Rev. Invest. Mar. (2012), 32(1): 74-84

ISSN: 1991-6086

CIM.UH
Centro de Investigaciones Marinas
Universidad de La Habana

Genetic diversity and demographic variation in *Farfantepenaeus notialis* (Pérez-Farfante, 1969) from southern Cuban platforms

Aymée Robainas-Barcia[1]* ◘, Erik García-Machado[1]

(1)Centro de Investigaciones Marinas, Universidad de La Habana, Calle 16, No. 114 entre 1ra y 3ra, Miramar, Playa, Ciudad Habana 11300, Cuba.
* Corresponding author: arbarcia@gmail.com
◘ Present address: Universidad de Barcelona, Diagonal 643, 08028 Barcelona, España.

ABSTRACT

Deciphering demographic process among local populations is particularly important for the sustainable management of natural populations. In this study, we used nuclear and mitochondrial markers in order to estimate the immigration rates and variations in effective population sizes of *Farfantepenaeus notialis* from the Batabanó and Ana María Gulfs. A fragment of 385 bp of the mtDNA non coding region and a 506 bp of the flanking regions of two microsatellite loci (*PnS03* and *PNS18*) were sequenced in individuals sampled during 1999 and 2003. The results confirmed the genetic subdivision between gulf populations and the high levels of genetic diversity in this species. The estimates of immigration between the studied localities at Ana María Gulf were relatively high and differed between type of markers and years. The analysis also evidenced that the species has experienced a sudden population expansion about 51 kya. However, the current Ana María Gulf population has experienced a reduction of its effective population size in few generations while show a positive growth rate. We hypothesize that the effect of the increasing migrations between localities probably influenced the observed estimates while have been not enough to counterbalance the demographic reduction of the population effective population size. In contrast, Batabanó Gulf population seems stable or decreasing as suggested by both types of markers. This results alert about the delicate situation of one of the most important fisheries resource in Cuba.

Key words: genetic demography; genetic diversity; pink shrimp; Penaeid

RESUMEN

Descifrar los procesos demográficos entre poblaciones locales es particularmente importante para el manejo sostenible de las poblaciones naturales. En este estudio, se utilizaron marcadores nucleares y mitocondriales para estimar las tasas de inmigración y el tamaño efectivo de *Farfantepenaeus notialis* de los golfos de Ana María y Batabanó. Un fragmento de 385pb de la región no codificadora del ADNmt y 506 pb de las regiones flanqueantes de dos *loci* microsatélites (*PnS03* y *PNS18*), fueron secuenciadas en individuos colectados durante los años 1999 y 2003. Los resultados confirmaron la subdivisión genética entre las poblaciones de ambos golfos y la alta diversidad genética de esta especie. Los estimados de inmigración entre localidades del Golfo de Ana María fueron altos y difirieron entre tipos de marcadores y años. El análisis demográfico evidenció que la especie experimentó una expansión súbita hace aproximadamente 51,000 años. Sin embargo, la población efectiva actual del Golfo de Ana María ha experimentado una reducción en su tamaño efectivo, mientras muestra una tasa de crecimiento positiva. En este sentido se establece la hipótesis de que el incremento de las migraciones en los últimos años ha influenciado los estimados de crecimiento mientras que no ha sido suficiente para contrabalancear la reducción del tamaño efectivo poblacional. Por otra parte, la población de golfo de Batabanó parece estable o se encuentra en decrecimiento según los estimados de *g* para ambos marcadores. Los resultados alertan acerca de la delicada situación demográfica que presenta uno de los mayores recursos pesqueros de Cuba.

Palabras clave: camarón rosado; demografía genética; diversidad genética; Penaeidae

INTRODUCTION

In conservation and management of natural exploited populations it is of fundamental significance to estimate the level of individual interchange between populations as well as their effective population sizes (Wang, 2004; Broquet and Petit, 2009). Molecular markers have been a good alternative to make population sizes inferences because the information contained in DNA can provide gene flow parameter estimates for different timescales. Methods based on coalescent theory can give long-term migration rates because they use the genealogical information contained in a sample of genes (Beerli and Felsenstein, 1999; Wilson and Rannala, 2003).

Molecular population genetic have provided geneticist with numerous markers to carry out such estimations. Among them, microsatellite loci have been widely used (Goldstein and Schlöterer, 1999).

However, it is known that these markers show two major difficulties. First, due to the replication-slippage mechanisms and high mutation rates microsatellites are prone to high levels of homoplasy (Estoup, 1995; Jarne and Lagoda, 1996).

Second, it is difficult, for a given level of population differentiation, to discriminate if divergence is the product of the balance between the genetic drift and the gene flow or the effect of population history in the absence of present day gene flow.

The shrimp fishery is the second most valuable fishery within Cuban waters. Around 85% of commercial catches are from *Farfantepenaeus notialis* (Joyce, 1999). However at present this species represents the 100% of the shrimp national landings (Sosa, personal communication).

Overfishing of this resource has lead to declining catches from the late 1970s, exacerbated by degraded estuarine nursery habitats due to reduced river outflow caused by extensive damn construction during the 1970s and 1980s, the increase of dry seasons, and fishing of juveniles (Baisre and Zamora, 1983; Baisre, 2000; Claro *et al.*, 2001; Baisre *et al.*, 2003; Sosa, 2006).

Population genetic variation and structure of *F. notialis* have been examined based on different molecular markers: isozymes and mtDNA RFLP (García-Machado *et al.*, 2001), isozymes (Robainas-Barcia *et al.*, 2005) and microsatellites (Robainas-Barcia *et al.*, 2008). These studies analyzing temporal population genetic diversity and population structure provided important conclusions about the instability of this specie's population structure.

García-Machado *et al.* (2001) analyzed a total of 25 allozyme loci in samples of shrimps sampled in March of 1995, from seven localities at Ana María Gulf; including samples from Batabanó Gulf. The study also analyzed the variation of 2027 bp segment of mtDNA including part of the COI and COIII genes.

