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Density of Associated Macrofauna of Black Corals (Anthozoa: Antipatharia) in Jagna, Bohol, Central Philippines

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Black corals or antipatharians (Anthozoa: Antipatharia) are abundantly found in the waters (depths of 15-40 m) of Jagna, Bohol, Central Philippines. However, inaccessibility due to SCUBA depth limitation has discouraged researchers from documenting the ecological role of antipatharians in hosting associated macrofauna. The study, conducted in August-September 2013 and February 2014 at 15-40 m depth, compared the density of macrofauna between whip and branching black corals among different colony sizes, across six sampling sites and different depth ranges. Each colony size of black corals was determined by writing a script under LabVIEW. Of the forty colonies observed (eight branching and 32 whip types), 68% (or 27 colonies) hosted associated macrofauna. Eighteen macrofauna taxa belonging to six phyla were identified: Porifera, Cnidaria (Anthozoa), Mollusca (Gastropoda and Bivalvia), Echinodermata (Crinoidea), Arthropoda (Cirripedia) and Chordata (Ascidiaceae and Actinopterygii). The average density of macrofauna ranged from 82 to 8,313 individuals/m^2, with counts ranging from 1 to 74 individuals. The crinoids were found to be the most abundant with 243 individuals and a density of 166 individuals/m^2. By generating data using Monte Carlo simulation and comparison by student t-test at 95%, p=0.05, a significant difference in the density of macrofauna between whip and branching black corals was found. However, there was no significant difference between density of macrofauna and sampling stations, depth, and colony size (Spearman R correlation and Kruskal-Wallis test; 95%, p=0.05). This lack of statistical difference suggests higher within group variance than between group variance. Black corals should be protected in the entire study site to promote biodiversity at depths and in areas not inhabited by scleractinian corals.

Keywords: biodiversity, Cnidarians, Crinoidea, LabVIEW, marine conservation, MPA

INTRODUCTION

Antipatharians are colonial anthozoans characterized by small polyps with 6 simple tentacles and a noncalcareous skeletal axis with colonies which can grow to a height of 10 cm to 2 m or more. These organisms are exclusively marine with some species thriving in areas with reduced light intensity and depths ranging from about 20 to 500 m (Opresko 2009). The bathymetric distributions and habitat preferences of some black corals species can be attributed to the interactions among abiotic and biotic factors (i.e., competition for space with stony corals in shallow water). As a consequence, these species show increasing abundance and diversity with depth (Tazioli et al. 2007).

Ecologically, antipatharians contribute to the three-dimensionality of the coral reefs and host a rich associated fauna like stalked barnacles and zoanthids

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(Bo et al. 2012). In North Sulawesi, Indonesia, they are very favorable environments for filter feeders (e.g. Crinoidea and Ophiuroidea), commensal decapods, parasitic invertebrates and fishes (Tazioli et al. 2007). However, despite their ecological importance, black corals are considered as one of the least studied groups within the non-scleractinian corals (Bo et al. 2009) mainly due to limited field studies (Tazioli et al. 2007) and the logistic constraints involving deep diving and its safety measures. Related studies generally concerning the ecology, population structure, distribution and abundance of black corals can be found in Warner (1981) for Trinidad, West Indies, Grange (1985) and Grange & Singleton (1988) for New Zealand, Sanchez (1999) for Colombia, Caribbean Sea, shallow-water of Caribbean Reef (Opresko & Sanchez 2005), Southern California (Tissot et al. 2006), North Sulawesi, Indonesia (Tazioli et al. 2007), Calabrian coast (South Italy, Tyrrhenian Sea) (Bo et al. 2009), Ecuador (Bo et al. 2012) and Colombian Caribbean (Santodomingo et al. 2013). While most of the aforementioned studies mainly focus on population structure, distribution and abundance, only few dealt with associated organisms of black coral colonies. Some of these studies include assessment of benthic invertebrates that form habitats in deep banks specifically on sponges, black corals, and gorgonians (Tissot et al. 2006), symbiotic associations involving black corals in the Marine Park of Bunaken, North Sulawesi, Indonesia (Tazioli et al. 2007), epibiotic zoanthids observed on the black coral colonies from Machalilla National Park, Ecuador (Bo et al. 2012), the epibiontic assemblages of *Antipathella subpinnata* of the central Mediterranean Sea (Bo et al. 2009) and the different invertebrates associated with Christmas tree black coral, *Antipathes dendrochristos* (Love et al. 2007).

