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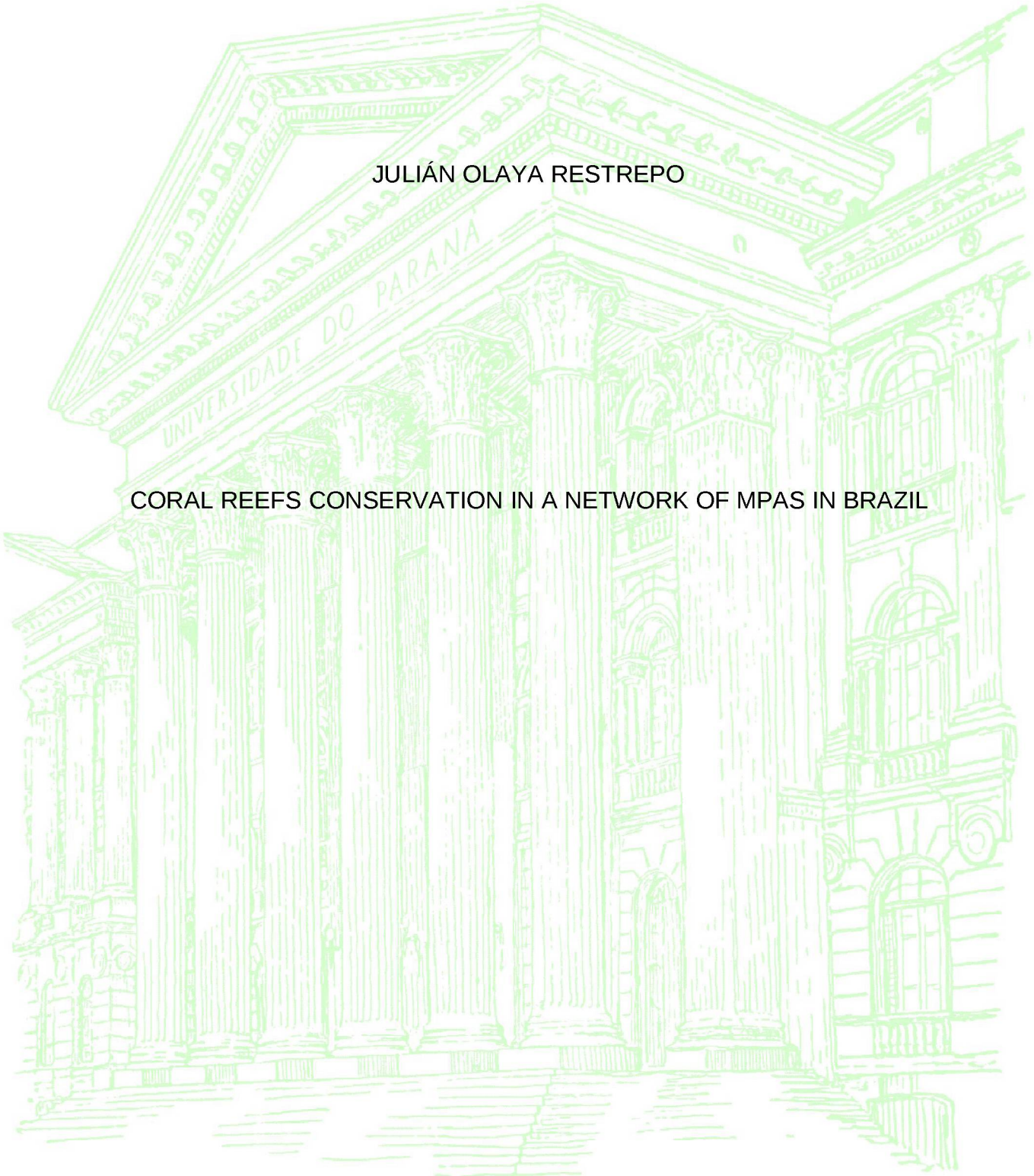

JULIÁN OLAYA RESTREPO

CORAL REEFS CONSERVATION IN A NETWORK OF MPAS IN BRAZIL

CURITIBA
2020

JULIÁN OLAYA RESTREPO

CORAL REEFS CONSERVATION IN A NETWORK OF MPAS IN BRAZIL

Tese apresentada ao Programa de Pós Graduação em Zoologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Zoologia

Orientador: Prof. Dr. Marcos Soares Barbeitos

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... para que tu Tomás, hijo mio, puedas ver y disfrutar de las bellezas naturales que todavía se pueden encontrar!

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“I can mention many moments that were unforgettable and revelatory. But the most single revelatory three minutes was the first time I put on scuba gear and dived on a coral reef. It's just the unbelievable fact that you can move in three dimensions”.

“If I can bicycle, I bicycle”.

David Attenborough

RESUMO

Os corais escleractíneos são os principais construtores dos recifes atuais e um dos ecossistemas mais ameaçados do mundo. No Brasil, eles são caracterizados por baixa riqueza, alto endemismo e grandes descontinuidades ao longo da costa. Atualmente, os recifes de coral estão ameaçados pelo branqueamento, sobrepesca, poluição, eliminação de resíduos, desenvolvimento costeiro, sedimentação, espécies exóticas, doenças, entre outros fatores. Uma forma eficaz de conservar a biodiversidade é a partir da implementação de áreas marinhas protegidas (AMPs). Estas áreas são espaços geográficos claramente definidos e geridos por meios legais, para alcançar os objetivos de conservação a longo prazo. Quando bem manejadas, as AMPs ajudam a manter a saúde do ecossistema, ao mesmo tempo em que asseguram o desenvolvimento social e econômico. Individualmente AMPs se beneficiam do trabalho em rede através de vínculos ecológicos e sociais, aumentando a conservação e a eficiência administrativa. As redes de áreas protegidas são sistemas compostos por uma série de AMPs individuais que estão cooperativamente conectadas e interagindo em vários níveis espaciais para alcançar os objetivos de conservação. Entretanto, para otimizar os benefícios das redes de AMPs, é importante entender que as áreas são sistemas sócio-ecológicos, sendo a resiliência o produto da integração entre três tipos de redes: 1) a rede ecológica, formada por assegurar ligações naturais entre e dentro das áreas; 2) a rede baseada na gestão, compreende um conjunto de AMPs que, trabalhando em conjunto, aumentam a eficácia da administração; e 3) a rede social, definida pelas interações entre a gestão das AMPs e os atores da sociedade civil, assegurando a representatividade efetiva da sociedade. Dessa forma, o sucesso dos seus objetivos de conservação dependem não só das suas características particulares, mas também das interações biológicas e administrativas com outras AMPs. Observar o sistema de AMPs a partir de uma perspectiva de rede permite-nos identificar atores relevantes e descrever os padrões das suas interações, fornecendo um modelo conceitual com uma forte base teórica. Aqui, utilizamos a teoria dos gráficos para descrever a topologia das redes que compõem o tripé sobre o qual se baseia a conservação das AMP. O objetivo principal foi aplicar a teoria dos gráficos e a análise das redes a um conjunto selecionado de AMPs no Brasil (dos níveis federal, estadual e municipal), com ênfase na conservação dos recifes de coral. Queríamos saber se os níveis de conectividade entre essas reservas correspondem a uma maior extensão ao apoio extrínseco (proveniente de grupos da sociedade civil, organizações governamentais e não governamentais, universidades, etc.) ou a interações recíprocas (cooperação entre AMPs). Para tratar destes assuntos, este documento foi dividido em três capítulos que facilitam a ordem sequencial do projeto. No primeiro capítulo, o objetivo foi desenvolver marcadores nucleares com altas taxas de evolução - polimorfismos uninucleotídeos (SNP) - adequados para avaliar a conectividade entre as espécies de cnidários, proporcionando uma ferramenta útil para avançar no entendimento dos vínculos ecológicos entre as AMPs brasileiras. É apresentado um protocolo otimizado de ddRADseq. No segundo capítulo, a rede baseada na gestão foi avaliada através dos padrões de interação e colaboração entre as AMP. Uma especial ênfase foi dada ao comportamento daquelas localizadas nas 18 áreas focais para desenvolver ações prioritárias para a conservação dos recifes de coral. Dados sobre 8 tipos de interações

(compartilhamento de equipamentos entre AMPs, participação recíproca em conselhos de gestão, apoio de pessoal, participação conjunta em atividades de treinamento formal, resolução de conflitos e desenvolvimento conjunto de programas de vigilância, pesquisa e monitoramento) entre AMPs foram coletados por meio de questionários online e tratados como camadas separadas em uma abordagem de análise de rede em multi-camadas. Por fim, o terceiro capítulo descreve o uso de uma abordagem multi-camadas para investigar as propriedades topológicas da rede de base social das AMPs. A coordenação e cooperação entre AMPs e atores interessados foram transformadas em laços relacionais com o objetivo de verificar se a rede de AMPs brasileiras tem sido eficaz para garantir a participação da sociedade civil no processo de tomada de decisões. De modo geral, detectamos níveis surpreendentes de conectividade dentro da rede de gestão das AMPs brasileiras, uma vez que elas não foram originalmente projetadas para apoiar a coordenação da gestão. Entretanto, também há muito espaço para aumentar a frequência e a relevância de outros tipos de interação entre a sociedade civil e a gestão das AMPs.

ABSTRACT

Scleractinian corals are the main builders of current reefs and one of the most threatened ecosystems in the world. In Brazil, they are characterized by low richness, high endemism and large discontinuities along the coast. Currently, coral reefs are threatened by bleaching, over-fishing, pollution, waste disposal, coastal development, sedimentation, exotic species, and diseases, among others. One effective way to conserve biodiversity is the implementation of marine protected areas (MPAs), that are clearly defined geographic spaces managed through legal means, to achieve long-term conservation goals. These areas, when well managed, help maintain ecosystem health while assuring social and economic development. Individual MPAs benefit from networking through ecological and social links, increasing conservation and administrative efficiency. Networks of protected areas are systems composed of a series of individual MPAs that are cooperatively connected and interacting at various spatial levels to achieve conservation objectives. However, to optimize the benefits of MPAs networks, it is important to understand that MPAs are socio-ecological systems, and resilience is the product of integration among three types of networks: 1) the ecological network formed by ensuring natural connections between and within sites; 2) the management-based network, comprises a collection of MPAs that working together enhance management effectiveness, and 3) the social network, defined by the interactions between the MPA's administration and civil society actors, ensure effective representativeness of society. Then, the successful achievements of its conservation goals depend not only on their particular characteristics but also on biological and administrative interactions with other MPAs. Looking at the system of MPAs from a network perspective, allow us to identify relevant actors and describe the patterns of their interactions, providing a conceptual model with a strong theoretical basis. Here, we used graph theory to describe the topology of the networks that make up the tripod upon which MPA conservation stands. The main objective was to apply graph theory and network analysis to a selected set of MPAs in Brazil (from the federal, state and municipal level), with special emphasis on the conservation of coral reefs. We wanted to know whether the levels of connectivity between these reserves correspond to a greater extend to extrinsic support (coming from civil society groups, governmental and non-governmental organizations, universities, etc.) or to reciprocal interactions (cooperation between MPAs). To address these, the proposal was divided into three chapters that facilitate the sequential order of the project. In the first chapter, the objective was to develop nuclear markers with high evolution rates - single-nucleotide polymorphisms (SNP) - suitable to evaluate connectivity among cnidarian species, providing a useful tool to advance in the understanding of the ecological linkages among Brazilian MPAs. An optimized ddRADseq protocol is presented. In the second chapter, the management-based network was evaluated through the patterns of interactions and collaboration among MPAs, making special emphasis on the behavior of those located in the 18 focal areas identified to develop priority actions for coral reefs conservation. Data on 8 types of interactions (equipment sharing among MPAs, reciprocal participation in management councils, staff support, joint participation in formal training activities, conflict resolution, and joint development of surveillance, research, and monitoring programs) among MPAs were collected through online questionnaires and

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Chapter 1

Optimization of a ddRADseq protocol for scleractinian corals and their validation in the characterization of genetic diversity and connectivity in the Brazilian coast

ABSTRACT

Researchers interested in answering questions on ecology, evolution or conservation of wild populations who wish to enter the field of population genetics need good quality genomic sequences, which allow the correct identification of suitable genetic markers to study processes such as mutation, mating patterns, gene flow and natural selection. Hence, the quality of the libraries sent for sequencing and, therefore, the results obtained, depends on a wide variety of theoretical and technical factors that, if carefully analyzed, can save time and money. This work presents a series of tests designed to optimize the ddRAD method to be used with cnidaria species for sequencing on the Illumina platform. This procedure uses two distinct restriction enzymes to fragment the genome, followed by the sequencing of fragments of known size. The technique allows the identification of hundreds and even thousands of single nucleotide polymorphisms (SNPs) that can be used as molecular markers in the study of patterns of genetic variability between populations. The tissue of 146 individuals belonging to 2 species of Cnidaria was collected in 5 marine protected areas (MPAs) along the Brazilian coast, hoping to simultaneously detect thousands of SNPs, which allow to evaluate the patterns of genetic variation of populations. Some of the most important factors affecting the quality of libraries were: 1) obtaining good quality DNA; 2) the appropriate selection of restriction enzymes for each taxonomic group, allowing to determine a good cost-benefit ratio between the sequencing price and the number of SNPs obtained; 3) a precise selection of the sizes of the fragments that will be sequenced, seeking to produce a uniform distribution around an average size (between 300 bp to 600 bp) suitable for sequencing on the Illumina HiSeq 2500 platform; 4) simultaneously incorporate a two-level combinatorial indexing scheme, so that each read can be identified and classified using bioinformatics; 5) an adequate number of PCR amplification cycles. The ddRAD method significantly reduces the economic cost, necessary biological material and time invested by the researchers. Therefore, it is hoped that the information presented here will make this method accessible to a wide range of biological questions and taxonomic groups.

Keywords: Illumina library. Sequencing. Bench protocol. SNP.

RESUMO

Pesquisadores interessados em responder perguntas em ecologia, evolução ou conservação de populações silvestres que desejam entrar no campo da genética de populações precisam de sequências genômicas de boa qualidade, que permitam a correta identificação de marcadores genéticos adequados para estudar processos como mutação, padrões de acasalamento, fluxo gênico e seleção natural. Porém, a qualidade das bibliotecas enviadas para sequenciamento e, portanto, dos resultados obtidos, depende de uma ampla variedade de fatores teóricos e técnicos que, se cuidadosamente analisados, podem economizar tempo e dinheiro. Este trabalho apresenta uma série de testes projetados para otimizar o método ddRAD e seu uso com espécies de cnidaria para sequenciamento na plataforma Illumina. Esse procedimento utiliza duas enzimas de restrição distintas para fragmentar o genoma, seguido do sequenciamento de fragmentos de tamanho conhecido. A técnica permite a identificação de centenas e até milhares de polimorfismos de nucleotídeo único (da sigla em inglês SNPs) que podem ser utilizados como marcadores moleculares no estudo dos padrões de variabilidade genética entre populações. O tecido de 146 indivíduos pertencentes a 2 espécies de Cnidários foi coletado em 5 localidades ao longo da costa brasileira, esperando detectar simultaneamente milhares de SNPs, que permitem avaliar os padrões de variação genética das populações. Alguns dos fatores mais importantes afetando a qualidade das bibliotecas foram: 1) a obtenção de DNA de boa qualidade; 2) a seleção adequada das enzimas de restrição para cada grupo taxonômico, permitindo determinar o número de leituras obtidas durante o sequenciamento é crítica para obter uma boa relação custo-benefício entre o preço de sequenciamento e o número de SNPs obtidos; 3) uma seleção precisa dos tamanhos dos fragmentos que serão sequenciados, procurando produzir uma distribuição uniforme em torno de um tamanho médio (entre 300 pb a 600 pb) apropriado para sequenciar em plataforma Illumina HiSeq 2500; 4) incorporar simultaneamente um esquema de indexação combinatório de dois níveis, para que cada leitura possa ser identificada e classificada antes da análise final dos dados e; 5) um número adequado de ciclos de amplificação por PCR. O método ddRAD reduz significativamente o custo econômico, material biológico necessário e tempo investido pelos pesquisadores. Por tanto, espera-se que as informações apresentadas aqui tornarem este método acessível a uma ampla série de perguntas biológicas e grupos taxonômicos.

Palavras-chave: Biblioteca Illumina. Sequenciamento. Protocolo de bancada. SNP

INTRODUCTION

The Brazilian coral fauna is characterized by low species richness and high endemism, with most of the species presenting affinities with Caribbean counterparts and related to a Tertiary coral fauna (Leão et al., 2003). The distribution of coral species in Brazil is fragmented and responds to geomorphological and environmental factors like turbidity and hydrodynamics (Correia, 2011). Several coastal rivers found along the coast lessen habitat availability for corals by reducing salinity and increasing turbidity, probably acting as drivers for their fragmented distribution.

In general, all natural populations exhibit some degree of genetic structure due to a variety of non-exclusive agents like environmental barriers and historical processes (Truelove et al., 2017; Planes and Fauvelot, 2002). This genetic subdivision reflects the gene flow between populations and has important consequences in the genetic component of individuals. Additionally, from an ecosystem point of view, it was suggested that coral reefs that exhibit high levels of demographic connectivity tend to show higher levels of diversity and resilience against environmental perturbations and can persist for wider spatial and temporal scales (Bellwood et al., 2004, Engelhard et al.,2017). Understanding how natural populations are genetically structured allows us to make inferences about some ecological determinants of variation that are important for the management and conservation of wild populations (Montalvo et al., 1997). Additionally, the identification of barriers affecting connectivity is essential in the delimitation of MPA networks with coherent biological justifications (Hedgecock et al., 2007; Lukoschek et al., 2016).

Most of cnidarians species have a complex life cycle involving at least two life stages -a free-swimming and a sedentary or sessile phase- that makes species to respond distinctly to ecological processes, and largely determine dispersal potential of the species, producing an asymmetric probability of connectivity (Magris et al., 2017). However, there is already compelling evidence showing that larval retention near local populations may be also of great importance (Cowen et al., 2000), and that correlation between geographic distance and genetic structuring is not always present (Neves et al., 2008). Those results presume that the scales of larval dispersal and connectivity

in cnidarians are often species-specific and almost always location-specific, owing to the great variety of factors involved in larval transportation (Coelho and Lasker, 2016).

MPAs that are suitable for one species or group of species may be inappropriate for other species because they have different requirements in terms of area and may exhibit different movement and dispersion abilities requiring different management measures (Palumbi, 2004). Additionally, the spatial and temporal scales of the biological processes that are intended to be protected rarely coincide with administrative boundaries, making it difficult to implement truly effective conservation actions. For that reason, the establishment of networks with coherent biological justification instead of isolated entities in the space is a major challenge (Steinberg et al., 2016). Well-designed networks of MPAs may be more efficient in maintaining ecosystem processes and connectivity (Lowry et al., 2009) while decreasing the administrative burden associated with the management of large reserves.

Porites astreoides is found in the Caribbean and Brazil and is considered to have a “secondary role” in the construction of Brazilian reefs, specially in the northeaster and eastern regions (Leão et al.,2003). This zooxanthellate brooder species has hermaphroditic and gonochoric colonies (Chornesky and Peters, 1987), but gonochoric male colonies are rarely found (Chornesky and Peters, 1987; Pires and Caparelli, 2002). The reproductive cycle occurs with both seasonal and lunar periodicity with overlapping months among different regions. For example it goes from October to February with the occurrence of several planulation events (Pires and Caparelli, 2002) and coincides with a rise in sea surface water temperature. In Jamaica, it was suggested that male gametes are spawned prior to the full moon and larvae are release near to the new moon (Chornesky and Peters, 1987). Within hermaphroditic colonies, individual polyps could be either male, female or hermaphroditic. Individual polyp fecundity vary widely among colonies, and the duration of the reproductive period of individual corals may vary also withing populations. Its distribution in Brazil encompasses the Atol das Rocas (03 52’ S - 33 49´´ W), Fernando de Noronha (3 51´ 13.71´´ S - 32 25´ 25.63´´ W) and Abrolhos archipelagoes (17 55´ 34´´ S - 38 56´ 07´´ W), and its continental distribution goes from the Cabo São Roque (5 29´ 00´´ S - 35 16´ 00´´ W) to the south of the Bahia state (17 43´ 55´´ S - 39 15´ 57.6´´ W), with the exception of Sergipe state, were no

corals have been registered (Amaral et al.,2009; Capel, 2012; Capel et al., 2011; Leão et al., 2003; Mayal et al., 2009; Pires et al., 1992; Pires and Caparelli, 2002; Souza, 2012) (Figure 1 and Figure 3).

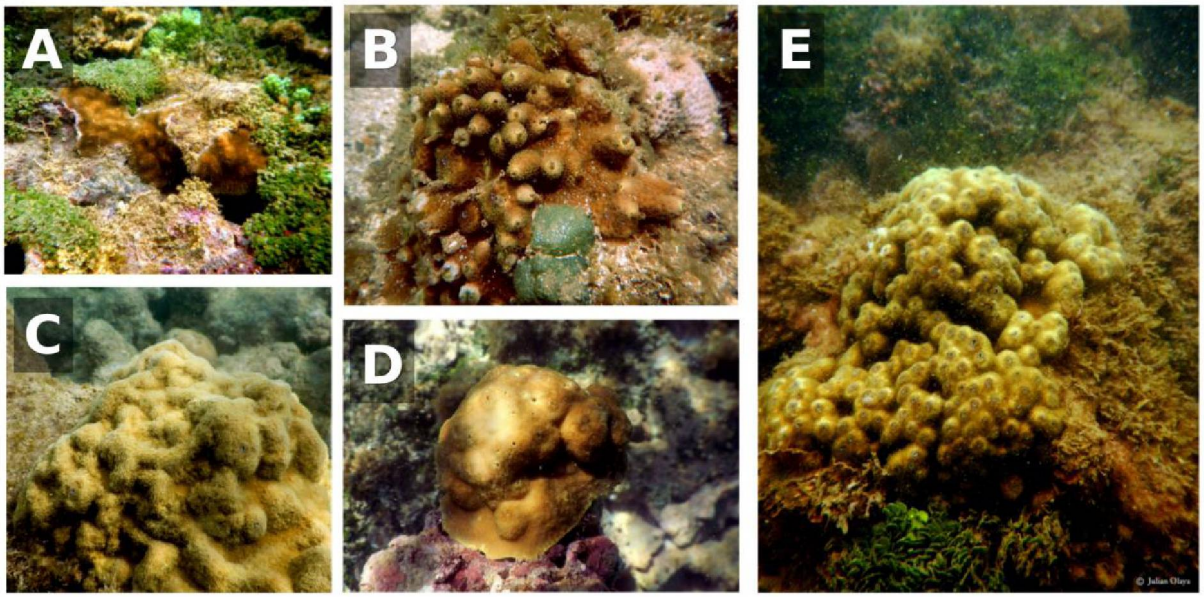

Figure 1 *Porites astreoides* from 4 different environmental protection area (APAs) along the Brazilian coast: A and E=APA das Ilhas de Tinharé and Boipeba, in Moreré beach; B= APA das Ilhas de Tinharé and Boipeba, Tassimirim; C=APA Recifes de Corais, Parrachos de Maracajaú; D= APA Costa dos Corais, Tamandaré..

Millepora alcicornis is one of the 19 species comprising the genus *Millepora*, included in the Class Hydrozoa; 12 of those species are distributed in the Indo-Pacific region and 7 in the Atlantic, including 3 endemic species from Brazil (Ruiz-Ramos et al., 2014; Souza et al., 2017). *M. alcicornis* colonies reproduce largely by fragmentation, and in Brazilian waters, due to their fast growing capacity, they assume the role that Acroporids species have on the Caribbean (Leão et al., 2003), generating a three-dimensional complexity on reefs and supporting critical ecological functions for other reef associated species from several taxa (Amaral et al., 2002; Garcia et al., 2009; Drury et al., 2016). Milleporids are easily found in coral reefs where they can occur in colonies up to 2 m in diameter. In Brazil, the species is known to be important reef builders, with colonies between 2 to 3 m in diameter, limited to a maximum depth of 30 m (Figure 2) (Amaral et al., 2002). Recent phylogenetic studies in the Pacific and Atlantic oceans (based on ITS-rDNA region) did no recover the same species previously delineated by morphological characters, suggesting that a

taxonomic revision of the genus is needed (Ruiz-Ramos et al., 2014; Takama et al., 2018). In Brazil, the species has been registered from Cabo Frio (22 52´ 44´´ S - 42 01´ 08´´ W) to Parcel do Manuel Luis (0 54´ 48´´ S - 44 19´ 19.10´´ W), including Guarapari, Abrolhos Islands, Maceió, Japaratinga, Tamandaré, Joao Pessoa, and Atol das Rocas (Amaral et al., 2002) (Figure 3).

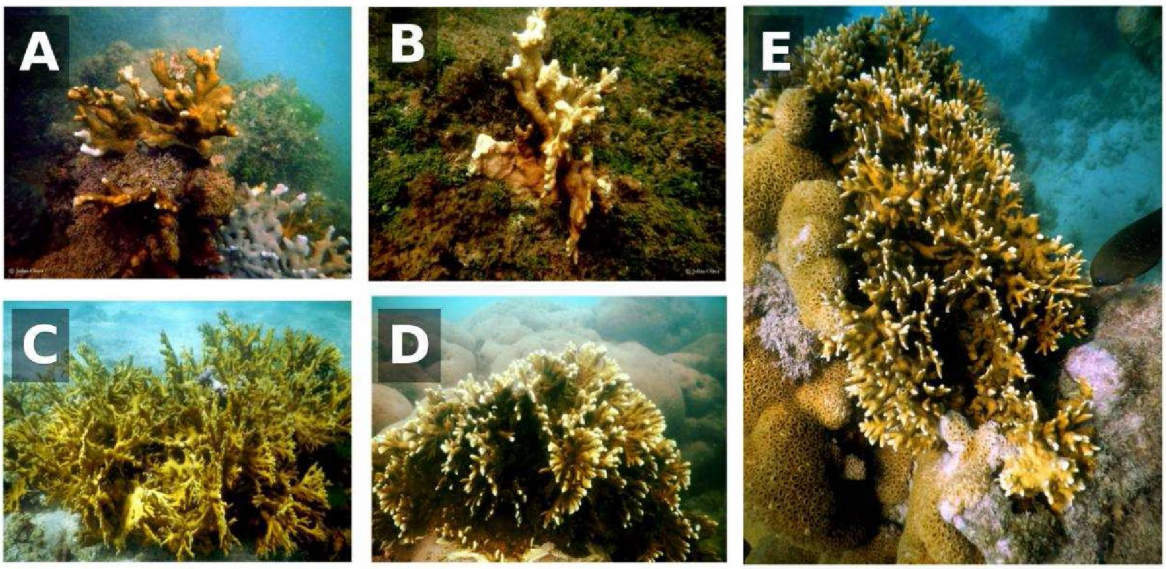

Figure 2 *Millepora alcicornis* from: A and B= APA das Ilhas de Tinharé e Boipeba, in Moreré beach; C= APA Costa dos Corais, Tamandaré; D= Parque Natural Municipal dos Corais de Armacao dos Búzios, in Tartaruga beach; E= APA Recifes de Corais, in Parrachos de Maracajaú.