The pattern of allozyme variation among localities revealed strong population structuring at Ana María Gulf, the differentiation pattern was concordant with the geographical position of the localities and the sea current pattern of the gulf. The mtDNA marker employed shown genetic homogeneity inside the Ana María Gulf but a pronounced differentiation among the two gulfs. Robainas-Barcia *et al.*, (2008) conducted a spatiotemporal genetic structure study using five microsatellites loci in the populations of pink shrimp from both gulfs, using samples from years 1995, 1999 and 2003. The results revealed high and stable genetic diversity levels over the eight years of the study, but also significant changes of allele frequencies overtime. As previously found (García-Machado *et al.*, 2001), it was detected a significant genetic differentiation between the gulfs of Ana María and Batabanó and a genetic structure inside the Ana María Gulf. However, it was demonstrated that the local populations appeared genetically homogenized since 1999. The change in the increase in frequency of catastrophic natural events i.e. hurricanes, which have impacted the Caribbean region since 1998. These events should have promoted changes in the sea current patterns favouring migration between localities within the gulfs (Robainas-Barcia *et al.*, 2005; 2008). Similar results were also observed for the white shrimp *Litopenaeus schmitti* in the Cuban platform, however, in its case drift appeared as the more likely cause because distance between populations were high and estimates of census and effective population sizes low (Borrell *et al.*, 2007).

The aforementioned studies arrived to important conclusions about *Farfantepenaeus notialis* population's genetic diversity and structure; however some questions regarding the demography of this important natural resource, as the magnitude and direction of gene flow among populations, and their effective size, remained unanswered. In this study, nuclear and mtDNA sequences from samples taken in 1999 and 2003 were analyzed in order to estimate directional gene flow and effective population sizes.

This data will provide us with the necessary information to infer the current genetic patterns.

MATERIALS AND METHODS

Sample collection and genetic analyses

DNA from individuals of *Farfantepenaeus notialis* sampled during October 1999 and June 2003 at different localities of Ana María and Batabanó Gulfs (see Robainas-Barcia *et al.*, 2008) (Figure 1, Table 1) was extracted with 10% Chelex® resin (Walsh et al., 1991) and conserved at -20°C.

A fragment of 385bp of the 5´region of the mtDNA non coding region from seventy-six individuals was sequenced and analyzed using the primers: FnotRNC F- 5´ AAGAACCAGCTAGGATAAAACTTT 3´ and FnotRNC R-5´GATCAAAGAACATTCTTTAACTAC 3´. Ninety individuals from the Gulfs of Batabanó and Ana María (Table 1) comprising those studied with the mitochondrial non-coding region were sequenced for 506 bp (aligned sites) of flanking regions of two previously described microsatellite loci *PnS03* (208-234 pb) and *PNS18* (277-279 pb). These loci were chosen regarding the length of the known flanking regions. Both loci were amplified following Robainas-Barcia *et al.* (2002): *PnS*03 using the primers (F- 5´ TGCTAAATAAAAGTTTCTCGGTGAG and R- 5´ACACCACGACAGTCGACAG), and *PnS*18 with the primers (F- 5´GTCTTATCAAAACCCAAAGG and R- 5´GAAAAAGATGTCTGTGGACG).

PCR for both microsatellite loci were carried out in a 50 µl reaction volume with approximately 40 ng of genomic DNA containing 1 U of Taq polymerase (Amersham), 1x buffer (Amersham), 0.2 µM of each primer and 0.12 mM of dNTPs. The reaction profile was 94°C for 4 min for initial denaturing, followed by 36 cycles at 94°C for 1 min, 48°C for 1 min, 72°C for 1 min and a final extension at 72°C for 7 min. The amplification products were sent to Macrogen Inc., for sequencing. Sequences were visually inspected and aligned using Clustal method implemented in BioEdit v7.0.1 (Hall, 1999).