All species of Antipatharians are protected under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) because they are being harvested for use in the jewelry trade (Green & Shirley 1999). In the Philippines, the presence of black corals is confirmed to occur, with reports of harvesting for making costumes jewelries. Green & Shirley (1999) documented that trading of black corals from 1982-1997 recorded a total of 72t and 7,100,000 pieces of black corals, with an average of 430,000 pieces of corals traded internationally per year. 55% of all imports (transaction recorded by weight) in this trade came from the Philippines with USA as the major importer. Despite this, there is still lack of studies on black corals in the Philippines, whether taxonomic or ecological (e.g. distribution, population structure, role in the ecosystem). This is also because the usual distribution of black corals tends to be at the relatively deeper portion which pose logistic constraints when conducting evaluation and monitoring. Furthermore, no efforts had been made to quantify the significance of these organisms in terms of their role as habitat for various macrofauna species at depths no longer dominated by scleratinian corals (e.g. <25 m depth). This could be a major reason why the ecological roles of antipatharians had never been an area of interest to resource managers in the Philippines.

In this study, the density of macrofauna associated with the black coral colonies in Jagna, Bohol, Central Philippines was assessed. The study aimed to know if macrofauna population density varies with depth, colony size of black corals, sampling stations and the general morphology of the corallum (e.g. whip or branching). Density was computed as count of the associated fauna per unit area of a black coral colony. The cross-sectional area of a black coral colony was generated using LabView computer program. Despite losing depth information in the 2-dimensional image of a 3-dimensional object, the maximum projected cross-sectional area of the colonies was calculated based on their optimum projection on the camera plane. The density is then calculated as the ratio of the number of associated organisms to the maximum cross-sectional area of a black coral colony. Associated fauna in this study is defined as the macrovertebrates and invertebrates that utilized the black coral colony either as habitat (e.g. means of attachments), feeding grounds, or potential nursery grounds. Preliminary repeated observations showed that certain species of fishes are residing in black corals as they are frequently seen hovering around it. The baseline information generated from this study could provide information to Local Government Units (LGUs) on the ecological role of black corals as habitats of other macrofauna.

MATERIALS AND METHODS

Description of the Study Site

This study was conducted by SCUBA diving at daytime, at depths of 15 to 40 meters. The sampling location was in Jagna, Bohol (9.6274170 N and 124.3580 E), 63 km away from Tagbilaran City, between barangay Ipil and Cantagay (Figure 1). The current in this area is strong (approximately 1.3m/sec) and the sunlight is relatively minimal but fishes were observed to be present utilizing the black coral area, possibly as a refuge from predators. Gorgonians and other soft corals can be found coexisting with the black corals.

Collection of Data

A reconnaissance dive was conducted prior to the actual survey to determine the exact geographic location and distributions of the black corals. Thereafter, a total of six

