SPRFMO
South Pacific Regional Fisheries Management Organisation

12th MEETING OF THE SCIENTIFIC COMMITTEE

30 September to 05 October 2024, Lima, Peru

SC 12 - SQ 04
SNPs for population genomics analysis of D gigas

Peru

South Pacific Regional Fisheries Management Organisation
12th Meeting of the Scientific Committee
Lima, Peru, 30 September to 05 October 2024

SNPs for the analysis of the population genomic structure of *Dosidicus gigas* collected from the Peruvian jurisdictional waters

by

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This report contains information on the jumbo flying squid stock and fishery in Peruvian jurisdictional waters that, we reiterate, the delegation of Peru, in use of its discretionary powers, voluntarily provides for the purpose of information and support to the scientific research work within the Scientific Committee of the SPRFMO. In doing so, while referring to Article 5 of the Convention on the Conservation and Management of High Seas Fishery Resources in the South Pacific Ocean and reiterating that Peru has not given the express consent contemplated in Article 20 (4) (a) (iii) of the Convention, Peru reaffirms that the decisions and conservation and management measures adopted by the SPRFMO Commission are not applicable within Peruvian jurisdictional waters.

2024

SUMMARY

There is a great interest in understanding the biological and genetic aspects of *Dosidicus* gigas in order to implement effective management strategies. Knowing the number of subpopulations and their distribution throughout their geographic range, is important because they could exhibit unique demographic characteristics and respond independently to fishing pressures and environmental changes. In this study, we used a ddRAD-seq approach to obtain more than 14,000 single nucleotide polymorphisms (SNPs), neutral and under selection loci, to characterize population structure potential selection signals. A total of 92 samples of *D. gigas* in mature stages, collected in Peruvian jurisdictional waters were evaluated, considering a spatial and temporal sampling criteria. According to neutral loci, comparing its geographic distribution, northern coastal and southern oceanic groups showed significant differences, and the groups were also discriminated in their latitudinal distribution. According to the neutral and under selection loci, differences between phenotypic groups were observed, even more so for the organisms more distant from the coast (oceanic group). An important difference was also observed with loci under selection between the different years sampling periods, 2018-2019 versus 2021-2022. Apparently, the variation observed with the neutral loci revealed differences according to the geographical location of the species, whereas the adaptive variation revealed phenotypic diversity.

1. INTRODUCTION

The Jumbo flying squid *Dosidicus gigas* holds a significant economic and social value both locally and regionally, as it is a vital resource for Peru's artisanal fishing fleet and one of the main oceanic and transboundary fisheries in the southeastern Pacific (Csirke et al., 2018). *D. gigas* is widely distributed in the Southern Pacific and exhibits a highly dynamic population structure, characterized by significant variations in size and spatial distribution (Anderson & Rodhouse, 2001; Taipe et al., 2001). For example, this species differed greatly in age structure, size-at-maturity, and growth of the soft body between females and males (Liu et al., 2015). It is known for its migratory behavior, which includes both vertical migrations up to depths of 1200 meters and horizontal migrations across significant distances. Along its geographical distribution, three phenotype-size groups have been distinguished in adult organisms, based on the mantle length at which they reach gonadal maturity: the small (130–260 and 140–340 mm of mantle length, ML), medium (240–420 and 280–600 mm ML), and large-phenotype size (>400–500 and 550–650 to 1000–1200 mm ML, for male and female, respectively) (Nigmatullin et al., 2001). For instance, studies have shown that smaller specimens are typically found further offshore and in lower latitudes, while larger individuals are more common closer to the coast and in higher latitudes. In Peruvian waters, Arguelles et al. (2011) mentioned that this species showed a significant change in size structure, and in the size they reached gonadal maturity.

In this sense, we evaluated *D. gigas* from the Peruvian jurisdictional water based on neutral and outlier SNPs obtained with ddRAD-Seq analysis, considering a spatial (along its latitudinal and longitudinal distribution) and temporal (from 2018 to 2019 for small and medium-sized phenotypes, and 2021-2022 for large-sized phenotype) sampling

criteria. Finally, we compared our results with previous reports using the mtDNA ND2 gene.

