Drivers for genetic structure at different geographic scales for Pacific red snapper (*Lutjanus peru*) and yellow snapper (*Lutjanus argentiventris*) in the tropical Eastern Pacific

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jfb.14656

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**Funding information:** This research was supported by grants from Consejo Nacional de Ciencia y Tecnología (CONACyT) to Noé Díaz-Viloria (CB2015-257019). Nicole Reguera-Rouzaud was recipient of a CONACyT scholarship (No. 703296).

**Abstract**

The tropical Eastern Pacific (TEP) is a highly dynamic region and a model system to study how habitat discontinuities affect the distribution of shorefishes, particularly for species that display ontogenetic habitat shifts, including snappers (Lutjanidae). To evaluate the genetic structure of the Pacific red snapper (*Lutjanus peru*) and the yellow snapper (*Lutjanus argentiventris*) throughout their distribution range along the TEP, 13 and 11 microsatellite loci were analyzed, respectively. The genetic diversity of *L. peru* (*N* = 446) and *L. argentiventris* (*N* = 170) was evaluated in 10 and five localities, respectively, showing slightly higher but non-significant values in the Gulf of California for both species. The genetic structure analysis identified the presence of significant genetic structure in both species, but the locations of the identified barriers for the gene flow differed between species. The principal driver for the genetic structure at large scales >2500 km was isolation by distance. At smaller scales (<250 km) the habitat discontinuity for juveniles and adults and the environmental differences throughout the distribution range represented potential barriers to gene flow between populations for both species.

**KEYWORDS:** Gulf of California, habitat discontinuity, isolation by distance, larval dispersal, microsatellites.
1 INTRODUCTION

Seascape connectivity (the connectedness among different habitats in a seascape) is essential for species that utilize more than one habitat during their life cycle (Weeks, 2017), and play an important role in population dynamics and genetic diversity (Blanco-Gonzalez et al., 2016; Cowen et al., 2007). Understanding how connectivity is maintained is crucial for effective fisheries management (Schunter et al., 2019) and conservation (Reis-Santos et al., 2018).

Some species of marine fishes present genetic breaks due to historical and contemporary processes that act as barriers to larval dispersal and gene flow (Blanco-Gonzalez et al., 2016; Hellberg et al., 2002). These include habitat discontinuities (Blanco-Gonzalez et al., 2016; Sandoval-Huerta et al., 2019; Torquato et al., 2019) and strong environmental gradients (García-De León et al., 2018; Sandoval-Huerta et al., 2019). Also, the life cycle of each species, including the breeding season, dispersal ability (Frisk et al., 2012; Treml et al., 2012), and the location of the habitats for juveniles and adults, play an important role in population demography, connectivity and the location of those genetic breaks (Berkström et al., 2020; Blanco-Gonzalez et al., 2016; Pascual et al., 2017).

The Pacific red snapper [Lutjanus peru (Nichols & Murphy, 1922)] and yellow snapper [L. argentiventris (Peters, 1869)] are considered important food and economic resources in several coastal communities along the tropical Eastern Pacific (TEP) (Gold et al., 2015). They share most of their distribution range from the Gulf of California (GC), Mexico to Peru. The only difference being that the distribution of L. peru starts from Magdalena Bay, Mexico, and L. argentiventris
from southern California, U.S.A. (Fischer et al., 1995; Zárate-Becerra et al., 2014) (SI 1).

The spawning period of these species varies throughout the GC and the TEP and is related to environmental conditions, principally temperature (Erisman et al., 2010; Gallardo-Cabello et al., 2010; Lucano-Ramirez et al., 2014; Piñón et al., 2009; Rojas et al., 2004; Trejos-Castillo et al., 2007; Vega et al., 2016; Zárate-Becerra et al., 2014). The planktonic larval period is approximately 30 days (Claro and Lindeman, 2008) and the adults display ontogenetic habitat shifts. The juvenile recruitment habitat of L. peru is a soft bottom (Rocha-Olivares, 1998) while in L. argentiventeris it is in mangroves (Aburto-Oropeza et al., 2009; Zapata and Herrón 2002). Once juveniles reach adulthood, both species occur in rocky reef habitats and seamounts (30 to 100 m depth for L. peru, 0 to 60 m for L. argentiventeris) (Fischer et al., 1995; Gallardo-Cabello et al., 2010; Green et al., 2015; Tinhan et al., 2014).

The planktonic larval phase (Peña et al., 2017; Zapata and Herrón, 2002) and ontogenetic habitat shifts (Berkström et al., 2020), together with physical factors like geographic distances, marine currents, habitat discontinuities and environmental differences (temperature and salinity) (Berkström et al., 2020; Berkström et al., 2012; Blanco-Gonzalez et al., 2016; García-De León et al., 2018; Reguera-Rouzaud et al., 2020) are the main processes determining genetic structure or homogenization of the populations (Reis-Santos et al., 2018; Schunter et al., 2019).
Several studies have identified how these physical factors facilitate or restrict gene flow between populations of rocky reef fishes (Riginos and Liggins, 2013). Genetic structure due to isolation by distance have been found at scales ≥1000 km (Dalongeville et al., 2018; Kennington et al., 2017), while on smaller scales the genetic structure is caused mainly by larval dispersal driven by ocean currents (Dalongeville et al., 2018; Perez-Enriquez and Taniguchi, 1999). The availability of nursery habitats near rocky reefs within a radius of 150 km from mangroves also increases the abundance and connectivity of snappers (Berkström et al., 2020). In this regard habitat discontinuities, strong environmental gradients and permanent oceanographic fronts have been reported to be physical barriers to gene flow (Blanco-Gonzalez et al., 2016; García-De León et al., 2018).

