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Diversity and Toxicity of Shallow Water Sponges in Tubajon Coastal Area, Laguindingan, Misamis Oriental

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ABSTRACT

Sponges are sessile organisms that thrive in unique physical and biological environments. In such conditions, their abundance and distribution are directly affected by disturbances and predations which they tend counter by producing toxic secondary metabolites. Tubajon, Laguindingan, Misamis Oriental is known to be a mangrove sanctuary, this study aimed to determine the diversity of shallow water sponges and further determine if they exhibit toxicity activity. A total of 136 individuals from seven species (Species A-G) of sponges were recorded in the five sampling sites covered. Sp-A ranked highest in abundance and were found in all sampling sites. Only Species A, B, C and G were found associated with the mangrove *Rhizophora sp*. (bakauan). However, in some site, species A and B were also found attached to dead logs and sea grasses while others, on the sediments. Based on the Canonical Correspondence Analysis (CCA), the abundance of species B, D, and E in sites 1 and 3 were positively affected by salinity, temperature, dissolved oxygen and pH. In contrast, the abundance of species G in site 5 was positively affected by humidity alone. Species A, F and C were not affected by these conditions since they were present in all sampling sites. Both methanol and aqueous extracts of Species A did cause brine shrimp lethality at concentrations at 500ppm and 1000ppn except methanol extract at 500ppm because of zero mortality after 24 hours. The aqueous extract gave the highest percent mortality of 50 at 100 ppm. Lethal concentrations (LC_{50}) after 24 hours for all extracts in both species were all the same at LC50> 1000ppm. It is recommended that more confirmation in identification using spicules and the use of other extraction methods and inclusion of antimicrobial assay be done for further studies.

Keywords : sponges, diversity, toxicity, brine shrimp lethality test assay, biodiversity

INTRODUCTION

Sponges are the oldest and simplest metazoans that have evolved 500 million years ago. Sponges are belonging to Phylum Porifera. Most sponges are predominantly marine invertebrates and have a simple body plan with perforations where water can pass through. They come in an incredible variety of shapes, sizes and colours (Lutta et al., 2008). Their diversity is mainly due to their adaptation to different environments, from tropical marine waters to polar areas (Dickey et al., 2012; Lavrov, 2011; Muller & Thankur, 2004).

One distinct group is the mangrove-associated sponges that constitute a large portion of the whole community of epibionts on the mangrove roots (Nagelkerken et al., 2007). During their adult stage, they bind onto stable substrates and become sedentary/sessile residents (Ruppert, Fox & Barnes, 2004). Thus, they are considered filter-feeders (Dickey et al., 2012; Lavrov, 2011; Myers, 2001; Taylor et al., 2007).

Because of this characteristic, sponges are apparently vulnerable to other organisms that threaten overgrowth, poisoning, and infection of predation. However, they are able to develop mechanisms to cope with intensive evolutionary pressure from competitors and predators by producing secondary metabolites (Thakur and Muller, 2004). They produce their own toxins through normal metabolism, or in collaboration with many microbes that live inside them. Whatever the source of these toxic chemicals is, many have been found to be highly toxic to other life forms. Thus, these sponges are armed with this arsenal of potent chemical defence agents which are of potential pharmacological interest to humans (Munro et al., 1994; Faulkner, 2000).

This study was conducted to determine the diversity and cytotoxicity of mangrove- associated sponges in Tubajon Coastal Area, Laguindingan, Misamis Oriental. Specifically, this study aimed to: (1) determine the diversity and relative abundance of mangrove-associated sponges in Tubajon, Laguindingan, Misamis Oriental; (2) determine the correlation between the diversity and abundance of

sponges with conditions such as pH, salinity, dissolve oxygen, water temperature, and wet and dry humidity; (3) determine the mangrove species in which these sponges are found associated with; and (4) evaluate the toxicity activity of each of the crude extract of the two most abundant sponge species collected using the Brine shrimp lethality assay.

There are about 7,000 known species of sponges worldwide with the Philippines only having less than 500 species documented. There is still a lot of sponges yet to be discovered in the Philippine waters, hence, the need to conduct this study.

Sponges have been recognized to be a rich source of novel compounds and bioactive secondary metabolites which are hoped to inhibit cancerous growths and other diseases, thus sponges become the focus of many medical and biochemical studies.

