Review article

GENETIC IDENTIFICATION OF FISHING STOCKS: NEW TOOLS FOR POPULATION STUDIES OF THE SPINY LOBSTER *Panulirus argus* (LATREILLE, 1804)

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**ABSTRACT**

Over the past years fishery managers and scientists have been addressing concerns on the spiny lobster *Panulirus argus* fisheries, due to unsustainable harvesting throughout the coastline of the Americas. Commercial fishing commonly overexploits stocks, and current landings of *P. argus* fisheries throughout the western central Atlantic indicate a resource that is being exploited beyond its limits. Knowledge regarding population subdivision is critical to sustainable fishery management and seems to be the correct approach as a problem-solving strategy in *P. argus* fisheries. The goal of this article is to provide baseline information in order to help researchers and fishery managers build knowledge base that would be used to facilitate the conservation and support the establishment of specific regional management policies for this valuable resource. With the advance of DNA technologies new approaches started to be applied on population genetic studies. Molecular ecologists have begun to use new techniques that allow them to subdivide a particular species into a number of genetically distinct stocks. The application of both microsatellite markers and DNA sequencing to the population genetics of *P. argus* is believed to be the method of choice in detecting heterogeneity and identifying lobster stocks. However, both genetic and ecological tools (e.g. fully understanding of hydrological patterns along the coast) should be integrated to efficiently manage *P. argus* fisheries in Brazil and in the Caribbean.

**Key words:** Population genetics, lobster fisheries, molecular markers, DNA.

IDENTIFICAÇÃO GENÉTICA DE ESTOQUES PESQUEIROS: NOVAS FERRAMENTAS PARA ESTUDOS POPULACIONAIS DA LAGOSTA ESPINHOSA *Panulirus argus* (LATREILLE, 1804)

**RESUMO**

Nos últimos anos gerentes de pesca e pesquisadores têm endereçado grande preocupação à pesca da lagosta vermelha *Panulirus argus*, devido principalmente à sua pesca insustentável em todo o litoral Atlântico dos Americas. A pesca comercial geralmente causa sobrepesca aos estoques, e dados atuais indicam um recurso sendo explorado além de seus limites em todo o Atlântico Ocidental. O conhecimento da estrutura genética populacional da lagosta *P. argus* é crítico à gerência sustentável da pesca e parece ser a abordagem correta tendo em vista soluções aos atuais problemas. O objetivo deste artigo é fornecer informações às investigadores e gerentes de pesca visando a construção de uma forte base de conhecimento que poderá ser utilizada na elaboração e no estabelecimento de regulamentações específicas à pesca deste valioso recurso pesqueiro. Com o avanço de tecnologias do DNA novas abordagens começaram a ser aplicadas em estudos genéticos populacionais. Ecologistas moleculares começaram a usar novas técnicas que permitem subdividir uma espécie particular em um número de estoques geneticamente distintos. A aplicação de marcadores microsatélites e/ou o sequenciamento do DNA à análise genética da *P. argus* é o método de escolha para detectar a heterogeneidade e a identificação de estoques de lagosta. Entretanto, ferramentas genéticas e ecológicas (e.g. padrões hidrológicos ao longo da costa) devem ser integrados para gerenciar eficientemente a pesca da lagosta *P. argus* na costa do Brasil e no mar do Caribe.

**Palavras chave:** Genética populacional, pesca da lagosta, marcadores moleculares, DNA.

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INTRODUCTION

The Crustacea is a remarkably diversified class, both morphologically and ecologically, and has a great number of species, more than 30,000. They can be found in seawater, estuarine and freshwater environments, but not all of them are aquatic. There are semi-terrestrial and even terrestrial crustaceans. Some are small microscopic organisms (brachiopods, copepods, ostracods, amphipods, isopods, etc.); others are large-sized species (decapods) such as crabs, lobsters and shrimps (BOWMAN; ABELE, 1982).

