.8	-
	-	-	0.1	-	-
Total kb	16.7	16.7	16.7	16.7	16.7

*Ban*I	A	B	C	D	E	F	G	H	I
	-	-	-	-	-	-	-	6.19	-
	3.94	3.94	3.94	3.94	3.94	3.94	-	-	3.94
	-	-	-	-	3.46	-	-	3.46	-
	2.41	-	2.41	2.41	2.41	2.41	2.41	2.41	2.41
	-	-	2.36	-	-	-	-	-	-
	2.31	2.31	2.31	2.31	2.31	2.31	2.31	2.31	2.31
	2.25	2.25	2.25	-	2.25	2.25	2.25	-	-
	-	-	-	-	-	-	2.07	-	-
	-	-	-	-	-	-	1.87	-	-
	-	1.85	-	-	-	-	-	-	-
	1.78	1.78	-	1.78	-	-	1.78	-	1.78
	-	-	-	-	-	1.75	-	-	-
	-	-	-	-	-	-	-	-	1.73
	1.68	1.68	1.68	1.68	-	1.68	1.68	-	1.68
	-	-	-	1.40	-	-	-	-	-
	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
	-	-	-	0.85	-	-	-	-	-
	0.58	0.58		0.58	0.58	0.58	0.58	0.58	0.58
	-	0.56	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	0.52
	-	-	-	-	-	0.03	-	-	-
Total kb	15.91	15.91	15.91	15.91	15.91	15.91	15.91	15.91	15.91

Appendix 2 - cont'd

*Ban*II	A	B	C	D	E	F	G	H	I
	-	5.8	5.8	5.8	5.8	-	5.8	5.8	5.8
	-	-	3.1	-	-	-	-	3.1	3.1
	3	-	-	-	-	3	-	-	-
	2.8	-	-	-	-	2.8	-	-	-
	1.9	1.9	1.9	1.9	1.9	1.9	1.9	-	1.9
	-	-	-	-	1.8	-	1.8	-	1.8
	1.7	1.7	-	1.7	-	1.7	1.7	-	-
	-	-	-	-	-	-	-	1.6	-
	-	-	-	-	1.5	-	-	-	-
	1.4	1.4	-	1.4	1.4	1.4	1.4	-	-
	-	1.3	1.3	1.3	-	-	-	1.3	-
	1.2	-	-	-	-	1.2	-	-	-
	0.9	0.9	0.9		0.9		0.9	0.9	0.9
	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	-	-	-	0.75	-	-	-	-	-
	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	-	-	-	-	-	0.65	-	-	-
	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
	0.5	0.5	0.5	0.5	-	0.5	-	0.5	-
	-	-	-	-	-	-	-	0.3	-
	-	-	-	-	-	0.25	-	-	-
	-	-	-	-	0.2	-	-	-	-
	-	-	-	0.15	-	-	-	-	-
	0.1	-	-	-	-	0.1	-	-	-
Total kb	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6

*Bgl*II	A	B	C	D
	-	-	4.2	-
	-	4.16	-	-
	3.9	3.9	-	-
	3.58	3.58	3.58	3.58
	3.58	-	3.58	3.58
	-	-	-	2.98
	1.72	1.72	1.72	1.72
	1.61	1.61	1.61	1.61
	1.33	1.33	1.33	1.33
	-	-	-	0.92
	0.58	-	0.58	0.58
	0.3	0.3	-	0.3
Total kb	16.6	16.6	16.6	16.6

Appendix 2 - cont'd

*Bst*EII	A	B	C	D	E
	-	-	-	9.06	-
	7.8	7.8	-	7.8	-
	-	-	-	-	-
	-	-	-	-	6.1
	5.1	-	5.1	-	5.1
	-	-	5	-	-
	-	4.13	-	-	-
	3.96	3.96	3.96	-	3.96
	-	-	2.8	-	-
	-	-	-	-	1.7
	-	0.97	-	-	-
Total kb	16.86	16.86	16.86	16.86	16.86

*Eco*RI	A	B	C	D	E
	7.85	7.85	-	7.85	7.85
	-	-	6.63	-	-
	3.7	3.7	3.7	-	-
	3.5	3.5	3.5	3.5	3.5
	-	-	-	-	3.15
	-	-	-	3	-
	-	1.47	-	-	-
	-	-	1.25	-	-
	0.97	-	0.97	0.97	0.97
	-	-	-	0.7	-
	-	-	-	-	0.55
	0.5	-	0.5	0.5	0.5
Total kb	16.52	16.52	16.52	16.52	16.52

Appendix 2 - cont'd

*Hae*II	A	B	C	D	E	G	H
	-	-	-	-	-	-	8.21
	7.7	-	7.7	7.7	7.7	7.7	-
	-	6.15	-	-	-	-	-
	4.76	4.76	4.76	4.76	-	4.76	4.76
	-	-	-	-	-	-	-
	-	-	-	-	3.86	-	-
	-	-	-	2.35	-	-	-
	-	-	-	-	-	-	-
	1.84	1.84	-	-	1.84	-	1.84
	1.64	1.64	1.64	1.64	1.64	1.64	1.64
	-	-	-	-	-	1.56	-
	-	1.55	-	-	-	-	-
	-	-	1.0	-	-	-	-
	-	-	-	-	0.9	-	-
	-	-	0.84	-	-	-	-
	0.51	0.51	0.51	-	0.51	0.51	-
	-	-	-	-	-	0.28	-
Total kb	16.45	16.45	16.45	16.45	16.45	16.45	16.45