In Brazil, studies addressing population genetics and connectivity in corals showed contrasting patterns of genetic structure among species. Souza et al. (2017) presented evidence of high levels of connectivity inside the Brazilian province for *M. alcicornis* despite significant genetic structuring among four biogeographic provinces - Caribbean, Brazilian, Ascención Island and Tropical Eastern Atlantic. This inter-regional differentiation was also observed in other 6 species of scleractinian corals (Nunes et al., 2011), except for *P. astreoides,* for which no structuring between Caribbean and Brazilian populations was observed. Within the Brazilian province, the results were also contrasting, with limited gene flow estimated for two brooding gonochoric species - *Favia fragrum* and *Siderastrea radians* -, and high gene flow among populations of the broadcasting hermaphroditic species *Montastrea cavernosa* and *S. siderea* (Nunes et al., 2011). These results led the authors to suggest that gene flow is in part determined by the reproductive strategies of species. However,

those results were obtained using few (and for *P. astreoides* only one) nuclear loci, and the same authors highlighted the need to evaluate regional connectivity using more genetic markers with higher evolutionary rates. The present study aims to optimize a protocol to develop nuclear markers with high evolution rates -single nucleotide polimorphisms (SNP)- suitable to answer phylogenetic questions and evaluate connectivity in two cnidarian species, *P. astreoides* and *M. alcicornis,* along the Brazilian coast.

Single Nucleotide Polymorphisms (SNPs) are the most abundant molecular markers in most genomes (Baird et al., 2008, Morin et al., 2009), and provides high resolution to population genetics and phylogenetic studies (Maroso et al., 2016, Loureiroet al., 2020). Although several methods for SNP discovery has been proposed (Vignal et al., 2002), the restriction site associated methods, known as RADseq, have shown to be an effective approach for the identification of thousands of SNPs at relatively low cost (Cariou et al., 2013). The RADseq family of methods share some basic steps, like starting with high-molecular-weight DNA, the use of digestion enzymes to fragment the DNA, and the need to add specific adapters for sequencing and barcode individual samples (Andrews et al., 2016). However, thanks to its flexibility several adaptations and modifications from the original protocol have been proposed (Baird et al., 2008). One of those methods is the Double Digest Restriction Associated DNA (ddRADseq) initially proposed by Petersen and colleagues in 2012. This technique provides a sub-sample of the genome generated by the action of two restriction enzymes and reduces the final cost and DNA inputs during library preparation when compared with other RADseq methods that use random shearing to fragment the genome. ddRADseq has the advantage of not requiring previous knowledge of the target genome (DaCosta and Sorenson, 2016), as it is the case of the two species object of this study. Additionally, the possibility to perform *in silico* digest to test enzymatic performance of several commercially available restriction enzymes, increases specificity and efficiency in capture a broad range of the target genome and the thousands of SNPs used as genetic markers (Etter et al., 2011).

This method has been used in a wide set of taxonomic groups like plants (Nazareno et al., 2017; Sakaguchi et al., 2017), fishes (Recknagel et al., 2015; Roman et al., 2018; Maroso et al., 2018), birds (Shultz et al., 2016), insects (Kozlov et

al., 2017; Macher et al., 2015), mollusca (Kess et al., 2016; Combosh et al., 2017), and corals (Devlin-Durante and Baums, 2017; Dimond et al., 2017). However, one flaw of RADSeq methods is the need to be adapted for each organism since they are species-specific (Baird et al., 2008), and adaptations during library preparation may largely affect the performance of the method.

In this section is presented an optimized ddRADseq protocol to efficiently identify SNPs in Scleractinian species. The data that can be latter used in population genetics analyses and phylogeographic studies. The main innovation in this protocol is the selection of the two enzyme pairs that produced a biased number of host (coral) fragments relative to endosymbiont genomes, increasing the chances to effectively sequence higher number of coral loci with better cost-benefit ratio, the design of specific primers to test success during the intermediate steps of adapter ligation and sequencing primers incorporation (i5 and i7), and optimized PCR reactions to effectively incorporate those specific primers and barcodes.

MATERIALS AND METHODS

Sample collection

Coral tissue from colonies of the species *M. alcicornis* and *P. astreoides* were collected between July 2016 and July 2017 in 5 MPAs along the Brazilian coast (Table 1). Additionally, few individuals of *M. braziliensis* and *P. branneri* were collected to be used as outgroups in future morphological and phylogeographic analysis. Colonies were randomly sampled by free diving and scuba diving, always making sure that collected colonies were separated by several meters to avoid sampling of clonal fragments. Immediately after sampling, the live tissue was preserved in a hyper-saturated DESS solution (DMSO, EDTA, and NaCl) renewed approximately every 6 hours to remove the mucus secreted by corals until samples were stored at -10 °C in lab conditions at the Federal University of Paraná (UFPR). Collections were made under the permanent license for the collection of zoological material No. 410011, issued by *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio).

The skeleton of sampled colonies was cleaned with a solution of sodium hypochlorite and dried for 15 hours before deposited in the collection of the Department of Zoology in the UFPR (Perez, 2018). Some colonies of *Porites* were used to implement a texture-based analysis of coral surfaces for species delimitation (work In prep). For that purpose, 15 colonies of *P. astreoides* and 12 of *P. branneri* were photographed in the Center for Electron Microscopy (CME) of the UFPR. Images were taken via scanning electron microscopy (JEOL JSM 6360-LV) with magnification of 1 mm (11x) covering more than one corallite, and with variable magnification in 500 µm (20x range) for individual corallites. Were taken 3 replicates per colony and scale (6 images per colony), totaling 162 images.

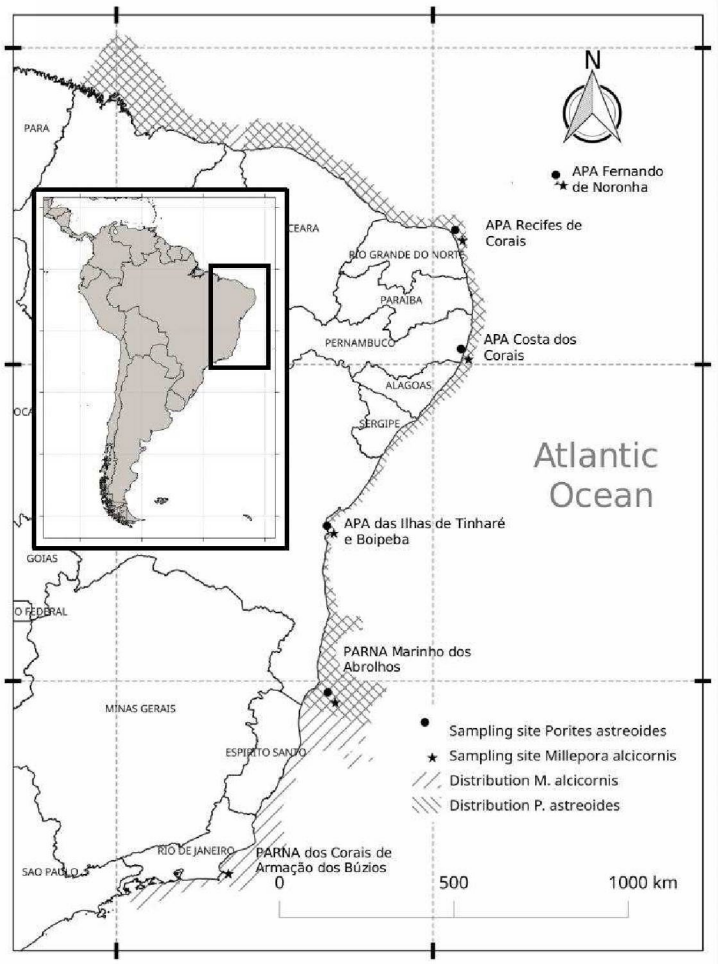

Figure 3 Map of the study area showing sampling sites and distribution of species along the Brazilian coast. The data on the distribution of species were obtained from IUCN red list (https://www.iucnredlist.org/)

Optimized ddRAD library preparation protocol

Selection of restriction enzymes

One critical step while performing ddRADseq methods is to decide the number of loci and individuals needed to achieve sufficient statistical power, meaning how many libraries and sequencing runs to perform in order to get a cost effective genotyping method. The selection of restriction enzymes determine the number of reads obtained during sequencing (Burns et al., 2017), and is critical for obtaining a good cost-benefit ratio between the sequencing price and the number of SNPs obtained. Lepais and Weir (2014), proposed that the ideal scenario is to find approximately 50.000 loci (of the desired size of approximately 150 pb) that would need 1.5 million reads per individual with depth coverage of 30X in an Illumina HiSeq 2500 run. Having that

scenario as a starting point were surveyed several commercially available restriction enzymes, seeking the pairs that yield between 10.000 to 50.000 fragments with an average size of <250 pb appropriate for Illumina library preparation, through the *in silico DNA* digestion of different cnidarian and symbiodinium species with a reference genome available.

Several commercially available restriction enzymes were initially classified according to the number of cutting sites along the genomes of the stony coral *Acropora digitifera* (~ 448 Mbp - Shinzato et al., 2011), and the hydrozoan *Hydra magnipapillata* (~ 852 Mbp - Chapman et al., 2010) employing a custom wrapper package (*RADSeq*), built around BioPerl's *Bio::Restriction* module (Stajich et al., 2002). As rare cutters were labeled those endonucleases that had between 2,500 and 500,000 restriction sites across the genome and as fast cutters those enzymes producing fragments with average sizes between 10 and 850 bp. Therefore, each enzyme was double classified targeting the genomes of the two cnidarian species. Then, was conducted an *in silico* ddRAD for all pairs of rare and fast cutters enzymes for the *A. digitifera* and *H. magnipapillata* genomes. Because the primary goal was to find enzyme pairs that would produce a biased number of host fragments with respect to endosymbiont genomes, were also digested the genomes of three symbiodinium species: *Symbiodinium minutum* (~610 Mbp, Shoguchi et al., 2013), *S. microadriaticum* (assembly ASM193914v1, ~808 Mbp) and *S. kawagutii* (assembly SymbB1_1.0, ~1180 Mbp - Aranda et al., 2016). For the final selection of the pair of restriction enzymes to use in the double digestion was taken into account, beyond the *in silico* results, the reaction condition (type of buffer, recommended temperature reaction), the possible undesired loss of adapters due to chimeric formation between complementary restriction cut sites, and the economic cost of the enzymes. Because this analysis was designed and implemented by professor Marcos Barbeitos, and will be published in a separate work, detailed analysis and results are not shown here.

DNA Extraction

DNA was extracted using two different methods: a commercial kit (Purelink genomic DNA Kit – Invitrogen) and Sera-Mag™ SpeedBeads (GE Healthcare). Initially, all samples were extracted using the commercial kit following manufactures protocol. DNA samples were then classified as "good", "med", or "low" quality

according to: 1) a clear band observed in 1.5 % agarose gel, indicating no degradation of DNA; 2) the purity ratios 260/280 and 260/230 measured with NanoDrop; and 3) DNA concentration quantified with Qubit (≥1.5 ng/µl). Those samples that do not obtain good quality DNA were re-extracted using SpeedBeads following manufacturer recommendations as follow: coral tissue was placed in a new 1.5 ml tube and filled with TE (10 mM Tris/1mM EDTA) until covering it completely and left it on the refrigerator for 2 h. Then, samples were centrifuged at 12.000 g for 5 min, and the supernatant discarded. We added 180 µl of digestion buffer to the samples and left them incubating in a water bath previously adjusted to 37 °C for 45 min. After that, 25 µL of Proteinase K was added and the solution mixed by pipetting up and down a few times before left it overnight at 37 °C. Then, samples were centrifuged at 12.000 g for 5 min and the supernatant transferred to a new 1.5 ml tube to proceed with the extraction with the SpeedBeads. The volume of SpeedBeads added was 2.7X proportional to the sample volume. The solution was briefly vortexed and incubated for 10 minutes at room temperature. The binding mixture was placed in the magnetic rack until the solution was clear. The supernatant (that contained unwanted biological and chemical elements) was aspirated and discarded without disturbing the bead pellet. Then, the beads were washed with the tube kept in the rack adding approximately 500 µl of freshly prepared 80% ethanol, or enough to cover the beads, and left it incubating for 1 min. The ethanol was aspirated and discarded, and the procedure repeated once. Caution was taken to aspirate as much wash solution as possible after the last wash. To dry the pellets and remove all ethanol residues, the beads were placed in a heater at 40 °C for around 3 min, taking care to not over-dry the pellet. Beads were re-suspended in 150 µl of TE, vortexed and returned to the heater at 40 °C for 15 minutes to complete extraction of the DNA from the beads. After that, the solution was vortexed and briefly centrifuged, and then placed in the magnetic rack to magnetically separate the beads. When the solution was clear, it was transfered to a new tube for storage and posterior library preparation.

Double-digestion

Were selected the combination of PacI + ApoI-HF to digest *Porites* species and AseI + ApoI to digest *Millepora* species. The reaction of the two endonucleases generates short double strand fragments of DNA that terminate at each end with a known restriction site (PacI= TTAAT, AseI=AT, ApoI=AATTC). Initially, single

digestion was performed to empirically see digestion patterns of the selected endonucleases in the target species, since the *in silico* experiment to select the restriction enzymes was run in the genome of different species. For that, 500 ng of good quality DNA from one individual of *P. astreoides* and *M. alcicornis* were digested with 1 µl (10 units) of PacI and AseI respectively incubating reaction at 37 °C for 5 h. Then, the parameters of starting mass of DNA and digestion time were tested with the objective to ensure consistent digestion reaction among samples. To test the effect of the starting amount of DNA, was performed a double-digestion over one sample of *M. alcicornis* with high quality DNA under similar reaction conditions. Finally, the most efficient digestion time was tested using one sample of *Favia fragum* using 3 U of each enzyme (PacI + ApoI-HF) and incubating the reaction at 37 °C for 1, 3 and 7 h respectively.

Adapter ligation

Specific adapters that recognize the restriction sites were designed to capture the DNA fragments obtained after double digestion, ensuring that only fragments flanked by both restriction cut overhangs are sequenced (see complete list of adapters in Supplementary Material 1). The 5' R1 adapter is composed by the PacI (*Porites* spp.) or AseI (*Millepora* spp.) recognition sites, followed by a specific 8-bp long index used to differentiate among the samples (8 indexes, D501-D508 standard Illumina adapters), and a 43 pb long sequence complementary to the i5 primer used to generate the Illumina sequencing library. The 3' R2 adapter is an "Y-yoke" adapter that increases the efficiency during library preparation by allowing each starting DNA molecule to serve as template for the two adapters (Glenn et al. 2016; Greigite 2009; Bentley et al. 2008; Hoffberg et al., 2016). The R2 adapter is composed by the ApoI recognition sequence, a sample-specific 8-bp index (12 indexes, D701-D712 standard Illumina adapters) and a 42-pb long sequence complementary to Illumina's i7 primer. We use a 5-fold molar excess of adapters to genomic DNA ends. This excess was calculated from the results of single enzyme digestions measured using Bioanalyzer (Agilent Technologies, Inc.) with the help of the ligation molarity calculator provided by Peterson et al., (2012). Effective adapter ligation is paramount because only fragments flanked by both restriction cut overhangs are sequenced. For that reason, two specific primers that recognize the R1 and R2 adapter respectively -forward

ACGACGCTCTTCCGATCT, and reverse ACGTGTGCTCTTCCGATC- were designed to test effective ligation by PCR amplification.

Pooling of samples

To reduce variability in library preparation between samples, we made pools by assembling together equal volumes of DNA from each sample (Baird et a., 2008). Libraries were pooled according to DNA integrity, ligation product concentration (measured with Qubit), and R1 and R2 adapter compatibility. To avoid problems during the demultiplexing process, it is extremely important not to mix samples containing the same adapter combination. We also avoided combining samples of the same species and collected from the same site in the same library.

Size selection

The double digestion reaction generates a wide range of fragment length, and the size selection step produces a uniform distribution of fragments within ideal size for sequencing in Illumina platforms (Andrews et al., 2016). We used SpeedBeads to isolate the desired DNA size in the range of 400-600 bp that represents 300-500 bp of genomic DNA. As a general rule, the lower the ratio SpeedBeads:DNA, the larger will be the final fragments bound to the beads. Using a higher ratio of SpeedBeads:DNA will bind smaller fragments. Size selection is comprised of two steps: 1) select and discard fragments bigger than 600 bp, and 2) select and retain fragments bigger than 400 bp. Recover this size range require adjust the amount of reagent (SpeedBeads) that is mixed with a fixed volume of sample. To achieve this and select the final ratio SpeedBeads:DNA to use, we subjected DNA ladder to a dual sided size selection with the following ratios:

Ladder volume (µl)	Round 1 (ratio / vol.)	Round 2 (ratio / vol.)
50	0.6X / 30 µl	0.2X / 10 µl
50	0.6X / 30 µl	0.3X / 15 µl
50	0.63X / 31.5 µl	0.2X / 10 µl
50	0.63X / 31.5 µl	0.3X / 15 µl
50	0.65X / 32.5 µl	0.2X / 10 µl
50	0.65X / 32.5 µl	0.3X / 15 µl

PCR amplification to generate Illumina sequencing libraries

After size selection, Illumina flow cell annealing sequences and one extra multiplexity index are incorporated into the libraries. In a first one-cycle PCR reaction is incorporated the i5 primer that recognizes the R1 adapters. This primer has the complementary oligo that anneals to the Illumina sequencing flow cell and also add an 8N degenerated index that helps to identify PCR duplicates (Hoffberg et a., 2016, Glenn et al., 2016). During a second PCR, a plate-specific i7 index is introduced increasing multiplexity capacity (Figure 4), and add the sequencing Illumina primers. Different PCR reaction conditions were tested aiming to increase the outcome of both reaction using the Phusion High-Fidelity DNA polymerase (NEB). In particular, improved specificity is of great importance during the first one-cycle PCR reaction to incorporate the i5 primer, and an increase in efficiency is desired during i7 incorporation and enrichment of the final product.

With that objective, we performed one experiment composed of 27 PCR reactions following an orthogonal array. We tested the effect of MgCl concentration, by using as treatments 1.5, 2.5 and 3.5 mM. Increasing in 1 mM from the manufacturers' recommendation (1.5 mM) seeking to increase yield but without loose in specificity (Ekman 1999). The effect of annealing temperature was tested increasing and reducing 4 °C from the estimated melting temperature for each i5 and i7. Finally, the final extension time of 6, 8 and 10 min were tested, hoping to detect an optimum extension time that may increment yield by increasing the probability of amplifying difficult templates (Ekman 1999).

Sample Multiplexity

Samples were arranged into five plates, in each of them was incorporated the specific i7 primer (see complete sequences in supplementary material 1) (Figure 4). This primer increasing the multiplexing capacity during a single sequencing run, allowing indexing up to 576 individuals: 8 index R1 x 12 index R2 x 5 plates with index i7 = 576) (Glenn et al. 2016).

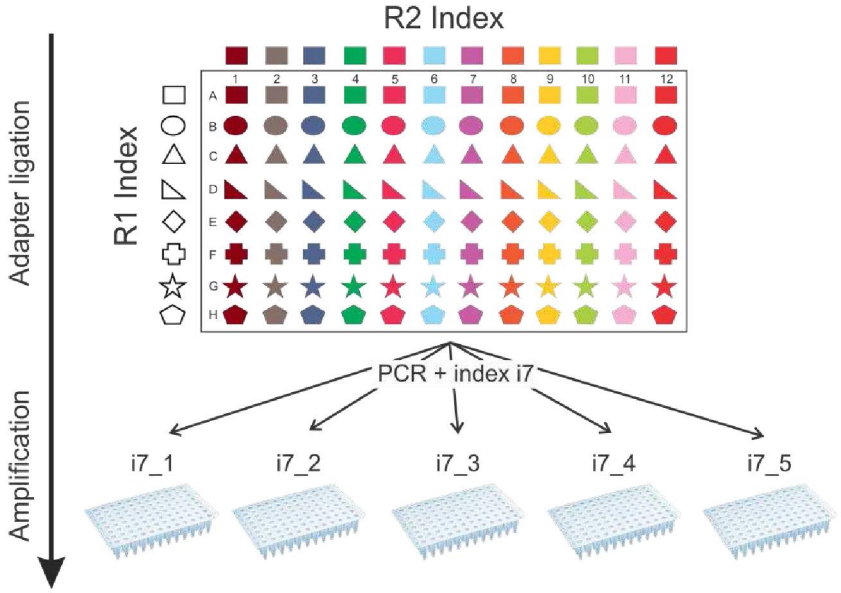

Figure 4 Multiplexing scheme. We synthesized a set of 8 adapters R1 and 12 adapters R2 that are incorporated into individual samples in a single plate. Then, we differentiate plates by one of the i7 primers. Samples are demultiplexed bioinformatically by the recognition of a combination of the 3 indexes (8-R1 x 12-R2 x 5 i7 = 576).

RESULTS AND DISCUSSION

Sample collection

Were collected 146 colonies belonging to 4 scleractinian species from 5 MPAs along the Brazilian coast (Table 1). All samples from Fernando de Noronha and the *M. alcicornis* from Abrolhos were donated by professor Dra. Carla Zilberberg from the Federal University of Rio de Janeiro (UFRJ).

Table 1 Number of sample colonies collected in 5 MPAs along the Brazilian coast.

Sampling site	*M. alcicornis*	*M. braziliensis*	*P. astreoides*	*P. branneri*
PARNA dos Abrolhos	10		14	5
PARNA Corais de Armacao dos Buzios	14			
APA das Ilhas de Tinhare e Boipeba	12		14	10
APA Recifes de Corais	11		13	
APA Costa dos Corais	11	2	12	
APA Fernando de Noronha	9		10	

Visual inspection of the images used for texture analysis suggested variability in corallite characters and shapes inside localities for both species. In Figure 5 and Figure 6, can be seen how some corallite characters -like the shape of the cup and wall structure- vary among individuals of *P. astreoides* within and between localities. *Porites branneri* also showed variability within and between the two localities where it was collected -the PARNA Dos Abrolhos and the APA Ilhas de Tinhare and Boipeba-. Similar intra-specific variation in corallite morphology has been reported in other *Porites* species from Jamaica (Brakel, 1977), and in *P. lobata* from the pacific ocean (Tisthammer et al., 2018). However, further detailed classification implementing a texture-based analysis is under development (work In prep.), and posterior molecular analysis using the SNPs discovered here will help to properly determine and quantify the significance of the observed variability.

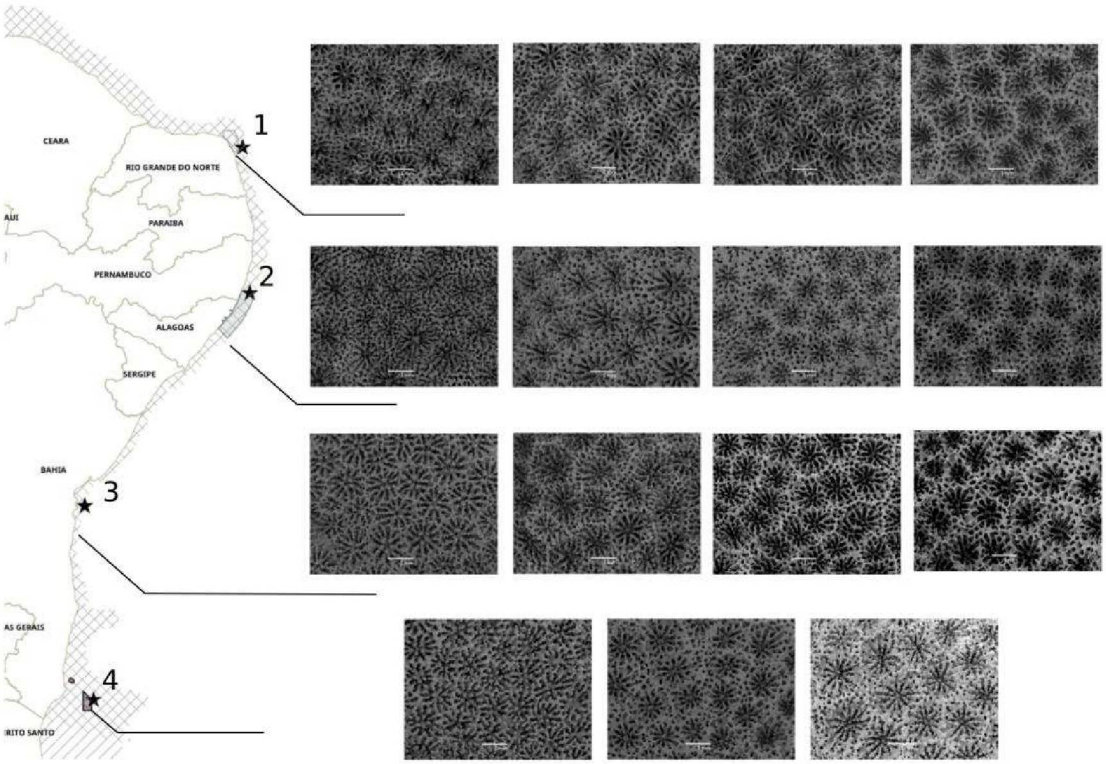

Figure 5 Scanning electron microscope images of *P. astreoides* at 11x magnification. Each picture represent one individual colony. 1) APA Recifes de Corais; 2) APA Costa dos Corais; 3) APA Ilhas de Tinhare e Boipeba; 4) PARNA dos Abrolhos.

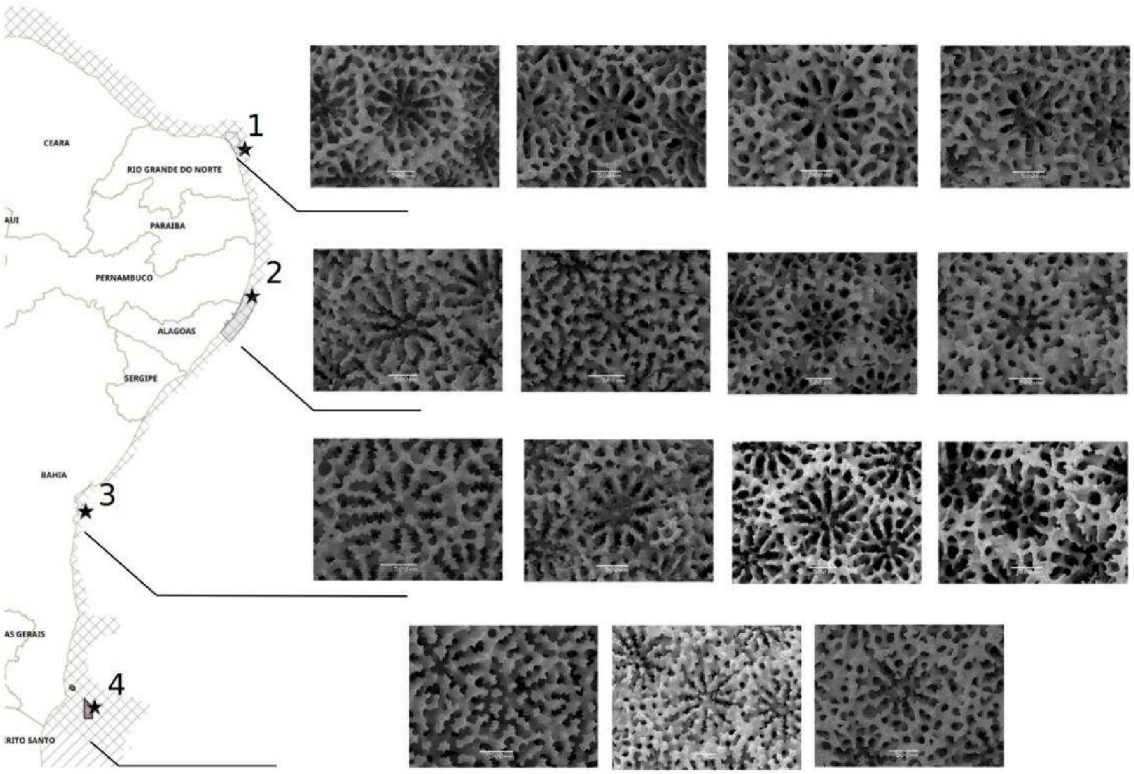

Figure 6 Scanning electron microscope images of *P. astreoides* at 20x magnification, detailing a single corallite.