Large crustaceans from the Decapoda order are among the species of most direct use to mankind. Some species of lobsters, shrimps, and crabs, are major sources of highly nutritional food, and support major key fisheries in many countries worldwide. For instance, edible spiny lobsters from the genus *Panulirus* are a major income source in the Brazilian and Caribbean fisheries, without mentioning the Penaeidae (shrimp) catches. In Brazil it is believed that more than 250 thousand people depend directly or indirectly on their fisheries (IBAMA, 1994; FAO/WECAFC/COPACO, 2003). In the western tropical Atlantic, including the Caribbean Sea and the Brazilian coastline, the lobster *Panulirus argus* is among the most abundant crustacean decapod species. Few other reptant decapods (i.e. crawling crustaceans) contribute to these regions’ crustacean fisheries as much as this lobster (HOLTHUIS, 1991; IBAMA, 1994).

Over the past ten years fishery managers and scientists have been addressing concerns on the spiny lobster *Panulirus argus* fisheries, due to unsustainable harvesting throughout the coastline of the Americas. This marine resource is being overexploited throughout much of its range (COCHRANE; CHAKALALL, 2001). Conservation strategies seem to be inefficient and outdated. This valuable organism, under specific regulations, is depleting, either having already reached unsustainable fishery levels, or very close to reaching this threshold. Current regulations include the prohibition on the harvest of berried females, the establishment of a minimum size for lobster capture, and a closed fishing season, among other directives. These regulations, however, were not able to protect lobster stocks from being overexploited. The eventual loss of lobster genetic diversity may contribute to the collapse of Panulirus fishing in Brazil and in The Caribbean, causing ecological and social impacts to the ecosystem and to fishery communities, respectively.

The goal of this article is to provide baseline information in order to help researchers and fishery managers build knowledge base that would be used to facilitate the conservation and support the establishment of specific regional management policies for this valuable marine resource.

BIOLOGY AND DISTRIBUTION OF SPINY LOBSTERS

The Palinuridae family, also known as spiny or rock lobsters, is classified in the phylum Arthropoda, the Crustacea class and Decapoda order. The family is made up of 49 species worldwide. Thirty-three of which have commercial value, and almost all belong to two genera only, *Panulirus* and *Jasus* (PHILLIPS et al., 1980; COBB; WANG, 1985; WILLIAMS et al., 1989). Palinurids inhabit tropical, subtropical and temperate waters. Adults of both genera are considered shallow water species (occasionally down to 90 m deep), inhabiting the seafloor, and often living among coral reefs and calcareous algae substrate (BLISS, 1982).

Within the genus *Panulirus* the Caribbean spiny lobster *P. argus* is distributed in the western central Atlantic (Figure 1). *Panulirus argus* have been found from the Gulf of Mexico to the Antilles and Bermuda, and as far north as Beaufort in North Carolina (U.S.), with its southernmost boundary of its distribution near Rio de Janeiro, Brazil (BLISS, 1982; HOL-
THUIS, 1991). This species is the most commercially-harvested Palinurid in American waters, and it is practically fished throughout its distribution. It supports major fisheries in the Caribbean and in Brazil with total landings around 30,000 metric tons per annum over the last decade, valued at hundreds of millions of dollars (COCHRANE; CHAKALALL, 2001). However, exploitation appears to have reached unsustained levels leading lobster production in the Caribbean and in Brazil at a significant decline (PHILLIPS et al., 1980; FONTELES-FILHO, 1994; COCHRANE; CHAKALALL, 2001). Therefore, major concern has been directed to the management of this important marine resource.

Figure 1. World distribution of the Caribbean spiny lobster *Panulirus argus* (Pa), based on Bliss (1982) and Holthuis (1991).