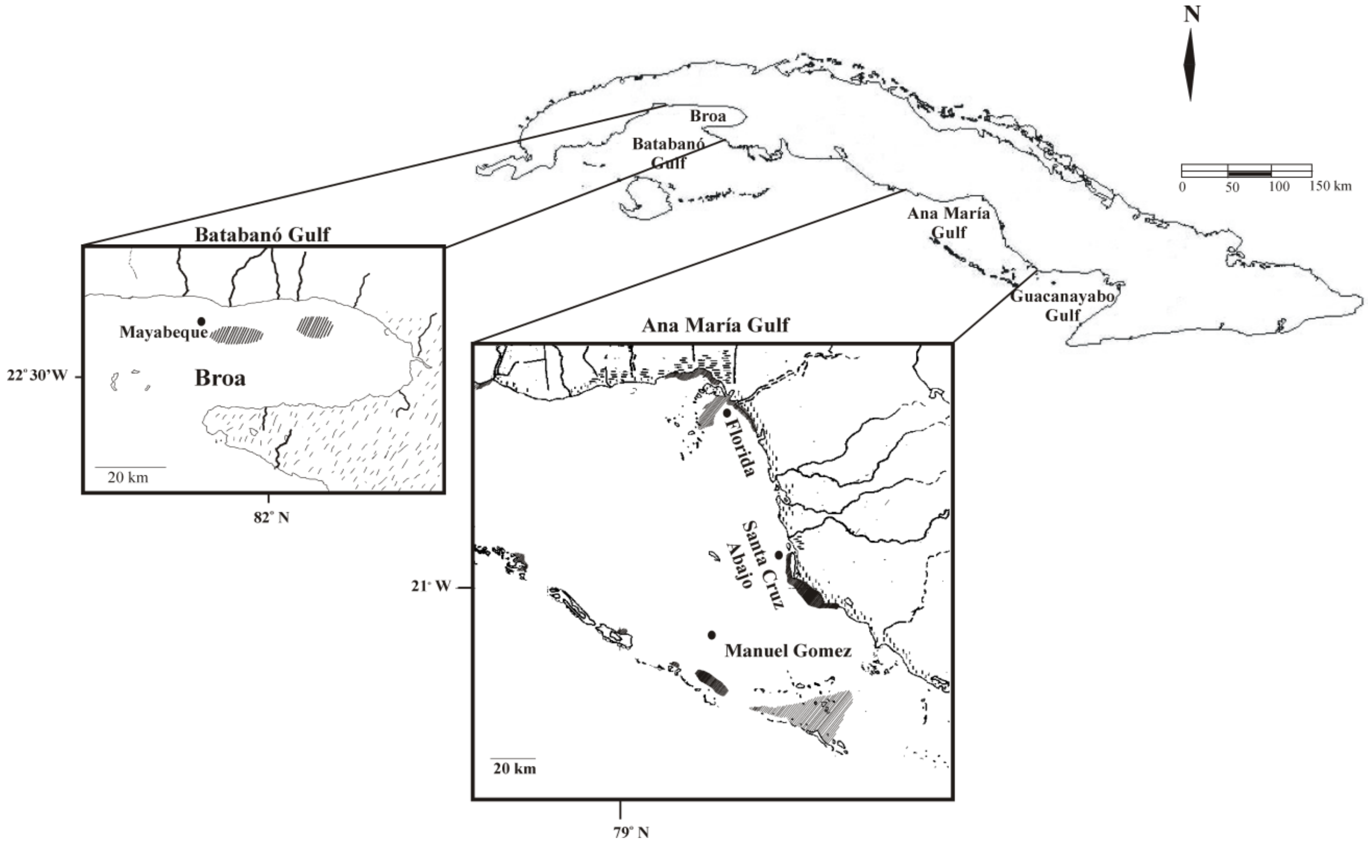

Figure 1. Sampling localities of *F. notialis* in Ana María and Batabanó Gulfs.

Neutrality and population differentiation

Deviations from neutrality were assessed using Tajima's *D* statistic (Tajima, 1989). Nucleotide diversity π (Nei, 1987), haplotype diversity (h) and their standard deviations were calculated using ARLEQUIN v 3.5 (Excoffier and Lischer, 2010).

To test for genetic differentiation among localities and regions a Principal Coordinates Analysis (PCoA) was carried out with the GenAlEx package (Peakall and Smouse, 2006) using pairwise Gamma$_{ST}$ estimates obtained using the DnaSP.v5 (Librado and Rozas, 2009).

Genetic diversity

Taking into account that the subdivided populations appears genetically homogenized in 1999 and 2003 (Robainas-Barcia *et al.*, 2005, 2008), we estimated the diversity and population size parameters only as overall means for each Gulfs.

However, for other parameter estimates, i.e. immigration rates, former local subpopulations found in 1995 (García-Machado *et al.*, 2001) were considered. The program Network 4.6.0.0 (Fluxus Technology Inc.) was used to elaborate median joining haplotype networks for each type of marker and for each year. In all cases the maximum parsimony calculation (MP) post-processing option was used to eliminate non-parsimonious links in the networks.

Demography

The program LAMARC v. 2.1.6 (Kuhner, 2006) was used to estimate different demographic parameters using the Bayesian approach. The estimates of population parameter θ (4Nmμ for nuclear and Nmμ for mitochondrial) were obtained. The immigration rates among localities at Ana María Gulf were estimated using the estimator $M=m/\mu$ (where m is the chance of immigration per individual per generation, and mμ is the chance of mutation per site per generation). In the case of the nuclear genes both sequenced loci were analysed as two independent partitions. For all calculations most of the default settings in LAMARC v. 2.1.6 were used on the first run. For the sub-sequent runs the number of short and long chains was set to 10 and 2 respectively, with 10000 sampled trees every 50 iterations and burn-in of 1000 trees, and 100000 sampled trees every 50 iterations and burn-in of 1000 trees. The migration model was unconstrained.

Note that in our case, the subpopulations are currently not genetically differentiated (Robainas-Barcia *et al.*, 2008), which violate one of the assumptions of always greater than one. Accordingly, the magnitudes were only analysed as relative values, to show general patterns, because the estimates can be inaccurate (Kuhner, 2006). The computer program jModelTest v. 0.1.1 (Posada, 2008) was used to determine the substitution model that best explain

Table 1. Estimates of genetic diversity and neutrality for *F. notialis* at Ana María and Batabanó gulfs using the sequences of the mtDNA NCR and flanking regions of the nuclear *PnS03* and *PnS18* loci.

mtDNA NCR							
Population	N	Variable sites	Number of haplotypes	h	π	*D*	*Fs*
Ana María 99	24	40	24	1.00 ± 0.012	0.020 ± 0.0109	-1.256 p=0.077	-21.65 p=0.000
Ana María 03	28	41	28	1.00 ± 0.009	0.020 ± 0.0112	-1.203 p=0.099	-21.77 p=0.000
Batabano 99	11	26	10	0.982 ± 0.046	0.018 ± 0.0105	-0.914 p=0.164	-3.38 p=0.045
Batabano 03	12	30	11	0.985 ± 0.040	0.020 ± 0.0115	-1.061 p=0.152	-3.80 p=0.031
Nuclear DNA							
Population	N	Variable sites	Number of alleles	h	π	*D*	*Fs*
Ana María 99	48	67	40	0.989 ± 0.008	0.0124 ± 0.0081	-1.932 p=0.011	-29.19 p=0.000
Ana María 03	28	68	27	0.997 ± 0.010	0.0130 ± 0.0110	-1.929 p=0.016	-12.70 p=0.000
Batabano 99	6	14	6	1.00 ± 0.096	0.0070 ± 0.0014	-0.062 p=0.499	-2.94 p=0.018
Batabano 03	8	17	7	0.964 ± 0.077	0.0083 ± 0.0016	0.321 p=0.636	-2.39 p=0.043

the evolution of the data. The corrected Bayesian information criterion (BIC; Schwartz, 1978) was used to select the models. When the model was not available in LAMARC, only GTR and F84 are implemented, the closest in the ranking with respect to the best model was used. The substitution probabilities, base frequencies and transition/transversion ratios were set for each calculation.

Exponential growth rate (g) of population size was tested using LAMARC v. 2.1.6 (Kuhner, 2006) and using the same parameterization described above.

The g ($\theta_t = \theta_{\text{present day}}\, e^{-gt}$, where $\theta_{\text{present day}}$ is the current θ and θ_t is the value of the parameter t time ago) when positive indicates that the population is growing, and when negative, the population is declining.

Demographic changes were also investigated by estimating Fu's *Fs* (Fu, 1997), which is sensible to detect demographic changes (i.e. population size expansion) in populations. The statistics and its statistical signification were obtained by 10000 coalescence simulations using DnaSP v. 5.10 (Librado and Rosas, 2009). In the case of nuclear gene data, in order to obtain accurate values of these parameters, we first estimated the recombination rate using γ estimator implemented in SITES v1.1 (Hey and Wakeley, 1997).

