Multiple Distant Origins for Green Sea Turtles Aggregating off Gorgona Island in the Colombian Eastern Pacific

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Abstract

Mitochondrial DNA analyses have been useful for resolving maternal lineages and migratory behavior to foraging grounds (FG) in sea turtles. However, little is known about source rookeries and haplotype composition of foraging green turtle aggregations in the southeastern Pacific. We used mitochondrial DNA control region sequences to identify the haplotype composition of 55 green turtles, Chelonia mydas, captured in foraging grounds of Gorgona National Park in the Colombian Pacific. Amplified fragments of the control region (457 bp) revealed the presence of seven haplotypes, with haplotype (h) and nucleotide (π) diversities of h = 0.300 ± 0.080 and π = 0.009 ± 0.005 respectively. The most common haplotype was CMP4 observed in 83% of individuals, followed by CMP22 (5%). The genetic composition of the Gorgona foraging population primarily comprised haplotypes that have been found at eastern Pacific rookeries including Mexico and the Galapagos, as well as haplotypes of unknown stock origin that likely originated from more distant western Pacific rookeries. Mixed stock analysis suggests that the Gorgona FG population is comprised mostly of animals from the Galapagos rookery (80%). Lagrangian drifter data showed that movement of turtles along the eastern Pacific coast and eastward from distant western and central Pacific sites was possible through passive drift. Our results highlight the importance of this protected area for conservation management of green turtles recruited from distant sites along the eastern Pacific Ocean.

Introduction

Genetic, tagging and satellite tracking studies have demonstrated that green turtles, Chelonia mydas, spend an early pelagic stage in the ocean, followed by a neritic stage in which juveniles settle in coastal foraging grounds (FG) [1–3]. These areas can be either shared with adults (and will constitute the adult residential foraging grounds where juvenile turtles will later spend their inter-reproductive periods), or be frequented only by juveniles, that will later shift to a different adult feeding area [4]. These FG are used as developmental areas, stopover habitats and refueling stations during turtles’ life cycles [5]. Sea turtle foraging aggregations commonly consist of genetically mixed stocks made up of turtles originating from different distant rookeries [6–9], although it was recently demonstrated that green turtle foraging populations in the central Pacific consisted of a single Hawaiian genetic stock [10]. Understanding the links between FG stocks and the breeding rookeries from which animals originate is of great importance to developing holistic conservation strategies for this trans-boundary species [10], and there is a need to pursue genetic studies to determine the stock composition at FG for green turtles in the eastern Pacific [8,11–13].

Green turtles of about 45–65 cm curved carapace length (CCL) forage all year round in the Marine Protected Area (MPA) of Gorgona Island National Park in Colombia (Figure 1). The island foraging aggregation is thought to comprise transient, mostly juvenile individuals that pass through Gorgona on their way from one FG to another. This is concluded from their diet, demographic structure and the low recapture rate of animals from year to year [14,15]. Further, the striking morphological differences in carapace shape and coloration (Figure 2), suggest that turtles converging on this small island might have their origins in multiple rookeries. In order to test this speculation and to identify the geographic regions from which turtles may have come, we characterized the genetic composition of Gorgona’s FG aggregation. The mtDNA control region has been used extensively to characterize stock structure and patterns of dispersal in sea turtles [9–11,16–19]. In this study we used mtDNA control region sequences to identify the genetic stock composition and infer the contributions from the eastern, central and western Pacific regions to the Gorgona FG aggregation. Information on the geographic origins of turtles at this FG has important conservation implications, because the significant mortality caused by illegal artisanal fleets operating around Gorgona in the Colombian
Figure 1. Location of Gorgona National Park in the Pacific. Dots represent locations from where haplotypes were identified, excluding an Australasian hypothetical rookery. Turtles were caught by hand in the coral reefs of La Azufrada and Playa Blanca on the east side of the island. doi:10.1371/journal.pone.0031486.g001

Figure 2. Observed variations in carapace color and shape of green turtle (Chelonia mydas) juveniles. Variations corresponding to the Australasian (CMP21, CMP 22 and CMP 97) and central/eastern Pacific (CMP4, CMP 8, CMP 5, CMP 17) haplotypes (left and right turtles, respectively) caught at the Gorgona foraging study site. Putative west Pacific turtles exhibited a much lighter golden-brown coloration with indentation in the lower carapace edges, in contrast to the darker “black” carapaces of the typical eastern Pacific individuals. Photo: Javier Rodriguez-Zuluaga. doi:10.1371/journal.pone.0031486.g002
Pacific [20,21] potentially affect breeding populations on a regional geographic scale [22].

In this phase of a long-term study as part of the Gorgona National Park Sea Turtle Action Plan, our main objectives were to carry out a genetic characterization of the green turtles foraging on Gorgona waters and to estimate the contribution from Pacific-wide stocks to the island. Knowledge from this investigation will be useful to better understand green turtle ecology, improve regional conservation management strategies and aid understanding of oceanographic currents and patterns that might play a significant role in green turtle dispersal within the Pacific Ocean.

Materials and Methods

Ethics statement

This study was conducted under research permit DTSO 0029 from the Unidad Administrativa Especial del Sistema de Parques Nacionales Naturales, contract for access to genetic resources issued by the Colombian Ministry of the Environment 28/03/2007 and ethics approval BSCI/2003/04 from Monash University.