LIFE CYCLE AND DISPERSAL OF *Panulirus argus*

The complex life history of *P. argus*, as is also characteristic for other Palinurids, consists of three phases: 1) a transparent leaf-like larvae called phyllosoma, which is also a long-lived (6-12 months) planktotrophic phase due to its 10-12 developmental stages; 2) a short-lived (1 month) metamorphosed swimming postlarvae called puerulus that resembles adult lobsters; and 3) a benthic juvenile-adult phase which is dominant in the biocenosis (Figure 2; RICHARDS; POTTHOFF, 1981; GLAHOLT; SEEB, 1992; BOOTH; PHILLIPS, 1994).

According to Phillips et al. (1980) and Lipcius; Cobb (1994) berried female lobsters migrate towards the outer continental shelf to spawn between 159,000 to 1,925,000 eggs (FAO 2000) in deeper waters than referred to above, providing a constant supply of pelagic larvae that disperse throughout its distribution, and then are carried by oceanic currents. After the phyllosome larvae phase, lobsters metamorphose into a brief oceanic phase, puerulus, in the open ocean at the edge of the continental shelf and migrate back to shore using specialized abdominal pleopods. Around a month later, the transparent pueruli settle in a suitable inshore substrate (structurally complex vegetation) and acquire dark pigmentation and molt into the first benthic juvenile stage within a few days of settlement, between 8 and 10 days afterwards. Then, juveniles start to aggregate in biotic and abiotic structures in protected areas, in which they are sheltered from predators. After about one year of benthic existence, late juvenile spiny lobsters start occupying extensive shallow (3-15 m) banks where food is more abundant. Two years after settlement, mature *P. argus* move seaward to reefs where mating and spawning will occur to start a new life cycle.
The strong flow of major oceanic surface currents in the Atlantic Ocean (e.g. current velocities up to 80 cm s\(^{-1}\) in the Caribbean basin; KINDER, 1983) indicates a high probability for the mixing of larval populations from different breeding regions (Figure 3). It is important to remember that the lobster phyllosoma is planktonic and its development takes place over a period from 9 to 12 months, during which larvae are carried 1500 km away from the coast. Despite the fact that *P. argus* has a teleplanic and long-lived larvae, which in turn favours dispersal and gene flow over large distances, phyllosoma can also be affected by local patterns of ocean circulation and experience loops and gyres, which could act as barriers to dispersal (PALUMB, 1994). Therefore, local current systems may be more important in larval entrainment and recruitment processes (HATELEY; SLEETER, 1993).

Figure 2. Life cycle of *Panulirus* lobsters. Adapted from Phillips et al. (1980).

Figure 3. Major oceanic surface currents in the Atlantic Ocean (after Emilsson 1959; Peterson & Stramma 1991; Castro & Miranda 1998; Knoppers et al. 1999). Solid arrows indicate main current patterns in the region.
INFLUENCE OF BIOGEOGRAPHICAL BOUNDARIES ON LARVAL DISPERSAL

The western tropical Atlantic is characterized by numerous gyres, eddies and seasonally varying currents (LYONS, 1981; YEUNG; MCGOWAN, 1991). Dispersal in the upper Caribbean Sea might be favoured by the oceanic current flows off Nicaragua coast and the northern portion of the Belize Barrier Reef up to the Yucatán Channel, which may carry larvae and post-larvae from Nicaragua and Belize to the south of Florida (MENZIES, 1981; FRATANTONI et al., 2000; EZER et al., 2003). The hypothesis that phyllosoma larvae recruitment (originating from populations in the lower Caribbean, transported via the Yucatán Channel) probably sustains the lobster populations in the Straits of Florida is supported by many studies (LYONS 1981; YEUNG; MCGOWAN, 1991; ACOSTA, 1997).

