UNIVERSIDAD SAN FRANCISCO DE QUITO

Colegio de Posgrados

"Genetic Diversity and Conservation of the Misty Grouper (*Hyporthodus mystacinus*) in the Galapagos Islands, Ecuador"

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Tesis de grado presentada como requisito para la obtención del título de Master en Ecología con mención en Ecología Tropical y Manejo de Recursos.

Quito, mayo 2012

Universidad San Francisco de Quito

Colegio de Postgrados

HOJA DE APROBACION DE TESIS

"Genetic Diversity and Conservation of the Misty Grouper (*Hyporthodus mystacinus*) in the Galapagos Islands, Ecuador"

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Dedicatoria

Quiero dedicar mis esfuerzos en mi tesis ante nada a Dios que siempre me ha bendecido; a mi madre Cyndy por siempre apoyarme y ayudarme en todo; a mi esposo Israel por todo su amor y cariño en los momentos más difíciles y a mi perrito paco por siempre hacerme sonreír.

Agradecimientos

Quiero Agradecer a Dios ante todas las personas por que si no fuera por Dios no podría haber hecho mi tesis. Gracias a mi financiamiento de Rufford Small Grants y de GAIAS-USFQ. A mi comité de tesis (Carlos Valle, Matt Craigg, y Venancio Arahana) por guiarme por el camino correcto. A mi madre Cyndy Leoro por siempre ayudarme. A mi esposo Israel Riofrío por su paciencia y ayuda. A mi suegra Inés Sabando por apoyarme en San Cristóbal, Galápagos. A el Doctor Franklin Pérez y el staff de su laboratorio One Lab que me ayudo con los análisis de laboratorio. A los Pescadores que me ayudaron a recolectar muestras. A Juan Carlos Murillo por su apoyo en el Parque Nacional Galápagos.

Resumen

Más del 90% de la pesca marina en todo el mundo ahora están sobre explotados o muy cerca a este punto. En el pasado, la sobrepesca fue ampliamente reconocido como teniendo un gran impacto sobre la diversidad y abundancia de especies, sin embargo, sus efectos sobre los ecosistemas marinos la diversidad genética de peces han sido ampliamente ignoradas. Los meros (*Serranidae*) son una familia de peces con una importancia comercial en muchas partes del mundo, así como en las Islas Galápagos. Las evaluaciones recientes de la familia sugieren que el grupo podría ser particularmente vulnerables a la pesca (GWSG 2007), y ha sido también sugieren que su diversidad genética puede verse amenazada debido a la sobrepesca (GWSG 2007). De acuerdo con el grupo de especialistas de los meros y pez (GWSG por sus siglas en Ingles), una evaluación de todas las especies de mero es necesaria para examinar la familia entera y darles prioridades de gestión y conservación de acuerdo a cada caso (GWSG 2007). Uno de los meros estudiado, la Misty Grouper *Epinephelus mystacinus* (Poey 1852), recientemente renombrada *Hyporthodus mystacinus* (Craig y Haistings 2007), ha sido descrito como un "misterioso" y "rara vez se ve" (especies de meros Schobernd 2004). *H. mystacinus* se clasificó como de Preocupación Menor en 2008 por la UICN y en el informe final GWSG. Esto se debe al hecho de que prácticamente nada se sabe acerca de la edad, el crecimiento y la reproducción de esta especie (Rocha et al. 2008, Heemstra y Randall, 1993). El informe final de GWSG dice que todos las especies clasificadas como DD y especies LC debe ser el objetivo inmediato de más de recopilación de datos, especialmente en el sudeste de Asia y las islas del Pacífico (GSWG 2007).Se encontró alta diversidad genética y gran flujo génico para *H. mystacinus* entre las localidades de las Islas Galápagos. Una alta diversidad genética se ha asociado tradicionalmente con la buena salud de las poblaciones, y sería una señal de un buen futuro para la pesca tradicional de la *H. mystacinus*. Por lo tanto, para la pesca de *H. mystacinus* pueda continuar a un nivel sostenible, es imprescindible mantener una alta diversidad genética a través de un buen plan de manejo. Es importante conservar la diversidad genética, ya que proporciona la materia prima para el mantenimiento de las especies sobre la evolución a lo largo del tiempo, y también es de particular relevancia en la actualidad en términos de proporcionar la base para responder a los rápidos cambios ambientales (cambio climático, por ejemplo), ya que la diversidad genética reducida se ha correlacionado con la disminución de la aptitud (Hoelzel et al. de 2002, Bell y Okamura, 2005).

Abstract

More than 90% of marine fisheries worldwide are now either overexploited or nearing this point. In the past, overfishing was widely recognized as impacting species diversity and abundance; however, its effects on marine fish genetic diversity have been largely ignored. The groupers (*Serranidae)* are a commercially important family of fish in many parts of the world as well as in the Galapagos Islands. Recent assessments of the family suggest that the group might be particularly vulnerable to fishing (GWSG 2007), and it has also been suggest that their genetic diversity may be threatened due to overfishing (GWSG 2007). According to the groupers and wrasse specialist group (GWSG), an assessment of all grouper species is needed to examine the sub-family as a whole and set conservation and management priorities as necessary (GWSG 2007). One of the groupers studied, the misty grouper *Epinephelus mystacinus* (Poey 1852), recently renamed *Hyporthodus mystacinus* (Craig and Haistings 2007), *has been* described as a "mysterious" and "rarely seen" grouper species (Schobernd 2004). *H. mystacinus* was categorized as Least Concern in 2008 by the IUCN and in the GWSG final report. This is due to the fact that virtually nothing is known about the age, growth, and reproduction of this species (Rocha *et al.* 2008, Heemstra & Randall 1993). The final report of the GWSG states that all larger DD and LC species should be the immediate focus of more data-gathering, especially in Southeast Asia and the Pacific islands (GSWG 2007). High genetic diversity and high gene flow for *H. mystacinus* was found among the localities in the Galapagos Islands. High genetic diversity has traditionally been associated with good health of populations, and would signal a good future for traditional fishing of *H. mystacinus*. Therefore, for fishing of *H. mystacinus* to continue at a sustainable level, it is imperative to maintain a high genetic diversity through a good management plan. It is important to conserve genetic diversity since it provides the raw material for the maintenance of species over longer evolutionary time-scales, and is also of particular relevance at present in terms of providing the basis for responses to rapid environmental change (e.g. climate), since reduced genetic diversity has been correlated with decreased fitness (Hoelzel *et al*. 2002, Bell and Okamura 2005).

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INTRODUCTION

Genetic diversity, the raw material for adaptive evolution, is fundamental for the conservation of threatened populations (Toro and Caballero 2005) like the overexploited fisheries. Low genetic diversity and increased homozygosity, which often leads to reduced fitness, resulting from small population size may become exacerbated among isolated or highly structured populations thus severely constraining their potential to respond by adapting to a changing environment (Lande 1982, Polans and Allard 1989, Caro and Laureson 1994, Amos and Harwood 1998).

