Genetic Connectivity in Scleractinian Corals across the Northern Gulf of Mexico: Oil/Gas Platforms, and Relationship to the Flower Garden Banks

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Introduction

Prior to the 1940s, the bottom of the Gulf of Mexico (GOM) was characterized primarily by terrigenous, sandy muds with low habitat diversity [1,2]. During that decade, offshore drilling for oil and gas began there and production platforms grew steadily in number, spreading southward across the continental shelf. Those platforms served as substrate for colonization of numerous marine organisms, and this process has continued [3–8]. These production platforms extend up from the bottom into the atmosphere, creating an island and providing hard substrate accreted by corals (non-reef-building) scleractinian corals on many of these platforms [5,10–17].

The only true coral reefs in the northern Gulf of Mexico are the Flower Garden Banks (FGB; NOAA Flower Garden Banks National Marine Sanctuary) [18], located ~180 km SE of Galveston, TX. The FGB are defined by two banks that approach the surface to within 18 m [19]: the East Bank (27°54'32'' N, 93°36’ W) and West Bank (27°52'27'' N, 93°48'47'' W) [1,20] (see Fig. 1). Calcium carbonate reefs have developed on their caps [20,21] which are productive [22] and healthy, being characterized by 24 species of hermatypic corals [18–20,23]. The closest reefs to the FGB are the Lobos-Tuxpan system, located 13 km off Cabo Rojo, Mexico [18] (Fig. 1), ~640 km away [24,25]. Other banks do exist on the northwestern GOM shelf, such as Stetson, Sonnier, 28 Fathoms, etc. and do possess scleractinian corals [19,22,25–29]. These banks are deeper, however, or occur in cooler waters and do not qualify as true coral reefs because they are not biogenic in origin (i.e., composed of calcium carbonate that has been accreted by corals). The FGB are now surrounded by hundreds of
platforms. It is possible that coral populations on the deeper banks could be a source of larvae that might colonize the platforms, but the abundance of coral on those banks is much lower than those on the FGB. The potential of the banks being a larval source for recruitment for the platforms is in probably relatively low.

In this study, we focused on one hermatypic (zooxanthellate and reef-building) scleractinian coral species and one ahermatypic (azooxanthellate and non-reef building) one which occur on the platforms in the northern GOM and also on the FGB. We attempted to determine the degree of genetic connectivity (or relatedness) among the natural and platform populations on a large geographic scale, covering most of the northern GOM. Through earlier surveys, we found that these species were abundant enough to provide sample sizes sufficient for meaningful comparative molecular genetic analysis. The corals were *Madracis decactis* (Lyman 1859; Pocilloporidae; hermatype) and *Tubastraea coccinea* (Lesson 1829; Dendrophylliidae; ahermatype). Both of these reproduce via brooding. *Madracis decactis* is a simultaneous hermaphrodite and planulates monthly between March and December, with a broad peak occurring from Sept. to Nov. [30]. *Tubastraea coccinea* produces planulae sexually [31] but can also produce them asexually [32,33]. It commonly produces numerous runners and is highly effective at producing new colonies asexually [34,35]. This invasive species was first observed to colonize the East Flower Garden Bank in 2002 [36].

*Tubastraea coccinea* is the single, most successful invasive coral and one of the most successful of all species to invade the Atlantic Ocean [37–40]. Other known invasive corals in the Caribbean are *Fungia scutaria* [41–43] and now *Tubastraea micranthus* (Ehrenberg 1834), a recent introduction to the region just south of the Mississippi River mouth (the Grand Isle – GI-lease area) [44]. Figueira de Paula and Creed [45] have also reported the introduction of *T. taguensis* to Brazil, along with that of *T. coccinea*. Thus, the total number of introduced coral species to the western Atlantic Ocean is now four. *T. coccinea* was first recorded in Puerto Rico in 1943 and then in Curacao, Netherlands Antilles in 1948, occurring on ships’ hulls [46]. It appeared in Belize and Mexico in the late 1990s and early 2000s [37]. Its spread progressed to Venezuela, northern Gulf of Mexico, and the Florida Keys [25,38,39]; Brazil [45]; and Colombia, Panama, the Bahamas, and throughout the Lesser and Greater Antilles [40]. In terms of sheer numbers, *Tubastraea coccinea* is clearly the most abundant scleractinian coral, hermatypic or ahermatypic, in the northern Gulf of Mexico on artificial substrata [14–16,25,47]. Hundreds of thousands of colonies may be found on a single platform (e.g., 28/m²) [14,48]. It has also been found on some of the deeper banks of the northern GOM, but its abundances are low there [26,28,49]. It was first observed to colonize the East Flower Garden Bank in 2002 [36]. Its abundances there are also low, where it only occurs cryptically. It would appear that *T. coccinea* is not as successful a competitor for space on natural well-established reefs as on artificial substrate.

The two target coral species are brooders and reproduce by producing fully developed larvae which have the capability to settle in $\approx4$hrs [50]. Recruits can become reproductively mature within 1–2 yrs of age. Brooders generally planulate on a monthly cycle and can release larvae up to 8–10 times per year (e.g., *Porites astreoides*) [51]. Planulae are released during a spawning event that may extend over a period of days. Data from some coral studies suggest that brooders are adapted for short-distance dispersal. Broadcast spawners (corals that release sperm and eggs into the water column for fertilization and larval development there), on the other hand, are believed to be more effective at longer-distance dispersal [see [52]]. The potential for long-range dispersal between planulae produced by these two types of corals (brooders and broadcasters) is most likely comparable once the planulae have become fully developed [53,54].

Here we examine genetic affinities among populations of the scleractinian corals *Madracis decactis* and *Tubastraea coccinea*, respectively. The populations sampled were from the Flower Garden Banks and a large number of platforms across the Gulf of Mexico.
continental shelf of the northern Gulf of Mexico. The platforms cover ~800 km of coastline from Matagorda Island, TX to Mobile, AL. The FGB occur west of the center of this range. We will examine the degree of connectivity between coral populations on the platforms in this region, and between those platforms and the natural reefs. We will also attempt to infer information about the comparative effectiveness of dispersal and colonization by these two brooding species, and relate it to an island-hopping strategy of colonization as described by MacArthur and Wilson ([55], also see [52,56–58]). We will also expand the original analyses of Atchison et al. [58,59], who conducted similar studies on Madracis decactis and Diploria strigosa (a broadcasting coral) in this region. We also utilize a more conservative statistical analytical approach to analyze the data, based on extensive simulations. The specific objectives of this study are: To determine the degree of genetic connectivity between adult coral populations of Madracis decactis (hermatype) and of Tubastrea coccinea (hermatype) on oil/gas platforms throughout the northern Gulf of Mexico and the Flower Garden Banks; to compare variation in genetic affinity between conspecific populations on different platforms; to determine the degree of genetic affinity between conspecific populations on the platforms vs. the FGB; and, to utilize data on patterns of genetic variation to infer comparative colonization potentials for these species.