2. MATERIALS AND METHODS

a. DNA extraction, ddRAD-seq, and SNP calling

A total of 92 samples were sampled from the Peruvian jurisdictional waters, according to the protocol described in SC7-SQ10, including the small (n=40), medium (n=31), and large (n=21) phenotype-size groups. The total DNA was extracted, and the quantity and quality of the extractions were evaluated. Concentrations were normalized, and the best samples were selected for ddRAD library construction. First, the PstI and ApeKI restriction enzymes digest the gDNA. Then, libraries were purified, pooled, and subjected to size selection for fragments ranging from 300 to 500 base pairs (bp). The sequencing was conducted using 150-bp pair-reads on the Illumina NexSeq 550 platform, generating between 5 and 28 million raw reads.

We used the Stacks v.2.65 (Catchen et al., 2013) modules from the read processing to the SNP calling steps, following the recommendations described by Rochette & Catchen (2017), Paris et al. (2017), and Cerca et al. (2021). For raw read processing, the paired-end reads were demultiplexed for each individual. Low-quality reads and internal adapters were filtered, and the remaining were trimmed to 120 bp using the *process_radtags* module. Additionally, the PCR clones were removed with the *clone_filter* module and one sample due to the low number of reads. For SNP calling, the *denovo_map.pl* wrapper was applied to all remaining samples after *de novo* parameters were defined on a small subset of samples. Additionally, the *population* module filtered loci present in a minimum of 80% of individuals in a population (-r) and a minimum of 15 populations (-p). The 1% minimum allele frequency and the maximum observed heterozygosity of 70% were applied to avoid false-positive SNP calls and the erroneous merging of paralogous loci, respectively. The VCF file was used to filter samples and loci with 20% percent of missing data, remove duplicated genotypes, and not polymorphic loci using 'adegenet' (Jombart, 2008; Jombart & Ahmed, 2011) and 'poppr' (Kamvar et al., 2014) packages in R Studio.

To identify potentially loci under selection, three software were used: Bayescan v2.1 (Foll & Gaggiotti, 2008), OutFLANK v0.2 (Whitlock & Lotterhos, 2014, 2015), and PCadapt v4.3.5 (Luu et al., 2016). Bayescan is based on the multinomial Dirichlet model, and default parameters with minor modifications were used. The other two methods were executed through R Studio with default parameters. The total number of outlier loci was extracted from the total variants into a new file to do independent analyses on neutral and adaptive loci.

2.2. Population genomics analysis

Population analyses were conducted for clean samples with high-quality SNPs, considering the comparison between phenotype-size groups, geographical distribution, and temporal discrimination. To analyze the adaptive variants separately, a subset with the total outlier loci from the three methods was obtained using R. The discriminant analysis of principal components (DAPC) was performed using the adegenet package. The pairwise Fst was calculated between each pair of populations based on 1000 permutations as implemented in the StAMPP R package (Pembleton et al., 2013). Three different hypotheses of groups' subdivision were evaluated using the analysis of molecular variance (AMOVA) using the Poppr R package. Samples were grouped according to their geographical distribution (06 populations) and including the phenotype-size groups at different geographical distributions (15 subpopulations). Analysis was also performed considering the number of sampling locations (latitudinal and longitudinal distribution), the sampling years (2018-2019 and 2021-2022), and the phenotype-size groups (small, medium, and large) as prior. Finally, fixation indexes were calculated with 1000 permutations. The significance level of multiple tests was adjusted using the false discovery rate (FDR) correction.

3. RESULTS

3.1. Geographic distribution grouping

Six populations were grouped using geographic distribution grouping, where 42,374 neutral and 433 outlier loci were identified. Using the neutral loci set, the pairwise Fst analysis showed significant differences (adjusted p values < 0.05) between the coastal and oceanic southern groups (SC-SO) and between the oceanic samples from the northern and central groups (CO-NO) (Fig. 1A). Considering two hypotheses of clustering based on: i) the geographical distribution and ii) the distance from the coast, all the Fis and Fit indices values were significant in the AMOVA analysis.