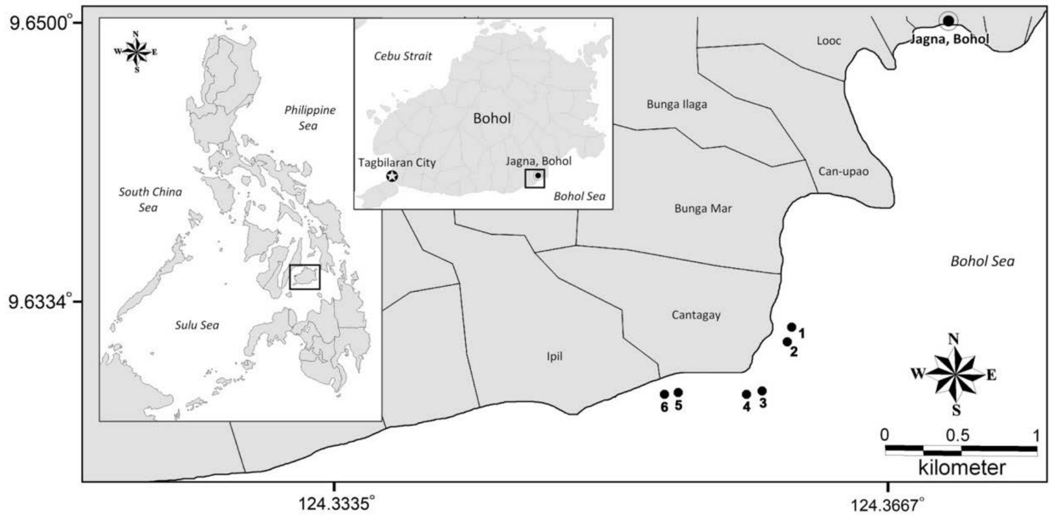

Figure 1. Map of Jagna, Bohol in Central Philippines, showing the six sampling areas.

Figure 2. A sample image of a black coral. A measuring tape was used as a scale bar and was needed during image analysis using LABView.

survey sites were chosen. Using a compass, two divers swam perpendicular to the shore going down from 15 m deep until 40 m. Since bottom time underwater was limited to only 10-15 minutes, the colonies encountered during each dive were considered. Any black coral colony situated every 5 kicks was photographed (with meter tape as scale bar) and associated macrofauna thriving in that colony were recorded, counted and photodocumented *in situ*. To obtain a calibration factor and ascertain its accuracy, the meter tape and the black coral should be of the same distance from the camera (Figure 2). For each colony, the following sets of information were gathered: morphological description of each species (branching or whip-like), associated macrofauna species, macrofauna count, dimension of the colony (area of the colony), depth where the colony was found. Sampling was conducted during the months of Aug 2013, Sept 2013, and Feb 2014.

Species of macrofauna included fishes that hover around the black coral and the different invertebrates that attaches itself to these colonies. Efforts were made to identify these organisms to the genus level, otherwise, higher taxonomic ranking was considered. Each species were counted individually but for the encrusting organisms (e.g. colonial anemones, sponges), they were taken as one.

Data Analysis

Determination of the area of a black coral colony

A computer vision program was developed using LabView to measure the physical dimensions of black coral colonies from underwater pictures. Pixel calibration was performed for all images individually to eliminate unwanted optical artifacts. The area of a black coral colony is calculated from the product of the measured maximum cross-sectional length and width. The maximum cross-sectional length is determined between the anterior part to the base of the colony, while the maximum cross-sectional width is between one lateral point to the next (Figure 2). Determination of the length and width of each colony were done three times, with the average of these three readings taken as the final value.

Statistical Analysis

Differences in the macrofauna density between whip and branching black corals were determined by generating data through Monte Carlo simulation assuming Gaussian distribution and comparison by student t-test. Using the measured mean and standard deviation of the macrofauna density between whip and branching black corals, a total of 667 data points were generated. Correlations between

