Comparative studies of *Echinometra mathaei* species complex (Echinoidea: Camarodonta: Echinometridae) from two sites in Western Visayas, Philippines (Taklong Island, Guimaras and Nabas, Aklan)

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Abstract

Echinometra mathaei is a species complex with its constituent reproductively-isolated species informally called A, B, C and D based on studies done in Okinawa and the Red Sea. Little research has been done on this genus, and to our knowledge no studies have been done on the *E. mathaei* complex in the Philippines. To help clarify species delineations in the *E. mathaei* complex, a comparative study was done between two localities in Western Visayas, Philippines: the Taklong Island National Marine Reserve, in Nueva Valencia, Guimaras and Barangay Unidos in Nabas, Aklan. Morphological characteristics (spine color, milled rings, and skin around the peristome) and tubefeet and gonad spicules were observed. Two or possibly three species of *Echinometra* were found in the two sites based on their morphology and spicules, namely: *Echinometra* sp. A, *Echinometra* sp. C, and *Echinometra* affinity C, which resembles sp. C but differs in the milled rings and gonad spicules. *Echinometra* sp. C and *E.* affinity C cannot be distinguished on the basis of field-visible characters, thus the two morphs are referred to as *Echinometra* VC for the purpose of field surveys. *Echinometra* VC and *Echinometra* sp. A exhibited differences in abundance (VC was much more common) and microhabitat (VC was restricted to rocky shores and never observed in coral communities). To study the abundance and distribution of Echinometra VC, 50 m by 2 m belt transects were surveyed along the rocky shores of both sites: two parallel transects (at 0 m and at 0.9 m) and a perpendicular transect (only in Nabas), each with three replicates. The transect data showed that the mean densities for 0m and 0.9 in Nabas are significantly higher in Taklong yielding p-values of 0.001 and 0.002, respectively, when analyzed using t-test. Of the two sites, only Nabas showed a significant

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difference between the mean densities at 0 m and 0.9 m, with the mean density at 0.9 m significantly higher than that of 0 m yielding a p-value of 0.02 when analyzed using two-sample t-test. A Poisson regression on the perpendicular transect data from Nabas showed a trend of increasing *Echinometra* density with increasing distance from the shore. In the future, DNA barcoding and cross-fertilization studies should be performed in order to confirm the species of *Echinometra* observed. Many factors can affect the density and distribution of *Echinometra*, so further studies must be conducted to explain observed differences in their distribution and abundance.

Introduction

Sea urchins are also important consumers of benthic algae on reefs, thus they mediate competition between corals and algae. Urchins also contribute to bioerosion, and the production and reworking of sediments and the burrowing and feeding behaviors of urchins moderate the balance between coral erosion and algal growth (reviewed in Johansson et al., 2013). *Echinometra* is one of the more abundant bioeroding sea urchin genera in the Indo-West Pacific (IWP). Earlier studies suggested that two species of this genus occurred in the IWP, namely *Echinometra mathaei* which was described by Blainville (1825) based on a specimen from Mauritius, and *Echinometra oblonga* which was also described in the same paper and recorded based on two rather small individuals. However, the type locality of *E. oblonga* was unknown. Döderlein (1906) transferred *E. oblonga* to a separate genus, *Mortensia oblonga* because of the gonad spicule morphology, but Mortensen (1943) considered these as morphs and gave a trinomial name, *E. mathaei oblonga*, thus synonymizing the two taxa. An extensive study by Kelso (1970) on the ecological distribution, test morphology, gonadal spicules, and gamete compatibility of these two morphs strongly suggested that they were separate species, *E. mathaei* and *E. oblonga* (Arakaki et al., 1998). On the basis of cross-fertilization experiments, morphology, and genetics, the presence of four types of *Echinometra mathaei* were reported in Okinawa, informally referred to as *Echinometra* species A, B, C, and D (Uehara and Shingaki, 1985; Palumbi and Metz, 1991). Members of the *E. mathaei* species complex also occur sympatrically across the IWP (Matsuoka and Hatanaka, 1991 as cited in Bronstein and Loya, 2013; Uehara et al., 1996; Arakaki et al., 1998; Landry et al., 2003). The species-level taxonomy of the genus has yet to be completed (Bronstein and Loya, 2013). The number of valid species in the genus *Echinometra* (Echinodermata: Echinoidea) have been a subject of debate in the scientific literature for over 180 years. Furthermore, the currently available morphological keys are of limited utility in delineating all species within this genus (Bronstein and Loya, 2013).

Echinometra mathaei scrapes surfaces in the process of grazing (Coppard and Campbell, 2006). Bioerosion by *Echinometra* at high densities has been a limiting factor for the growth and survival of coral ecosystems (Bronstein and Loya, 2013). To our knowledge, in the Philippines no studies on *Echinometra* have been conducted, thus there are no data regarding the occurrence of members of the *E. mathaei* species complex, neither is the abundance and distribution of species known. The checklist of Philippine sea urchins of Mooi and Munguia (2014) reported seven species from the family Echinometridae, with *E. mathaei* the sole species reported for the genus *Echinometra*. It was not specified in their report, however, if the un-named members of the species complex occur in the country. Therefore, there is a need to study *Echinometra* in the Philippines.

The study was conducted in two sites in the Western Visayas region of the Philippines, namely Taklong Island National Marine Reserve in Nueva Valencia, province of Guimaras and Barangay Unidos, Nabas in the province of Aklan. The general objective of this study is to describe and compare the morphology and distribution of *Echinometra* in Taklong Island National Marine Reserve, Guimaras and in Barangay Unidos in the municipality of Nabas in order to: (i) identify the species or species complex composition of *Echinometra* in the two sites of collection based on the morphological characteristics and the spicules from the gonads and tubefeet, (ii) determine the abundance of *Echinometra* populations in the two sites, and (iii) find out if there are microhabitat differences among *Echinometra* species complex within and between the two sites.

Materials & Methods

Sample Collection

<u>Filing of Necessary Permits</u>

Before the intended date of sample collection, we applied for the necessary permits to the respective agencies or organizations responsible for granting access and permission to sample in Taklong Island. A courtesy visit to the Nueva Valencia Barangay Hall was also conducted to inform the officials of the intended plan of action. Printed thesis proposals were provided to the officials. The Unidos Barangay Hall was visited to ask permission for samples collection.