*Hin*dII	A	B	C	D	E	G	H	I	J	K
	-	-	-	-	4.92	-	4.92	-	-	-
	4.4	4.4	-	4.4	-	4.4	-	4.4	4.4	4.4
	-	-	3.56	-	-	-	-	-	-	-
	3.36	3.36	3.36	3.36	3.36	-	3.36	3.36	-	3.36
	-	-	-	-	-	3.18	-	-	-	-
	-	-	-	-	-	-	-	-	-	2.85
	2.3	2.3	2.3	-	2.3	2.3	2.3	2.3	2.3	-
	2.2	-	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
	-	-	-	-	-	-	-	-	2.08	-
	-	1.5	-	-	-	-	-	-	-	-
	-	-	-	1.49	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	1.28	-
	-	-	-	-	-	-	1.18	-	-	-
	-	-	-	-	-	-	-	1.07	-	-
	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
	-	-	0.84	-	-	-	-	-	-	-
	-	-	-	0.81	-	-	-	-	-	-
	-	0.7	-	-	-	-	-	-	-	-
	0.7	0.7	0.7	0.7	0.7	0.7	-	0.7	0.7	0.7
	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
	0.55	0.55	0.55	0.55	0.55	0.55	0.55	-	0.55	-
	0.52	0.52	0.52	0.52	-	0.52	-	-	0.52	0.52
	0.48	0.48	0.48	0.48	0.48	0.48	-	0.48	0.48	0.48
	-	-	-	-	-	0.18	-	-	-	-
Total kb	15.96	15.96	15.96	15.96	15.96	15.96	15.96	15.96	15.96	15.96

Appendix 3. *Argopecten gibbus.* MtDNA fragment length estimates, in kilobases, for six restriction endonucleases: *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II. Fragment sizes can only be reliably estimated to the nearest 0.1 kb, but figures are provided to the next decimal place to indicate additivity and distinguish bands of similar sizes. Lowercase letters designate different haplotypes for each given enzyme.

*Ava*I	a	b
	6.3	-
	-	3.6
	3.4	3.4
	3.0	3.0
	2.8	2.8
	-	2.7
	1.0	1.0
Total kb	16.5	16.5

*Ban*I	a	b	c	d	e
	-	5.0	5.0	-	-
	3.3	-	-	3.3	3.3
	2.9	2.9	2.9	2.9	2.9
	-	-	-	-	2.3
	-	-	-	2.0	-
	1.9	1.9	1.9	1.9	-
	1.7	-	-	1.7	1.7
	-	-	1.6	-	-
	1.5	1.5	1.5	1.5	1.5
	1.4	1.4	1.4	-	1.4
	1.4	1.4	-	1.4	1.4
	0.8	0.8	0.8	0.8	0.8
	0.6	0.6	0.6	-	0.6
	0.4	0.4	0.4	0.4	-
	0.2	0.2	-	0.2	0.2
Total kb	16.1	16.1	16.1	16.1	16.1

*Bgl*II	a	b	c	d	e	f	g
	-	-	-	-	-	-	14.8
	-	9.6	9.6	-	-	9.6	-
	8.0	-	-	8.0	8.0	-	-
	-	-	-	-	6.3	6.3	-
	-	-	5.2	5.2	-	-	-
	4.3	4.3	-	-	-	-	-
	2.0	2.0	2.0	2.0	-	-	2.0
	1.6	-	-	1.6	1.6	-	-
	0.9	0.9	-	-	0.9	0.9	-
Total kb	16.8	16.8	16.8	16.8	16.8	16.8	16.8

Appendix 3 - cont'd

*Bst*EII	a	b
	12.0	-
	-	9.2
	4.4	4.4
	-	2.8
Total kb	16.4	16.4

*Eco*RI	a	b	c
	-	11.6	-
	8.0	-	8.0
	4.4	4.4	-
	-	-	3.7
	3.6	-	3.6
	-	-	0.7
	0.5	0.5	0.5
Total kb	16.5	16.5	16.5

*Hae*II	a	b	c	d	e	f	g
	4.6	4.6	4.6	4.6	4.6	4.6	4.6
	-	-	-	3.7	-	-	-
	-	-	-	-	-	-	3.1
	2.9	2.9	2.9	-	2.9	2.9	2.9
	2.7	-	2.7	2.7	2.7	-	-
	-	2.4	-	-	-	-	-
	-	-	-	-	-	1.8	-
	1.7	1.7	1.7	1.7	-	1.7	1.7
	1.58	1.58	1.58	1.58	1.58	1.58	1.58
	-	-	1.55	-	-	-	-
	-	-	-	-	1.54	-	-
	1.45	1.45	-	1.45	1.45	1.45	1.45
	-	-	-	-	-	0.9	-
	0.8	0.8	0.8	-	0.8	0.8	0.8
	0.4	0.4	0.4	0.4	0.4	0.4	-
	-	0.3	-	-	-	-	-
	-	-	-	-	0.16	-	-
	0.1	0.1	-	0.1	0.1	0.1	0.1
Total kb	16.23	16.23	16.23	16.23	16.23	16.23	16.23

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VITA

Sandra Grace Blake

Born in Wakefield, Rhode Island, on September 20, 1968. Graduated from Zephyrhills High School, Zephyrhills, Florida, in 1986. Received a Bachelor of Arts degree in biology from Reed College in 1990. Entered the master's program at the College of William and Mary, School of Marine Science, in 1991.