For mtDNA, we also made a mismatch distribution analysis, where the number of pairwise differences between haplotypes was plotted according to their relative frequencies to determine if populations of either gulf had experienced a sudden population expansion (Slatkin and Hudson, 1991; Roger and Harpending, 1992). Time elapsed since population expansion was estimated using the equation: $t = \tau/4\mu k$ (Rogers and Harpending, 1992), where τ is the time measured in units of mutational time, k is the sequence length and μ is the mutation rate per nucleotide. The mutation rate used was 19% per million years, estimated for the mtDNA non coding region of *Farfantepenaeus aztecus* and *Litopenaeus setiferus* (MacMillen-Jackson and Bert, 2004).

RESULTS

Population differentiation

The agreement of the pattern of DNA polymorphism with the neutral model at each locus was evaluated by the Tajima's D tests and the results are summarized in (Table 1). For both mtDNA NCR and nuclear loci most of the values were negative and the tests failed to detect any departure from expected proportions under neutrality assumptions. The exception was for nuclear loci from Ana María Gulf samples where values were negatives but significantly different from the expected.

The PCoA was carried out using pairwise Gamma$_{ST}$ estimates (Figure 2). For both, the mtDNA NCR and nuclear loci, the first coordinates accounted for 55.97% and 52.7% of the variance indicating genetic subdivision between the Batabanó and Ana María Gulfs. As expected from previous results little differences were observed between localities at Ana María Gulf.

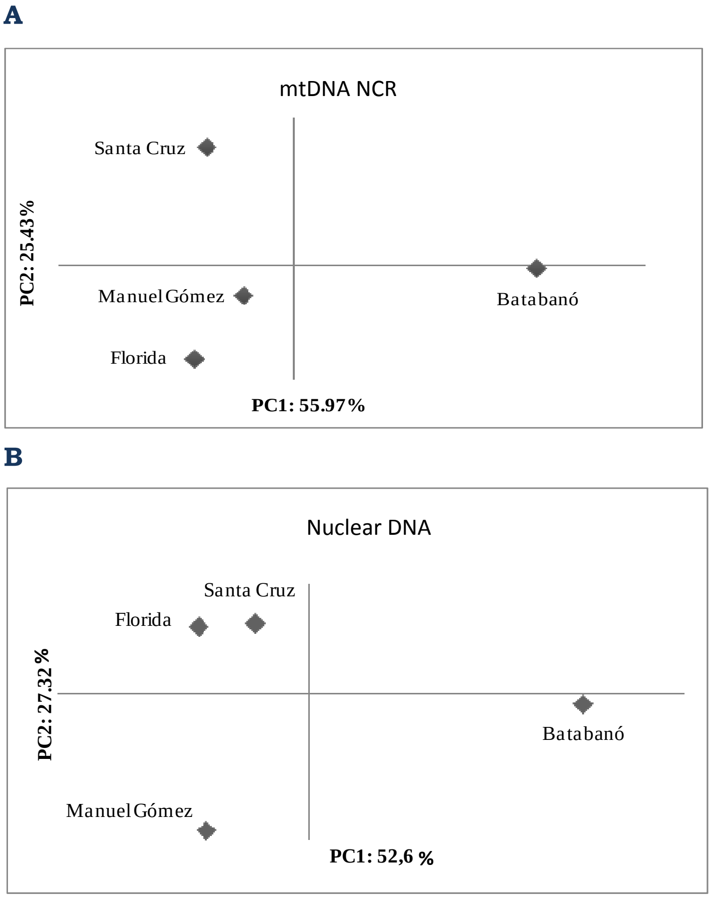

Figure 2. Principal Coordinate Analysis (PCoA) of *F. notialis* sampling sites for the mtDNA NCR (A) and the nuclear DNA (B).

Genetic diversity

The mtDNA NCR segment (385bp) presented 137 variable sites defining a total of 73 haplotypes for the 76 sequenced individuals. Considered together, for both years 70 haplotypes were unique, and the minimum number of mutations steps relying haplotypes was 2 (Figure 3). Only one haplotype was share between the Ana María and Batabanó Gulfs in 1999, but none in 2003. The NCR haplotype was high for each gulf in both years, with estimates of haplotype diversity (*h*) ranging from 0.9822 to 1 (Table 1). Similarly, the nucleotide diversity was also high, ranging from 0.018 to 0.020, with a mean estimate of $\pi = 0.019 \pm 0.004$.

There were no evidence for geographic association between haplotype relationships and their spatial location (Figure 3).

The aligned 506 bp of concatenated flanking regions of nuclear loci *PnS03* and *PnS18* have 166 polymorphic sites, considering indels (there were 30 and 7 unshared indels in Ana María and Batabanó Gulfs) as a fifth state character, a total of 80 alleles were defined for the 90 sequenced individuals. A great number of exclusive alleles were found separated by one or more mutational steps, with a mean value of 4.679 ± 0.88 differences. Three alleles were shared among Ana María and Batabanó Gulfs (Figure 3). The average estimates of number of differences among alleles within each gulf were 4.799 ± 0.896 at Ana María, and 4.111±1.209 at Batabanó. As for the mtDNA NCR the haplotypes relationships showed no association with their geographic distribution (Figure 3).

Diversity estimates were high with haplotype diversity (*h*) ranging from 0.964 to 1 and nucleotide diversity estimates also high ranging from 0.007 to 0.013. However, mean estimates per locality differs independently of the year. Ana María Gulf, although not statistically significant (t= 0.777, p> 0.05), presented higher estimates of nucleotide diversity ($\pi = 0.0127 \pm 0.009$) than Batabanó Gulf ($\pi = 0.0075 \pm 0.0029$) (Table 2).

Demography

Effective population sizes and exponential population growth test

The mtDNA NCR sequence variation agree with the substitution model HKY+I+Γ, with gamma shape value $\alpha = 0.7$ and invariant I= 0.606. However, for parameter estimation with LAMARC the closes ranked model, GTR+G (α= 0.16), was used with an estimated transition/transversion ratio of 7.95.