In Brazilian waters, two major oceanic circulation systems are found along the 7,490 km (about 4,650 miles) coastline: the North Brazil Current (NBC) and the Brazil Current (BC). Both systems are originated from the South Equatorial Current (SEC) bifurcation. As it approaches the most eastern part of Brazil, the South Equatorial Current, bifurcates into two branches giving origin to these two important western boundary currents (Figure 3). The NBC, an extension of the South Equatorial Current coming from the East, flows to the Northern Hemisphere, parallel to Brazil’s semi-arid North Coast, and eventually crosses the equator entering the Caribbean. Its salinities are as low as 35 ‰ at times, and it has an annual range in surface temperature of 26º to 28º C. The Brazil Current turns south along the eastern coast of South America from Cape St. Roque, Brazil, to about 30º - 40º S latitude. The current is characterized by warm temperatures that vary from 19º to 27º C and a high salinity that averages up to 37 ‰ (STRAMMA; PETERSON, 1990; STRAMMA; ENGLAND, 1999).

The Amazon River outflow carries out sediments and planktonic larvae from an estuarine environment. It affects a large region in the tropical Atlantic and its influence can be extended for more than 100 km from the river’s mouth, across the continental shelf (CALEF; GRICE, 1967; HULBURT; CORWIN, 1969). The water masses drifting northwards from the Amazon River lower overall salinity of denser sea water in the region and therefore may become a natural boundary to more sensitive aquatic organisms from larvae to adult (e.g. lobsters among many other invertebrates).

The SEC bifurcation variability, along with the massive Amazon River outflow, the most voluminous source of freshwater outflow in the world, may play a significant role for the shelf fauna, mainly in the dispersion of planktrophic organisms (FLOETER; GASPARINI, 2000).

The oceanic currents within the Caribbean Sea and off the Brazilian coast, together with the Amazon outflow might influence directly, or indirectly, coastal processes and biological productivity. Currents can play a significant role on the transport of aquatic organisms (e.g. larvae) for long distances and also may keep them restricted to certain areas (RO-EMMIC; MCGOWAN, 1995). The Amazon River outflow influence over the shelf, limits the extended Caribbean and Brazilian provinces (FLOETER; GASPARINI, 2000). The shelf waters closed circulation prevents the transport of pelagic larvae to the areas that are unfavourable for their settling (CHEKUNOVA, 1972). All these factors may be responsible for genetically-defined boundaries for a large number of species, even though ocean mixing is believed to be sufficiently frequent and effective to decrease the influence of these natural boundaries.

Population subdivision and species diversification in many marine planktonic species, however, may find an explanation on ocean circulation patterns and the outflow of large rivers into the continental shelf.
LOBSTER FISHERIES AND GENETIC DIVERSITY

Commercial fishing commonly overexploits stocks, and current landings of *P. argus* fisheries throughout the western central Atlantic indicate a resource that is being exploited beyond its limits (COCHRANE; CHAKALALL, 2001). The intense fishing in certain habitats can cause the elimination of distinct stocks, which are genetically adapted to a specific environment (WILLIAMS et al., 1989). The result is a loss in diversity and the adaptive potential of a species. In order to maintain this very valuable resource and its fisheries it is essential that the stocks are all managed responsibly and sustainably. Serial mismanagement of fisheries extends beyond the effects of overexploitation in damaging marine biodiversity (AGARDY, 2000). Therefore, there is an urgent need to control and reduce the fishing effort in the lobster fisheries, as well as to study the genetic diversity and population structure of lobster populations. Knowledge regarding population subdivision is critical to sustainable fishery management. The more we understand about the structure of populations, the more something can be done to preserve commercial fishing resources. For example, to provide data on the maximum sustainable yield (MSY) it has been recognized that it is first essential to reveal patterns of population structuring in case any exist (EVANS; EVANS, 1995). Genetic analyses could be aimed at determining population structure. The information on stock structure has important implications for resource managers working to rebuild fisheries that have been overfished. Differences in stock structure can suggest that groups within a species constitute separate populations with distinct gene pools. The knowledge of the genetic structure of *P. argus* could aid fishery managers in estimating the contribution of new recruits from local and foreign sources. It is necessary to know if there are distinct stocks and, if so, how big the stocks are. Another important question that can be answered is if the stocks are discrete or if there is some interchange between them. This information will definitely impact the management of lobster fisheries in Brazil. If the Brazilian lobster population can be divided into distinct stocks, it may be found that the current restrictions can be relaxed or refined, or even redesigned. Resource managers may be able to design a fishing season that involves the harvest of all of the available stocks to a sustainable level and avoids the decimation of a particular stock, since different stocks have different biological characteristics, such as feeding habits, growth rates, yields, mortality rates, migration, reproduction, fecundity, intraspecific relationship, etc. (POLLOCK, 1993; FONTELES-FILHO, 1994).