Specifically in *L. peru*, previous phylogeographic studies using mitochondrial DNA have reported panmixia. This is probably explained by wide larval dispersal over long temporal scales in the southern GC (Rocha-Olivares and Sandoval-Castillo, 2003) and through its distribution range in the TEP (Hernández-Álvarez et al., 2020). In contrast, a microsatellite study in the southern GC found low but significant levels of population structure explained by the strong asymmetry in the oceanic currents (Munguia-Vega et al., 2018a). In *L. argentiventris*, Reguera-Rouzaud et al. (2020) reported no significant genetic differentiation among peninsular and mainland sites in the southern GC, however, a metapopulation structure was present due to the presence of eddies in contact with the two coast lines.
We hypothesized that connectivity in *L. peru* and *L. argentiventris* at large scales (>150 km) is driven by isolation by distance patterns, where extensive areas of non-optimal habitat for the recruitment of juveniles and adults and strong environmental gradients could act as barriers to gene flow. Thus, the goal of this investigation was to evaluate the genetic structure of *L. peru* and *L. argentiventris* in the TEP and how the environmental factors such as the patchy distribution of juvenile and adult habitats and the oceanographic conditions throughout their distribution range can affect patterns of gene flow.

Microsatellite markers were used because they are very informative to infer fine-scale population structure (Liu *et al.*, 2005) and are sensitive to recent demographic events (Fischer *et al.*, 2017). In addition, a seascape connectivity approach was taken to better understand the implications of the ontogenetic habitat shifts and the habitat patches in the genetic structure of both species.

2 MATERIALS AND METHODS

Fin clips of *L. peru* from 10 locations (*n* = 446) and *L. argentiventris* from five locations (*n* = 171) were collected along the TEP from México, Panamá and Colombia (Figure 1, SI 2) and preserved in 80% ethanol for subsequent genetic analyzes. DNA extraction was carried out with the modified salt extraction method of Lopera-Barrero *et al.* (2008).

Fourteen (SI 3) and 12 (SI 4) microsatellite loci were amplified for *L. peru* (Paz-García *et al.*, 2016, Perez-Enriquez *et al.*, 2020) and *L. argentiventris* (Perez-
Enriquez et al., 2020, Reguera-Rouzaud et al., 2020), respectively. For the polymerase chain reaction (PCR) a touchdown protocol (Munguía-Vega et al., 2013) was used with a fluorescent label M13 primer (Schuelke, 2000) in a thermal cycler (Applied Biosystems MiniAmp Plus). The PCR products were sent for fragment analysis to the University of Arizona Genetics Core (U.S.A.).

2.2 Genetic diversity and genetic structure

The genotyping for both species was done in GeneMarker (SoftGenetics 2012) and classified into bins where absolute allele lengths were converted into allele classes with FLEXIBIN (Amos et al., 2007). Individuals that had >10% of missing data in all loci were eliminated. The Hardy-Weinberg equilibrium test (HWE) was carried out in Genepop (Raymond and Rousset, 1995), and the linkage disequilibrium (LD) was evaluated in Fstat (Goudet, 1995). The level of significance of multiple tests in HWE and LD was adjusted (\(\alpha = 0.05\)) with the sequential Bonferroni approach (Rice, 1989) for each locality. Null allele frequencies were estimated with the Brookfield method in Micro-checker (Van Oosterhout et al., 2004). To evaluate the effect of null alleles in the population structure, the loci that had a mean frequency of null alleles \(\geq 0.05\) were the candidates to be removed from the data set. The number of alleles per locus (\(Na\)), effective number of alleles (\(Nef\)), the expected (\(He\)) and observed (\(Ho\)) heterozygosities, and the allelic frequencies were estimated with GeneAlEx (Peakall and Smouse, 2012). To test for significant differences in the diversity indexes for all localities for \(L. peru\) and \(L. argentiventris\), a non-parametric Kruskal-Wallis test was done for \(Na, Nef, Ho\) and \(He\).
To assess the statistical power to detect genetic population differences, with the set of microsatellite loci used in this study, the software POWSIM (Ryman and Palm, 2006) was implemented. Thirteen and 11 microsatellite loci for *L. peru* and *L. argentiventris*, respectively, were evaluated with the following recommended parameters by the POWSIM manual: Fisher exact test (10,000 dememorizations, 1000 batches, 10,000 iterations per batch), 200 replicates and effective population size of 2000. Thirty-two and 28 generations of drift were chosen according to predefined $F_{ST}$ values of 0.008 for *L. peru*, and 0.009 for *L. argentiventris*, respectively. These $F_{ST}$ values were the smallest values that showed significant differences in the pairwise $F_{ST}$ analysis.

Genetic population difference was assessed with the global $F_{ST}$ (Weir and Cockerham, 1984), pairwise $F_{ST}$, and global and hierarchical analysis of molecular variance (AMOVA) among locations with the software ARLEQUIN ver. 3.5.1.2. (Excoffier *et al.*, 2005). For the hierarchical AMOVA, several combinations of groups were tested; only the combination with the highest explained variance was chosen. The levels of significance of multiple tests in the pairwise $F_{ST}$ were adjusted ($\alpha = 0.05$) with the sequential Bonferroni approach (Rice, 1989).

To determine the population structure the software STRUCTURE (Pritchard *et al.*, 2000) was run with the following parameters: admixture model, allele frequencies correlated and 1,000,000 Markov Chain Monte Carlo (MCMC) of which the first 25% were discarded as burn-in. Based on the groups formed by the hierarchical AMOVA, $K = 1$ to 4 for *L. argentiventris* and $K = 1$ to 7 for *L. peru* were
chosen, with 10 iterations per K (Hubisz et al., 2009). The most likely K was selected by using CLUMPAK (Kopelman et al., 2015).

A discriminant analysis of principal components (DAPC) with Adegenet for R was also used (Jombart et al., 2010; Jombart et al., 2008) The DAPC was divided into two steps, first, a principal component analysis (PCa) was used and then the DAPC. For L. peru, 200 PCa and four clusters indicated by the Bayesian information criterion (BIC) were retained, and for the DAPC 100 PCa and nine eigenvalues were retained. For L. argentiventris, 120 PCa and three clusters indicated by the BIC were retained, and for the DAPC 60 PCa and four eigenvalues were retained.

To assess isolation by distance for L. peru and L. argentiventris, a correlation between the genetic ($F_{ST}$) and geographic distances (km) was carried out by a Mantel test with 10,000 permutations in the software ARLEQUIN.