No significant differences between populations were found using the neutral loci set (Table 1). On the other hand, considering the loci under selection, the significant difference was observed among the northern coastal and the southern oceanic (NC-SO) groups (Fig. 1B). In concordance, a significant difference (Fct=0.00826) was observed for latitudinal distribution between the northern, central, and southern groups in the AMOVA results.

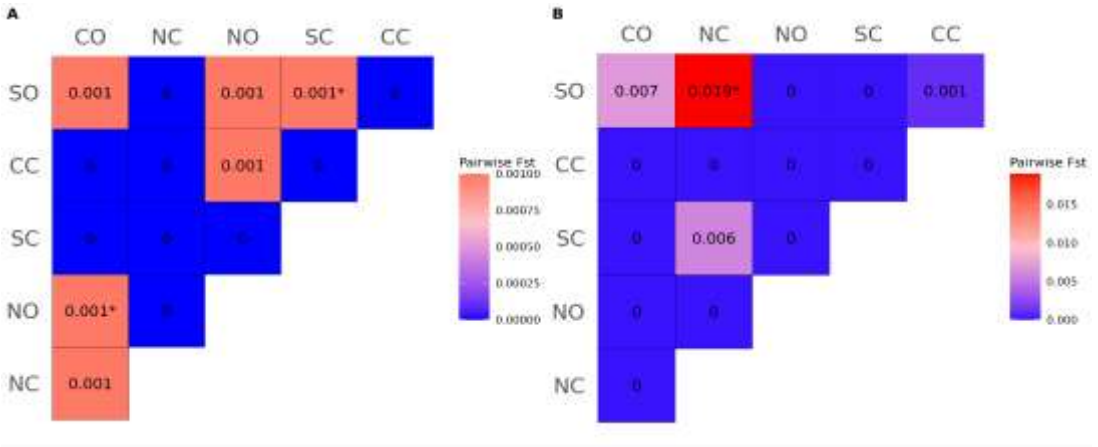

Figure 1. Heatmaps of pairwise Fst comparison between the six populations grouped according to their geographical distribution, using the neutral (A) and the outlier set of loci (B). The numbers are Fst values. Asterisk means significant differences, adjusted p values < 0.05. Population names are composed of two letters: the first stands for northern (N), central (C), and southern (S) samples; and the second for coastal (C) and oceanic (O).

Table 1. AMOVA analysis for 6 populations according to their geographical distribution, and the grouping criteria for latitudinal and longitudinal distribution, based on neutral (n=42374) and outlier (n=433) loci. Bold numbers indicate values with p<0.05.

Source of variation	Variance components	Percentage of variation	Fixation indices (p-value)
Considering neutral loci			
a. Grouping hypothesis: North vs Central vs South			
Between populations	0.7	0.01	Fct = 0.00013 (0.34)
Between subpopulation within pop	0.47	0.01	Fsc = 0.0009 (0.40)
Between samples within pop	1139.58	21.65	**Fis = 0.21651 (0.00)**
Within samples	4123.89	78.33	**Fit = 0.21668 (0.00)**
b. Grouping hypothesis: Coastal vs Oceanic			
Between populations	-0.26	0.00	Fct = -0.0005 (0.39)
Between subpopulation within pop	1.19	0.02	Fsc = 0.0023 (0.27)
Between samples within pop	1139.58	21.65	**Fis = 0.21651 (0.00)**
Within samples	4123.89	78.34	**Fit = 0.21665 (0.00)**
Considering loci under selection			
a. Grouping hypothesis: North vs Central vs South			
Between populations	0.52	0.83	**Fct = 0.00826 (0.00)**
Between subpopulation within pop	-0.92	-1.47	Fsc = -0.01480 (0.93)
Between samples within pop	15.97	25.60	**Fis = 0.25435 (0.00)**
Within samples	46.82	75.04	**Fit = 0.24956 (0.00)**
b. Grouping hypothesis: Coastal vs Oceanic			
Between populations	-0.21	-0.34	Fct = -0.00337 (0.79)
Between subpopulation within pop	-0.38	-0.61	Fsc = 0.00606 (0.79)
Between samples within pop	15.97	25.68	**Fis = 0.25435 (0.00)**
Within samples	46.82	75.27	**Fit = 0.24731 (0.00)**

3.2. Phenotypes by geographic distribution grouping

A total of fifteen groups were obtained when the three phenotypes were considered in different geographical distributions. When neutral loci were considered for the pairwise Fst analysis, the significant difference was mainly between the groups more distant to the coast (the large and medium-size groups of the oceanic groups from northern and southern Peru).