Optimized ddRAD library preparation

DNA extraction

DNA extraction is the first critical step during library preparation when chosen restriction site associated protocols (Graham et al., 2015). Our results suggest that the DNA extraction protocols performed differently among species, so it is recommended to empirically search for the more efficient method in regards target species to increase the quality and quantity DNA, which is required for optimal restriction endonuclease digestion (Etter et al., 2011).

In total were performed 238 extraction procedures, 184 with the commercial kit and 54 with the SpeedBeads. The 55% of collected samples performed well with the commercial kit and obtained good quality band in the agarose gel after the first extraction procedure (Figure 7). However, subsequent extractions were performed with SpeedBeads because it reduce extraction cost per sample, its expected to produce superior yield, and eliminate the purification step needed before digestion with restriction enzymes. With both methods were obtained samples with very large (>100 ng/µl) and very low (<0.001 ng/µl) DNA concentrations. However, the SpeedBeads yield a slightly higher median of DNA concentrations than the commercial kit (Figure 8). Although, those differences were obtained only for the two *Porites* species (Figure 8). These differences in yields of DNA after coral DNA extraction in different species were previously reported in other works, and were associated with differences in microbial community structure among coral species (Rohwer et al., 2002, Santos et al., 2012, Weber et al., 2017).

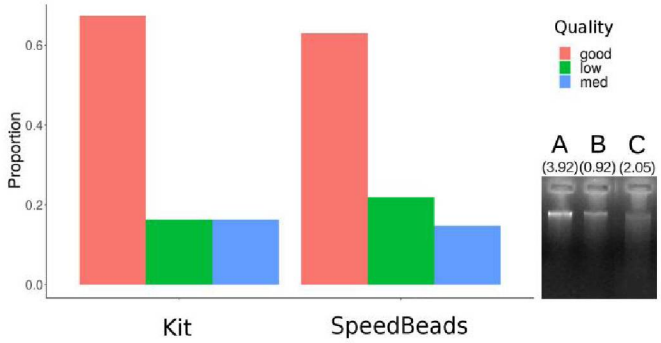

Figure 7 Band quality proportions after extraction procedures with commercial kit and Speedbeads. An example of agarose gel electrophoresis of DNA is shown: A= “good” quality band, B= “med”, and C= “low” quality band. Values in parenthesis are concentration values (ng/µl) measured with Qubit.

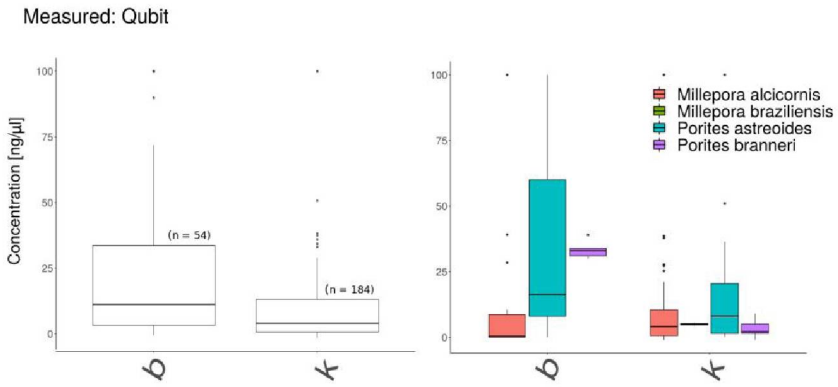

Figure 8 DNA concentration obtained with two methods: k= commercial kit and b= SpeedBeads, in four cnidarian species. On the left, is compared the total concentration obtained by each method; on the right the results were disaggregated by specie.

The purity ratio 260/280 in both methods obtained on average values that are higher than suggested for pure DNA, and in both cases close to 2, that is the expected value for pure RNA. This general deviation for the 260/280 ratio toward higher than expected values may probably respond to a high ratio of Adenine composition observed in cnidarian genomes (Kitahara et al., 2014), nucleotide that exhibits the higher absorbance ratio (www.nanodrop.com). The SpeedBeads seem to produce more consistent results with a narrow range of variation, showing a higher density peak around the mean value (Figure 9). For the ratio 260/230, both methods showed values lower than suggested for pure DNA, indicating some sort of organic contamination, probably with reagents used in the isolation procedure. However, only those samples with extreme deviation for the expected values were re-extracted.

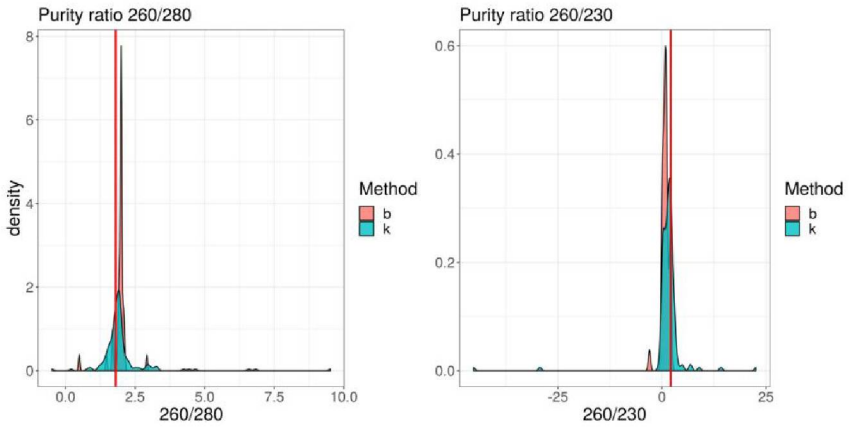

Figure 9 Density plot of the purity ratios 260/280 and 260/230 measured with NanoDrops. The red line shows the generally expected values for pure DNA (~1.8 in ratio 260/280, and 2.0 in the ratio 260/230). b= SpeedBeads; k=Commencial kit

In total, 27 samples -16 *M. alcicornis*, 7 *P. astreoides* and 4 *P. branneri*- did not passed the quality control after several extraction attempts, and were excluded for further analysis.

Double-digestion

The selection of restriction enzymes determines the number of reads obtained during sequencing (Burns et al., 2017), and is critical for obtaining a good ratio between the sequencing costs and the number of SNPs. If the digestion is not consistent among samples, it can cause difficulties in fragment recovery and variable coverage during sequencing, leading to loci drop-out.

The test for single enzyme digestion produced satisfactory results of a right tale distribution of fragment sizes (Figure 10), and the double digestion test showed that starting with as little as 25 ng of high quality DNA could be enough to recover sufficient DNA within appropriate sizes (Figure 11). However, we decided to digest between 100-1000 ng when possible to increase the chance to recover higher quantities of usable fragments.

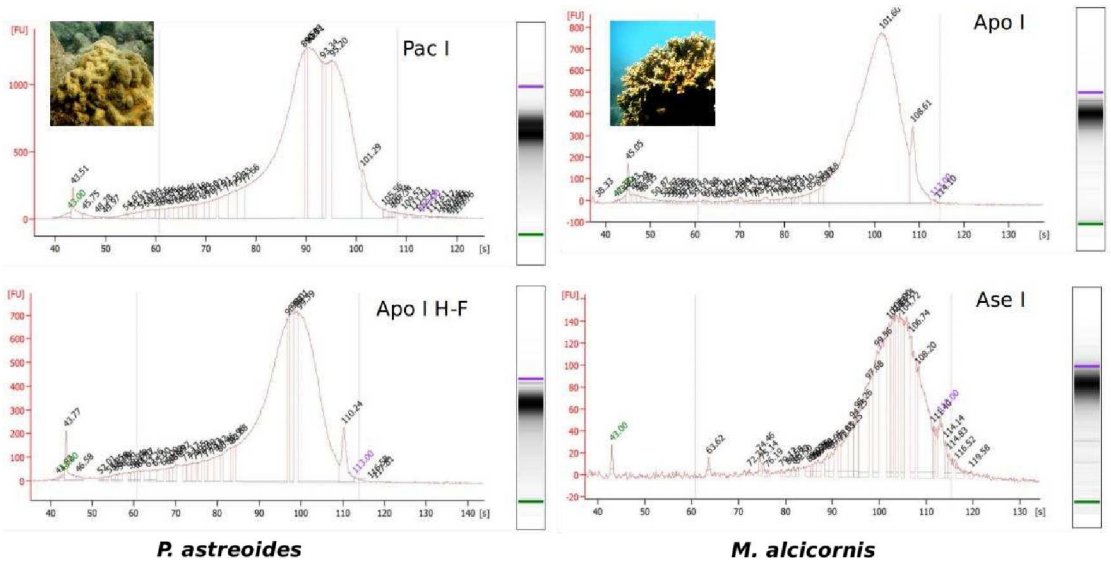

Figure 10 Single digestion results obtained for *P. astreoides* (left panel) and *M. alcicornis* (right panel), run in Angilent Bioanalyzer 2100. The x axis represents the time need for fragments to migrate (in seconds), larger fragments require more time for migration.

The results for the optimum digestion time showed that a reaction time of 7 h produced a peak of smaller fragments (and closer to our desired size) when compared with 1 h of digestion (Figure 11). Unfortunately, due to technical problems, it was not possible to observe the results for the 3 h treatment. However, the digestion time was standardized to 5 h to save handwork time, and following the successful results obtained during individual enzyme digestion for 5 h (Figure 10), and the successful results obtained by Dimond et al., (2017) digesting *Porites* samples using similar digestion time

To optimize the reaction, it is ideal to use enzymes that have compatible reaction buffers and activation temperatures, but this process can be adapted when needed (check the manufacturer recommendations). The AseI and ApoI are supplied with NEBuffer 3.1, however, to achieve 100% efficiency they should be incubated at different temperatures - 37 °C for AseI and 50 °C for ApoI. In that case, a sequential digestion protocol was defined for *Millepora* species, adding first the AseI and incubating for 2.5 h, and then adding the same amount of ApoI and incubating for another 2.5 h (see final protocol for details).

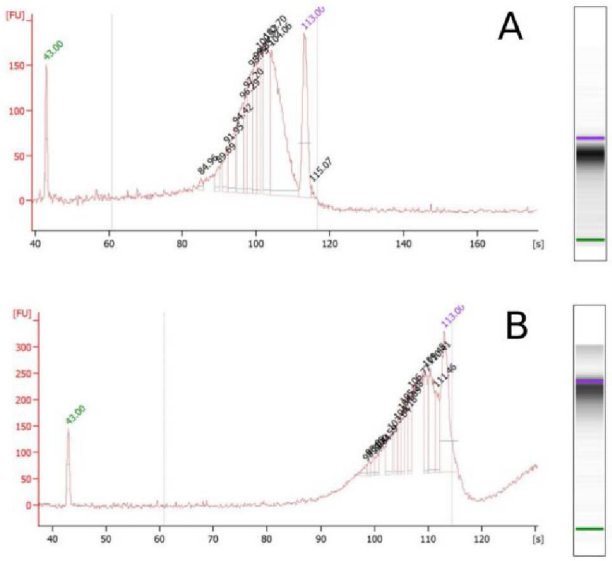

Figure 11 Different digestion times visualized in Angilent Bioanalyzer 2100. We run reaction with 3 U of each enzyme (PacI + ApoI-HF) and incubating at 37 °C. Digestion for 7 h (A) peaked at 100 s, while the digestion for 1 h (B) peaked at 110 s (larger fragment sizes). The x axis is the time needed for fragments to migrate in seconds, larger fragments require more time for migration.

Difficulties during digestion are usually due to the use of degraded material, or the presence of inhibitors after DNA extraction, especially when using silica-based DNA

extraction kits. To avoid these problems it is recommended to perform rigorous quality control after DNA extraction and check band integrity in agarose gel, purify extracted material with SpeedBeads (or use it to extract the DNA), and use high fidelity (HF) version of enzymes when available (e.g. we did not use ApoI-HF to digest *Millepora* samples due to buffer incompatibility with the AseI, but it was used to digest *Porites* samples). After DNA extraction and quality control check, 119 samples entered the double-digest reaction.

Adapter ligation

Ligation reaction was set up in 40 µl reaction volume, adding 1 µl of the (5 µM) working stock solution of each adapter (R1 and R2), 4 µl of 10X T4 DNA ligase buffer, 0.25 µl (or 100 U) of T4 DNA ligase, and 33.75 µl of digested DNA. Input DNA was maximized ligating as much DNA mass as possible for all samples. Reactions were incubated for 30 min at 25 °C and heat inactivated at 65 ºC for 10 min, and then decreasingly cooled at a rate of 2 °C per 90 sec until room temperature. It is important to keep T4 ligase in ice during the procedure, and always add it at last to prevent degradation. Effective ligation can be tested by PCR amplification using the specifically designed primers.

Size selection

DNA ladder (diluted 1:20) was subjected to dual size selection with given ratios of SpeedBead volume to sample volume (Figure 12). The best result was obtained with the ratios 0.63X for the right sided size selection, and 0.2X for the left sized size selection. The first part of the procedure (0.63X) select fragments bigger than ~600 pb which bind to the beads and are then discarded (“R” in Figure 12). The second part of the procedure (0.2X) select fragments larger than ~300 pb which bind to the beads and are retained (“DSS” in Figure 12). The standard washing and elution operations were performed to isolate the purified and size-selected DNA fraction in 20 µl of elution buffer. Because a consistent size selection across libraries is essential when using magnetic beads (Andrews et al., 2016), the selected ratio was then tested using the DNA of one sample of *M. alcicornis* (initial concentration of 38.6 ng/ul) comparing different initial input DNA: 1000 ng; 50 ng; 25 ng. The size-selected product was run in Angilent Bioanalyzer 2100 (Figure 13).

PCR amplification to generate Illumina sequencing libraries

The results in the PCR experiment suggest using 47 °C as annealing temperature during i5 incorporation, because, despite yield similar mean concentration than 51 °C (the estimated melting temperature for i5 primer), the ratio signal to noise factor is much lower (Figure 14-A). For the concentration of MgCl, we decide to use 2.5 mM (higher than manufacturer recommendation) prioritizing a higher mean in yield, even though presented higher signal to noise ratio when compared with 1.5 mM (Figure 14-B). The final extension time selected was 8 min, because presented the lower signal to noise ratio and the final mean concentration was just slightly lower (Figure 14-C).

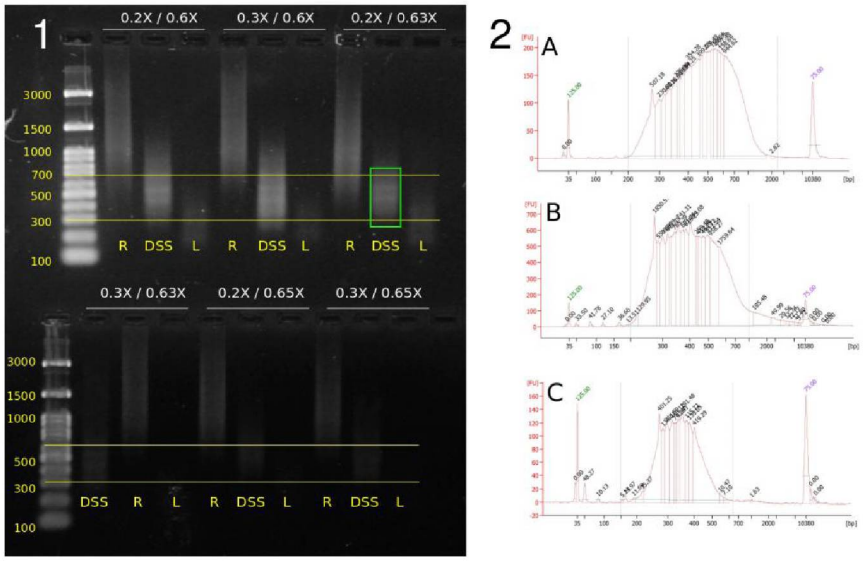

Figure 12 Size selection results. 1) DNA ladder subjected to dual size selection testing different ratios SpeedBeads:DNA. DSS= fragments retained after dual size selection; R= fragments discarded during the first round (Right sided); L= fragments that bind to the beads and discarded during the second round (Left sided). 2) Size selection results after double-digestion with AseI + ApoI on one sample of *M. alcicornis* (initial concentration of 38.6 ng/ul). Input DNA subjected to size selection: 2-A=1000 ng; 2-B= 50 ng; 2-C= 25 ng. The size-selected product was run in Angilent Bioanalyzer 2100; the x axis is the size of the fragments in base pair.

The results for the PCR conditions to incorporate the i7 primer, suggest using 64 °C as annealing temperature (3 °C below the melting temperature for i7 primer), because despite the occurrence of a linear increase in the mean concentration with the increment in the annealing temperature, obtaining higher mean at 68 °C, the ratio of signal to noise was much lower at 64 °C. Using 1.5 mM of MgCl we obtained higher mean concentration and lower signal to noise ratio, and we decided to keep this

concentration as recommended by the manufacturer. And finally, we decided to use 8 minutes for the final extension time because it yields the higher concentration mean and a slightly lower signal to noise ratio than using 6 min.

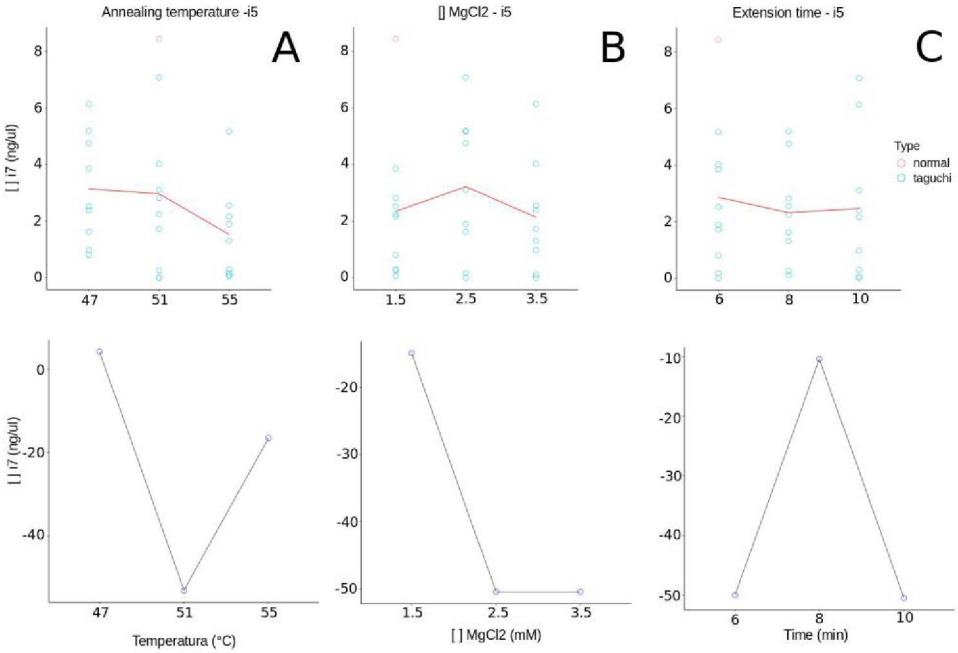

Figure 13 Results of the PCR optimization conditions experiment for i5 primer incorporation. Upper graphs represent the concentration values measured with Qubit. The red line shows the means. Lower graphs represent the ration signal to noise.

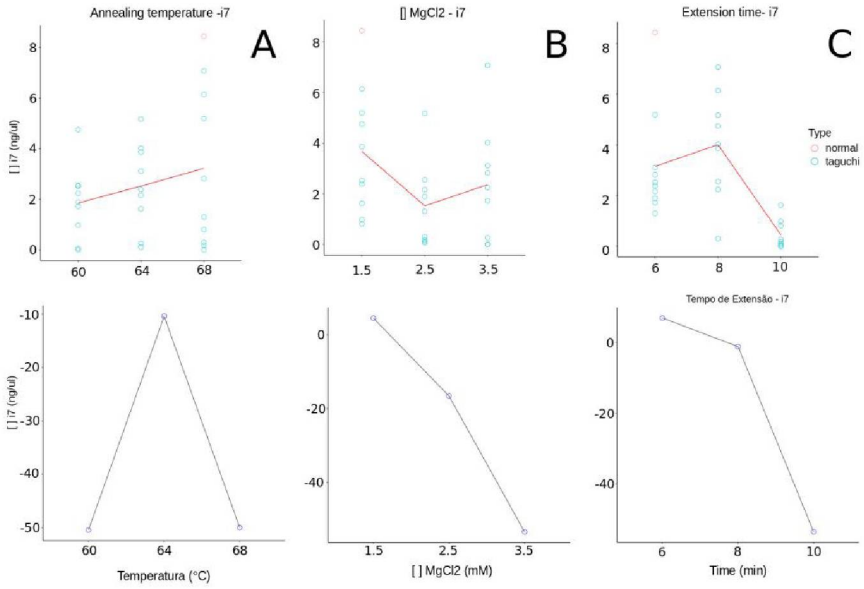

Figure 14 Results of the PCR optimization conditions experiment for the i7 primer incorporation

ddRADseq Bench Protocol

We propose the following optimized protocol to prepare ddRADseq libraries to efficiently identify SNPs in Scleractinian species:

Double Digestion with Restriction Enzymes

Materials

- Thermocycler
- Magnetic plate
- Qubit Fluorometer
- Filter pipette tips (box should be autoclaved, but not the tips if they are DNAse free)
- Styrofoam box
- Ice
- PCR plates

Reagents

- Restriction enzymes: PacI + ApoI-HF, AseI + ApoI
- Restriction enzymes buffers: SmartCut, NEBuffer 3.1
- SpeedBeads
- Nuclease-free water

Notes:

- *Remove* SpeedBeads *from refrigerator 60 min before the cleaning and let it reach room temperature; Mark tubes for digestion and quantification prior to digestion.*
- *Enzymes must be placed in ice during the procedure.*

Procedures

For ***Porites spp.*** use the combination PacI+ApoI-HF:

1. Set up reaction (50µl) for the digestion in a 0.6 ml PCR tube:
   a) Add ~43 µl of DNA (between 100 to 1000 ng);
   b) Add 5 µl of (1X) CutSmart Buffer;
   c) Add 3 units of PacI;
   d) Add 3 units of ApoI-HF;
   e) Add appropriate volume of nuclease-free water;
   f) Incubate at 37 °C for 5 h in a PCR machine;
   g) Let the reaction slowly cool down to room temperature;
   h) Proceed with cleaning with SpeedBeads and elude in 40 µl of TE;
   i) Measure concentrations with the Qubit Fluorometer (dsDNA HS Assay Kit);

For *Millepora spp* use the combination AseI + ApoI:

1. Set up reaction (50 µl) for the sequential digestion in a 0.6 ml PCR tube:

***Note: DO NOT** make a MasterMix of both restriction enzymes! They have different activation temperatures and must be added separately.*

a) Add ~43 µl of DNA (between 100 to 1000 ng);

b) Add 5 µl of NEBuffer 3.1;

c) Add 3 U of AseI;

d) Add appropriate volume of nuclease-free water;

e) Incubate at 37 °C for 2.5 h in a PCR machine;

f) Add 3 units of ApoI;

g) Incubate at 50 °C for 2.5 h in a PCR machine;

h) Let the reaction cool slowly down to room temperature;

i) Proceed with cleaning with magnetic beads and elude in 30 µl of TE;

j) Measure concentrations with the Qubit Fluorometer (dsDNA HS Assay Kit);

SAFE STOP POINT: *You can safely stop the protocol here.*

Adapter ligation

Materials

- Ice
- Styrofoam box
- 0.2 ml microcentrifuge tubes or PCR plate
- Filtered pipette tips

Reagents

- T4 DNA ligase (NEB)
- T4 DNA ligase buffer 10X (supplied with T4 DNA ligase)
- R1 and R2 annealed working stock solution at 5 µM
- Nuclease-free water

Procedures

Notes:

- *Ligation procedure must be made in tubes of 0.2 ml.*
- *Keep T4 Ligase in ice during the procedure. Pipetting must be done slowly because of the viscosity of T4 and to prevent formation of bubbles.*
- *The T4 DNA Ligase Buffer should be thawed at room temperature. Remove from freezer before start the procedure.*
- *Samples must be arranged into five PCR plates, where the R1 and R2 adapters will be added in a specific order.*
- Gently mix Master Mix by tapping the tubes. Whenever possible keep on ice.

Ligation conditions for a 40 µl reaction volume:

1. Set up reaction in a 0.2 ml tube:
2. Add 33.75 µl of digested DNA

Note: Follow the molarity calculation spreadsheet provided by Peterson et al., (2012) for the adapter concentration calculation!!

3. Add 1 µl of the working stock solution of the R1 adapter at 5 µM;
4. Add 1 µl of the working stock solution of the R2 adapter at 5 µM;

Note: You can make a mix of T4 Ligase + buffer with the appropriate volume to complete all the samples. Always account from pipetting errors.* ***T4 DNA ligase should be added least to prevent degradation.*

5. Add 4 µl of 10X T4 DNA ligase buffer per sample;
6. Add 0.25 µl (100 U) of T4 DNA Ligase per sample;
7. Gently mix the reaction by tapping the tubes;
8. Place the tubes in the thermocycler;
9. Incubate for 30 min at room temperature (25 C);
10. Heat inactivate at 65 C for 10 min;
11. After the heat-kill, cool the solution 2 C per 90 sec until it reaches room temperature;
12. Proceed with cleaning with magnetic beads, elute in 45 µl (2 µl for concentration quantification, 20 µl for next step of pooling, and 20 µl for storage in case need to repeat experiment);
13. Measure concentrations with the Qubit Fluorometer (dsDNA HS Assay Kit).

SAFE STOP POINT: *you can safely stop the protocol here.*

Testing for effective adapter ligation:

Following standard protocols, successful ligation of adapters can only be inferred after incorporation of i5 and i7 primers, when valuable amounts of DNA material, reagents, and time have been already spent. To avoid such a lost, 2 primers that recognize the R1 and R2 adapters were designed using the AMUSER web server tool (Genee et al., 2015). Successful amplification after standard PCR experiments will reveal success in ligation.