Gene flow was estimated for both species with the method of private alleles [average number of migrants between locations ($Nm$)]: $e^{(\ln(pl)+2.44)/-0.505}$ (Slatkin, 1985) where $pl$ was the mean frequency of private alleles.

2.3 Habitat distribution and niche modelling

The influence of the habitat discontinuity on the genetic structure for juveniles (mangroves and soft bottoms) and adults (rocky reefs) of L. peru and L. argentiventris was evaluated. Shapefile layers of rocky reefs, mangroves and soft bottoms which form the GC and the TEP were obtained from Halpern et al. (2019), Munguia-Vega et al. (2018b) and the global mangrove watch (GMW 2016;
https://www.globalmangrovewatch.org/). Only the mangrove shapefile had to be joined in 75 km quadrants due to a large amount of information (>30,000 polygons). The shapefile habitats used by *L. peru* were bounded by the 100 m isobath and for *L. argentiventris* by the 60 m isobath, which are the maximum depths at which they can be found. In the software QGIS, the plugin for CONEFOR (Pascual-Hortal and Saura, 2006) was used to measure the distance between polygons for each habitat. The threshold distance for connecting polygons was 150 km and the maximum threshold for link removal from the analysis was 151 km. This is the estimated distance for larval dispersal at 20 – 26 days of the pelagic stage for several snappers such as *L. peru* (Munguia-Vega et al., 2018a), *L. argentiventris* (Reguera-Rouzaud et al., 2020), *L. campechanus* (Poey, 1860) (Johnson and Perry 2020), *L. synagris* (L., 1758), *L. analis* (Cuvier, 1828), *L. jocu* (Bloch & Schneider, 1801), *L griseus* (L., 1758) and *L. cyanopterus* (Cuvier, 1828) (Kough et al., 2016). With this information, a graphical theoretical approach (Treml et al., 2008) was used in the software GEPHI (Bastian et al., 2009) to construct a spatial network of polygons connected by links representing the habitat patches that were connected.

The niche modelling was done to compensate for the lack of genetic samples from southern Mexico to Colombia, where populations of *L. peru* and *L. argentiventris* potentially could be present and represent an important link between sampled sites. The occurrences of *L. peru* (113) and *L. argentiventris* (571) were downloaded from the global biodiversity information facility (GBIF; www.gbif.org) with the following filters: human observation, material sample and preserved.
specimen. Environmental data based on monthly averages of bio-ORACLE were used [temperature (°C), salinity, chlorophyll-a (mg m3-1), dissolved oxygen concentration (mol m3⁻¹), pH, silicate (mol m3⁻¹), phosphate (mol m3⁻¹) and nitrate (mol m3⁻¹)], from 2000 to 2014 (Assis et al., 2018).

To obtain the probability of occurrence for both species, the occurrences and oceanographic data were processed in ModestR (García-Roselló et al., 2013). Only the GBIF records from the adjacent Pacific (Conquista Agraria, only for L. peru) and from Gulf of California to Colombia were taken into account. The data were filtered following García-Roselló et al., (2014). A density map to calculate the extent of occurrence of the species was used, the percentage of contribution of each oceanographic variable was assessed, and finally, the niche of occurrence was calculated based on the extent of occurrence (García-Roselló et al., 2013, 2014).

To evaluate if the environmental differences between the ecoregions (Magdalena transition, Cortezian, Mexican tropical Pacific, Chiapas-Nicaragua, Nicoya and Panamá Bight) (Spalding et al., 2007) could represent a barrier to the gene flow, the bio-ORACLE oceanographic variables were extracted for each occurrence for both species and processed with a discriminant analysis in RWizard (http://www.ipez.es/RWizard/).

2.1 ETHICAL STATEMENT

In Mexico, Panama and Colombia the samples were taken by local fishermen. In Mexico we took the samples from the fishing cooperatives. Samples from Panama
and Colombia were imported to Mexico with permits B00.02.04.657/2017 and B00.02.04.0020-2019, respectively.

3 RESULTS

3.1 Genetic diversity

For *L. peru* the mean values of the number of alleles (13.9 – 19.5), effective alleles (8.1 – 12.2), private alleles (0.3 - 1.5), and the observed and expected heterozygositities (0.757 - 0.843, 0.805 - 0.886, respectively) showed high levels of genetic diversity, and higher values in the GC (Espiritu Santo Island, Loreto, San Bruno, Guaymas and Topolobampo) and Conquista Agraria (Table 1). The differences however were not statistically significant (*P > 0.05*). After the HWE test, 31 combinations (locus and location) from 140, were statistically significant after the sequential Bonferroni test (*P < 0.0038* and *P < 0.005*) (SI 3). From the 91 tests to prove LD for each locality, only two combinations (*Lupe63-Hogu25* from Loreto and *Lupe39-Lgut34* for Colombia) were statistically significant after the sequential Bonferroni test (*P < 0.0005*). Evidence of null alleles was found in all microsatellite loci but not consistently in all populations (-0.33 to 0.42 frequency range). *Lupe65*, *Lgut21*, *Hogu25* and *Lgut15* presented high null alleles frequencies, but *Lgut15* had null alleles in all locations and for this reason, was removed from the data set (for detailed information see SI 3); therefore, all the subsequent analyzes were done with 13 microsatellite loci.
For *L. argentiventris* *Lupe*34 was removed from the data set due to an unreliable pattern in the electropherograms and non-specific amplification at the samples of Colima. Hence all the analysis were done with 11 microsatellite loci. The mean values of the number of alleles (9.4 – 11.2), effective alleles (5.3 - 5.9), private alleles (0.3 - 1.5), and the observed and expected heterozygosities (0.651 - 0.774, 0.704 - 0.755, respectively), showed medium levels of genetic diversity, and did not present a clear latitudinal pattern. Only the *Na* showed slightly higher values in the GC (Espiritu Santo Island, San Bruno and Altata). The differences however were not statistically significant (*P* > 0.05) (Table 1). After the HWE test, four combinations (locus and locality) from 60 presented statistically significant deviations after the sequential Bonferroni test (*P* < 0.03) (SI 4). From the 66 LD tests for each locality, one loci combination (*Lupe*44-*Larg*27 from San Bruno) was in linkage disequilibrium (*P* < 0.005). Finally, evidence of null alleles was detected in a few locations (-0.31 to 0.17 range frequency); *Lupe*61 was the microsatellite with the highest frequencies.