Materials and Methods

Study Site

We initiated a set of field surveys followed by laboratory analyses. Surveys were conducted by SCUBA on offshore platforms extending between Madagorda Island, TX and Mobile, AL – a distance of 780 km. We sampled an area from ~20 km offshore to the edge of the continental shelf and beyond. Platforms were sampled along four cross-shelf transects spaced at approximately equal intervals along the GOM coast (Fig. 2; Table 1). Transect 1 ran SE from Matagorda Island, Texas and included 8 platforms; Transect 2 ran S from Port Arthur/Lake Sabine, LA (7 platforms); Transect 3 ran S from Timbalier Island, LA (7 platforms); and Transect 4 ran SW from Mobile, Alabama (6 platforms).

These transects were chosen, firstly because they covered the breadth and width of the shelf where platforms exist, particularly the shelf edge. Secondly, they cover enough of the shelf to potentially provide northern boundary information regarding coral colonization and survival. In addition, they would provide information on coral colonization and growth on platforms near the shelf edge.

Sample Collection

We chartered a dive vessel (M/V Fling, Freeport, Texas) and conducted surveys over a period of three years (Fig. 2; Table 1). Surveys were conducted between 5 and 37 m depth with teams of SCUBA divers during the summer and fall seasons of 2004–2007. All platforms surveyed had been deployed for 15–26 years, since it has been determined that a minimum of 15 yrs is associated with the development of substantial adult coral populations [25]. (Data on distribution, abundance, and species diversity of corals in this region may be found in a previous publication) [47]. At the East and West FGB, coral tissue samples were collected by SCUBA divers haphazardly.

Tissue samples, two cm² in area, were collected by SCUBA divers from the growing edge of adult corals of the two target species using small hammers and chisels. Tissue was collected from

Figure 2. Map of the oil and gas platforms in the northern Gulf of Mexico. Four cross-shelf transects (I – IV, west to east) were used to examine scleractinian corals on a sub-set of oil/gas production platforms along each transect. Small squares represent platforms. Large dots represent study platforms sampled for corals; see Table 1 for specific names, latitudes, longitudes, and lease area names of platforms. The transects ran generally SE from Matagorda Island, Texas; 5 from Port Arthur/Lake Sabine, LA; 5 from Timbalier Island, LA; and 3W from Mobile, Alabama. The oval represents a region encompassing the Flower Garden Banks and 13 platforms sampled in an earlier study for coral community development and population genetics [25].

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Amplified Fragment Length Polymorphisms (AFLPs) have been used successfully to estimate migration rates [64], species boundaries [65,66], and degree of parental contributions to populations [67]. Use of AFLPs is not ideal for all population genetic applications [60]. They do, however, perform extraordinarily well for population assignment or allocation studies [62,68–71], where the number of polymorphic loci is more important than allelic diversity [72]. In this case, 117 polymorphic markers were generated and utilized for the study. Only those samples yielding readable markers were included in the study, defining the sample sizes for each site (see Table 3 for a summary).

It is possible that some genetic variation detected using AFLPs may not be derived from the target organism [60]. This has been an area of concern with corals, which possess endosymbiotic zooxanthellae. Here, however, we have used zooxanthella-specific PCR primers to confirm for each sample that any contamination by zooxanthellar DNA occurs at levels far below those necessary for AFLP (i.e., 5–10 pg of zooxanthellar DNA in a background of coral DNA) [69].

Preparation of Coral Tissue Lysates for Genetic Analysis

DNA was isolated by macerating samples lightly in SED buffer and spinning at 16Xg for 5 min to pellet the zooxanthellae from the homogenate. The DNA was then purified using the Wizard® SV Genomic DNA Purification System (Promega Corporation, Madison, WI), following the manufacturer’s instructions for animal tissue. All samples were checked for zooxanthellae DNA contamination using the PCR techniques described in Brazeau et al. [69] and Atchison et al. [58,59].

Amplified Fragment Length Polymorphisms (AFLPs)

Amplified Fragment Length Polymorphism (AFLP) is a DNA-“fingerprinting” technique [60] that detects polymorphisms based upon the selective PCR amplification of a subset of numerous restriction fragments generated by two different restriction enzymes [61,62]. The AFLPs tend to be highly polymorphic, but they are not co-dominantly expressed. They are commonly used in studies of commercial crop species and other economically important species; but they have not been widely used in animal studies [63]. This is surprising, since AFLPs provide abundant polymorphic markers relatively quickly for any species of interest.

Preparation of Coral Tissue Lysates for Genetic Analysis

The samples were returned to the ship and sealed in plastic freezer bags containing SED high-salt buffer (saturated NaCl, 250 mM EDTA, pH 7.5, 20% DMSO) to preserve the DNA. This preservative allows tissue samples to be stored at room temperature, eliminating the need for storage in liquid nitrogen. The samples were then placed in additional SED buffer, placed in ice chests, and returned to the laboratory.

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The AFLP technique, like other similar molecular genetic techniques, generate a subset of markers from a large population of markers. Of the subset obtained from a given AFLP experiment, a portion is often sensitive to specific reaction conditions. Thus, extra caution is required in processing samples through all procedural steps to maximize repeatability of results. Here, we processed samples in large lots containing members from all populations. This helped to distribute any error potentially introduced by reaction conditions uniformly between populations in an unbiased fashion. Also, all PCR reactions were replicated using one machine and the same thermal cycle profiles.

