Diversity and ecological structure of vibrios in benthic and pelagic habitats along a latitudinal gradient in the Southwest Atlantic Ocean

Luciane Chimetto Tonon, Bruno Sergio Silva, Ana Paula Moreira, Cecilia V P Pereira, Nelson Alves Junior, Giselle Cavalcanti, Gizele D. Garcia, Rubens Lopes, Cristiane Thompson, Fabiano Thompson

We analyzed the diversity and population structure of the 775 *Vibrio* isolates from different locations of the southwestern Atlantic Ocean (SAO), including St. Peter and St. Paul Archipelago (SPSPA), Abrolhos Bank (AB) and St. Sebastian region (SS), between 2005 and 2010. In this study, 195 novel isolates, obtained from seawater and major benthic organisms (rhodoliths and corals), were compared with a collection of 580 isolates previously characterized (available at www.taxvibrio.lncc.br). The isolates were distributed in 8 major habitat spectra according to AdaptML analysis on the basis of *pyrH* phylogenetic reconstruction and ecological information, such as isolation source (i.e. corals: *Madracis decactis*, *Mussismilia braziliensis*, *M. hispida*, *Phyllogorgia dilatata*, *Scolymia wellsi*; zoanthids: *Palythoa caribaeorum*, *P. variabilis* and *Zoanthus solanderi*; fireworm: *Hermodice carunculata*; rhodolith; water and sediment) and sampling site regions (SPSPA, AB and SS). Ecologically distinct groups were discerned through AdaptML, which finds phylogenetic groups that are significantly different in their spectra of habitat preferences. Some habitat spectra suggested ecological specialization, with habitat spectra 2, 3, and 4 corresponding to specialization on SPSPA, AB, and SS, respectively. This match between habitat and location may reflect a minor exchange of *Vibrio* populations between geographically isolated benthic systems. Moreover, we found several widespread *Vibrio* species predominantly from water column, and different populations of a single *Vibrio* species from *H. carunculata* in ecologically distinct groups (H-1 and H-8 respectively). On the other hand, AdaptML detected phylogenetic groups that are found in both the benthos and in open water. The ecological grouping observed suggests dispersal and connectivity between the benthic and pelagic systems in AB. This study is a first attempt to characterize the biogeographic distribution of vibrios in both seawater and several benthic hosts in the SAO. The benthopelagic coupling observed here stands out the importance of vibrios in the global ocean health.
Introduction

The microorganisms' distribution in space does not occur evenly, but generally, it is efficiently because local environmental conditions selects specific populations to become relatively more abundant. In situations where this may be a good approximation
of the primary mechanisms creating population structure, one can invoke a niche theory
to understand how organisms interact with other species and their environment (Tilman,
1982). Under this model, competition with co-existing organisms is an important
determinant of which species are present in specific environment (Hibbing et al., 2010).
Over evolutionary time scales, competition can cause species to adapt alternative life-
styles, e.g. to attach to different hosts or different substrates in the same hosts. Such
trade-offs are a necessary result of constraints on cellular machinery, such as cell-
surface proteins and enzymatic capacity or genome size, resulting in specialized
populations with limited niche overlap (Preheim, 2010).

Microbes associated with benthic holobionts, such as corals, play key roles in the health
of their hosts (Rosenberg et al., 2007). Vibrios represent a significant component of the
culturable microbiomes of marine hosts and plankton. Their proliferation and population
abundance can be induced by multiple factors including increased water temperature
related to global climate change, which can cause impacts in ecosystem structure,
including Vibrio-associated diseases (Baker-Austin et al. 2012, Vezzulli et al. 2012).
However, when vibrios' proliferation is not favored, they are able to find refuge in
suitable reservoirs to survive, making use of their extensive adaptive capabilities. For
instance, vibrios can change from free-swimming cells to "swarmer cells" that prosper in
more viscous environments such as biofilms (McCarter 1999), or switch from an active
stage to a dormant, viable but not culturable (VBNC) stage, but yet may still be very
potent opportunists if favorable conditions recur (McDougald & Kjelleberg 2006).

Previous studies suggest that the proliferation of vibrios in the plankton results in lethal
vibrio infections in the benthos, suggesting mechanisms of benthopelagic coupling. In
other words, these events reveal a causal relationship between water-column and benthic processes, which may influence the health of global ocean (Vezzulli et al, 2010; 2012). For instance, an increase in seawater temperature (average range 21.0 °C to 24.3°C) appears to induce the growth of certain vibrios (e.g. *V. harveyi* related species) and the concomitant mass mortality of the gastropod *Haliotis discus hannai* in northern Japan (Fukui et al., 2010). It has also been shown that the ocean warming observed in the last decades has induced a significant increase in the abundance and range of vibrios in a long-term study in the North Sea (Vezzulli et al., 2012). Moreover, increases of organic material used by *Vibrio* for energy may be additional factor in determining *Vibrio* dynamics. Phosphorous, for example, seems to influence the abundance of planktonic vibrios according to a metagenomic study of bacterioplankton diversity in a tropical bay. According to this study, nutrient limitation effects can be observed at community (metagenomic) and population levels (total prokaryote and vibrio counts) (Gregoracci et al., 2012). Vibrios display broad metabolic ranges and enzyme activities that enable them to use a wide variety of carbon sources (Thompson & Polz 2006). Nevertheless, it is not clear whether vibrioplanktonic cells are genetically and ecologically coupled to vibrio cells that live in association with holobionts.

We previously reported massive mortality of the major coral reef builder, *Mussismilia braziliensis*, and we isolated potential causative agents (Francini-Fo. et al., 2008; Alves et al., 2010). According to these former studies, the diseases affecting corals were tissue necrosis in *Phyllogorgia dillatata*, bleaching in *M. hispida* and white plague and bleaching in *M. braziliensis*. Most of the isolated vibrios fell into the Harveyi clade (Sawabe et al., 2007, 2013) and *V. coralliilyticus*. The vibrio isolates of these studies encompassed strains originating from both apparently healthy and diseased corals and
had high pathogenic potential for different animals. *V. alginolyticus* 40B, *V. communis* 1DA3 and *V. coralliilyticus* 2DA3 caused 25–88% mortality in the model organism *Drosophila melanogaster* (Alves et al., 2010). However, the possible ecologic structure and genetic connectivity among vibrios from the coastal-oceanic and benthic-pelagic systems of the SAO remains unclear.

Studies performed in subtropical areas indicate that coastal vibrioplanktonic communities are finely structured in discrete phylogenetic clusters, revealing the co-occurrence of several hundreds of closely related populations (Thompson et al., 2005). Sympatric differentiation may be due to niche partitioning and specialization, with the association of different groups of bacterioplanktonic species with different habitats (zooplankton, particles and water) in the same geographic location. Hunt et al. (2008) showed that some vibrio species appeared to occur only in association with plankton, whereas other species appeared to be exclusively free-living.