<u>Sample Collection and Microhabitat Survey</u>

Samples were collected on October 2018 from Taklong Island National Marine Reserve in the Municipality of Nueva Valencia, Province of Guimaras and on November and December 2018 from Barangay Unidos in the Municipality of Nabas, Province of Aklan. The specimens were located by snorkeling or walking along the shoreline of the sampling site.

All possible microhabitats (rocky shore: sub-to-intertidal zones and coral reef) were surveyed. The urchins were then excavated from their burrows using a hammer and a chisel and were individually labelled with a unique field tag using Dymo tape attached to a cable tie. The urchins were then placed inside a container filled with seawater and brought back to the laboratory.

<u>On-Site Data Gathering</u>

Upon the obtaining of specimen, on-site data were recorded and written on a slate. Data such as time, description of habitat and the specimens' locations and estimated depth of collection were recorded.

Recording of Morphological Characteristics

The urchins' morphological characters were then individually observed following the array of characters in Arakaki et al. (1998). The test size (length, width, and height) was estimated using a ruler due to the unavailability of thin-blade Vernier calipers. The spine colors were recorded, as well as the presence or absence of the white tips. Milled rings were then classified as bright or dark. Due to the subjectivity of the color of the milled rings, the milled rings of *Echinometra* sp. A was used as the basis for "bright" milled rings. The skin on peristome was also observed and were classified as either dark or bright. Table 1 shows the summary of the morphological characteristics of the *Echinometra* species.

Photography of Specimens

The specimens were placed in a tank with the bottom surface covered in black textile to improve visibility of details. The tank was then filled with seawater such that the urchin spines were covered. Two automatic flashes were installed beside the tank to provide maximum lighting and the camera flash was covered by a paper to avoid reflection. Both oral and aboral sides were photographed. The specimens were photographed using a Nikon D700 Digital Single Lens Reflex (DSLR) camera with a 60 mm f2.8 Nikkor macro lens.

Gonad and Tubefeet Spicule Observation

To observe the gonad spicule morphology, a piece of gonad tissue approximately 1 cm by 1 cm in size was obtained using scissors. The gonad tissues were individually soaked in store-bought commercial bleach to dissolve the unnecessary tissues, rinsed with distilled water, placed on a slide, and covered with a cover slip. The slide was then examined under the microscope and the types of gonad spicules were identified following the papers of Arakaki and colleagues (1998) and Bronstein and Loya (2013). For the tubefeet spicules, tubefeet were clipped and soaked in bleach for a few seconds in order to have a good visual of the spicules. It was then rinsed with distilled water, placed on a slide and covered with a cover slip. Photos of the spicules of each specimen were then taken through a compound microscope. Table 2 shows the summary of the spicule characteristics based on Arakaki and colleagues (1998).

Preservation of Specimens

The specimens were placed inside glass jars and filled with analytical grade ethyl alcohol for preservation of most morphological characteristics. The jars were then labelled according to the respective specimen code.

Transect Survey

Belt transect surveys were conducted on December 2018 in Nabas and February 2019 in Taklong Island (satellite images of both sites in Appendix B). At each site, *Echinometra* abundance was surveyed parallel to shore at 0 m and parallel to shore at 0.9 m Additionally, at Nabas, urchin density was also surveyed perpendicular to the shoreline from the tide mark outwards.

For each type of survey, three replicates of 50 meter by 2 meter belt transects were surveyed. Thus a total of 6 transects were surveyed in Taklong Island (three at 0 m and three at 0.9 m) and 9 transects for Nabas (three each at 0 m and 0.9 m, and three perpendicular transects). When doing the belt transect survey, a one-meter stick was used by the researcher to survey each side of the transect (i.e. one meter per side of the transect). The transects were then surveyed by counting the number of *Echinometra* one meter to the left and to the right side of the transect tape. In recording the data, the number of individuals per meter of transect length was tallied, to ensure that no individuals were missed in the counting. An assistant aided the researcher in laying and surveying the belt transects in Nabas.

Statistical Analysis

In order to determine if there is significant difference between the mean densities at two depths, a two-sample t-test was run in Microsoft Excel 2013. For the perpendicular transect data, the replicates were binned every 10 meters and their mean densities were calculated. Poisson regression was used in order to find out the effect of increasing distance from the shore on the abundance of *Echinometra.* Poisson regression is used when the outcome variable is a count (i.e. model for count data) and this statistical test is a characterization of complete randomness which excludes any form of dependence between events (Trivedi, 2014).

Results

Morphological Characteristics

The pilot study identified a minimum of two or possibly three *Echinometra* species, based on field characters. These 2-3 species were present in both Taklong and Nabas. A summary of the raw data is provided in Appendix A.

Field identification of *Echinometra* sp. A was unambiguous because of its distinctive white-tipped spines (Fig. 1 A-B). Lab examinations confirmed that *Echinometra* sp. A from the two sites are consistent in their morphological characteristics. Aside from white-tipped spines, the sp. A individuals collected from both sites has bright milled rings and dark skin around the peristome (Table 3). Only six *Echinometra* sp. A individuals were collected, and overall it was not as common as other species.

The more commonly observed type of *Echinometra* displayed various colored spines lacking white tips, and bright-to-faded milled rings at the base of their spines (termed various-colored *Echinometra* or *Echinometra* VC from hereon; Fig. 1 C-F). Various-colored *Echinometra* proved difficult to identify, whether in field or in the lab. This is because aside from the variation in spine color, it was not possible to observe the peristomeal skin while in the field, and the peristomeal skin color quickly changed in the lab. Moreover, the milled rings proved to be difficult to classify, because in addition to the visual subjectivity, most scientific literatures used as reference in this paper did not specify the method of classifying the milled rings as either bright or faded. Various-colored *Echinometra* had a wide variety of spine colors (brown, white, pink, green) and also had heterogeneous morphological character states (Table 3).