### Table 2. A list of platforms sampled and number of coral colonies sampled per platform in each transect/sector across the northern Gulf of Mexico.

| Species    | Transect Number/Sector | Platform | No. Coral Samples |
|------------|------------------------|----------|-------------------|
| Tubastraea | Transect 1 Western Sector | BA-133-A | 16 |
| cocinea    | GB-189-A | 31 |
|            | Transect 2 Near Western Sector | GB-236-A | 7 |
|            | ST-188-A | 14 |
|            | ST-190-A | 14 |
|            | ST-262-A | 0 |
|            | ST-277-A | 14 |
|            | ST-292-A | 17 |
|            | ST-295-A | 19 |
|            | Transect 3 Central Sector | GB-236 | 14 |
|            | ST-190-A | 14 |
| Madracis   | Transect 2 Near Western Sector | GB-236 | 14 |
| decactis   | Transect 3 | ST-277-A | 9 |
|            | Central Sector | ST-292-A | 21 |
|            | ST-295-A | 30 |
|            | Transect 4 | MP-144-A | 16 |
|            | Near Eastern Sector | MP-144-B | 12 |
|            | MP-265-A | 46 |
|            | MP-288-A | 27 |
|            | MP-289-B | 31 |

Numbers are provided for two target coral species – *Madracis decactis* and *Tubastraea coccinea*. Data were combined in the Main Pass (MP) lease area in the case of the former species due to small sample sizes per platform. Inviable samples not included in analysis shown in italics. doi:10.1371/journal.pone.0030144.t002

### Table 3. Sequences of the adapters and primers used in the AFLP protocol.

| Name           | Sequence                              |
|----------------|---------------------------------------|
| Adapters EcoRI | EcoF 5′-CTCGTAGACTGCTACCT ACC         |
|                | EcoR 5′-AATTGTCACGAGCTCTAC             |
| Adapters MseI  | MseF 5′-GACGATGAGTCCTGAG              |
|                | MseR 5′-TACTCAGACGTCTAC               |
| Pre-selective Primer | EcoRI A 5′-GACTGCTGATTACCT AATC A |
| Pre-selective Primer | MseI C 5′-GATGATCGTCTGAG TAC C |
| Selective Primers (Set 1) | EcoRI 5′-GACTGCTGATCAATTC ACT       |
|                | MseI 5′-GATGATCGTCTGAGTAA CAG        |
| Selective Primers (Set 2) | EcoRI 5′-GACTGCTGATCAATTC ACC |
|                | MseI 5′-GATGATCGTCTGAGTAA CTT        |

Genomic Coral DNA digestion and adapter ligation

A restriction-ligation “master mix” was prepared using the following reagents (measures are per sample): 1.1 μl T4 DNA ligase, 10X buffer (30 mM Tris-HCl, pH 7.8/10 mM MgCl₂, 10 mM dithiothreitol (DTT)/1 mM ATP), 1.1 μl of 0.5 M NaCl, 0.1 μl bovine serum albumin (BSA; 1 mg/ml), 1.0 μl MseI adapters (50 μM), 1.0 μl EcoRI adapter (5 μM), 0.25 μl MseI I (4 U/μl; New England BioLabs, Beverly, MA), 0.25 μl of EcoRI (20 U/μl; New England BioLabs), and 0.33 μl of Taq ligase (3 U/μl; 10 mM Tris-HCl, pH 7.0/50 mM KCl/1 mM DTT/0.1 mM EDTA/50% glycerol). Sequences for the MseI and EcoRI adapters and PCR primers are listed in Table 3. To each new 1.7 ml tube, 5.5 μl of the restriction-ligation mixture plus 5.5 μl (500 ng genomic DNA) of the purified genomic was added, centrifuged for 15 s, and incubated at room temperature overnight. At the end of the restriction-ligation reaction, 189 μl of TE buffer (10 mM Tris-HCl, pH 8.0/0.1 mM EDTA) was added (10-fold dilution), serving as the template for the next-step, pre-selective amplification.

Pre-selective (PS) Amplification of Coral DNA

A second “master mix” was made for pre-selection (PS) amplification, using the following reagents (per sample measure given): 8.1 μl of nuclease-free water, 2.0 μl of 10X PCR buffer (15 mM MgCl₂ in buffer), 0.8 μl of 5 mM dNTPs, 2.0 μl of EcoRI PS primer (2.75 μM), 2.0 μl of MseI PS primer (2.75 μM), and 0.1 μl of Thermostable (Taq) DNA polymerase (5 U/μl), for a total volume of 15.0 μl. Fifteen μl of the pre-selective amplification master mix was combined with 5 μl of each of the diluted restriction ligation reaction in a 0.5 ml tube. Samples were vortexed and centrifuged for 15 s. Amplification was performed using a 2-min initial incubation at 72°C, followed by 20 cycles of 20 s denaturation at 94°C, 30 s annealing at 56°C, and 2 min extension at 72°C. Last steps were 2 min final extension at 72°C, and 30 min final incubation at 60°C. After the cycling was completed, 180 μl of TE buffer was added to each tube, which consisted of the templates for the final step, selective amplification.

Selective Amplification of Coral DNA

In the final step, a selective amplification “master mix” was made, containing the following components: 8.1 μl of nuclease-free water, 2.0 μl of 10X PCR buffer (with MgCl₂ at 15 mM), 0.8 μl of 5 mM dNTPs, 2.0 μl of EcoRI selective primer

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(Please select and see all nucleotides listed in bold).
(0.46 μM), 2.0 μl of MseI selective primer (2.75 μM), and 0.1 μl of Taq DNA polymerase (5 U/μl) for a total volume of 15.0 μl. To each 0.5 ml micro-centrifuge tube, 5 μl of the diluted pre-selection PCR reaction was added to each corresponding tube, mixed, and centrifuged for 15 s. Samples were placed in the thermocycler, and the cycle profile was performed as indicated: 2 min initial denaturation at 94°C, followed by 1 cycle of 20 s denaturation at 94°C, 30 s annealing at 66°C, and 2 min extension at 72°C. Next, there were 9 cycles: 20 s at 94°C, initial 30 s at 66°C (reduced 1°C/cycle), and 2 min at 72°C. Final cycle consisted of 20 cycles: 20 s at 94°C, 30 s at 56°C, and 2 min at 72°C, followed by a 30 min final incubation at 60°C.

Products for the selective PCR reactions were run on an Amersham MegaBACE 1000 96 capillary sequencer at the University of Florida’s Interdisciplinary Center for Biotechnology Research. Resulting electropherograms were analyzed using SoftGenetics GeneMarker® (ver 1.51) for bands ranging from 50 to 400 bp in size in 20 bp increments.

The Selective PCR reactions were repeated three times for each sample. These “repeat” reactions were run on different days with populations mixed in each run. Bands were considered present if they appeared in two of the three runs; conversely, bands were scored as absent if two out of the three reactions yielded no band. Of the bands included in the study, >90% yielded the same result in all three PCR runs. These inclusion criteria helped to identify bands that were sensitive to reaction conditions.