The fishing grounds of the spiny lobster *P. argus* throughout its distribution have been proposed to be divided into four major areas based on the nature of coastal shelf and oceanic currents present in the region (FAO, 2000; COCHRANE; CHAKALALL, 2001): 1) Northern stock: Bermuda, Bahamas, northern Cuba, Saint Lucia, the Turks and Caicos Islands and the United States of America (Florida); 2) North Central stock: Belize, southwestern Cuba and Mexico; 3) South Central stock: Colombia, Honduras, Jamaica and Nicaragua; and 4) Southern stock: Brazil, Venezuela, the Dominican Republic and the Lesser Antilles Islands. Other three sub-areas could also be determined in Brazil considering the work developed by IBAMA’s Fisheries Statistics Project within The Lobster Working Group (FAO/WECAFC/COPACO, 2003). These administrative sub-areas include the following: Area I: Amapá to Ceará; Area II: Rio Grande do Norte to Alagoas; and Area III: Bahia to Espírito Santo. Alternatively, if the hypothesis that the spiny lobster stock comprises a single panmictic population is considered, the monitoring of genetic diversity of these fishing stocks would be useful as they might respond differently to local exploitation and management. The genetic identification of conservation units and their coordinated management are here of paramount importance to the conservation of a species under intense exploitation. Failure to do so might contribute to a long-term reduction in genetic variability, which may also play an extra role in the fishery collapse of one of the America’s most important fishing resource,
as observed in other aquatic species (BECKLEY; van der LINGEN, 1999). This would cause incalculable consequences for thousands of people that depend on this activity. However, if more than one stock exists, distinctively periods of closed fishing season should differ from population to population depending on biological characteristics, and regulations should be applied in agreement with each lobster stock.

GENETICS AND STOCK IDENTIFICATION: ATTEMPTS TO REVEAL POPULATION SUBDIVISION

At this point, we find it necessary to define the terms stock and local population/subpopulation, which are frequently used throughout this article, in order to comprehend the reason why it is sometimes used indistinctively in this study of genetic population structure. The term stock is usually applied by fishery biologists referring to breeding units with temporal and spatial wholeness, and may be considered equivalent to local population/subpopulation defined by evolutionary ecologists as a group of interbreeding individuals of the same species subject to limited dispersal and/or migration to different areas, living at the same time-period (CARVALHO; HAUSER, 1994). Therefore, it is considered that within a stock or local population genetic homogeneity exists. However, between stocks or populations, in which genetic isolation occurs due to limited gene flow, genetic divergence may occur with time (THORPE et al., 2000).

Biochemical polymorphism based on protein electrophoresis has been used to seek the molecular characterization of different spiny lobster stocks since late 1970s. Menzies & Kerrigan (1979) have quantified allozyme heterogeneity between *P. argus* populations in Central America and those off the Florida coast. Menzies (1981) evidenced possible population subdivision between Belizean *P. argus* and those of Elliot Key, Boca Raton, Dry Tortugas and the Virgin Islands, owing to a small gene flow between the Belize and Florida areas. Glahtol & Seeb (1992) suggest, however, that the Belizean population of *P. argus* is derived from a common genetic pool. Hately & Sleeter (1993) investigating spiny lobster populations from south Florida and Bermuda were unable to detect genetic subdivision. Ogawa et al. (1991), using several protein systems, investigated spiny lobster populations off the Brazilian coast, theoretically isolated by opposite ocean currents (North Brazil and Brazil). No evidence of subdivision among different fishery stocks was found between *P. argus* samples collected off Ceará and Bahia coastlines.