Populations with low genetic diversity are more vulnerable to demographic and environmental stochasticity, which could cause this population to face several genetic threats (Amos and Harwood 1998, Lande 1998, Blomqvist *et al*. 2010). In addition, due to a small population size mating becomes restricted and inbreeding becomes more likely. Small and/or isolated populations lose the genetic variation necessary to respond to environmental challenges (Lande 1976, 1982, 1993). Of these processes, inbreeding poses a more immediate threat, whereas genetic drift and mutation accumulation affect the population in the long term (Lande 1993, 1994, Blomqvist *et al*. 2010). Low genetic diversity can pose a direct threat to conservation of a species. Low genetic diversity can result from a small population size, although this is not always the case since besides genetic drift low genetic diversity can also be attributed to sweepstakes recruitment, or selective sweeps (Lande 1982, 1998). The genetic diversity of a population can tell us a lot about the state or heath of a population, and it can also help estimate the size of the population (Skaug 2001).

Another critical component of any study of genetic diversity of a population is to quantify rates of exchange, or population connectivity, among subpopulations of said organism (Hastings and Harrison 1994). Patterns of connectivity among subpopulations of marine organisms are determined by interactions between biological phenomena including life history characteristics and larval behavior, and physical processes of advection and diffusion (Cowen *et al*. 2000, Cowen 2002). Population connectivity is relevant to a fundamental understanding of marine ecological processes and is directly applicable to critical human and environmental impact on the fisheries (Cowen 2002, Gilg and Hilbish 2003). An understanding of population connectivity could potentially benefit management strategies including marine protected areas (MPAs), yet information on this subject is lacking to make proper fisheries management decision (Hastings and Harrison 1994). In order to fill this knowledge void, the nature of the connectivity problem will require a diverse toolbox of techniques of molecular and genetic research (among others) (Cowen *et al*. 2000, Cowen 2002, Gilg and Hilbish 2003).

The role of genetic diversity and connectivity has been an issue largely neglected by fisheries ecologists and fisheries conservation biologists. This may be partly due to the fact that genetic theory suggests that significant loss of genetic diversity only occurs in very small populations while the ‘‘collapsed’ stocks are made up by several million individuals (Hauser et al. 2002). However, during the past decade questions relating to biological diversity, genetic vulnerability, narrowing of the gene base of important cultivars and the loss of germplasm of commercially important species have received increasing attention (Brown 1983). An in-depth knowledge of genetics is urgent because more than 90% of marine fisheries worldwide

are now either overexploited or nearing this point (Worm *et al*. 2009). In the past, overfishing was widely recognized as impacting species diversity and abundance; however, its effects on marine fish genetic diversity have been largely ignored.

The groupers (*Serranidae)* are a commercially important family of fish in many parts of the world as well as in the Galapagos Islands. According to the groupers and wrasse specialist group (GWSG), an initial assessment of all grouper species is needed to examine the sub-family as a whole and set conservation and management priorities as necessary (GWSG 2007). Recent assessments of the family suggest that the group might be particularly vulnerable to fishing (GWSG 2007), and it has also been suggested that their genetic diversity may be threatened due to overfishing (GWSG 2007). One of the groupers studied, the misty grouper *Epinephelus mystacinus* (Poey 1852), was recently renamed *Hyporthodus mystacinus* (Craig and Hastings 2007) and was categorized as Least Concern in 2008 by the IUCN and in the GWSG final report. This is due to the fact that virtually nothing is known about the age, growth, and reproduction of this species (Rocha *et al.* 2008, Heemstra & Randall 1993). The final report of the GWSG states that all larger Data Deficient (DD) and Least Concern (LC) species should be the immediate focus of more data-gathering, especially in Southeast Asia and the Pacific islands (GSWG 2007).

A study by Hauser *et al* (2002) of the New Zealand snapper (*Pagrus auratus*) fisheries pointed out that threats to the genetic diversity of marine fish populations have so far been largely neglected partially due to the fact that even ‘‘collapsed’’ stocks usually consist of several million individuals, whereas population genetics theory suggests that only very small

populations suffer significant loss of genetic diversity(Hauser *et al*. 2002). Hauser *et al* concluded that if such low ratios of genetically effective population size to number of fish in a population are commonplace in marine species, many exploited marine fish stocks may be in danger of losing genetic variability, potentially resulting in reduced adaptability, population resistance, and productivity (Hauser *et al*. 2002).

This study focuses on the population genetics of the misty grouper (*H. mystacinus)* on the Galapagos Islands a presumably small closed population on the way to becoming overfished or at least in the process of becoming an important commercial fishery both worldwide and in the Galapagos (Murillo *et al*. 2003, Molina *et al*. 2004). Specifically, the objective of this study is to assess the misty grouper population's genetic health by analyzing the population genetic variability throughout the Galapagos archipelago using microsatellites. Based on the results from genetic analyses I aim to suggest possible management alternatives to the local conservation authority (the Galapagos National Park) that would prevent overfishing of *H. mystacinus*. Finally, this study had a social aspect, in which I created an environmental awareness of the shifting baselines among the younger generations of fishermen, in hopes that this would help conservation efforts of *H. mystacinus* as a species and as a commercially important fishery.

METHODS

Research Site and Sample Collection

The sampling of 108 fin clippings of misty groupers *H. mystacinus* through the Archipelago was conducted by Galapagos local fishermen during the February to April 2011 fishing season fishermen in Puerto Baquerizo Moreno, San Cristobal Island, Galapagos, Ecuador. Efforts were made to involve fishermen from Santa Cruz and Isabela Islands but the main source of fish samples come from landings on San Cristobal Island where most landings usually take place (Murillo *et al*. 2003, Molina *et al*. 2004) Tissue samples were collected from six localities of the Galapagos Archipelago (Figure 1): Banco, Genovesa, Isanco, La Oso, Pinta, and Darwin/Wolf. Several attempts were made to obtain samples from the mainland, but they were unsuccessful due to the fact that fishermen on the mainland do not fish *H. mystacinus* commercially. All of the fishing of *H. mystacinus* occurred at seamounts, according to the GPS coordinates given by the fishermen (see Figure 4)

The fin clips taken from specimens captured by the local fishermen, were placed in plastic containers with 95% ethanol and stored at room temperature. The fishermen took GPS locations of the fishing sites of *H. mystacinus*, which were then overlaid with Bathymetry maps, to determine habitat selection or preferences. Unfortunately some of the samples were lost and the final data analysis was conducted on the remaining 88 samples. Samples were transported to mainland Ecuador and analyses were conducted at "One Lab" in Ballenita, Province of Santa Elena, Ecuador.