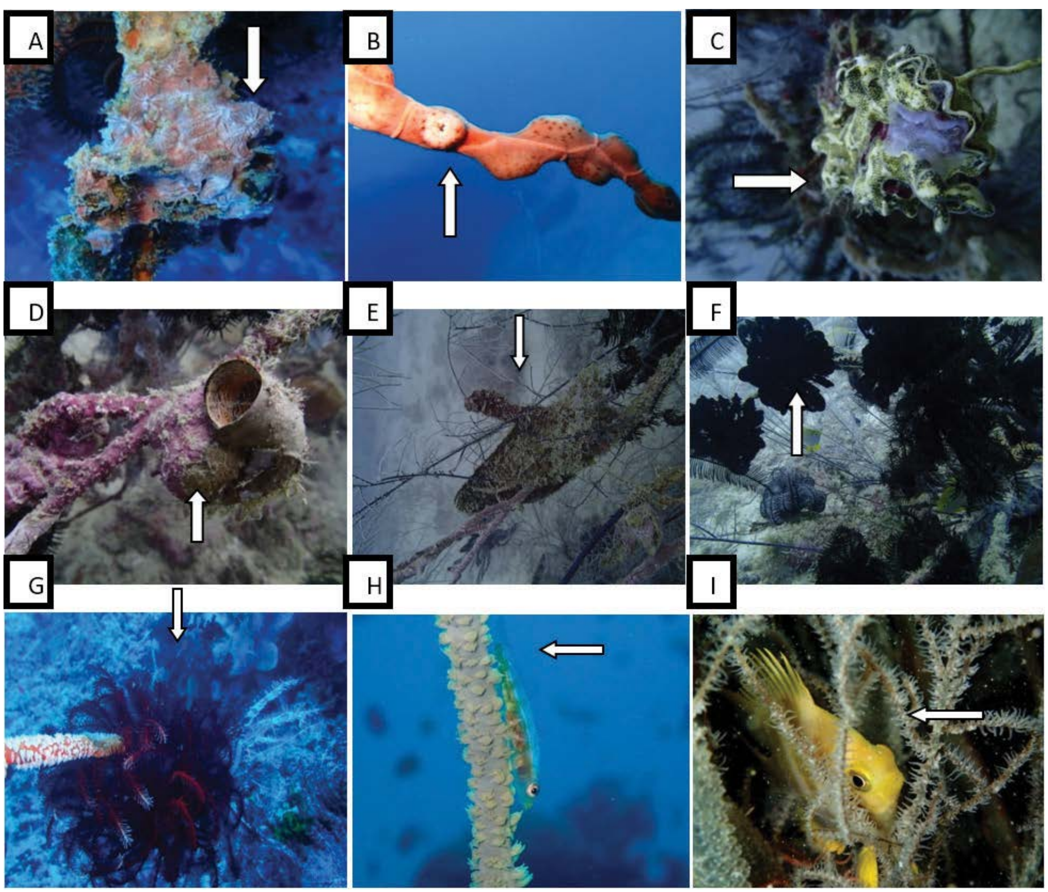

Figure 3. Examples of associated macrofauna found in the black coral colonies. (A) sponge encrusting the whip black coral; (B) Anemone that is colonial covering the entire colony of the whip black coral; (C) the unidentified cnidarians; (D) Unidentified gastropod that is associated only with the branching black corals; (E) Oyster attached to the branching black corals; (F) The crinoids inhabiting the branching black corals; (G) Crinoids found in the largest whip black coral; (H) Goby inhabiting in the whip black corals; (I) Damselfish that is observed in the branching black corals. *(Photocredits: J. Horowitz)*

Table 1. Counts of associated macrofauna of the whip and branching black corals.

Associated Species	Whip	Branching	Total
Phylum Porifera	10	7	**17**
Phylum Cnidaria			**8**
Class Anthozoa			
Anemone 1 (colonial)	4		
Anemone 2	1		
Soft corals	2		
Unidentified species		1	
Phylum Mollusca			**41**
Class Gastropoda			
Limpets	1		
Slug	1		
Unidentified gastropod		13	
Class Bivalvia			
Oyster	7	19	
Phylum Echinodermata			**244**
Class Crinoidea			
Featherstar	34	209	
Class Ophiuroidea			
Brittle star		1	
Phylum Arthropoda			**3**
Subclass Cirripedia			
Barnacle 1		1	
Stalked barnacle	2		
Phylum Chordata			**56**
Class Ascidiacea			
Tunicate	1		
Class Actinopterygii			
Family Pomancentridae		31	
Family Gobiidae	6		
Family Labridae		6	
Family Centriscidae		9	
Family Pomacanthidae	3		

macrofauna density and colony size, between depth and macrofauna density were tested using Spearman R correlation. Kruskal-Wallis test was used to determine difference between macrofaunal density across various depth ranges and sampling stations. The significant level was set to 95% (p=0.05).

RESULTS

There were 40 black coral colonies surveyed from depths of 15 to 40 m. The spatial distribution tended to show defined composition as each of these sites either predominantly contain all the branching or the whip types. However, it was noted that there were more whip type (32 colonies) compared to the branching black corals (8 colonies). From all the colonies counted, 27 (or 68%) hosted associated macrofauna, where 100% of the branching type and 48% of the whip type were observed to contain associated organisms. These 40 colonies had a total area of 1.46 m^2, as analyzed by image analysis using LabVIEW.