With the advance of DNA manipulation technologies, as well as the utilization of the polymerase chain reaction, new approaches started to be applied on population genetic studies (e.g. restriction analysis of mitochondrial DNA [RFLP] and PCR followed by direct sequencing). Other PCR-based markers (RAPDs) indicate the possible existence of two sub-populations of *P. argus* off the coasts of Brazil: one located from Pernambuco to Bahia and another from Ceará to Pará (CARREIRO, 2001; FAO/WECAFC/COPACO 2003). However, the sample survey conducted on the previous studies is too small to be sure on their findings.

Despite the fact that *P. argus* mtDNA reveals high levels of restriction-site polymorphism (KOMM et al., 1982; MCLEAN et al., 1983), no evidence of genetic differentiation among populations of *P. argus* distributed throughout the Caribbean was found (SILBERMAN et al., 1994a; 1994b). Mitochondrial DNA sequencing analysis, however, was able to reveal high levels of sequence divergence between *P. argus* samples collected in the Caribbean Sea and those from the Brazilian coast (SARVER et al., 1998).

Allozyme and restriction-site polymorphism (RFLP) data from the studies cited above have produced incongruent results, some indicating population differentiation over small geographical areas, whereas others suggesting the existence of a panmictic population, for closely related species, or even for the same species (i.e. *P. argus*). These results shed
doubts on the validity of these techniques and indicate that more convincing approaches should be employed to assess lobster genetic population structure.

Molecular ecologists have begun to use relatively new techniques that allow them to subdivide a particular species into a number of genetically distinct stocks and to determine the amount of intermixing that takes place among those stocks. The direct sequencing of the hypervariable domain in the mitochondrial control region and microsatellites have become popular for population genetic studies (DINIZ et al., 2004; DINIZ et al., 2005a; DINIZ et al., 2005b). These genetic markers are much more sensitive than allozyme and RFLP data for studying population processes like genetic drift and bottleneck events (RUZZANTE et al., 1996). The application of both microsatellite markers and mitochondrial DNA sequencing to the population genetics of the spiny lobster *P. argus* seems to be the method of choice to detect heterogeneity and identifying stocks of a marine organism with great dispersal capability.

**USE OF GENETIC MARKERS IN MOLECULAR ECOLOGY STUDIES**

The relatively new developments in molecular genetics and new DNA-based techniques have provided the necessary basis for the appearance of a variety of markers with different advantages and disadvantages with respect to the examination of genetic changes in the genome. As population discriminators, a great deal of markers employs techniques that involve the use of polymerase chain reaction (PCR) to amplify very rapidly evolving sections of the nuclear (nDNA) or mitochondrial DNA (mtDNA). Extensive literature provides a detailed explanation on the benefits and the role of the most used molecular markers to date [e.g., RFLP (restriction fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), microsatellites, DNA sequence polymorphism, etc...], applied to population genetics (SUNNUCKS, 2000; WARD, 2002; AVISE, 2004). The efficiency of these markers seems to be connected to both the species being studied and each markers’ evolutionary genetic features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, etc. The choice of the most appropriate markers should also be based on aspects such as: 1) genetic expertise of the research group involved in the project; 2) technical facilities available; 3) research budget and time limitations, and 4) the quality and type of tissue that can be collected (MITTON, 1994).

In recent years, however, the use of microsatellite DNA loci (nuclear) and DNA sequencing (of hypervariable mitochondrial regions) represents the most extensively used approach widely applied to questions related to micro-scale processes among conspecific populations (AVISE, 2004).