Figure 3. Genetic affinity in *Madracis decactis* coral populations on oil and gas platforms across the continental shelf in the northern Gulf of Mexico. Genetic affinity value (degree of relatedness) determined by the population genetics analytical software STRUCTURE. The reference population was the West Flower Garden Bank; i.e., the population against which all other members of all other populations were compared. The peak in the west implies that corals on platforms in that region were most likely derived from the Flower Garden Banks. Population differentiation is evident in the east, on either side of the Mississippi River mouth. The point-depression south of Terrebonne Bay, LA may represent a population drawn from outside of the northern Gulf of Mexico. Note: The orientation of the map has been reversed to east-to-west in order to facilitate viewing of the topography of the three-dimensional pattern generated by the data. This reveals fine-scale structure that would otherwise be hidden using a southerly view. The reader is viewing the region from the north, with east being on the left and west on the right.

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Statistical Analyses

Two statistical analyses were used to assess population differentiation: AFLPOP population allocation analysis (V. 1.1; 73), a statistical analytical procedure designed particularly to analyze data generated by AFLPs, and STRUCTURE V 2.0 [74]. The development of these techniques and their application to the analysis of coral population genetics have been described elsewhere [57–59,65,66,69–71,75].

The AFLPOP analysis uses AFLP presence/absence data to calculate log-likelihood values for any individual’s membership in a reference population, based upon their banding patterns. The reference population is that target population (e.g., from one platform) against which all other colonies from other sites are compared for genetic affinity. Each individual is allocated to the population showing the highest likelihood for that genotype [64,73]. These assignment tests have been successfully used to estimate long-distance dispersal [64]. When the individual is assigned to a population different than the site from which it was collected, it is interpreted as evidence of dispersal. One major advantage of the method is that populations do not have to be sampled exhaustively [64]. In an AFLPOP simulation, an individual is chosen randomly from the entire population, population marker frequencies are calculated without that individual, and then the individual is assigned to the new data set. This simulation was repeated 500 times for each run. Average assignments to a given site were subsequently calculated as a percent value, based on 10 repeats of these 500 iterations.
Table 4. Genetic affinities in populations of the coral Madracis decactis.  

(a) Madracis decactis Transects III & IV  

| Percentage (%) of Colonies | W. of Miss. River | E. of Miss. River |
|---------------------------|------------------|------------------|
| ST-295                    | 100%             | 0.3%             |
| ST-297                    | 0                | 100%             |
| ST-292                    | 0.2%             | 99.7%            |
| MP - All                  | 0                | 100%             |

(b) Log-Likelihood = 1  

| Percentage (%) of Colonies | W. of Miss. River | E. of Miss. River |
|---------------------------|------------------|------------------|
| ST-295                    | 99%              | 0                |
| ST-277                    | 0                | 100%             |
| ST-292                    | 0.1%             | 99.4%            |
| MP - All                  | 0                | 100%             |
| CNM                       | 0.6%             | 0.6%             |

(a) Genetic affinities on oil/gas platforms in the northern Gulf of Mexico along two transects – one south of Terrebonne Bay, LA (II), west of the Mississippi River mouth, and another south of Mobile, AL (IV), east of it. Data were combined in the Main Pass (MP) lease area due to small sample sizes per platform, to facilitate comparison. Data analyzed via AFLPOP, with a log-likelihood value set at 1.0. Note extraordinarily low levels of high self-assignment to home populations and lack of recognition of neighboring populations. An indication of geographic isolation of these coral populations and a possible indication of different larval sources. (b) Same, but data analyzed with a log-likelihood value set at 1.0. Resulting pattern almost identical to analysis performed with more liberal 0 setting. doi:10.1371/journal.pone.0030144.t004

The AFLPOP program allows the user to set a log-likelihood threshold for each assignment. If set to 0.0, a colony will always be assigned to the population with the highest likelihood value. Atchison [57] and Atchison et al. [58,59] found that this may yield misleading results. This is because individual assignment does not take into account that multiple sites may have nearly equal likelihood values. Here, we also performed simulations using 1.0 as the comparative log-likelihood threshold in the analysis. This approach is more conservative, though at the cost of some information. With the threshold set to 1.0, a colony would not be assigned to a population unless the probability of the given assignment was 10 times more likely than the next most probable assignment. If this threshold was not met, the individual was not assigned to any population and is designated “Criteria for allocation Not Met” (CNM). This does not imply that the sample could not be assigned to a population; it means that there may be at least two populations with nearly equivalent probabilities of assignment. It could also mean that the individual fits none of the populations well and may be derived from an outside population (see [58,59]). In this study, we focused on cases where clear assignments have been made.

STRUCTURE uses Bayesian techniques and Monte Carlo simulations to assign samples to populations. Unlike AFLPOP, in which assignment is based solely upon marker frequencies, STRUCTURE makes assignments that minimize deviations from the Hardy-Weinberg (H-W) equilibrium, which assumes that the population giving rise to the recruits constitutes a large, randomly mating population. Using this approach, the program calculates probabilities of individual assignment, estimates of FST, and probable paternity, grand-paternity, etc. relationships. The program can accommodate dominant marker data such as those generated by the AFLP technique. Data were analyzed using a burn-in period of 500,000 iterations, followed by another 100,000 Markov Chain Monte Carlo (MCMC) repetitions.

STRUCTURE also calls for definition of the parameter MIGPRIOR before running. This parameter was the prior probability of a spat being identified as coming from an external source. It was run at both levels, for comparative purposes – 0.05 and 0.50, taking into account different potential estimated migration rates.

We chose not to use AMOVA for our analysis. Although AFLP data can be treated like allelic data, since these markers are codominant and multi-locus, we chose to take a more conservative approach by using genetic assignment tests. Genetic assignment tests are also more appropriate to the types of questions we have addressed here. Finally, AFLP data can be problematic in attempting to estimate “allele” frequency distributions among populations, plus classic parameters like Nm and FST can in themselves be problematic as indirect genetic indices of assessing population connectivity see [76–79]).