Among the *Echinometra* VC, 28 individuals (18 from Nabas and 10 from Taklong) were identified as *Echinometra* sp. C since they have spines without a white tip, bright milled rings, and bright skin around the peristome; all of which are characteristics of species C as described by Arakaki et al. (1998).

Another notable finding in the study are the eleven individuals (three from Taklong Island and eight from Nabas) that possessed a character state that did not quite conform to those of species A, B, and C as listed in Table 1. These 11 individuals had bright milled rings, dark skin around the peristome, and no white tips on their spines (Fig. 1 E-F). The colors of their spines also varied (brown, yellowish-green, white). Because the morphological characteristics (aside from the peristomeal skin) resemble that of species C, these individuals are named *Echinometra* affinity C from hereon.

Gonad and Tubefeet Spicules

Spicules from both the tubefeet and gonads are categorized into four types based on Bronstein and Loya (2013): bihamate, needle, triradiate, and presence of multiple spicule types at once (namely triradiate, needle, and irregular spicules; Figure 2). Spicule types proved to be as variable as spine color. As was reported by Arakaki et al. (1998) for Okinawan *Echinometra*, the spicule types either occur alone or with another type of spicule.

The *Echinometra* sp. A tubefeet and gonad spicules were consistent across the two sites, Nabas and Taklong Island. All six *Echinometra* sp. A collected from the two sites possessed only needle spicules in their gonads and only bihamate spicules were found in the tubefeet (Table 3).

Echinometra sp. C were characterized by the consistent presence of triradiate spicules in both the tubefeet and gonads. In the tubefeet of eight individuals (n=5 in Nabas and n=3 in Taklong), triradiate and bihamate spicules co-occurred. Likewise, in the gonads, multiple spicule types were found eight individuals (n=5 in Nabas and n=3 in Taklong) and both triradiate and needle spicules were found in 14 individuals (n=9 in Nabas and n=5 in Taklong; Table 3).

Echinometra affinity C at both sites had only triradiate spicules in the tubefeet. For the gonads, multiple spicules were found in five individuals from Nabas, while triradiate and needle spicules co-occurred in six individuals (n=3 in Nabas; n=3 in Taklong) (Table 3).

Microhabitat

Taklong Island and Nabas both have habitats suitable for *Echinometra*. There is presence of rocky shore and fringing coral reef, the typical habitats of *Echinometra* species. In Taklong, all (n=15) of the collected various-colored *Echinometra* were from the rocky shores. Two *Echinometra* sp. A were found in Taklong, with one collected at the rocky shore area and the other one collected from a burrow in the coral reefs.

The Nabas *Echinometra* showed similar patterns as those of Taklong. All of the various-colored *Echinometra* that were collected for further study (n=30) were found in the rocky shores. Among the four *Echinometra* sp. A, two were collected from the rocky shores and two from the coral reef. Table 4 shows that various-colored *Echinometra* are limited to the rocky shores, while *Echinometra* sp. A, while much rarer, are found equally on the coral reef and on the rocky shores.

Surveys of *Echinometra* density and distribution

<u>Parallel Transects</u>

Figure 3 presents the *Echinometra* density data for the parallel transects. Densities in Nabas are higher than in Taklong, regardless of the depth surveyed using t-test (Table 6). In both Taklong and Nabas, the mean density at a depth of 0.9 m is higher than mean density at 0 m. Two-sample t-test shows that the densities at 0 m and 0.9 m in Taklong are not significantly different, while in Nabas densities at the two depths are significantly different (Table 5). Raw data from the parallel transects can be seen in Appendices C-D.

<u>Perpendicular Transects (Nabas)</u>

Urchin density (expressed as number of individuals per m^2) from the perpendicular transects in Nabas were binned every 10 meters, and the average of these bins were calculated to show the trend in the densities of *Echinometra* with increasing distance from the shore. Figure 4 shows the line graph of the mean densities of the bins, and the average densities for every bin in Nabas are summarized in Table 7. Raw data from the perpendicula transects can be seen in Appendix E.

A Poisson regression was used to find out the count of *Echinometra* per meter of the perpendicular transect. The Poisson regression shows a 95% coefficient interval of 1.04-1.05 (Table 8). This means that there is a 5% increase in the count of *Echinometra* for each meter increased perpendicularly or the count of *Echinometra* is 1.05 times higher for each meter increased (Table 8).

Discussion

Morphology and Spicules

Echinometra sp. A has a prominent feature, the presence of white-tipped major spines. Hence, field identification of this species is possible. All *Echinometra* sp. A individuals showed homogeneity in their external morphological characters and spicules from both the tubefeet and gonads. *Echinometra* sp. A from both Taklong and Nabas have bright milled rings, dark skin around the peristome, bihamate tubefeet spicules, and needle gonad spicules which are the same characteristics of *Echinometra* sp. A in Okinawa (Arakaki et al., 1998). Therefore, we can say that the bihamate spicules in the tubefeet and needle spicules in the gonads are the distinguishing characteristics of this species, in addition to the white-tipped spines. However, it must be taken into consideration that that sample size of *Echinometra* sp. A is ten times lower than the sample size of the *Echinometra* sp. VC (*Echinometra* sp. C and *Echinometra* affinity C combined). This might explain why no variation was observed in species A, while a lot of variations were noted in *Echinometra* sp. VC.

Echinometra sp. C individuals (n=28) possessed bright milled rings, bright skin around the peristome, and the consistent presence of triradiate spicules in their tubefeet and gonads. Since the morphological characteristics and spicules in the tubefeet and gonads of these individuals conform to the character state of *Echinometra* sp. C as listed in Table 1 and 2, it is likely that these are the same with the Okinawan *Echinometra* sp. C reported by Arakaki et al. (1998).

The *Echinometra* affinity C constitute another subset of the various-colored *Echinometra*. These affinity C individuals have dark peristomeal skin, and only exhibited triradiate type of spicules in the tubefeet. Variability in gonad spicule types was observed, however triradiate spicules are consistently present in the gonads. Since the character state of these individuals does not unambiguously conform to the character state of either species B or C, it might be another undescribed species. In order to determine what species this is, cross-fertilization and genetic studies must be conducted.