Genetic Analysis

Following the procedures described in Craig *et al.* (2009), DNA from the sample tissues were extracted using the DNEasy isolation kit (Qiagen) following manufacturer's protocols. The

subsequent extracted DNA was archived at -20°C. Primers developed by Ramirez *et al* (2006) for a sister species (*Epinephelus guttatus*) were initially tested on *H. mystacinus.* Due to poor results from all but two of the primers (RHCA002 and RHCA007), new primers were developed from the microsatellite loci identified by Ramirez *et al* (2006) and using the software websat (Martins *et al*. 2009). Out of the 38 primers designed 12 were successful in amplifying loci for *H. mystacinus* and yielded a higher consistent allelic variation (see Table 1). Touchdown Polymerase Chain Reaction (PCR) was performed in an ABI 9700 384 well twin block thermocycler to amplify an approximately 74-378 base pair (bp). Reactions were conducted in a total volume of 4µl using the conditions described as follows: 25 ng DNA, 1X green PCR buffer, 200µM each dNTP's, 2µM $MgCl_2$, 1µg/µl bovine serum albumin (BSA), 0.3µM each primer and 0.72 units *Taq* DNA polymerase (*Go Flexi Taq* - Promega). The touchdown PCR program consisted of an initial cycle at 95°C for five minutes, then 20 cycles: of 92°C for 30 seconds, 65°C with a 1°C decrease per cycle for 30 seconds and 65°C for 60 seconds; followed by 39 cycles of 92°C for 30 seconds, 45°C for 30 seconds, and 70°C for 60 seconds, then finally one cycle of 72°C for 7 minutes.

Polymorphisms were visualized on denaturing gels (6% polyacrylamide-5M urea). The electrophoresis was performed in CBS Scientific dual adjustable height sequencing vertical electrophoresis chambers. The gels were developed using the silver staining technique (Benbouza *et al.* 2006; Creste *et al.* 2001). The molecular weight (MW) and number of tandem repeats data were calculated using "Gene Profiler ver 4.05" from Scanalytics (Scanalytics Inc. 2011).

Statistical Analyses

The data extracted from Gene Profiler was analyzed using GeneALEx 6.41 (Peakall and Smouse 2006), and Arlequin 3.5 (Excoffier and Lischer 2010) to determine allele frequencies, unique or private alleles, fixation index, heterozygosity, allelic richness, Hardy Weinberg equilibrium, AMOVA and population structure (Nei 1978, Slatkin 1985, Frankham *et al*. 2002, Hanski and Gaggiotti 2004, Freeland 2005).

Social Impact

Through informal surveys I investigated about historical fishing: approximately how many "mero" or *H. mystacinus* were fished, how big they were, and why has the fishing tendency moved from the *M.olfax* to *H. mystacinus*. Theses informal surveys were conducted in both the Galapagos Archipelago and in fishing towns throughout the mainland. Informal surveys were conducted with 28 local fishermen regarding their fishing tendencies. They were asked what species they used to fish more, either *Mycteroperca olfax* (Galapagos grouper, locally known as the Galapagos bacalao) or *H. mystacinus* (Galapagos grouper, known locally as mero); how much of it they used to fish compared to now (high levels, medium levels or low levels); have they changed species; if they did change species, why they changed species; have they noticed a change in the size of fish they are catching.

RESULTS

GENETIC DIVERSITY

Allelic frequencies are shown in Figure 2. Figure 2 locus RHGATA076 is the locus with greatest number of alleles (18 different alleles) also known as the locus with the greatest allelic diversity.

Figures 2 locus RHGATA015, RHGATA032, RHGATA053, and RHGATA133 also have a great allelic diversity ranging from 11 to 13 different alleles each. Figure 2 locus RHCA002, RHGATA067, and RHGATA 106 have the lowest allelic diversity with only 3 alleles. In Figure 2 locus RHCA002, allele 10 occurs in very low frequencies in the Wolf & Darwin locality, and allele 16 is about to become fixed. Throughout Figure 2 locus RHCA002 we can observe that allele 16 appears in greater frequencies in all of the local populations. In Figure 2 locus RHGATA067, allele 10 is most frequent in all of the local populations, and allele 17 is only observed in the Pinta locality. In Figure 2 locus RHGATA106, allele 31 apears in very low frequencies in the Banco and Isanco localities and will more than likely soon be lost in the other local populations for its frequency is very low; the other two alleles (10 and 22) have similar frequency rates. In Figure 2 RHGATA065, allele 12 appears with greatest frequency which could eventually lead to the loss of the other alleles present. Figure 2 RHGATA 118, in Banco allele 13 has become fixed while allele 16 has been lost in all of the localities except Genovesa and Pinta, in which it is barely present.

GENETIC STRUCTURE

Genetic variation between populations was analyzed as the frequency of private alleles. Private alleles were found in all of the localities except for Isanco (Table 2 and Figure 2). Pinta has the greatest amount of private alleles (11 alleles) seen across 8 different loci followed by La Oso with 7 private alleles see in 6 different loci. Wolf & Darwin have 6 private alleles in 5 different loci. Banco has 3 private alleles over 3 different loci. Genovesa has only 1 private allele. The private alleles which appear in the greatest frequency belong to the Banco locality (Table 2 and Figure 2).

The results of the Analysis at the Inter-Population level can be seen in Table 3. The mean number of alleles per locus varied little across the five localities ranging from 3.00 to 6.17 with the lowest number of alleles per locus being found in Isanco. Pinta shows the greatest number of polymorphic loci (11 alleles), followed closely by Isanco with 9 polymorphic loci. The localities with the lowest polymorphic loci are: La Oso and Genovesa with only 2 polymorphic loci, and Banco with 3 polymorphic loci. Mean observed heterozigosity ranged from 0.49242 to 0.61136 and were similar across locations with the exception of the Banco locality which showed substantially lower heterozygosity (Table 3). Generally, observed heterozygosities (H_o) were only slightly lower than expected heterozygosities (H_E), with the exception the Banco locality in which the difference was much greater. Banco showed the greatest genetic diversity (0.823232) among the other localities, while Genovesa had the lowest levels of genetic diversity (0.614805) (Nei 1978)(Table3). Wolf &Darwin locus RHCA007, Genovesa locus RHCA007, and La Oso locus RHCA007,andGenovesa locus RHAGATA118 all have a significant heterozygote deficiencies at $p<0.001$ (Table 4). Other significant heterozygote deficiencies at $p<0.05$ were found in the following localities: Wolf &

Darwin locus RHGATA015 and RHGATA065; Banco locus RHCA007; Pinta locus RHCA002, RHGATA67, and RHGATA118; and Genovesa locus RHCA002 (Table 4).

Microsatellite analyses showed low levels of allelic structuring (Table 5 and Figure 3). Only 1.57% of the total microsatellite DNA diversity was explained by the variance among population groups, which means there is very little variation among local populations within regions (ALPR) and among regions (AR) due to great gene flow among the local populations. The largest proportion of variation was explained by the variance among individuals within local populations and even greater variability when comparing each individual. Since there is not a statistically significant variation between localities, the differences may be explained by variation between individuals.