Results

Relationship between Genetic Distance and Geographical Distance for Madracis decactis

In examining Madracis decactis, the results derived from STRUCTURE revealed a clear pattern. The highest levels of genetic affinity occurred between coral populations on platforms in Transect II, off of Port Arthur/Lake Sabine, TX, and on platforms around the FGB (Fig. 3). This affinity peaked in the west and then decreased steadily and to the east, to Transect III, south of Terrebonne Bay. Affinity then increased slightly in Transect IV, off Mobile, AL. A point depression in genetic affinity occurred near the edge of the continental shelf in Transect III (off Terrebonne Bay, LA).

We compared genetic affinities of Madracis decactis populations between the two sides of the Mississippi River mouth to determine whether the river plume – a major oceanographic feature - could be acting as a geographic barrier to dispersal. When analyzing the genetic data using AFLPOP, with a log-likelihood threshold value of “0” (all colonies must be assigned to a population), it became clear that not only was there little similarity between the two populations, there was also little similarity among platform populations within a transect (Table 4a). When this analysis was repeated using a log-likelihood threshold value of 1.0 (a colony must be 10X more likely to belong to a given population than another before being assigned there), a much more conservative approach, there was still very little difference in the results (Table 4b). Both analyses indicated minimal levels of dispersal between these two sets of populations – between or within transects.
Relationship between Genetic Distance and Geographical Distance for *Tubastrea coccinea*

The analytical program STRUCTURE yielded clear results for *Tubastrea coccinea*. In general, the genetic affinity values in this species were higher across the continental shelf than those for *Madracis decactis* (Fig. 4; see Y-axis). Although genetic affinity generally decreased from west to east, there was substantial local variation in this pattern. Firstly, genetic affinities dropped highly significantly east of the Mississippi River, in Transect IV. In addition, there was an anomalous point depression in genetic affinity in *T. coccinea* at the edge of the continental shelf in Transect III (off Terrebonne Bay, LA).

When one makes a direct comparison of genetic affinities of populations occurring in Transect III (off Terrebonne Bay, LA) vs. Transect IV (off Mobile, AL) using AFLPOP with a log-likelihood threshold value of 0.0 (forcing assignment), there was once again little similarity among populations across the mouth of the Mississippi River (Table 5a). A higher degree of recognition occurred between populations of Madracis decactis within these transects. When this analysis was repeated using a log-likelihood threshold value of 1.0 (more conservative), self-assignment to home populations decreased and more colonies were assigned to the CNM (Criteria Not Met) category (Table 5b). In general, dispersal appeared to be broader in *Tubastrea coccinea* than in *Madracis decactis*.

Discussion

The high degree of connectivity between populations of *Madracis decactis* on platforms in the western GOM, as determined by STRUCTURE, confirms that these populations most likely originated from the Flower Garden Banks (FGB). The platform populations show high affinity for those on the FGB and those on platforms around them. Also, as the platform populations become more distant from those in the west and the FGB, they exhibit less genetic affinity to each other. The slight increase in genetic affinity in the eastern sector, beyond the mouth of the Mississippi River mouth, underscores the lack of affinity between the populations on the eastern and western sides of the river. The mouth of the Mississippi River appears to represent a strong geographic barrier to larval dispersal, as is known to be the case in other organisms in the vicinity of river mouths, particularly the Mississippi (e.g. bivalves and other organisms; see [80–84]) because of its physical and chemical characteristics. Success of fertilization of eggs is known to be affected by low salinities [85,86], as is planular development, settlement, and survival [87]; but see [88]. Other related factors affect planular survival and settlement are sedimentation [89] and increased nutrients [90,91].

The anomalous point depression in genetic affinity observed in *Madracis decactis* in Transect II, off Terrebonne Bay, LA suggests that some *Madracis decactis* larvae may have been introduced to this region by more than one means – from more than one region - or
more than one time. In the first case, larvae may have been introduced from the Caribbean via the Loop Current entering the Gulf of Mexico through the Yucatan Straits, from the Caribbean, proceeding north to the Mobile, AL region (Transect IV; Fig. 5). Alternatively, *Madracis decactis* larvae may have been introduced via a jet current from the northern portion of the Yucatan Peninsula [92]. In addition, hurricanes can promote larval dispersal over long distances by producing high speed currents that could move larvae from the Caribbean to the northern GOM [93]. In any case, it is clear that this *Madracis* population is different from all other *M. decactis* populations sampled in the northern GOM.

The geographic pattern of genetic affinity exhibited by *Tubastraea coccinea* (as determined by STRUCTURE) had general similarities to that of *Madracis decactis*; that is, there was a general decrease in affinity from west to east. The variations in this pattern observed in *M. decactis*, however, were much more striking in *T. coccinea*. Firstly, the strong decrease in genetic affinity near the mouth of the Mississippi River indicates a sharp differentiation between that far-eastern population and all of those to the west of the Mississippi River mouth. In addition, a deep point depression in genetic affinity of *T. coccinea* was also noted in Transect II. It was similar to that observed in *Madracis decactis*, but more marked. The population on that platform, was clearly unrelated to the others in the northern GOM. This may represent a second introduction of this species to the northern Gulf of Mexico. Potential sources include the Caribbean, as described above, or a second successful introduction to the western Atlantic from a commercial vessel from the Indo-Pacific. Once again, in either case, this population is unrelated to the others in this region. The Mississippi River appears to represent a formidable east-west barrier to coral larval dispersal in this region. This was evidenced through the lack of genetic affinity between coral populations on either side. This pattern was evident in two distantly related coral species - *Madracis decactis* and *Tubastraea coccinea*.

There are two possible explanations for this anomaly which are not mutually exclusive. The first is that the lower salinities, higher sediment, and higher nutrient concentrations associated

| Table 5. Genetic affinities in populations of the coral *Tubastraea coccinea*. |
| --- |
| a. *Tubastraea coccinea* |
| Transects III & IV |
| Percentage (%) of Colonies on these Platforms |
| W. of Miss. River | E. of Miss. River |
| Allocated to | ST & SS pops | MP-144 | MP-236 | MP-265 | MP-288 | MP-289 |
| ST & SS pops | 100% | 0 | 0 | 0 | 0 | 0 |
| MP-144 | 0 | 70 | 0.1 | 8.6 | 4.2 | 9.3 |
| MP-236 | 0 | 0.6 | 99.7 | 0.2 | 0 | 0.5 |
| MP-265 | 0 | 12.6 | 0.1 | 90.3 | 0.4 | 0.6 |
| MP-288 | 0 | 4.6 | 0 | 0.3 | 94.8 | 0.8 |
| MP-289 | 0 | 12.2 | 0.1 | 0.6 | 0.6 | 88.8 |