3.2 Genetic structure

The statistical power of the 13 and 11 microsatellite loci for *L. peru* and *L. argentiventris*, respectively was 100% in both cases. In *L. peru*, the global AMOVA with (*F*<sub>ST</sub> = 0.0148, *P* = 0) and without (*F*<sub>ST</sub> = 0.0159, *P* = 0) the three loci with high null allele frequencies (*Lupe*65, *Lgut*21 and *Hogu*25), did not show significant differences, therefore, the 13 loci were used in all the subsequent analyses. The pairwise *F*<sub>ST</sub> among distant locations showed significant differences that ranged from 0.009 to 0.034, but the *F*<sub>ST</sub> values between close locations in the GC (San
Bruno, Espiritu Santo Island, Guaymas and Topolobampo) and adjacent Pacific (Conquista Agraria), showed non-significant values that ranged from 0.000 to 0.003 ($F_{ST} = 0.0019, P = 0.76$). Comparisons between Oaxaca-Panama and Panama-Colombia showed no differences (Table 2).

The hierarchical AMOVA between the four groups (1 = Conquista Agraria, San Bruno, Loreto, Espiritu Santo Island, Guaymas, and Topolobampo, 2 = Nayarit, 3 = Oaxaca, and 4 = Panama and Colombia) did show significant differences ($F_{ST} = 0.0225, P = 0$) (SI 5).

The analysis of STRUCTURE suggested that the populations of *L. peru* were distributed in four genetic groups. However, two well-defined groups were identified, one corresponding to Conquista Agraria and the localities inside of the GC, and the other one corresponding to Nayarit, Oaxaca, Panama and Colombia. The most marked differences were between the locations north of Topolobampo and those south of Nayarit (SI 6).

The DAPC showed a slightly different result as three groups were observed (membership probabilities in parenthesis). The first group was formed by Conquista Agraria (0.57) and the localities inside the GC [San Bruno (0.84), Loreto (0.73), Espiritu Santo Island (0.7), Guaymas (0.71) and Topolobampo (0.8)]. The second group was Nayarit (0.89) and Oaxaca (0.89) and the last one was Panama (0.81) and Colombia (0.87), with Panama showing some shared alleles with Oaxaca (Figure 2a).
For *L. argentiventris* the global AMOVA with \( F_{ST} = 0.017, P = 0 \) and without the locus with high null alleles frequencies \( F_{ST} = 0.019, P = 0 \) showed the same results. Since the effect of *Lupe61* did not show significant differences, the 11 loci were used in all the subsequent analyzes. The pairwise \( F_{ST} \) ranged from 0.005 to 0.037. The localities inside the GC (San Bruno, Espiritu Santo Island and Altata) also showed genetic structure \( F_{ST} = 0.012, P = 0 \), except the pair of locations Espiritu Santo Island-Altata (Table 3).

The hierarchical AMOVA between four groups (1 = San Bruno, 2 = Espiritu Santo and Altata, 3 = Colima and 4 = Panama) was also significant \( F_{ST} = 0.019, P = 0 \) (SI 5).

The STRUCTURE analysis suggested that the populations of *L. argentiventris* were distributed in two genetic groups, but all these populations were admixed (SI 7). However, the DAPC showed three groups: one group made up of Espiritu Santo Island (0.88) and Altata (0.84), the second group of Colima (0.92) and San Bruno (0.91), where Colima shared alleles with Altata, and the third group was formed only by Panama (0.78) (Figure 2b).

The Mantel test, for *L. peru* indicated isolation by distance in a TEP scale \( R^2 = 0.51, P = 0.0007 \) (Figure 3a). However, on a scale with only Mexican locations the isolation by distance was non-significant \( R^2 = 0.38, P = 1 \) (Figure 3b). The same occurred for *L. argentiventris*, in a TEP scale, there was isolation by distance \( R^2 = 0.52, P = 0.07 \) (Figure 3c), but in a Mexico scale the isolation was non-significant \( R^2= 0.01, P = 1 \) (Figure 3d).
Regarding the gene flow based on private alleles, for *L. peru*, the locations in Mexico showed higher number of migrants among locations (*Nm* = 25 - 55) than those observed in Panama (*Nm* = 5) and Colombia (*Nm* = 17) (Table 4). For *L. argentiventris*, the locations in the GC (San Bruno, Espiritu Santo Island and Altata) showed a higher gene flow (*Nm* = 17 – 24) than Colima (*Nm* = 14) and Panama (*Nm* = 15).

3.3 Habitat distribution and niche modelling

The distribution of the rocky reef habitat bounded by the isobath of 100 m for *L. peru* was shaped by eight patches. The largest patches were found in the GC, outside the GC on the continental coast, and between the countries of Costa Rica and Panama. These areas were well connected between the reef patches, the connections were from 100s to approximately 1,000 km of longitude. In this sense, the most important gaps were located in the central part of Mexico with an extent of 400 km (e.g. between Nayarit and Michoacan). Another one was found between the central part of Mexico (Guerrero) to El Salvador in Central America with an extent of approximately 1,500 km. Minor gaps were also found with an extent of 150 to 300 km (e.g. between the GC and Nayarit) (Figure 4a).

The soft bottom was distributed in two habitat patches of large area, one from Mexico to northern Costa Rica and the other from Panama to Colombia. Only one gap was detected between southern Costa Rica and northern Panama with an extension of 320 km (Figure 4b).
For *L. argentiventris*, the distribution of the rocky reef habitat bounded by the isobath of 60 m was composed of nine patches. The largest patches were observed in the GC, outside of the GC on the mainland coast, and from southern Costa Rica to Panamá. The patches ranged from 100s to approximately 1,000 km of longitude. The most important gaps were located similarly to those of *L. peru*, in the central part of México (*e.g.* between Nayarit and Michoacan), and from central Mexico (Guerrero) to El Salvador. Some minor gaps were present with an extent of 150 to 320 km (*e.g.* in the middle and southern part of the continental side of the GC, Nicaragua, and in the north of Costa Rica) (Figure 4c).