Overall the global AMOVA F_{ST} values of the metapopulation has a genetic differentiation expected under random mating according to the F_{ST} values, but they are not significant ($p > 0.05$; Table 6). Where a F_{ST} value from 0 - 0.05 indicates low genetic differentiation, 0.05 – 0.15 indicates moderate genetic differentiation, 0.15 – 0.25 indicates great genetic differentiation and values above 0.25 indicate very great genetic differentiation .There appears to be a reduction in heterozygosity of an individual due to non-random mating within each population according to the F_{IS} value ($p<0.001$; Table 6). F_{IS} values range from -1 to 1, in which: negative values or values close to -1 indicate an excess in heterozygosity due to negative assortative mating; positive values or values close to 1 indicate inbreeding or undetected null alleles; values close to 0 are expected under random mating. Pairwise Population F_{ST} Values (Weir and Cockerham1984) are seen in Table 7. The highest F_{ST} values

are seen between the Banco and Isanco localities, while the lowest F_{ST} values are seen between Pinta and La Oso. Of these pairwise populations F_{ST} values, none of them had statistically significant differences among the localities. The localities specific F_{IS} indices show no statistically significant p values (Table 8)

DISCUSSION

High genetic diversity has traditionally been associated with good health of populations, and would signal a good future for traditional fishing of *H. mystacinus*. Therefore, for fishing of *H. mystacinus* to continue at a sustainable level, it is imperative to maintain a high genetic diversity through a good management plan.

H. mystacinus has been found dwelling off of slopes and deep shelf waters (on rocky pinnacles and ledges); based on its deep habitat and apparent constant catches, *H. mystacinus* is currently not experiencing a significant decline. However, it was strongly suggested by grouper experts that continued monitoring is required since little is known of its biology and because it is a target of commercial fishery (GWSG 2007, Rocha *et al*. 2008). According to Matt Craig's report to the IUCN red list, there are no studies on the abundance of *H. mystacinus* (Rocha *et al.* 2008). In the eastern Pacific, it is reported to be found from the Galapagos Islands to the Paramount Seamount (north of the Galapagos and west of Columbia and south west of Panama; N 3° 20' 0" W 90° 45' 0") and all the way to coastal Ecuador.

However, a preliminary study conducted by Craig (personal communication) has led to the thought that the Eastern Pacific population may be a distinct species from that in the Atlantic.

Little consideration has been given to the genetic composition of populations associated with marine reserves—the protected areas category intended to preserve specific species, communities or habitats. In the Galapagos marine reserve, fishing pressures within the reserve, in what could be considered a small closed population, could result in inbreeding and loss of genetic diversity due to overfishing, thus increasing the risk of extinction of these small populations (Blomqvist *et al*. 2010). In this context, genetic information is becoming increasingly important in ecology and conservation biology.

The variance component of genetic diversity at the individual level both within and between individuals suggests no inbreeding or assortative mating within each location. Our results suggest great levels of gene flow among the localities, due to the fact that there are no real geographic barriers to separate the individuals from one locality from the next. Also supporting the high levels of global diversity and within local population genetic diversity are the high levels of allelic diversity and high levels of private alleles found within each locality The low genetic structuring found is congruent with the high levels of gene flow among localities. The highest level of genetic diversity found in Banco may be due to its central location between all of the other localities which is in agreement with Nei (1978)

These high levels of genetic diversity suggest a healthy population as well as high population connectivity within the Galapagos;. The highest levels of diversity are found within

individuals and therefore among local populations (63.45% and 34.33% respectably), yet due to the great level of gene flow among local populations of localities, there is a low genetic differentiation among local populations and within regions (0.65% and 1.57% respectably). Nonetheless, precautions must be taken in order to preserve these high levels of heterozygosity, genetic diversity and gene flow among the localities of the Galapagos Marine Reserve. One of the reasons why there are such high levels of diversity within individuals and such low levels among regions is because of the high levels of gene flow among localities which also explains the high levels of heterozygosis. In order to maintain theses high levels of genetic diversity, gene flow among localities must be maintained.

Historically, the Galapagos Islands fisheries have been focused on the *M. olfax,* but in recent years the trend has moved to a fishery focused on the *H. mystacinus*. According to local fishermen, the switch occurred because they can no longer fish *M. olfax,* in the abundance that they used to. Its population started to decline with overfishing (due to its demand) and the remaining population is now found in deeper waters, which gave the fishermen no other choice but to switch to another fish. The information provided by the fisherman was confirmed by the Galapagos Marine Reserve (GMR) report by Murillo *et al* (2003) and Molina *et al* (2004) since the coastal species of grouper demersal fish are decreasing (specifically *M. olfax),* people are increasingly relying on species of demersal fish found in the seamounts ("bajos" in Spanish) such as the *H. mystacinus*. Also the amount of *H. mystacinus* being caught is greater now, not because the population has increased but because there is now a greater focus on this species. The information given by the local fishermen are supported by the reports from the Galapagos National Park (GNP), monitoring of fish landings from 2003 which show that the

most predominant species fished was the *H. mystacinus* (Molina *et al*. 2004). In 2003, *H. mystacinus* fisheries alone represented 95.4 tons, which is 25% of all fisheries (Murillo *et al*. 2003, Molina *et al*. 2004). This surpassed by far the *M. olfax*, which only had 59.7 tons, making *H. mystacinus* the most economically valuable fish in that year (Molina *et al*. 2004). This could mean that if no precautions are taken, the *H. mystacinus* could follow the path of the endemic *M. olfax*. It must be noted that older and younger generations of fishermen are familiar with the concept of the shifting baseline (also known as sliding baseline) in which the current generation of fishermen think that the "normal" size of fisheries is much smaller than that of previous generations (Pauly 1995). This is very important when it comes to conservation of the species.

As a member of the Serranidae family, the *H. mystacinus* is a hermaphrodite, more specifically a protogynous hermaphrodite, which means that the individuals change sex from females to a few dominant males (Nelson 1994). According to the local fishermen females are the smaller ones and live in much shallower waters. In Isla de la Plata, part of Machalilla National Park in mainland Ecuador, *H. mystacinus* has been observed and photographed at depth of only 15 meters, with sizes ranging from 40-60 cm. The *H. mystacinus* being caught commercially in Galapagos is said to be the male of the species and is caught at depth of 140-200 meters and with lengths up to 135 cm. This means that all the samples from Galapagos used in my study were taken off of large males. If all of the larger males are being caught, then how will the smaller females be fertilized? Not enough is known about *H. mystacinus* about when or what causes the sex change to occur, therefore, if all of the males of the species are being extracted from the metapopulation, it could have serious effects on the population size. The fact that

they are protogynous hermaphrodites also implies the need for special management of the species with regards to the allowable size to be caught. Furthermore, a study revealed that among the females, older individuals of some fish species produce larvae that have substantially better survival potential than do larvae from younger fishes (Birkeland and Dayton 2005). If this were applied to *H. mystacinus,* this could mean that the larger females are more important in producing viable offspring than the smaller ones. The combination of these two factors, their hermaphroditic characteristic and the more optimal production of the larger individuals which usually tend to have exponentially greater fecundity is important since commercial and traditional fisheries often target the larger fish. The protection of larger or older individuals is necessary for the sustainability of species currently exploited by humans (Birkeland and Dayton 2005).