Little is known about the vibrioplankton population diversity and structure in the SAO, and whether vibrioplankton diversity is linked to benthic compartments in this region. Oceanic islands, and reef systems, such as Saint Peter and Saint Paul Archipelago (SPSPA) and Abrolhos Bank (AB) are important environments located in the SAO. SPSPA is constituted by five emerged summits of the Mid-Atlantic Ridge approximately 1000 km off the coast of Natal (Moreira et al., 2013). It is a biodiversity hotspot. AB is an extension of the continental shelf off the south of the Brazilian State Bahia (17 - 20° S), corresponding to approximately 45,000 km² (Amado-Filho et al., 2012). This bank comprises the world’s largest rhodolith bed (aggregates of non-geniculate crustose coralline algae nodules) forming large expanses of hard bottom habitat with
approximately 21,000 km², contributing to the SAO as a nursery place, nutrient producer and carbonate storage (Cavalcanti et al., 2013a; 2013b). Rhodolith beds stand together with kelp beds, seagrass meadows, and crustose coralline algae reefs as one of the world's four largest macrophyte-dominated benthic communities (Foster 2001). AB is a particularly nutrient rich reef system, with higher nutrients concentration levels than reef systems less influenced by river estuaries (Bruce et al., 2012). SPSPA and AB are under the influence of water masses formed by the warm and salty Tropical Water (TW), with temperature values ranging from 22 to 27 °C, and salinity values ranging from 36.5 to 37 (Castro and Miranda, 1998; MMA, 2006). The benthic communities occurring in the different islands may also be locally a source of nutrients in the SAO. SS is a sea passage 25 km long, 2-7 km wide and 40m maximum depth located between the island of São Sebastião (municipality of Ilhabela) and the mainland (São Sebastião), on the coast upstate São Paulo, southeastern Brazil. The currents in the channel are directed by the wind and the water temperature range is 15 - 28 °C. The region is influenced by both the warm waters of the Brazil Current (22-28ºC) that goes down to the south and, the cold (<13ºC) and saltier (~ 36 psu) South Atlantic Central Waters (ACAS (Oliveira & Marques, 2007).

Based on analysis of the diversity and population structure of vibrios, by using a collection of new isolates originated from seawater, sediment, rhodoliths, coral (Scolymia welsii), and previously characterized strains (Chimetto et al., 2009; Alves et al., 2010; Moreira et al., 2013) from different environments located in the SAO, we investigated (i) the habitat spectra of Vibrio populations in the SAO; (ii) whether these vibrio population were generalist- and/or specialist-adapted; and (iii) if there was connectivity among the benthic-pelagic systems in AB, the largest South Atlantic
reef complex. We performed the taxonomic characterization of the isolates using the reliable taxonomic marker gene pyrH (which has higher discriminatory power than 16S rRNA sequences, allowing the distinction of closely related Vibrio species) (Thompson et al. 2005, 2007) and inferred ecological associations by using a mathematical model (AdaptML) (David, 2010; Hunt et al., 2008).

Material and Methods

Sources of Isolation

In total, 775 vibrio strains, isolated between 2005 and 2010, and identified by means of pyrH sequences, were analyzed. Information on locations and sources are detailed in Table S1 (strain list). The geographic span represents over 3,000 Km, including the oceanic SPSPA, AB, and the southeastern Brazilian Saint Sebastian channel (SS) (Figure 1, map). For the 195 novel isolates, sources were: sediments from the Buracas (N=25) (for a full description of the so called Buracas reef system see Cavalcanti et al., 2013a); rhodoliths (N=76) (for a full description of the rhodoliths holobiont see Cavalcanti et al., 2013b) from 27 and 43m depth; seawater from AB, from 10 and 150 m depth (N=76), including two locations (station 61: closer to shore, and station 65: oceanic), and S. welsii mucus (N=18), from SPSPA. The remainder 580 strains were obtained previously (Chimetto et al., 2009; Alves et al., 2010; Moreira et al., 2013) and isolated from: corals (Madracis decactis, Mussismilia braziliensis, M. hispida, Phylogorgia dilatata); zoanthids (Palythoa caribaeorum, P. variabilis and Zoanthus solanderi), and fireworm (Hermodice carunculata). These previously characterized data contributed to increase the number of habitat categories under comparison as well as their geographic extent, allowing a more comprehensive evaluation of potential
ecological and genetic relationships among locations. Sampling permit Sisbio n. 24732-1 was issued by the Ministry of Environment Institute Chico Mendes (ICMBio).

Isolation and preservation of vibrios

Vibrios originating from the water column were obtained from inoculation, performed on board the RV Prof. W. Besnard, in July 2007, in AB. Samples were collected with a rosette system in three depths (10, 75 and 150 m) in station 65 (17° 0' 36" S; 36° 59' 56.4" W - oceanic), and at 10 m in station 61 (17° 0' 3.6" S; 39° 0' 0" W - on the shelf). Samples from sequential filtration (200 mL) in 3 µm and 0.22 µm filters and aliquots of unfiltered seawater (1 mL) were plated onto the culture medium thiosulfate-citrate-bile salt-sucrose Agar (TCBS) (Oxoid) to obtain vibrios strains. Plates were incubated at 26 - 28ºC for 48-72 h. Similarly, in independent cruises to SPSPA (00° 56' N; 29° 22' W) and the Buracas reefs (27m deep: 17° 81' 33.0" S; 38° 23' 74.4" W and 43m deep: 17° 81' 39.9" S; 38° 24' 30.6" W) in 2010, aliquots of coral mucus (S. welsii,) and rhodoliths were surveyed. Rodoliths were crushed and homogenized (0.1 g) in sterile saline buffer (3% NaCl, SSB). Coral mucus was 10-fold serially diluted in SSB. Homogenates (0.1 mL) were plated in triplicates in TCBS at 28C°. Isolates were purified at Federal University of Rio de Janeiro (UFRJ). The pure cultures were maintained in vials with Tryptic Soy Broth (Oxoid) with 3% NaCl or Marine Broth media, both supplied with 20% (v/v) glycerol, and preserved at −80 °C.

Taxonomic characterization

Characterization of all vibrio isolates was obtained by pyrH sequencing (80F and 530R primers) as described previously (Thompson et al., 2005). DNA extraction was
performed based on Pitcher et al. (1989). PCR sequencing reactions, consensus sequences determination and alignment were performed as in Moreira et al. (2013). Phylogenetic trees were built in MEGA 5 (Tamura et al., 2011). The topology of the tree was based on neighbor-joining method. Distance estimations were obtained according to Kimura-2-parameter and Maximum Composite Likelihood model. Bootstrap percentages were used after 1000 replications. To analyze the ecological grouping multifasta file was converted in MEGA 5 to PHYLIP 3.0 format. The .phy file was used as input to PhyML 2.44 (Guindon and Gascuel, 2003; Guindon et al., 2010) where the suitable tree was generated and again used as input file for the AdaptML software (David, 2010; Hunt et al., 2008). Tree figures were generated using the interactive Tree of Life web application (itol.embl.de) (Letunic and Bork, 2007).