The mangroves did not present gaps throughout their distribution, but in the peninsula and the middle part of the continental side of the GC the mangrove patches were very small, <1 km of longitude (Figure 4d).

Regarding the probability of occurrence throughout the distribution range of *L. peru*, the occurrences were well distributed in Mexico although there was a gap of information principally in Central America and Colombia. The main environmental variables that contributed to the analysis were salinity with 26.98%, chlorophyll-a 21.33%, phosphates 16.88% and oxygen with 15.65%. Five places where the species was most likely to occur were found. These were two in the GC with a probability of 0.6, between Sinaloa and northern Jalisco with 0.96, between Michoacan and Guerrero 0.74 and from Oaxaca to Guatemala with probability ranging from 0.86 to 0.52 (Figure 5a).

For *L. argentiventris*, the occurrences were well distributed in all the study areas. The environmental variables that best explained these occurrences were

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silicates with 28.34%, chlorophyll-a 15.53%, salinity 14.84%, pH 10.28% and temperature with 10.41%. Four places where the species was most likely to occur were found: two in the GC with probabilities ranging from 0.72 to 0.79, one in Nayarit to Michoacan with a probability of 0.54, and in Oaxaca to Guatemala with 0.9 (Figure 5b).

The discriminant analysis showed that the ecoregions of the Magdalena transition, Cortezian, Mexican tropical Pacific, Chiapas-Nicaragua, Nicoya and Panama Bight had significant environmental differences between them. The first canonical analysis explained 78.12% of the variance and the second 16.84%. Together, they explained 94.96% of the variance. The principal oceanographic variables that explained the differences between the ecoregions were: pH, chlorophyll-a, temperature, salinity and phosphates (Figure 6).

4 DISCUSSION

Our analyses identified the presence of significant genetic structure in L. peru and L. argentiventris along the TEP. Despite the two species belonging to the same genus, displaying a similar geographic distribution, the co-occurrence of adult populations at rocky reefs and both showing ontogenetic habitat shifts during their life cycle, the location of the identified barriers to gene flow differed between them. The drivers for the genetic structure were relevant at different geographical scales. While isolation by distance was evident at larger scales (>2500 km), at local scales (<250 km) the discontinuities in the distinct habitats used by juveniles and adults of each species, the different environmental gradients and the oceanographic conditions were the most important drivers for the genetic structure in the GC and
the TEP. Different drivers may be acting in combination at different geographic scales all at the same time, making the genetic patterns that were found difficult to explain if the spatial scales were ignored in the analysis.

4.1 Genetic structure at large scales

On a large scale (>2500 km), isolation by distance prevented gene flow, where nearby populations exchanged more migrants, similar to previous reports (Dalongeville et al. 2018).

Contrary to our study, Hernández-Álvarez et al. (2020) did not find genetic differences in L. peru, along the TEP coasts. The differences could be explained because they used mtDNA, while we used microsatellites, markers that have higher mutation rates than the mtDNA, which have the potential to provide contemporary estimates of migration and the power to distinguish relatively high rates of gene flow from panmixia (Selkoe and Toonen, 2006). We observed that the STRUCTURE clustering method was unable to solve some fine-scale population structures (Eble et al., 2011) and it detected only the uppermost hierarchical levels (Evanno et al. 2005) compared to DAPC.

Although the genetic brake found between central Mexico (Colima - Oaxaca) and Central America (Panama) could be an artefact of the sampling design, the same break has been observed previously and has been attributed to isolation by distance, lack of suitable habitat and environmental differences, principally temperature and salinity (García-De León et al., 2018; Pliego-Cardenas et al., 2020; Rodríguez-Zárate et al., 2018; Sandoval-Huerta et al., 2019). For both
species, the genetic break can be explained by the habitat discontinuities for juveniles (soft bottoms for *L. peru*) and adults (rocky reef for both species), that agrees with the previously identified Central American Gap (Craig *et al.*, 2006; Hastings, 2000). In addition, we observed low probability of occurrence around the gap for *L. argentiniventris* and strong differences in temperature, salinity, pH and chlorophyll-*a* between the Mexican Tropical Pacific and Panama Bight. The TEP is well known for the strong upwelling system present at the Tehuantepec isthmus (Kessler, 2006), which can be stressful to larvae and adult fishes (Sandoval-Huerta *et al.*, 2019). The habitat gap and contrasting oceanographic conditions in this area also limit gene flow in other species, including the reef-associated *Elacatinus puncticulatus* (Ginsburg, 1938) (Gobiidae) (Sandoval-Huerta *et al.*, 2019).

Barriers to gene flow imposed by strong selection and local adaptation between populations could also be detectable in neutral markers (Kirk and Freeland, 2011; Sexton *et al.*, 2013). Examples include strong environmental gradients in the structure of Pacific hake [*Merluccius productus* Ayres, 1855)] estimated with microsatellites (García-De León *et al.*, 2018), and environmental differences or high selective pressure pre- or post-settlement in the structure of *Thaleichthys pacificus* (Richardson, 1836), and lobsters *Homarus americanus* and *Sagmariasus verreauxi* detected with neutral SNP datasets (Benestan *et al.*, 2016; Candy *et al.*, 2015; Woodings *et al.*, 2018).