Similarly, for nuclear genes the optimal substitution model was TPM3uf+I+G, with gamma shape value of a = 0.23, but again the GTR+G (α= 0.017) was used. The transition/transversion ratio estimated from the data was 1.11.

In (Table 2) are depicted the effective population sizes per geographic region and per year. As observed, for most of the cases mtDNA NCR estimates were higher than the values obtained from nuclear loci sequences, however, support intervals

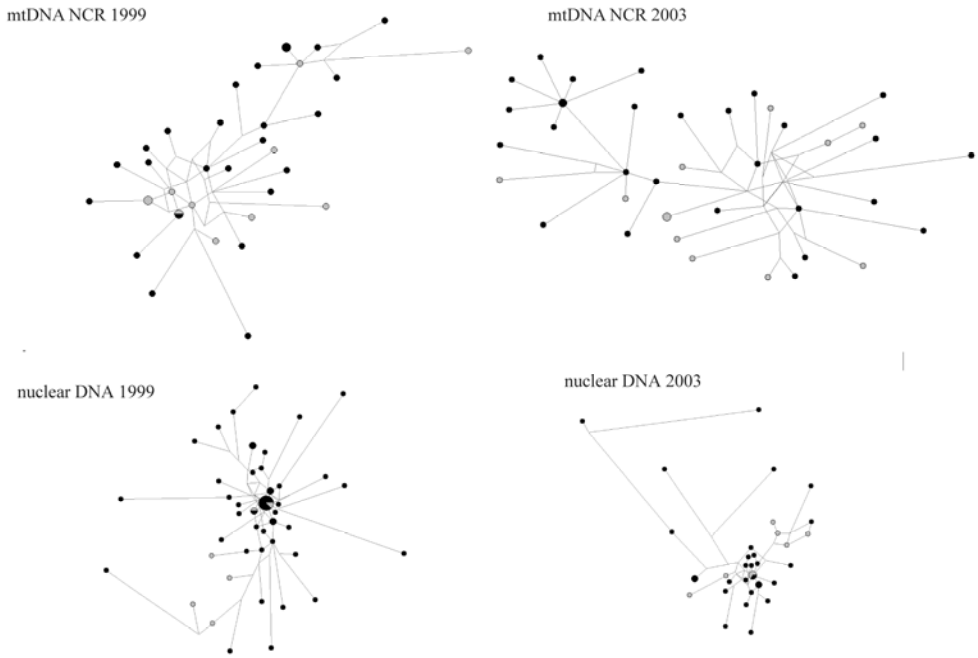

Figure 3. Haplotype networks reconstructed using the mtDNA NCR and nuclear DNA sequences of *F. notialis* sampled in 1999 and 2003 at Ana María and Batabanó Gulfs. The colours in circles (haplotypes) indicate the Gulf where they were found (black and gray for Ana María and Batabanó, respectively). The length of the connecting branches and the size of the circles are proportional to the number of changes and the number of individuals, respectively.

Table 2. Effective population size (Θ) and exponential growth estimation (g) of *F. notialis* in Ana María and Batabanó Gulfs.

Gulf	Θ	Support intervals	g	Support intervals
		mtDNA NCR		
Batabanó 1999	2.012	0.402/2.299	-455	-482/74
Batabanó 2003	1.631	0.231/2.269	- 448	-447/89
Ana María 1999	0.189	0.034/1.910	241	103/330
Ana María 2003	1.916	0.053/2.231	235	110/345
		Nuclear DNA		
Batabanó 1999	0.143	0.016/4.897	6	-450/45
Batabanó 2003	0.1076	0.027/3.329	-26	-442/13
Ana María 1999	0.165	0.101/0.346	31	16/83
Ana María 2003	0.0371	0.017/0.085	94	56/149

are too wide to consider any difference for comparisons.

Nonetheless, it should be noted, that diversity the estimates obtained for Ana María Gulf from nuclear loci were more accurate, as suggested by narrower support intervals, than those obtained for Batabanó Gulf, probably due to better sampling sizes used (see Table 2). In this case, the inferred size for Ana María Gulf in 1999 was about five times higher (Θ= 0.165, 0.101/0.346) than the estimate from 2003 (Θ= 0.0371, 0.017/0.085).

The analysis of the exponential population growth rates (g) clearly evidenced two tendencies (Table 2).

The positive values of g obtained for both types of markers at Ana María Gulf were signature for population growth. In contrast, the negative values obtained for Batabanó Gulf seems to indicate that the population is drastically declining. Although the value obtained from nuclear markers in Batabanó in 1999 is positive, it is small and the support intervals are largely skewed to negative values, also suggesting no growth or declining.

Sudden population expansion test

The trend of negative values obtained for the Tajima's

D test (Table 1) may be possible indicators of a past bottleneck, followed by a sudden population expansion. This was further evaluated by the analysis of Fu's *Fs* statistic, which is highly sensitive to population size changes (Fu, 1997). Strongly negative and significant values were observed for Ana María Gulf samples while also negative but smaller, and in some cases marginally significant values, were obtained for Batabanó Gulf (Table 1).

The analysis of demographic changes, according to the distribution of pairwise differences (mismatch distribution) for the mtDNA NCR was carried out pooling the samples of each year for each gulf. The mutational time since population expansion (τ) was 7.26 for Batabanó and 7.91 for Ana María Gulf samples, with a mean estimate of 7.58 with a confidence interval for the 95% confidence interval of 4.84 and 9.37 (Table 3). The estimated time for the coalescence of *F. notialis* haplotypes suggested that the samples from Batabanó and Ana María Gulfs had experienced a sudden growth approximately 51,000 (33,000/64,000) years ago.

Table 3. Summary of demographic parameters of the mismatch distribution analysis of the mtDNA NCR.

Parameter	Ana María Gulf	Batabanó Gulf
θ_0	0 (0 - 2.55)	0 (0 - 4.22)
τ	7.910 (4.941 - 9.598)	7.258 (2.51 - 10.95)
SSD	0.00188 p= 0.4799	0.00449 p= 0.6912

Immigration estimates

The immigration rates between sampling localities in Ana María Gulf are shown graphically in (Figure 4). The observed pattern varies between markers and overtime. The estimates of *M* from the mtDNA NCR sequences were in all cases higher than 28 and reach 205 from Santa Cruz Abajo to Florida in 2003. For nuclear genes, values were low in 1999 with only important exchanges between Santa Cruz Abajo to Florida. However, immigration rates were increased for 2003 when values were higher than 49 immigrants among localities.