Ecologic grouping of vibrios

Clusters of vibrios' sequences were obtained with the software AdaptML as described previously (David, 2010; Hunt et al., 2008). In brief, the software combines genetic information embedded in sequence-based phylogenies and information about the ecology, herein source and place of isolation, in order to identify genetically- and ecologically-distinct bacterial populations. This quantitative model (AdaptML) uses a Hidden Markov Model to predict the phylogenetic bounds of ecologically distinct populations, and their habitat composition (distribution among environmental categories). AdaptML algorithm can account for environmental parameter discretization schemes and is based on the model concept of a habitat (a place and related features that determines microbial distribution). Habitats are characterized by discrete probability distributions describing the likelihood that a strain adapted to a habitat will be sampled from a given ecological state (e.g. at a particular location in the water column or in a
specific host). Habitats are not defined *a priori* but rather learned directly from the sequence phylogeny and ecological data using an Expectation Maximization routine. Once habitats are defined, a maximum likelihood model is used for the evolution of habitat association on the tree (David, 2010; Hunt et al., 2008). The habitat-learning and clustering steps of AdaptML were performed using the default settings. Confident assignments are show for ecological populations predicted by the model. The model threshold value was set at 0.05 and *Photobacterium* was used as out-group. The bootstrap percentages analysis was rerun 100 times with the same phylogenetic tree to verify the stability of the predictions. Circular tree figure was drawn using the online iTOL software (Letunic and Bork, 2007). To prevent numerical instabilities in AdaptML’s maximum likelihood computations, branches with zero length were assigned the minimal observed non-zero branch length: 0.001. Clades supported in 80% of bootstraps were shown. The visualization of the distribution of *Vibrio* groups in all habitats (Fig. S2) was generated by the online tool Many Eyes (IBM Many Eyes Project; Viégas et al., 2007).

All gene sequences obtained in this study are available through the website TAXVIBRIO ([http://www.taxvibrio.lncc.br/](http://www.taxvibrio.lncc.br/)). The GenBank accession numbers for the *pyrH* sequences reported in this study are KC871632 – 720; KJ154031 - 48; EU251514 – 1689; EU716656 – 7075; GU186166 – 6371; KC871598 - 1720.

**Results**

**Taxonomic assignment of the vibrio isolates**

The taxonomic characterization of the isolates was mainly based on the phylogenetic position of *pyrH* gene sequences and its similarities in relation to the closest type strain
of Vibrio species. The pyrH gene has shown to be a reliable taxonomic marker for the Vibrio group, even able to discriminate closely related species. (Thompson et al. 2005, 2007). However, in some cases we also performed a Multilocus sequence Analysis (MLSA) of housekeeping genes and whole genome sequence (Chimetto et al., 2009, Moreira et al., 2014). Most of the vibrio isolates were V. communis (21.9%), V. mediterranei/V. shiloi (19.7%), V. harveyi (12.4%), V. alginolyticus (9.5%), V. campbellii (7.7%). Other prevailing groups were V. maritimus (4.5%), V. tubiashii (3.5%), V. coralliilyticus (3.1%), V. pelagius (2.5%), V. diabolicus (2.2%) and V. chagasii (1.8%). In addition, 22 strains (2.8%) were identified as candidate new Vibrio species based on 16S rRNA (data not shown) and pyrH gene sequence similarities. Ecological populations predicted by the AdaptML model totalized 19 Vibrio groups clustered accordingly Fig. 2. Most species found in the water column (i.e. V. communis, V. campbellii, V. harveyi, V. maritimus, V. pelagius and V. diabolicus) were also isolated from different invertebrate hosts (Table 1 and S1). However, some species as V. hepatarius and a unique strain of V. alfacsensis were found only in water samples, whilst V. rotiferianus only in the benthos, coral and zoanthid samples (M. hispida, M. braziliensis, P. dilatata and P. caribaeorum). Some species were retrieved from a single given host. V. sinaloensis was found only in the coral Mussismilia (hispida and M. braziliensis), as well as V. fortis was associated only with M. hispida. V. shiloi was found mainly in association with the fireworm H. carunculata and also with M. hispida in SS. V. furnissii was only associated with H. carunculata. We observed low counts (colony forming units - CFU) in the water column (typically <10^2 per mL) compared with the abundance of vibrios in reef waters (up to 10^4 CFU mL^{-1}) (Bruce et al., 2012) and in the coral mucus (10^6 CFU mL^{-1}) (Alves et al., 2010; Moreira et al., 2013).
Partitioning of vibrio isolates according to their genetic and ecological similarity

The *Vibrio* isolates were distributed in 8 habitat spectra. Herein habitat is a spectrum of environment types over which a given population may be isolated from (Table S2; Figure 2 and S1). The three studied areas (i.e. SPSPA, AB and SS) and their distribution in the composition of each habitat spectrum is shown in Figure S1. Some spectrum of habitat were mainly composed by categories from an unique geographic region as in habitats 1 and 3 (H-1, H-3) (from AB), habitats 2 and 8 (H-2, H-8) (from SPSPA) and habitat 4 (H-4) (from SS). Although habitats 5, 6 and 7 (H-5, H-6, H-7) seemed to be more variable, geographic predominance was observed for H-6, dominated by categories from AB (67%), followed by those from SPSPA (25%) and SS (8%) (Fig. S1). H-1 was mainly composed of strains isolated from water (86%), H-2 from *M. decactis* (92%), H-3 from *M. braziliensis* (72%), H-4 from *M. hispida* (69%), H-5 from *Mussismilia (hispida and brasiliensis)* (72%), H-6 from rhodolith (44%), H-7 from *P. caribaeorum* (50%) and H-8 from *H. carunculata* (94%) (Figure3).

Although the observation of these different microenvironments in SAO was based on single sampling the correlation found among the inferred habitat spectra, sampling sites, isolation sources and the associations with all ancestors of the vibrios studied indicated structured populations (Figure 2). The predominant environmental category in the composition of each predicted habitat spectrum can be clearly visualized in Figure 3.

In more detail, H-1 was characterized mostly by vibrios from AB seawater (86%) and it was divided into 7 groups occupied by different *Vibrio* species (Fig. 2 marked with asterisk, and Table S2). *V. pelagius* (group 1); *V. maritimus* (groups 2 and 3); *V. hepatarius* (group 4), and *V. communis, V. campbellii* and *V. diabolicus* (groups 5-7). H-2
was composed mostly of isolates from *M. decactis* - SPSPA (92%) and from AB seawater (5%), *V. campbellii* (N=42), candidate *Vibrio* sp. nov (N=19) and *V. maritimus* (N=15) were the most frequently found species (Fig. S3 and Table S2). Two clusters of *V. maritimus*, one from AB seawater and the other from SPSPA *M. decactis*, were clearly distinguished (Fig. S3). H-6 had mainly (57%) vibrios from Buracas - AB (44%: rhodolith, 13%: sediment) and from seawater (6%) (Fig. S3). Species highlighted were *V. harveyi*, *V. communis, V. coralliilyticus, V. tubiashii* and candidate *Vibrio* sp. nov. (Figure S2). H-7 was mainly represented by the same *Vibrio* species found in H-6, except for potential new *Vibrio* species and the presence of *V. rotiferianus*. The hosts observed in this habitat were *P. caribaeorum* (50%) and *M. hispida* (12%) both from SS; *P. dilatata* (23%: Recife de Fora), *M. braziliensis* (10%: Saint Barbara Island), rhodoliths (3%: Buracas), and water (2%: AB) (Fig. 3 and Table S2).