Microhabitat, Density, and Distribution

In Nabas, two *Echinometra* sp. A were found on the coral communities and the other two in the rocky shores. In Taklong, one *E.* sp. A was found in the coral communities and the other in the rocky shore. The individuals found in the rocky shores co-occurred in the same microhabitat as *Echinometra* sp. VC. They were found in burrows in close proximity to the burrows of the *Echinometra* sp. VC. *Echinometra* sp. A can, therefore, be found in the coral communities or co-occur with other *Echinometra* species.

The *Echinometra* sp. VC, on the other hand, were found in burrows in the rocky shore area in both Taklong and Nabas, and never in the coral communities. The burrows of the *Echinometra* sp. VC (Figure 5A) were observed in close proximity with each other (Figure 5B). Such aggregations in *Echinometra* were observed by Tsuchiya and Nishihara (1985) in Okinawa and McClanahan et al. (1999) in northern Tanzania. A study by Russo (1977) in Hawaii showed that *Echinometra* burrows were found in the rocky shore areas where the urchins feed on drift algae and detritus. Such a habitat provides the *Echinometra* shelter against intruders (Grunbaum et al., 1978; Neil, 1998). In Okinawa, Arakaki et al. (1998) reported that *Echinometra* sp. C were found in the intertidal zones while Tsuchiya and Nishihara (1984) reported the presence of *Echinometra* sp. B and C in quieter waters. In the case of the current study, the distribution of *Echinometra* sp. VC from Nabas and Taklong agreed with previous reports in inhabiting both exposed and sheltered rocky shore areas.

The density of *Echinometra* VC at 0.9 m is greater than that at 0 m in both sites. However, this difference is only significant in Nabas (with a p-value of 0.02). Moreover, the distribution in Nabas showed a pattern of increasing density with increasing distance from the shore. The highest mean density is recorded at 40-50 m from the shoreline.

Overall, the density of *Echinometra* VC was about five to eight times higher in Nabas than in Taklong. Furthermore, the mean densities at 0m and 0.9m in Nabas are significantly higher in Taklong yielding p-values of 0.001 and 0.002, respectively, when analyzed using t-test. There are numerous factors that may affect the density and distribution of *Echinometra* in their microhabitats such as food availability and predation pressure (Hay, 1984; McClanahan, 1995 as cited in Johansson et al., 2013), wave action, larval supply, water flow, eutrophication, habitat complexity, sedimentation (Johansson et al., 2013), and environmental factors such as desiccation and availability of crevices (McClanahan and Muthiga, 2007). In order to determine which factors affect the densities of *Echinometra* and to explain the results of the surveys, extensive studies about the different factors that affect the population of *Echinometra* must be conducted in the future.

Conclusion

To our knowledge this is the first study reporting on the presence of members of the *E. mathaei* species complex in the Philippines. Based on morphology and tubefeet and gonad spicule types, there are two or possibly three *Echinometra* species present in Taklong Island, Guimaras and Nabas, Aklan. These species are: *Echinometra* sp. A, *Echinometra* sp. C, and a potentially new morph, *Echinometra* affinity C. However, the distinguishing character of *E.* affinity C (i.e., the pigmentation of the peristomeal skin) is not wholly reliable, thus whether or not *E.* affinity C is a distinct species is still uncertain and should be further studied.

Echinometra sp. A can be found in coral communities and can also cohabitate with *Echinometra* sp. VC. However, the sample size of sp. A in this study is relatively low, so determining if this species exhibits a habitat preference needs further investigation. The *Echinometra* sp. VC, on the other hand, were all collected in the rocky shores, and never on the fringing reefs, so it is highly possible that they exhibit a habitat preference. Different factors may be able to explain these differences in microhabitat distribution and densities and in order to fully determine which factors these are and to interpret the results in this manuscript, further studies with regards to these factors must be conducted.

After a careful evaluation of the findings and analysis of the data, the researchers would like to suggest several areas of study for future researchers:

- Since morphological characteristics are not wholly unambiguous in delineating *Echinometra* species, genetic studies and cross-fertilization experiments must be performed. Such studies will establish whether *E.* affinity C is truly a separate species.
- The factors affecting the density and distribution of *Echinometra* ought to be investigated. For instance, why is the density much higher in Nabas than in Taklong? Are density differences explained by habitat differences, or other factors?
- The low density of *Echinometra* sp. A is intriguing. Future studies can be conducted in order to provide explanation for such findings.

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References

Arakaki Y, Uehara T, Fagoonee I. 1998. Comparative Studies of the Genus *Echinometra* from Okinawa and Mauritius. Zool Sci 15: 159-168.

Blainville HM. 1825. Dictionnaire des sciences naturelles, dans lequel un traité méthodiquement des différens êtres de la nature *(par plusieurs professeurs du Jardin du Roi ..., Georges Cuvier, ed.)*. Paris. Vol 37:93–120

Bronstein O, Loya Y. 2013. The Taxonomy and Phylogeny of *Echinometra* (Camarodonta: Echinometridae) from the Red Sea and Western Indian Ocean. PLoS ONE 8(10).

Coppard S, Campbell A. 2006. Taxonomic Significance of test morphology in the echinoid genera *Diadema* Gray, 1825 and *Echinothrix* Peters 1835 (Echinodermata). Zoosystema 28(1): 93-112.

Döderlein L. 1906. Die Echinoiden der deutschen Tiefsee-Expedition. Wiss Ergeb dtsch Tiefsee-Exped 5: 61-290.

Grunbaum H, Bergman G, Abbott D, Ogden J. 1978. Intraspecific agonistic behavior in the rock-boring sea urchin *Echinometra lucunter* (Echinodermata: Echinoidea). Bull Mar Sci 28: 181-188.

Hay M. 1984. Patterns of fish urchin grazing on Caribbean coral reefs:- are previous results typical?. Ecol 65: 446-454.

Johansson CL, Bellwood DR, Depczynski M, Hoey A. 2013. The distribution of the sea urchin *Echinometra mathaei* (de Blainville) and its predators on Ningaloo Reef: The Implication for top-down control in intact reef system. J Exp Mar Bio Eco 442: 29-46.