N = 500, 10x

| b. Log-Likelihood = 1 |
| Percentage (%) of Colonies on these Platforms |
| W. of Miss. River | E. of Miss. River |
| Allocated to | ST & SS pops | MP-144 | MP-236 | MP-265 | MP-288 | MP-289 |
| ST & SS pops | 100% | 0 | 0 | 0 | 0 | 0 |
| MP-144 | 0 | 21.4 | 0 | 0.8 | 0.2 | 0.4 |
| MP-236 | 0 | 0.4 | 98.4 | 0 | 0 | 0 |
| MP-265 | 0 | 1.6 | 0 | 58.6 | 0 | 0 |
| MP-288 | 0 | 1 | 0 | 0 | 82.8 | 0.2 |
| MP-289 | 0 | 2.2 | 0 | 0 | 0 | 57.8 |
| CNM | 0 | 73.4 | 1.6 | 40.6 | 17 | 41.6 |

N = 500, 10x

(a) Genetic affinities on oil/gas platforms in the northern Gulf of Mexico along two transects – one south of Terrebonne Bay, LA (III), west of the Mississippi River mouth, and another south of Mobile, AL (IV), east of it. Platforms on the western side combined to provide sufficient sample size for comparison. Data analyzed via AFLPOP, with a log-likelihood value set at 0. (b) Same, but using a log-likelihood value of 1.0. Note how self-assignment arguments have diminished in magnitude, indicating much greater levels of cross-assignment, varying greatly from analysis performed with more liberal 0 setting. This indicates a broader dispersal capacity for *T. coccinea* vs. *M. decactis* (see Table 4).

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with the Mississippi River plume [94–97] may decrease coral larval survivorship levels in this region [90,91]. This would result in increased geographic isolation of the coral populations. The second is that coral larvae from the Caribbean may be derived from elsewhere, carrying with them a different genetic identity. Larvae could have been transported north by the Caribbean Current into the northern Gulf of Mexico via the Loop Current, major oceanographic feature in this region. Eddies that break off from the Loop Current can traverse the Main Pass (MP) region (near eastern sector, Transect IV), prior to moving W-SW over the next 6 mos or so [see 98 for an overview of these features].

Our results also revealed that the invasive ahermatypic *Tubastraea coccinea* has higher larval dispersal and recruitment capabilities than *Madracis decactis*. The latter species is clearly the most abundant and widely dispersed native hermatypic species on the platforms, yet its populations showed almost no genetic affinity to each other when compared across the two sides of the Mississippi River mouth. In addition, the populations showed little if any affinity to each other within a transect on a given side. Populations of *T. coccinea*, although exhibiting a similar high degree of separation across the river mouth, showed more recognition between platforms within a transect – suggesting higher dispersal between them.

These results support the findings from our earlier study [25,58,69] - that populations of *Madracis decactis* and other corals are highly isolated on these platforms and that their variation from each other most likely results from Founder Effect (see [99] for a discussion of similar patterns in deep-sea corals). This phenomenon is a by-product of colonization by a small number of larvae – a sample-size effect. That is, the local population resulted from arrival of a small sub-group of larvae from the mother FGB population, carrying with it a genetic identity specific to this sub-group. With time, the population continued to grow in size, possibly experiencing genetic drift, to produce a larger sub-population with a different genetic signature [100,101].

The higher dispersal rates exhibited by *Tubastraea coccinea* may be one of the major reasons this species has been so successful in its distribution throughout the Greater Caribbean region since its introduction 70 yrs ago. Its reproductive, larval dispersal, and recruitment capabilities are high – higher than our dominant native species. Its dispersal capabilities are also better than those of one of the only other successfully introduced coral species – *Fungia scutaria* [41–43]. Indeed, with reproductive characteristics like these (including rapid asexual reproduction and growth), the only reason that this species has not over-run our natural coral reefs is that it apparently does not compete well for space in natural Caribbean systems – only in artificial habitats (breakwaters, platforms, bridge pilings, etc.).

The second inference that may be drawn from these genetic affinity patterns is that some other Indo-Pacific species may be similarly better adapted to reproduce and disperse than our native species, and that all precautions should be taken to eliminate them should they be successfully introduced in our waters. Sammarco et al. [44] recently reported the new introduction of a closely related species – *Tubastrea micranthus* – into the northern GOM in the Grand Isle offshore oil/gas lease area – S-SW of the mouth of the Mississippi River. This area borders on commercial shipping lanes (safety fairways) leading to Port Fourchon, and New Orleans via the Mississippi River. We are currently attempting to determine the extent of the invasion [102]. Nonetheless, a rapid eradication should be considered for such species because of the possibility of success of such an action if taken early [103], the decreasing probability of success of eradication with time [104,105], and major problems that can arise if one waits far too long to proceed with eradication [106,107].
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References

1. Rezk R, Bright TJ, McGrall DW (1983) Reefs and banks of the northwestern Gulf of Mexico: Their geological, biological, and physical dynamics: Final report. US Dept of the Interior, Minerals Management Service, Outer Continental Shelf and Offshore Energy; New Orleans, LA. 139 p.

2. Scarborough-Bull A (1989) Some comparisons between communities beneath offshore platforms and other platforms: Anadarko Petroleum – J. Davidson, S. Huthcock, S. Jensen; Apache Oil – J. Bordelon, G. Thibodaux; Chevron-Texaco Exploration and Production – D. Lucas, K. Mire, S. Ulm; Coastal Oil and Gas, Devon Oil – B. Gary, R. Hebert, V. Miley, B. Moody; Dominion Exploration and Production – B. Ventura, K. Schlogel, M. Sledge; El Paso Energy – C. Thornton, S. Lesker; Forcenergy/Forest Oil – W. Fontenot, W. Myers, G. Ruiz; Kerr-McGee Oil and Gas – C. Bradford; Merit Energy – T. Lambert, P. Mailherber; Newfield Exploration – A. Comeaux, E. Haas, M. Prosper, J. Zernell; Noble Energy – S. Shuman; Offshore Energy; UNOCAL – D. Ruskan, M. Hebert; Samedan Oil – R. Bennis, S. Berryhall, S. Mulhert, T. Spitznegerber, P. Tallos; and W&T Offshore – S. Schroeder; United Space Alliance – A. Knight. We thank J. Benzie for suggesting the use of AFLPs on coral tissue for the molecular genetics.