Ligation Forward primer: ACGACGCTCTTCCGATCT
Tm: 55.6 C
GC content: 52.63%
nt: 18

Ligation Reverse primer: ACGTGTGCTCTTCCGATC

Tm: 55.5 C
GC content: 52.63%
nt: 18

Pooling samples

After adapter ligation, pools are made by assembling together equal amounts of DNA from each sample according to R1 and R2 adapter combination (multiplexing) and the i7 index that will be added in the incorporation step.

√ The combined library concentration of the pool should be 5 nM or higher.

Procedure
Create pools according with pooling criteria:

1. Use different multiplexing indexes for each sample you wish to pool
2. Quantify concentration of ligation product using Qubit (ng/µl);
3. Pool the same amount for each sample in fentomoles (calculated by multiplying the concentration (nM) by the volume to be added;

4. Add 20 µl of ligation product of the sample with the lowest DNA concentration (this is an arbitrary volume) in a new tube, and calculate the concentration in nM:

$$\mathbf{nM_{low} = (\ C_{low} / 660 \times 1069\ bp) \times 10^6}$$

Where C_{low} is the lowest DNA concentration (ng/µl), and nM_{low} is the calculated concentration in nM.

5. Calculate the number of fentomoles for the lowest concentration sample in the 20 µl volume:

$$\mathbf{fentomoles_{low} = 20\ \mu l \times nM_{low}}$$

6. Now the fentomole value for the lowest concentration sample will be fixed in the next steps;
7. Calculate the necessary volume of the other samples (V_i) to add to the pool using the following equation:

$$\mathbf{V_i = fentomoles_{low}/nM_i}$$

Where nM_i is the concentration in nM for each of the other samples and $fentomoles_{low}$ is the fixed value for the lowest concentration sample.

8. Do the same process for all samples to be added in the same pool;
9. Use one aliquote of the pool to continue the next steps.

***WARNING:** take extremely care not to mix samples with the same adapter combinations.*

Size selection

Materials
➢ Pipette tips (use 100ul (yellow) to remove the isopropanol)

- PCR tubes
- Magnetic plate

Reagents
- SpeedBeads
- Freshly prepared isopropanol (80%)
- Nuclease-free water

Procedures

***Note:** SpeedBeads Mix should be use at room temperature. Remove Mix from the refrigerator at least 60 minutes before start the procedure.* Ensure reagent is fully homogenized and equilibrated before use!

The first part of the procedure selects fragments bigger than 600 bp, which will bind to the beads.

1. (Not so) Gently shake the SpeedBeads bottle to re-suspend any magnetic particles that may have settled.
2. Add 40 µl of the pooled samples;
3. Add the volume of SpeedBeads equivalent to **0.2X** the library volume **dispensed by reverse pipetting**:

   $V_{Serapure} = V_{library} * \mathbf{0.2X}$

4. **Vortex** the binding mixture, then leave to **incubate** at room temperature for 10 minutes;
5. Place the tubes on the magnetic plate for at least 2 minutes (or until the solution is clear) to separate beads from the solution;
6. Carefully **transfer the supernatant** (the volume of supernatant is equivalent to the volume of library used + volume of SpeedBeads) to a new tube;

***Caution:** DO NOT disturb the bead pallet and DO NOT discard the supernatant!*

The second part of the procedure selects fragments bigger than 400bp, which **will bind to the beads**.

7. In the new tube, the volume of SpeedBeads to be added is equivalent of **0.63X** the initial volume of DNA:

   $V_{Serapure} = V_{library} * \mathbf{0.63X}$

8. Mix well by pipetting up and down (be careful not to create bubbles);
9. Incubate for 5 minutes at room temperature;
10. Place the PCR plate on the magnetic plate for at least 2 minutes (or until the solution is clear) to separate beads from the solution;
11. Carefully aspirate and **discard the supernatant** (DO NOT disturb the bead pellet). The DNA targets will be bound to the beads;

***Note:** do not remove the PCR plate from the magnetic stand.*

12. Add 200 µl of freshly prepared 80% isopropanol. **Do not** add directly into the beads, use the opposite wall of the tube to avoid dislodging the pallet;
13. Incubate 1 minute at room temperature;
14. Carefully aspirate and **discard the supernatant** (DO NOT disturb the bead pellet);
15. Repeat the washing step one more time;
16. Take care to aspirate as much wash solution as possible after the last wash;
17. Dry beads in a tube heater at 40 °C for 5 to 10 minutes;

***Caution:** DO NOT over-dry the beads. This may result in permanent binding of the DNA to the beads and lower recovery of DNA target.*

18. Resuspend the dried beads in 30 µl of nuclease-free water mixing the solution until beads are completely eluted in the solution *(DO NOT scrap the walls of the tube);*
19. Incubate for 2 min at room temperature;
20. Return the tube to the tube heater at 40 °C for 15 min to complete extraction of the DNA from the beads;
21. Place the PCR tube on the magnetic plate for at least 2 minutes (or until the solution is clear) to separate beads from the solution;
22. Without disturbing the bead pellet, transfer the supernatant to a PCR tube and proceed with library preparation.

***Note:** be careful not to transfer any bead along with the supernatant. If you disturb the bead pallet, mix the solution again and return the tube to magnetic plate until beads are fully separated from the supernatant.*

***SAFE STOP POINT:** you can safely stop the protocol here.*

PCR amplification to generate Illumina sequencing libraries

We performs PCR reactions with Phusion® High-Fidelity DNA Polymerase (NEB).

Materials

- Thermocycler
- PCR plate
- Pipette tips
- Ice
- Styrofoam box

Reagents

- Ligation product (DNA + adapters)
- Primer i5 8N (degenerate)
- i7 and p5 primers (the p5 primer is used as forward primer)
- Nuclease-free water
- Phusion-HF PCR kit

It is extremely important to assemble all reaction components on ice and quickly transferring

the reactions to a thermocycler preheated to the denaturation temperature (95°C). All components should be mixed and centrifuged prior to use (except the polymerase). It is important to add Polymerase last, in order to prevent any primer degradation.

Procedures for i5 incorporation

One-cycle PCR Reaction to add the i5-8N degenerated index.

In a 25 µl reaction volume:

1. In a new tube add 9,4 µl of pooling samples;

2. Make a Master Mix of Phusion-HF polymerase and i5 primer:
    a. Add 2.5 µl of i5 primer at 5 µM (concentration at reaction = 0.5 µM) for each sample;
    b. Add 12.5 µl of Phusion-HF Master Mix for each sample;
    c. Add 0.6 µl MgCl (to obtain final reaction concentration of 2.5 mM)

3. Add 15 µl of the Master Mix to each sample;
4. Gently mix the reaction and spin down in microcentrifuge;
5. Transfer tubes to PCR machine;

6. Incubate at 95°C for 2 min.;
7. 1 cycle of:
    a. Denaturation 98 °C for 20 sec;
    b. Annealing 47 °C for 15 sec;
    c. Extension 72 °C for 60 sec;
8. Final extension 72 °C for 8 min;

9. Clean reaction with magnetic beads following Magnetic Beads Cleaning, final elution in 20 µl of ddH2O.

Testing for effective i5 incorporation:

One additional primer (complementary to the i5) was designed to test for successful incorporation of i5 primer by running standard PCR reactions in combination with the previously designed Ligation Reverse primer:

Incorporation Forward primer: AATGATACGGCGACCAC
Tm: 60.1 C
GC content: 52.9%
nt : 17

Ligation Reverse primer: ACGTGTGCTCTTCCGATC
Tm: 55.5 C
GC content: 52.63%
nt: 18

Procedures for i7 index and iTru5 primer incorporation

At least three-cycle PCR reaction is needed to incorporate the i7 index (we propose 10 cycles to additionally enrich the quantity of the final libraries). To increase yield without incorporate PCR bias, we recommend split each pool into 3 aliquots and perform individual PCR reactions as follow:

In a 25 µl reaction volume:

1. Add 7.5 µl of the pooled ligated DNA with i5;
2. Make a Master Mix of Phusion HF Master Mix, i7 index and iTru5 primer:
    a. Add 2.5 µl of i7 primer at 5µM (concentration at reaction = 0.5 µM) for each sample;
    b. Add 2.5 µl of iTru5 primer at 5 µM (concentration at reaction = 0.5 µM) for each sample;
    c. Add 12.5 µl of Phusion-HF Master Mix for each sample;
3. Add 17.5 µl of the Master Mix to each sample;
4. Gently mix the reaction and spin down in microcentrifuge;
5. Transfer tubes to PCR machine;
6. Incubate at 95 C for 2 min;
7. Ten (10) cycles of:
    a. Denaturation 98 °C for 20 sec;
    b. Annealing 64 °C for 15 sec;
    c. Extension 72 °C for 60 sec;
    d. Final extension 72 °C for 8 min.
8. Clean reaction with magnetic beads following Magnetic Beads Cleaning.
9. Measure concentrations with the Qubit Fluorometer (dsDNA HS Assay Kit).

***Note:** Now your library is ready! To check for fragment size and distribution you can do a Bioanalyzer run. The next steps are for library enrichment, and must be performed only if necessary.

Procedures for library enrichment using iTru5 and iTru7 primers.

Maximum of six-cycle PCR reaction to enrich the library.

In a 25 µl reaction volume:

1. Add 7.5 µl of the library;
2. Make a Master Mix of Phusion-HF Master Mix, iTru5 and iTru7 primers:
    a. Add 2.5 µl of iTru5 primer at 5 µM (concentration at reaction = 0.5 µM) for each sample;
    b. Add 2.5 µl of iTru7 primer at 5 uM (concentration at reaction = 0.5 µM) for each sample;
    c. Add 12.5 µl of Phusion-HF Master Mix for each sample;
3. Add 17.5 µl of the Master Mix to each sample;

4. Gently mix the reaction and spin down in microcentrifuge;
5. Transfer tubes to PCR machine;
6. Incubate at 95 C for 2 min;
7. Up to six-cycles of:
    d. Denaturation 98 °C for 20 sec;
    e. Annealing 61 °C for 15 sec;
    f. Extension 72 °C for 60 sec;
    g. Final extension 72 °C for 6 min.

8. Clean reaction with magnetic beads following Magnetic Beads Cleaning.
9. Measure concentrations with the Qubit Fluorometer (dsDNA HS Assay Kit).

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Chapter 2

Properties of a Network of MPAs in the Brazilian coast: case study for coral reefs conservation

ABSTRACT

Conservation effectiveness of MPAs depends not only on their particular characteristics but also on biological and administrative interactions with other MPAs. Achieving effective collaboration among MPAs managers is crucial to confront complex environmental problems like the ones faced in coral reef conservation. The Brazilian government implemented since 2014, the National Plan for Conservation of Coral reefs (PAN Corais) to tackle specific issues related with corals conservation. Yet, its practical effects have barely been evaluated. Here, we used graph theory to characterize the patterns of interactions among MPAs along the Brazilian coast, with special emphasis on those located in the 18 focal areas prioritized for PAN Corais's actions. Data on 8 types of interactions (equipment sharing among MPAs, reciprocal participation in management councils, staff support, joint participation in formal training activities, conflict resolution, and joint development of surveillance, research and monitoring programs) among MPAs were collected through online questionnaires and treated as separate layers. The network encompassed 51 nodes (MPAs) and 131 edges (interactions). It was not fully connected and we detected significantly different levels of connectivity and diffusion capacities among its low-density layers. While this may increase the diversity of management practices, it may also limit the spread of information such as alerts about coral bleaching, disease outbreaks or simply the direct sharing of successful experiences. Geographic proximity between MPA increases the likelihood of interactions corroborating the hypothesis that neighborliness is the main driver of link building in socio-ecological systems. There was clear interdependence between participation in management councils and conflict resolution, confirming the utility of this space as an instrument for local governance. Additionally, was detected a correlation between the development of research activities and implementation of surveillance programs were strongly correlated, suggesting that technical knowledge is being directly transferred to surveillance programs. Network connectivity has been evenly distributed across all types of interactions, conferring high resilience to the management-based network against the removal or loss of specific types of interactions. PAN Corais seems to be promoting positive interactions in the surveillance, monitoring and research layers, as observed by high number of prioritized areas performing such activities. However, this situation was not observed in relational ties among prioritized and not prioritized areas and interactions are mainly restricted to a regional (statewide) level. We call for further efforts to strengthen the ties between targeted and non-targeted areas and also to extend the geographic scope of the Brazilian MPA network.

Keywords: Connectivity. Multilayer analysis. Management and cooperation. Social network analysis.

RESUMO

A eficácia da conservação das AMPs não depende apenas das suas características particulares mas também das interações biológicas e administrativas com outras AMPs. Conseguir uma colaboração eficaz entre os gestores das AMPs é crucial para enfrentar problemas ambientais complexos, como os enfrentados na conservação dos recifes de coral. O governo brasileiro implementou desde 2014, o Plano Nacional de Conservação dos recifes de coral (PAN Corais) para tratar de questões específicas relacionadas à conservação dos corais. No entanto, seus efeitos práticos pouco foram avaliados. Aqui, utilizamos a teoria dos gráficos para caracterizar os padrões de interação entre as AMPs ao longo da costa brasileira, com ênfase especial para aquelas localizadas nas 18 áreas focais priorizadas para as ações do PAN Corais. Dados sobre 8 tipos de interações (compartilhamento de equipamentos entre MPAs, participação recíproca em conselhos gestores, apoio de pessoal, participação conjunta em atividades de treinamento formal, resolução de conflitos e desenvolvimento conjunto de programas de vigilância, pesquisa e monitoramento) entre AMPs foram coletados por meio de questionários online e tratados como camadas separadas usando a metodologia de redes de interação multi-camada. A rede abrangeu 51 nós (MPAs) e 131 bordas (interações). Ela não estava totalmente conectada e detectamos níveis significativamente diferentes de conectividade e capacidade de difusão entre suas camadas de baixa densidade. Embora isto possa aumentar a diversidade das práticas de gestão, também pode limitar a disseminação de informações como alertas sobre branqueamento de corais, surtos de doenças ou simplesmente o compartilhamento direto de experiências bem sucedidas. A proximidade geográfica entre AMPs aumenta a probabilidade de interacções, corroborando a hipótese de que a vizinhança é o principal motor da construção de ligações nos sistemas sócio-ecológicos. Houve uma clara interdependência entre a participação em conselhos de gestão e a resolução de conflitos, confirmando a utilidade deste espaço como instrumento de governança local. Além disso, foi detectada uma correlação entre o desenvolvimento de atividades de pesquisa e a implementação de programas de vigilância, o que sugere que o conhecimento técnico está sendo transferido diretamente para programas de vigilância. A conectividade de rede foi distribuída igualmente em todos os tipos de interações, conferindo alta resiliência à rede baseada em gerenciamento contra a remoção ou perda de tipos específicos de interações. A PAN Corais parece estar promovendo interações positivas nas camadas de vigilância, monitoramento e pesquisa, conforme observado pelo alto número de áreas priorizadas que realizam tais atividades. Contudo, esta situação não foi observada em laços relacionais entre áreas priorizadas e não priorizadas e as interações estão restritas principalmente a um nível regional (estadual). Fazemos um apelo para maiores esforços no sentido de fortalecer os laços entre as áreas priorizadas e não priorizadas e também para ampliar o escopo geográfico da rede brasileira de AMPs.

Palavras-chave: Conectividade. Análise multicamada. Gestão e cooperação. Redes de interação

INTRODUCTION

Marine Protected Areas (MPAs) have been established with the aim to protect biological diversity and ecosystem services important for human well being and to maintain biodiversity in the long term, especially in current times of increasing anthropogenic disturbances. Effective conservation of MPAs is complex due to disparate goals and interests as to the use of marine resources (Cowen et al., 2017; Magris et al., 2017), making the design and establishment of MPA networks a touchy issue.

A network of MPAs must be strategically planned to operate cooperatively at various spatial scales. MPAs are viewed within this framework as socio-ecological systems and the network's resilience is conferred by a combination of environmental (e.g. marine currents, gene flow, life history of species, geology, etc.), administrative (e.g. institutional support, economic and human resources, equipment, etc.) and social features (civil society) that are expected to act synergistically to beget conservation efficiency (Magris et al., 2014; Cumming et al., 2015; Janssen et al., 2006). The systemic behavior arising from the non-linear interaction of all its components means that the whole is more that the sum of its parts (Mitchell, 2006).

The three types of networks emerging from the features mentioned above, need to be integrated and coordinated to maximize the potential benefits. The social network is defined by the interactions between MPA management and civil society groups, and may improve conservation effectiveness by ensuring participation of society in the decision-making process (Bodin, 2017). The management-based network is made up by interaction among staff in charge of the MPAs, whose management effectiveness on a larger ecosystem-base scale may be improved through coordinated actions and shared knowledge (Treml et al., 2015; Bodin, 2017), promoting collaboration in surveillance activities, passing new regulation and conservation guidelines, outlining resource and data sharing strategies, etc. Lastly, the ecological network must ensure that biological exchange among sites enhance ecological functions and connectivity among populations either via larval or adult stages (Magris et al.,2014).

Achieving efficient integration among political, social and ecological processes is not a trivial problem, recognized in the specialized literature as the “problem of fit” (Ekstrom and Young, 2009; Bergsten et al., 2014; Treml et al., 2015). If the fit is high, political and management decisions are efficiently supporting ecological function across MPAs. If two areas are biologically accessible in the network (i.e. there is a biological link between them) it is important that interested actors maintain good communication, since the vitality of an MPA can be a function of another. For instance, coral recruitment in a MPA under federal administration may comes from reefs located in another MPA under municipal authority. Preservation efforts in the first area will be of little consequence if the second area is poorly conserved. The awareness of biological interdependence between these two areas may lead the federal agents to propose joint conservation actions with the local agency. As an example, Crowder et al. (2006) mention how the lack of clarity about different roles among governmental agencies in the United States prevented the implementation of a network of MPAs in California, causing serious mismatches in ocean governance.

Different hypothesis about what drives the building of ties among MPAs have been proposed. For example, geographic proximity of actors may affect the probability of creating administrative and social links; if MPAs are located near to each other or in the same ecoregion, they will likely share similar social and environmental problems and the likelihood of cooperative actions is expected to increase (Gerber et al., 2013, Maciejewski and Cumming 2015, Bodin, 2017). MPAs run by the same organization (homophily) may present more interactions due to staff exchange and manifest trust when sharing resources (Maciejewski and Cumming 2015). However, certain particularities regarding ecological and social dynamics may produce different conservation outcomes (Alexander and Armitange, 2015), making it imperative to evaluate such interactions in the context of specific regions.

Brazilian system of MPAs and coral reefs conservation

Scleractinian corals are the main builders of current reefs and one of the most threatened ecosystems in the world. In Brazil, they are characterized by low richness, high endemism and large discontinuities along the coast (Leite Prates, 2006) and are currently threatened by bleaching, over-fishing, pollution, waste disposal, coastal

development, sedimentation, exotic species and diseases, among others (Leão et al., 2016, Zilberberg et al., 2016). Acting together, these factors reduce the health and resilience of the ecosystem (Grimsditch and Salm 2006; Leão et al.,2016; dos Santos Brandão et al., 2017).

As of July 1st 2019, there were 194 MPAs listed in the Brazilian National Registry of Conservation Units (CNUC), covering 964.153 km^2 and representing 26.5% of territorial sea. Approximately 57% of MPAs allow sustainable use and 42% are no-take areas (data from CNUC/MMA). About half of them (51%) have instituted a management council, but roughly 62% do not have a management plan, hindering the implementation of an effectively managed, ecologically representative and well connected system of MPAs (Magris et al., 2013). Additionally, several financial, operational and administrative flaws were reported by stakeholders and MPA managers during the implementation of the National System of Conservation Units (SNUC), including insufficient financial and technical resources, inadequate commitment to enforce management, and absence of coordination among agencies (Gerhardinger et al., 2011; dos Santos Brandão et al., 2017).

Coral reefs and cnidarian species are known to be present in approximately 44 Brazilian MPAs, however, up to now only 18 MPAs have been specifically created to protect coral reefs (6 under federal, 8 state and 4 municipal administration) and a few more (summing up to 27 MPAs) mention corals as conservation target in official documents. The Brazilian government, implemented the National Plans for Conservation of Coral reefs (PAN Corais) under the supervision of the National Institute for Conservation of Biodiversity “Chico Mendes” (ICMBio). A complete description and history of the PAN Corais can be found in (Zilberberg et al., 2016). This program seek to improve the conservation status of coral reefs in 18 prioritized areas along the Brazilian coast where we identifyed 33 declared MPAs. Specifically, the PAN Corais seeks to promote ecosystem integrity by the identification of priority areas for conservation, increase declaration no-take areas, strengthen surveillance and monitoring programs in coral reef environments, stimulate sustainable use of marine resources, increase knowledge on corals reefs habitats, prevent the invasion of exotic species, among others.

In this work, we evaluated the patterns of interactions and collaboration among MPAs located along the Brazilian coast, making especial emphasis on the behavior of those located in the 18 focal areas identified to develop priority actions for coral reefs conservation in the PAN Corais. Those areas include MPAs from the federal, state and municipal levels, including the oceanic islands, ranging from coral to sandstone reefs to rocky shores. For that purpose, we used graph theory and multi-layered network analysis to investigate the general topological properties of the management-based networks of MPAs in Brazil. The patterns of relational ties were analyzed with focus on the coordination and cooperation among managers, and we discuss the possible effects of the PAN Corais and geographic proximity of MPAs on management collaboration.

MATERIALS AND METHODS

Data collection

To quantify management-based interactions, we used an on-line questionnaire sent to the managers of the protected areas. Whenever possible, MPA personnel was invited over phone to fill out the questionnaire, otherwise, emails providing information on the project were sent out. In the first section, the questionnaire was designed to provide an overview of the conservation unit, followed by 8 questions regarding management interactions with other MPAs (see complete questionnaire in appendix 2). The strength of interactions were quantified in a 5-point Likert scale with respect to the perceived relevance for management or its frequency (Table 2).

Table 2 Likert scales used to quantify the strength of interactions.

Relevance	Frequency	Weight
Irrelevant	Very rarely (once in the last 5 years or less)	1
Not very significant	Rarely (once every 2 years)	2
Significant	Regularly (once a year)	3
Very significant	Frequently (every 6 moths)	4
Fundamental	Very Frequently (once a month or more)	5

Site selection: the subset of MPAs the questionnaires were sent to were selected (1) by the presence of coral reefs or other marine-related ecosystems (e.g. mangroves, coastal zones), (2) to cover a broad range of the Brazilian coast, (3) to include MPAs belonging to the three government levels (federal, state and municipal level), and (4) to include MPAs from different management categories (i.e. no-take or sustainable use).

Network definition

The management network was made up by interactions among staff in charge of the MPAs such as: equipment sharing among MPAs, reciprocal participation in management councils, staff support, and joint participation in formal training activities, conflict resolution, and joint development of surveillance, research and monitoring programs. Nodes had two attributes: the governmental level of the overseeing agency (i.e. federal, state or municipal) and its participation in the PAN Corais. Edges'

attributes were type of interaction (the layer to which it belongs) and the strength. The management-based network was defined as a weighted, undirected, edge colored network. Here we assume the same definition of color as the label or type of each interaction as proposed by Kivela et al., (2014).

Multi-layer approach

The different types of interactions were treated as separate layers in a multilayer network approach. Embedded into interaction (edges) weight are importance, cost and distance. This means that not all layers are equally important for all actors, which may be more prone to more active participation in one layer than in others depending on particular interests or expertise. The nature of each interaction (e.g transfer of knowledge and information versus equipment loan) imply different economic and/or energetic costs. Thus, some information may be lost when only the aggregate single layer (monoplex) network is evaluated, and that is why we favor a multiplex approach in this paper, despite its increased analytical complexity (Battiston et al., 2014, Kivelä et al., 2014, De Domenico 2015). Statistical analyses and network visualization were performed with the aid of the packages *iGraph* (Csardi and Nepusz, 2006) and *muxViz* (2.0.1) (De Domenico et al. 2015) implemented in R version 3.6.1.

Centrality and connectivity of MPAs

The network was topologically described through the quantification node centrality and connectivity across the different layers, characteristics that make the system more responsive to information and/or physical flows (Janssen et al., 2006, De Domenico et al., 2015). The levels of connectivity were represented by two aspects of the graph as suggested by Janssen et al., (2006): the density of the layers calculated as the ratio between the number of edges and the number of possible edges, and reachability, quantified as the network diameter or the numbers of steps needed to go from one node to any other. In this work the diameter is represented by the longest “geodesic” path found in the graph via breadth-first search algorithm. The centrality of nodes were summarized with 3 indexes: 1) the multiplexity index quantifies the proportion layers where the node appears, identifying versatile actors that are

participating in several types of interactions; 2) the strength degree is a function of the number of interactions and the sum of their strengths (i.e. relevance for management); and 3) PageRank versatility, that ranks nodes assuming that important nodes are more likely to interact with other important nodes, highlighting influential actors whose reach extends beyond just their direct connections (de Domenico et al., 2015). Centrality values were calculated following the tensorial representation of multilayer networks proposed by De Domenico et al., (2015) and represented graphically with the annular visualization tool in *muxViz* which allows to compare the centrality information encoded in the multilayered structure. This analysis makes use of the Jensen-Shannon divergence correlation method for ordering the rings, and nodes were sorted according to rank obtained in the centrality indexes, following the same radial trajectory (de Domenico et al., 2015).

Correlation of layers

We used *muxViz* and *ggplot2* to quantify and visualize dependency among different types of interactions within the network according to four similarity measures proposed by De Domenico et al., (2015a and 2015b): (a) mean global node and (b) edge overlapping or the fraction of nodes and edges which are common to all layers, (c) inter-layer assortativity, which is the Pearson correlation between the strength of nodes and their counterparts in the other layers, for all pair of layers, and (d) the inter-layer similarity obtained by computing a shorter path matrix between the all pairs nodes for each layer and then comparing the resulting distance matrices to each other using Frobenius distance.

Functional connectivity

To profile functional connectivity in the network, we removed all the edges associated with every layer in a stepwise fashion (i.e. one at a time) computing, at each step, three measures describing overall connectivity in the network: (1) the number of components, (2) the order, represented as the number of nodes in the largest component, and (3) the diameter of the largest component (Urban and Keitt, 2001). Because these measures are sensitive to the removal order, the process was

randomized 1000 times. Results where graphically summarized as dispersion plots of connectivity measures versus number of interaction remaining.

Modularity

A community structure analysis was performed over the aggregate layer of the network to detect modules that were more densely connected. This analysis identifies nodes whose reciprocal interactions were more frequent and relevant than interactions with nodes outside of their module. We ran an exploratory modularity analysis using a simulated annealing (*spin-glass*) algorithm with no *a priori* hypothesis about its structuring. Networks were visualized using the force-directed layout algorithm by Fruchterman and Reingold. These analyses were conducted using the *iGraph* package.

RESULTS

A total of 21 MPAs answered the questionnaires, covering almost the entire Brazilian coast (from Ceará to Rio Grande do Sul, Figure 15). Of those, 11 are located in focal areas prioritized by the PAN Corais (Zilberberg et al., 2016), six were no-take (3 Natural Parks, 2 Wildlife Refuges and 1 Biological Reserve) and 15 were sustainable-use areas (9 Environmental Protection Areas, 4 Extractive Reserves and 2 Marine State Parks - supplementary material 2).

