THE POPULATION GENOMICS OF ROOSTERFISH (Nematistius pectoralis) ALONG THE EASTERN PACIFIC

Non-technical Summary

The Roosterfish is highly sought for its strong fighting abilities and striking beauty that is largely defined the presence of a "rooster comb", formed by seven very long spines on the first dorsal fin, and by a silvery reflective and bluish to gray pigmentation on the head and body and four very prominent black bars, one running between their eyes, another across the posterior end of the head, with two more that begin at the base of the dorsal fin curving along the body sides towards the tail. Roosterfish is found exclusively along the warm (73-88°F; 23–31°C) shallow coastal waters of the Eastern Pacific Ocean, from San Clemente in Southern California to San Lorenzo Island, Peru, including the Gulf of California and Galapagos Island (Fig.1). They may spend most of their time between the sea surface and a depth of 12 m, and while all age classes are considered neritic, juveniles prefer the shoreline but as they mature their range includes reefs and sand bars not far from the coast.

Figure 1. Range of the Roosterfish

Roosterfish is an important resource that contributes significantly to local economies favored by ecotourists such as Baja California Sur and Central America where it is targeted by inshore anglers practicing catch-and-release sportfishing, but also by artisanal local fisheries. To guarantee the future health of Roosterfish populations throughout its range, it is necessary to implement conservation measures. However, knowledge about the biology and fisheries, necessary to implement sound management practices, is sparse. For Roosterfish, fisheries statistics are limited to catch series along time, and our understanding of this species migrations consists of a single study. While we know that for most part Roosterfish movements appear to be limited in scale (9-26 mi; 15 to 42 km), there is no knowledge about the level of connectivity of this species, and such information is required if sound management practices are to be implemented throughout its range. Understanding the need to close this information gap, the IGFA implemented the Roosterfish Research Program (https://igfa.org/roosterfish-research-program/) focusing on answering several questions.

1. What is the contemporary population structure of roosterfish throughout their range, and what are the most appropriate population management units locally and internationally?
2. What is the effective population size of roosterfish?
3. What is the historical gene flow of roosterfish?
4. How connected is the roosterfish population? From Mexico to Peru, do roosterfish make up one large stock or multiple localized stocks?
5. Should this species be managed by individual countries or as a single large stock across its range?
6. What indications do we have for how roosterfish will cope with future climatic conditions along its range?

To begin answering some of these questions requires the involvement of experts in fisheries and genomics working in collaboration with sportfishermen, sport fleet operators, and government agencies assisting in obtaining the samples for genetic analysis. Here we report the successful collaboration funded by IGFA that included Dr. Sofía Ortega García of CICIMAR, Mexico and Dr. Jaime Alvarado-Bremer of Texas A&M University (TAMU). Tissue samples for genetic analysis, which consisted of fin clips from the Rooster Comb as a minimally invasive approach that poses no harm to the fish were obtained with the assistance of PISCES Sports Fleet in Mexico, Mr. Jorge Sinibaldi in Guatemala, Mr. Tom Olivo, Silena Ceballos Castro, Diego Camacho Aguilar, CONAGEBIO and INCOPESCA in Costa Rica, and numerous sportfishermen throughout the study area. As a result of this extremely successful collaborative effort, a total of 69 Roosterfish were collected in Baja California Sur, 64 in Guatemala and 53 in Costa Rica (Fig. 2).

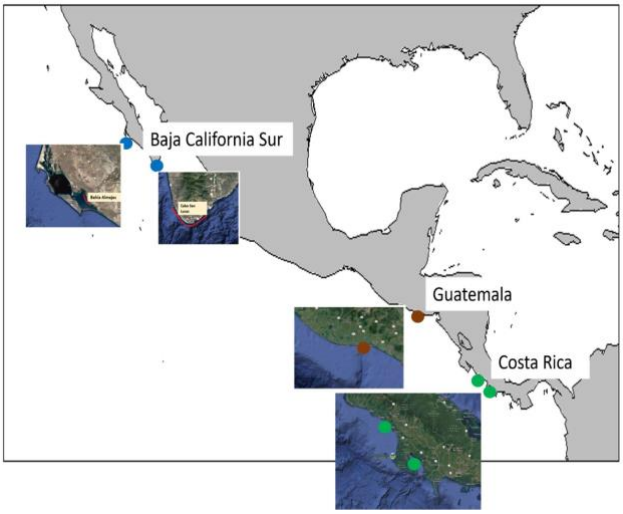

Figure 3. Collaborative sampling effort to obtain Roosterfish samples for genetic studies