STUDY AREA AND METHODOLOGY

Survey of the Sampling Site

The barangay of Tubajon is located within the Municipality of Laguindingan, belonging to the Province of Misamis Oriental, with an area of 237.39 hectares (Department of Agriculture Regional Field Unit 10, 2012). The mangrove forest sanctuary is situated adjacent to the barangay, and is a protected area under the supervision and the protection of the Department of Environment and Natural Resources (Figure 1). Figure 2 is a photograph of the mangroves of the study area.

Figure 1. Map (left) and satellite image (right) of the mangrove forest sanctuary and the sampling sites located at Brgy. Tubajon, Laguindingan, Misamis Oriental, Philippines ($8^{O}37'23.28"$ N $124^{O}28'48.32"$ E)

Figure 2. Photograph of the study area showing mangrove trees with its prop roots where the sponges are attached.

Ethics Statement

A letter of request for sampling was submitted to incumbent local chief executive of the municipality of Laguindingan for approval. The approved letter was presented to the Department of Environment and Natural Resources annex office supervising the Mangrove Forest Sanctuary during sampling, and so, the researchers had to carry out this study.

Data Collection

This study did employ a stratified sampling method. An imaginary line was established parallel to the shore. The imaginary line was divided into five segments, and within each segment sampling sites were chosen. In each site, ten meter (10m) transect lines perpendicular to the imaginary line was established. Three one meter by one meter ($1m^2$) quadrat was laid along the transect line with four meter (4m) distance from one to the other.

Sponge species were visually identified in the field. In situ photographs of each sponge were taken to document natural colour and general morphological description. For all species, 2 or more voucher specimens were collected for closer examination and for taxonomic identification following standard protocol. Sponges were identified based on spicule characters, when present, and skeletal structure, then preserved in 70% ethanol. Proper labels for collected samples were followed (place and date of collection) and each sample was placed separately in a clean container. Ideally, the specimens were identified to species level, however, many of the surveyed sponges may have belonged to novel or little-known species and rare genera, so these were assigned to morphospecies for formal description. Ecological conditions such as salinity, temperature, pH, dissolved oxygen, relative humidity, and total suspended solid were measured twice in each location at each transect in both sampling periods with a multimeter. Where possible, measurements were made at the surface, and 1 and 2 m depths.

Preparation of Methanol Extracts

The Species A and Species B, the most abundant sponges collected and identified were dried by freeze drier and powdered. About 50 g of powder was soaked into pure methanol for 48hrs at room temperature. The obtained solution was filtered and concentrated using the rotary evaporator and freeze drier. The extracts were stored on a fumer to further concentrate it.

Preparation of the Crude Extracts

About 200 grams of the species A and species B were boiled in 500ml of distilled water for about 5 minutes. The solution obtained was filtered and was concentrated by using steam bath and freeze drier.

Hatching of Brine Shrimp Eggs

Brine shrimp eggs were obtained and prepared in the Chemistry Department, CSM, Mindanao State University – Iligan Institute of Technology. The brine shrimp eggs were hatched using sterilized sea water. The sterilized seawater was filtered and poured into a small plastic container (hatching chamber). The container used for brine shrimp hatching, consisted of two unequal chambers with several holes on the divider in between. This would enable the hatched brine shrimp to migrate from the hatching compartment into the illuminated compartment (Sharma et. al., 2013; Pisutthanan et al., 2004). The shrimps were allowed to hatch and mature as nauplii (larva). After two days, the hatched brine shrimps were ready for BSLT (Olowa &

Nuneza, 2013).

Brine Shrimp Lethality Assay (BSLA)

For a rapid and convenient preliminary assessment of toxicity of the sponge extract, BSLA was employed. Brine shrimp (*Artemia salina*) eggs/cysts were allowed to hatch in sterile seawater (after incubation for 24 hours under illumination) at room temperature (Meyer et. al, 1982). Hatched nauplii were collected using Pasteur pipet. Ten nauplii were delivered to each 5-mL crude extract (dissolved in sterile seawater) at concentrations of 100, 500, and 1000 ppm. The tests for each concentration were done in triplicates. The numbers of the dead and alive nauplii were recorded after 6 and 24 hours. The positive standard used was methanol. Nauplii were considered dead if no internal or external movement observed for 30 seconds. The percent mortality of the brine shrimps and the LC50 (Median Lethal Concentration) values of each crude extract were then calculated. This was done by plotting the percent mortality against the logarithm of the concentration of the crude extract.

$$\text{Percent Mortaliy} = \frac{Accumulated\ Dead}{Accumulated\ Alive + Accumulated\ Dead}\ X\ 100\%$$

Statistical Tools

Canonical Correspondence Analysis (CCA) and biodiversity indices were applied using the software Paleontological Statistical Tool (PAST) to determine the relationship of the different physicochemical parameters to the abundance of species.