As for conservation of the species, ideally, considering that the local fishermen are fishing within a marine reserve, the Galapagos Marine Reserve (GMR), the Galapagos National Park (GNP) should only allow fishing for local consumption and not for exportation to the main land or international consumption. But due to the fact that this is the fishermen's livelihood, other conservation efforts could be taken into consideration.

Genetic diversity has been shown to be directly correlated with the species fitness and population size (Lande 1994, Hauser *et al*. 2002, Reed and Frankham 2003). If no proper management steps are taken, more than likely we will see a collapse of the *H. mystacinus* fisheries within the GMR as has been see worldwide in other fisheries (Caro and Laurenson 1994, Bell and Okamura 2005, Birkeland and Dayton 2005). According to the 2006-2007

Galapagos Report, marine resources including *M. olfax*, have declined precipitously over the years (Galapagos Report 2006-2007). In fact in 2007 the IUCN considered *M. olfax* to be a vulnerable species (VU) (Galapagos Report 2006-2007). Overall the amount in tones fished of *M. olfax.* is steadily decreasing, while amount in tones of *H.* mystacinus is generally increasing (see Table 9) (Murillo *et al*. 2003.)

An alternative would be to set aside no take zones on seamounts where *Serranidae* are usually found. Connectivity between all of the localities will likely maintain levels of genetic diversity, heterozygosity and gene flow in spite of an overfishing of local populations at seamounts. The *H. mystacinus* is considered to be a metapopulation in Galapagos, which means that each one of the localities has the possibility of going extinct. The optimal harvest regime depends on the endogenous source-sink dynamics, which are determined by differences in population levels across space, as well as on the biological mechanisms acting on dispersal (Sanchirico *et al.* 2005) Therefore the key to understanding the optimal management of marine species is knowledge of dispersal and geneflow (Gerber *et al*., 2003; Guichard *et al.* 2004). It must first be determined where the source of this metapopulation is located and where the sink is located, and in order to accomplish this, further research is needed on the mainland. Taking current into account, we could assume that the mainland could be the source of the Galapagos metapopulation, but since there is no evidence of this yet, I therefore would suggest for the GMR to either set aside some of the seamounts as no take zones in which all fishing is prohibited or to set a maximum amount of fish that can be taken from any one of these seamounts in order to prevent depletion or overfishing of the species, as was the case with the endemic *M. olfax*. Another option would be to rotate the no

take seamounts on a yearly basis; i.e. in one year allow fishing in the Banco locality and setting the Isanco locality as no take zone and the following year reversing them. With each passing year, *H. mystacinus* fisheries are becoming more important commercially to the local economy in the Galapagos Islands. The Galapagos' white fishery includes the exploitation of different demersal and coastal pelagic fish species (68 species) with the groupers family being the most important (Murillo *et al*. 2003, Molina *et al*. 2004). There are no recent estimates of the state of this fishery, but it is thought that the resource could be overexploited (Murillo *et al*. 2003, Molina *et al*. 2004). Since the coastal species of grouper are decreasing (specifically *M. olfax),* people are increasingly relying on species of demersal fish found in the seamounts or "bajos") such as the *H. mystacinus* (Murillo *et al*. 2003, Molina *et al*. 2004). According to reports from the Galapagos National Park (GNP), monitoring of fish landings from 2003 shows that the most predominant species fished was the *H. mystacinus* (Molina *et al*. 2004). In 2003, *H. mystacinus* fisheries alone represented 95.4 tons, which is 25% of all fisheries. This surpassed by far the *M. olfax,* which only had 59.7 tons, making *H. mystacinus* the most economically valuable fish in that year (Molina *et al*. 2004).

It is important to conserve genetic diversity since it provides the raw material for the maintenance of species over longer evolutionary time-scales, and is also of particular relevance at present in terms of providing the basis for responses to rapid environmental change (e.g. climate), since reduced genetic diversity has been correlated with decreased fitness (Hoelzel *et al*. 2002, Bell and Okamura 2005).

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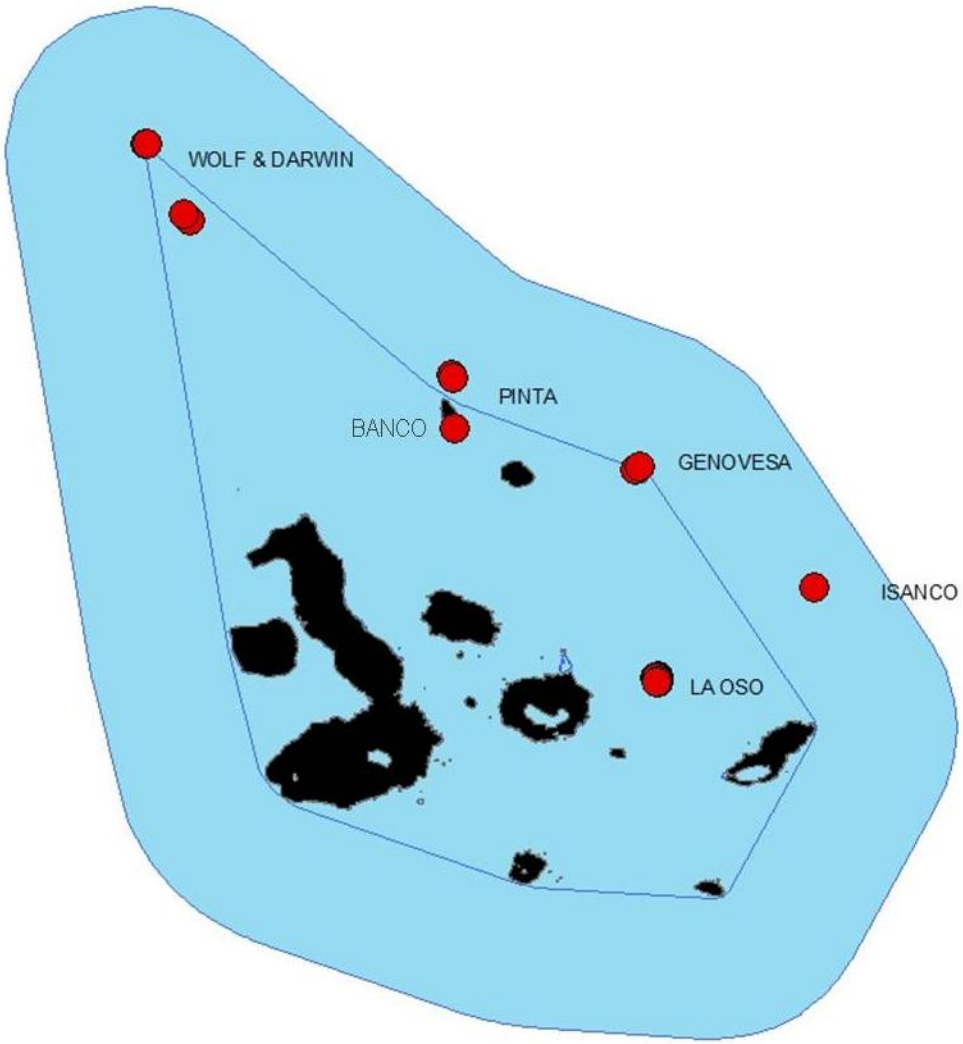