The genetic and genomic analyses conducted in Dr. Alvarado-Bremer's lab at TAMU included the analysis of microsatellite markers and of single nucleotide polymorphisms (SNPs). The analysis of microsatellite markers, which are short copies of DNA sequence motifs repeated in tandem, was conducted because of their strong record in identifying population structure in many species, and because a set of markers for Roosterfish was available in the literature. In addition, the quality of the DNA required for microsatellite analysis is not very high, which was a concern when genetic samples were not going to be collected by scientists. A drawback of microsatellite analysis is the large amount of work required in the laboratory to generate size polymorphism data. Conversely, the analysis of SNPs involved generating billions of base pairs of DNA sequence for a representative sample of Roosterfish from each locality, but the amount of laboratory work required is comparatively small. The major constrains in the analysis of SNP's are that it requires very high quality of DNA, and the enormous computational power required to conduct bioinformatic analyses to identify SNPs. However, all these requirements are rewarded by the relative ease of obtaining thousands of SNPs several times more informative than microsatellite data.

The analysis of nine microsatellite markers identified differences between the sample from Baja California Sur from those collected in Central America (Fig. 2). Given the large geographic between Baja California and Central America, which spans at least 2,200 km (1367 mi), these results may not be surprising. However, identifying genetic differences in marine fishes with microsatellites is not that common because the ocean acts as a conveyer belt that facilitates the contact between populations either by the active migration of fishes, or due to the dispersal of eggs and or larvae by ocean currents. It is worth mentioning that this is the first report that identifies population structuring in Roosterfish, and that result alone has scientific merit as it helps close the information gap on the biology of this species. However, there were two additional results obtained with microsatellites worth mentioning. The first, which was particularly surprising, was the genetic differentiation of Panama from the other Central American samples, which in turn appear not to be different from each other. This result is particularly puzzling given the relative proximity between Coiba Island, Panama and Puerto Jimenez, Costa Rica (200 km; 125 mi). The second surprising finding obtained with microsatellites is the apparently depauperate levels of genetic variation observed which suggest that Roosterfish populations suffered a very dramatic decline in the past that eroded large amounts of genetic variation. This and other questions included in the IGFA's Roosterfish Program will require further evaluation, some of which will be included as part of the Master's of Science thesis of Ms. Emily Castillo, a graduate student conducting research at TAMU under the direction of Dr. Alvarado-Bremer, with the assistance of undergraduate student Savanna Watson (Fig.

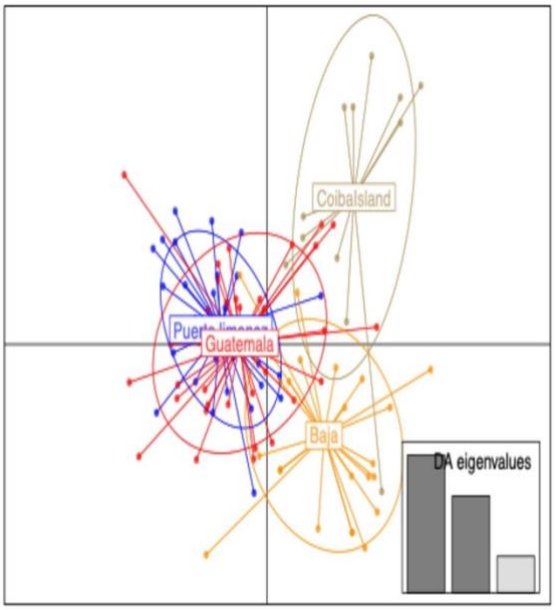

Figure 2. Comparison of Roosterfish samples based on microsatellite data.

