The Coral Holobiont Madracis Decactis Associates With Multiple Types of Symbiodiniaceae

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Research Article

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Abstract

The scleractinian reef building coral *Madracis decactis* is a cosmopolitan species. Understanding host–symbiont associations is critical for assessing the coral's habitat occupancy and it's response to environmental changes. In this study, we performed fine grained phylogenetic analyzes of Symbiodiniaceae algae associated with *Madracis* across a broad latitudinal gradient in the SW Atlantic (Abrolhos Bank, and St. Peter and St. Paul Archipelago). Previous studies have argued that *Madracis* is a specialist coral, with colonies harboring a single symbiont type B. However, these previous studies have not precisely addressed if *Madracis* is colonized by several types of Symbiodiniaceae simultaneously, or if whether this coral is a specialist. The hypothesis that *Madracis* is a generalist coral host was evaluated in the present study. A total of 1.9 million reads of ITS2 nuclear ribosomal DNA were obtained by Illumina MiSeq sequencing. ITS2 sequences were classified into Clades A, B, and C in Abrolhos, and only clade B symbionts recorded in St. Peter and St. Paul. This study also demonstrate that a single *Madracis* colony can host different symbiont types with >30 Symbiodiniaceae species. Abrolhos corals presented a higher photosynthetic potential as a possible result of co-occurrence of multiple Symbiodiniaceae in a single coral colony. Multiple clades of Symbiodiniaceae possibly confer coral hosts with broader environmental tolerance and ability to occupy diverse or changing habitats.

1. Introduction

Symbiodiniaceae dinoflagellates belonging to the genus *Symbiodinium* (former *Symbiodinium* Clade A) and other related phylogenetic lineages are pivotal cnidarian endosymbionts [1]. These are important primary producers and also form a crucial symbiosis with reef-building corals [2]. Global and local environmental disturbances may cause losses of symbionts from the hosts (coral bleaching), with potential mortality for the coral [3, 4]. Currently, nine evolutionary lineages of Symbiodiniaceae (clades A to I), each with many subgroups (subclades), are recognized based on relatively conserved markers [5, 6]. The ability to harbor different Symbiodiniaceae clades and subclades may provide an effective way to withstand severe and extended stressful conditions [7, 8]. The scleractinian reef building coral genus *Madracis* inhabits shallow and mesophotic regions across the West Atlantic. Species-specific studies documented only Symbiodiniaceae type B (with different subgenus, B7 and B13) in species of *Madracis* in Caribbean, Mexico and the Bahamas [9–10–11–12, 13] and they suggest that the brooding coral is reproductively isolated and specialist. However, the fine grained diversity of Symbiodiniaceae associated with the reef-building coral *Madracis* is remained unknown.

The present study analyzed the diversity of Symbiodiniaceae associated with *Madracis decactis* (only species of *Madracis* in Brazil) across a broad latitudinal gradient (Abrolhos, and St. Peter and St. Paul). Abrolhos is a large continental reef complex that consists of a topological barrier for the southward warm waters of the Brazil Current. While reefs in the Northeastern Region are composed mostly by patch or elongated banks on a narrow continental shelf (~ 50 km), those in the central Brazilian coast are predominantly formed by large platforms and isolated pinnacles with expanded tops [14, 15]. St. Peter and St. Paul comprises the eastern most Brazilian oceanic islands and is one of the smallest and most isolated tropical oceanic islands in the world. The islets are devoid of shore and consist entirely of steep drop-offs extending to 60–150 m depth [16], with the most limited area of shallow habitat among oceanic islands (~200m^2) [17]. *Madracis decactis* is an important contributor of reef structures in Abrolhos, while it is the dominant scleractinian in the rocky reefs of the St. Peter and St. Paul [18]. Previous studies on *M. decactis* did not assess species-specific diversity and indicated the exclusive presence of clade C in shaded habitats (pinnacles) in the Abrolhos, a pattern similar to that recorded in other regions [19, 20], and the presence of the clade A from shallow well-lit waters of the Abrolhos [21], again corroborating previous studies [22, 10].