The management-based network encompassed 51 MPAs (i.e., the 21 with respondent management plus the non-respondent areas that they interact with), and 131 edges distributed through 8 layers. Five MPAs that answered the questionnaire had no interactions whatsoever: the environmental protection area (APA) of Jenipabu and APA Litoral Sul de Sergipe, plus 3 areas prioritized in the PAN Corais, namely, APA Tinharé e Boipeba, APA Fernando de Noronha-RSPSP, and APA Recifes de Corais. Thus, when only active nodes are considered, the fraction of MPAs located within prioritized areas in the PAN Corais is 43% (22).

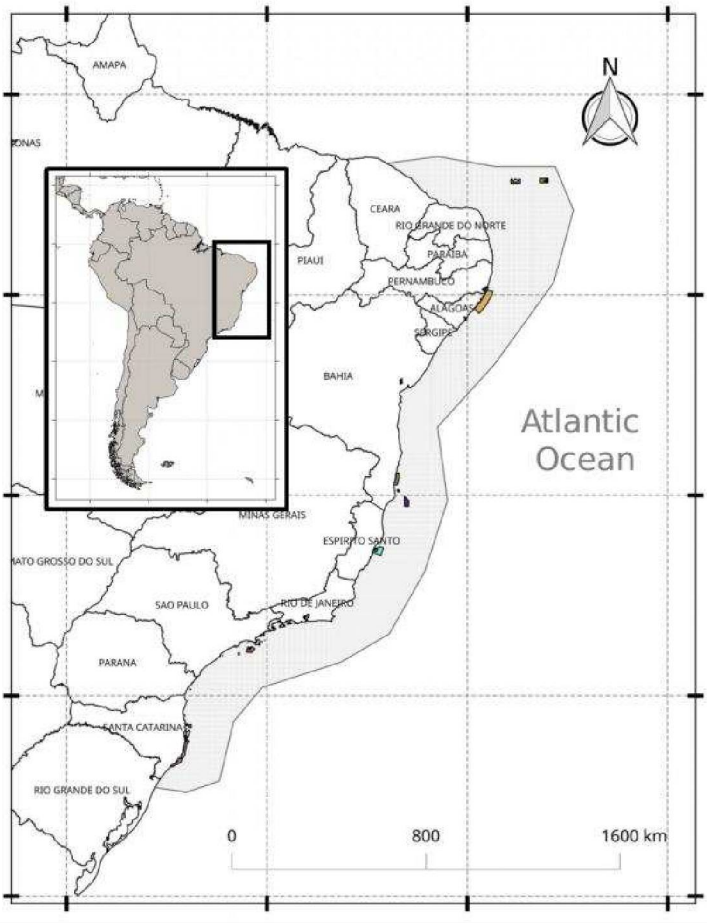

Figure 15 Geographic location of MPAs that answered the questionnaire.

Graphing the number of interactions (edges) in all managerial activities (layers) against the number of MPAs (nodes) revealed a linear relationship (Figure 16). Most MPAs either interact with one or two MPAs or do not interact at all (i.e. are absent from the layer). If all MPAs interacted with every other MPA in the network, the number of edges would increase with the square of the number of nodes present in a layer. Remarkably, this pattern holds regardless of the type of interaction considered, as evidenced by the high value of the correlation coefficient (r = 0.89). The number of participating MPAs in one individual layer does not necessarily represented an increase in the number of connections (i.e. cooperative interactions among MPAs) or strength (i.e. frequency). For example, the "monitoring" layer had lower number of nodes (i.e. participating MPAs) than the "staff support" and "conflict resolution", but all three layers showed similar number of connections. On the other hand, "formal training" involved fewer MPAs (i.e. lower number of nodes) but these interactions were perceived as more relevant for management (i.e. edges had higher strength) than the aforementioned ones (Table 3).

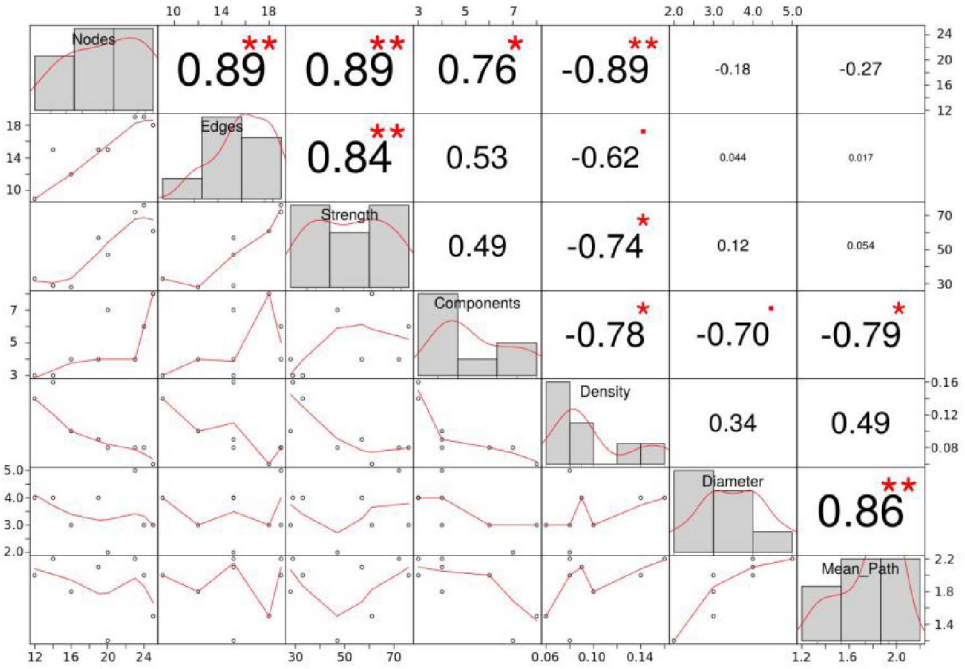

Figure 16 Correlation matrix for intralayer variables. Lines in below-diagonal scatterplots were fit using locally estimated scatterplot smoothing (loess). Pearson correlations (significant ones with stars) are displayed in above-diagonal graphs.

Layers varied considerably with respect to topology (sizes and density), hence showing different levels of connectivity and diffusion capacities (Janssen et al., 2006 - Figure 17). Surveillance, equipment loan and management council layers were more

similar in terms of the number of nodes and edges, however, varied considerably in their topologies (Table 3 and Figure 17). The conflict resolution layer had the lowest diameter formed by many small components, which may reduce node reachability. The aggregate network exhibited the lowest density and highest diameter and it has one large component and five isolated nodes representing the 5 MPAs that do not reported interactions in this network.

Table 3 intralayer summary statistics for the management-based network of MPAs in Brazil. The density of the layers was calculated as the ratio of the number of edges and the number of possible edges. The diameter correspond to the numbers of steps needed to go from one node to any other in the largest component.

Layer	Nodes	Edges	strength	Density	Diameter	Mean Path
Aggregate	51	59	403	0.05	8	3.9
Management council	25	18	61	0.06	3	1.5
Surveillance	24	19	76	0.07	3	2.0
Equipment loan	23	19	72	0.08	5	2.2
Conflict resolution	20	15	47	0.08	2	1.2
Staff support	19	15	57	0.09	4	2.1
Research	16	12	28	0.10	3	1.8
Monitoring	14	15	29	0.16	4	2.2
Formal training	12	9	33	0.14	4	2.0

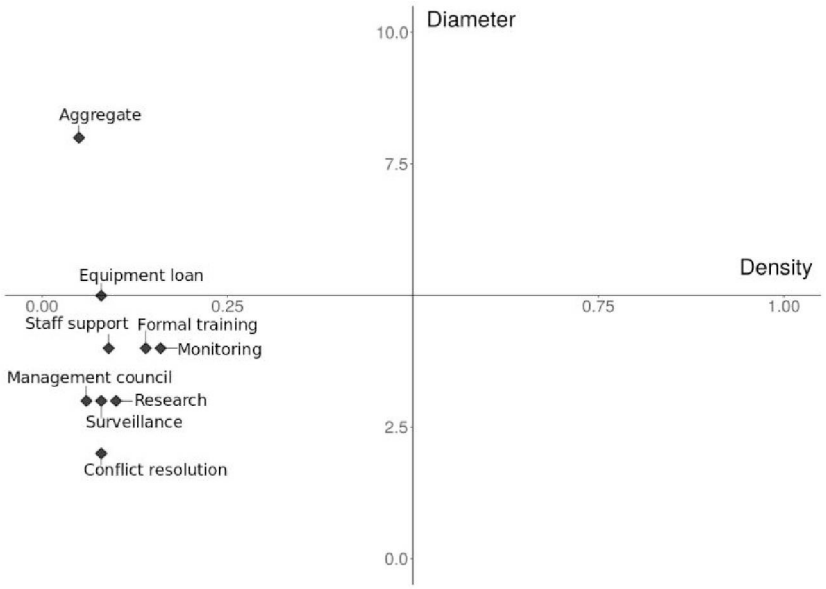

Figure 17 Multilayer connectivity space defined by density (the ratio of the number of edges and the number of possible edges) and reachability (the network diameter or the numbers of steps needed to go from one node to any other).

Node degree centrality

Only 2 MPAs had some sort of interaction with at least one other MPA in all of their management activities, i.e., only two nodes were active in all layers (multiplexity = 1.0). Those areas make up the management module formed by the no-take wildlife refuge (RVS) of Santa Cruz (54) and the sustainable-use APA Costa das Algas (16), in which the former is embedded. Both MPAs are overseen by the same manager, who naturally reported reciprocal interactions between them in all activities. Other MPAs with higher values of multiplexity were the Laje de Santos state marine park (PEM) and the RVS Archipelago dos Alcatrazes (both located in São Paulo state) and the Abrolhos national park (PARNA) (Figure 18), that were active in 7 out of the 8 layers. The latter three MPAs were identified as "high priority" in the PAN Corais. Interestingly, the most versatile MPAs were not the ones with the highest centrality. APA Costa dos Corais (27) and the extractive reserve (RESEX) of Corumbau (6), ranked 6 and 11 in multiplexity participating in 6 and 5 layers respectively, were the two more prominent MPAs with respect to strength and PageRank centrality when considering the full multilayer structure (Figure 18).

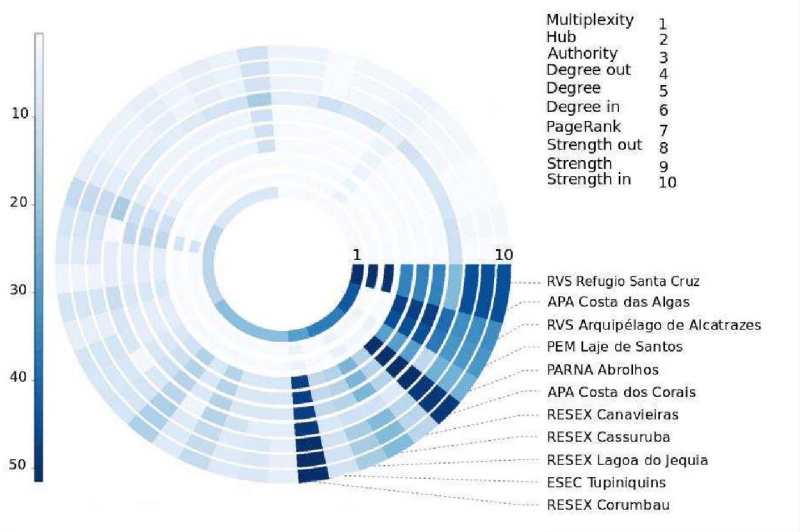

Figure 18 Annular visualization of the multiplexity versatility of nodes in the management-based network. Each ring represents a centrality descriptor, numbered from 1 to 10 from the inside out (see legend). Each sector corresponds to a MPA (only the top 11 are shown). The membership of the node in each ring corresponds to centrality score in the scale on the left. The thickness of a ring is proportional to its information entropy.

The RESEX Corumbau (6) ranked first in the strength centrality as a result of strong participation in monitoring, research, and equipment loan (Figure 19). The APA Costa dos Corais (27) ranked second in multiplexity and the aggregate network due to strong interactions with other MPAs with regard to staff support, formal joint training, and participation in the management councils. These two MPAs also appeared with the highest PageRank versatility which means that their influence can be extended over other MPAs beyond their direct connections. In terms of strength, the RVS Santa Cruz (54) and the APA Costa das Algas (16) ranked third and fourth respectively. However, they presented lower PageRank values as a consequence of their exclusively reciprocal interactions in five of the layers. In contrast, RVS Arquipélago dos Alcatrazes (39) and PEM Laje de Santos (38), also neighbouring MPAs in the same state, stood out with high PagePank versatility. The Arquipélago dos Alcatrazes is a federal MPA with an important role in the management council layer, while the state park of Laje de Santos has strong participation in training, research, and surveillance (Figure 19). Visual inspection of node centrality (Figure 19) reveals that some patterns are conserved across layers and that strength and PageRank in the multiplex network are correlated with strength and PageRank in the aggregate network. This means that having a high centrality in the aggregate layer requires a MPA to be active in several layers.

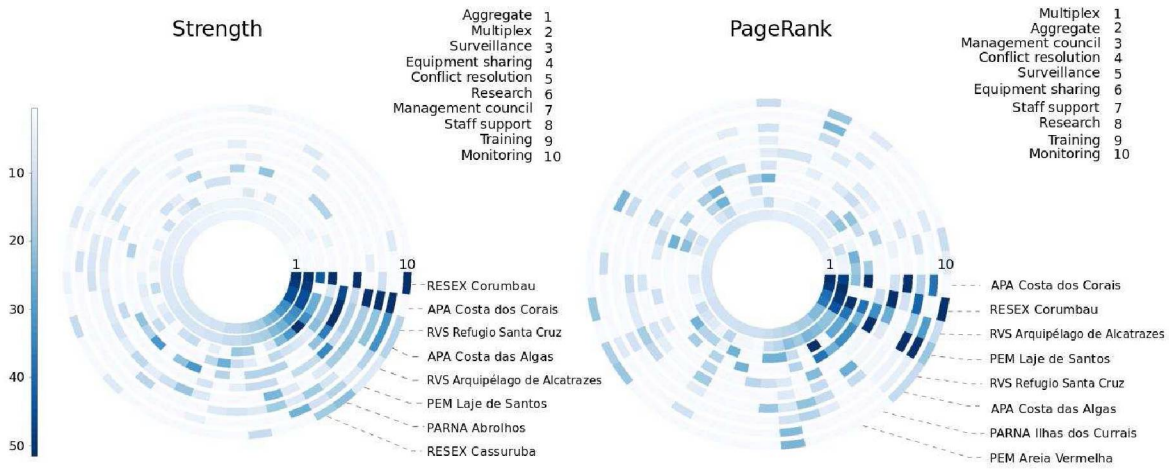

Figure 19 Annular visualization of strength and Page-rank centrality considering the whole multplexity structure.

Intralayer centrality

Aggregate layer

The aggregated network was composed of 51 MPAs and 59 edges with an average degree of 2.31 interactions per node. The network was not fully connected, composed by one large component and 5 isolated nodes (Figure 20). Additionally the aggregate network presented the higher density among the layers, and the larger mean path (3.93 steps) (see supplementary material 3-Tab. 1). A Kolmogov-Smirnov test suggest that the cumulative degree frequency follows a power law distribution (p-value = 0.76) (Figure 21), characteristic of a “small-world network”, i.e. most of the relevant links dispersed around a few nodes (MPAs) (Janssen et al., 2006, Barabási 2007). The majority of the nodes located at the center of the Fruchterman-Reingold layout are prioritized by the PAN Corais (Figure 20), indicating how they are playing a central role in maintaining the overall management connectivity.

The APAs Corumbau (6) and Costa dos Corais (27) were the most active and prominent nodes in strength and PageRank centrality (Figure 19 and Figure 20). The RVS Refugio Santa Cruz (54) and the APA Costa das Algas (16) also scored high in strength values, however, that was the consequence of strong reciprocal interactions among them, as they were only linked to the network throughout interactions with other two MPAs and hence had low PageRank centrality. The RVS Arquipelago dos Alcatrazes (39) and PEM Laje de Santos (38) obtained a high PageRank versatility, linking important components in the network.

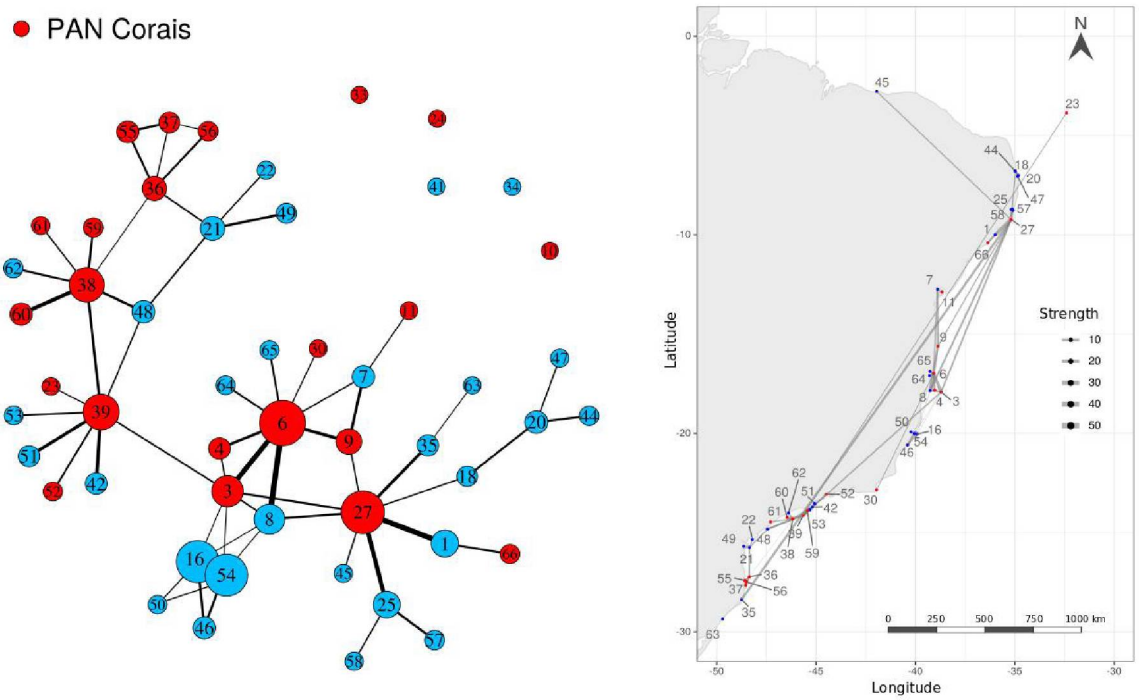

Figure 20 Network visualization for the aggregate network. Node sizes and edges width are proportional to the centrality values and interaction strengths, respectively. The location of nodes in the right panel correspond to the geographical centroid of the MPA polygon in the Brazilian coast. Red nodes are MPAs located inside prioritized areas in the PAN Corais.

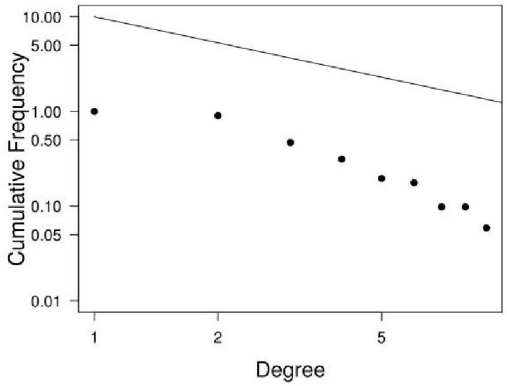

Figure 21 Cumulative frequency of nodes in the management-based network with a given degree, using the GenInd function implemented in the NetIndices package in R. The line represent the expected power law distribution. A large disparity on node degree is observed in the network with a very few nodes having more than 5 interactions.

Surveillance

The PEM laje de Santos (38) ranked fist in strength centrality, interacting with other 3 federal and 3 estadual AMPs, all located in the São Paulo state (Figure 22-A). One of those interactions was with Arquipélago dos Alcatrazes (39), ranked third in strength centrality, which interacts with 3 other MPAs forming a larger and centralized component in the layer. These results suggest an important effort in São Paulo state

to coordinate surveillance activities among MPAs. The second largest component include interactions among MPAs located in two focal areas of the PAN Corais, the RESEX Canavieras, RESEX Corumbau and the PARNA dos Abrolhos also indicating some degree of coordination aimed at protecting coral reefs in Bahia.

Management Council

This layer is composed by the highest number of nodes (n=25) dispersed as several small components ranging in size from 2 to 5 nodes each (Figure 22-B). APA Costa dos Corais (27) ranked first in the strength centrality followed by APA Guadalupe (25) with which it reciprocally interacts, forming a single component. RVS Arquipelago dos Alcatraces (39) and PEM Areia Vermelha (20) ranked first in PageRank versatility, both participating in different components. The high number of small components suggests that participation in management council takes place among geographically close areas, except for the link between APA Costa dos Corais and APA Baleia Franca (35), an interaction that is maintained across different layers.

Monitoring

This layer presented the highest density (Table 3). RESEX Corumbau (6) ranked first with respect to strength and PageRank centrality and showed the same interactions as in the research layer (Figure 22-C). PARNA dos Abrolhos (3) also plays an important role in this layer, ranking second for both centrality indexes, taking part in monitoring programs with four other MPAs and enlarging the geographical scope and density of the layer by linking one module of MPAs from Bahia with the RVS Arquipelago dos Alcatrazes, located in São Paulo state.

Research

This layer was mainly composed by two geographically aggregated, star-like components, with important participation of PAN Corais prioritized MPAs (Figure 23-D). RESEX Corumbau (6) again played a central role in one of the components and obtained the highest strength, sharing research activities with 4 MPAs from Bahia state that also protect coral reefs environments. PEM Laje de Santos (38) ranked first in this same layer and also had the highest PageRank prominence, participating in research activities with 5 other MPAs.

Formal training

The APA Costa dos Corais (27) showed the higher strength degree, and the PEM Laje de Santos (38) (the only MPA from the state sphere participating in this layer) ranked first in PagRank centrality (Figure 22-E). Despite its low number of nodes (12) this layer had geographically distant interactions like the one between APA Baleia Franca (35) and APA Costa dos Corais (27) and the one between RVS Archipelago dos Alcatrazes (39) and the PARNA Fernando de Noronha (23) (Figure 22-E).

Equipment sharing

Equipment sharing was reported in 22 MPAs. RESEX Corumbau (6) once more highlighted as the most prominent node in this layer, due to the strong interactions with other MPAs in Bahia and with the APA do Pau-Basil (30) located in São Paulo state (Figure 22-F). Other relevant MPAs sharing equipment were RVS Archipelago dos Alcatrazes (39), a federal MPA interacting with 3 other state MPAs in close geographic range and PARNA Ilha dos Currais (21), a federal no-take area that interacts with 3 other federal MPAs in the same component (Figure 22-F).

Staff Support

APA Costa dos Corais (27) plays a prominent role in the staff support layer, with the highest strength and PageRank values. It also interacts with several MPAs from different states, some as far as with the APA Baleia Franca (35) more than 3000 km to the south (Figure 22-G). Most of the MPAs that Costas dos Corais interacts with do not participate in the PAN Corais, suggesting that the support may be related to a wider set of management issues besides coral reefs conservation. The second largest component found in this layer was formed by 4 federal MPAs, located in very close geographic proximity protecting reef environments in southern Brazil (Figure 22-G), specifically in the islands and rocky shores of São Francisco do Sul, Tamboretes and Florianópolis, in Santa Catarina state.

Conflict resolution

The highest strength centrality in this layer was obtained by the management module formed by the RVS Santa Cruz (54) and APA Costa das Algas (16) (Figure 22-H). APA Costa dos Corais (27) also had important participation, ranking third in strength centrality and first in PageRank versatility. Arquipélago dos Alcatrazes (39)

and PEM Areia Vermelha (20) again showed active participation obtaining PageRank values as high as in the management council layer (Figure 22-B).

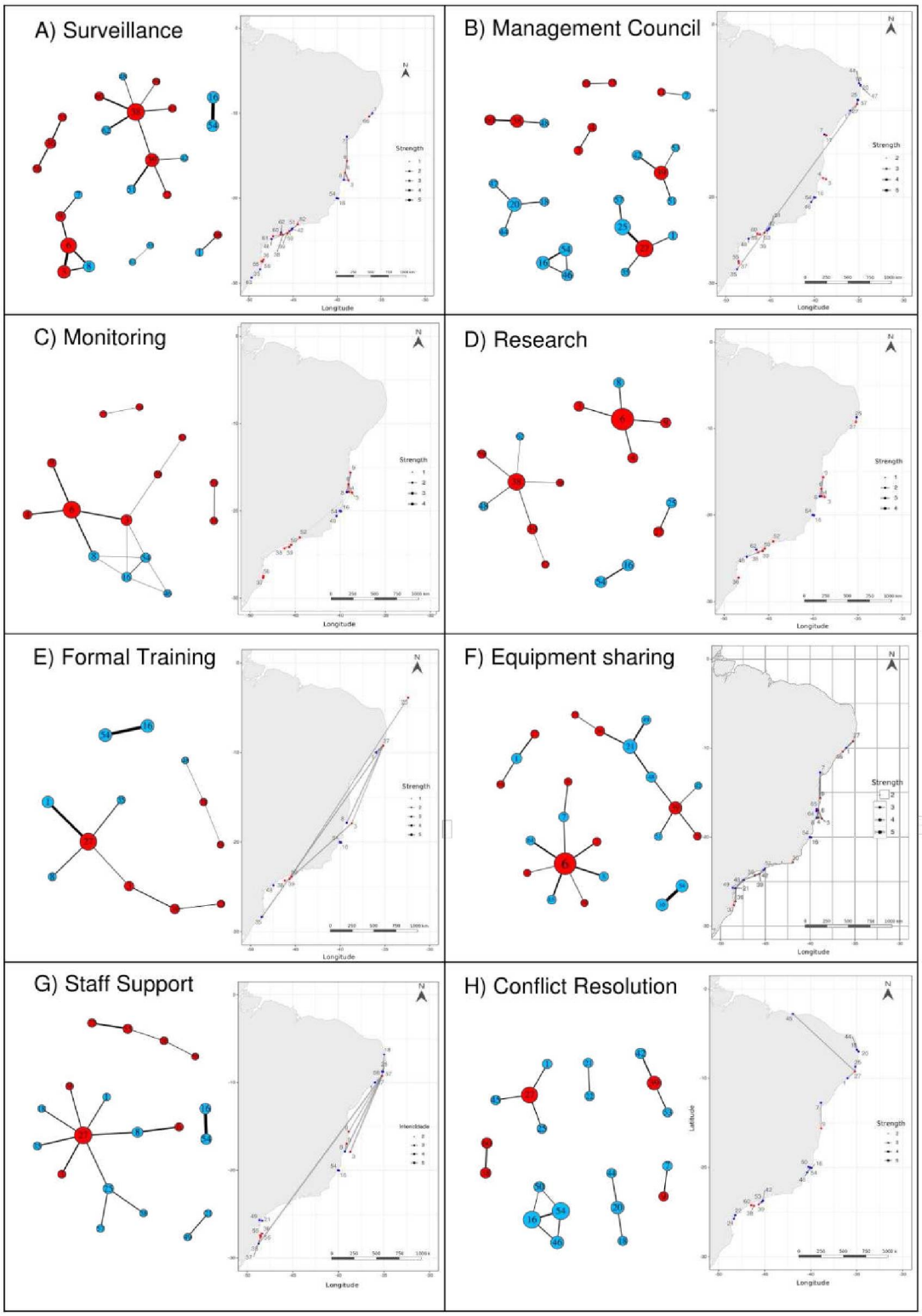

Figure 22 Intralayer node centrality. Red nodes are MPAs located inside prioritized areas in the PAN Corais. The size of nodes is proportional to strength centrality, and edges width is proportional to strength of the interaction.