**Microsatellite analysis**

Microsatellites are simple DNA sequences that are repeated several times at various points in the organism’s DNA (Figure 4). Such repeats are highly variable, enabling that location (polymorphic loci) be used as a marker. Their core-repeat units are short, ranging from two to six base pairs in length (TAUTZ; RENZ, 1984; JARNE; LAGODA, 1996), though di-, tri- and tetra-nucleotides are the most common. Microsatellites have much more information than allozymes; yet offer the same advantages of analysis. Ambiguity (RAPD and AFLP) and scarcity (RFLP) are not problems with microsatellites, given that appropriate enrichment technologies on microsatellite development are followed. The technical expertise required for detection and scoring/analysis once the polymorphic loci are identified is basically similar for all the methods. DNA microsatellites are probably the most powerful class of markers in genomic analysis for assessing population structure, linkage, and parentage and
relatedness. Their attributes of co-dominant Mendelian inheritance and high mutation rate make them appealing for use on closely related populations (BARON et al., 1992).

Microsatellites are abundant and widely distributed throughout eukaryote genomes, therefore, these short tandem repeats can be readily developed for use on genetic studies. The microsatellite polymorphism is detected using PCR amplification and acrylamide gel electrophoresis. The results can be visualised using silver staining, autoradiography or fluorescently labelled PCR primers with automated sequencing, the latter being more currently used.

Notwithstanding the fact that microsatellite primers already exist for Nephropidae lobsters (TAM; KORNFIELD, 1996; STREIFF et al., 2001), primers developed for one species seldom amplify homologous regions in a different species, even if it belongs to the same genus. To date, a total of 19 microsatellite DNA loci have been reported in *P. argus* (DINIZ et al., 2004; DINIZ et al., 2005b). Expectations on the utility of these loci are drawn from heterozygosity estimates.

Figure 4. Sequencing chromatogram of a section of the *Panulirus argus* nuclear DNA containing a short tandem repeat region [(TCTA)$_{10}$] and part of its unique flanking sequence (Diniz et al. 2004).

**Mitochondrial DNA sequencing**

The mitochondrial genome of invertebrates is a circular molecule and is 15.5–18 Kb in length. In contrast to the diploid nature of nuclear DNA, the mitochondrial genome is usually haploid. This is because the mitochondrial genome is maternally inherited, and thus the genotype of any individual is identical to that of its female parent. As a result, the genome does not undergo recombination (AVISE, 2004) and consequently variation accumulates by mutation alone. For instance, the mtDNA of the spiny lobster *Panulirus japonicus* is 15,717 bp and contains a non-coding region and 37 genes: 13 genes for proteins of electron transport [cytochrome oxidase subunits I–III (COX-1 – COX-3), cytochrome b (Cyt-b), NADH dehydrogenase subunits 1–6 and 4L (ND1–ND6 and ND4L), ATP synthase subunits 6 and 8 (atp6 and atp8)], 2 for rRNAs [large- and small-subunit rRNAs (LSUrRNA – 16S and SSUrRNA – 12S, respectively)], and 22 for transfer RNAs (Figure 5, YAMAUCHI et al., 2002).

DNA sequencing has become far more accessible due to the ease of amplifying specific polymorphic regions in the genome, especially those in the mitochondrial DNA. This cytoplasmic genome is frequently used in population studies to assess genetic connections over a variety of geographic scales due to the presence of regions with a rapid rate of evolution. The non-coding segment within the structure of the mitochondrial genome, called control region (i.e. it carries the genetic signals needed for replication and transcription), is usually the fastest evolving region in the mtDNA of invertebrates (AVISE, 2000; BILLINGTON, 2003). It is believed that the control region in decapods is divided into three polymorphic domains set apart by two stretches with no intraspecific variability (GRABOWSKI; STUCK,
The domains neighbouring coding genes in the mtDNA are hypervariable with a higher base substitution rate than the central domains (GRABOWSKI et al., 2004). For this reason, the control region has recently been used with success for population surveys (CHU et al., 2003; MCMILLEN-JACKSON; BERT, 2003; 2004) and therefore, it is anticipated that the hypervariable domain within the mitochondrial control region of the spiny lobster *P. argus* would be useful as a genetic marker in population genetics of the species. Primers for the CR of the mtDNA and its hypervariable domain are already developed (DINIZ et al., 2005a). These authors have also characterized the most variable domain in the control region in the spiny lobster *P. argus*. The domain shows all the variability expected to be found in the *P. argus* control region and its utility for population genetic studies was also discussed.