Coral species are considered generalists when associated with more than one symbiont clade [1]. The knowledge about the diversity of symbionts of shallow water scleractinian corals in the south Atlantic has been largely neglected, and it has been mainly restricted to the report of clades A (*Symbiodinium*), B (*Breviolum*), C (*Cladocopium*) [23–26–19, 20]. *Cladocopium* Clade C is one that confers the most resistance to bleaching in scleractinians and has high resistance and resilience to environmental disturbances [8, 23]. *Symbiodinium* A was found to be associated with high temperatures [20]. South Atlantic corals occupy greater depth zones, are tolerant to higher turbidity and nutrification, are morphologically resistant, engaged in flexible symbiotic associations, and most importantly, are less affected by bleaching [25]. The south Atlantic has proportionally more generalist coral species. For species that have been evaluated for symbiont diversity, approximately 60% of Symbiodiniaceae phylotypes in the South Atlantic are generalists, such as *Favia, Millepora, Mussismilia* and *Siderastrea* [25]. Meanwhile, *Montastraea* and *Madracis* were considered as specialists [25]. However, a previous study suggested *Madracis decactis* metagenomes from St. Peters and St. Paul had Clades A1 and F [27].

The aim of this study was to evaluate the diversity of Symbiodiniaceae in *Madracis decactis* in Abrolhos and in St. Peter and St. Paul. The presence of possible new endosymbionts lineages was also analyzed. The results of this study showcase that *M. decactis* exhibits some degree of flexibility in its symbiotic association with zooxanthellae as a generalist. In addition, high Symbiodiniaceae diversity may lead to greater resistance to environmental changes and plasticity in habitat occupancy, as well as less bleaching susceptibility.

2. Material And Methods

2.1. Sample collection and DNA extraction

Madracis decactis corals were collected at 10-15m depth (28–29 ºC) in Parcel dos Abrolhos (PAB) reef (17°57'32.7"S, 38°30'20.3"W), and 25–35 m in St. Peter and St. Paul (22–24 ºC) (00°56'N; 29°22'W) in 2014 (Fig. 1a) as described in Silva-Lima et al, [21] and Moreira et al. [27], respectively. Specimens were collected and immediately stored in liquid nitrogen. DNA extractions were conducted with the CTAB method [28]. In total, five samples from Abrolhos (M1, M2, M3, M4 and M5) and two samples from St. Peter and St. Paul (M18 and M22) were obtained (Table 1). The samples are from different coral colonies. Quantity and quality of extracted DNA samples were checked by Nanodrop spectrophotometer and 1% agarose gel electrophoresis. The nuclear ribosomal Internal Transcribed Spacer ITS2 region was amplified by PCR using the primer pair ITS2alg-F, 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTG-AATTGCAGAACTCCGTG and ITS2alg-R, 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACA-GCCTCCGCTTACTTATATGCTT [13], with an initial denaturation for 5 min at 94 ºC; followed by 35 cycles of 94 ºC for 50 s, 57 ºC for 50 s and 72 ºC for 90 s; followed by a final extension at 72 ºC for 7 min. All PCRs were conducted in a 25 µl final volume with 1µl of genomic DNA, 5 µl of buffer, 0.5 µl of dNTPs, 1 µl of 10 µmol/µl for each primer, 0.125 µl of 5 U/µl Taq DNA polymerase, and 15.87µl of ddH2O.

Table 1
The statistical analysis of ITS2 from SymPortal. St. Peter and St. Paul Archipelago (SPSPA) and Abrolhos Bank (AB).

Sample ID / Region	No. reads	Mean Length of reads	No. lineages	No. genera	No. Clade A	No. Clade B	No. Clade C	Types of Clade A	Types of Clade B	Types of Clade C
M18/SPSPA	235887	264.05	1	1	0	235887	0	0	30	0
M22/SPSPA	179631	265.61	1	1	0	179631	0	0	25	0
M1/AB	247925	263.37	3	3	9	247797	119	1	38	7
M2/AB	287007	259.89	3	3	73	286837	97	2	46	5
M3/AB	257303	266.26	2	2	0	209	257094	0	16	16
M4/AB	321935	262.17	2	2	0	321531	404	0	35	19
M5/AB	383134	266.2	3	3	13	124	382997	2	12	18

2.2. Sequencing

All samples were sequenced using the Illumina MiSeq platform. Quantification of samples in Qubit (ng / microliter), construction of the libraries using Nextera XT kit (except the stage of tagmentation because they are amplicons), quantification of libraries in Qubit (check concentration), Bioanalyzer (check size), QPCR (Real time quantitative PCR), Preparation of the Pool with the libraries to be sequenced [29].