4.2 Genetic structure at local scales

In line with multiple other previous studies, we confirmed that habitat discontinuities can prevent gene flow between populations at small spatial scales (< 250 km)
The most important barrier to gene flow in *L. peru* was found between the GC and Nayarit (Figure 4a), where a gap of suitable rocky reef habitat was found which has been previously identified as the Sinaloan gap (Craig et al., 2006; Hastings, 2000). Other species including *Tripterygion delaisi* (Cadenat & Blache, 1970) (Tripterygiidae), show a genetic brake related to this kind of habitat gap (Schunter et al., 2019). Other potential factors include environmental differences between the Cortezian and Mexican Tropical Pacific ecoregions and mesoscale eddies and the upwelling zone at the mouth of the GC, where the California Current, the Mexican Coastal Current and the Gulf of California Current converge (Sandoval-Huerta et al., 2019; Zamudio et al., 2008). Interestingly, the Sinaloan gap had no effect on the genetic structure of *L. argentiventris*, which could be explained by the connectivity of juveniles due to the continuity of the mangrove habitat along this coast. The barrier found for *L. peru* between Nayarit and Oaxaca could also be explained by the discontinuities of rocky habitat (>150 km). *Lutjanus peru* does not exhibit extensive migrations (Rocha-Olivares and Sandoval-Castillo, 2003), and its adult home range has been estimated to be between 5 to 10 km (Munguía-Vega et al. 2018b; Green et al., 2015). We found genetic structure inside the GC for *L. argentiventris*, between San Bruno and Espiritu Santo Island, in contrast to *L. peru* where we observed genetic homogeneity. The genetic structure in *L. argentiventris* could be explained by the small number and size (< 1 km) of
mangrove patches in the western coast of the GC for the recruitment of juveniles (Munguia-Vega et al., 2018a).

Besides the gaps in habitat, other studies have found that at scales <1,000 km the larval dispersal and recruitment were the main processes affecting genetic structure (Berkström et al., 2020; Dalongeville et al., 2018; Pascual et al., 2017; Schunter et al., 2019). The general lack of genetic structure in the GC could be related to seasonally-reversing surface currents and mesoscale eddies (Lavin and Marinone, 2003) that transport fish larvae, creating different patterns of metapopulation structure that tend to homogenize allele frequencies (Beldade et al., 2014; Cisneros-Mata et al., 2018; Lodeiros et al., 2016; Munguia-Vega et al., 2014, 2018a; Reguera-Rouzaud et al., 2020; Soria et al., 2012). However, swimming behaviour, different settlement success and topography, like the narrow continental shelf or very steep slopes, could also contribute to genetic differences (Sefc et al., 2020).

Larval dispersal via a cyclonic eddy between Panama and Colombia could also explain the genetic homogeneity found within the Panama Bight ecoregion in *L. peru*. The velocity of the current in this eddy is ~ 30 cm s⁻¹; which would imply that *L. peru* larvae could arrive from the northern part of Colombia to Panama in ~10 days (Chaigneau et al., 2006; Peña et al., 2017). For *L. argentiventris*, larval dispersal in a stepping stone fashion via the Mexican coastal current could help explain the lack of structure between Colima, San Bruno and Altata. This current, during spring and autumn, reaches seasonal-average velocities for approximately...
10 cm s⁻¹ and is extended from the Tehuantepec Gulf to Mazatlan, Sinaloa (Gómez-Valdivia et al., 2015)

Future analysis with SNP loci distributed in coding and non-coding regions of the genome will be necessary to have a better understanding of how the structure of the populations could be influenced by the environmental variables through the ecoregions. On the other hand, since the larval dispersal is an important driver for gene flow the use of oceanographic models could help in elucidating patterns of genetic structure.

ACKNOWLEDGMENTS

We thank Diana Cecilia Escobedo Urías from CIDII-Guasave for providing housing and transportation during our stay in Sinaloa, Vicente Hernández C. (CRIP Mazatlán), Oswaldo Morales-Pacheco (CRIP Salina Cruz), Deivis Samuel Palacios-Salgado, Marco Antonio Porchas-Cornejo, Armando Hernández López, Elias Montaño, A.M. Millán (Universidad del Valle, Colombia) and J.A. Clarós (Universidad Marítima Internacional de Panamá, Panama) for the facilitation of the samples. Also, we are grateful to the fishermen and the fishing cooperatives: Horacio Fierro, San Carlos, La Salina, and Mariscos Baja Sur, for providing support during sampling.

SUPPORTING INFORMATION

S1. Distribution map of Lutjanus peru (red line) and Lutjanus argentiventris (yellow line).
SI 2. Location, simple size and collection year for *Lutjanus peru* and *Lutjanus argentiventris*.

SI 3. Genetic diversity of *Lutjanus peru* by population.

SI 4. Genetic diversity of *Lutjanus argentiventris* by population.

SI 5. Table of hierarchical AMOVA for *Lutjanus peru* with 13 microsatellites and *Lutjanus argentiventris* with 11 microsatellites.

SI 6. Average of membership coefficient and $\Delta K$ from *Lutjanus peru*.

SI 7. Average of membership coefficient (above) and $\Delta K$ (below) from *Lutjanus argentiventris*.

**AUTHOR CONTRIBUTIONS**

Study design: N.R.R., N.D.V, A.M.V and R.P.E.; preparation of the manuscript: N.R.R.; funding: N.D.V.; samples acquisition; N.D.V., R.P.E. and E.E.B.; laboratory analysis and data interpretation: N.R.R and A.M.V. All authors contributed in drafting the manuscript, to the scientific discussion, and reviewed and approved the manuscript.

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Halpern, B. S., Frazier, M., Afflerbach, J., Lowndes, J. S., Micheli, F., O'Hara, C.,


Paz-García, D. A., Munguía-Vega, A., Plomozo-Lugo, T., & Hudson-Weaver, A. (2016). Characterization of 32 microsatellite loci for the Pacific red snapper...
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Comparative Biology, 52(4), 525–537.


Significance Statement

Being *L. peru* and *L. argentiventer* important fish resources, there is not enough information about what the drivers of population genetic structures are at different spatial scales, such as the isolation by distance, habitat discontinuities and the strong environmental gradients influencing both species along the Gulf of California and the Tropical Eastern Pacific.
**TABLE 1** Average genetic diversity for each population from 13 microsatellites of *Lutjanus peru* and 11 microsatellites of *Lutjanus argentiventris*.