RESULTS

A total of 136 colonies from seven species (Fig. 3) of sponges were recorded in the five sampling sites (Table 1). The highest number of individuals was both recorded in Site 1 and Site 2. The highest species richness (6) was documented in Site 1 and the lowest species richness (3) was recorded in Site 5. Species A (Green) ranked highest in abundance and were found in all sampling sites while species G yellowish white was found only in site 5. Only species A, B, C and G were found to be associated with the mangrove *Rhizophora* sp. ("bakauan") since they were all found attached to its prop roots (Fig. 4) in some sites, however species A and B were also found attached to dead logs and sea grasses while the remaining species were found growing on the sediments.

Table 1. Species Composition, Number of Individuals and Relative Abundance of Sponges In Five Different Sampling Sites.

Sponges	Number of Colonies (Relative Abundance in %) Sampling Sites					
	1	2	3	4	5	Total
Sp-A Green	20(62.5)	23(71.9)	10(40)	13(72.22)	20(69)	86(63.24)
Sp-B Pink	6(18.8)	1(3.13)	11(44)	-	-	18(13.24)
Sp-C Brown Thorny	1(3.13)	6(18.75)	2(8)	2(11.11)	1(6.9)	12(8.82)
Sp-D White Thin	2(6.25)	-	-	-	-	2(1.47)
Sp-E White Huge	2(6.25)	1(3.13)	1(4)	2(11.11)	-	6(4.41)
Sp-F White Muddy	1(3.12)	1(3.13)	1(4)	1(5.6)	-	4(2.94)
Sp-G Yellowish White	-	-	-	-	8(27.6)	8(5.9)
Total number of individuals	**32**	**32**	**25**	**18**	**29**	**136**
Total number of species	**6**	**5**	**5**	**4**	**3**	**7**

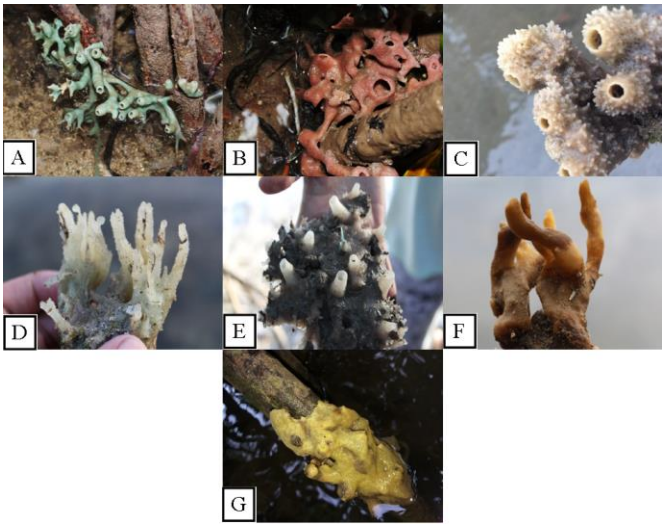

Figure 3. Seven species (A-G) of sponges found in five sampling sites in Tubajon, Laguindingan, Misamis Oriental.

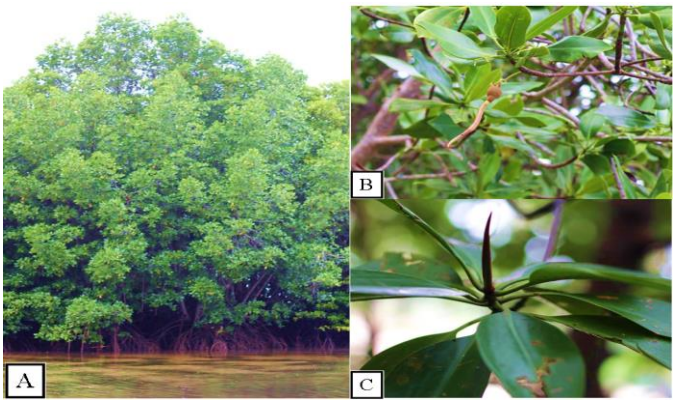

Figure 4. Photograph of the mangrove *Rhizophora* sp. A) whole plant B) fruit and C) shoot.