Similarly, when loci under selection were analyzed, the Fst pairwise analysis showed that mainly the significant differences were among phenotype-sizes from the organisms more distant from the coast (oceanic group). In contrast, no significant Fst was found between the coastal populations. Although the majority of populations seem to be grouped into a single cluster, DAPC shows that large phenotype-size of the central oceanic population (COG) was the group discriminated from the others. Moreover, the grouping hypothesis showed significant values between populations of the three phenotypes, considering the neutral (Fct = 0.00054, p value 0.0466) and the outlier (Fct = 0.01831, p value 0.008) loci. Also, it is important to mention that an important (Fct = 0.01720, p value = 0.06) difference was observed between different sampling periods of years, 2018-2019 vs. 2021–2022.

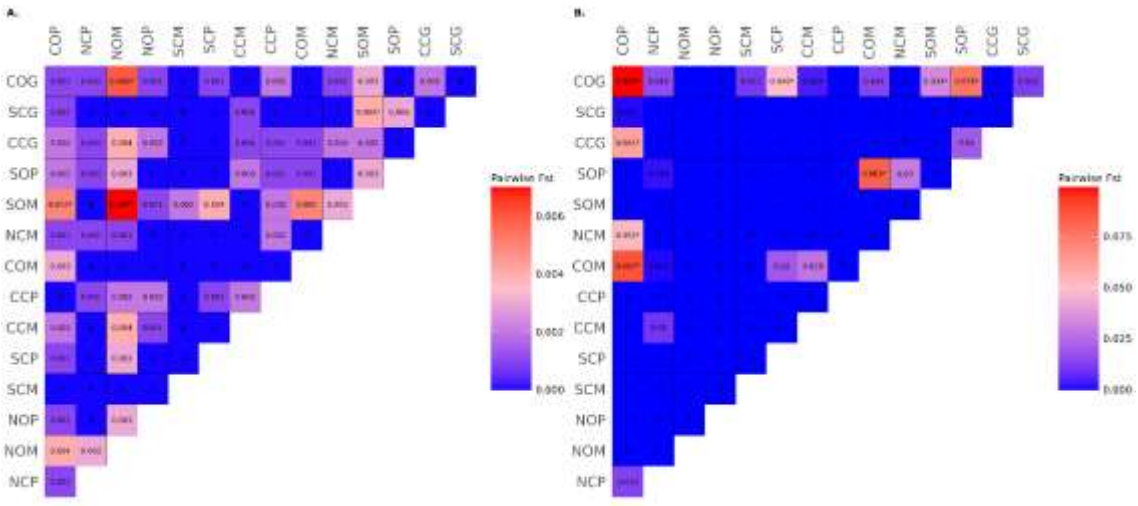

Figure 2. Heatmaps of pairwise Fst comparison between 15 populations, corresponding to the 3 phenotypes in different geographical distributions, using the neutral (A) and the outlier set of loci (B). The numbers are Fst values. Asterisk means significant differences, adjusted p values < 0.05. Population names are composed of three letters: the first stands for northern (N), central (C), and southern (S) samples; the second for coastal (C) and oceanic (O); and the third for small (P), medium (M), and large sizes (G).

Table 2. AMOVA analysis for 15 populations according to the three phenotype-size groups in different geographical distributions grouping based on latitudinal distribution (a), distance from the coast (b), phenotype-size groups (c), and sampling year periods (d), based on neutral (n=14519) and outlier (n=154) loci. Bold numbers indicate p values with p<0.06.