Study site and sample collection

Turtles were captured by hand while snorkeling at night in water up to 7 m depth in coral reefs of Gorgona National Park. (2°56’–3°02’N, 78°10’–78°13’W). This 9 km long and 2.5 km wide island with a total protected area of 617 km² (including surrounding waters) is located 56 km from the mainland town of Guapi in the southern Colombian Pacific coast. The island is surrounded by near-shore coral reefs where green turtles are found throughout the year. Samples were taken from 55 green turtles ranging from 42.7 to 77.6 cm minimum curved carapace length (mCCL), measured from the nuchal scute to the posterior notch at midline between the supracaudals; mean = 61.2±S.D. (8.2 cm) which were subsequently tagged in both front flippers with Inconel 1005-681S tags [National Band & Tag Co.], using standard techniques [23]. About of 2–3 mm of skin tissue was removed from the neck or shoulder of each animal with a sterile scalpel following standard methodology [24]. Skin samples were preserved in a 20% DMDSO solution saturated with NaCl and processed in the Laboratory of Molecular Biology and Tissue Bank of the Colombiano Alexander von Humboldt Biodiversity Research Institute (IAvH), in Cali, Colombia.

DNA extraction, PCR amplification and sequencing

DNA was extracted from samples using a Qiagen DNeasy kit (Qiagen, Germantown MD, USA) and DNA concentration and quality was visualized by electrophoresis of 5 μl on a 0.8% agarose gel stained with EtBr. We amplified a 550 base pair fragment of the mtDNA control region using primers LTCM2 and HDCM2 [25]. For the polymerase chain reaction (PCR) we used 2 μl of template DNA in 25 μl reaction volumes containing 50 ng of genomic DNA, 1 μl of Taq polymerase (Perkin-Elmer/Cetus, Norwalk, PA), 10 μM of each primer, 5 μM dNTPs [Deoxynucleotide Triphosphates], 25 μM MgCl₂ and Buffer 10× (KCl 500 mM and 200 mM Tris-HCl, pH 8.4). The reaction consisted of 35 cycles of a thermocycler (PTC-100, MJ Research, Waltham MA, USA) applying 20 s at 94°C, 20 s at 53°C and 20 s at 72°C. The initial denaturing (94°C) and last extension (72°C) cycles were of 2 minutes each [26]. Reactions were verified in 1% agarose electrophoresis gel and successful PCR products were then purified using the Poly Ethylene Glycol [27] method (20% PEG 8000, 2.5 M NaCl) prior to sequencing in both directions in a Multichannel Capillary Electrophoresis sequencer (ABI 310 - 3100).

MtDNA haplotype characterization and data analysis

The mtDNA sequences were edited and assembled using Chromas Pro v1.34 (Technelysium Pty Ltd, Tewantin QLD, Australia) and trimmed before alignment with CLUSTAL X v1.74 [28]. Sequences were aligned and compared to reference sequences in order to identify haplotypes. Each nucleotide change encountered in an individual sequence was considered a different haplotype. Haplotypes encountered in Gorgona National Park were compared with Pacific Ocean sea turtle haplotypes reported in the National Marine Fisheries Service - Southwest Fisheries Science Center (NMFS-SWFSC) Marine Turtle Research Program website (http://swfsc.noaa.gov/prd-turtles.aspx). In order to allow comparisons with published studies in other FG and nesting stocks in the Pacific basin sequences were truncated at an internal 304 bp segment used universally by others [10,19,29] (Table 1).

A selection of haplotypes from Central, Eastern Pacific [30,31] and Australian rookery clades [19] as well as from regional foraging grounds in Japan (haplotypes CMJ12, CMJ3 GenBank acc no. AB472311, AB472302; [32], Palmyra Atoll (CMP109; acc. no. GU12196), and French Polynesia (CMPo1, CMMp3; acc. no. EF555564, EF555566) were included to help detect probable geographic origins for orphan haplotypes found at the Gorgona FG (GPC5, GPC6, GPC7).

A 10 bp insertion at position 355 in haplotypes E1, E2 and CMJ12 was coded as a single substitution for further analyses. Haplotype frequencies, Nei’s [33] haplotype diversity (h) and nucleotide diversity (π) from the control region sequences were calculated using Arlequin v.3.11 [34]. The phylogenetic relationships between Gorgona and reference haplotypes data from Dethmers et al [19] were inferred from MEGA4 [35] using the Neighbor-Joining method [36] on the basis of genetic distances computed using the Tamura-Nei model and a Pacific loggerhead (Caretta caretta) sequence as the outgroup (GenBank acc. no. U22261). Genetic differentiation between the Gorgona foraging aggregation and eastern Pacific nesting and foraging aggregations, was quantified using 10,000 permutations for ΦST [37] under the Tamura-Nei model [38]. All computations were carried out by the program Arlequin v.3.11 [34].

Rookery contributions to the Gorgona green turtle aggregation were estimated using Bayesian Mixed Stock Analyses (MSA) as implemented in the many-to-many model described by Bolker et al. [7] using both weighted and unweighted applications. Source haplotype profiles for the MSA were taken from eastern (Michoacan, Revillagigedos and Galapagos) and central (Hawaii Pacific rookeries [10,30]. Source population size (as nests/yr) was calculated prior information in the weighted model for Michoacan, Mexico [39], Galapagos, Ecuador [40,41] and French Frigate Shoals, Hawaii USA [42]. Identifying potential origins for turtles with orphan haplotypes was attempted on the basis of sequence similarities using the phylogenetic relationships mentioned previously.