In Table 2, the Shannon's Diversity index classifies the area to have low diversity if the index value is below 1; moderate if between 1 and 2; and, high diversity if the value is greater than 2. Moderate

species diversity was observed in sampling sites 1 and 3 while low species diversity was in sites 2, 4 and 5. Site 5 had the most even distribution. Evenness value was influenced mainly by the competition of species for food and territory within an area. Hence, competition of species in this site was less compared to other sites. Low evenness value (E = 0.4803) and high dominance value (D = 0.5547) in Site 2 are due to the high abundance of *Sp A green.*

Table 2. Biodiversity indices in five sampling sites.

Biodiversity Indices	Sampling Sites in Tubajon, Misamis Occidental				
	Site 1	Site 2	Site 3	Site 4	Site 5
Species Richness	6	5	5	4	3
Shannon's Diversity (H')	1.171	0.8761	1.187	0.8839	0.7276
Dominance (D)	0.4355	0.5547	0.3632	0.5494	0.5529
Evenness (E)	0.5374	0.4803	0.6557	0.6051	0.6901

Figure 5 shows the effect of water temperature, relative humidity, dissolved oxygen, salinity and pH on the abundance of species in different sampling sites. It can be seen that the abundance of species B, D, and E in site 1 and site 3 were positively affected by salinity, temperature, dissolved oxygen and pH. In contrast to this, abundance of species G in site 5 was positively affected by humidity. Species A, F and C were not affected by these conditions since they were found in all sampling sites.

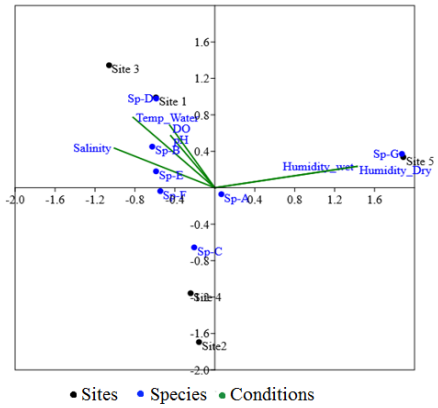

Figure5.Ordination diag\ram showing five environmental factors: temperature, relative humidity, dissolved oxygen, salinity and pH.

Brine Shrimp Lethality Assay

Table 3 shows that both methanol and aqueous extracts of Sp-A did cause brine shrimp lethality at concentrations 500 and 1000 ppm except methanol because of zero mortality at 500 ppm after 24 hours. The aqueous extract gave the highest mortality of 50% at 1000 ppm. The same trend was observed in Sp-B (Table 4) that only at 500 and 1000 ppm exhibited %mortality but was higher in methanol extract at 21%, unlike, that of Sp-A in aqueous extract.

Table 3. Brine Shrimp Larvae Mortality After 24-hours Exposure to Different Concentrations of Decoction and Methanol Extracts of Sp-A (Green)

Species A (Green)	Mortality at Varying Concentration (%)			LC_{50} (ppm)
	100	500	1000	
Methanol Extract	0	0	33.3	LC_{50} > 1000ppm
Aqueous Extract	0	8.51	50	LC_{50} > 1000ppm

Table 4. Brine Shrimp Larvae Mortality After 24-hours Exposure to Different Concentrations of Decoction and Methanol Extracts of Sp-B (Red)

Species B (Red)	Mortality at Varying Concentration (%)			LC_{50}
	100	500	1000	
Methanol Extract	0	5.36	21.21	LC_{50} > 1000ppm
Aqueous Extract	0	3.45	12.5	LC_{50} > 1000ppm

No difference in lethal concentration (LC_{50}) after 24 hours for all extracts in both species since they all had LC50 >1000 ppm (Tables 3 and 4) even if Sp-A aqueous extract had higher % brine shrimp mortality as shown in Fig. 6.