Correlation of layers

Node overlapping among layers was relatively low, with the exception of management council and conflict resolution that had 32.6% of shared nodes and clustered together. All the other layers were grouped in a second cluster (Figure 23 - A). Participation in surveillance programs always showed similarities close or above 20%, reaching a maximum of 32% of shared nodes (equipment loan). In terms of edge overlapping two main clusters were observed (Figure 23 - B). One grouped MPAs co-participating in management council and conflict resolution (sharing 59.7% of edges) and the staff support and official training layers (46.7% of edges). The other main cluster was formed by research and monitoring layers sharing more than half (59.6%) of edges and 44.6% of edges between equipment loan and surveillance. These main clusters were also recovered when looking at the assortativity of layers calculated by the Pearson correlation (Figure 23 - C). These results suggest that participation in management councils, formal training, staff support and conflict resolution make up one module of correlated activities, while the other includes research, monitoring, equipment loan and participation in surveillance programs.

Research and participation in surveillance programs presented the highest assortativity (r =0.83), followed by participation in management councils and conflict resolution (r = 0.69), confirming the interdependence between these layers. Analysis of similarity by shorter path allows for detection of similarity between layers by measuring how distant nodes would be if they were equally accessible through the different layers. No significant clustering was detected, but equipment loan was the most dissimilar layer, presenting the longest path to all other layers due to its longer linear component (Figure 22 - D). On the other hand, the highest similarity was again between participation in management council and conflict resolution (0.78), probably because these two layers composed by several small components (Figure 22-B and H).

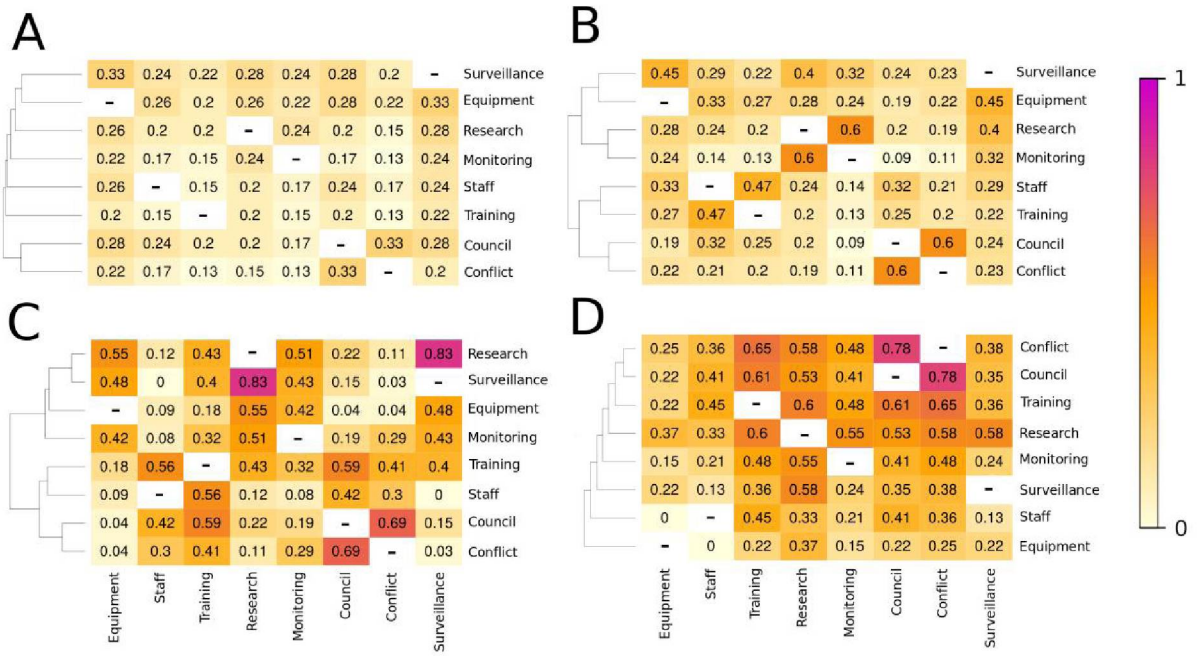

Figure 23 Correlation among layers of the management-based network of MPAs. A) Mean node overlapping, representing the proportion of nodes shared between layers; B) mean edge overlapping, representing the proportion of shared edges between layers; C) Inter-assortativity by Pearson correlation; D) Inter-similarity by shortest-path distance among nodes. A value of 1 means complete similarity among layers.

Functional connectivity

All relational ties evaluated here seem to contribute similarly to the overall connectivity in the management-based network. No sharp drops in connectivity were detected when the different types of interactions were randomly excluded (Figure 24). The number of components increases almost linearly, from a few when all 8 types of interactions were considered to more than 35 when only one interaction remains. The order of the largest component also decreased steadily. The diameter of the graphs initially presented some variation, but dropped steadily in size after 5 interactions were removed. The observed levels of functional connectivity may be conferring high resilience to the network against edge loss, reducing the dependency of the system on any particular type of interaction.

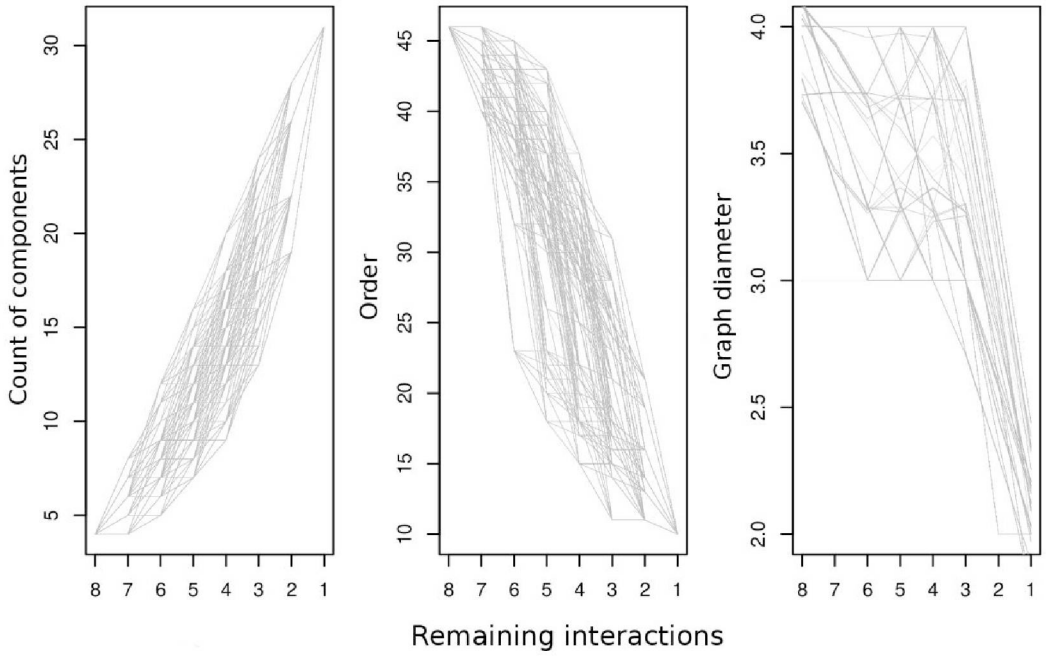

Figure 24 Edge thinning of the management-based network. The plots track changes in three different network statistics (y-axis) as edges representing each type of interaction are removed until none is left in the network (x-axis). Each experiment was randomly replicated 1000 times. Count of components represent the number of components remaining in the graph, i.e. nodes connected by at least on edge; the order is the number of nodes in the largest component, and the graph diameter of the largest component. The lack of abrupt shifts in all curves indicates that there is no single type of interaction that is significantly more frequent than others in the network.

Modularity analysis

Modularity analysis indicates that geographic proximity between MPAs may increases the likelihood of interactions. After removing isolated nodes, modularity was low (0.11) and the network was clustered, consisting in 8 communities ranging in size from 3 to 10 nodes (Figure 26; Csárdi et al., 2016). In most cases, subgroups were comprised by MPAs located in a single state. Examples are subgroups 1, 2, 4, 6 and 7 (Figure 26). Other subgroups include MPAs located in two different states like subgroup 3 that clustered several MPAs in Bahia with Rio the Janeiro's APA do Pau-Brasil, and the subgroup 8, that clustered several MPAs in São Paulo with Pernambuco's PARNA Fernando de Noronha, more than 2000 km away. Finally, the subgroup 5 aggregates MPAs in 5 different states, from APA da Baleia Franca in Rio Grande do Sul to APA Delta do Parnaiba located in Maranhão (Figure 26).

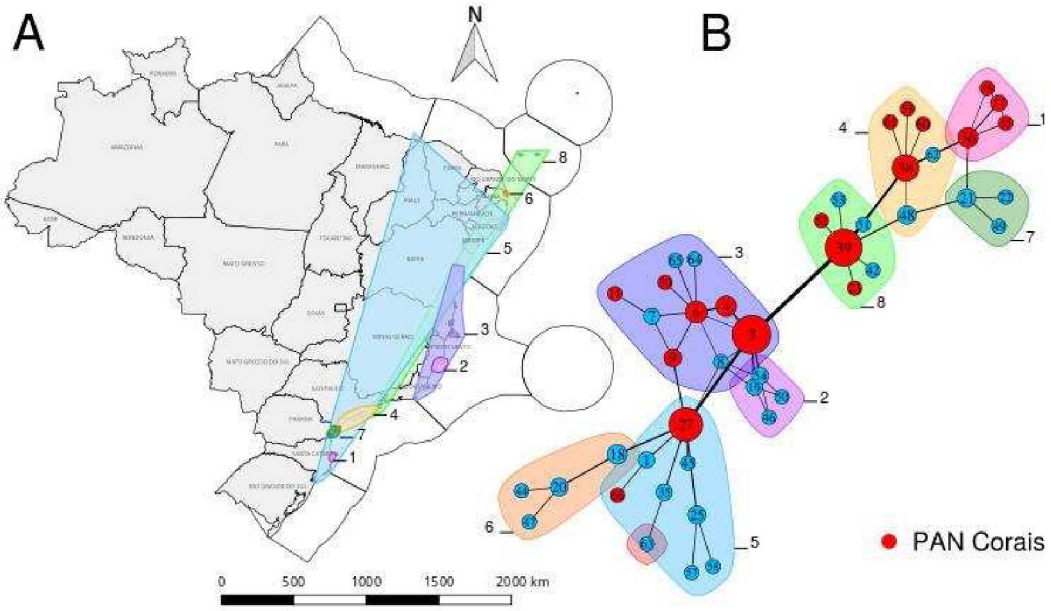

Figure 25 Modularity results for the management-based network. A. Geographic distribution of modules along the coast. Black lines delimit marine ecoregions as defined for the Tropical Southwestern Atlantic province (Spalding et al., 2007). Colored polygons correspond to the network modules represented in the graph. B. Topological representation of modules. Red nodes are the MPAs located inside PAN Corais prioritized areas and blue nodes are otherwise. Size of nodes and width of edges are proportional to betweenness centrality, respectively.

Four management modules were identified in which at least 50% of MPAs are prioritized in the PAN Corais (Figure 25-B), indicating that such management modules may be playing an important role in conservation. Module 3 displays strong equipment sharing, research, and monitoring interactions (Figure 22-C, D and H respectively) among MPA located in two focal areas of the PAN Corais: 1) from RESEX Canavieiras to the south of RESEX Corumbau, and 2) Abrolhos's bank. Furthermore, this management module is covering all of the Eastern Brazilian coastal ecoregion (Figure 25-A), the most extensive and richest area of coral reefs in Brazil (Leão and Kikuchi, 2001). The grouping of PARNA Fernando de Noronha with MPAs in São Paulo state was due to formal training interactions. Additionally, the southern islands and rocky shores in São Francisco do Sul, Tamboretes, and Florianópolis in Santa Catarina state, seem to be benefit from the presence of management module 1, composed by 4 MPAs with staff support and surveillance activities interactions. On the other hand, the subgroup 5 that include MPAs from several states cooperate in management councils and conflict resolution. However, most of the MPAs included in this module do not participate in the PAN Corais, suggesting that the main driver of tie building is not coral reef conservation.

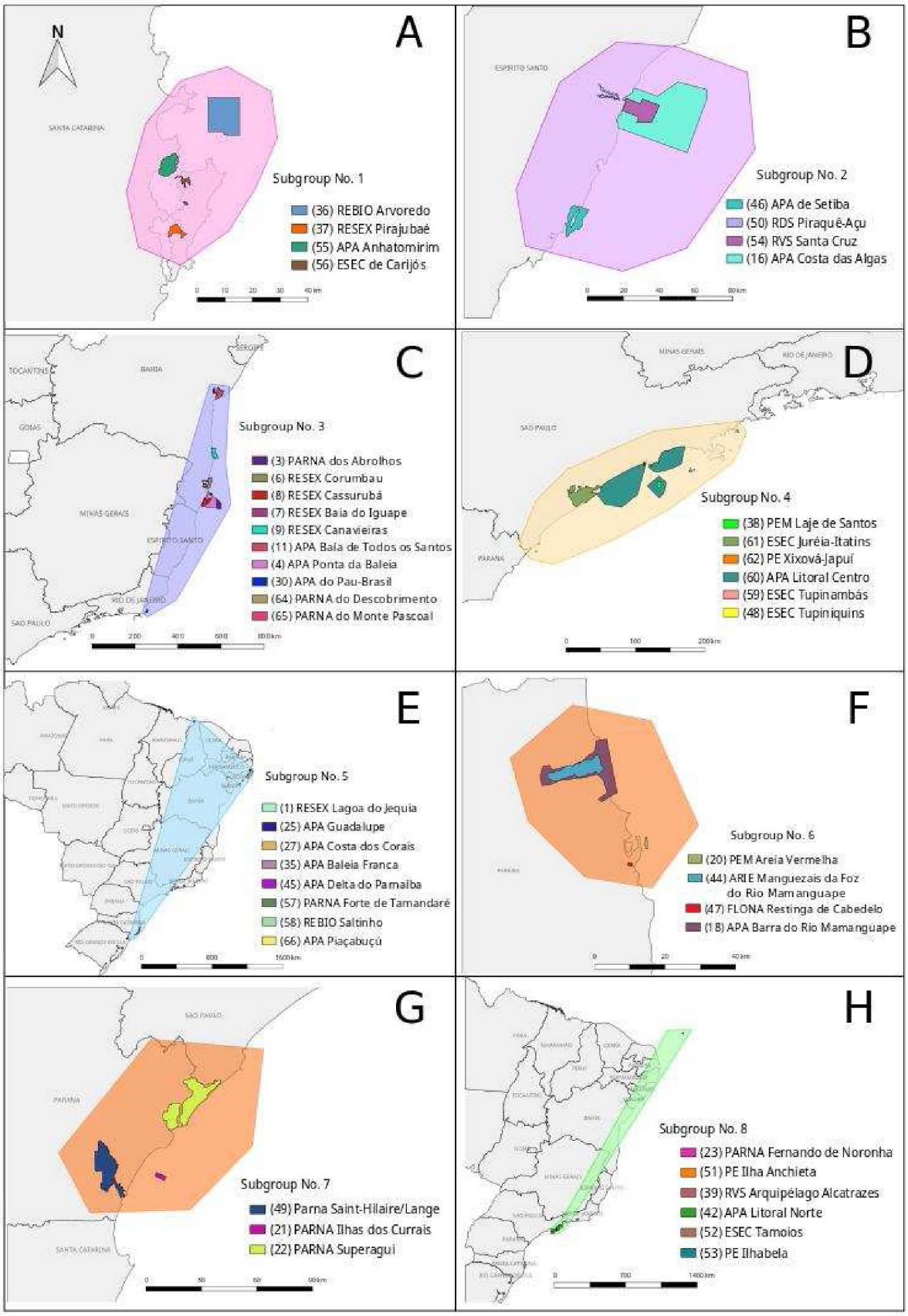

Figure 26 Geographic representation every subgroup founded in the modularity analyses. Number inside parenthesis indicate the subgroup in Figure 26.

DISCUSSION

Geographic proximity, and not centralized planning seems to be driving interactions that make up the management-based network of MPAs in Brazil, as reported elsewhere (e.g. Maciejewski and Cumming, 2015). In most cases, subgroups were comprised by MPAs located within a single state, regardless the level of the overseeing governmental agency or participation on the PAN Corais (Figure 25). The detection of MPA management modules is fundamental in conservation since they may become natural conduits for coordination and control efforts during local implementation of the conservation measures proposed at higher managerial levels. Such modules may also lead to cooperation emergence and persistence among MPAs, enhancing the resilience of the protected areas. However, in spite of those modules, the Brazilian network of MPAs is still made up by low-density layers. The aggregate layer has the features of the so called “small-world networks”, that have low average path length and high levels of clustering (Janssen et al., 2006, Barabási 2007, Zhang et al., 2019), with most of the activity centered in few MPAs. This pattern was also described for two networks of private protected areas in South Africa (Maciejewski and Cumming, 2015) and suggests that non-neighbouring nodes may be reached by a small number of steps (Colchester 2016), conferring potential for information flow through the network. Yet, this scale-free feature may make the system vulnerable to “hub removal” i.e., the most versatile MPAs, as they play a vital role in maintaining the currently observed levels of connectivity (Albert et al., 2000). A dense graph has the number of edges close to the maximal possible number of edges (close to 1) and all of the surveyed layers were far from that (Table 3). Low-density networks may foster diversity in management practices, tailored by diverse sets of environmental problems and different views about the use of the natural resources (Boding and Norberg, 2005). This may also limit the direct exchange of experiences, learning opportunities and the flow of knowledge among management (Janssen et al., 2006; Pretty and Ward, 2001). Node overlapping among layers was also relatively low (see multiplexity in Figure 18 and Figure 19) suggesting low capability of the MPAs to simultaneously perform several activities, as it was already observed during SNUC implementation (Gerhardinger et al., 2011; dos Santos Brandão et al., 2017).

We also provided evidence that the implementation of the National Plan for Conservation of Coral reefs (PAN Corais) could be enhancing collaboration among MPAs at a regional scale, but not at the national level, particularly in building specific ties in the network. The wider participation of MPAs prioritized by the PAN Corais in the surveillance and monitoring layers may be the outcome of efforts made to control and monitor fishing activities in coralline environments as part of the Specific Objective 2 (see Zilberberg et al., 2016). However, those areas are scattered across different components within those layers, indicating the need to increase coordination of surveillance and monitoring efforts that could extend these activities to the national level. Additionally, the targeting Specific Objective 4 of the PAN Corais ("increase knowledge about poorly studied reef environments") seems to be enhancing connectivity in the research layer (Figure 22 D) as seen by the development of joint research activities among prioritized MPAs. Two sustainable-use MPAs prioritized in the PAN Corais (APA Costa dos Corais and the RESEX Corumbau) stood out in strength and PageRank centrality, giving them a prominent position inside the network (Figure 19). However, both areas showed some sort of specialization with respect to the type of interaction. While APA Costa dos Corais was more prone to participate in layers related to flow of knowledge and information among staff members. These layers are management council, conflict resolution, staff support and formal training and formed one of the clusters recovered by the the interlayer assortativity analysis (Figure 23-C). RESEX Corumbau played a more relevant role in the second cluster recovered in that analysis, which is characterized by technical or logistics-related layers, (surveillance, research, monitoring, and equipment loan - Figure 19 and Figure 23-C). Parsing these differences among MPAs is a complex task due to their historical, political and administrative components, demanding an in-depth view of the individual MPAs' differences in profile and expertise of staff members.

Participation in the PAN Corais do not seem to be the main driver for interactions in the other layers. Out of the 33 MPAs inside focal areas, 5 did not reported any kind of interaction with any other MPAs (isolated nodes) and 9 were not mentioned by any respondent and were not active in the network. Some of those areas are recognized by their high diversity of coral and reef environments, and their inclusion in the network is essential. Examples are the first Brazilian MPA, the Biological Reserve (REBIO) of Atol das Rocas, the only atoll in the Southwestern Atlantic (Almeida et al.,

2000), Parcel do Manoel Luis State Park, recognized as RAMSAR site since the year 2000 and APA of the Archipelago of Trindade and Martim Vaz plus the Natural Monument (MONAT) of the islands Trindade and Martim Vaz and Monte Carlo Columbia, that together constitute the largest MPA in Brazil.

We observed a clear interdependence between participation in the management council and conflict resolution. This correlation was recovered in 3 of the 4 indexes evaluated (Figure 23), and confirms the value of the management councils as the instance in which environmental conflicts are settled in Brazil. For instance, Nobre and Schiavetti (2013) described how the deliberative management council in the RESEX Cassurubá, is a democratic space that gives voice to different actors and was instrumental to the establishment of fishing regulation in the protected area. However, the constitution of a management council is not enough to create mutual understanding among interested actors and historical reasons may lessen the resolution of environmental problems. This was observed in the APA Jenipabu, one of the isolated nodes in this work. Soares et al. (2018) reported that after more than 12 years management council institution, few actions were developed, and the APA was unable to implement effective surveillance programs, leading to “a high degree of unsustainability”.

The high similarity between research and monitoring layers (sharing 24% and 60% of nodes and edges respectively, Figure 23-A), highlight the correlation between those activities. This suggests that when two MPAs are performing joint research activities, the chances to implement a joint monitoring program increases (and vice verse). Research layer also showed high pearson correlation the implementation of surveillance programs in the network. This inter-dependency suggests that technical knowledge is reflected in the implementation of more relevant surveillance programs, reinforcing the importance of investing in science-based studies to guide conservation actions (Drummond et al., 2011, Simpfendorfer et al., 2011, Cvitanovic et al., 2013).

The functional connectivity analysis showed that all relational ties contributed similarly to the overall connectivity of the network, conferring high resilience to the management-based network against the removal or loss of specific types of interactions (Urban and Keitt 2001). This is a positive feature of the network because

the progressive loss of relational ties among MPA management is likely due to undergoing political changes in Brazil. Such changes may have a profound impact on the injection of economic and human resources into conservation, eroding the capacity of MPAs to effectively develop all needed activities to ensure effective governance (levis et al., 2020)

CONCLUSION

To our knowledge, this is the first attempt to analyze the dynamics of marine conservation in Brazil from a social network perspective. The results suggest that interactions among MPA managerial staff are organically formed and have strong historical components, whose details are beyond the reach of our methods. Network analysis revealed that such interactions resulted in management modules that are mostly locally formed, although some surprising long-range interactions were also detected, particularly with MPAs that were exceptionally active in more than one layer. We suggest that these MPAs may enhance coordination of conservation efforts by acting as conduits of conservation policies formulated as higher levels, such as those outlined by the PAN Corais, whose positive effects on conservation were also detected in our analysis. While every attempt to include additional MPAs in the network and to foster further interactions across layers is desirable, this may be hard to achieve due to the sheer geographical scale of the network and scarcity of funding, which will likely be aggravated in coming years. Focusing on those versatile MPAs to effect change within and beyond their modules may potentialize the limited resources available to marine conservation in Brazil. We also highlight the importance of further research into those MPAs with the goal of understanding the specific reason that made them outstanding in the first place

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Chapter 3

Brazilian network of MPAs: a Social-based perspective

ABSTRACT

Understand the role social actors play during the establishment and implementation of MPAs and MPAs networks is of great importance and may increase conservation effectiveness by ensuring the participation of society in the decision-making process. To achieve this, the Brazilian government implemented the National System of Conservation Units (SNUC) establishing the mechanisms to regulate social participation in management, modifying the governance system and the way the government and social organizations interact. However, to date, there are no quantitative studies on the effective inclusion of the different social actors in the management and conservation of MPAs in Brazil. In this work, we used graph theory and multi-layer network analysis to investigate the patterns of interactions among MPAs and civil society groups. To quantify such interactions, an on-line questionnaire was sent to the managers of the protected areas with questions regarding participation of social actors during environmental education and formal training activities, participation in management councils, joint development of surveillance, research, monitoring, and volunteer programs. Two visual approaches were implemented, one allowing to identify individual nodes performances, and other with social actors aggregated by the sectors they belong to providing a wider view of the role of different social sectors play in the MPA network. The social-based network was composed of 141 nodes interacting through 181 edges distributed in seven layers. All responding MPAs (n=21) reported interactions with social actors. The governmental agencies appear as an important sector, summing up the highest strength degree and playing a central role connecting different subgroups in the graph. The participation in the management council was the most active layer with the presence of 104 nodes (73.7% of total nodes) and 102 edges (56.3%). Some sort of specialization regarding the type of interaction the different actors get involved in was observed, with the government agencies dominating the surveillance and formal training activities, the research layer dominated by universities, and the monitoring activities mostly performed by universities and NGOs. The community groups only showed outstanding representation in the layer of environmental education. The similarity among layers was very low in terms of node and edge overlapping, always lower than 10% of shared nodes, and a maximum of 21% of similar edges. A clear and abrupt transition from connected to disconnected was observed every time the layer of participation in the management council was removed indicating a strong dependency on this layer to maintain the currents levels of connectivity. Modularity analysis identifies 12 management modules, mostly composed of one individual MPA and the social actors with whom it interacts at the local level. These results showed that the management councils are important tools the Brazilian government is implementing to ensure social participation in the management of protected areas. However, this may not materialize yet in integrated management actions, and efforts should be made to increase participation of the different sectors of society, especially the local

communities, in the decision-making process intending to strengthen governance and increase the resilience of the system.