Figure 1. Galápagos Marine Reserve and sample sites. The localities of Wolf & Darwin, Pinta, Banco, Genovesa, Isanco and La Oso. These were then divided into 3 zones: North West(Wolf & Darwin), Central (Pinta, Banco and Genovesa) and South East(La Oso and Isanco)

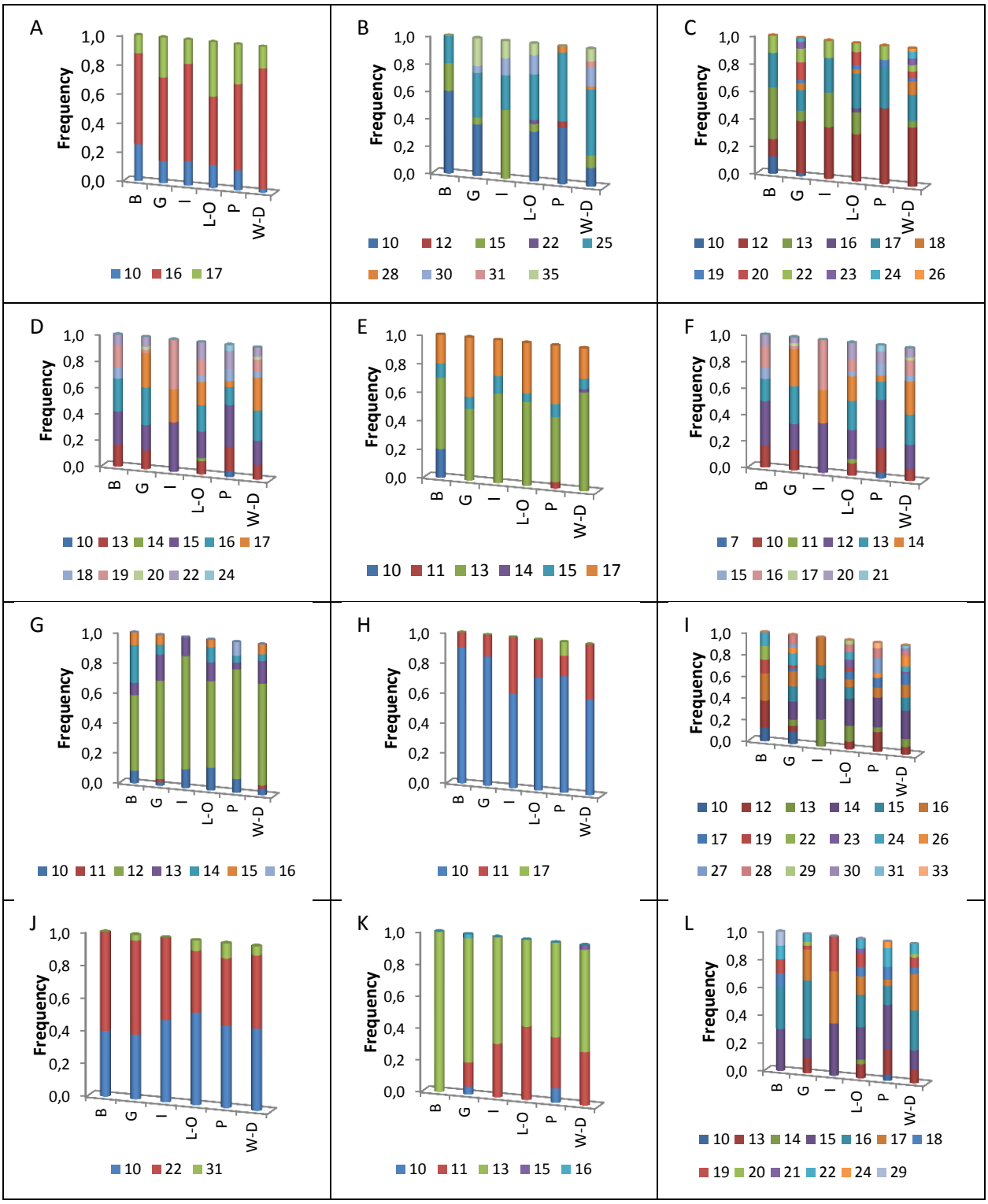

Figure 2. Allele Frequency for Local populations: Banco (B), Genovesa (G), Isanco (I), La Oso (L-O), Pinta (P), and Wolf & Darwin (W-D). A=Locus RHCA002, B= Locus RHCA007, C = Locus RHGATA015, D = Locus RHGATA032, E = Locus RHGATA035, F = Locus RHGATA053, G = Locus RHGATA065, H = Locus RHGATA067, I = Locus RHGATA076, J = Locus RHGATA106, K = Locus RHGATA118, and L = Locus RHGATA133.