Results

Haplotype composition

Seven haplotypes (GPC1-7) based on 26 variable sites were identified from the 55 sequences of green turtles sampled at Gorgona (Table 1). All have been previously described [10,19] although no nesting origin has been reported for haplotypes GPC5, GPC6 and GPC7 (CMP21, CMP22, and CMP97, respectively). The most common haplotype at Gorgona was

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Table 1. mtDNA control region haplotype (384 bp) frequencies for Gorgona National Park foraging grounds, compared with other published East and central Pacific rookeries and foraging grounds.

| Gorgona designation | std SWFSC haplotype nomenclature | GENBANK Accession numbers | East and central Pacific rookeries | East and central Pacific foraging grounds |
|---------------------|----------------------------------|---------------------------|-----------------------------------|-------------------------------------|
|                     |                                  |                           | Mexico | Ecuador | Hawaii | Hawaii | Colombia |
|                     |                                  |                           |        |         |        |        |          |
| CMP1                |                                  |                           | 1      | 156     | 477    | 76     | Gorgona  |
| CMP2                |                                  |                           | 34     | 82      | 16     |        |          |
| CMP3                |                                  |                           | 11     | 39      | 112    | 22     |          |
| GPC1                | CMP4                             | AY382323                  | 82     | 23      | 95     | 1      | 46       |
| CMP5                |                                  |                           | 34     |         |        |        |          |
| CMP6                |                                  |                           | 50     |         |        |        |          |
| CMP7                |                                  |                           | 2      |         |        |        |          |
| GPC3                | CMP8                             | AY382326                  | 2      |         |        | 1      |          |
| CMP9                |                                  |                           | 2      |         |        |        |          |
| CMP10               |                                  |                           | 1      |         |        |        |          |
| CMP11               |                                  |                           | 1      |         |        |        |          |
| CMP12               |                                  |                           | 3      |         |        |        |          |
| CMP13               |                                  |                           | 1      |         |        |        |          |
| GPC2                | CMP15                            | AY540063                  | 3      |         |        | 1      |          |
| GPC4                | CMP17                            | AY540065                  |        |         |        |        |          |
| CMP20               |                                  |                           |        |         |        | 1      |          |
| GPC6                | CMP21                            | AY540069                  |        |         |        | 1      |          |
| GPC5                | CMP22                            | AY540070                  |        |         |        | 3      |          |
| GPC7                | CMP97                            | FJ268479                  |        |         |        | 2      |          |
| Total haplotypes    |                                  |                           | 5      | 8       | 2      | 3      | 5       |
| No. of animals      |                                  |                           | 123    | 90      | 98     | 229    | 673     |
| Nesting pop. size (nests.yr⁻¹) |             |                           | 1,395 [39] | 90 [40] | 1,650 [41] | 400 [42] | N/A     | N/A     |

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Galapagos turtles (converted from original SCL) [43], we assumed 0.011 green turtles sampled in central (CMP15 and CMP17) and haplotypes falling within phylogenetic clades of sequences from different from green-black and included yellowish–brown, pale sampled turtles (15%, n = 8) had variable carapace colorations common East Pacific haplotypes CMP4 and CMP8. Remaining animals in San Diego Bay have more variable carapace shape and black, which is commonly associated with the eastern Pacific green color for most of the sampled turtles (85%, n = 47) was greenish - carapaces of individuals captured at the Gorgona FG. Carapace h = 0.46, NH = 5; Table 2), the nucleotide diversity (within the range reported for other green turtle foraging habitats in French Polynesia (CMPo3 and CMPo1) and Japan (CMJ3) [32]. The sequence for haplotype GPC6 (CMP21; from a single individual) is identical to that from haplotypes E2 [19] and CMJ12 [32] except for a 10 bp insertion at position 355 that these latter sequences have and that is absent in GPC6. Haplotype E2 was reported from two rookeries in Micronesia (Elato and Ngulu Atolls) [19].

Genetic and morphological diversity

Although the haplotype diversity (h = 0.30 ± 0.080) and the number of resolved haplotypes (NH = 7) at Gorgona FG falls within the range reported for other green turtle foraging aggregations (Atlantic h = 0.18–0.77, NH = 2–13; Hawaii h = 0.46, NH = 5; Table 2), the nucleotide diversity (π) 0.011 ± 0.006 is the second highest recorded for the species at a FG.

The mean mCCL for the 55 sampled turtles was 61.2 ± 8.2 cm and ranged between 42.7 and 77.6 cm. On the basis of a classification for nesting female sizes of over 75 cm CCL for Galapagos turtles (converted from original SCL) [43], we assumed the presence of a total of 47 juveniles (<70 cm CCL), 7 sub adults (70–75 cm CCL) and one large juvenile of unspecified sex (>75 cm CCL) in our sample.

There were characteristic morphological differences amongst carapaces of individuals captured at the Gorgona FG. Carapace color for most of the sampled turtles (85%, n = 47) was greenish - black, which is commonly associated with the eastern Pacific green turtles [44], although the Revillagigedo nesters and foraging animals in San Diego Bay have more variable carapace shape and coloration [40,45–47]. All of these turtles were associated with the common East Pacific haplotypes CMP4 and CMP8. Remaining sampled turtles (15%, n = 8) had variable carapace colorations different from green-black and included yellowish–brown, pale green and dark yellow (Figure 3), and were associated with haplotypes falling within phylogenetic clades of sequences from green turtles sampled in central (CMP15 and CMP17) and western Pacific (CMP21, CMP22 and CMP97) rookeries.