Author Contributions

Conceived and designed the experiments: PWS DAG. Analyzed the data: PWS DAB. Contributed reagents/materials/analysis tools: PWS DAG. Wrote the paper: PWS DAG. J. Bary, V. Miley, B. Moody;
33. Shearer TL (2008) Range expansion of an introduced coral: Investigating the source and ecological impact of the invasion. 2008 Ocean Sciences Meeting: From the Watershed to the Global Ocean, Orlando, FL (USA), 2–7 Mar 2008.

34. Vermeij MJ (2005) A novel growth strategy allows Tubastrea coccinea to escape small-scale adverse conditions and start over again. Coral Reefs 24: 442–444.

35. Pagad S (2007) A novel growth strategy allows Tubastrea coccinea (coral). Global invasive species database. Invasive species specialist group, IUCN Species Survival Commission. Available: http://www.isg.org/database/species/ecology.asp?si = a1006&fr = 1&rn = 792&kr = FR. Accessed 2011 Dec 19.

36. National Oceanic and Atmospheric Administration. (2008) Flower Garden Banks 2008 condition report. NOAA Flower Garden Banks National Marine Sanctuary, Galveston, TX. http://sanctuaries.noaa.gov/science/condition/ligths/pressrelease.html.

37. Fenner D (1999) New observations on the stony coral (Scleractinia, Milleporidae, and Stylasteridae) species of Belize (Central America) and Cozumel (Mexico). Bull Mar Sci 64: 143–154.

38. Fenner D (2001) Biogeography of three Caribbean corals (Scleractinia) and the invasion of Tubastrea coccinea into the Gulf of Mexico. Bull Mar Sci 69: 1175–1189.

39. Fenner D, Banks K (2004) Orange cup coral Tubastrea coccinea invades Florida and the Flower Garden Banks, northwestern Gulf of Mexico. Coral Reefs 23: 503–507.

40. Humann P, DeLoach N (2002) Reef coral identification: Florida, Caribbean, Bahamas. New World Publ, Jacksonville, Fla.

41. Lujanreese TC, Lee S, Bush S, Bruno, JF (2005) Persistence of non-Caribbean algal symbionts in Indo-Pacific mushroom corals released to Jamaica 35 years ago. Coral Reefs 24: 157–159.

42. Lang JC (1972) pers. comm.

43. Sammarco PW (1973) pers. obs.

44. Sammarco PW, Porter SA, Cairns SD (2010) New invasive coral species for the northern Gulf of Mexico. Bull Mar Sci 74: 175–183.

45. Cairns SD (2006) Revision of the shallow-water azooxanthellate Scleractinia of the western Atlantic. Studies Nat Hist Canibb Reg 75: 1–240.

46. Sammarco, et al. (2011) Submitted.

47. Sammarco PW (2008) Gulf drifting platforms as an environmental asset: Long-term artificial reefs and sites for coral recruitment/Determining the Geographical Distribution, Max, Depth, and Genetic Affinities of Corals on Offshore Platforms, Northern Gulf of Mexico US Dept. Interior, Minerals Management Service, Environmental Section New Orleans, LA, Annual Report.

48. Schnall GP, Hickerson EL (2006) Ecosystem approaches to the identification and characterization of a network of reefs and banks in the northwestern Gulf of Mexico. EOS, Trans. Am. Geophys. Union 87 (36), suppl. 153–158.

49. Harrison PL, Wallace CC (1990) Reproduction, dispersal, and recruitment of scleractinian corals. In: Dubinsky Z, ed, Coral Reef Ecosystems of the World, Vol 25, Elsevier Sci Publ Co, Inc, New York, pp 135–208.

50. McPeek MP (1998) Timing of larval release by Parrot astreus in the northern Florida Keys. Coral Reefs 17: 369–375.

51. Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, Acropora palmata. Molec Ecol 14: 157–170.

52. Sammarco PW, Andrews JC (1989) The Helix experiment: Differential localized dispersal and recruitment patterns in Great Barrier Reef corals. Limnol Oceanog 34: 896–912.

53. Sammarco PW (1994) Larval dispersal and recruitment processes in Great Barrier Reef corals: Analysis and synthesis. In: Sammarco PW, Heron ML, eds, The Bio-Physcs of Marine Larval Dispersal American Geophysical Union, Washington, D.C. pp 55–72.

54. MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, NJ. 209 p.

55. Malhagati F, Becari C, Casu D, Cussi M, Sartor P, et al. (2002) Allygene genetic variation and gene flow in Octopus vulgaris (Cephalopoda, Octopodidae) from the Mediterranean Sea. Bull Mar Sci 71: 473–486.

56. Achion AD (2003) Offshore oil and gas platforms as stepping stones for expansion of coral communities: A molecular genetic analysis. MSc thesis, Dept Oceanography & Coastal Sciences, Louisiana State University, Baton Rouge, LA. 86 p.

57. Achion AD, Sammarco PW, Brazeau DA (2006) Genetic connectivity in corals in the Flower Garden Banks and surrounding oil/gas platforms, Gulf of Mexico. J Exp Mar Biol Ecol 365: 1–12.

58. Achion AD, Sammarco PW, Brazeau DA (2006) Genetic affinities of coral populations between the Flower Garden Banks and oil and gas platforms in the northern Gulf of Mexico: Preliminary data. Proc 10th Intl Coral Reef Symp, Fort Lauderdale, FL, July 2008. Abstract.

59. Brazeau DA, Sammarco PW, Atchison AD (2008) Genetic structure in coral reef recruitment: Evidence of extreme patchiness in settlement. Proc 11th Int Coral Reef Symposium, Fort Lauderdale, FL, July 2008. Abstract.

60. Bernardette L, Duchene (2002) Individual-based genotype analysis in studies of parentage and population assignment: How many loci, how many alleles? In, Fish Aquat Sci 57: 324–337.

61. Duchene P, Bernardette L (2002) AFLPOP: A computer program for and real population assignment using AFLP markers. Mol Ecol Notes 2: 380–383.

62. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–955.

63. Amar K-O, Douek J, Rabinowitz C, Rinkevich B (2008) Employing of the Amplified Fragment Length Polymorphism (AFLP) methodology as an efficient population genetic tool for symbiotic cnidarians. Mar Biotechnol 10: 350–357.

64. Whitting MC, McCauley DE (1999) Direct measures of gene flow and migration: FST doesn't equal 1/(4 Nm). Heredity 82: 117–125.