4) who was responsible of isolating DNA and initially characterized the microsatellite markers prior to Emily's arrival. The academic formation of future geneticists was an integral component of this project, and this was made possible with the support provided by IGFA. More importantly, the impact on the future of these two students, largely because of the participation in this project, has been transforming. After completing her Master's, Emily wants to pursue a doctorate degree in fisheries genetics, and Savanna is looking forward to starting her own Master's of Science studies in the same field.

Figure 4. Graduate student Emily Castillo (right) and undergraduate student Savanna Watson (left) viewing the results of the analysis of microsatellite markers at TAMU.

The results of the analysis of SNP's were even more informative than those obtained with microsatellites. SNP's were obtained by conducting double-digest Restriction Associated DNA (ddRAD) sequencing, and on an average we obtained about 8.3 million reads about 150 bp long per individual. Accordingly, roughly 1.2 billion base pairs (bp) of DNA sequence of very high quality (Q>30) for each of the 41 Roosterfish specimens characterized. A major concern at the beginning of this study was the ability to involve sportfishermen as citizen scientists collecting samples for genomic analysis. The high high-quality values of sequence data are a testament of success of the collaboration between sportfishermen and scientists. After applying multiple filters to the SNP data aimed to minimize any potential biases that would affect the interpretation of the pertinent statistics, a total of 14,366 SNPs were retained, and the results of the analysis of these data is presented in Figure 5. The most relevant finding is the strong differentiation of the sample from Baja California from the samples from Guatemala (two sampling years) and Puerto Jimenez, Costa Rica. In addition, the two Central American locals can be readily differentiated from each other, a level of differentiation not displayed by microsatellites. Further, the lack of differentiation at a temporal level for two sampling years (Y1 and Y2) in Guatemala, indicates that the genetic signals are stable. Unfortunately, we could not include in this analysis the sample from Panama due to the poor quality of its DNA. Future studies should assess the level of differentiation using SNPs to verify the results obtained with microsatellites.

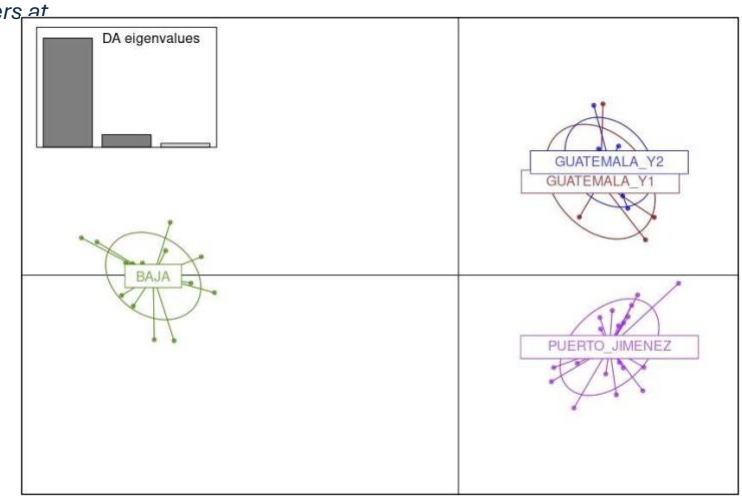

Figure 5. Results of the analysis of SNP genomic data showing the differentiation of the samples from Baja California, Guatemala and Costa Rica (Puerto Jimenez);

The rejection of a single population throughout Roosterfish's range has important implications towards fisheries management. The fact that each of the localities assessed was different from all others implies that the level of exchange of individuals among populations (gene flow) is extremely low. Accordingly, the data presented here suggest that each subpopulation is for most part self-sustaining, and under such scenario, if any given subpopulation was to be overexploited could result in population levels so low that may lead towards local extinction of Roosterfish from those waters.

In conclusion, for the first time the existence of subpopulations of Roosterfish is demonstrated. Future studies should expand to other localities throughout Roosterfish's range to include samples from Ecuador, as well as other localities along the continental coast of Mexico (e.g., Puerto Vallarta, Ixtapa, and Puerto

Escondido) and Panama. Information regarding the level of population structuring and gene flow would be particularly useful to improve the management practices of this ecologically important and charismatic fish.

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