Genetic differentiation

When comparing the Gorgona FG haplotype profiles with those from green turtle aggregations of central and eastern Pacific habitats (Table 1) both the exact test of differentiation (results not shown) and the θST values revealed the Gorgona turtles as being statistically distinct (θST 0.12–0.58) all P < 0.01; Table 3). Notably, the level of differentiation was lowest when comparing against eastern Pacific aggregations and the lowest genetic distance value was found when comparing with the Galapagos rookery.

Mixed Stock Analysis

To conduct the mixed stock analysis, we removed the orphan haplotypes (6 of the total of 55 individuals sampled), all of which consisted of sequences phylogenetically associated with the Central-Eastern (CMP17) and Western Pacific (CMP21, CMP22 and CMP97) lineages. With the remaining set of samples, the mixed stock analysis (MSA) identified and quantified contributions to our study site from genetically characterized Eastern (Michoacan, Revillagigedo, Galapagos) and Central Pacific (Hawaii) rookeries (Figure 4). Weighting the analysis by abundances of the source populations, the major contributing rookeries from these regions appeared to be Galapagos 80% (55–96%, 95% confidence interval) and Michoacan 15% (2–38%, 95% confidence interval), while Revillagigedo and Hawaii appeared to not contribute significantly (<2%). The proportions of contribution by rookery remained the same when the MSA was run with uninformative priors.

Discussion

The results of this study provide genetic evidence to support previous speculation that green turtle stock at Gorgona FG is composed of individuals recruited from multiple nesting sites in the Pacific [14,15]. These findings contribute important information to updating the Colombian Sea Turtle Action Plan, and for strengthening protection of the Eastern Pacific Tropical Marine Corridor (CMAR). They also highlight the value of inferences that can be drawn by considering the foraging strategies, population size-class composition and residence of turtles in non-nesting habitats for the implementation of national and regional wildlife policy planning.

Genetic composition and diversity of Gorgona foraging aggregation

The haplotype (h) and nucleotide (π) diversities indicate the Gorgona FG contains turtles that probably originate from a broad geographic range, since this parameter (π) increases with sequence divergence. The seven haplotypes identified in Gorgona FG suggests that this MPA represents a critical site potentially used as developmental and/or stopover habitat by a number of green turtle stocks from both sides of the Pacific Ocean basin. The western Pacific haplotypes may be rare, but could be present at some of the under-sampled eastern Pacific nesting sites or others that have not yet been surveyed. By maintaining the health and richness of Gorgona’s marine habitats these FG can help sustain a large number of turtles from different breeding origins, considering that over 700 individuals have been tagged since 2001 (Amorocho, unpublished data).

The genetic diversity estimated for the Gorgona foraging grounds is within the ranges for green turtle feeding aggregations in other FG of the Pacific [10,19,48] and Atlantic sites [6,8,23,49,50] as shown in Table 2. The overall haplotype (h = 0.300) and nucleotide (π = 0.080) diversities were relatively low, due to the dominance of haplotype CMP4, present in 46 (83%) of the 55 turtles. Despite the limited number of sampled individuals and comparatively low number of haplotypes (seven), high nucleotide differences were observed within the Gorgona aggregation. The π value was also high compared to other localities in the Atlantic such as Brazil (Ubata 0.002; Almofala 0.006), Tortuguero in Costa Rica (0.003) and other foraging sites in the wider Caribbean (0.000–0.005) (Table 2). The relatively high haplotype and nucleotide diversity found in our study indicate that Gorgona is an important place for green turtles in order to maintain population and genetic variability in the eastern
Pacific, contributing to diversity of Regional Management Units (RMUs) [51].

Role of sea surface currents
To complement the MSA results and to help explain genetic links between the major regional green turtle rookeries and the Gorgona FG, we explored plausible regional dispersal routes by ocean currents (Figure 5), using the trajectories of satellite-tracked Lagrangian drifter buoys (http://www.aoml.noaa.gov/). The location of Revillagigedo and Michoacan rookeries places them at a junction between the southerly moving California Current and the North Equatorial Current flowing due West [52]. Drifter data is scarce in this general area, but tracks were recovered that are consistent with drift scenarios for small juvenile green turtles from these rookeries to the Gorgona area. These tracks follow different paths, but terminate in a region very close to Gorgona (Figure 6), as did a drifter deployed East of Galapagos, consistent with the finding of Michoacan and Galapagos haplotypes in the Gorgona FG. Thus, although we did not find genetic contribution from Revillagigedo, there is the potential for it to be present. No drifter deployed in the vicinity of FFS-Hawaii reached the general Gorgona area and oceanic current transport of buoy drifters from Western Pacific habitats to Gorgona appeared to be more likely than from Hawaii, despite being much further away. Transport through the North Pacific Drift was discounted as being too cold for green turtles [53]. Two tracks were found terminating relatively close to Gorgona that suggest that passive transport from Western Pacific habitats would take around 2 years, but is possible (Figure 7), providing a potential route into the Gorgona aggregation for animals from these distant populations. It must be

Table 2. Comparison of mtDNA control region sequence diversities in the Gorgona green turtle FG with the species’ published nesting and foraging aggregations, measured as haplotype diversity and nucleotide diversity.