DISCUSSION

The present study provides new data about the historical and present time demography of the populations of the species *Farfantepenaeus notialis* in the southern platforms of Cuban archipelago.

Particularly, evidencen that the populations have been subjected to changes in effective size and mition rates across the time that support previous observation from genetic and demographic studies.

Population differentiation

Although the analysis of the population structure was not a goal of this study the simple inference of population differentiation conducted by using a PCoA was congruent with previous studies using allozyme, mtDNA RFLP and microsatellite markers that showed a stable significant partition between Ana María and Batabanó Gulfs pink shrimp populations (García-Machado *et al*., 2001; Robainas-Barcia *et al*., 2005, 2008). The genetic homogenization through migration may have happen at relatively small geographic scale (i.e. intra platform) but for longer distances it looks unlikely. It seems that geographic accidents like deep Gulfs are effective barriers for dispersal in this species (García-Machado *et al*., 2001). In contrast, other authors have detected, by using mark and release techniques that shrimps are enable to migrate long distance across a continental shelf influenced by seasonal changes. This is the case of the brown shrimp, *Farfantepenaeus aztecus* and the pink shrimp *Farfantepenaeus duorarum* in the western part of the Gulf of Mexico (Sheridan *et al*., 1987). This probably explains the lack of population structure in another species, *Litopenaeus setiferus*, across the Gulf and the eastern coast of USA (Ball and Chapman, 2003).

The analysis of the flanking sequence of two microsatellite loci *PnS03* and *PnS18*, revealed a significant polymorphism, undetected when only electromorphs were considered. Notably, there were insertion deletions (indels) (not shown) in the flanking regions of microsatellite loci in *F. notialis* that were unshared between Ana María and Batabanó Gulfs. These elements are a potential source of size homoplasy when correcting length differences of electromorphs and shaped the differences between alleles. This is called Molecular Accesible Size Homoplasy (MASH) (Angers *et al*., 2000) and may had affected the observed level of polymorphism obtained from microsatellite analysis in our previous study (Robainas-Barcia *et al*., 2008) by reducing the observed number of alleles, the proportion of heterozygous individuals and also diminishing genetic differentiation among the compared populations.

Demographic inferences

The patterns observed at the different parameters estimated are relatively complex and should be analyzed integrally, particularly because we were unable to study samples from 1995 when intra-gulf population subdivision was detected (García-Machado *et al*., 2001). Populations from both Gulfs seem to have experienced a sudden demographic expansion, suggested by Fu's *Fs* (Table 3) and the unimodal distribution resulting from mismatch analyses (Table 3). The high number of changes accumulated through

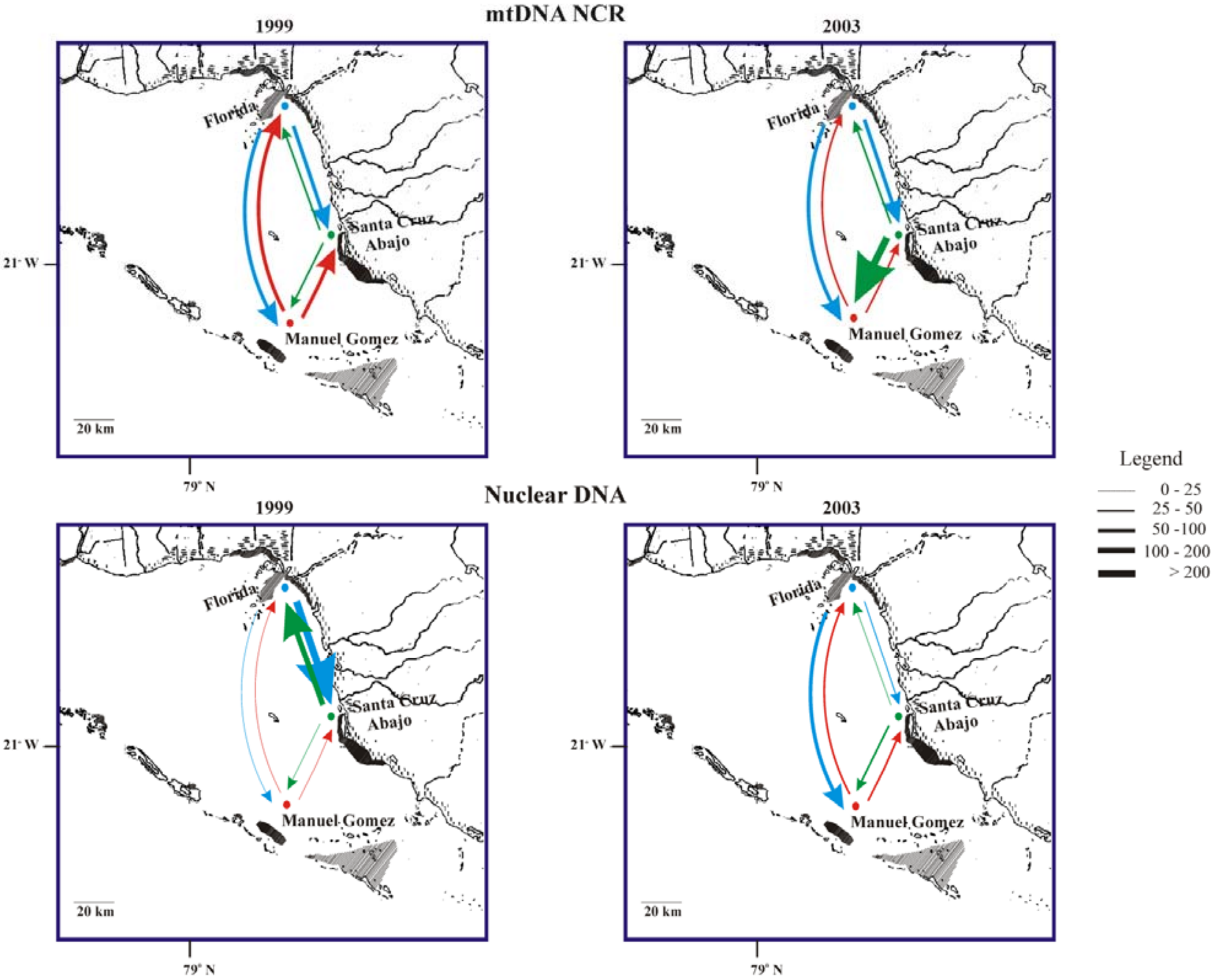

Figure 4. Schematic representation of the immigration rates between sampling localities in Ana María Gulf.

time, as exhibited also by the haplotype networks (Figure 2), suggested that this was a past expansion event that according to the estimates from the mtDNA NCR dated back about 51 kya. The population time expansion estimate could be associated to a major glacial cycle that when finished produced a rise of sea level episode 52 kya (Chappell, 2002). The increment of the sea temperatures after this glacial cycle would favour the expansion of species populations with a tropical distribution.