H-3 encompassed mostly isolates associated with *Mussismilia* (78%), mainly *M. braziliensis* (68%) (Roi-Roi reef - AB). The species adapted to this habitat spectrum were *V. coralliilyticus, V. harveyi, V. communis, V. sinaloensis* and *V. tubiashii* (Figure S3). H-4 was mostly represented by benthic animals from SS channel (85%). The main host (72.5%) was *M. hispida* (69%: SS, 2%: AB) *M. braziliensis was also represented* (1.5%: AB). *Vibrio* species observed in H-4 were *V. alginolyticus, V. communis, V. campbellii, V. tubiashii, V. chagasii* and *V. sinaloensis* (Fig. S2). H-5 was composed of environmental categories from AB (61%) and SS (37%) (Fig. 3 and Table S2). The dominant category was the host *Mussismilia* (72%), including both species: *M. hispida* (46%: AB and SS) and *M. braziliensis* (26%: AB). *V. communis* and *V. alginolyticus* were the dominant species (Fig. S2). H-8 comprised mostly *V. shiloi* associated with *H.*
carunculata in SPSPA (94%) A few V. shiloi strains were associated with M. hispida in SS (6%) (Fig. 3).

Connectivity among the benthic-pelagic systems in Abrolhos bank.

The presence of identical pyrH sequences of vibrios from planktonic and coral reinforce the hypothesis of connectivity (Fig. S3). For instance V. communis (PEL4D from 150m depth and R-680 from M. hispida, G35, G52 from rhodoliths), (PEL103A from 10m depth and R-239, R-264 from M. hispida); V. harveyi (PEL36B from 10m depth and 1DA5 from P. dilatata); V. campbellii (PEL44A from 10m depth and 42A from M. hispida, PEL45A from 10m deep and A-391 from M. decactis); V. diabolicus (PEL41D from 150m depth and 4D2 from P. dilatata); V. pelagius (PEL22B from 10m depth and 28A2 from M. hispida) and V. chagasii (PEL47A from 75m depth and PA10 from M. braziliensis and 1DA1 from P. dilatata) (Figure S3). Samples from water clustered with samples from benthos are highlights (*) in Figure 2.

On the other hand, some clusters of planktonic strains seemed to have unique pyrH gene sequences (i.e. V. maritimus group, PEL21 (A, B, C, E and F - station 61,10 m); PEL102 (A e B), PEL106A, PEL111A, PEL121C, PEL122A, PEL124A and PEL125 (A and B); and V. pelagius group PEL115 (A, B, C, D, E, F, G, H and J - station 65, 150m) (Figure S3). The distribution pattern of the main Vibrio species from benthic and pelagic sources in AB based on evolutionary history inferred by using the Neighbor-Joining method of pyrH gene sequence (532 positions) can be visualized in the Fig.S4.
A more targeted AdaptML analysis was performed by dividing the isolation sources in generic hosts from benthic and pelagic, to explore the extent and significance of the coupling events. It resulted in a very similar cluster distribution of the populations of vibrios. Again, isolates from Abrolhos Bank, both from open water and benthos, were present in the same branches. However, the number of habitat spectra established were reduced to six (H1-H6). (Fig.S5).

Discussion

Vibrio population distribution in the Southeastern Atlantic

The predicted habitats spectrum were dispersed along a spatial gradient ca. 3,000 km, from the coastal southeastern SS to the most distant from coast Brazilian archipelago, located above the Equator (SPSPA). Ecologically coherent groups were associated with seawater (H-1), corals [M. decactis (H-2), M. braziliensis (H-3), M. hispida from SS (H-4), both Mussismilia species (M. braziliensis and M. hispida) (H-5)], rodoliths and sediment (H-6), zoanthids - P. dilatata and P. caribaeorum (H-7), and the polichaete H. carunculata (H-8).

Vibrios' behavior showed a wide spectrum, from approaching a true generalist to a strict specialist. The dominant group – the Communis cluster, was present in all habitats spectrum, except for H-8 (mainly composed by the host fireworm), and in 10 out of the 12 samples analyzed. This suggests that these hosts may serve as a reservoir for V. communis' populations when their abundance in seawater decreases. The exceptions were the zoanthid (Z. solanderi) and the fireworm (H. carunculata). Nevertheless it's worth to note that only V. alginolyticus inhabited Z. solanderi, according to this study,
thus raising the possibility that antagonists among its populations (or the animal itself) could be effective against vibrios. Apart the exceptions, *V. communis*’ populations showed a true generalist behavior. *V. communis* strains were isolated from multiple independent samples and thus do not represent clonal expansion, suggesting that this may reflect a true habitat switch. Moreover, *V. communis* appeared to have ecologically diversified, possibly by invading new niches or partitioning resources at increasingly fine scales in a similar way to *V. splendidus* in the northwestern Atlantic coast (Preheim et al., 2011, Hunt et al., 2008). Despite recognized as a generalist, *V. splendidus* was not represented in this study, what might reflect its low tolerance to high temperatures (> 21 °C) (Materna et al., 2012). The other host that seemed unavailable to *V. communis* was the fireworm in SPSPA. Contrasting to the high diversity that corals harbored, this host was dominated by *V. shiloi* (n=143) and some *V. furnissii* strains (n=4). *V. shiloi* was also present in corals (*M. hispida*) in SS. SPSPA and SS are the extremes of the latitudinal gradient uncovered. Corals and the fireworm appear to define the habitat spectrum for *V. shiloi*. Both host associations were previously observed in the Eastern Mediterranean (Sussman et al., 2003), suggesting they are stable and that population-habitat linkage is highly predictable for *V. shiloi*. Moreover, in this survey we found *V. shiloi* associated with corals, in a human impacted coastal area (SS), and with fireworms in the oceanic SPSPA.

Populations of vibrios in the SAO and those in the northwestern Atlantic showed narrow intersection. In addition to *V. splendidus*, other vibrios found in the American northern coast were *V. rumoiensis*, *V. alginolyticus*, *V. fischerillogei*, *V. penaeicida*, *V. superstes*, *V. aestuarianus*, *V. ordalii*, *V. breoganii*, *V. crassostreae*, *V. kanaloae*, *V. tasmaniensis*, *V. gigantis*, and *V. cyclitrophicus* (Preheim et al., 2011; Hunt et al., 2008). The only
common group is *V. alginolyticus*. It was associated with zooplankton and also displayed free-living style in the coastal northern hemisphere (Hunt et al., 2008). In this study *V. alginolyticus* was also found in coastal areas of AB and SS, but associated with almost all cnidarians surveyed, and not in the seawater. Variation in host association and life style may reflect genome heterogeneity, possibly due to a large set of flexible genes. In members of the *Vibrionaceae*, small-scale differences in environmental conditions based on microenvironment and season have been shown to drive lineage adaptation (Hunt et al., 2008) and presumably genome content. A representative study targeting *V. alginolyticus* genomes (n=192) from the Chinese coast revealed high prevalence of mobile genetic elements, including integrating conjugative elements (ICEs), superintegron-like cassettes (SICs), insertion sequences (ISs), and two types of transposase genes (valT1 and valT2). Moreover, BLAST searches and phylogenetic analysis of the ICE, SIC, IS elements and transposase genes showed that the corresponding homologues were bacterial derived from extensive sources, indicating intensive exchange with environmental bacteria (Luo et al., 2012). Indeed, horizontal gene transfer (HGT) mechanism has played an important role in bacterial evolution, facilitating the origins of bacterial diversity and adaptation to new ecological niches (Wiedenbeck and Cohan, 2011). An important feature shared by the habitats where *V. alginolyticus* was common is the vicinity to coast, thus to human activities and nutrient enrichment (SS, AB and Plum Island Estuary, NE Massachusetts).