<table>
<thead>
<tr>
<th>Species/locations</th>
<th><em>N</em></th>
<th><em>Na</em></th>
<th><em>Nef</em></th>
<th><em>Npa</em></th>
<th><em>Ho</em></th>
<th><em>He</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L. peru</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAA</td>
<td>43.5</td>
<td>19.5</td>
<td>11.9</td>
<td>1.2</td>
<td>0.843</td>
<td>0.886</td>
</tr>
<tr>
<td>SBO</td>
<td>42.3</td>
<td>17.8</td>
<td>10.6</td>
<td>0.3</td>
<td>0.818</td>
<td>0.872</td>
</tr>
<tr>
<td>LTO</td>
<td>40.8</td>
<td>18.0</td>
<td>11.1</td>
<td>0.7</td>
<td>0.838</td>
<td>0.877</td>
</tr>
<tr>
<td>ESI</td>
<td>46.7</td>
<td>18.2</td>
<td>11.0</td>
<td>0.4</td>
<td>0.803</td>
<td>0.873</td>
</tr>
<tr>
<td>GUA</td>
<td>40.7</td>
<td>19.3</td>
<td>12.2</td>
<td>0.7</td>
<td>0.832</td>
<td>0.885</td>
</tr>
<tr>
<td>TOP</td>
<td>40.2</td>
<td>17.0</td>
<td>11.1</td>
<td>0.2</td>
<td>0.832</td>
<td>0.873</td>
</tr>
<tr>
<td>NAY</td>
<td>47.4</td>
<td>17.5</td>
<td>10.5</td>
<td>0.3</td>
<td>0.799</td>
<td>0.845</td>
</tr>
<tr>
<td>OAX</td>
<td>45.4</td>
<td>16.9</td>
<td>10.2</td>
<td>0.3</td>
<td>0.757</td>
<td>0.853</td>
</tr>
<tr>
<td>PAN</td>
<td>25.5</td>
<td>13.8</td>
<td>8.7</td>
<td>0.1</td>
<td>0.789</td>
<td>0.831</td>
</tr>
<tr>
<td>COL</td>
<td>23.3</td>
<td>12.9</td>
<td>8.1</td>
<td>0.1</td>
<td>0.797</td>
<td>0.805</td>
</tr>
<tr>
<td><strong>Global diversity (all samples)</strong></td>
<td>395.7</td>
<td>26.8</td>
<td>12.9</td>
<td>-</td>
<td>0.811</td>
<td>0.885</td>
</tr>
<tr>
<td><strong>L. argentiventris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBO</td>
<td>35.5</td>
<td>10.8</td>
<td>5.4</td>
<td>1.5</td>
<td>0.655</td>
<td>0.704</td>
</tr>
<tr>
<td>ESI</td>
<td>24.4</td>
<td>10.0</td>
<td>5.3</td>
<td>0.5</td>
<td>0.690</td>
<td>0.719</td>
</tr>
<tr>
<td>ALT</td>
<td>30.8</td>
<td>11.2</td>
<td>5.9</td>
<td>1.5</td>
<td>0.677</td>
<td>0.715</td>
</tr>
<tr>
<td>CMA</td>
<td>26.5</td>
<td>9.4</td>
<td>5.5</td>
<td>0.5</td>
<td>0.651</td>
<td>0.706</td>
</tr>
<tr>
<td>PAN</td>
<td>22.9</td>
<td>9.5</td>
<td>5.9</td>
<td>0.3</td>
<td>0.774</td>
<td>0.755</td>
</tr>
<tr>
<td><strong>Global diversity (all samples)</strong></td>
<td>139.8</td>
<td>17.2</td>
<td>7.1</td>
<td>-</td>
<td>0.704</td>
<td>7.757</td>
</tr>
</tbody>
</table>

Sample size (*N*), number of alleles (*Na*), number of effective alleles (*Nef*), number of private alleles (*Npa*), observed and expected heterozygosities (*Ho, He*). The average takes into account all the locations as a single population. CAA = Conquista Agraria, SBO = San Bruno, LTO = Loreto, ESI = Espíritu Santo Island, GUA = Guaymas, TOP = Topolobampo, ALT = Altata, NAY = Nayarit, CMA = Colima, OAX = Oaxaca, PAN = Panama and COL = Colombia.
TABLE 2  Pairwise $F_{ST}$ from samples comparisons of *Lutjanus peru* with 13 loci.

Above the dashed diagonal are the $F_{ST}$ values and below this are the $P$ values. The asterisks represent the significant $P$ values after the Bonferroni correction ($P < 0.0044$).

<table>
<thead>
<tr>
<th></th>
<th>CAA</th>
<th>SBO</th>
<th>LTO</th>
<th>ESI</th>
<th>GUA</th>
<th>TOP</th>
<th>NAY</th>
<th>OAX</th>
<th>PAN</th>
<th>COL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAA</td>
<td>-</td>
<td>-0.000</td>
<td>-0.000</td>
<td>-0.000</td>
<td>0.001</td>
<td>0.001</td>
<td>0.027</td>
<td>0.021</td>
<td>0.023</td>
<td>0.030</td>
</tr>
<tr>
<td>SBO</td>
<td>0.694</td>
<td>-</td>
<td>0.002</td>
<td>0.001</td>
<td>0.002</td>
<td>0.006</td>
<td>0.028</td>
<td>0.021</td>
<td>0.020</td>
<td>0.033</td>
</tr>
<tr>
<td>LTO</td>
<td>0.734</td>
<td>0.234</td>
<td>-</td>
<td>0.002</td>
<td>0.000</td>
<td>-0.000</td>
<td>0.026</td>
<td>0.022</td>
<td>0.025</td>
<td>0.034</td>
</tr>
<tr>
<td>ESI</td>
<td>0.735</td>
<td>0.329</td>
<td>0.230</td>
<td>-</td>
<td>0.004</td>
<td>0.003</td>
<td>0.029</td>
<td>0.021</td>
<td>0.022</td>
<td>0.029</td>
</tr>
<tr>
<td>GUA</td>
<td>0.413</td>
<td>0.263</td>
<td>0.578</td>
<td>0.032</td>
<td>-</td>
<td>0.003</td>
<td>0.024</td>
<td>0.017</td>
<td>0.021</td>
<td>0.030</td>
</tr>
<tr>
<td>TOP</td>
<td>0.345</td>
<td>0.004</td>
<td>0.636</td>
<td>0.075</td>
<td>0.103</td>
<td>-</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
<td>0.032</td>
</tr>
<tr>
<td>NAY</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>-</td>
<td>0.009</td>
<td>0.012</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>OAX</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>-</td>
<td>0.005</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>PAN</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.063</td>
<td>-</td>
<td>0.005</td>
</tr>
<tr>
<td>COL</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.080</td>
</tr>
</tbody>
</table>