In Ana María Gulf the occurrence of subpopulations in 1995 and the perturbation of this subdivision in following years (Robainas-Barcia *et al.*, 2005, 2008) have induced changes in the spectrum of genetic variation. The increased gene flow between localities suggested from previous analysis (Robainas-Barcia *et al.*, 2008) was further supported here by the estimates of bidirectional immigration rates. The localities analyzed (formerly distinguished as Subpo-pulations), have exhibited exchanges of individuals sufficient to produce a homogenization of the frequency of the haplotype and allele variants allover the area (see Figure 4) (Vucetich and Waite, 2001) and might explain the temporal instability of the genetic structure of this species (Wang and Whitlock, 2003).

In this sense previous analysis evidenced an increase of migration rates between localities (Robainas-Barcia *et al.*, 2008), and the results obtained here additionally indicated that the migration occurs with similar intensity regardless the geographic position of the sampled site.

Nonetheless, other localities within these gulfs, which were identified as genetically differentiated populations and that could be significant nodes for deciphering the overall migration pattern, were not included in this study. For this reason the estimates of immigration rates should be considered cautiously (Beerli, 2004).

The estimates of the effective population sizes obtained at the two studied years for the mtDNA NCR do not differed between years and regions. In this case support intervals are large and completely overlap preventing further analysis of this parameter. Similarly has occurred with the estimates obtained from nuclear loci sequences from Batabanó samples.

However, an interesting outcome was obtained for Ana María Gulf; a shrink of the effective population size was observed in 2003 (Θ= 0.037, 0.017/0.085) with respect to 1999 (Θ= 0.17, 0.101/0.346). The values of nucleotide diversity (π) were also congruent with this observation (Table 1).

This result contrast, with the estimates of the exponential growth rates that indicated that Ana María Gulf population have been exponentially growing and Batabanó Gulf population is stable or decreasing as suggested by both type of markers (Table 3). In the case of Ana María Gulf we hypothesize that the increased effect of migrations between localities probably influenced the observed trend while have been not enough to counterbalance the demographic reduction of the population.

Batabanó Gulf however, has always represented a small stock for the fisheries of this species.

The annual landings of shrimp in the Cuban shelf were estimated around 6000 MT during 1976 to 1980. However, they have been reduced progressively in the Ana María Gulf and completely collapsed in the Batabanó Gulf (Baisre y Zamora, 1983; Baisre, 2000). In spite of the fisheries regulations the 4800 MT captured during the 1980s, were reduced by 1998 to 1239 MT (Sosa et al., 1999) and in recent years it have reached as maximum approximately 968 TM (Sosa personal communication). This decline was a result of the overfishing as well as the effect of environmental changes caused by natural (e.g. variation in raining regimes) and anthropogenic perturbations (e.g. construction of dams, habitat degradation by fishing devises and pollution) (Baisre and Zamora, 1983; Páez *et al.*, 1996, 1997; Baisre, 2000; Sosa, 2006).

Overall, the results presented here supply further evidence about the delicate situation of the exploited pink shrimp *F. notialis*, an important fisheries resource for Cuba. A reduction in the census population size in exploited natural populations is frequently not necessarily correlated with a reduction of the genetic diversity and its effective population size (Maruyama and Fuerst, 1985; Moyer *et al.*, 2005; Reed, 2007). This could be expected for a large population that exhibit random mating systems like is the case of penaeid shrimps.

However, a significant variation of their genetic structure and variability can reduce temporarily or permanently the population size, and in some occasions ends up with the species' extinction (Höglund, 2009).

In this sense, the tendencies observed in *F. notialis* call the attention about the conservation status of the species since a reduction in an effective population size of *F. notialis* was estimated in the largest and most productive zone for this species in Cuba in spite of the high migration levels estimated between the studied localities. Efforts have been made to introduce fishing devises more selective for size, however, the degradation of the habitat by these devises itself, and the uncontrolled effect of environmental variations remain challenging the future of the pink shrimp fisheries.

Acknowledgements

We want to thank Anna Pérez Beloborodova for technical assistance. This research was supported by grants from the International Foundation for Science, Stockholm, Sweden to EGM.

REFERENCES

Angers, B., Estoup, A., Jarne, P. (2000) Microsatellite size homoplasy, SSCP and population structure: a case study in the freshwater snail *Bulinus truncates*. *Molecular Biology and Evolu-tion* **17**(12): 1926-1932.

Baisre, J. (2000) *Crónica de la pesca marítima en Cuba (1935-1995). Análisis de las tendencias y el potencial pesquero.* FAO Documento Técnico de Pesca 394. 27 pp.

Baisre, J., Booth, S., Zeller, D. (2003) Cuban fisheries catches within FAO area 31 (Western Central Atlantic): 1950 – 1999. *Fisheries Centre Re-search Reports* **11**(6): 133-139.

Baisre, J., Zamora, A. (1983) Las pesquerías cubanas de camarón, antecedentes históricos, situación actual y perspectivas. Ministerio de la Industria Pesquera, Centro de Investigaciones Pesqueras. Cuba. 62 pp.

Ball, A.O., Chapman, R.W. (2003) Population genetic analysis of white shrimp, *Litopenaeus setiferus*, using microsatellite genetic markers. *Molecular Ecology* **12**, 2319–2330.

Beerli, P. (2004) Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology* **13** (4): 827-36.

Beerli, P. ,Felsenstein, J. (1999) Maximum likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics* **152**, 763–773.