When we look to the evolutionary history of the generalist and specialist vibrio populations found in this study, two distinct clades defined by MLSA of 8 housekeeping genes can be highlighted: Harveyi and Mediterranei respectively (Sawabe et al., 2007, 2013). Harveyi clade is composed by 9 species mainly associate with seawater, salt
marsh mud, marine animal and mucus of the coral. In some cases, distinguishing species and strains within this clade is hard task in taxonomy, because the presence of recombination between closely related species. Meditteranei clade is composed by 4 species mainly found in habitats as warm seawater and coral mucus (Sawabe et al., 2007, 2013; Moreira et al., 2014 – V. madracius sp novel). Although, the clades are not phylogenetic very closely related, both possess typically pathogenic species in aquatic environments (Reshef et al., 2008; Ruwandeepika et al., 2010; 2011).

We also observed geographic influence in H-4, since it included M. hispida from SS, but not from AB. H-5, 6, 7 and 8 were mainly composed of benthic organisms from combinations of two locations, revealing some connectivity across a spatial scale might also occur. H-5 showed connectivity between AB and SS. H-6 showed connectivity between AB and SPSPA. H-7 showed connectivity between SS and SPSPA.

The influence of temporal dimension in habitat distribution is visualized in Fig.S6. Although not all regions were sampled all years, there was prevalence of 2010 strains in H-2, H-6 and H-8; and of 2007 in H-1 and H-3. On the other hand, strains from 2005, 2006, 2007 and 2010 were present in H-4, H-5 and H-7.

Habitat delineation and taxonomy are congruent

We observed a good congruence between the ecologic grouping generated by the AdaptML and the currently recognized Vibrio species. However, the AdaptML provided further refinement of the species into subspecific groups that may reflect niche partitioning. For instance, we found groups of V. maritimus associated with the seawater and with the coral M. decactis in SPSPA. We defined ecologic groups of vibrios that live...
in the water column and in association with the benthic organisms. Some species (such as *V. harveyi*, *V. aliyonyticus* and *V. communis*) occupied different habitat spectra, whereas other species (such as *V. hepatarius*, *V. rotiferianus* and *V. brasiliensis*) appeared to be restricted to one habitat (Figure S2). Some habitat spectra (e.g. H-1) can be defined by multiple species (e.g. *V. communis*, *V. campbellii*, *V. diabolicus*, *V. pelagius*, *V. hepatarius*, *V. maritimus* and *V. chagasii*). These species are widespread in the water column (up to 150 m depth), representing 86% of the isolates in this habitat spectrum.

The ecological grouping observed in this study suggests dispersal and connectivity among the benthic-pelagic systems in AB. The distribution pattern of the *Vibrio* species from benthic and pelagic sources in AB based on evolutionary history inferred by using the Neighbor-Joining method of pyrH gene sequence (Fig.S4) corroborates with this hypothesis. Genetic coherence among the strains from SS, SPSPA and AB also contributes with the coupling idea. Conspecific identical isolates (e.g. PEL4D and R-680, G35, G52; PEL 103A and R-239, R-264; PEL36B and 1DA5; and others), based on pyrH sequences, originated from the pelagic and benthic systems reinforced the idea of connectivity. Even if AdaptML mistakenly have pooled open water specialists and benthic specialists into one population, these identities (of pyrH sequences from benthic and pelagic isolates) are strong evidence of benthopelagic coupling. It is noteworthy identical pyrH sequences from both compartments and among distantly located isolates, since this gene is one of the most divergent among the pool of housekeeping genes employed for vibrios’ MLSA (Thompson et al. 2005, 2007). Isolates from both open water and benthic sites were also detected when the AdaptML analysis was based on
generic hosts (benthic and pelagic). Moreover, similar cluster distribution of vibrios populations were observed in both parameters analyzed (Fig. 2 and S5).

In spite of the low CFU counts observed in the water column, we suggest that dispersal through the seawater may be important for the persistence of vibrios in the environment. In reef waters, dispersal may be promoted by the shedding of bacteria by the coral host, as a mechanism to regulate the abundance of associated bacteria (Garren & Azam, 2012). The presence of both strategies in vibrios highlights their adaptation to thrive in both oligotrophic (e.g. water column) and copiotrophic (e.g. coral mucus, organic matter particles) environments, and illustrates the genome plasticity of this ubiquitous group. Furthermore, several vibrios observed in the plankton of the AB may have a pathogenic potential to corals. However, we did not recover some known coral pathogens (e.g. V. coralliilyticus and V. shiloi) in our pelagic survey, suggesting that some vibrio species may have evolved into associated habitats, as the coral holobiont, for example, as observed in the H-8. It was demonstrated that V. shiloi and V. coralliilyticus use chemotaxis to find their coral hosts, by sensing a β-D-galactopyranoside-containing receptor and the metabolite dimethylsulfiniopropionate (DMSP), respectively, both present in the coral mucus (Toren et al., 1998; Garren et al., 2013). V. coralliilyticus employs also chemokinesis and its swimming ability is noteworthy (Winn et al., 2013; Garren et al., 2013). These vibrios may thus have a higher host association frequency. Interestingly, V. madracius retrieved only from the coral M. decactis, might indicate a new ecological role of this bacterium in this host. This recently described species (Moreira et al., 2014) is closed related to V. Mediterranei/shiloi, known for the pathogenicity.
Influence of benthopelagic coupling in the coral reef health

We observed that several vibrios associated with the seawater and with benthic organisms (corals) formed a cohesive ecologic unity, indicating the connectivity between the benthic-pelagic compartments. Benthic communities obtain their energy through primary production from the benthic compartment and, to a lesser extent, from the overlying water column. Thereupon, the distribution and abundance of planktonic microbes may be dependent on benthic processes, which affect the transfer of organic material between benthic and pelagic systems (Fowler & Knauer 1986). Bacteria and phytoplankton production are also stimulated by resuspension of nutrients from the seabed into the photic zone, which in turn stimulates zooplankton production, and so on up the food chain (Wainright 1987). In the present study, we reinforce the power of ecologic theory already developed for the study of vibrioplankton from temperate areas (Materna et al., 2012; Szabo et al. 2013). The genetic connectivity observed among the vibrios originated from the seawater and coral hosts in the SAO illustrates the potential influence of the pelagic system in the coral reef systems health. In a scenario of increasing abundance of vibrios, mediated by higher global oceanic temperatures, the pathogenic potential of some *Vibrio* groups may lead to increased incidence of diseases in the marine realm. For instance, *V. vulnificus* implicated in outbreaks were linked to climate change in Israel (Paz et al., 2007), as well as *V. parahaemolyticus* outbreaks documented in Alaska and linked to the consumption of raw seafood followed by episodes of increased seawater temperature, pinpointing a link between climate change and disease (McLaughlin et al 2005; Martinez-Urtaza et al., 2008).

Conclusions
This study was a first attempt to characterize the diversity and the ecological structure of vibrios in several benthic hosts along a latitudinal gradient in the SAO. The occurrence of vibrios from the benthic systems from SPSPA, AB, and SS in the habitats 2, 3 and 4, respectively reinforces the hypothesis that each benthic system may have its own microbiome. Moreover, V. communis' populations showed a true generalist behavior, whilst V. shiloi was confirmed as specialist, associated to H. carunculata and corals. AdaptML analysis generated a good congruence between ecologic grouping and the currently recognized Vibrio species, with further refinement of the species reflecting niche partitioning. Vibrios might occupy the pelagic and the holobiont habitats, indicating coupling between these microbes and their benthic hosts. The benthic pelagic coupling observed in AB, which is the largest South Atlantic reef complex, may suggest the importance of vibrios in the global ocean health.