Kelso D. 1970. A Comparative Morphological and Ecological Study of Two Species of Sea Urchins, Genus *Echinometra* in Hawaii. PhD dissertation, Department of Zoology, University of Hawaii, Honolulu.

Landry C, Geyer LB, Arakaki Y, Uehara T, Palumbi SR (2003) Recent speciation in the Indo-West Pacific: rapid evolution of gamete recognition and sperm morphology in cryptic species of sea urchin.

Matsuoka N, Hatanaka T. 1991. Molecular evidence for the existence of four sibling species within the sea urchin, *Echinometra mathaei* in Japanese waters and their evolutionary relationships. Zool Sci 8:121-133.Bak R. 1990. Patterns of Echinoid bioerosion in 2 Pacific coral reef lagoons. Mar Ecol Prog Ser 66: 267-272.

McClanahan T, Muthiga N, Kamakuru A, Machario R. 1999. The effects of marine parks and fishing on the coral reefs of northern Tanzania. Biol Cons 89:161-162.

McClanahan T, Muthiga N. 2007. Ecology of *Echinometra*. Wild Conser Soc 15: 297-317

McClanahan T. 1995. Fish predators and scavengers of the sea urchin *Echinometra mathaei* in Kenya coral reef marine parks. Environ Biol Fish 43: 187-193.

Mooi R. 2014. Sea Urchins of the Philippines. In: Williams, Gary C., and Terrence M. Gosliner, eds. 2014. The Coral Triangle: The 2011 Hearst Philippine Biodiversity Expedition. California Academy of Sciences, San Francisco, California 94118, USA.

Mortensen T. 1943. A monograph of Echinoidea. Vol. III, 3.Camarodonta. II. Echinoidea, Strongylocentrotidae, Parasaleniidae, Echinometridae. CA Reitzel, Copenhagen, pp 277-439.

Neil J. 1998. Experimental analysis of burrow defense in *Echinometra mathaei* (de Blainville) on Indo-West Pacific reef flat. J Exp Mar Biol Ecol 115: 127-138.

Palumbi S, Metz E. 1991. Strong reproductive isolation between closely related tropical sea urchins (genus *Echinometra*). Mol Ecol 9: 1391-1400.

Russo A. 1977. Water Flow and the distribution and abundance of echinoids (genus *Echinometra*) on an Hawaiian Reef. Mar Freshw Res 28: 693-702.

Trivedi P. 2014. Models for Count Data. Encyclopedia of Health Economics pp. 306-311.

Tsuchiya M, Nishihara M. 1984. Ecological distribution of two types of sea urchin *E. mathaei* (Blainville) in Okinawa Reef Flat. Galaxea 3: 131-143.

Tsuchiya M, Nishihara M. 1985. Agonistic behavior and its effect on the dispersion pattern of two types of sea urchins, *Echinometra mathaei* (Blainville). Galaxea 4: 37-48.

Uehara T, Shingaki M. 1985. Taxonomic studies in the four types of sea urchins, *Echinometra mathaei*, from Okinawa, Japan. Zool Sci 2:1009.

Uehara T , Shingaki M, Taira K (1986) Taxonomic studies in the sea urchin, genus Echinometra, from Okinawa and Hawaii. Zool Sci 3: 1114.

Figure 1

A-B *Echinometra* sp. A (aboral-oral view of JRUNI014); C-D Various-Colored *Echinometra* (aboral-oral view of JRUNI020); E-F Various-colored *Echinometra* affinity C (aboral-oral view of JRUNI024).

