Genetic monitoring of the Mexican four-eyed octopus *Octopus maya* population: New insights and perspectives for the fishery management

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ABSTRACT

The *Octopus maya* fishery is one of the most important among the octopod fisheries in America. Recent studies about the wild population of this species, have contributed to understand that population variations and dynamics are highly influenced by temperature. The distribution area of this species could be divided in two well-differentiated thermal zones: an eastern zone influenced by the upwelling pulses, and a western zone with no upwelling influence. There is evidence suggesting that variations in population parameters could be linked to these thermal zones. However the fishery is still managed as one single unit, and there is no control of the capture limit. In this study we analyzed the multilocus microsatellite genotypes of wild *O. maya* across its distribution area to find out if the population is structured, and if the structure matches the mentioned thermal zones. Additionally, the heterozygosity was compared between samples from 2010 and 2015 to monitor changes related to the high fishing pressure. Results show that *O. maya* population is structured in two clusters that match with the different thermal zones in the distribution area. Moreover, significant genic differentiation was detected between these thermal zones. On the other hand, despite the high fishing pressure over this species, there is no significant difference in heterozygosity between 2010 and 2015 samples, and no inbreeding was detected, showing that genetic diversity is still high. We recommend the continuous monitoring of heterozygosity in this species, and a separate fishery management for Campeche (western) and Yucatan (eastern) subpopulations.

1. Introduction

The octopus fishery in the Yucatan Peninsula (Mexico) is the largest in the American continent; this fishery relies on two species, *Octopus maya* and *O. vulgaris,* and however *O. maya* represents the major part of the total catch (Galindo-Cortés et al., 2014; Gamboa-Álvarez et al., 2015). *O. maya* is a very well-studied species, and evidence suggest that there are differences in some population parameters between the western zone (in front of Campeche State), and the eastern zone (in front of Yucatan State), such as: size (Cabrera et al., 2012), abundance, catchability (Gamboa-Álvarez et al., 2015), sexual maturation (Ángeles-González et al., 2017) and reproductive season (Ávila-Poveda et al.,

2016). Moreover, a preliminary genetic analysis working with 4 heterologous microsatellites showed 2 loci with significant differentiation between eastern and western locations (Juárez et al., 2010). Another difference is that El Niño Southern Oscillation (ENSO) has an influence in the productivity of the western fishery locations but not in the eastern locations (Gamboa-Álvarez et al., 2015; Ángeles-González et al., 2017). Therefore, differences between western and eastern population features may be influenced by the different thermal conditions between these regions (Ángeles-González et al., 2017). The eastern edge of Yucatan Peninsula differs from the western zone, by the presence of upwelling pulses along the year. In summer, the upwelling maintains lower sea surface temperature (SST) offshore along the Yucatan State
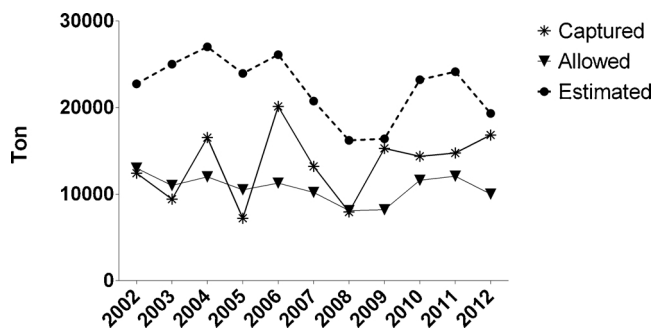

Fig. 1. Comparison of total estimated biomass, allowed biomass to be captured, and captured biomass for *O. maya* during 10 fishing seasons.

(23–27.5 °C) in comparison with the western zone with no influence of the upwelling (Zavala-Hidalgo et al., 2006; Enríquez et al., 2013; Noyola et al., 2015). In the western zone the offshore SST in summer can reach 30 °C (Noyola et al., 2015). Recently, it was observed in experimental conditions that temperatures above 27 °C, can reduce the reproductive success of females (Juárez et al., 2015), but also the metabolic performance of embryos and hatchlings (Caamal-Monsreal et al., 2016; Juárez et al., 2016). This could explain why thermal anomalies such as ENSO have a stronger effect over the fishery productivity in the western zone. However, in spite of the differences detected in the *O. maya* population features between western and eastern zones, the fishery has been managed as one single unit. Additionally, another management challenge, but related to the high fishing pressure, is that the maximum quote allowed has been frequently exceeded (Galindo-Cortés et al., 2014) (Fig. 1). In fact, in 2009 the estimated total biomass and the captured biomass was almost the same (SAGARPA, 2013). Such fishing pressure could cause changes in the genetic diversity of the population. So it is important to monitor this population parameter in order to detect a possible reduction. In this sense, and considering that only 4 neutral loci have been analyzed in this population (Juárez et al., 2010), it is necessary to go further in the monitoring of population dynamics using neutral markers as microsatellites. This kind of genetic markers have been successful in the identification of population structure and in the assessment of genetic diversity and heterozygosis of many cephalopod species around the world (Shaw et al., 1999; Adcock et al., 1999; Greatorex et al., 2000; Murphy et al., 2002; Casu et al., 2002; Pérez-Losada et al., 2002; Cabranes et al., 2007; Doubleday et al., 2009; Juárez et al., 2010). Therefore, in response to the mentioned management challenges, the aim of this study is to assess if the population is genetically structured, by incrementing the number of neutral loci analyzed. Moreover, the number of alleles and heterozygosity between samples of 2010 and 2015 from Sisal were also compared, to detect if the high fishing pressure has caused a reduction in the genetic diversity of this species in that lapse of time. Previous results planted the hypothesis that Sisal zone is a place of passage of octopus, migrating from the warmer zones to the cooler ones, where the upwelling has influence (Gamboa-Álvarez et al., 2015; Ángeles-González et al., 2017), making Sisal a key marine area to assess the genetic diversity of *O. maya* population.