65. Hellberg M, Burton RS, Neigel J, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. Bull Mar Sci 70: 273–290.

66. Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar EcolProg Ser 393: 1–12.

67. Lowe WH, Allenford IW (2010) What can genetics tell us about population connectivity? Molec Ecol 19: 3038–3051.

68. Adamkewiczi SL, Harasewych MG (1996) Systematics and biogeography of the genus Dnas (Bivalvia: Donacidae) in eastern North America. Am Malacol Bull 13: 97–103.

69. Tunnell JW, Felder DA, Earle SA, eds (2009) The Gulf of Mexico origins, waters, and biota: Biodiversity. Texas A&M University Press, Corpus Christi, TX.

70. Brandle MC, Guiher T, Pyron RA, Wimne CT, Burbirk FT (2010) Does dispersal across an aquatic geographic barrier obscure phylogeographic structure in the diamond-backed watersnake (Nerodia rhombifera)? Molec Ecol Notes 10: 324–326.

71. Aronson RB, ed (2007) Geological approaches to coral reef ecology. Ecol Studies 192. Springer, New York.

72. Olausmoja MJ (2010) Isolation on the West Florida Shelf with implications for red tides and pollutant dispersal in the Gulf of Mexico. Nonlinear Processes Geophys 17: 693–696. Available: http://www.nonlin-processes-geophys.net/17/693/2010/npg-17-693-2010.pdf. Accessed 2011 Dec 19.

73. Pyron RA, Burbirk FT (2010) Hard and soft allopatry: Physically and ecologically mediated modes of geographic speciation. J Biogeogr 37: 2005–2015. 85 p.

74. Esler AZ, Lugo-Fernandez A (2007) Influence of terrigenous runoff on offshore coral reefs. In, Aronson RB (Editor), Geological approaches to coral reef ecology, Ecol Studies 192. Springer, New York, pp 126–160.

75. Vermeij MJ, Fogarty ND, Miller MW (2006) Pelagic conditions affect larval survival, growth, and settlement patterns of the Caribbean coral Montastraea faveolata. Mar Ecol Prog Ser 310: 119–128.

76. Edmundson CH (1941) Behavior of coral planulae under altered saline and thermal conditions. Oec Pap Bishop Mus 10: 283–304.

77. Hodgson G (1998) Sedimentary processes: A modern and the settlement of larvae of the reef coral Pocillopora damicornis. Coral Reefs 7: 41–43.

78. Basham KM, Sammarco PW, Snell T (2002) Effects of temperature on fertilization success and embryogenesis in Diphylia intermedia (Coelenterata, Scleractinia). Mar Biol 141: 479–489.

79. Basham KM, Sammarco PW (2003) Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral. Mar Biol 142: 241–252.
92. Lugo-Fernandez A (2006) Travel times of passive drifters from the western Caribbean to the Gulf of Mexico and Florida Bahamas. Gulf Mex Sci 2006: 61–67.

93. Lugo-Fernandez A, Gravois M (2010) Understanding impacts of tropical storms and hurricanes on submerged bank reefs and coral communities in the northwestern Gulf of Mexico. Cont Shelf Res 30: 1226–1240.

94. Rahalais NN, Turner RE, Justic D, Dortch Q, Wiseman WJ (1996) Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf Estuar Coasts 19: 386–407.

95. Dagg MJ, Breed GA (2003) Biological effects of Mississippi River nitrogen on the northern Gulf of Mexico – A review and synthesis. J Mar Syst 43: 133–152.

96. Lohrenz SE, Redalje DG, Cai W-J, Acker J, Dagg M (2008) A retrospective analysis of nutrients and phytoplankton productivity in the Mississippi River plume. Cont Shelf Res 28: 1466–1475, Available: http://www.sciencedirect.com/science/article/pii/S0278434308001271 Accessed 2011 Dec 19.

97. Dagg MJ, Bianchi TS, McKee BA, Powell R (2008) Fates of dissolved and particulate materials from the Mississippi River immediately after discharge into the northern Gulf of Mexico, USA during a period of low wind-stress. Cont Shelf Res 28: 1127–1137.

98. Oey L-Y, Ezer T, Lee H-C (2005) Loop current, rings, and related circulation in the Gulf of Mexico: A review of numerical models and future challenges. In, ed. Sturges W and Lugo-Fernandez A, eds, Circulation in the Gulf of Mexico: Observations and models, Geophys Monograph Ser 161, Am Geophys Union, Washington, D. C.

99. Hellberg ME (2007) Footprints on water: The genetic wake of dispersal among reefs. Coral Reefs 26: 463–473.

100. Futuyma DJ (1983) Evolutionary Biology. Sinauer Assocs, Inc, Sunderland MA, 763+ p.

101. Mettler LE, Gregg TG, Schaffer H (1988) Population genetics and evolution (2nd ed.), Prentice Hall, Upper Saddle River, New Jersey. 235 p.

102. Sammarco, et al. (2011) work in progress.

103. Fitzhugh K, Roue GW (1999) A remarkable new genus and species of fanworm (Polychaeta; Sabellidae; Sabellinae) associated with marine gastropods. Invert Biol 118: 357–390.

104. Simberloff D (2000) No reserve is an island. Marine reserves and non-indigenous species. Bull Mar Sci 66: 567–580.

105. Hewitt CL, Campbell ML, McEnnulty F, Moore KM, Murfet NB, et al. (2005) Efficacy of physical removal of a marine pest: The introduced kelp Undaria pinnatifida in a Tasmanian marine reserve. Biol Invas 7: 251–263, Available: doi:10.1007/s10530-004-0739-y http://www.mendeley.com/research/efficacy-of-physical-removal-of-a-marine-pest-the-introduced-kelp-undaria-pinnatifida-in-a-tasmanian-marine-reserve/ Accessed 2011 Dec 19.

106. Bergstrom DM, Lucieer A, Kidder K, Wasley J, Behlin L, et al. (2009) Indirect effects of invasive species removal devastate World Heritage Island. J Appl Ecol 46: 73–81, Available: doi:10.1111/j.1365–2664.2008.01601.x http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2008.01601.x/abstract Accessed 2011 Dec 19.

107. Casey M (2009) Species eradication backfires big time. CBS News, Jan. 13, 2009, Available: http://www.cbsnews.com/stories/2009/01/13/tech/mainl719190.shtml Accessed 2011 Dec 19.