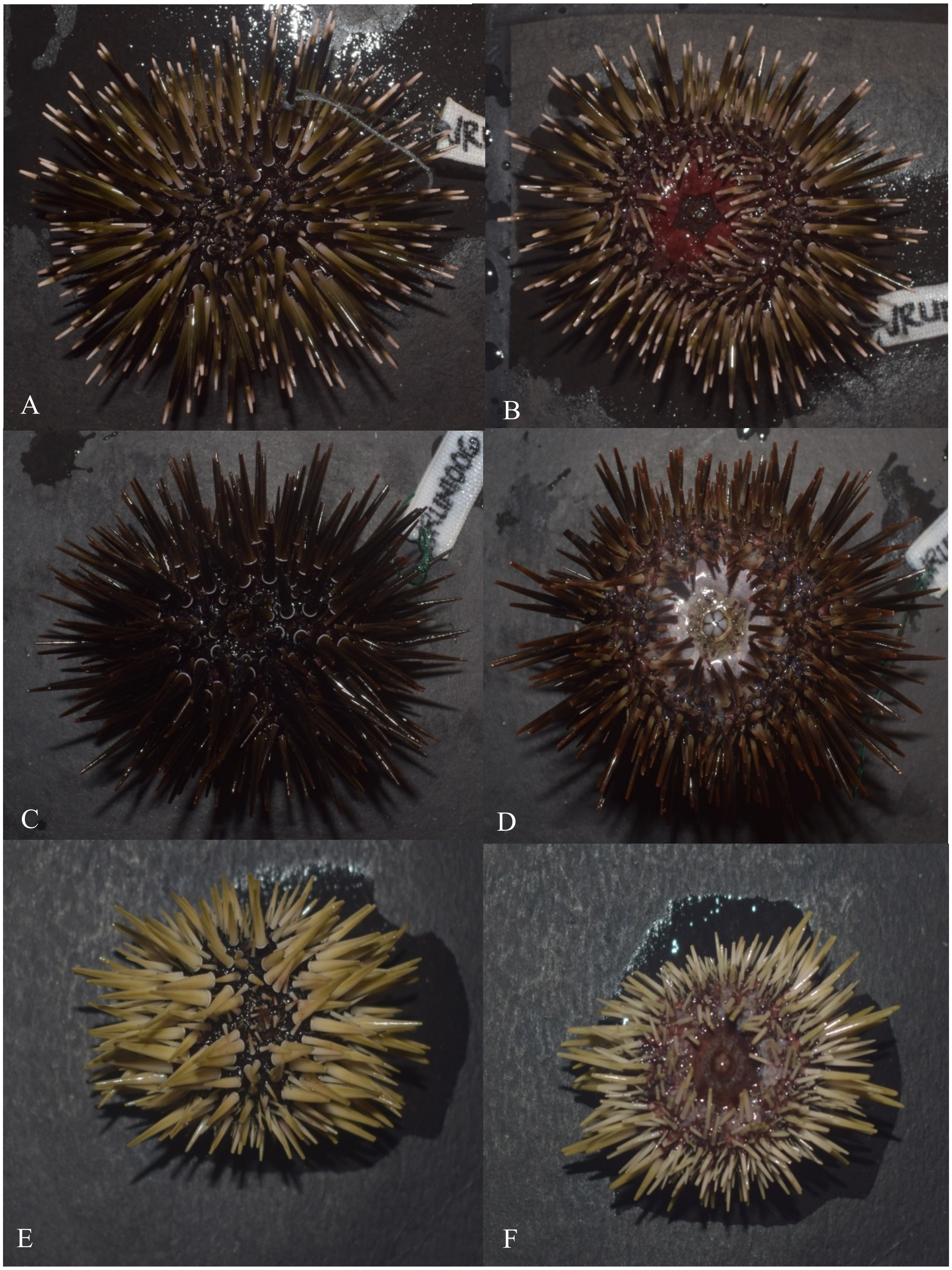

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Figure 2

(A-C) Triradiate spicules in the gonads; (D) Needle spicules in the gonads; (E-F) Irregular spicules in the gonads; (G) Bihamate spicules in the tubefeet

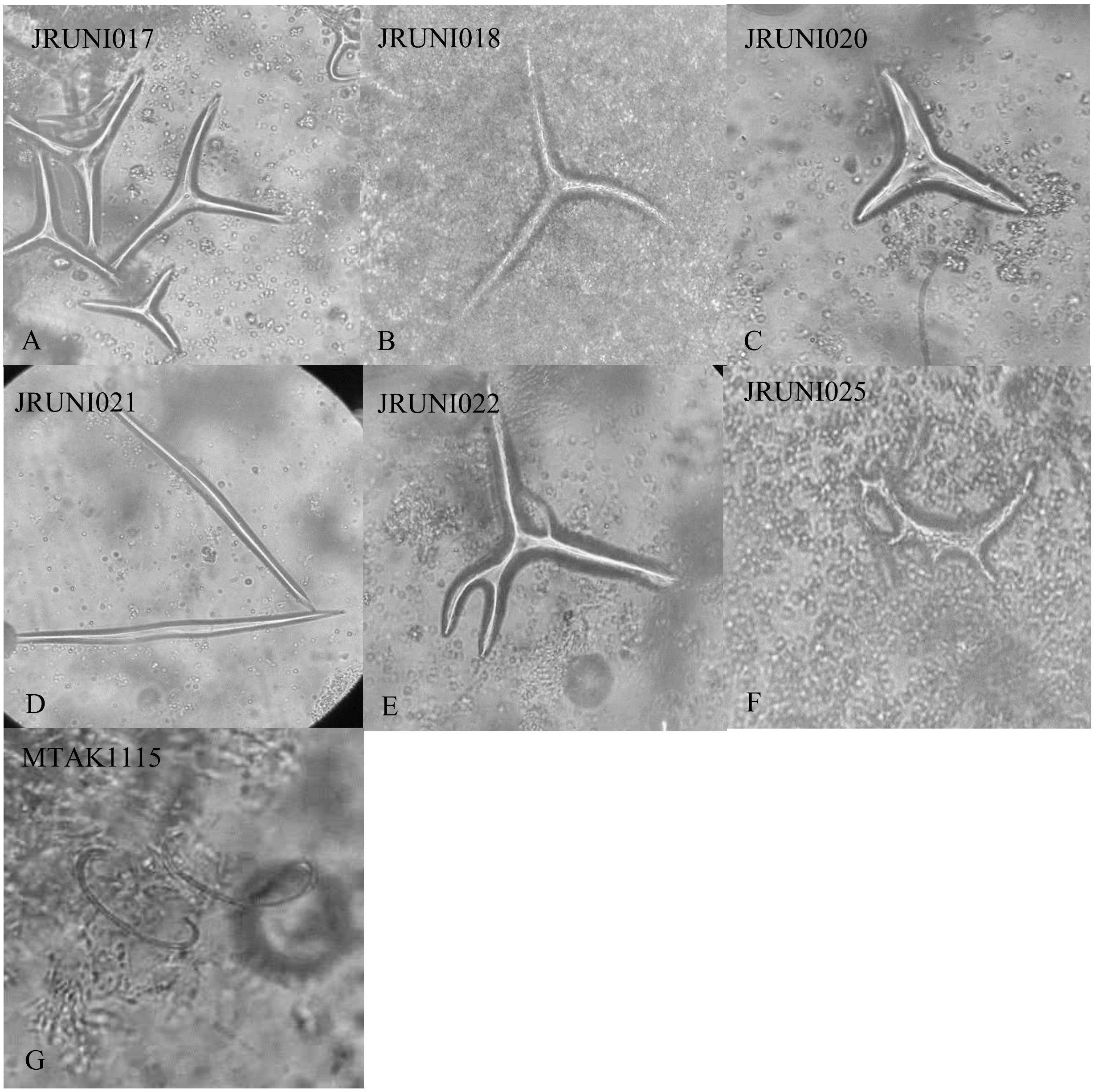

Figure 3

Mean densities of *Echinometra* VC at 0 m and 0.9 m in both Taklong Island and Nabas. Vertical lines are standard deviations.