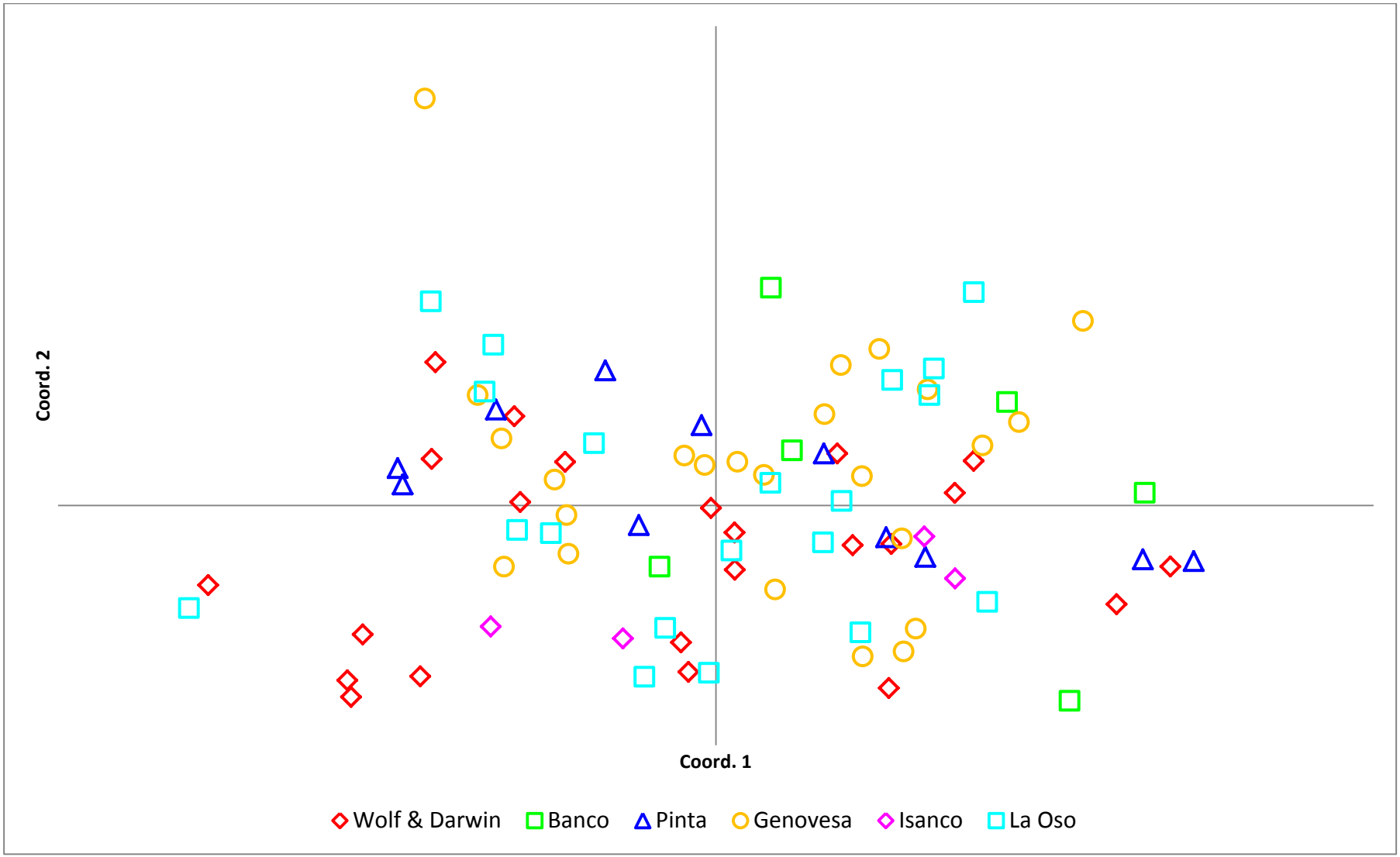

Figure 3 . Principal coordinantes analysis (PCoA)

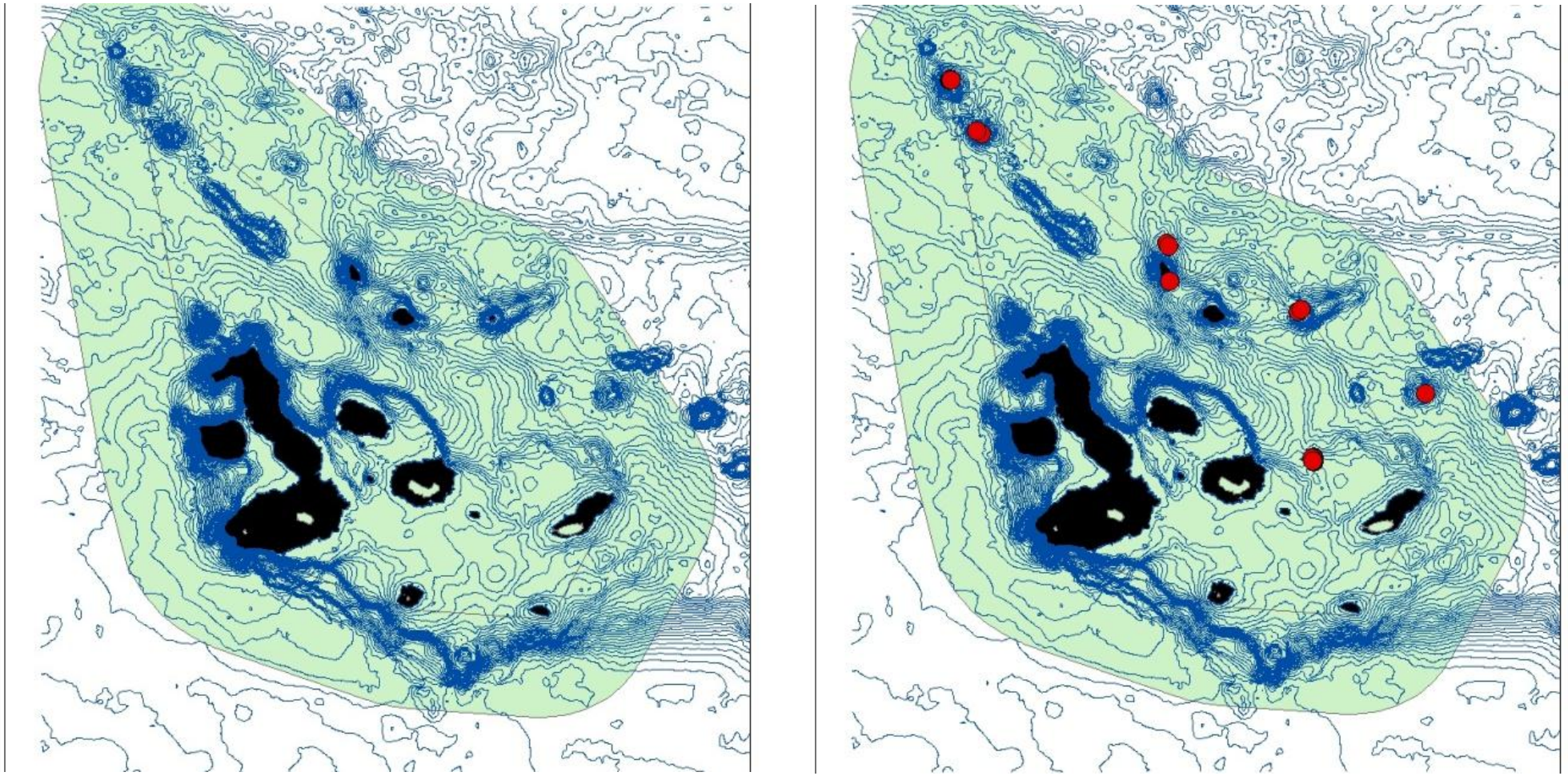

Figure 4. Bathymetry map indicating seamounts and sites where samples were taken. Map on the left only indicates contour lines of bathymetry. Map on the right indicates with a red dot where samples were collected. Sample site match seamount locations

Table 1. Twelve microsatellite loci and PCR primers used on *Hyporthodus mystacinus*. RHCA002 and RHCA007 developed by Ramirez *et al* (2006) RHGATA primers were developed as part of this study using microsatellites loci from an *Epinephelus guttatus* identified by Ramirez *et al* (2006) and using the websat software(Martins et al. 2009).