Key words: Socio-ecological system; multilayer networks, marine governance

RESUMO

Entender o papel dos atores sociais durante o estabelecimento e implementação de AMPs e redes de MPAs é de grande importância e pode aumentar a eficácia da conservação, garantindo a participação da sociedade no processo de tomada de decisões. Para tanto, o governo brasileiro implementou o Sistema Nacional de Unidades de Conservação (SNUC) estabelecendo os mecanismos para regular a participação social na gestão, modificando o sistema de governança e a forma como o governo e as organizações sociais interagem. Entretanto, até o momento, não existem estudos quantitativos sobre a efetiva inclusão dos diferentes atores sociais na gestão e conservação das AMPs no Brasil. Neste trabalho, utilizamos a teoria dos gráficos e a análise de redes em multicamadas para investigar os padrões de interação entre as AMPs e grupos da sociedade civil. Para quantificar tais interações, um questionário on-line foi enviado aos gestores das áreas protegidas com perguntas sobre a participação dos atores sociais durante as atividades de educação ambiental e capacitação formal, participação em conselhos de gestão, desenvolvimento conjunto de programas de vigilância, pesquisa, monitoramento e voluntariado. Duas abordagens visuais foram implementadas, uma permitindo identificar o desempenho de nós individuais, e outra com atores sociais agregados pelos setores aos quais pertencem, proporcionando uma visão mais ampla do papel dos diferentes setores sociais na rede AMP. A rede de base social foi composta por 141 nós interagindo através de 181 bordas distribuídas em sete camadas. Todas as AMPs respondentes (n=21) relataram interações com os atores sociais. As agências governamentais aparecem como um setor importante, constituindo o mais alto grau de força e desempenhando um papel central conectando diferentes subgrupos no gráfico. A participação no conselho gestor foi a camada mais ativa, com a presença de 104 nós (73,7% do total de nós) e 102 bordas (56,3%). Observou-se algum tipo de especialização quanto ao tipo de interação em que os diferentes atores se envolvem, com os órgãos governamentais dominando as atividades de vigilância e treinamento formal, a camada de pesquisa dominada pelas universidades e as atividades de monitoramento realizadas em sua maioria por universidades e ONGs. Os grupos comunitários só mostraram uma representação destacada na camada de educação ambiental. A semelhança entre as camadas foi muito baixa em termos de sobreposição de nós e bordas, sempre inferior a 10% dos nós compartilhados, e um máximo de 21% de bordas semelhantes. Uma transição clara e abrupta de conectado para desconectado foi observada toda vez que a camada de participação no conselho gestor foi removida, indicando uma forte dependência desta camada para manter os níveis atuais de conectividade. A análise de modularidade identifica 12 módulos de gestão, compostos na sua maioria por uma AMP individual e os atores sociais com os quais interage a nível local. Esses resultados mostraram que os conselhos de gestão são ferramentas importantes que o governo brasileiro está implementando para garantir a participação social na gestão das áreas protegidas. Entretanto, isso pode ainda não se materializar em ações de gestão integrada, e esforços devem ser feitos para aumentar a participação dos diferentes setores da

sociedade, especialmente das comunidades locais, no processo de tomada de decisão que pretende fortalecer a governança e aumentar a resiliência do sistema.

Palavras-chave: Sistema socio-ecológico. Rede multicamada. Governanca marinha

iNTRODUCTION

Marine Protected Areas (MPAs) are globally recognized as important management tools supporting ecosystem-based conservation, and during the last decades their numbers have increased dramatically worldwide (Thorpe et al., 2011). These areas, when well managed, help maintain ecosystem health while assuring social and economic development (IUCN-WCPA, 2008). However, difficulties may arise, because different groups with stakes on protected areas management (e.g. government agencies, community groups, NGOs) may not coordinate efforts and/or share resources, hindering the achievement of conservation goals.

Individual MPAs benefit from networking through ecological and social links, increasing conservation and administrative efficiency (Roberts and Hawkins 2000). Maintaining MPA networks requires good governance so that all affected actors have a say in the decision-making process (Graham et al., 2003). Understanding the role that social actors play during the establishment and implementation of MPAs and MPAs networks is of great importance, especially when such participation is considered relevant for the achievement of Aichi Biodiversity Targets (CBD 2013). The social component of MPA networks may improve conservation effectiveness by empowering communities and thus enhancing cooperation in addressing environmental problems as management learn and share experiences with social actors (Bodin, 2017; Bustamante and Vanzella-Khouri, 2011). Additionally, the management of individual MPAs is enhanced as knowledge is shared across the network. However, not all networks are equally structured and particularities of ecological and social dynamics may produce different conservation outcomes (Alexander and Armitange, 2015).

Marine governance in Brazil is challenging due to its complexity, large extension and jurisdictional overlaps. Brazilian MPAs date back to 1979 when the Biological Reserve of Atol das Rocas was created. Since then the system has grown steadily in size and scope, demanding more resources and integrated planning with a higher systemic view (Drummond et al., 2011). To achieve this goal, the National System of Conservation Units (SNUC) was instituted in July 2000 with the enactment of the Law No. 9,985. SNUC was conceived thinking that protected areas (PAs) must be planned and managed in a way that encourages integration among them. Additionally, it established mechanisms that regulate the participation in management, seeking to improve the relationship between the state, citizens and the environment. One innovation introduced by SNUC was to divide the PAs into two groups based on their objectives and the type of resource use: areas for integral protection (“no-take”) and

sustainable use areas, that attempt to make conservation compatible with resource exploitation.

Furthermore, different initiatives have been instituted by the Brazilian government to strengthen the MPAs system, address the Aichi Biodiversity Targets and stimulate civil society participation and integration into conservation. Some of those initiatives deal specifically with marine and coastal areas, such as: the National Plans for Conservation of Endangered Species (PAN - designed to identify and guide priority actions to combat threats against endangered species), the (discontinued) Program on Network of Marine and Coastal Conservation Units (RUMAR), which was strongly focused on the integration of the MPAs and their infrastructure (Figueroa et al., 2016), besides multilateral-projects developed by the Brazilian government agencies in cooperation with international organizations, private institutions, and civil society. Two examples of those are the TerraMar and GEFmar projects, geared towards building networks that increase ecological representativeness, operational capacity and management effectiveness through research, surveillance and monitoring (www.mma.gov.br/). However, several problems arose during the implementation of the Brazilian system of MPAs and their effective consequences are largely unknown (Gerhardinger et al., 2011). To date, there are no quantitative studies on the effective inclusion of the different social actors in the management and conservation of MPAs in Brazil.

Looking at the system of Brazilian MPAs from a network perspective allow us to identify relevant actors and describe patterns in their interactions (Alexander and Armitange, 2015). This approach provides a conceptual model with theoretical basis strongly grounded on social network analysis (Wasserman and Faust 1994). A MPA network is defined by its actors (nodes) connected via selected ties (edges) defined with respect to similarity (e.g., geographical location, government level), relationship (trust), interactions (e.g., knowledge exchange, formal training) or flow (e.g., information, equipment) (Borgatti et al., 2009). Additionally, descriptive statistics of network structures (e.g., centrality, modularity) allows to establish a quantitative relationship among features, processes and outcomes (Alexander and Armitange, 2015). The objective of this work was to apply this approach to investigate the topological properties of the social-based network of MPAs in Brazil. Coordination and cooperation among MPAs and interested actors were transformed into relational ties with the goal of verifying if the network of Brazilian MPAs has been effective in ensuring participation of civil society in the decision-making process.

MATERIALS AND METHODS

To quantify social interactions, we used an on-line questionnaire sent to the managers of the protected areas. Whenever possible, MPA personnel was invited over phone to fill out the questionnaire. Otherwise, e-mails providing information on the project were sent out. The first section of questionnaire was designed to provide an overview of the conservation unit, followed by 6 questions regarding interactions with civil society groups (see complete questionnaire in appendix 1). The strength of interactions were quantified in a 5-point Likert scale with respect to the perceived relevance for management or its frequency (Table 4).

Table 4 likert scale table to quantify the strength (relevance) of interaction.

Relevance	Frequency	Strength
Irrelevant	Very rarely (once in the last 5 years or less)	1
Not very significant	Rarely (once every 2 years)	2
Significant	Regularly (once a year)	3
Very significant	Frequently (every 6 moths)	4
Fundamental	Very Frequently (once a month or more)	5

Site selection: the subset of MPAs the questionnaires were sent to were selected (1) by the presence of coral reefs or other marine-related ecosystems (e.g. mangroves, coastal zones), (2) to cover a broad range of the Brazilian coast, (3) to include MPAs belonging to the three government spheres (federal, state and municipal level), and (4) to include MPAs from different management categories (i.e. no-take or sustainable use).

Definition of the Social-based network

The social network is composed of the interactions between MPA management and civil society sectors, forming two different sets of nodes. All lines in the graph are among nodes belonging to the different subsets, generating an interaction matrix of m nodes (= MPAs) with n nodes (= social actors), a n x m matrix. MPAs interactions with social groups were represented by edges defined in terms of participation in environmental education activities, joint development and/or participation in management councils, surveillance, research, monitoring and volunteer programs.

The social actors were grouped in one of the following sectors, set as node attributes: NGO, governmental agencies, universities, community groups, or “other” (industry, tour operators and local schools were grouped together due to their low representativeness). Edge attributes were interaction’s type and strength (representing the relevance of interactions scored according to the Table 2). This weighted network was treated as an undirected graph because answers from the questionnaire were obtained only from a subset of MPA managers, making impossible to evaluate the full extent of reciprocity in all interactions that occur in the network.

The different types of interactions were considered as separate layers in a multilayer network approach as explained in chapter 2. In summary, at the MPA leve the focus was on node centrality summarized with 3 indexes: 1) the multiplexity index; 2) the strength degree; and 3) PageRank centrality. Centrality values were calculated following the tensorial representation of multilayer networks proposed by De Domenico et al., (2015). At the network level, we considered: (1) the patterns of intra-layer connectivity, that allows to identify how responsive the system is for to the information and/or physical flows (Janssen et al., 2006, De Domenico et al., 2015); (2) layers interdependencies according to four similarity measures proposed by De Domenico et al., (2015a and 2015b); (3) the functional connectivity, to identify the types of interactions that contribute more for the observed levels of connectivity, and (4) the modularity or community detection to identify management clusters formed by strongly connected MPAs (see methods in Chapter 2 for details). Two visual approaches were implemented, one allowing the quantification of single node statistics, and other with social actors aggregated by sectors providing a broader view of each sector’s role on the network.

RESULTS

After more than 78 phone calls and 65 e-mail messages sent to 66 protected areas, 21 answers to the questionnaire were obtained. The spatial location of those respondent MPAs covering almost the entire Brazilian coast (from Ceará to Rio Grande do Sul, see Figure 15). Six of those were no-take areas (3 Natural Parks, 2 Wildlife Refuges and 1 Biological Reserve) and 15 were sustainable-use (9 Environmental Protection Areas, 4 Extractive Reserve and 2 Marine State Park) (Suppelementary material 2).

The social-based network was composed of 141 nodes interacting through 181 edges distributed in seven layers. All responding MPAs (n=21) reported interactions with social actors. The government agencies were the most represented type of social actor in the network with 35 nodes, followed by NGOs with 27 nodes (Table 5)().

Table 5 Social sectors represented in the network interacting with the 21 respondent MPAs. Are shown the number of nodes and edges; *Number of AMPs with which each social sector interacts; and the average strength of interactions withing social sector. The total strength is obtained summing up all edeges.

Social actors	Nodes	Edges	Number of MPAs*	Average strength	Total Strength
Government agency	35	55	15	3.69	203
NGOs	27	34	13	3.41	116
Community group	23	30	11	3.83	115
Universities	20	43	18	3.27	141
Other	15	20	10	3.70	74

The aggregate social network was obtained by summing, across layers, edge strengths connecting the same nodes. It was composed by 141 nodes and 150 edges (Figure 27). The network is not fully connected presenting 6 components: one large component with 124 nodes and 5 small ones with 5, 4, 3, 3 and 2 nodes respectively. The mean number of links per node was 2.12 and the main distance in the network was 4.6 steps. A Kolmogov-Smirnov test suggest that the cumulative degree frequency follows a power law distribution (p-value=0.99) (Figure 28) with most of the relevant links concentrated around a few nodes. This is characteristic of the so-called small-world networks (Janssen et al., 2006, Barabási 2007), suggesting few dominant actors with relatively high degree of centrality. The top 5 MPAs were the same in stength and PageRank centrality rankings. PARNA dos Abrolhos (PA3) was the most prominent node, followed by RESEX Baia do Iguape (PA7), APA Baleia Franca

(PA35). Government is the most important sector, with the highest aggregate strength and playing a central role connecting different subgroups in the graph (Figure 27). The Environmental Police Department (BPAmb) and the national institute in charge of executing and promoting SNUC actions, The Chico Mendes Institute for Biodiversity Conservation (ICMBio) connect MPAs on a national level (Figure 27-A), although is unclear if their actions provide flow of information and/or resources across the network.

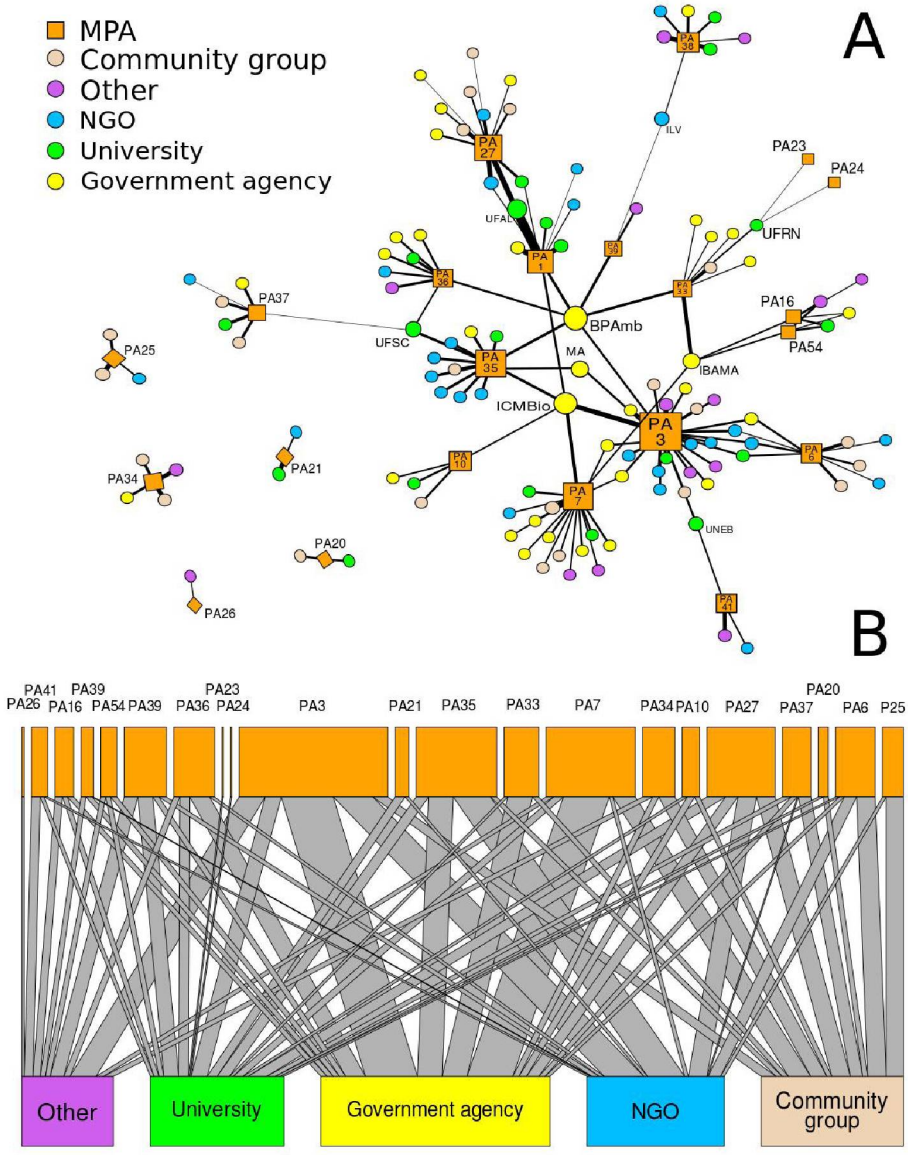

Figure 27 Visualization of the aggregate social network of MPAs. A) Visualization representing individual nodes centrality. Edges thickness and node sizes are proportional to strength values. Only names of nodes with high centrality are shown for simplicity. ILV= Laje Vida Institute; BPAmb= Environmental Police Department; MA= Brazilian Navy; ICMBio= Chico Mendez Institute for Biodiversity Conservation; IBAMA=Brazilian Institute of the Environment and Renewable Resources; UFSC= Federal University of Santa Catarina; UFAL= Federal University of Alagoas; UFRN= Federal University of Rio Grande do Norte; UNEB= Bahia State University. B) bipartite visualization aggregating the edges strength by the category of the social actors. The size of the square is proportional to aggregate strength centrality for the social sector.

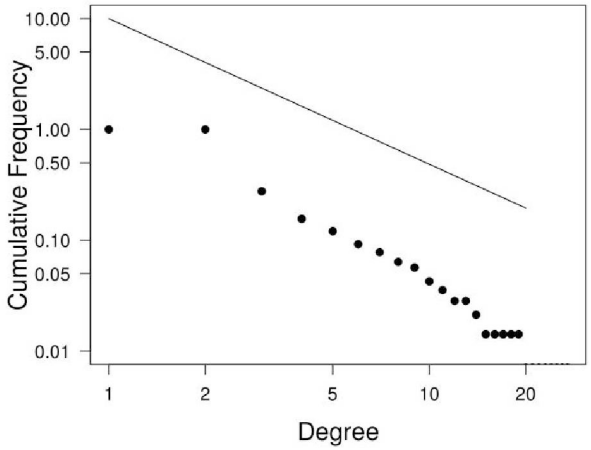

Figure 28 Cumulative frequency of nodes in the social network with a given degree using the GenInd function implemented in the NetIndices package in R. The line represent the expected power law distribution. A large disparity on node degree is observed in the network with a very few nodes having more than 10 interactions

However, the social network of MPAs varied in several characteristics within layers. Participation in the management council was the most active layer with 104 out of 141 nodes (73.7%) and 102 out of 150 edges (68%), and displayed the higher strength and link density among layers (supplementary material 3). Other active layers were participation in environmental education, research and monitoring with 36, 25 and 24 active nodes respectively. The layer of formal training presented the lowest number of nodes and edges, composed by a unique three-way (triadic) interaction.

Figure 29 summarizes layer topologies along the density (fraction of possible edges present in the layer) and diameter (number of steps needed to go from node to any other) axis. Aggregate and participation in the management council showed identical configurations with high diameter and low densities. Monitoring, volunteering and and environmental education layers have low density and the lowest diameter. These layers have several small components composed by individual MPAs interacting with social actors only at the local level. Research and surveillance also have low densities, but slightly higher diameters, indicating formation of larger components with participation of more social actors on those layers, suggesting integration at regional scales. The formal training layer have the highest density, but it represents a unique triadic interaction among one governmental agency with two different MPAs -RESEX Baia do Iguape and APA Ilhas de Tinhare e Boipeba -. Finally,

we highlight the absence of layers characterized by high density (interactions with several MPAs) and diameter (countrywide span).

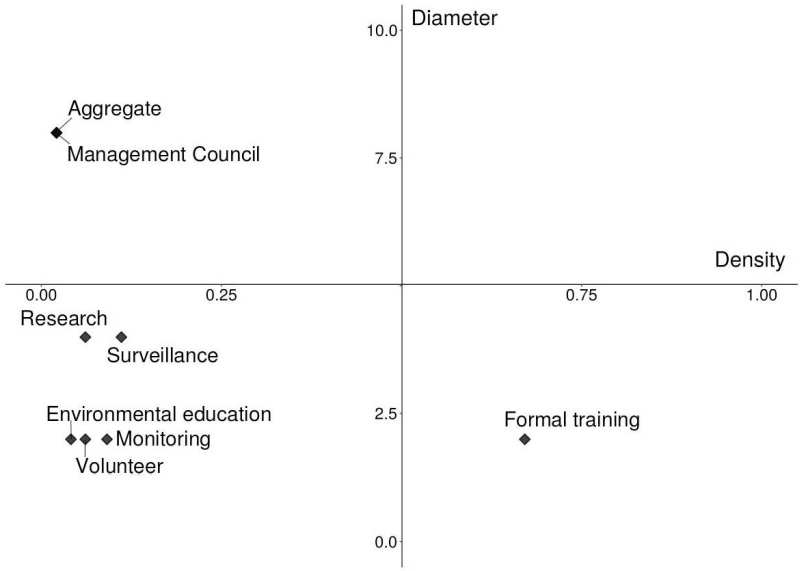

Figure 29 Intra-layer connectivity represented as function of the diameter of the graphs and its density.

Node degree centrality

Three centrality indexes were used to identify the MPAs playing more versatile roles within the social network. The RESEX Lagoa de Jequia (PA1) was the top node, participating in 6 layers (multiplexity = 0.85), followed by APA Litoral Sul de Sergipe (PA41), REBIO de Arvoredo (PA36), and APA Costa dos Corais (PA27), all participating in 5 layers (multiplexity = 0.71). PARNA dos Abrolhos (PA3) ranked 7 in multiplexity (active in 4 layers), but was first in degree, strength, and PageRank centrality when considering the whole multiplexity structure (Figure 30). The more versatile social actor was the Federal University of Alagoas (UFAL - SA60), ranked 8 in multiplexity (0.57), participating in 4 out of the 7 layers, yet all interactions were with two of the most versatile MPAs (APA Costa dos Corais and RESEX Lagoa de Jequiá), both located in Alagoas state. Other social actors with relatively high multiplexity (0.42) participating in 3 layers were ICMBio, 3 universities (The University of Bahia, UEBA, SA86, The Federal Institute of Alagoas IFAL (SA79) and The Federal University of Paraná, UFPR, SA44), and one NGO (Instituto Biota de Conservação).

PARNA dos Abrolhos (PA3) ranked first in strength and PageRank centrality in both the aggregate and multiplexity networks (Figure 30), as a result of a prominent role in management council and monitoring layers, in which it interacts with 18 and 7 different social actors respectively (Figure 30). The second-ranked node for these two centrality measures was the RESEX Baia do Iguape (PA7), as a result of an important participation in management council layer, where it interacts with 19 social actors. There were, however, only two additional interactions, one in the formal training layer with the ICMBio, and other in environmental education with local communities. RESEX Lagoa de Jequiá (PA1) and the APA Costa dos Corais (PA27), two MPAs with high multiplexity indices, ranked third and fourth in strength and PageRank centrality, respectively. Lagoa de Jequiá had an important participation in the research, environmental education, surveillance and volunteering layer whereas Costas dos Corais was prominent in research and surveillance (Figure 30). Top three social actors in strength were: ICMBio (SA87), with important contributions to the volunteer and formal training layers; UFAL (SA60) interacting with RESEX Lagoa de Jequia (4 layers) and with the APA Costa dos Corais (2 layers) and the Environmental Police Department (SA54) with an (expected) important role in the surveillance layer, interacting with 4 MPAs (Figure 32-B) but also participating in the management councils of 2 other MPAs.

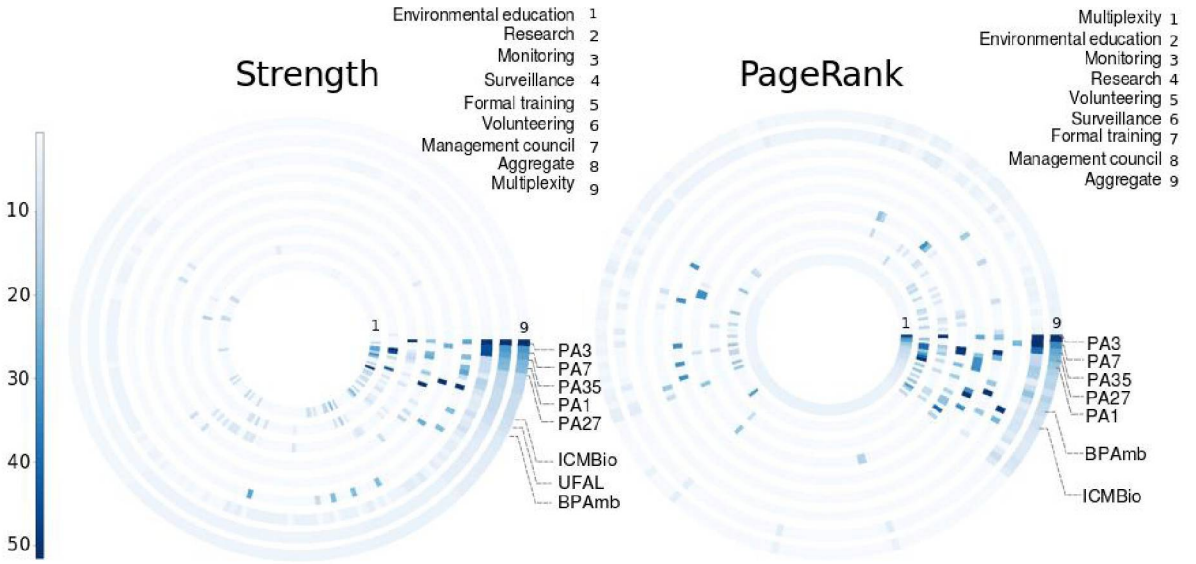

Figure 30 Annular visualization of strength and PageRank centrality of nodes. Each ring represents one type of interaction, including the aggregate layer and the multiplexity structure. Each cell corresponds to a node, with cells following the same radial trajectory corresponding to the same node. Colors code the value for the centrality index. Only the top 5 of the MPAs and the first social actors of the top 20 in each index are shown.

Intra-layer centrality

The different layers showed variation with respect to topology and node centrality across all layers surveyed in this study, summarizes in Figure 31.

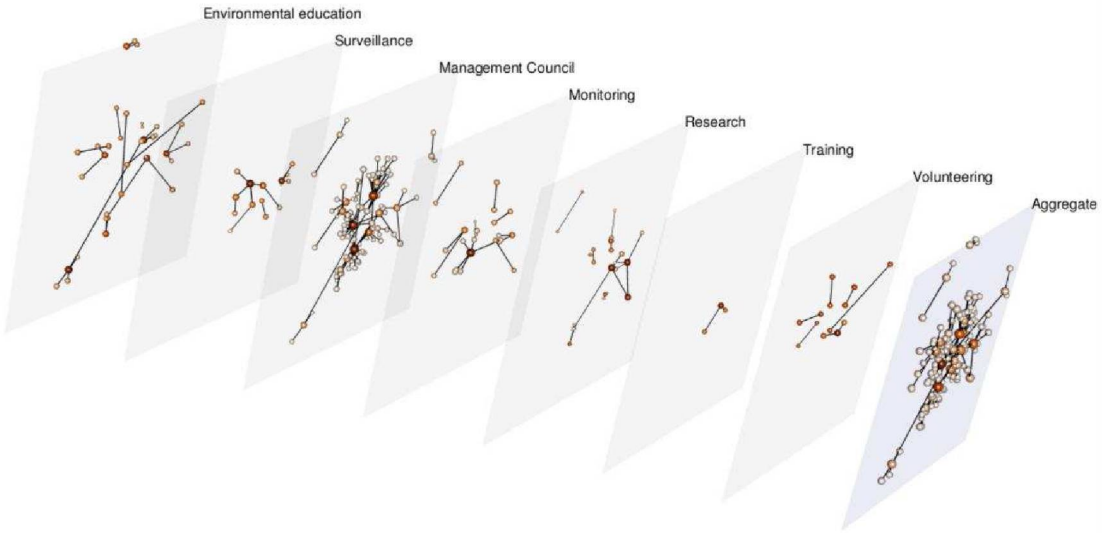

Figure 31 Intra-layer topologies of the social network of MPAs in Brazil. The size of the nodes represent the strength value in each layer, and the color is proportional to PageRank centrality, darker nodes with higher values. The size of the edges is proportional to the strength of the interaction. The position of nodes is conserved along the layers.