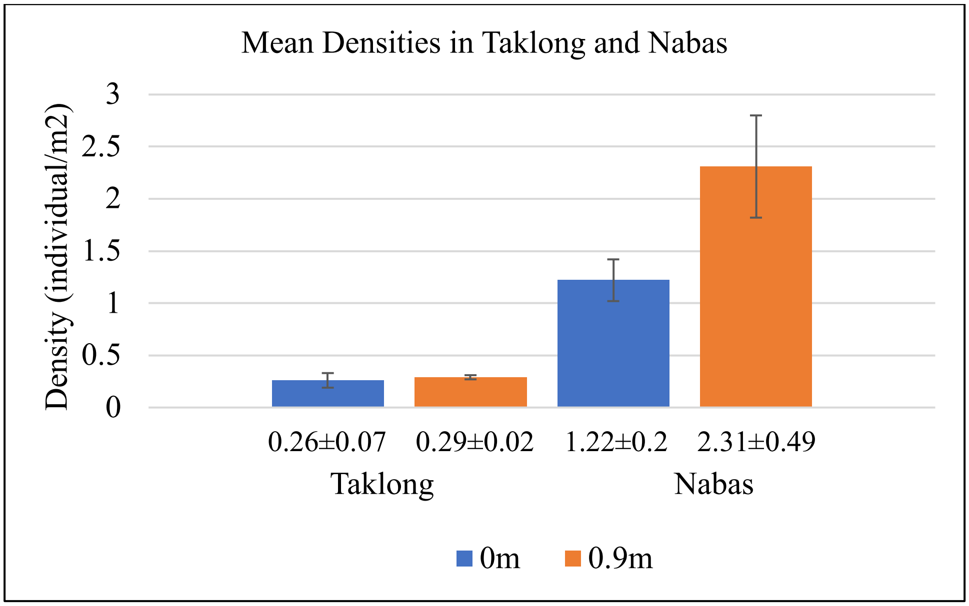

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Figure 4

Mean densities of *Echinometra* with increasing distance from the Nabas shoreline, binned every 10 meters. Vertical lines are standard deviations.

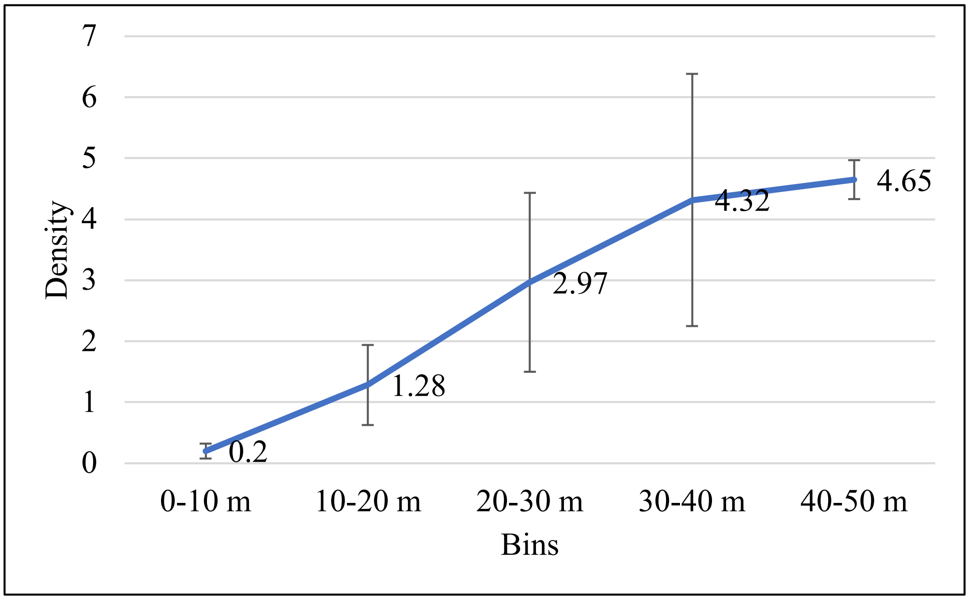

Figure 5

(A)*Echinometra* sp. VC in its burrow in the rocky shore, (B) *Echinometra* sp. VC forming aggregations. Burrows of individuals can be seen in close proximity with each other.