Eighteen species of macrofauna belonging to six phyla were counted at all six sampling stations (Table 1 and Figure 3). Seventeen occurrences of different species of sponges (Phylum Porifera) were observed to have encrusted the colonies of black corals. Most were frequently seen in branching black corals, attaching themeselves to the branches but they were also present especially in the bigger whip black coral. Three species belonging to Phylum Cnidaria were found in the black coral colony. Anemones were found encrusted in four whip black corals, notably killing the specific portion that was being colonized (Figure 3B). Soft corals were found at the base of a big whip black coral while an unidentified cnidarian attached itself to one of the branching black coral colony. Four species of molluscs were seen attaching themselves to the colonies of whip, branching, or both types of black corals. The limpets and slugs were only found in the whip type while the unidentified gastropods with 13 individuals (Figure 3D) were associated only with the colonies of the branching type. The most number of counts within this phylum were the bivalves (oyster) with 26 individuals and were found in both types of black corals (Figure 3E). Phylum Echinodermata, specifically the crinoids (feather stars) had the most number of individuals counted at 243, having a density of 166 individuals/m^2 (Figures 3F & 3G). These species were seen attached to the colonies regardless of whether it was branching or whip, and tended to dominate within the black coral colonies. The members of Phylum Arthropoda had the least number of counts with only three individuals comprising mainly of barnacles. Majority of the species belonging to Phylum chordata were mostly fishes, either directly or indirectly associated with the black coral colonies. Those observed to frequently stay near or on the colonies were the gobies, found only in the whip black corals (Figure 3H) and the damselfishes that tended to occur in groups, apparently utilizing the branching black corals as their habitats (Figure 3I).

The density of macrofauna ranged from 82 to 8,313 individuals/m2 with an average of 640 individuals/m^2.Across different groups, the echinoderms had the highest density averaging at 346 individuals/m^2 while the lowest was the arthropods averaging at 6 individuals/m^2 (Figure 4A). Between the types of black corals, the macrofauna density for the branching was 428 individuals/m^2 while those associated with the whip black corals had an average density of 689 individuals/m^2 (Figure 4B). In terms of depths, average density was from 112 to 2,392 individuals/m^2 (Figure 4C). The highest value was found at a depth of 36-40 m and was composed of crinoids, anemones, oysters, and wrasses while the lowest density was at 30-35 m with only one limpet observed. Among sampling stations, Station 5 had the highest average density at 1,920 individuals/m^2 and were inhabited by sponges, anemones, and crinoids. Station 6 had the 2nd highest average density at 803 individuals/m^2 but contained a diverse fauna compared to Station 6. These macrofauna were crinoids, stalked barnacles, sponges, bivalves, slugs, soft corals, and gobies. Both of these stations were composed of whip black corals. Station 4, which contained branching black corals, showed the lowest average density of 137 individuals/m^2 but were also relatively diverse despite the low number of black