2.3. Amplicon Sequencing Analysis with SymPortal

Demultiplexed forward and reverse fastq files were analyzed locally with SymPortal [30] and were subjected to standard sequence quality control protocols implemented with MOTHUR 1.39.5 [31], the BLAST suite of executables [32], and minimum entropy decomposition [33] to filter non-Symbiodiniaceae and sequencing artifacts from the dataset [30]. Sequences were clustered by clade. Sequences occurring in a sufficient number of samples within both, the dataset being analyzed and the entire database of samples run through SymPortal, were identified as DIVs which were then used to characterize ITS2 type profiles [30]. All sequences were queried against the SymPortal database using the nucleotide BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Best hits were used to classify ITS2 sequences into subclades. Sequence alignments were performed with Muscle [34]. Phylogenetic reconstruction with a maximum likelihood model was inferred with Fast Tree 2 [35]. Nodal support tested by bootstrap analysis (1000 times).

2.4. Coral physiological analyzes

Field measurement of photosynthetic performance of *M. decactis* colonies were made in AB and SPSPA using an Underwater Fluorometer (Diving PAM, WALZ). Yield was estimated by applying a saturation light pulses of 800 µmol photons m − 2 s − 1, 0.8 s width actinic light, to previously dark-adapted samples. Analysis of covariance (ANCOVA) was used to evaluate differences in photosynthetic yield (Y) of corals (Y = Fm − Fo/Fm; Fm: maximum fluorescence, Fo: initial fluorescence) between regions, while controlling for the effect of depth which was included as covariate in the model [36].

3. Results And Discussion

A total of 1.9 million ITS2 nuclear ribosomal DNA reads were obtained (Table 1). Three Clades and eight subclades of Symbiodiniaceae were detected (Table 1 and Supplementary Table S1). Symbiodiniaceae of clades A, B and C occurred in Abrolhos, while Symbiodiniaceae B was found in St. Peter and St. Paul (Fig. 1a and 1b). Moreover, the ITS2 results identified variants within the clades A, B and C (Figs. 1c and 2). The results presented here suggest the dominant Symbiodiniaceae types in St. Peters and St. Paul corals belong to Clade B. Meanwhile, Clade C is the dominant type in Abrolhos. These differences may be related to co-evolutionary processes to enhance holobiont survival.

The Analysis of Covariance (ANCOVA) showed a significant difference in photosynthetic yield between Abrolhos and St. Peter and St. Paul corals (Fig. 3), with higher values recorded at Abrolhos (DF, 1; MS, 0.054; F, 6.20; p, 0.013). The effect sampling depth was not significant (DF, 1; MS, 0.002; F, 0.24; p, 0.619). The higher photosynthetic potential in Abrolhos than in St, Peter and St. Paul is a possible result of multiple Symbiodiniaceae co-occurrence in a single coral colony in Abrolhos (Fig. 1a and 1b). Many species of coral harbor multiple clades of Symbiodiniaceae, and the symbiont composition may vary both within and/or between colonies depending on environmental conditions and geographic location [19–13–37, 38]. The enhanced symbiont diversity observed in Abrolhos, represented by multiple clades of Symbiodiniaceae (A, B, and C), possibly leads to greater resistance to environmental changes and plasticity in habitat occupancy.