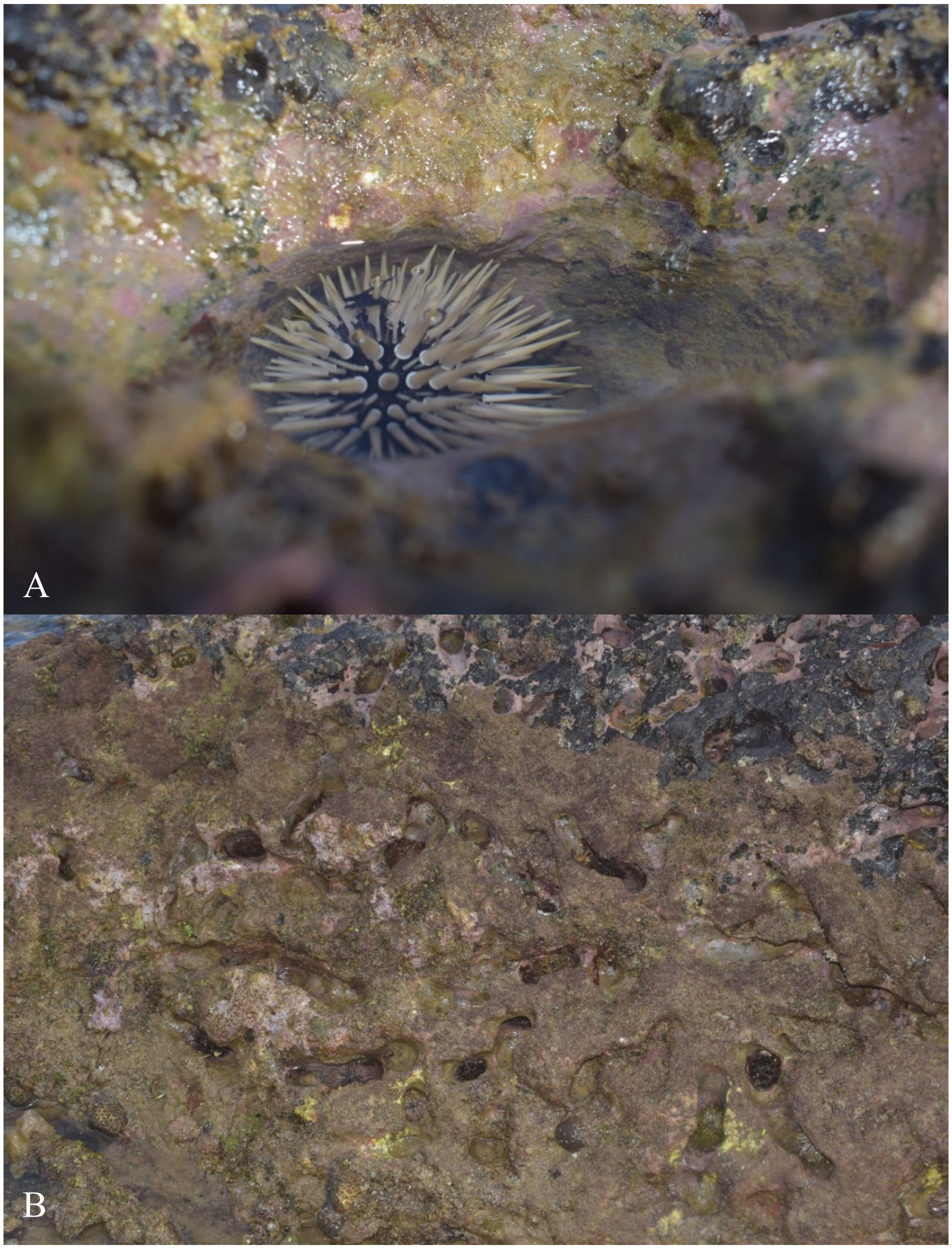

Table 1

Summary of morphological characteristics of *Echinometra* species based on Arakaki et al. (1998)

Species	White tipped spines (+/-)	Milled Rings	Skin around the peristome
Echinometra sp. A	+	Bright	Dark
Echinometra sp. B	- Spines w/ various colors	Dark	Dark
Echinometra sp. C	- Spines w/ various colors	Bright	Bright
Echinometra sp. D (=*E. oblonga*)	- Spines deep black	Dark	Dark

Table 2

Summary of the major types of spicules in each *Echinometra* species based on Arakaki et al. (1998)

	Spicules	
Species	Gonad	Tubefeet
Echinometra sp. A	Needle	Bihamate
Echinometra sp. B	Needle	Bihamate
Echinometra sp. C	Triradiate	Triradiate
Echinometra sp. D (=*E. oblonga*)	Triradiate	Triradiate

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Table 3

Morphological characteristics and spicules of *Echinometra* in both sites. N= total number of individuals collected, n= number of *Echinometra* individuals possessing a specific character states. E. sp. A=*Echinometra* sp. A, E. sp.VC includes sp. C and affinity C (E. aff. C). Multiple spicule type means that triradiate, needle and irregular spicules co-occur. Percentage (%)=number of the individuals per site possessing a specific character state (n/N).

					Spicules		
Site		**Spine Color**	**Milled Rings**	**Skin of Peristome**	**Tubefeet**	**Gonad**	**Percentage in population**
Nabas, Aklan	N=30						
E. sp. A	n=4	With White tip	Bright	Dark	Bihamate	Needle	100%
E. sp. VC	n=26						
	E. sp. C						
	n=4	Various colors	Bright	Bright	Triradiate	-----	15.38%
	n=9	Various colors	Bright	Bright	Triradiate	Triradiate Needle	34.61%
	n=5	Various colors	Bright	Bright	Triradiate Bihamate	Multiple	19.23%
	E. aff. C						
	n=5	Various colors	Bright	Dark	Triradiate	Multiple	19.23%
	n=3	Various colors	Bright	Dark	Triradiate	Triradiate Needle	11.54%
Taklong Island	N=15						
E. sp. A	n=2	With White tip	Bright	Dark	Bihamate	Needle	100%
E. sp. VC	n=13						
	E. sp. C						
	n=3	Various colors	Bright	Bright	Triradiate Bihamate	Multiple	23.08%
	n=5	Various colors	Bright	Bright	Triradiate	Triradiate Needle	38.46%
	n=2	Various colors	Bright	Bright	Triradiate	-----	15.38%
	E. aff. C						
	n=3	Various colors	Bright	Dark	Triradiate	Triradiate Needle	23.08%

Table 4

Distribution and abundance of *Echinometra* in their habitats in Taklong Island and Nabas, as determined through reef-walking and snorkeling. *Echinometra* sp. VC denotes *Echinometra* with various colors and *E.* sp. A denotes white-tipped *Echinometra* sp. A.

	Microhabitat	
Site	Rocky Shore	Coral Reef
Taklong Island n=15	13 VC *Echinometra* 1 *Echinometra* sp. A	*1 Echinometra* sp. A
Nabas n=30	26 VC *Echinometra* *2 Echinometra* sp. A	*2 Echinometra* sp. A

Table 5

Summary of the two-sample t-test results comparing the mean densities at two depths in both sites. Analyzed using 95% confidence level; indicates significance with 0.05 alpha level.

Site	Depth Mean Density ± Standard Deviation		
	0 m	0.9 m	p-value
Taklong	0.26 ± 0.07	0.29 ± 0.02	0.20
Nabas	1.22 ± 0.20	2.31 ± 0.49	**0.02**

Table 6

Summary of the two-sample t-test results comparing the mean densities at 0m and 0.9m between the two sites. Analyzed using 95% confidence level; indicates significance with 0.05 alpha level.

Site	Depth Mean Density ± Standard Deviation		
	Taklong	Nabas	p-value
0m	0.26 ± 0.07	1.22 ± 0.20	**0.001**
0.9m	0.29 ± 0.02	2.31 ± 0.49	**0.002**

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Table 7

Mean counts and mean densities of the bins of the perpendicular transect in Nabas.
Note: Rep.= Replicate; +/-= denotes standard deviation

	Echinometra count			Density of each Replicates (urchins / m^2)			
Bins (m)	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Average Densities
0-10	4	1	7	0.2	0.05	0.35	0.2±0.12
10-20	44	19	14	2.2	0.95	0.7	1.28±0.66
20-30	27	53	98	1.35	2.65	4.9	2.97±1.47
30-40	82	139	38	4.1	6.95	1.9	4.32±2.07
40-50	102	88	89	5.1	4.4	4.45	4.65±0.32

Table 8

Poisson model count with respect to perpendicular distance from the shoreline in Nabas

Coefficient	1.05
95% Confidence Interval	1.04-1.05
P-value	**0.0001***

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