Borrell, Y.J., Arenal, F., Mbemba, Z.M., Santana, O., Díaz-Férnandez, R., Vázquez, E., Blanco, G., Sánchez, J.A., Espinosa, G. (2007) Spatial and temporal genetic analysis of the Cuban white shrimp *Penaeus* (*Litopenaeus*) *schmitti*. *Aquaculture* **272**(Supplement 1): S125-S138.

Broquet, T., Petit, E.J. (2009) Molecular estimation of dispersal for ecology and population genetics. *Annu. Rev. Ecol. Evol. Syst.* **40**, 193–216.

Chappell, J. (2002) Sea level changes forced ice breakouts in the last glacial cycle: new results from coral terraces, *Quaternary Science Review* **10**, 1229–1240.

Claro, R., Baisre, J.A. , Lindeman, K.C., García-Arteaga, J.P. (2001) Cuban Fisheries: Historical Trends and Current Status. *In*: *Ecology of the Marine Fishes of Cuba*. (R. Claro, K.C. Lindeman and L.R. Parenti, *eds*.). Smithsonian Institution Press, Washington. pp. 194-219.

Estoup, A. (1995) Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). *Mol. Biol. Evol.* **12**, 1074–1084.

Excoffier, L., Lischer, H.E.L. (2010) Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitch-hiking and background selection. *Genetics* **147**, 915-925.

García-Machado, E., Robainas, A., Espinosa, G., Oliva, M., Paez, J., Verdecia, N., Monnerot, M. (2001) Allozyme and mitochondrial DNA variation in cuban populations of the shrimp *Farfantepenaeus notialis* (Crustacea: Decápoda). *Marine Biology* **138**(4): 701-707.

Goldstein, D.B. , Schlöterer, C. (1999) *Micro-satellites Evolution and Applications*. Oxford University Press, Inc. New York.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* **41**, 95-98.

Hey, J., Wakeley, J. (1997) A coalescent estimator of the population recombination rate. *Genetics* **145**, 833-846.

Höglund, J. (2009) *Evolutionary conservation genetics*. Oxford University Press, USA.

Joyce, I.T. (1999) Socialism and the fishery: a case study of the Cuban shrimp fishery. *The Cana-dian Geographer* **43**(1): 53-69.

Jarne, P., Lagoda, J.L. (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**, 424-429.

Kuhner, M.K. (2006) "LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters." *Bioinformatics* **22**(6), 768-770.

Librado, P., Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451-1452.

MacMillen-Jacson, A.L., Bert, T. (2004) Genetic diversity in the mtDNA control region and population structure in the pink shrimp *Farfantepenaeus duorarum. Journal of Crustacean Biology* **24**(1): 101-109.

Maruyama, T., Fuerst, P.A. (1985) Population bottlenecks and non equilibrium models in population genetics. 11. Number of alleles in a small population that was forms by a recent bottleneck. *Genetics* **111**, 675-689.

Moyer, G.R., Winemiller, K.O., McPhee, M.V., Turner, T.F. (2005) Historical demography, selection, and coalescence of mitochondrial and nuclear genes in *Prochilodus* species of norther South America. *Evolution* **59**, 599-610.

Páez, J., Font, L., Sosa, M., Morenza, M. (1996) Las pesquerías de camarón en la plataforma cubana. *FAO Fish Report* **544**, 155 – 179.

Páez, J. (1997) The role of the ecological and anthropological factors in the last three stages of Cuban shrimp fisheries. V Meeting of the WECAFC ad hoc Shirmp and Groupfish Working Group of Guianas-Brazil Continental Shelf and CFRAMP Shirmp and Groundfish subproject specification Workshop,155-179 pp.

Peakall, R., Smouse, P.E. (2006) GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288 - 295.

Posada, D. (2008) jModelTest: Phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253-1256.

Robainas-Barcia, A., Blanco, G., Sánchez, J.A., Monnerot, M., Solignac M., Gracía-Machado, E. (2008) Spatiotemporal genetic differentiation of Cuban natural populations of the pink shrimp *Farfantepenaeus notialis. Genetica* **133**, 283-294.

Robainas-Barcia, A., Espinosa, G., Hernández, D., García-Machado, E. (2005) Temporal variation of the population structure and genetic diver-sity of *Farfantepenaeus notialis* assessed by allozyme loci. *Molecular Ecology* **14**, 2933-2942.

Reed, D.H. (2007) Natural selection and genetic diversity. *Heredity* **99**, 1–2.

Rogers, A.R., Harpending, H. (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* **9**, 552-569.

Slatkin, M, Hudson, R.R. (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing population. *Genetics* **129**, 555-562.

Sosa, M. (2006) Las pesquerías de arrastre de cama marón en Cuba. *FAO Reports. Disponible en:* *ftp://ftp.fao.org/fi/document/rebyc/cuba/* .

Sosa, M., Iglesias M, Castro PO (1999) Contribución al conocimiento de la dinámica poblacional del camarón rosado (*Farfantepenaeus notialis*) en el Golfo de Ana María. *Taller 40 Aniversario del Centro de Investigaciones Pesqueras*. Cuba.

Schwarz, G., 1978. Estimating the dimension of a model. *The Annals of Statistics* **6**, 461-464.

Sheridan, P.F., Patella, F.J., Baxter, N., Emiliani D.A. (1987) Movements of brown shrimp, *Penaeus aztecus*, and pink shrimp, *P. duorarum*, relative to the U.S. - Mexico border in the western Gulf of Mexico. *Marine Fisheries Review* **49**(1): 14-19.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymer-phism. *Genetics* **123**, 585-595.

Vucetich, J.A., Waite, T.A. (2001) Migration and inbreeding: the importance of recipient population size for genetic management. *Conservation Genetics* **2**, 167-171.

Walsh, P. S, Metzger, D.A., Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**, 506–510.

Wang, J. (2004) Application of the one-migrant-per-generation rule to conservation and management. *Conservation Biology* **18**, 332-343.

Wilson, G.A., Rannala, B. (2003) Bayesian inference of recent migration rates using multi-locus genotypes. *Genetics* **163**, 1177–1191

Wang, J., Whitlock, M.C. (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**, 429-446.

Recibido: 26/09/2011
Aceptado: 08/02/2012