Acknowledgements

The authors thank Pedro Meirelles comments and technical support of Oswaldo Maia and Milene MA Mesquita.

Supplemental Information

Supplemental information for this article can be found online

Funding Statement

The authors thank CNPq, FAPERJ, and CAPES for the grants.

References
1. Alves Jr N, Neto OSM, Silva BSO, de Moura RL, Francini-Filho RB, Barreira e Castro C, Paranhos R, Bitner-Mathà BC, Kruger RH, Vicente ACP, Thompson CC, Thompson FL. 2010. Diversity and pathogenic potential of vibrios isolated from Abrolhos Bank corals. Environmental Microbiology Reports 2:90-95. DOI: 10.1111/j.1758-2229.2009.00101.x

2. Amado-Filho GM, Moura RL, Bastos AC, Salgado LT, Sumida PY, Guth AZ, Francini-Filho RB, Pereira-Filho GH, Abrantes DP, Brasileiro PS, Bahia RG, Leal RN, Kaufman L, Kleypas JA, Farina M, Thompson FL. 2012. Rhodolith beds are major CaCO3 bio-factories in the tropical South West Atlantic. PLoS One 7(4): e35171. DOI: 10.1371/journal.pone.0035171

3. Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, Martinez-Urtaza J. 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. Nature Climate Change 3:73-77. DOI: 10.1038/nclimate1628

4. Bruce T, Meirelles PM, Garcia G, Paranhos R, Rezende CE, de Moura RL, Filho RF, Coni EO, Vasconcelos AT, Amado Filho G, Hatay M, Schmieder R, Edwards R, Dinsdale E, Thompson FL. 2012. Abrolhos Bank Reef Health Evaluated by Means of Water Quality, Microbial Diversity, Benthic Cover, and Fish Biomass Data. PLoS ONE 7(6): e36687. DOI: 10.1371/journal.pone.0036687

5. Castro BM, Miranda LB. 1998. Physical oceanography of the western Atlantic continental shelf located between 4º N and 34º S, in: Robinson, A.R., Brink, K.H. (Eds.), The Sea. 11, John Wiley and Sons, New York, pp. 209-252.

6. Cavalcanti G S, Gregoracci GB, Moura RL, Amado-Filho GM, Bastos AC, Francini-Filho R, Paranhos R, Ferreira, CM, Ghisolfi RD, Kruger R, Guth A, Z, Sumida PYG, Bruce T, Maia-Neto O, Santos EO, lida T, Thompson FL. 2013a. Sinkhole-like structures as bioproductivity hotspots in the Abrolhos Bank. Continental Shelf Research 70:126–134.DOI: 10.1016/j.bbr.2011.03.031

7. Cavalcanti GS, Gregoracci GB, Dos Santos EO, Silveira CB, Meirelles PM, Longo L, Gotoh K, Nakamura S, lida T, Sawabe T, Rezende CE, Francini-Filho RB, Moura RL, Amado-Filho GM, Thompson FL. 2013b. Physiologic and metagenomic attributes of the rhodoliths forming the largest CaCO(3) bed in the South Atlantic Ocean. ISME J 8(1):52-62. DOI: 10.1038/ismej.2013.133.

8. Chimetto LA, Brocchi M, Gondo ML, Thompson CC, Gomez-Gil B, Thompson FL. 2009. Genomic diversity of vibrios associated with the Brazilian coral Mussismilia hispida and its sympatric zoanthids (Palythoa caribaeorum, P. variabilis, and Zoanthus solanderi). Journal of Applied Microbiology 106: 1818-1826.DOI: 10.1111/j.1365-2672.2009.04419.x

9. David LA. 2010. Novel Phylogenetic Approaches to Problems in Microbial Genomics. Thesis. MIT. USA

10. Fowler SW, Knauer GA. 1986. Role of large sinking particles in the transport of elements and organic compounds through the water. Prog Oceanogr 16:147

11. Foster MS. 2001. Rhodoliths: between rocks and soft places. J Phycol 37: 659–667. DOI: 10.1046/j.1529-8817.2001.00195.x

12. Francini-Filho RB, Moura RL, Thompson FL, Reis RM, Kaufman L, Kikuchi RK, Leão ZM. 2008. Diseases leading to accelerated decline of reef corals in the...
largest South Atlantic reef complex (Abrolhos Bank, eastern Brazil). *Mar Pollut Bull* 56(5):1008-14. DOI: 10.1016/j.marpolbul.2008.02.013

13. Fukui Y, Saitoh S, Sawabe T. 2010. Environmental determinants correlated to *Vibrio harveyi*-mediated death of marine gastropods. *Environ Microbiol* 12(1):124-33.DOI: 10.1111/j.1462-2920.2009.02052.x.

14. Garren M, Azam F. 2012. Corals shed bacteria as a potential mechanism of resilience to organic matter enrichment. *ISME J* 6(6):1159-65. DOI: 10.1038/ismej.2011.180.

15. Garren M, Son K, Raina JB, Rusconi R, Menolascina F, Shapiro OH, Tout J, Bourne DG, Seymour JR, Stocker R. 2013. A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. *ISME J* [Epub ahead of print]. DOI: 10.1038/ismej.2013.210.

16. Gregoracci GB, Nascimento JR, Cabral AS, Paranhos R, Valentin JL, Thompson CC, Thompson FL. 2012. Structuring of bacterioplankton diversity in a large tropical bay. *PLoS One* 7(2):e31408.DOI: 10.1371/journal.pone.0031408

17. Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52: 696–704.DOI: 10.1080/10635150390235520.

18. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59: 307-21.

19. Hibbing ME, Fuqua C, Parsek MR, Peterson SB. 2010. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 8(1):15-25. DOI: 10.1038/nrmicro2259.

20. Hunt DE, David LA, Gevers D, Preheim SP, Alm, EJ, Polz MF. 2008. Resource partitioning and sympatric differentiation among closely related bacterioplankton. *Science* 320(5879):1081-5.DOI: 10.1126/science.1157890.

21. Letunick I, Bork P. 2007. Interactive Tree Of Life (ITOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23(1):127-8.DOI: 10.1093/bioinformatics/btl529.

22. Luo P, Jiang H, Wang Y, Su T, Hu C, Ren C, Jiang X. 2012. Prevalence of mobile genetic elements and transposase genes in *Vibrio alginolyticus* from the southern coastal region of China and their role in horizontal gene transfer. *Int Microbiol* 15(4):201-10. DOI: 10.2436/20.1501.01.173

23. Martinez-Urtaza J, Huapaya B, Gavilan RG, Blanco-Abad V, Ansede-Bermejo J, Cadarso-Suarez C, Figueiras A, Trinanes J. 2008. Emergence of asiatic Vibrio diseases in South America in phase with El Niño. *Epidemiology* 19:829–837. DOI: 10.1097/EDE.0b013e3181883d43.