The results of the present study demonstrate that *M. decactis* is a generalist coral that can associate with multiple clades of Symbiodiniaceae, including clades A and C, besides the B type previously recorded [43–44–9–10–11, 13]. *M. decactis* corals live in lower euphotic and upper mesophotic areas in St. Peter and St. Paul (25–60 m), and in both, shallow (5–15 m) and mesophotic depths (30–65 m) in Abrolhos. Clade B lineages have been found associated with a high diversity of hosts and commonly in deeper reef regions. A shift in the association from clade C to clade B with increase in latitude has been observed in colonies of *Plesiastrea versipora* along the northeastern and southeastern coast of Australia [39]. Furthermore, clade B, commonly found in temperate regions, may be better adapted to low-light and low temperature conditions [22–40, 41–42]. In addition, the ability of lineages of *Symbiodinium* A to efficiently synthesize photoprotectors as mycosporine-like amino acids [21, 24] seems to allow them to occupy shallow areas associated with high temperatures [20] in marine environments such as the shallow reefs of Abrolhos. On the other hand, *Cladocopium* spp. (clade C) may be correlated with high seawater turbidity, a typical feature of Abrolhos reefs. Presence of clade C in Abrolhos may be a consequence of living high-turbidity localities [19, 20]. Generalist phylotypes such as A4 (*Symbiodinium linucheae*), C1 (*Cladocopium goreaui*), and C3 are commonly found in South Atlantic locations that undergo changes in temperature (24–28°C) and other abiotic factors [45]. The occurrence of multiple clades of Symbiodiniaceae in *Madracis* corals may contribute to higher coral resilience [46–47–19–13–37, 38].

4. Conclusion

South Atlantic *Madracis decactis* is a generalist coral and contains an ample diversity of Symbiodiniaceae types that may contribute to coral holobiont health. Collectively, south Atlantic corals are not only typical generalists, but also tend to associate with generalist symbionts. This symbiotic flexibility may allow for adaptive shifts in the holobiont community, which is critical in increasing resistance and resilience to bleaching. It has adapted for thriving in turbid environments that may prove critical for surviving the increasing impacts of climate change and mass bleaching episodes.

Declarations

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Availability of Data and Material

Not applicable.

Code Availability

Not applicable.

Authors’ Contributions

Tooba Varasteh conceived the study design, DNA extractions and PCR, the bioinformatics analysis, and discussion of the results and drafted the manuscript. Vinícius Salazar performed the bioinformatics analyses. Ronaldo Francini-Filho, Jean Swings, Diogo Tschoeke, Gizele Garcia, and Cristiane Thompson participated in the discussion of the results and drafted manuscript. Fabiano Lopes Thompson participated in the acquisition of funding and conceived the study design, discussion of the results, and draft of the manuscript.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare they have no conflict of interest.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