Source of variation	Neutral loci			Outlier loci		
	Variance components	Percentage of variation (%)	Fixation indices (p-value)	Variance components	Percentage of variation (%)	Fixation indices (p-value)
a. Grouping hypothesis: North vs. Central vs. South						
Between populations	-0.57	-0.03	Fct = -0.00032 (0.8681)	0.00	0.00	Fct = -0.00003 (0.69)
Between subpopulation within pop	1.45	0.08	Fsc = 0.00083 (0.1279)	-0.42	-1.62	Fsc =-0.01624 (0.82)
Between samples within pop	274.34	15.67	**Fis = 0.15678 (0.0020)**	5.39	20.97	**Fis = 0.20632 (0.00)**
Within samples	1475.57	84.28	**Fit = 0.15720 (0.0020)**	20.73	80.66	**Fit = 0.19340 (0.00)**
b. Grouping hypothesis: Coastal vs. Oceanic						
Between populations	-0.04	0.00	Fct = 0.00003 (0.5345)	-0.05	-0.19	Fct = -0.00190 (0.82)
Between subpopulation within pop	1.07	0.06	Fsc = 0.00061 (0.1945)	-0.39	-1.53	Fsc = -0.01523 (0.83)
Between samples within pop	274.34	15.67	**Fis = 0.15678 (0.0020)**	5.39	20.99	**Fis = 0.20632 (0.00)**
Within samples	1475.57	84.27	**Fit = 0.15727 (0.0020)**	20.73	80.73	**Fit = 0.19270 (0.00)**
c. Grouping hypothesis: Small vs. Medium vs. Large						
Between populations	0.94	0.05	**Fct = 0.00054 (0.0466)**	0.47	1.83	**Fct = 0.01831 (0.008)**
Between subpopulation within pop	0.39	0.02	Fsc = 0.00022 (0.3157)	-0.75	-2.89	Fsc = -0.02946 (0.99)
Between samples within pop	274.34	15.67	**Fis = 0.15678 (0.00020)**	5.39	20.85	**Fis = 0.20632 (0.00)**
Within samples	**1475.57**	**84.26**	**Fit = 0.15742 (0.0020)**	20.73	80.21	**Fit = 0.19270 (0.00)**
d. Grouping hypothesis: Sampling years 2018-2019 vs. 2021–2022						
Between populations	0.52	0.03	Fct = 0.00030 (0.2358)	0.45	1.72	**Fct = 0.01720 (0.06)**
Between subpopulation within pop	0.84	0.05	Fsc = 0.00048 (0.2358)	-0.59	-2.27	Fsc = -0.02306 (0.95)
Between samples within pop	274.34	15.67	**Fis = 0.15678 (0.0020)**	5.39	20.74	**Fis = 0.20632 (0.00)**
Within samples	1475.57	84.26	**Fit = 0.15743 (0.0020)**	20.73	79.80	**Fit = 0.20198 (0.00)**

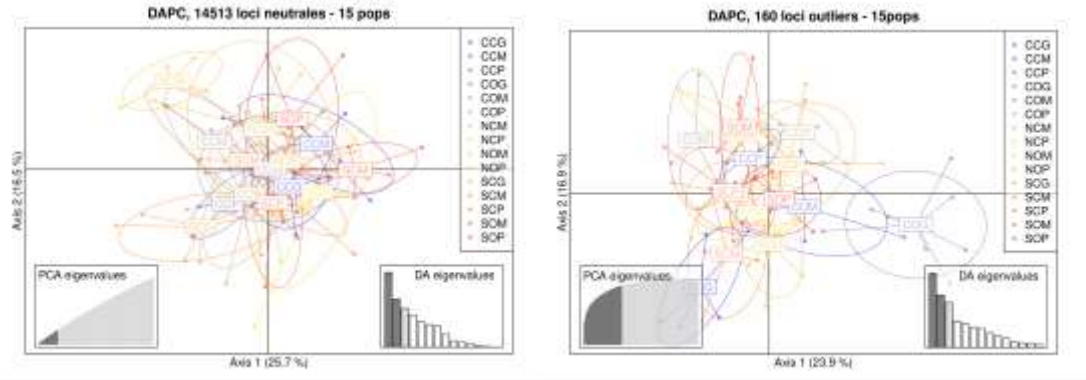

Figure 3. Discriminant analysis of principal components (DAPC) analysis of all individuals using the neutral (left) and the outlier (right) datasets. Population names are composed of three letters: the first stands for northern (N), central (C), and southern (S) samples; the second for coastal (C) and oceanic (O); and the third for small (P), medium (M), and large sizes (G).