2. Method

2.1. Sampling

Octopuses were captured by artisanal fishing gear during 2010 fishing season from 2 locations in the Campeche State representing the region without the upwelling influence: Sabancuy (n = 33) and Campeche (n = 33); as well as 3 locations in the upwelling-influenced region in the Yucatan State: Sisal (n = 36), Dzilam (n = 30) and Río Lagartos (n = 26) (Fig. 2). An additional sample of 27 individuals was obtained from Sisal in the 2015 fishing season. Captures were carried out approximately at 10 m depth.

2.2. Genotyping

For all samples, DNA was extracted from arm muscle tissue. For the 2010 samples, the genomic DNA was extracted via the phenol:chloroform method; for the 2015 samples genomic DNA was extracted by using the DNeasy Blood and Tissue kit (Qiagen) following manufacturer's instructions. DNA was quantified in a NanoDrop spectrophotometer (Thermo Fisher Scientific). The DNA integrity was assessed by electrophoresis in 1.5% agarose (85 V for 40 min). The amplification of eight microsatellite loci (accession numbers: KC602399.1, KC602400.1, KC602402.1, KC602403.1, KC602405.1, KC602406.1, KC602407.1, KC602409.1) were carried out in a thermal cycler (Eppendorf Mastercycler) using fluorescent labeled forward primers, following same PCR conditions as described in Juárez et al., 2013. Two positive controls and one negative control from each population were included in the genotyping analysis for verifying genotyping consistency. For allele visualization, PCR products were analyzed by commercial fragment analysis service (SeqXcel Inc., USA for 2010 samples and Carver Biotechnology Center, University of Illinois, USA for 2015 samples).

2.3. Data analysis

The sampling locations were grouped in two thermal zones. The group one is composed by Sabancuy and Campeche which represent the no-upwelling (western) zone and where SST can reach 30 °C. The group two is composed by Sisal, Dzilam and Río Lagartos representing the upwelling-influenced (eastern) zone, where temperature is below 27 °C most of the year (Fig. 2). A data matrix was built with the multilocus genotypes of each indivudual. This matrix was first analyzed with Microchecker v2.2.3 to detect null alleles and scoring errors. Then, departures from the Hardy-Weinberg (HW) equilibrium were tested for all locations in Arlequin v3.5.2.2, and for each locus in GenePop v4. The number of alleles in each sampling location were compared by using a one-way ANOVA, and multiple comparisons were corrected by using the Tukey test. The Fis index was estimated for all locations and Fst was estimated for the grouped locations with Fstat v2.9.3.2. A hierarchical AMOVA was performed in Arlequin v3.5.2.2 for the grouped locations, and the statistical significance was tested by bootstrapping. An exact test of genic differentiation was performed among the groups with GenePop v4. P values < 0.05 were considered significant in all the analyses. Finally, each individual was probabilistically assigned to a population by using Structure v2.3.4, inferring migrants from the data matrix by implementing the admixture model, and simulating from 1 to 5 clusters (*k*) with 30 replicates. The *ΔK* statistic (Evanno et al., 2005) was implemented to identify the number of *k* that best fits the data, by using Structure Harvestrer v0.6.94 (Earl and von Holdt, 2012).

3. Results

In spite of the presence of null alleles in the locus Omy4-11 (accession number KC602405.1), each analyzed locus is in H-W equilibrium (Table 1) as well as the multilocus genotypes in all the locations, including the 2015 sample from Sisal (Fig. 3, all values in Supplementary material 1). There are no significant differences in the number of alleles observed among the locations, and no significant differences between Sisal 2010 and Sisal 2015 (Fig. 4). Specific Fis indices per location were not significant (Table 2). When locations were grouped by thermal zones (zone one: Sabancuy-Campeche, zone two: Sisal-Dzilam-Río Lagartos) the Fst was significant between these zones. Moreover, the exact test of genic differentiation was also significant between these zones (Table 3). The probability for the number of clusters (*k*) composing the population was highest for *k* = 2 (Supplementary material 2). The assignment of individuals to putative clusters,