| Geographic site      | No. of haplotypes | Haplotype diversity (H) ± s.e. | Nucleotide diversity (π) ± s.e. | Sample size (animals) | Source |
|----------------------|-------------------|---------------------------------|---------------------------------|-----------------------|--------|
| **Nesting grounds**  |                   |                                 |                                 |                       |        |
| **ATLANTIC**         |                   |                                 |                                 |                       |        |
| Wider Caribbean      |                   |                                 |                                 |                       |        |
| Florida, USA         | 11                | 0.61 ± 0.103                    | 0.001 ± 0.001                   | 44                    | [25]   |
| Quintana Roo, Mexico | 7                 | 0.82 ± 0.058                    | 0.006                           | 20                    | [50]   |
| Isla Aves, Venezuela | 2                 | 0.25 ± 0.18                     | 0.005                           | 8                     | [50]   |
| Surinam               | 15                | 0.25 ± 0.141                    | 0.0006                          | 15                    | [50]   |
| Tortuguero, Costa Rica| 5                 | 0.16 ± 0.020                    | 0.003 ± 0.002                   | 433                   | [49]   |
| **PACIFIC**          |                   |                                 |                                 |                       |        |
| Australasia region   | 25                | 0.88 ± 0.010                    | 0.041 ± 0.020                   | 714                   | [19]   |
| Michoacan, Mexico    | 5                 | 0.48 ± 0.040                    | 0.003 ± 0.002                   | 123                   | [30]   |
| Revillagigedo, Mexico| 8                 | 0.61 ± 0.042                    | 0.003 ± 0.002                   | 90                    | [31]   |
| FFS Hawaii, USA      | 3                 | 0.49 ± 0.032                    | 0.003 ± 0.002                   | 229                   | [31]   |
| **Foraging grounds** |                   |                                 |                                 |                       |        |
| **ATLANTIC**         |                   |                                 |                                 |                       |        |
| Almofala, Brasil     | 13                | 0.71 ± 0.030                    | 0.006 ± 0.000                   | 117                   | [16]   |
| Ubatuba, Brasil      | 10                | 0.44 ± 0.056                    | 0.002 ± 0.001                   | 113                   | [16]   |
| North Carolina, USA  | 12                | 0.72 ± 0.030                    | 0.005 ± 0.003                   | 106                   | [6]    |
| Florida, USA         | 6                 | 0.48 ± 0.066                    | 0.003 ± 0.002                   | 62                    | [63]   |
| Bahamas              | 6                 | 0.37 ± 0.065                    | 0.006 ± 0.000                   | 79                    | [25]   |
| Barbados             | 8                 | 0.77 ± 0.029                    | 0.010 ± 0.005                   | 60                    | [64]   |
| Nicaragua            | 2                 | 0.18 ± 0.062                    | 0.003 ± 0.002                   | 60                    | [65]   |
| **PACIFIC**          |                   |                                 |                                 |                       |        |
| FFS Hawaii, USA      | 5                 | 0.46 ± 0.020                    | 0.002 ± 0.002                   | 673                   | [31]   |
| strandings FFS Hawaii, USA | 4 | 0.51 ± 0.044 | 0.003 ± 0.002 | 115 | [31] |
| Yaeyama Islands, Japan | 11            | 0.842                           | 0.024                           | 142                   | [32]   |
| Gorgona, Colombia    | 7                 | 0.30 ± 0.080                    | 0.011 ± 0.006                   | 55                    | this study |

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noted that current systems in the Eastern Pacific are very variable [52], as is the direction and scope of potential transport by the North Equatorial Countercurrent, so drift of turtles to Gorgona from distant regions may be sporadic. This preliminary analysis suggests that currents could be responsible for the transport of green turtle juveniles towards Gorgona, but more in-depth studies are needed to further determine how dispersal is influenced by currents.

### Dispersal, recruitment and migratory behavior of Gorgona turtles

Our findings reinforce what has been suggested in other sea turtle studies; that the recruitment into FG is influenced by oceanic mixing of individuals during their multi-year pelagic stage [6,54]. Equatorial currents may be an important vehicle for dispersal of green turtle post-hatchlings from western to eastern Pacific regions. After arriving at neritic areas such as Gorgona, turtles likely spend some time recovering from the transoceanic phase and storing resources to continue travel to further developmental and mating grounds. Our results indicate that most (estimated 55–96%) of Gorgona green turtles come from nesting beaches of the Galapagos, with potential contribution (estimated 2–38%) from rookeries in Michoacan State (Mexico). This suggests the existence of marine gateways, or paths connecting Michoacan State and Galapagos nesting rookeries with the FG of Gorgona and Galapagos [55,56] in the south-eastern Pacific. These three sites are part of the same green turtle management unit at a regional scale [51]. The results of our Gorgona MSA also provide new information related to potential linkages between eastern Pacific FG and distant nesting populations from FFS-Hawaii rookery (Figure 1, Table 2). The presence of western Pacific haplotypes has also been noted in green turtles foraging around the Galapagos Islands [56], suggesting further connectivity between these regional foraging populations. More comprehensive work including all the key Pacific rookeries and new genetic markers should provide more accurate estimates of the stock composition and connectivity between the regional nesting and foraging aggregations [7,57–59].

However, the Gorgona turtles with western Pacific haplotypes may merely be drifters transported by currents to the eastern Pacific that do not return to their natal beaches to breed. Gorgona and other habitats along the eastern Pacific would therefore act as genetic ‘sinks’ for these individuals. The Lagrangian drifter data show that it is possible for the eastward drift to occur over the vast distance to reach Gorgona, as has been shown for transatlantic movements of green turtle juveniles [3].