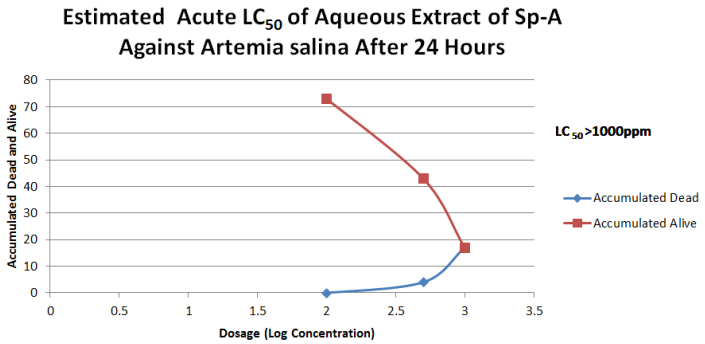

Figure 6. LC_{50} of Sp-A Aqueuos Extract against *Artemia salina* After 24 Hours

DISCUSSION

Distribution and Abundance of Sponges

Compared to other studies conducted of about diversity of sponges in mangrove ecosystems, the number of species and the abundance of each species in this study are low. For, instance, the study of Alcolado (1991) at Punta del Este, Cuba, estimated 50–80 individual sponges per meter of shoreline, while the study of Engel and Pawlik (2007) in the Florida Keys, U.S.A., counted 1195 sponges comprising ten species that occupied 73.5% of available mangrove root space. The low species number and low abundance of each species is probably due to the age of the mangrove area which is below 30 years when this study was conducted. In addition, these mangrove areas were man-made and were planted with one species of mangrove only.

The diversity and abundance of mangrove sponges may have been affected by different disturbances present in the area. These disturbances include the residents' activities such as grazing on molluscs, as well as, conditions such as light and wave actions. According to Longakit (2005), these disturbances may play an important role affecting sponge distribution and abundance. Wave stress may limit the colonization and growth of sponges by generating substrate instability, high turbidity and turbulence (Diaz *et al.,* 1985). In this study, the wave action may be the reason why most sponges in the study area were found in areas not directly affected by wave actions. The study of four mangrove islands in Belize, Farnsworth and Ellison (1996) found that sponge diversity and abundance was greatest on the leeward rather than the windward side of islands where wave action is seen. As primarily nearshore, estuarine habitats, mangroves are strongly influenced by abiotic factors such as freshwater runoff, sedimentation, and rapid temperature fluctuations from the influence of sun and wind on tidally driven shallow water (Nagelkerken, 2007). Moreover, other abiotic factors such as salinity, pH, water temperature, dissolved oxygen and humidity may also affect distribution and abundance of sponges. In this study, however, there were only slight differences of the conditions among the sites. Though, the results of the study suggest that species of sponges were affected by specific conditions, it is hard to say that the slight differences of conditions are affecting the distribution and abundance of sponges among the sites.

BRINE SHRIMP LETHALITY TEST (BSLT) ASSAY

The researchers used the two most abundant and most common sponges present in the study area against the brine shrimp since their abundance maybe an indicator of resistance against different environmental conditions and predators. Since sponges are sessile organism, they produce secondary metabolites to defend themselves from predators (Hussain, 2012). However, as shown in the results, the extracts were non-toxic (LC_{50} > 1000ppm). The results were interpreted using the criterion of sponges' toxicity set by Clarkson (2004): LC50 >1000 ppm is nontoxic, LC50 500-1000 ppm is low toxic, LC50 100-500 µg/mL is medium toxic, and LC50 0-100 ppm is highly toxic. The non-toxic results of the study could be due to the type and methods of extractions used. Extraction method like solvent extraction and partitioning maybe used for further toxicity studies of these species. Nevertheless, it is important to note that the methanol and aqueous extract of both species (Sp-A and Sp-B) showed mortality against the brine shrimp under 1000 ppm concentration and after 24 hours, especially, the 50% mortality showed by the aqueous extract of Sp-A. Moreover, comparing the percentage mortality from both extraction methods did not show pattern in terms of which extraction would exhibit higher percentage mortality. This could be due to the type of secondary metabolites present in each sponge. The kind of secondary metabolites sponges produced is associated to the kind of organisms preying on them (Hussain, 2012).

RECOMMENDATIONS

This study recommends the following: (1) identification of the sponges using their spicules which was done initially but needs further confirmation; (2) inclusion of all species sampled for screening against toxicity and antimicrobial assay; (3) method of extraction used should be appropriate with the specific metabolite present

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