24. Materna AC, Friedman J, Bauer C, David C, Chen S, Huang IB, Gillens A, ClarkeMcLaughlin JB, DePaola A, Bopp CA, Martinek KA, Napoliili NP, Allison CG, Murray SL, Thompson EC, Bird MM, Middaugh JP. 2005. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *New Eng J Med* 353:1463–1470. DOI: 10.1056/NEJMoa051594.
25. McCarter L. 1999. The multiple identities of *Vibrio parahaemolyticus*. *Journal of Molecular Microbiology and Biotechnology* 1:51-57
26. McDougald D, Kjelleberg S. 2006. Adaptive responses of *Vibrios*. In: Thompson FL, Austin B, Swings J (eds) *The biology of Vibrios*. American Society for Microbiology, Washington D.C., p 133-155
27. Ministério do Meio Ambiente – MMA. Programa REVIZEE: avaliação do potencial sustentável de recursos vivos na zona econômica exclusiva: relatório executivo. 2006. Brasília, Brasil. 280 p. ISBN: 85-7738-027-0.
28. Moreira APB, Chimetto Tonon LA, Valle P, Pereira C, Alves N, Amado-Filho G M, Francini-Filho RB, Paranhos R, Thompson FL. 2013. Culturable Heterotrophic Bacteria Associated with Healthy and Bleached Scleractinian Madracis decactis and the Fireworm Hermodice carunculata from the Remote St. Peter and St. Paul Archipelago, Brazil. *Current Microbiology* 68(1):38-46. DOI: 10.1007/s00284-013-0435-1.
29. Moreira AP, Duytschaever G, Tonon LAC, Dias GM, Mesquita M, Cnockaert M, Francini-Filho RB, DeVos P, Thompson CC, Thompson FL. 2014. *Vibrio madracificus* sp. nov. isolated from *Madracis decactis* (Scleractinia) in St Peter & St Paul Archipelago, Mid-Atlantic Ridge, Brazil. *Curr Microbiol* 69(4):405-11. DOI: 10.1007/s00284-014-0600-1.
30. Oliveira OMP, Marques AC. 2007. Dinâmica sazonal das massas de água no canal de São Sebastião (SE Brasil) de março de 2005 a maio de 2006. *XII Congresso Latino-Americano de Ciências do Mar - XII COLACMAR*
31. Paz S, Bisharat N, Paz E, Kidar O, Cohen D. 2007. Climate change and the emergence of *Vibrio vulnificus* disease in Israel. *Environ Res* 103:390–396. DOI: 10.1016/j.envres.2006.07.002
32. Pitcher DG, Saunders NA, Owen RJ. 1989. Rapid Extraction of Bacterial Genomic DNA with Guanidium Thiocyanate. *Letters in Applied Microbiology* 8:151–156. DOI: 10.1111/j.1472-765X.1989.tb00262.x
33. Preheim SP. 2010. Ecology and population structure of Vibrionaceae in the coastal ocean. MIT thesis.
34. Preheim SP, Boucher Y, Wildschutte H, David LA, Veneziano D, Alm EJ, Polz MF. 2011. Metapopulation structure of Vibrionaceae among coastal marine invertebrates. *Environ Microbiol* 13(1):265-75. DOI: 10.1111/j.1462-2920.2010.02328.x
35. Reshef L, Ron E, Rosenberg E. 2008. Genome analysis of the coral bleaching pathogen *Vibrio shiloi*. *Arch Microbiol* 190(2):185-94. DOI: 10.1007/s00203-008-0388-0.
36. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007. The role of microorganisms in coral health, disease e evolution. *Nat Rev Microbiol* 5: 355–362.DOI: 10.1038/nrmicro1635.
37. Ruwandeepika HA, Defoirdt T, Bhowmick PP, Shekar M, Bossier P, Karunasagar I. 2010. Presence of typical and atypical virulence genes in vibrio isolates belonging to the Harveyi clade. *J Appl Microbiol* 109(3):888-99. DOI: 10.1111/j.1365-2672.2010.04715.x.
38. Ruwandeepika HA, Defoirdt T, Bhowmick PP, Karunasagar I, Karunasagar I, Bossier P. 2011. In vitro and in vivo expression of virulence genes in Vibrio isolates belonging to the Harveyi clade in relation to their virulence towards gnotobiotic brine shrimp (Artemia franciscana). *Environ Microbiol* **13(2)**:506-17. DOI: 10.1111/j.1462-2920.2010.02354.x.

39. Materna AC, Friedman J, Bauer C, David C, Chen S, Huang IB, Gillens A, Clarke SA, Polz MF, Alm EJ. 2012. Shape and evolution of the fundamental niche in marine *Vibrio*. *ISME J* **6(12)**:2168-77. DOI: 10.1038/ismej.2012.65.

40. Sawabe T, Kita-Tsukamoto K, Thompson FL. 2007. Inferring the evolutionary history of vibrios by means of multilocus sequence analysis. *J Bacteriol* **189**:7932–7936. DOI: 10.1128/JB.00693-07.

41. Sawabe T, Ogura Y, Matsumura Y, Feng G, Amin AR, Mino S, Nakagawa S, Sawabe T, Kumar R, Fukui Y. 2013. Updating the *Vibrio* clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of *Vibrio tritonius* sp. nov. *Front Microbiol* **4**:414. DOI: 10.3389/fmicb.2013.00414.

42. Sussman M, Loya Y, Fine M, Rosenberg E. 2003. The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. *Environ Microbiol* **5(4)**:250-5. DOI: 10.1046/j.1462-2920.2003.00424.x

43. Szabo G, Preheim SP, Kauffman KM, David LA, Shapiro J, Alm EJ, Polz MF. 2013. Reproducibility of *Vibrionaceae* population structure in coastal bacterioplankton. *ISME J* **7(3)**:509-19. DOI: 10.1038/ismej.2012.134.

44. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol Biol Evol* **28(10)**:2731-9. DOI:10.1093/molbev/msr121.

45. Thompson FL, Gevers D, Thompson CC, Dawyndt P, Naser S, Hoste B, Munn CB, Swings J. 2005. Phylogeny and molecular identification of vibrios on the basis of multilocus sequence analysis. *Appl Environ Microbiol* **71(9)**:5107-15.DOI: 10.1128/j.syapm.2011.09.001.

46. Thompson FL, Gomez-Gil B, Vasconcelos AT, Sawabe T. 2007. Multilocus sequence analysis reveals that *Vibrio harveyi* and *V. campbellii* are distinct species. *Appl Environ Microbiol* **73**: 4279-85. DOI: 10.1128/AEM.00020-07

47. Thompson JR, Polz MF. 2006. Dynamics of *Vibrio* populations and their role in environmental nutrient cycling. In: Thompson FL, Austin B, Swings J (eds) The biology of *Vibrios*. American Society for Microbiology, Washington D.C., p 190 - 203

48. Thompson JR, Pacocha S, Pharino C, Klepac-Ceraj V, Hunt DE, Benoit J, Sarma-Rupavtarm R, Distel DL, Polz MF. 2005. Genotypic diversity within a natural coastal bacterioplankton population. *Science* **307(5713)**:1311-3. DOI:10.1126/science.1106028.

49. Tilman D. 1982. Resource competition and community structure. *Princeton University Press, Princeton.*
50. Toren A, Landau L, Kushmaro A, Loya Y, Rosenberg E. 1998. Effect of temperature on adhesion of *Vibrio* strain AK-1 to *Oculina patagonica* and on coral bleaching. *Appl Environ Microbiol* 64:1379–1384.