Some evidence of morphotype and size class similarities between Gorgona and Galapagos green turtles (from where a large proportion of the individuals at Gorgona FG originate) suggest

| Type of habitat     | Population | Tamura-Nei \(\phi_{ST}\) | Source     | P value |
|---------------------|------------|---------------------------|------------|---------|
| Rookeries           | Michoacan  | 0.144                     | [30]       | <.001   |
|                     | Revillagigedo | 0.281                  | [31]       | <.001   |
|                     | Galapagos  | 0.116                     |            | <.01    |
|                     | Hawaii FFS | 0.497                     |            | <.001   |
| Foraging grounds    | Hawaii     | 0.575                     | [31]       | <.001   |
|                     | Hawaii (strandings) | 0.429       |            | <.001   |

![Graph of mixed stock estimates](https://example.com/figure4.png)

**Figure 4.** Bayesian mixed stock estimates of contributions to the Gorgona foraging ground by central and eastern Pacific rookeries, after excluding putative western Pacific ‘orphan' haplotypes. Dark bars represent results using source population sizes (nests/yr) information; light bars without source population size. Source haplotype profiles used were obtained for Michoacan and Revillagigedo (Mexico), Galapagos (Ecuador) and French-Frigate Shoals Hawaii (USA). Error bars represent 97.5% and 2.5% percentile intervals.

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Figure 5. Mean surface ocean currents in the Pacific region 15°N–24°S 100°E–60°W from NOAA Ocean Surface Current Analyses-Real Time program. (a) Averages for July–December 1997 (during a very intense El Niño) indicating the strong and extensive eastward flowing North Equatorial Counter-current (NEECC) as a major feature of the current pattern. (b) Averages for July–December 2000 (common, non-El Niño conditions) showing the presence of the NECC as weaker and reduced compared to the westward flowing Equatorial current. Red overlay vector arrows indicate eastward flows; blue arrows indicate westward flows. Colored contour plots indicate current speed (meters per second) according to scale on the right. Map and information downloaded from http://www.oscar.noaa.gov/datadisplay/. doi:10.1371/journal.pone.0031486.g005

Figure 6. Satellite-tracked drifter buoy trajectories demonstrating potential ocean current pathways linking green turtle breeding areas in the Eastern Pacific with the Gorgona foraging site. Tracks from three drifters deployed near Eastern Pacific breeding ground heading towards the vicinity of the Gorgona study site (red cross). RE = Revillagigedo Islands, Mexico; MI = Michoacan, Mexico; GA = Galapagos Islands, Ecuador; Gor = Gorgona Island, Colombia. Rectangle in broken lines highlight area with frequent eddies provoking recurrent looped tracks with increased speed (about 2X average) but longer entrainment within the current system. Countries’ EEZ boundaries are indicated with two-letter abbreviations. Drifter data from NOAA/AOML Global Lagrangian Drifter Data (http://www.aoml.noaa.gov/envsids/gld/krig/parttrk_id_temporal.php). doi:10.1371/journal.pone.0031486.g006
migration or drifting between FG through the eastern Pacific pelagic and/or neritic habitats. Migratory paths for Galapagos post-nesting green turtles including, oceanic migration to Central America, residency within the Galapagos and travel into oceanic waters southwest of the Galapagos [55] are consistent with this suggestion. Consequently, a more comprehensive MSA is needed to determine if the CMP4 haplotype is present in other eastern Pacific FG or rookeries that have not yet been studied. The large error for the Michoacan and Galapagos contributions to the Gorgona MSA are probably due to the low haplotype diversity observed in this study and the sharing of haplotype CMP4 between Michoacan and Galapagos. Improvement in the accuracy of the MSA will require increased sample sizes, the inclusion of additional molecular markers and an expanded baseline of potential regional sources. Satellite or GPS telemetry will contribute to better understanding the dispersal and migratory movements of green turtle juveniles after they leave Gorgona. The mapped routes of satellite tracked animals supported by flipper tagging data will be a useful tool in designing accurate plans for the species conservation management in the Pacific. In order to fully identify the nesting origin of reported haplotypes in Gorgona, more breeding beaches need to be surveyed along the eastern tropical Pacific coast and molecular assignments developed to determine the percentage and contribution of haplotypes from each Pacific site to Gorgona’s green turtle aggregation. Surveys must also target females that occasionally breed at Palmeras beach in southwest Gorgona Island [60] and at the northern Colombian Pacific beach of La Cuevita, to test the possibility that these nesting sites are the origin of orphan haplotypes. Green turtles foraging in Gorgona could also be recruited from nesting beaches of coastal Machalilla National Park [61] in Ecuador or the Galapagos islands [62].

Conservation implications
Haplotypes identified so far have provided valuable information to the Colombian environmental authorities for the establishment of multinational strategies such as the Eastern Tropical Pacific Marine Corridor (CMAR). This is a marine conservation regional initiative carry out by the governments of Ecuador, Colombia, Panama and Costa Rica to protect MPAs of Galapagos (Ecuador); Malpelo and Gorgona (Colombia); Coiba (Panama) and Cocos (Costa Rica). Acknowledging the presence of individuals mainly from Galapagos in Ecuador and Mexico demonstrates to the Colombian government the need to sign the Inter American Convention for Sea Turtle Protection (IAC). The accomplishment of these transnational agreements would help not only to ensure the survival of sea turtles in the Pacific Ocean but also the coordinated management of other marine trans-boundary resources [10]. The convergence at Gorgona FG of green turtles from distinct, distant rookeries and the role it plays providing shelter and food is important for conservation management in the eastern Pacific and for the linkage between MPAs and protected nesting beaches along the CMAR. However, the role of Gorgona and other eastern Pacific FG in the ecology of western Pacific green turtle populations still remains unclear.