4. DISCUSSION

The SNPs analysis of *D. gigas* from Peruvian jurisdictional waters revealed differences in population discrimination between neutral and under selection loci, even more so when taking into account groups according to their geographic distribution, phenotypes, and the years sampled.

In the past, some genetic studies have indicated a lack of significant genetic differentiation between these size groups (Sandoval-Castellanos et al., 2009; Staaf et al., 2010), suggesting that environmental factors rather than genetic isolation may drive the observed phenotypic variations. It is important to mention that these studies were performed using molecular markers such as mtDNA sequences and microsatellites, which do not reflect recent events, without also including temporal criteria. It is known that SNPs increases the power to reveal recent variability, which could reflect recent evolutionary and selection responses, being this a great advantage for the population structure analysis (Walters & Schwartz, 2020).

Considering the neutral loci and individuals grouped only according to their geographic distribution, the northern coastal and the southern oceanic groups showed significant differences, while the AMOVA revealed latitudinal discrimination of the groups. Likewise, when mtDNA ND2 analysis was performed, a significant genetic differentiation was observed between latitudinal distribution groups (SC10-SQ09_rev1).

On the other hand, when we included the geographically distributed phenotypes and compared neutral and under selection loci, significant differences were observed between phenotype groups, even more so for the organisms more distant from the coast (oceanic group). In addition, the large phenotype-size of the central oceanic

population (COG) was the group discriminated from the others (with DAPC). This has been previously reported for the ND2 gene of mtDNA, where there is a differentiation between the coastal and oceanic groups, also related to the phenotype-large size group (SC11–SQ06) Morphology-based reports mention that the population structure of *D. gigas* changes in response to environmental factors (Csirke et al., 2015; Argüelles and Taipe, 2018), in addition to the fact that longitudinally, small phenotype organisms are known to be more distant from the coast. In addition, it should be mentioned that this species possesses high phenotypic plasticity, which allows it to adapt to changing environmental conditions effectively. The adults show high tolerance to abiotic changes such as temperature, water pressure, among others, with extensive vertical and horizontal migrations and a complex pattern (Boyle & Boletzky 1996). Thus, the geographic differentiation of morphometric variables seems to reflect the effects of environmental variability.

A visual summary of all pairwise Fst significant differences considering both sample clustering (6 and 15 populations) on SNPs and the previous results from the mtDNA ND2 locus is shown in Fig. 4. Briefly, the number of significant pairwise Fst differences was higher when grouping 15 populations, mainly between the central and southern oceanic populations, where only three comparisons are between coastal and oceanic samples. A similar pattern is observed with ND2 sequence analyses but with more interlongitudinal differences, where the COG population contrasts with all southern coastal populations (SCP, SCG, SCM) and the CCG population. Overall, higher Fst values were obtained using the outlier versus the neutral dataset in all comparisons.

We also highlight that a difference ($p=0.06$) was observed between different year sampling periods, 2018-2019 vs 2021-2022. Importantly, large phenotypes were collected during 2021-2022. This could be related to the different clusters observed in relation to temperature changes (Fig. 5). Csirke et al. (2018) mentioned that differences in size and ages at sexual maturity and their relative spatial segregation could be related to high annual and interannual variability in oceanographic conditions, which could determine changes in growth rate due to changes in food availability for early life stages. In fact, environmental variability was thought to be responsible for morphometric variations of different geographic populations in intraspecific soft and hard tissue growth (Arkhipkin, 1996; Yi et al., 2012; Liu et al., 2013). The expansion range of *D. gigas* has been correlated with environmental change and dietary variation (Argüelles et al., 2012; Stewart et al., 2014). So, the important difference and high fixation index value among different sampling years observed with markers under selection reveal that there is an influence of environmental changes on the population structure.