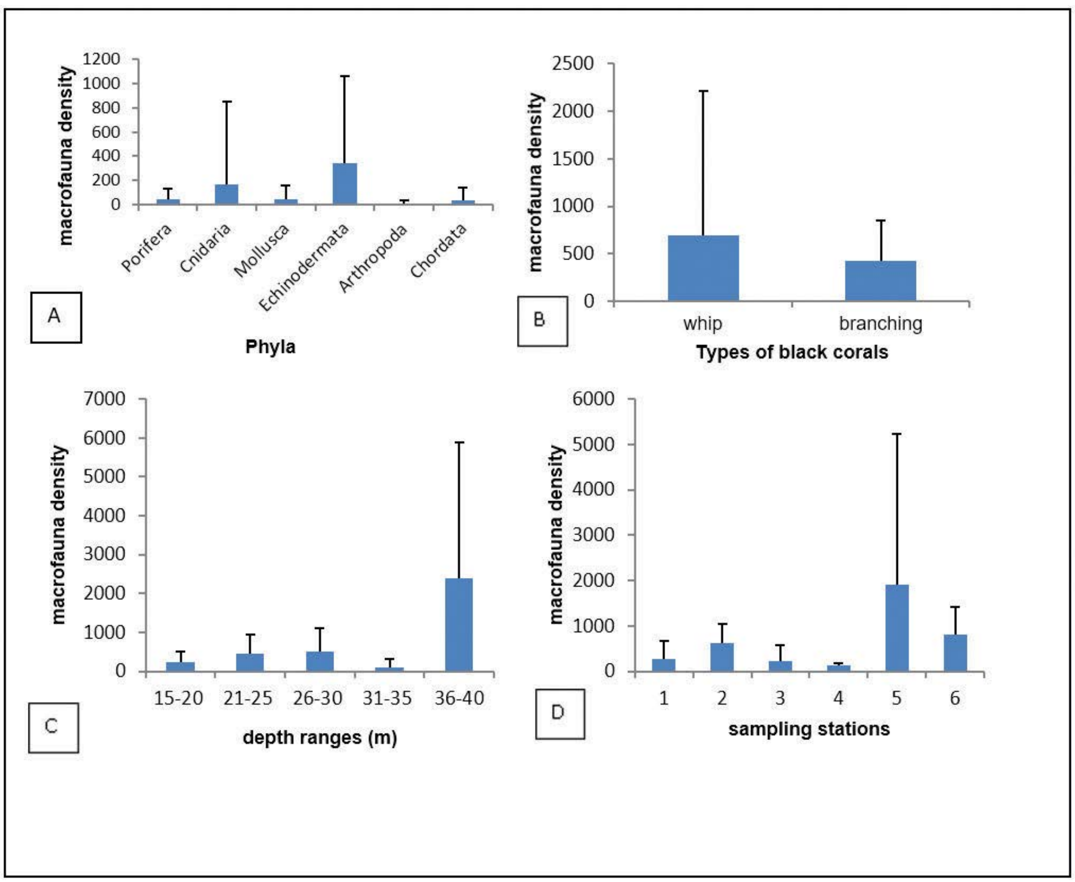

Figure 4. Density of associated macrofauna (in ind./m^2). (A) different phyla; (B) between whip and branching black corals; (C) different depth ranges; (D) different sampling stations. Error bars are SD.

coral colonies found in this site (Figure 4D). Damselfishes, wrasses, crinoids, brittlestars, bivalves, and sponges were observed in this station.

Statistically, there was a significant difference between the macrofauna density of whip and branching black corals ($p < 0.05$). However, there was no significant difference between the macrofauna density across sampling stations ($H=8.1778$; $p= 0.1467$), density at varying depth ranges ($R=0.1601$; $p= 0.3236$), and density at different colony sizes ($R=0.1772$; $p=0.2738$).

DISCUSSION

Data suggest that from depths of 15-40 m, across all sampling sites, black corals, regardless of types, can be considered favorable habitat for certain macrofauna. It appeared that black coral colonies served as essential habitats of these organisms either by direct or indirect associations such as utilizing colonies as habitat, feeding ground, or shelter area. One reason is because these depth ranges are relatively devoid of scleractinian corals, making the black corals suitable for direct or symbiotic associations with other species. In effect, they provide ecological services to other macrofauna in Jagna, Bohol.

The associated macrofauna of black coral in Jagna, Bohol is similar to other species documented in other areas of the world. These include crinoids (Tazioli et al. 2007; Warner 1981; Love et al. 2007), bivalves (Tazioli et al.2007; Bo et al. 2012; Warner 1981), gastropods (Warner 1981), anemones (Warner 1981; Love et al. 2007), barnacles (Bo et al. 2012 and Warner 1981), brittle stars (Tazioli et al. 2007; Love et al. 2007), sponges (Bo et al. 2012; Warner 1981), and fishes (Tazioli et al., 2007). Stalked barnacles were considered the most common associated species in Ecuador (Bo et al. 2012) and Trinidad (Warner 1981) and sea anemones as the most abundant organisms seen to attach to *Antipathes dendrochristos* in California (Love et al. 2007). Our results showed that crinoids were the most abundant and common associated macrofauna. All of these organisms tended to encrust, attach or seek shelter in the black coral colonies.