Our genetic findings combined with ongoing mark–recapture studies and satellite tracking will be helpful for connecting MPAs in a broader scale for better implementation of regional sea turtle management plans. This study is the first step toward characterizing a FG in Colombia and together with further movement tracking studies will be relevant to elucidate linkages between nesting and FG for improvement of current and the design of new MPAs. In addition, more genetic surveys of green turtle nesting beaches are required and together with comprehensive MSA will help ensure that the ecological role of Gorgona and other marine and coastal protected areas along the eastern Pacific region are recognized.

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Author Contributions
Conceived and designed the experiments: RR DA. Performed the experiments: DA. Analyzed the data: RR DA FAD. Contributed reagents/materials/analysis tools: RR DA FAD. Wrote the paper: RR DA FAD.

References
1. Luschi P, Hays GC, Papi F (2003) A review of long-distance movements by marine turtles, and the possible role of ocean currents. OIKOS 103: 293–302.
2. Meylan A (1982) Sea turtle migration - evidence from tag returns. In: Bjorndal KA, ed. Biology and Conservation of Sea Turtles. Washington, D.C.: Smithsonian Institution Press. pp 91–100.
3. Monne-Arregui C, López-Jurado LF, Rico C, Marco A, López P, et al. (2010) Evidence from genetic and Lagrangian drifter data for transatlantic transport of small juvenile green turtles. Journal of Biogeography 37: 1752–1766.
4. Musick JA, Limpus CJ (1997) Habitat utilization and migration in juvenile sea turtles. In: Lutz FL, Musick JA, eds. The Biology of Sea Turtles. Boca Raton: CRC Press. pp 159–164.
5. Broderick AC, Goiney MS, Fuller WJ, Glen F, Godley DJ (2007) Fidelity and over-wintering of sea turtles. Proceedings of the Royal Society B 274: 1333–1338.
6. Bass A, Epperly SP, Brau-McNeill J (2006) Green turtle Chelonia mydas foraging and nesting aggregations in the Caribbean and Atlantic; impact of currents and behavior on dispersal. Journal of Heredity 97: 346–354.
7. Bolker BM, Okuyama T, Bjorndal KA, Bolten AB (2007) Incorporating multiple mixed-stocks in mixed stock analysis: 'many-to-many' analyses. Molecular Ecology. 685–695.
8. Bowen BW, Meylan A, Ross PJ, Limpus CJ, Balaz GH, et al. (1992) Global population structure and natural history of the green turtle (Chelonia mydas) in terms of maternal phylogeny. Evolution 46: 865–881.
9. Bowen BW, Grant VS, Hillea-Storr, Shaver DJ, Bjorndal KA, et al. (2007) Mixed-stock analysis reveals the migrations of juvenilehawksbill turtles (Eretmochelys imbricata) in the Caribbean Sea. Molecular Ecology 16: 49–60.
10. Dutton P, Squire D (2006) Reconciling biodiversity with fishing: a holistic strategy for Pacific sea turtle recovery. Oceans Development & International Law 39: 200–222.
11. Bowen BW, Karl SA (2000) Meeting report: taxonomic status of the East Pacific green turtle (Chelonia mydas agassizii). Contributions to the Marine Biology and Environmental Science Library. 11: 35–45.
12. Volker K, Brooks LB, Nichols WJ (2010) Long-term feeding populations in the Yaeyama Islands, Japan. Zool Sci 27: 14–18.
13. Nei M (1987) Molecular Evolutionary Genetics. New York: Columbia University Press. 436 p.
14. Excoffier L, Laval G, Schneider S (2000) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.
15. Tamura K, Dudley J, Nei M, Kumaar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.
16. Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology Evolution 4: 406–425.
17. Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from distance matrices in DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479–491.
18. Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.
19. Seminoff JA, Schroeder B, McPherson S, Possardt E, Biib K (2007) Green sea turtle (Chelonia mydas) 3-year review: Summary and evaluation. National Marine Fisheries Service and US Fish and Wildlife Service Report 130. 150 p.
20. Jaraiz-Ceron JA, Sarti-Martinez AL, Dutton PH (2002) First study of the green/black turtles of the Revillagigedo Archipelago: a unique nesting stock in the Eastern Pacific. In: Seminoff JA, ed. Proceedings of the Twenty Second Annual Symposium on Sea Turtle Biology and Conservation. Miami: U.S. Dept. Commerce, NOAA Tech. Memo. NMFS-SEFSC-503. 336 p.
21. Balaz GH, Chaloupkova M (2006) Recovery trend over 3 years at the Hawaiian green turtle rookery of French Frigate Shoals. Atoll Res Bull 543: 147–158.
22. Hirth HF (1997) Synopsis of the biological data on the green turtle, Chelonia mydas, (Linnaeus 1758). United States Fish and Wildlife Service. 120 p.
23. Balaz GH, Chaloupkova M (2006) Recovery trend over 32 years at the Hawaiian green turtle rookery of French Frigate Shoals. Atoll Res Bull 543: 147–158.
24. Pritchard PCH, Moritmer J (1999) Taxonomy, external morphology, and species identification. In: Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M, eds. Research and Management Techniques for the Conservation of Sea Turtles. Blanchard: IUCN/SSC Marine Turtle Specialist Group. pp 21–40.
45. Dutton PH (2007) Molecular ecology of the eastern Pacific green turtle. In: Seminoff JA, ed. Proceedings of the 22nd Annual Symposium on Sea Turtle Biology and Conservation: U.S. Dept. Commer. NOAA Tech. Memo. NMFS-SEFSC-503. 336 p.