Figure 4. Summary of all significant pairwise Fst comparisons on SNPs (A and B) and ND2 mtDNA locus (C and D) analysis. Solid and dashed lines between two populations indicate significant pairwise Fst differences, where dashed lines show comparisons between coastal and oceanic populations. The number of individuals is indicated in parenthesis. The red and blue colors represent analyses with outlier and neutral loci, respectively, whereas green is for the ND2 mtDNA locus.

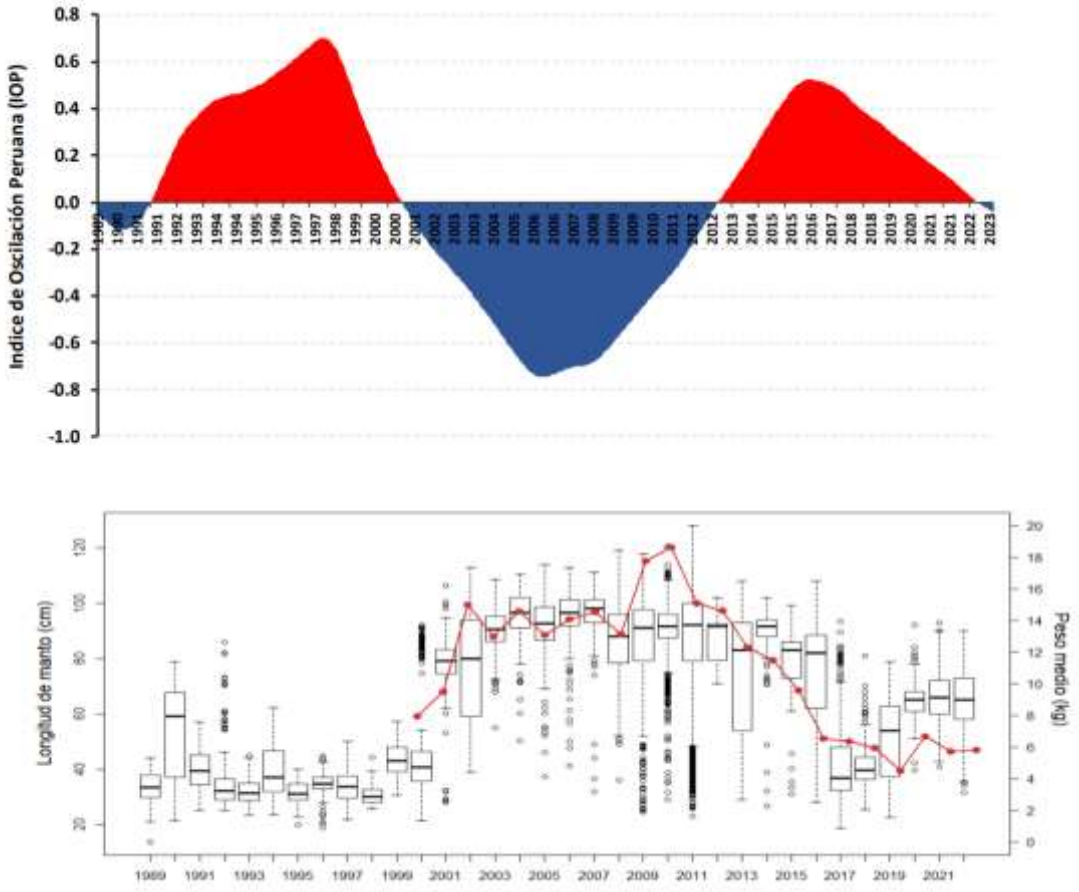

Figure 5. Annual variation in mantle length (gray bars) of mature female giant squid recorded in the industrial fishery, artisanal fishery, and during research cruises in Peruvian waters, average body weight (red line) and interannual variation of the Peruvian Oscillation Index (PIO) for the period 1989-2022. (IMARPE).

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