ID	Locus	Accession Number	Primer Sequence Forward	Primer Sequence Reverse
A	RHCA002	DQ223785	CTCGTTACCACATCGGGACT	AAGGGCATGATGGGAAATG
B	RHCA007	DQ223786	CAGAAACATCTCCCCCAAAA	CTGGCAGAGCAATTAGAGGC
C	RHGATA015	DQ223821	TGTTCCCATGTGTCTGCTTTAG	TGTTCCCATGTGTCTGCTTTAG
D	RHGATA032	DQ223824	ATGTGCATTTATGGAGTTTCCC	ATGTGCATTTATGGAGTTTCCC
E	RHGATA035	DQ223826	CAGGTCAGCTCTCCCATGATAC	CAGGTCAGCTCTCCCATGATAC
F	RHGATA053	DQ223829	ATGTGCATTTATGGAGTTTCCC	ATGTGCATTTATGGAGTTTCCC
G	RHGATA065	DQ223834	AGGGAGCCACACACAGATAAAG	AGGGAGCCACACACAGATAAAG
H	RHGATA067	DQ223836	GCCAGCCACATACACACG	GCCAGCCACATACACACG
I	RHGATA076	DQ223837	CCATTTACTGTGGAGGTGACAG	CCATTTACTGTGGAGGTGACAG
J	RHGATA106	DQ223843	TAACTGACACATGGACTGACCC	TAACTGACACATGGACTGACCC
K	RHGATA118	DQ223844	CCTGTGGTTAAAGAGACAATCG	CCTGTGGTTAAAGAGACAATCG
L	RHGATA133	DQ223850	ATGTGCATTTATGGAGTTTCCC	ATGTGCATTTATGGAGTTTCCC

Table 2. Summary of Private alleles by localities.

Localities	Locus	Number of alleles (NA)	Number of private alleles (NP)	Private alleles (frequencies)
Wolf & Darwin	RHCA007	7	1	31 (0.045)
	RHGATA015	10	1	26 (0.024)
	RHGATA035	4	1	14 (0.024)
	RHGATA076	12	2	30 (0.024) 31 (0.024)
	RHGATA118	3	1	15 (0.028)
Banco	RHGATA035	4	1	10 (0.200)
	RHGATA076	6	1	22 (0.125)
	RHGATA133	6	1	29 (0.100)
Pinta	RHCA007	4	1	12 (0.045)
	RHGATA032	8	2	10 (0.045) 24 (0.045)
	RHGATA035	4	1	11 (0.045)
	RHGATA053	8	2	7 (0.045) 21 (0.045)
	RHGATA065	5	1	16 (0.091)
	RHGATA067	4	1	17 (0.091)
	RHGATA076	9	1	33 (0.045)
	RHGATA133	8	2	10 (0.045) 24 (0.045)
Genovesa	RHGATA118	4	1	16 (0.025)
La Osa	RHCA007	6	1	22 (0.028)
	RHGATA015	8	1	16 (0.031)
	RHGATA032	8	1	14 (0.025)
	RHGATA053	8	1	11 (0.031)
	RHGATA076	11	1	29 (0.036)
	RHGATA133	9	2	14 (0.033) 21 (0.033)

Table 3. Summary of the Analysis at the Inter Population Level; number of Polyorphic Loci (PL), mean number of alleles (A), Observed Heterozygosity (Ho), Expected Heterozygosity (He) and Genetic Diversity (GD Nei 78) at 12 microsatellite loci for the 6 Local Populations of *Hyporthodus mystacinusI.* Where N = sample size and L = number of loci analyzed.

	N	L	PL	A	Ho	He	GD (NEI 78)
Wolf&Darwin	23	12	5	6.167	0.60712	0.64652	0.634
Banco	6	12	3	4.364	0.49242	0.71074	0.823232
Pinta	11	12	11	5.167	0.61136	0.6597	0.662731
Genovesa	24	12	2	5.75	0.55289	0.64274	0.614805
Isanco	4	12	9	3	0.542	0.665	0.694
La-Oso	20	12	2	5.667	0.6112	0.6942	0.7506

Table 4. Significant heterozygote deficiencies of each locality

Locality	**Locus**	**DF**	**ChiSq**	**Prob**	**Signif**
Wolf & Darwin	RHCA007	21	75.715	0.000	***
Wolf & Darwin	RHGATA015	45	68.169	0.014	*
Wolf & Darwin	RHGATA065	15	25.268	0.046	*
Banco	RHCA007	3	10.000	0.019	*
Pinta	RHCA002	3	10.877	0.012	*
Pinta	RHGATA67	3	11.343	0.010	*
Pinta	RHGATA118	3	11.108	0.011	*
Genovesa	RHCA002	3	10.514	0.015	*
Genovesa	RHCA007	10	74.669	0.000	***
Genovesa	RHGATA118	6	27.141	0.000	***
La Oso	RHCA007	15	44.722	0.000	***

(*** = $p<0.001$; * = $p<0.05$)

Table 5. Summary of AMOVA comparing molecular variance of the 6 local populations within the 3 regions (North West, Central and South East). Among Regions (AR), Among Local Populations within Regions (ALPR), Among Individuals within Local Populations (AILP), with Individuals (WI)

Source	**df**	**SS**	**MS**	**Est. Var.**	**%**
AR	2	741.327	370.664	3.541	1.57%
ALPR	3	659.529	219.843	0	0.65%
AILP	82	22139.57	269.995	70.512	34.33%
WI	88	11349.352	128.97	128.97	63.45%
Total	175	34889.778		203.024	100.00%

Table 6. Global AMOVA F_{ST} values

F-Statistics	Value	P(rand >= data)
Fst	0.005	0.271
Fis	0.353	0

Table 7. Pairwise Population Fst Values (Weir and Cockerham1984)

Wolf & Darwin	Banco	Pinta	Genovesa	Isanco	La Oso	
0.000						Wolf & Darwin
0.064	0.000					Banco
0.027	0.054	0.000				Pinta
0.022	0.037	0.024	0.000			Genovesa
0.030	0.077	0.051	0.050	0.000		Isanco
0.018	0.054	0.017	0.019	0.035	0.000	La Oso

Table 8. Localities specific F_{IS} indices (1023 permutations)

Localities	F_{IS}	P value (Rand $F_{IS} \geq$ Obs F_{IS})
Wolf&Darwin	-0.04043	0.795699
Banco	0.29293	0.047898
Pinta	0.0411	0.423265
Genovesa	-0.16029	0.982405
Isanco	-0.28571	1
La-Oso	0.06912	0.257087

Table 9. Table adapted from Murillo *et al*. 2003. Quantity in tonnes of Bacalao and Mero caught during 1997 untill 2002.

Family	Scientific Name	Local Name	1997	1998	1999	2000	2001	2002
Serranidae	*M. olfax*	Bacalao	118	145	92	45	61.5	30
Serranidae	*H. mystacinus*	Mero	31	18	10	1.2	25.8	41.6