46. Dutton PH, Davis SK, McDonald DL, Guerra TG (1994) A genetic study to determine the origin of sea turtles in San Diego Bay, California. In: Bjorndal KA, Bolten AB, Johnson DA, Eliazar PJ, eds. Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation. Hilton Head: U.S. Dept. Commer. NOAA Tech. Memo. NMFS-SEFSC-341. 337 p.

47. Eguchi T, Seminoff JA, LeRoux R, Dutton PH, Dutton DL (2010) Abundance and survival rates of green turtles in an urban environment: coexistence of humans and an endangered species. Mar Biol 157: 1869–1877.

48. FitzSimmons NN, Limpus CJ, Norman JA, Goldizen AR, Miller JD (1997) Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proceedings of the National Academy of Sciences, USA 94: 8912–8917.

49. Bjorndal KA, Bolten AB, Troeng S (2005) Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Marine Biology 147: 1449–1457.

50. Encalada SE, Lahanas PN, Bjorndal KA, Bolten AB, Miyamoto MM, et al. (1996) Phylogeography and population structure of the Atlantic and Mediterranean green turtle (Chelonia mydas): a mitochondrial DNA control region sequence assessment. Molecular Ecology 5: 473–483.

51. Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB, et al. (2010) Regional Management Units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5: doi:10.1371/journal.pone.0015465.

52. Kessler WS (2006) The circulation of the eastern tropical Pacific: A review. Progress in Oceanography 69: 181–217.

53. Davenport J (1997) Temperature and the life-history strategies of sea turtles. Journal of Thermal Biology 22: 479–488.

54. Velez-Zuazo X, Ramos WD, Van Dam RP, Diez CE, Abreu-Grobois A, et al. (2008) Dispersal, recruitment and migratory behaviour in a hawksbill sea turtle aggregation. Molecular Ecology 17: 839–853.

55. Seminoff JA, Zarate P, Coyne MS, Foley DG, Parker D, et al. (2008) Post-nesting migrations of Galapagos green turtles Chelonia mydas in relation to oceanographic conditions: integrating satellite telemetry with remotely sensed ocean data. Endangered Species Research 4: 57–72.

56. Zarate P, Seminoff JA, Dutton PH (2007) Assessment of sea turtle foraging areas in the Galapagos Islands. In: Mast RB, Hutchinson B, Hutchinson A, eds. Proceedings of the Twenty-Fourth Annual Symposium on Sea Turtle Biology and Conservation: U.S. Dept. Commer. NOAA Tech. Memo. NMFS-SEFSC-567. 128 p.

57. Dutton PH, Frey A (2008) Characterization of polymorphic microsatellite markers for the green turtle (Chelonia mydas). Molecular Ecology Resources 9: 354–356.

58. Roden SE, Dutton PH, Morin PA (2009) Characterization of single nucleotide polymorphism markers for the green sea turtle (Chelonia mydas). Molecular Ecology Resources 9: 1053–1060.

59. Hansen L, Zarate P, Chang S, Balazs GH, Surii L, et al. (2007) Stock structure and gene flow among green turtle nesting populations in the eastern Pacific based on microsatellite analysis. In: Mast RB, Hutchinson B, Hutchinson A, eds. Proceedings of the Twenty-Fourth Annual Symposium on Sea Turtle Biology and Conservation: U.S. Dept. Commer. NOAA Tech. Memo. NMFS-SEFSC-567. 128 p.

60. Amoracho DF, Sánchez F, Quiroga D (2001) El encanto de las tortugas marinas en el Parque Nacional Natural Gorgona. In: Barrios L, López-Victoria M, eds. Gorgona marina: contribución al conocimiento de una isla única. Bogotá-Cali-Córdoba: Instituto de Investigaciones Marinas y Costeras de Colombia. pp 141–148.

61. Baquer Gallegos A, Prina Mosquera M, Muñoz Pérez JP, Álvarez V (2008) Anidación de tortugas marinas en en las playas del Parque Nacional Malalá en 2008: Una nueva área de anidación de tortugas carey (Eretmochelys imbricata) en el Pacífico oriental. In: Kelez S, van Oordt F, de Paz N, Forsberg K, eds. II Simposio de Tortugas Marinas en el Pacífico Oriental. Lima: Oceanica. pp 116.

62. Zarate P, Carrión J Evaluación de las áreas de alimentación de las tortugas marinas en las Islas Galápagos: 2000–2006. Fundación Charles Darwin.

63. Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (Chelonia mydas) from the east central Florida coast: evidence from mtDNA markers. Herpetologica 56: 357–367.

64. Luke K, Horrocks JA, LeRoux RA, Dutton PH (2004) Origins of green turtle (Chelonia mydas) feeding aggregations around Barbados, West Indies. Marine Biology 144: 799–805.

65. Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (Chelonia mydas) from the east central Florida coast: evidence from mtDNA markers. Herpetologica 56: 357–367.