*P < 0.0044

Conquista Agraria (CAA), San Bruno (SBO), Loreto (LTO), Espiritu Santo Island (ESI), Guaymas (GUA), Topolobampo (TOP), Nayarit (NAY), Oaxaca (OAX), Panama (PAN) and Colombia (COL).
**TABLE 3** Pairwise $F_{ST}$ from samples comparisons of *Lutjanus argentiventris* with 11 loci. Above the dashed diagonal are the $F_{ST}$ values and below this are the $P$ values. The asterisks represent the significant $P$ values after the Bonferroni correction ($P < 0.015$).

<table>
<thead>
<tr>
<th></th>
<th>SBO</th>
<th>ESI</th>
<th>ALT</th>
<th>CMA</th>
<th>PAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBO</td>
<td>-</td>
<td>0.023</td>
<td>0.009</td>
<td>0.006</td>
<td>0.028</td>
</tr>
<tr>
<td>ESI</td>
<td>&lt;0.001*</td>
<td>-</td>
<td>0.005</td>
<td>0.019</td>
<td>0.037</td>
</tr>
<tr>
<td>ALT</td>
<td>0.015*</td>
<td>0.116</td>
<td>-</td>
<td>0.006</td>
<td>0.025</td>
</tr>
<tr>
<td>CMA</td>
<td>0.087</td>
<td>0.001*</td>
<td>0.100</td>
<td>-</td>
<td>0.027</td>
</tr>
<tr>
<td>PAN</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>-</td>
</tr>
</tbody>
</table>

*P < 0.015

San Bruno (SBO), Espiritu Santo Island (ESI), Altata (ALT), Colima (CMA) and Panama (PAN).
TABLE 4 Gene flow (average number of migrants between populations, $Nm$) based on private alleles for *Lutjanus peru* and *Lutjanus argentiventris*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>$Nm$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CAA</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>SBO</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>LTO</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>ESI</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>GUA</td>
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<td></td>
<td>TOP</td>
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<tr>
<td></td>
<td>NAY</td>
<td>43</td>
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<tr>
<td></td>
<td>OAX</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>PAN</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>COL</td>
<td>17</td>
</tr>
<tr>
<td><em>L. peru</em></td>
<td>SBO</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>ESI</td>
<td>17</td>
</tr>
<tr>
<td><em>L. argentiventris</em></td>
<td>ALT</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>CMA</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>PAN</td>
<td>15</td>
</tr>
</tbody>
</table>

Conquista Agraria (CAA), San Bruno (SBO), Loreto (LTO), Espiritu Santo Island (ESI), Guaymas (GUA), Topolobampo (TOP), Nayarit (NAY), Oaxaca (OAX), Panama (PAN) and Colombia (COL), Altata (ALT) and Colima (CMA).
**Figure legends**

**Figure 1.** Sampling sites for *Lutjanus peru* (circles), *Lutjanus argentiventris* (triangles) and common sites for both species (squares). San Bruno (SBO), Loreto (LTO), Espiritu Santo Island (ESI), Conquista Agraria (CAA), Guaymas (GUA), Topolobampo (TOP), Altata (ALT), Nayarit (NAY), Colima (CMA), Oaxaca (OAX), Panama (PAN), Colombia (COL). The black line in the map represent the borders of the countries and the grey line the states of Mexico.

**Figure 2.** Discriminant analysis of principal components for a) *Lutjanus peru* and b) *Lutjanus argentiventris*. Conquista Agraria (CAA), San Bruno (SBO), Loreto (LTO), Espiritu Santo Island (ESI), Guaymas (GUA), Topolobampo (TOP), Nayarit (NAY), Colima (CMA), Oaxaca (OAX), Panama (PAN) and Colombia (COL).

**Figure 3.** Mantel test for *Lutjanus peru* (above) a) all localities, b) without Panama and Colombia and *Lutjanus argentiventris* (below) c) all localities, d) without Panama.

**Figure 4.** Links between habitat patches. a) Rocky reefs and b) soft bottom bounded to the 100 m isobath for *Lutjanus peru*, and c) rocky reef and d) mangroves bounded to the 60 m isobath for *Lutjanus argentiventris*. Each colour represents the connections between patches by a maximum distance of 150 km. Sonora (SON), Sinaloa (SIN), Nayarit (NAY), Michoacan (MIC), Guerrero (GUE), Oaxaca (OAX), El Salvador (ES), Nicaragua (NIC), Costa Rica (CR), Panama (PAN) and Colombia (COL).

**Figure 5.** Probability of occurrence for a) *Lutjanus peru* and b) *Lutjanus argentiventris*. The colour scale represents the full probability of occurrence for both species.
species and the black dots are the GBIF records. Sinaloa (SIN), Nayarit (NAY), Jalisco (JAL), Michoacan (MIC), Guerrero (GUE), Oaxaca (OAX) and Guatemala (GUA).

**Figure 6.** Discriminant analysis of ecoregions of *Lutjanus peru* and *Lutjanus argentiventris*. Magdalena transition (MaT), Cortezian (Ctz), Mexican tropical Pacific (MTP), Chiapas-Nicaragua (Ch-Ni), Nicoya (Nya) and Panama bright (PaB). Dissolved oxygen concentration (DOC), chlorophyll-a (Chl). The dots represent outliers.