Certain ecological relationships likewise occur between the macrofauna and black corals. For example, parasitic association also occurs between zoanthids and black corals and can be exemplified by the sea anemone found in the study site that attaches itself to the whip corals to the extent of covering the entire colony, eventually killing the polyps. The death of the colony may be due to its polyps being completely covered by the anemone, making feeding impossible. Excoffon et al. (2009) documented that the sea anemone *Nemanthus californicus* settled and colonized on the branches of the black coral *Myriopathes panamensis*, while Tazioli et al. (2007) observed the same genus of anemone in solitary or groups attaching itself to the branches of *Antipathes* sp. In the study site, however, only the whip black corals were observed to be colonized by this type of sea anemone and none were observed from all the branching types, contrary to the findings of Excoffon et al. (2009) and Tazioli et al. (2007). Generally, this kind of association is said to affect the survival of antipatharians as exemplified by the results of Bo et al. (2012) wherein 38% of antipatharian colonies hosted zoanthids and 7% of these colonized colonies were completely covered. Association between fishes and black corals was also observed to occur such as damselfishes (Pomacentridae) and gobies (Gobiidae) where the former were seen to be resident of the branching *Antipathes* spp. while the latter literally stayed on the surface of the whip black corals. Tazioli et. al. (2007) explained that Pomacentridae and Gobiidae utilized the black coral colonies for reproduction. The damselfish could lay its eggs on the black coral skeleton at the base of large colonies while the male prepared the nest. Gobies also exhibit obligate association with the black corals, nesting on antipatharian host.

The density of macrofauna species in whip black corals is statistically different compared to that of the branching type. It showed higher density of macrofauna but with lesser diversity and count per colony. In the study area, 37.5% of the whip corals could hold more than one (1) associated species per colony despite the small average colony area (0.00409 m^2) relative to the branching black corals (0.165766 m^2), accounting to the former's high density value. However, results further showed that the diversity is lower because its colony shape is not conducive as shelter to other species. On the other hand, the branching black corals may have lesser density but the diversity and the number of associated macrofauna is greater due to its size and the 3-dimensional arborescent colony shape, making it ideal habitat for various animals (Tazioli et al. 2007). Furthermore, the macrofauna diversity within a single colony may offer other ecological services to other macrofauna species due to the presence of other functional groups, enabling other organisms to survive and increasing diversity.

The two general types of black corals are capable of hosting various macrofauna, and the absence of statistical significance of macrofauna density with regards to depth, colony size, and sampling sites suggests that density of associated macrofauna was very variable spatially across the factors mentioned. In terms of the type of black corals present, there is statistical difference between the associated species found in whip black corals and branching black corals. It is noteworthy to mention that in this study there were more whip black corals than branching ones but it was revealed that regardless of type, they could be considered as habitats especially in areas where no scleractinian corals are present.

The ecological role of black corals in hosting several associated organisms had already been long recognized. Tazioli et al. (2007) noted that they must be considered a keystone group due to their symbiotic relationships with black corals. Regardless of whether the black coral is dead or alive, studies also showed that there is a distinct association that exists between black corals and other species. For instance, there were 2,554 individuals of invertebrates found living on the dead colony of *Antipathes dendrochristos* (Love et al. 2007). In the deeper portion (50-100 m), the black coral *Antipathella subpinnata* of the Mediterranean Sea showed the same ecological role as it creates 3-dimensional habitat to other organisms (Bo et al. 2009). In Jagna, Bohol, our results likewise demonstrated that certain organisms were directly utilizing the black corals mainly as shelter. Given this, black corals therefore promote biodiversity especially in deeper areas with little or no scleratinian corals present. Its role as habitat to various species could have resulted in the coexistence with other species within a colony as shown by the diversity of macrofauna in the branching black corals. From the results obtained, a call to preserve and protect the black corals is urgently needed as they show equal capacity to potentially host associated macrofauna. Lastly, the Marine Protected Area of Jagna, Bohol, situated in proximity to the study site, must be extended to accommodate the black corals found in this area.

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