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Figures

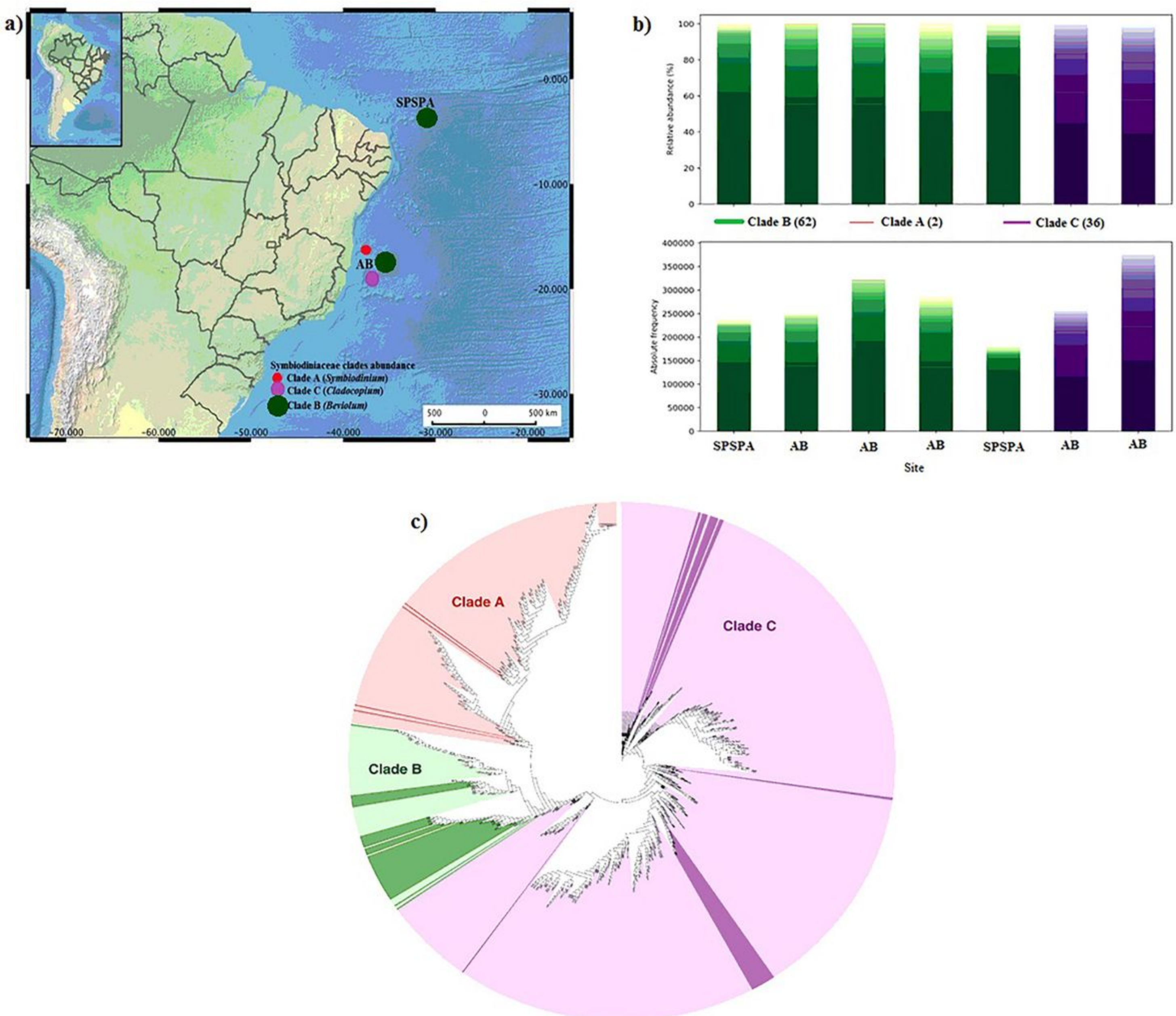

Figure 1

a) Study sites with Symbiodiniaceae clades abundance. St. Peter and St. Paul Archipelago (SPSPA) and Abrolhos Bank (AB) (Map modified from Tonon et al, [48]; b) Clade abundance between AB and SPSPA; c) Clades A, B and C were verified. Bold colors are samples in the present study. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

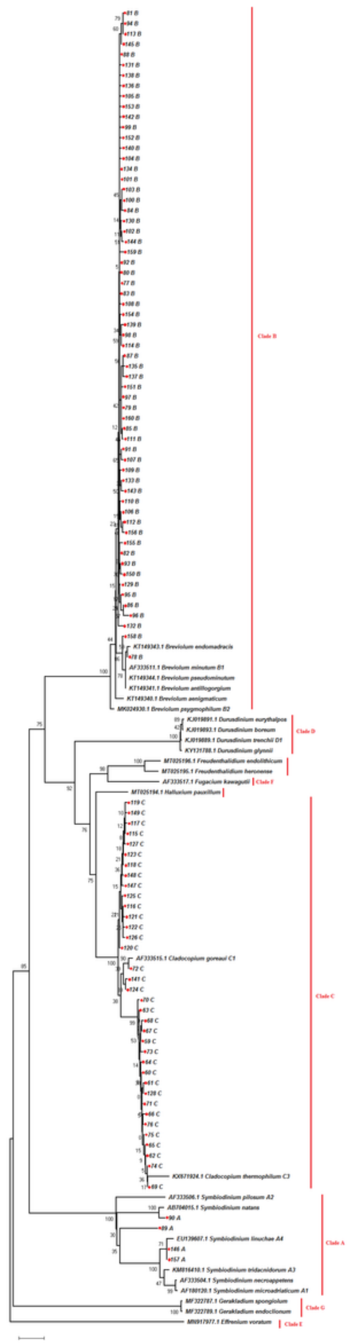

Figure 2

Maximum likelihood tree of sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. This analysis involved 127 nucleotide sequences. There were a total of 839 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [48]. Samples in the present study are shown in red circle.