Figure 5. Schematic representation of the complete mitochondrial genome of *Panulirus* showing distribution of coding regions (2 rRNA, 22 tRNA and 13 protein coding regions) and the non-coding control region (modified from Yamauchi et al. 2002).

**FINAL CONSIDERATIONS AND FUTURE WORK**

Like many other marine species, the spiny lobster *Panulirus argus* is experiencing a long-term decline in countries with intensive fisheries for this crustacean. Because fishing has the potential to affect the genetic structure and diversity of a species, an understanding of the population structure is especially important in affected species. The genetic information generated from new genetic tools (e.g. molecular markers) may offer new insights to assess microevolutionary processes, refuting or corroborating with the existence of different lobster populations; thus possibly supporting the establishment of specific regional management policies.

The spatial arrangement of genetic variation of a marine taxon with a large population size and potential for large-scale dispersal is dependent on the amount of genetic exchange (gene flow) between different populations. The higher the amount of genetic differentiation between populations (usually triggered by extrinsic barriers), the smaller the genetic exchange between these separated groups, and consequently the larger must be the intrinsic barriers that contribute to the observed reproductive isolation (GROSBERG; CUNNING-
HAM, 2001; PALUMBI, 1994). Other forces also help shaping the genetic structure of populations and influencing changes in genetic diversity. Drift and selection play an important role in forming spatial or temporal patterns, and intraspecific differentiation. However, no evidence exists for changes in diversity due to drift on stable and large marine populations, since gene flow tends to mitigate the effects of drift and selection on divergence among populations. Understanding microevolutionary processes is, therefore, dependent on the quantification of how genetically effective migration interacts with these forces (BOHONAK, 1999).

In Brazil, as in many other countries, stock assessment is still based on the catch/effort data (CPUE) and annual catch of spiny lobsters for the entire fishery. Given the shape of the Brazilian northeastern coastline, it was believed that the South Equatorial Current bifurcation phenomenon (i.e. the split of the SEC, most likely around the archipelago of Fernando de Noronha, into the Brazil Current (BC) flowing to the south and the North Brazil Current (NBC) flowing northwestward along the northern coastline of Brazil, Figure 3), would be responsible for significant genetic sub-division of the *P. argus* populations in this region into two distinct stocks (northern and southern). However, for caution reasons and the impossibility of sorting out lobster catch according to its origin, using conventional approaches, the adoption of only one management unit along the coastline of Brazil was taken for purpose of management (FONTELES-FILHO, 1994; PAIVA, 1974).

It is recommended, however, that both genetic (i.e. microsatellites and DNA sequencing) and ecological tools (i.e. fully understanding of hydrological patterns along the coast, the changes produced by the environment on patterns of larval dispersal and juvenile recruitment, the frequency of spawning, and mortality and growth parameters) are integrated to efficiently manage *P. argus* fisheries in Brazil.

It is also valid to add that the use of distinct markers to quantify genetic variation/divergence not only reduces the risk of erroneous assumptions regarding patterns of population structure of a species, but also provides a powerful method of identifying the factors contributing to the genetic structure/evolutionary relationships. Because not all portions of the genome capture the same information regarding processes of genetic diversification, comparisons of the patterns of diversity revealed by different markers can be highly informative (AVISE, 2004).

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