51. Vezzulli L, Previati M, Pruzzo C, Marchese A, Bourne DG, Cerrano C, *Vibrio*Sea Consortium. 2010. *Vibrio* infections triggering mass mortality events in a warming Mediterranean Sea. *Environmental Microbiology* 12: 2007–2019. DOI: 10.1111/j.1462-2920.2010.02209.x

52. Vezzulli L, Brettar I, Pezzati E, Reid PC, Colwell RR, Höfle MG, Pruzzo C. 2012. Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios. *ISME J* 6(1):21-30. DOI: 10.1038/ismej.2011.89.

53. Wiedenbeck J, Cohan FM. 2011. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiol Rev* 35(5):957-76. DOI: 10.1111/j.1574-6976.2011.00292.x. Review.

54. Viégas FB, Wattenberg M, Ham FV, Kriss J, McKeon M. 2007. Many Eyes: A Site for Visualization at Internet Scale. *Infovis* 13(6): 1121-1128. DOI: 10.1109/TVCG.2007.70577

55. Winn KM, Bourne DG, Mitchell JG. 2013. *Vibrio coralliilyticus* search patterns across an oxygen gradient. *PLoS One* 8(7):e67975. DOI: 10.1371/journal.pone.0067975.
Figures Legend.

Figure 1. Map of Brazil with sampling regions depicted. Microenvironments are highlighted in each sampling site. A= Saint Peter and Saint Paul Archipelago. Hosts investigated *Hermodice Carunculata*, *Scolymia wellsi* and *Madracis decactis*. B= Abrolhos Bank. Sediment, rhodolith, water, *Mussismilia brasiliensis*, *M. hispida* and *Phylogorgia dilatata*. C= Saint Sebastian region. *M. hispida*, *Zoanthus solanderi*, *Palythoa caribaeorum* and *P. variabilis*.

Figure 2. Inferred habitat associations for all ancestors of sequenced *Vibrio* strains. The rings surrounding the tree represent the isolation source (outer) and the sampling site (inner) from which strains were isolated. The maximum likelihood assignment of nodes to habitats is shown for clades supported by bootstraps > 80%. Colored circles on each branch indicate the habitat spectrum assignment (H1-H8) for the node immediately below that branch (see above legend for color scheme). Branch lengths are adjusted to aid visualization and do not represent evolutionary distances.* Highlights the isolation source water.

Figure 3. Distribution of the environmental categories that compose each of the 8 habitats predicted by AdapML. Distributions are normalized by the total number of isolates in each environmental category to reduce the effect of uneven sampling.
Table 1. Detection of vibrios species found in seawater and at the same time in other hosts investigated in this study. ND = Not detected

Supplementary Material

Supplementary Figure S1. Distribution of the studied regions [Saint Peter and Saint Paul Archipelago (SPSPA), Abrolhos Bank (AB) and Saint Sebastian channel (SS)] in each habitat composition defined by AdaptML approach. Scale represents percentage.

Supplementary Figure S2. Vibrio species diversity in the habitats. The figure shows how the 775 Vibrio strains are distributed in each habitat. The side of the circle represents the proportion of total strains in each group. Others = represents Vibrio species with low abundance found in that group. Figure generated through Many Eyes website (Viegas et al., 2007).

Supplementary Figure S3. PyrH tree of vibrios from plankton, rhodoliths and corals. Phylogenetic tree based on the neighbor-joining method using pyrH gene sequences showing the relationships among representative Vibrio species from plankton (blue color), rhodoliths (red color) and corals (black). Type strains of Vibrio were included (bold black). Distance estimations were obtained according to the Kimura-2-parameter model. Bootstrap percentages after 1000 replications are shown. Divergence bar estimated at 2%. Depth is indicated for planktonic strains.

Supplementary Figure S4. Evolutionary history inferred by using the Neighbor-Joining method based on 532 positions of pyrH gene sequence in the final dataset. The bootstrap test (1000 replicates) are shown next to the branches. The evolutionary
distances were computed using the Maximum Composite Likelihood method and are in
the units of the number of base substitutions per site. The analysis involved 316
nucleotide sequences including only strains from AB region and type strains of each
represented species. All ambiguous positions were removed for each sequence pair.
White and red circles represent strains from benthic source and blue circles from
pelagic.

Supplementary Figure S5. Inferred habitat associations for all ancestors of sequenced
Vibrio strains from AB region. The rings surrounding the tree represent the isolation
source (outer) and the collection point (inner) from which strains were isolated. The
maximum likelihood assignment of nodes to habitats is shown for clades supported by bootstraps
> 80%. Colored circles on each branch indicate the habitat assignment (H1-H6). Branch
lengths were adjusted to aid visualization and do not represent evolutionary distances.

Supplementary Figure S6. Habitat distribution according to sampling time. The values
represents percentages.

Supplementary Table S1. Strain list.
Supplementary Table S2. Habitat assignment.
Brazil Map showing the sampling regions

Microenvironments are highlighted in each sampling site. A= Saint Peter and Saint Paul Archipelago. Hosts investigated Hermodice Carunculata, Scolymia wellsi and Madracis decactis. B= Abrolhos Bank. Sediment, rhodolith, water, Mussismilia brasiliensis, M. hispida and Phylogorgia dilatata. C= Saint Sebastian region. M. hispida, Zoanthus solanderi, Palythoa caribaeorum and P. variabilis.
Inferred habitat associations for all ancestors of sequenced *Vibrio* strains.

The rings surrounding the tree represent the isolation source (outer) and the sampling site (inner) from which strains were isolated. The maximum likelihood assignment of nodes to habitats is shown for all nodes, regardless of the confidence of each prediction. Colored circles on each branch indicate the habitat spectrum assignment (H1-H8) for the node immediately below that branch (see above legend for color scheme). Branch lengths are adjusted to aid visualization and do not represent evolutionary distances.* Highlights the isolation source water.
Distribution of the environmental categories that compose each of the 8 habitats predicted by AdapML.

Distributions are normalized by the total number of isolates in each environmental category to reduce the effect of uneven sampling.
Table 1 (on next page)

Detection of vibrios species found in seawater and at the same time in other hosts investigated in this study.

ND = Not detected.
Table 1. Detection of vibrios species found in seawater and at the same time in other hosts investigated in this study. ND = Not detected

| Species         | M. hispida | P. dilatada | M. decactis | M. brasiiliensis | P. caribaeorum | S. wellsii | rhodolith | P. variabilis | sediment |
|-----------------|------------|-------------|-------------|------------------|----------------|------------|-----------|--------------|----------|
| V. communi      | X          | X           | X           | X                | X              | X          | X         | X            | ND       |
| V. harveyi      | X          | X           | X           | X                | X              | X          | X         | ND           | X        |
| V. campbellii   | X          | X           | X           | X                | X              | ND         | ND        | ND           | ND       |
| V. chagasi      | X          | X           | X           | X                | ND             | ND         | ND        | ND           | X        |
| V. pelagius     | X          | X           | X           | ND               | ND             | ND         | ND        | ND           | ND       |
| V. diabolicus   | X          | X           | ND          | ND               | ND             | ND         | ND        | ND           | ND       |