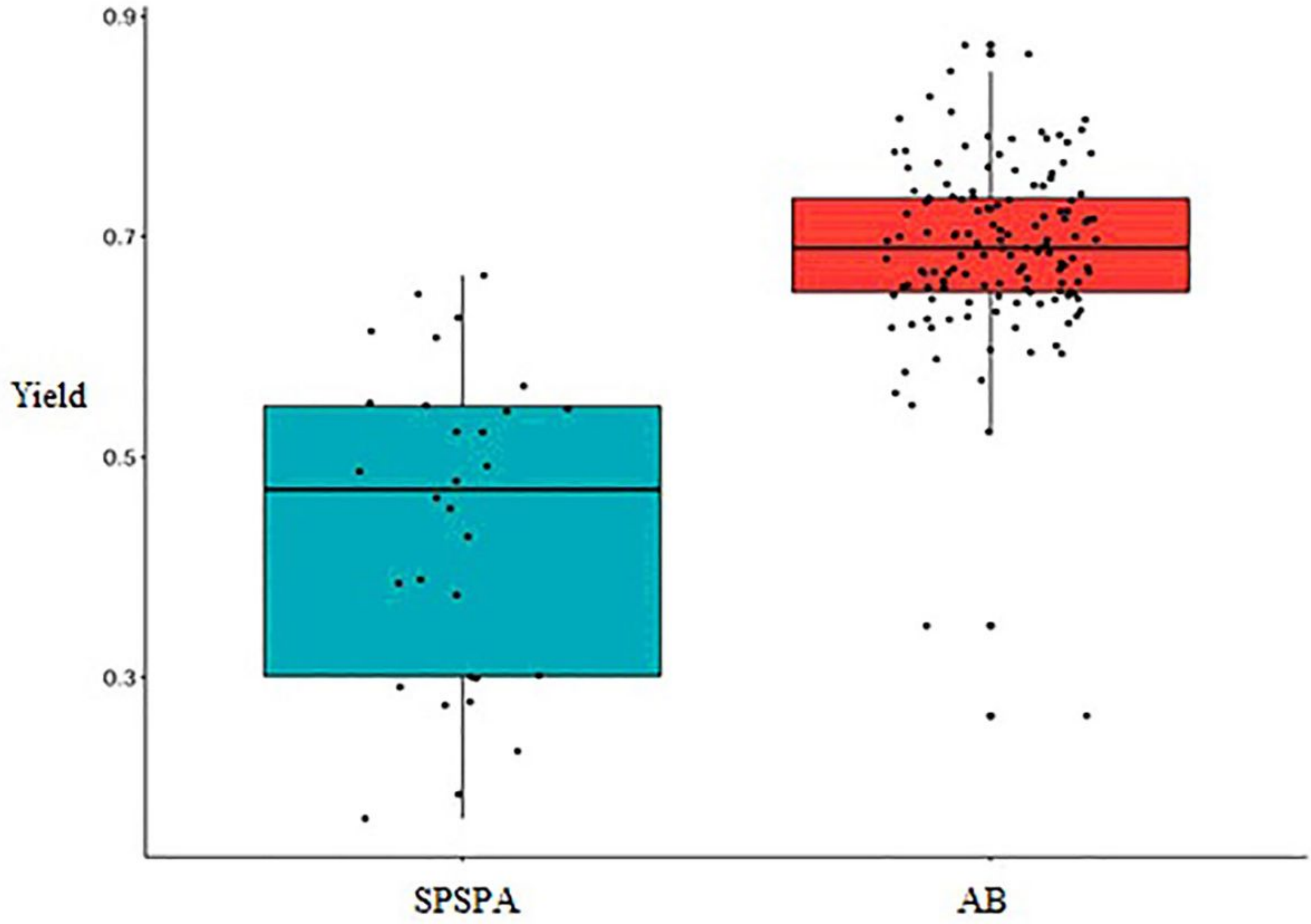

Figure 3

Differences in Madracis decactis yield according to sampling locations, St. Peter and St. Paul Archipelago (SPSPA) and Abrolhos Bank (AB).

Supplementary Files

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- SupplementaryInformation.pdf