Environmental Education

Out of the 21 surveyed MPAs, 13 reported participation in environmental education activities with 23 different social nodes. All type of social actors participated in environmental education activities, with a noteworthy participation of community groups (Figure 32-A). APA Jenipabu (PA34) presented the higher strength value and ranked first in PageRank centrality, followed by REBIO Arvoredo (PA36), RESEX Lagoa de Jequiá (PA1) and APA Costa dos Corais (PA27) all with degree of 3. In general, this layer was formed by many small components composed by one MPA interacting with local actors. The only social actors interacting with more than one MPA were municipal schools from Aracruz (SA24, Figure 32-A), participating in environmental education activities developed in nearby RVS Santa Cruz (PA54), which is embedded in APA Costa das Algas (PA16). These results suggest that all environmental education activities are occurring at a local scale.

Surveillance

In this layer, 6 MPAs interacted with 9 social actors, and almost all of them were government agencies (Figure 32-B), with only one community group (Community Association of Tinharé e Boipeba Island) interacting with the APA Ilhas de Tinharé e Boipeba (PA10). BAPamb (SA54) was the more prominent actor, presenting the highest strength centrality and ranked first in PageRank centrality. The environmental police supports surveillance activities in 4 different MPAs, 3 of which (PA3, PA39, and PA33) were prioritized in the PAN Corais. This indicates advances in the control and monitoring fishing activities in coralline areas. APA Costa dos Corais (PA27), which spans more than one state border, ranks second in strength and PageRank centrality, receiving support from the two state (SA20 and SA34) and two federal environmental agencies (SA58 and SA42).

Management council

Participation in management council was the most active layer (73.7% and 56.3% of all nodes and edges - Figure 32-C). All types of social actors were involved, but government agencies have slightly stronger participation. Three MPAs stand out in strength and PageRank centrality, having the most numerous and diverse participation of social actors during their management councils: PARNA dos Abrolhos (PA3), RESEX Baia do Iguape (PA7) and the APA Baleia Franca (PA35). The Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) has top centrality degree and highest strength, taking part in the management council of 4 different MPAs. It is followed by ICMBio, which participates in the management council of the three most prominent MPAs, ranking first in PageRank centrality among social actors.

Monitoring

Eight MPAs reported monitoring activities with participation of social actors, being universities were the most prominent, followed by NGOs. Notably, none reported participation of local community groups. PARNA dos Abrolhos (PA3) has top strength, degree and PageRank centrality (Figure 32-D). It interacts with 7 nodes in a star-like component with an important contribution from NGOs with focus on different taxonomic groups and ecosystems (marine mammals, birds, sea turtles and coral

reefs). The other components of the graph were composed of individual MPAs developing monitoring activities with different universities, and only two government agencies, namely The São Paulo State Fisheries Institute (IP/SP) and The National Center for Bird Conservation (Cemave) interacted with APA Laje de Santos (PA38) and PARNA dos Abrolhos, respectively. This difference in the behaviour between universities targeting several MPAs and NGOs working mostly in one particular area suggests that individual attributes of MPAs (biological diversity, administrative feature, geographic location, etc.) are the chief causes of partner choice when planning and implementing monitoring efforts (Bodin et al., 2019). For example, it could be more likely for NGOs provide economic support if the MPA is known as a renowned and famous hot-spots of biodiversity (such as PARNA dos Abrolhos, that harbors all the shallow water species registered for Brazil), while universities tend to do field work in the states where they are located.

Research

The research layer is made up by the interactions of 11 MPAs with 14 social actors: 10 universities, 3 NGOs and 1 community group (Figure 32-E). APA Costa dos Corais (AP27) was top ranking in degree and PageRank centrality due to its interactions with the 3 different social sectors, including the only community group found in this layer, Peixe Boi association, a locale-base group that works to ensure environmental preservation through tourism, observation and environmental education. RESEX Lagoa de Jequiá (PA1) ranked first in strength (it interacts with 4 universities) and second in PageRank centrality. Five universities performed research activities in two different MPAs but only the UFAL stands out with high strength and PageRank centrality.

Formal training

This layer presented the lowest overall participation: ICMBio interacts with APA Ilhas de Tinharé e Boipeba (PA10) and the RESEX Baia de Iguape (PA7); in both cases, edges's strength = 3 (Figure 32-F).

Volunteering

The volunteer layer was composed of 5 two-way (dyadic) interactions and one triad; thus, this activity is performer mainly at the local scale. ICMBio (SA87), that has a volunteer program, was the only node linking two MPAs: RESEX Lagoa de Jequiá (PA1) and PARNA dos Abrolhos (PA3) (Figure 32-G). The social actors classified in this work as “others” had the highest strength. They included were the municipal and state basic education institutions from Sergipe state, that interact with APA Litoral Sul de Sergipe (PA41), some diving operators volunteering in the REBIO Arvoredo (PA36), and The Subaquatic Monitors Group, interacting with the PEM Laje de Santos (PA38).

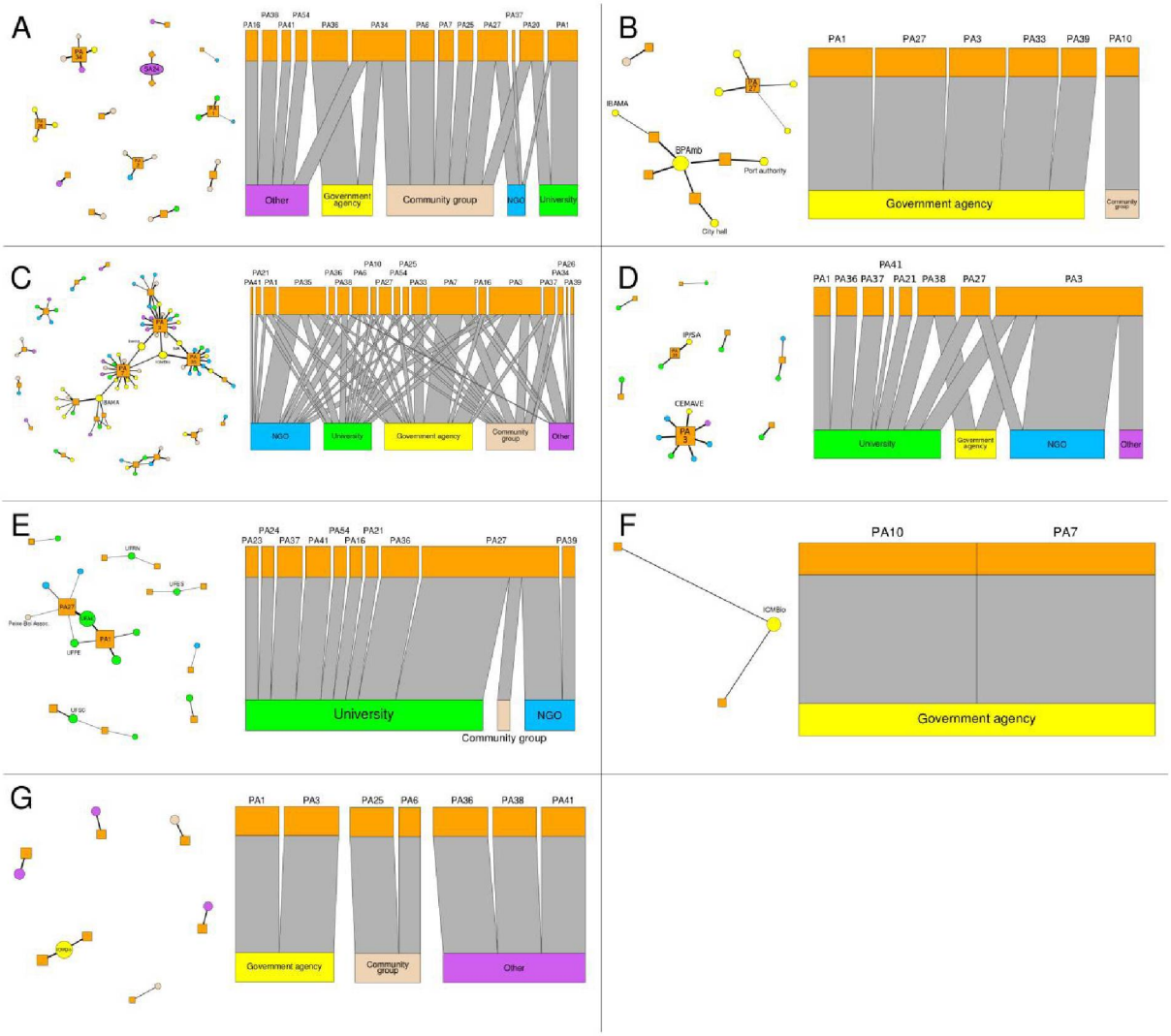

Figure 32 Intralayer variation in the social network of MPAs. Each panel presentin one layer (type of interactions), with the graph on the left representing individual nodes centrality, and on the left bipartite visualization aggregating the edges strength by the category of the social actor. Edges thickness and node sizes are proportional to strength values. A= Environmental education; B= Surveillance; C= Management council; D= Monitoring; E= Research; F= Formal training; G= volunteering.

Correlation of Layers

The similarity among layers was very low in terms of nodes (1-9% overlap, Figure 40-A) and edges (0-22, Figure 33-B), with average similarity values of 3.1% and 17.8% respectively. Spearman correlations among the strength of nodes and their counterparts in other layers show some patterns (Figure 33-C). Two main cluster were observed: one made up by volunteering and formal training (the latter showing null correlation with most of the other layers) and the second formed by two sub groups, one including research, surveillance and environmental education and another formed by monitoring and participation in management council; the latter two layers have the highest correlation values (0.5). In terms of the shortest path between all pairs of nodes (Figure 33-D), participation in management councils stands alone, due to its long diameter (the shorter path distance between nodes was 8 steps in a component with 124 nodes). All the other layers were extremely similar, as expected, since most of them were formed by several small components.

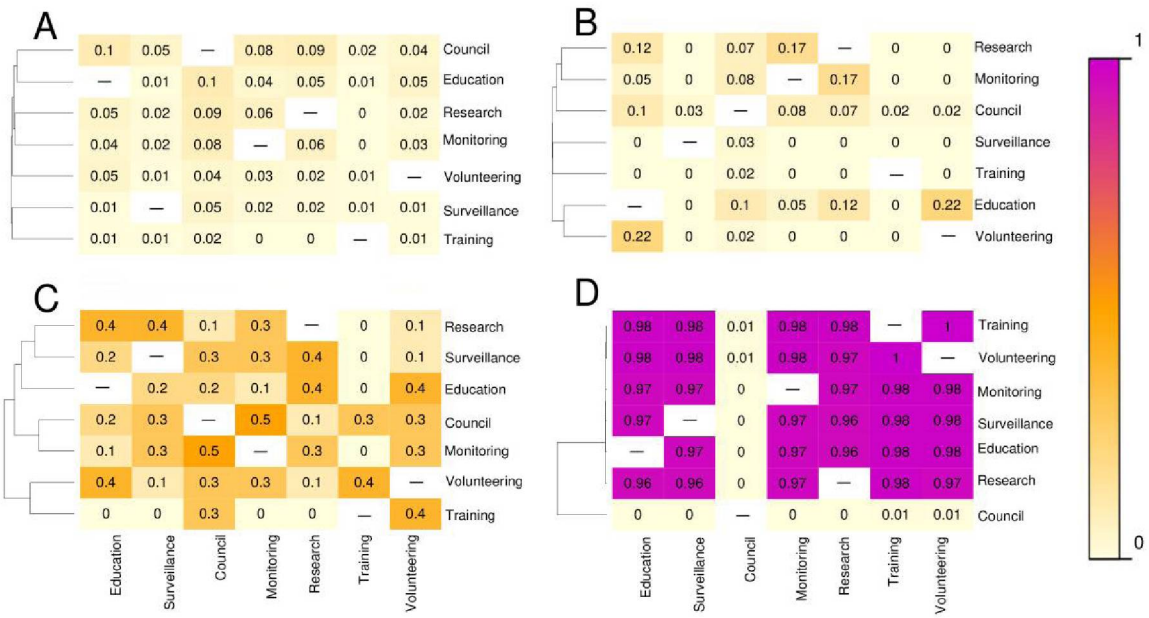

Figure 33 Correlation among layers of the social network of MPAs. A) Mean node overlapping, representing the proportion of nodes shared between layers; B) mean edge overlapping, representing the proporion of edges shared between layers; C) Inter-assortativity by pearson correlation; D) Inter-similarity by shortest-path distance among nodes.

Functional connectivity

A clear and abrupt transition from connected to disconnected was observed in the social network every time the layer of participation in the management council was removed, suggesting that this layer alone is responsible for most of the network connectivity. Figure 34 illustrates that not only the number of components increases but also the size of those components decreases steeply after elimination of the management council layer. The trends in the graph diameter show some increase during initial remove of edges, which results by the replacement of direct path among distant nodes by longer stepping-stone paths, dropping sharply whenever these stepping-stone paths are lost (Urban and Keit, 2001).

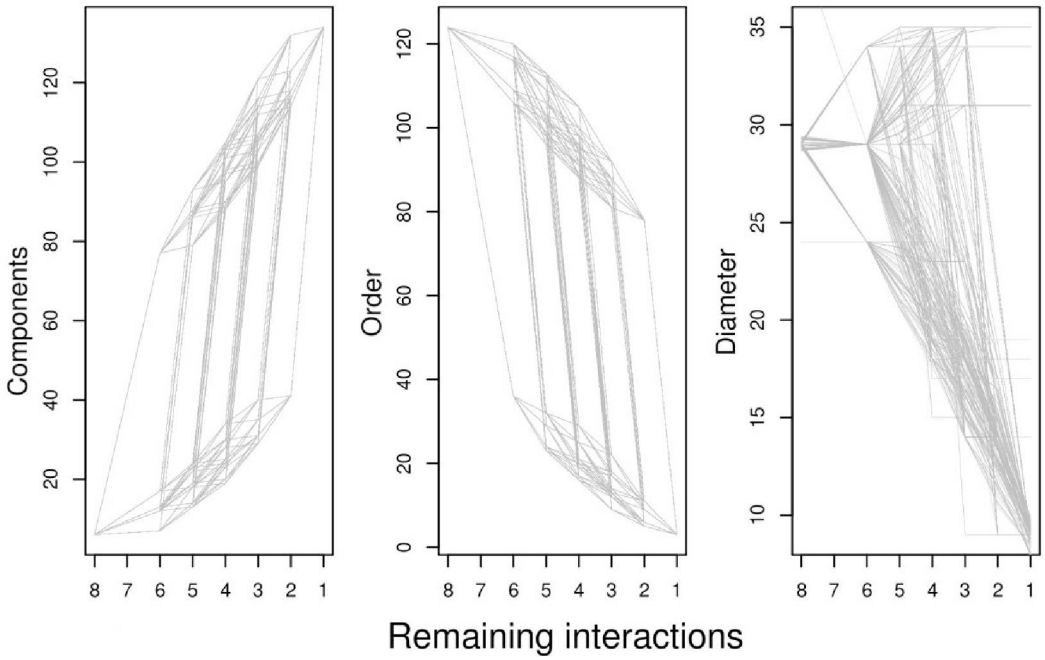

Figure 34 Edge thinning. Trends in the number of components, the number of nodes in the largest component (order), and the diameter of the graphs as the type of interactions were sequentially removed. The process was randomly repeated 1000 times.

Modularity analysis

The social-based network of MPAs had a modularity value of 0.19 and consisted of 12 subgroups, ranging in size from 4 to 21 nodes (Figure 35). Most of subgroups were made up of one MPA and the social actors with whom it interacts at the local

level, resembling the management council topology. Two governmental agencies (BPAmb and ICMBio) play an important role in connecting several modules at the national level. This results supports the hypothesis that geographic proximity among actors with interest in MPA management may increase their interaction probability (Maciejewski and Cumming 2015).

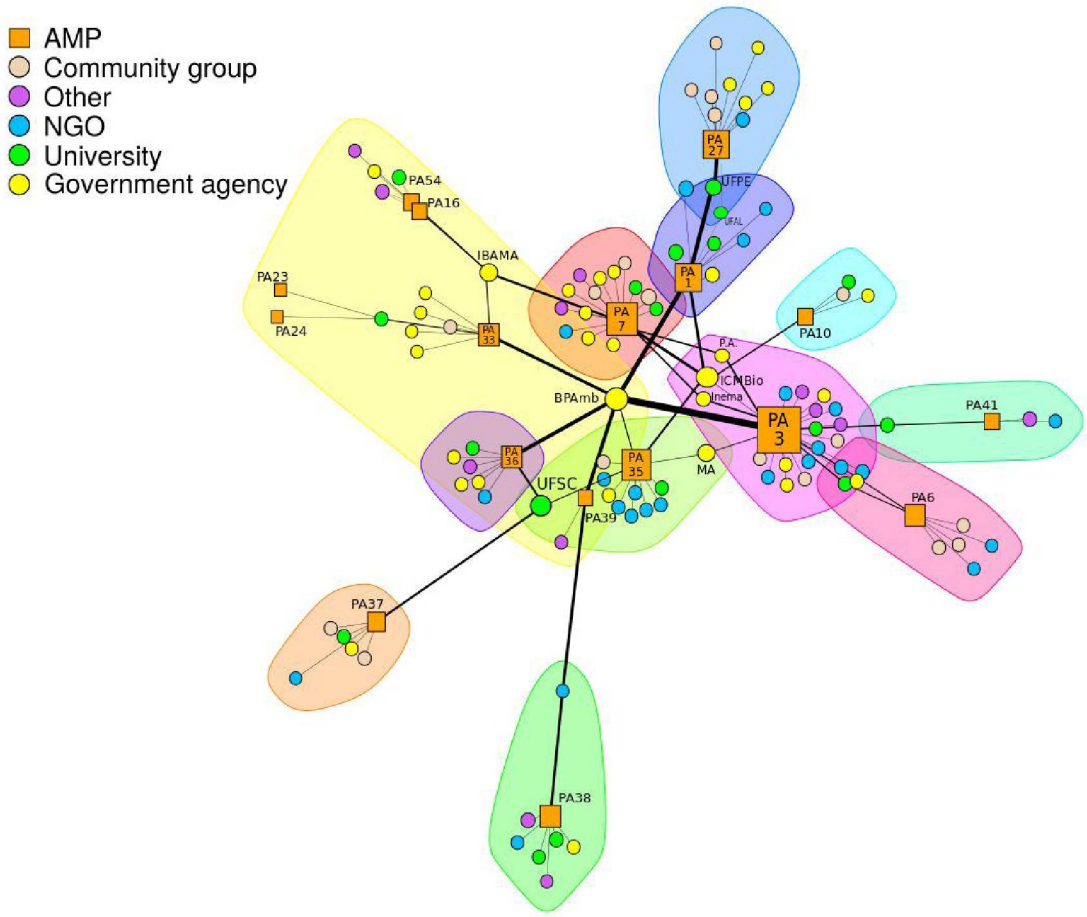

Figure 35 Modularity results for social network of MPAs. Analysis was performed sing a simulated annealing (*spin-glass*) algorithm with no *a priori* hypothesis about its structuring. Networks were visualized using the force-directed layout algorithm by Fruchterman and Reingold. MA= Brazilian Navy; P.A.= Porth Authority; BPAmb = Environmental Police Department, UFSC= Federal University of Santa Catarina, UFPE= Federal University of Pernambuco. Modularity =0.19

DISCUSSION

The snapshot of Brazilain MPAs captured by this study shows that only management councils (Figure 32-C) have strong participation of all social actors (GOs, NGOs, universities, schools, civil society groups, etc.), followed by activities involving environmental education (Figure 32-A). All other activities (Figure 32-B and D-G) are dominated by one or two actors: universities and NGOs are the most important when it comes to research and monitoring, whereas surveillance and formal training are the turf of governmental (including law enforcement) agencies. These also play a major part in volunteering activities, together with organized civil society, elementary and high schools. This first analytical description of the social network of MPAs in Brazil also reveals the largely local nature of interactions, lending support to the hypothesis that geographic proximity is the most important factor for tie formation in socio-ecological systems (Maciejewski and Cumming 2015). In spite of the high interlayer variability, the aggregate displays evident small-world network features, characterized by low average path length and high levels of clustering (Janssen et al., 2006, Barabási 2007, Zhang et al., 2019) as evidenced by the power law distribution of cumulative degree frequency (Figure 28) and the absence of interactions that occur across several MPAs at a national level (upper right quadrant in Figure 29).

These results suggest that participation of social actors in MPA management is not a target that is being systematically pursued as mandated by the now 20-year old SNUC. Rather, these likely arose organically due to historical (political and/or administrative) reasons and hence have limited scope and breadth. The fact that community groups only showed outstanding representation in the environmental education layer suggests that their are located at the receiving end and not yet at the center of the decision-making process. This contrast with initiatives such as the declaration of an Exclusive artisanal fishing zone in the Northern Pacific Ocean in Colombia (ZEPA by its name in Spanish), considered a successful co-management scheme with a self-regulatory logic that emerged after years of involvement of local fishermen in the gathering fishery data in conjunction with several institutions (Diaz et al., 2016). Those data were subsequently used to support the official resolution No. 002650/2008 that prevents industrial vessels from fishing near their coast.

No single MPA or social actor was active in all the layers. MPAs with highest centrality were PARNA dos Abrolhos, RESEX Baia do Iguape, RESEX Lagoa de Jequiá and APA Costa dos Corais. And 2 of them - PARNA dos Abrolhos, and Costa dos Corais - are currently focal areas under the GEFmar, TerraMar and PANCorais projects which could explain to some extent its high management capacity. PARNA dos Abrolhos has outstanding social participation in its management council and monitoring activities whereas APA Costa dos Corais invites diverse actors into its surveillance, research and environmental education activities. These results may suggest that capacity building along SNUC guidelines is happening within individual MPAs, but integrated planning with a higher systemic view is still lacking (Drummond et al., 2011). Similar conclusions were yield by Figueroa et al. (2016), who assessed the effective integration among federal MPAs in Santa Catarina State after the Center for Integrated Management of Marine and Coastal Conservation Units was created. The authors found that despite the elements needed for integration are available in official guidelines, there is still a separation of society from the discussion on the territorial management of the region, and can not be considered as an integrative management approach (Figueroa et al., 2016).

With the exception of formal training, spearheaded by a national reaching environmental agency (ICMBio), all other layers were located on the low density end of the spectrum (Figure 29). With the exception of participation in management councils, every other layer also had small diameters (Figure 29). This is somewhat expected in the case of monitoring and surveillance, due to the continuous nature of these tasks (although the environmental police has national reach and few MPAs reported conducting surveillance activities with them - Figure 32-B). The small diameter detected in the surveillance layer could even be beneficial because it increases the potential for the formation of local management clusters and hence more efficient surveillance programs at local and regional level. However, this should not be the case when it comes to other activities. Universities, that could provide volunteers to more than one MPA in the country, were absent from this layer (Figure 32-G). This is striking given the national reach of other initiatives such as TAMAR, Projeto Peixe-Boi, Meros do Brasil, etc. that routinely recruit undergrads from several institutions. Universities also play a major role in monitoring, along NGOs (Figure 32-D) and dominate research activities (Figure 32-E) but have only a minor role in environmental education (Figure 32-A), which is mainly in charge of community groups and local schools. Taken all together, these

results suggest that cutting edge conservation knowledge is not flowing to where it should be applied. While research findings may be shared through scientific meetings and publications, the low density and limited diameter of environmental education, research and monitoring layers (Figure 29) means that knowledge and experiences are not being directly shared throughout or across these layers (Janssen et al., 2006).

The only exception to the small-world topology was the management council layer, as evidenced, for instance, by the high centrality of MPAs with the larger participation of social actors in their councils (PARNA dos Abrolhos, RESEX Baía do Iguape and the APA Baleia Franca - Figure 32-C). The low similarity in node and edge overlapping across all layers (Figure 33 A and B) result from the fact that connectivity in the network exists mainly through participation in management councils, as clearly illustrated by the results of the edge thinning analysis: subtracting this layer from the network makes it fall apart (Figure 34). Although most social actors tend participate in only one council, the few governmental agencies, universities, and NGOs that participate in more than one (Figure 32-C) are responsible for the remarkable diameter of this layer (Figure 29 and Figure 31), making it an isolated cluster from the small-components layer in Figure 33-D. According to SNUC, implementation of management councils is fundamental for the management of protected areas, but some constraints must still exist, since only 51% of MPAs have legally instituted their councils (data from National Register of conservation Units CNUC/MMA). Assortativity analysis shows that participation in the management council and monitoring are the layers with the strongest correlations (Figure 33-C). This means that the direct participation of social actors in the management of MPAs is usually translated into monitoring activities. This highlights the importance of including the dominant actors in monitoring (universities and NGOs), into those councils. Another important correlation was observed between the development of research activities and the implementation of surveillance programs (Figure 33-C), supporting knowledge transfer into the implementation of surveillance programs and reinforcing the importance of investing in science-based studies to guide conservation actions (Drummond et al., 2011).

CONCLUSIONS

Effective participation of society during MPAs management is of great importance to achieve long-term conservation objectives. Our results support the implementation of management councils as spaces where the different social sectors come together to discuss environmental problems in Brazil, as it is ordered by law. However, the low participation of local communities in other initiatives suggests a marginal role during the management process, and reflects difficulties to translate their participation into integrated management actions. We detected a clear dependency of the social network of MPAs in the management council to maintain its currents levels of connectivity. These results imply susceptibility of the system to the removal of relational ties and highlight the need to preserve and promote the implementation of management councils. But, at the same time, effort should be made to increase the participation of the different sectors of society (especially the local communities) in all other activities to improve the resilience of the system.

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Apendix 1. On-line questionnare sent to the MPAs managers to obtain information on management-based and social interactions.

Questions management-based interactions:

1. Has the AMP received support in terms of equipment loans by another MPA in the last 5 years? From which MPA(s)?
2. Has the AMP received support by staff (formal staff) from another MPA in the last 5 years? From which MPA(s)?
3. Has the AMP received formal training (held after being in the position) related to MPA management with participation of staff from other MPAs? With which MPA(s)?
4. Have you developed research projects in partnership with another MPA in the last 5 years? With which MPA(s)?
5. Have you run monitoring programs in partnership with other MPAs in the last 5 years? With which MPA(s)?
6. Have you been on the management board of any MPAs in the last 5 years? In which MPA(s)?
7. Have you participated in the resolution of conflicts of some MPAs in the last 5 years? In which MPA(s)?
8. Do you run a joint surveillance program with another MPA? With which MPA(s)?

Questions social interactions:

1. Did you receive management support for planning or implementation from civil society groups in the last 5 years? From which group?
2. Have you developed environmental education programs or activities with civil society groups in the last 5 years? With which group?
3. Have you developed research projects in partnership with civil society groups in the last 5 years? With which group?
4. Did you receive support from volunteers who are part of a civil society group in the last 5 years? From which group?
5. Have you run monitoring programs in partnership with civil society groups in the last 5 years? With which group?
6. Have you implemented a joint surveillance program with civil society groups in the last 5 years? With which group?